"Strategic Approaches to Strengthen Academic and Industrial Collaboration"

Hosted by: Pharmacy Institutions of Madhya Pradesh,
## Oral Session

**PA: Pharmaceutics and Pharmaceutical Technology**

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PD: Pharmacology, Toxicology, Clinical Pharmacy, Pharmacoepidemiology and Pharmacovigilance

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PE: Pharmaceutical Education, Hospital Pharmacy and Community Pharmacy, Professional Pharmacy, and Pharmaceutical Management and Marketing

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- **PA:** Pharmaceutics and Pharmaceutical Technology
- **PB:** Medicinal Chemistry, Pharmaceutical Analysis, Quality Assurance and Drug Regulatory Affairs
- **PC:** Pharmacognosy and Phytochemistry, Pharmaceutical Biotechnology
- **PD:** Pharmacology, Toxicology, Clinical Pharmacy, Pharmacoepidemiology and Pharmacovigilance
- **PE:** Pharmaceutical Education, Hospital Pharmacy, Community Pharmacy, Professional Pharmacy, Pharmaceutical Management and Pharma Marketing
Gastro retentive floating microparticles for oral delivery of Metformin Hydrochloride

Manoj Kumar and J.S Dangi.
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Abstract: The purpose of this research was to develop a novel Metformin hydrochloride (MH) loaded gastro retentive multi particulate delivery system with floating ability. Prepared microspheres were with defined morphology and pores were clearly visible [1]. The mean particle sizes were found to be between 490±32µm for formulations containing calcium silicate in between 50-60 mg. Their true densities ranged between 1.81±0.19g/cm3 for all the formulations. The formulation porosity of particles was found to be in acceptable range i.e. 60-80%. The Compressibility index ware 14.28±1.21 Formulated micro particles powder had adequate flow ability expressed in terms of angle of repose (≤ 30%). Floating micro particles showed excellent in vitro buoyancy and release pattern. The proposed formulation strategy was based on the development of porous carrier based floating micro particulate carrier system to enhance the bioavailability of MH.

Introduction:
Metformin hydrochloride is an oral hypoglycemic drug used to improve glucose tolerance in patients with type-II non-insulin-dependent diabetes mellitus. Its low bioavailability, short half-life and absorption at the upper gastrointestinal (GI) tract, requiring suitable delivery systems providing sustained and complete release during stomach-to-jejunum transit [2]. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

Material and Methods:
Materials: Metformin hydrochloride (MH) and Calcium silicate (CS) was kindly donated by by M/s Cadila Pharmaceuticals Ltd. (Jammu, India) and M/s Gattefosse (St Priest, Cedex, France) respectively. EudragitS-100 (ES-100) was purchased from S.D. Fine Chemical Limited Mumbai, India). All other chemicals were of analytical reagent grade and were used without further purification.

Methods:
Preparation of MH loaded microsphere: Drug absorbed with calcium silicate (CS) porous system has been prepared as per the reported method of [3] with slight modification. The floating microspheres were prepared by emulsion solvent diffusion technique consisting of (i) CS as porous carrier; (ii) MH an oral hypoglycemic agent; and (iii) Eudragit S as polymer. The microspheres of
metformin without porous carrier were also prepared using same method for comparative study. The effect of process variables like polymer concentration, stirring rate, temperature, amount of porous carrier and concentration of aqueous phase on the particle size, buoyancy, drug entrapment efficiency and drug release were studied.

**Characterization of floating microspheres:** The microspheres were subjected to extensive characterization such as particle size, morphology and different micrometric properties. The size of particles was measured by microscopic technique. The morphology of the micro particles and calcium silicate were studied by scanning electron microscopy (SEM) (Philips CM12, Eindhoven Netherlands). The entrapment efficiency and floating behavior of micro particles was determined by as previously reported [4]. Drug release profiles were generated for each micro particle formulation in terms of cumulative drug release (%, w/w) vs time. Drug release mechanism from the micro particles was analyzed using in vitro dissolution data fitted to zero order, first order, Higuchi release model and Peppas model.

### Results and Discussion:

Improved floating behavior of the microspheres may be due to the porous nature of the microspheres and entrapment of CS of low true density. Euragit S acrylic polymer based micro particles containing CS as porous carrier remained buoyant but because of the high aqueous solubility of metformin the release could not be sustained. The mean particle size was found to be between 490±32µm for formulations containing CS in between 50-60 mg. Their true density of formulation was 1.81±0.19g/cm$^3$ for all the formulations. The porosity of micro particles was found to be in acceptable range of 60-80%. The compressibility index were 14.28±1.21 Prepared formulations showed the angle of repose 41.2±3º. Thus non-aggregated floating microspheres were produced. Drug loading & encapsulation efficiency was found 25.47±10% & 75.36±12% respectively Release kinetic parameters are shown in Table 1.

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<th>Higuchi-matrix $r^2$</th>
<th>Peppas-Korsmeyer $r^2$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non floating</td>
<td>0.9871</td>
<td>0.9072</td>
<td>0.9933</td>
<td>0.9922</td>
<td>0.666</td>
</tr>
<tr>
<td>Floating</td>
<td>0.9933</td>
<td>0.9537</td>
<td>0.9935</td>
<td>0.9953</td>
<td>0.743</td>
</tr>
</tbody>
</table>

The spherical in nature as evident by scanning electron microscopy and their internal structure showed small cavities which responsible for floating behaviour of micro particles. All formulations showed good floating ability and more than 80% of particles float for at least 4 h. The pattern followed the Higuchi matrix model and Peppas- Korsmeyer model. The presence of calcium silicate in micro particles reduces the drug release of drug cumulatively released in 8 h, this showed sustained
release ability of microspheres.

**Conclusion:**

From the present study it is concluded that the calcium silicate based floating microparticles could be used for sustained release of MH in gastric transit which may improve bioavailability and eventually lead to better patient compliance.

**Acknowledgments:**

Authors are thankful to Birla Institute of Technology, Ranchi, India for providing scanning electron microscopy facilities.

**References:**


Evaluation of uptake potential by dendritic cells pulsed antigen loaded elastic liposomes

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2School of Biological Sciences, Dr. H. S. Gour Central University, Sagar (M.P.), India.
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Abstract: Transcutaneous immunization (TCI) is a promising modality with both practical and immunological merits. This non-invasive mode of delivery has the ability to target the rich immunologically milieu of skin. It can offer an effective immune response systemic as well as mucosal and also has the capacity to develop effective vaccine for safe and effective delivery. In the proposed study antigen loaded elastic liposomes were used for better uptake potential by dendritic cells (DCs). Elastic liposomes were prepared by hand shaking vesicles method and characterized for various parameters like vesicles size, shape and entrapment efficiency. Better uptake potential of the system by DCs was studied by flow cytometry and internalization of vesicles by spectral bio-imaging. Results indicated the better uptake potential of system and suggested its use for vaccine delivery via skin.

Introduction:
Transcutaneous immunization (TCI) a form of non-invasive or needle free vaccination is a new that employs topical application of antigen on the skin that can evoke potent antibody and cell-mediated immune responses. This modern technique requires simple introduction of antigens to the host and possess both practical as well as immunological merits. Dendritic cells (DC) are antigen presenting cells (APCs), which gained attention owing to their potential for initiation of strong T-cell-dependent immune response. DCs play the major role in the generation of immune response to antigenic proteins in the skin. These cells phagocytose the antigen that migrates to the draining lymph node where the antigen is presented to T-cells for immune response induction [1]. Elastic liposomes potential carriers for TCI, consist of lipid mixture and membrane softeners. This mixture imparts flexibility to the elastic liposomal membranes and cause penetration through skin channels that are opened by the carriers. Present study was aimed to prepare and investigate the uptake potential of elastic liposomes by DCs which was studied by flow cytometry and spectral bio-imaging.

Materials and Methods:
Soya phosphatidyl choline (SPC) was purchased from Sigma, USA. Span-60 was purchased from Himedia, India. All other solvents were of HPLC grade and triple distilled water was used wherever required.
Preparation and characterization of vesicular system: Elastic liposomes were prepared by method reported by Paul et al. with slight modifications [2]. Briefly SPC was combined with Span 60 in phosphate buffer (pH 6.5) containing antigen solution. Likewise FITC-BSA and rhodamine B BSA-labeled vesicular system were also formulated for Spectral bio-imaging and FACS study. Prepared formulation was characterized for vesicle size, shape, polydispersity index, and entrapment efficiency.

Uptake potential evaluation of elastic liposomes: Antigen loaded rhodamine B labeled formulation was incubated with DCs and uptake kinetics of the system by cells was studied after definite time intervals. Finally elastic liposomal formulation uptake by DCs was studied by flow cytometer (BD FACS Calibur, USA). DCs pulsed with elastic liposomes was kept as control [3].

Internalization study by spectral bio-imaging: FITC-BSA labeled elastic liposomal formulation was incubated with DCs. After definite time period slide was prepared. Plain cells without treatment were kept as control. Observations were taken by fluorescence microscope (Axioscope, Carl Zeiss, Germany).

Results and Discussion:

Preparation and characterization of vesicular system: Elastic vesicles were prepared with SPC and span 60 (85:15 % w/w) and characterized for various parameters. Vesicles were found to be nanometer in size, spherical and low polydispersity index and showed homogeneous dispersion (Table 1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Elastic liposomal formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mean vesicles size</td>
<td>150±6.5</td>
</tr>
<tr>
<td>2.</td>
<td>Polydispersity index</td>
<td>0.045</td>
</tr>
<tr>
<td>3.</td>
<td>Vesicular shape</td>
<td>Spherical</td>
</tr>
<tr>
<td>4.</td>
<td>% Entrapment efficiency</td>
<td>64.2±1.5</td>
</tr>
</tbody>
</table>

Uptake potential evaluation of elastic liposomes: Uptake kinetics of antigen loaded elastic liposomal formulation was studied. Kinetics was represented in terms of mean fluorescence intensity versus counts. It was determined at definite time intervals (Figure 1 A).

Internalization study by spectral bio-imaging: Qualitative uptake of the system by the cells was investigated by spectral bio-imaging. Intracellular delivery of the system is clearly depicted in Figure 1 B.
Figure 1 A. Uptake kinetics of elastic liposomes
a. DCs population  b. Control  c. DCs after 60 min. incubation
d. DCs after 180 min. incubation  e. DCs after 300 min. incubation
f. DCs after 480 min. incubation

(a) Control  (b) DCs incubated with elastic liposomes

Figure 1 B Spectral bio-imaging analysis of DCs

Conclusion:
Elastic liposomal formulation showed spherical shape, optimum size. Better uptake and internalization was confirmed by flow cytometry and spectral bio-imaging analysis. Results suggested that system can be explored for effective vaccine delivery.

Acknowledgement:
Author is indebted to BMHRC, Bhopal (M.P.) for carrying out the flow cytometric analysis.

References:
Development and characterization of mucoadhesive beads containing antidiabetic drug

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Abstract: Mucoadhesive carriers are one of the promising carrier systems for the bioavailability enhancement of various drugs. They prolong the residence time of the dosage form leading to increased absorption and ultimately improved drug bioavailability. Present study deals with the formulation and optimization of Mucoadhesive beads containing Metformin HCl. Beads were developed through gel method and optimized using $3^2$ factorial designs for variables i.e; stirring speed and drug polymer ratio. Carrier system were characterized for particles size and drug loading efficiency and found to be 832 nm and 89.30% respectively. Further, micro beads were characterized using SEM, FTIR and in vitro drug release study. Based on these results and previous literature, 2-3 folds enhancement in bioavailability could be expected which will leads to effective management of diabetes.

Introduction:
Diabetes mellitus (DM) is a metabolic endocrine disorder. It is mainly characterized by hyperglycemia and is associated with the imbalance in carbohydrate, protein and lipid metabolisms [1]. In the present study a multiple unit swellable controlled release formulation of Metformin hydrochloride will be developed as one of the approach for gastro retentive drug delivery systems. This type of system capable of swelling to a size that prevents their passage through the pylorus; as a result, the dosage form is retained in the stomach for a longer period of time. The swelling is usually results from osmotic absorption of water. The dosage form is small enough to be swallowed, and swells in gastric liquid [2].

Materials and Methods:

Development of Jack fruit seed starch Beads
As reported previously with slightly modification method, the metformin loaded JFSS nanoparticles were prepared by solvent evaporation method [3, 4]. Different concentration (Table 1) of JFSS was dissolved in 1M NaOH Solution. Polymeric solution was stirred properly; drug was added in to it and mixed with continuous stirring. The cross linking was carried out by adding 3% calcium chloride solution under constant stirring. Stirring was continued at 70-80°C for 5h on the magnetic stirrer at room temperature for different time. The hardened beads were washed twice with distilled water and collected by filtration. Formulation was optimized for various process variables i.e. drug polymer ratio, time of stirring which could affect the preparation is shown in Table 1.
Characterization of Metformin loaded beads

- Morphology
- Particle size
- Percentage yield
- Drug entrapment in JFSS Nanoparticles
- Micromeritic study
- Swelling properties
- In-vitro drug release studies

Results and Discussion:

All the formulations showed good results with respect to particles size, drug entrapment, size and particles size distribution (Table 1). Percentage drug entrapment of MET beads showed 76 to 89% respectively. All prepared formulations were found to be uniform and spherical in shape. Micromeretic studies revealed that the tapped density values range from 0.50 to 0.31 g cm⁻³ while the apparent density is 0.47 to 0.57 g cm⁻³.

Table 1 Formulation code and independent variables.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug:Polymer ratio</th>
<th>Stirring speed</th>
<th>Particle size (nm)</th>
<th>Entrapment efficiency %</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>1000</td>
<td>991.39±0.92</td>
<td>83.67±0.57</td>
<td>85.52±0.48</td>
</tr>
<tr>
<td>F2</td>
<td>1:2</td>
<td>2000</td>
<td>967.54±0.65</td>
<td>79.56±0.29</td>
<td>83.27±0.28</td>
</tr>
<tr>
<td>F3</td>
<td>1:3</td>
<td>3000</td>
<td>908.54±0.84</td>
<td>82.31±0.48</td>
<td>77.8±0.74</td>
</tr>
<tr>
<td>F4</td>
<td>1:2</td>
<td>2000</td>
<td>861.34±0.38</td>
<td>76.48±0.85</td>
<td>86.45±0.30</td>
</tr>
<tr>
<td>F5</td>
<td>1:3</td>
<td>3000</td>
<td>832±0.47</td>
<td>89.04±0.43</td>
<td>89.27±0.37</td>
</tr>
</tbody>
</table>

Differential Scanning Calorimetry is a thermal analytical technique. Apart from the measurement of heat, DSC can also be used to measure heat flow rate (power) and to identify characteristic temperatures of a reaction as well as its transition. The thermogram of MET, Jack fruit starch and formulation were recorded in differential scanning calorimeter to characterize the solid state of drug in beads and to know any existing interaction between the polymer and drug. The DSC thermographs of this formulation showed a sharp peak at 232.98°C for pure MET and jack fruit starch exhibited an endothermic peak at 88.14°C.

The release characteristics of beads were studied at 37±0.5°C in 20 ml of simulated gastric fluid (pH-1.2) for 24h. The data obtained in the In-vitro release studies was concluded that increase in concentration of MET results in declined cumulative % drug release.
**Figure 1** *In-vitro* release profile of MET from JFSS beads in Simulated Gastric fluid at 1.2 pH

**Conclusion:**

In a nut shell, it may be concluded that the jack fruit starch polymer beads have the potential of providing the gastro-retentive delivery of metformin hydrochloride in a controlled manner.

**References:**

Formulation and evaluation of directly compressible agglomerates of Telmisartan

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Abstract: The present work is aimed to formulate spherical agglomerates and enhance the micromeritic properties, solubility and dissolution rate of Telmisartan, a poorly water soluble anti-hypertensive drug. The poor water solubility and poor micromeritic properties of Telmisartan lead to low dissolution rate and poor flow during tableting. Telmisartan spherical agglomerates were prepared by spherical agglomeration techniques using a quasi emulsion solvent diffusion method consisting of chloroform and water as good solvent and bad solvent respectively. PVP K30 and PEG 6000 in different concentration were used as hydrophilic polymers in agglomeration process.

Introduction:
Tablet is the major oral solid dosage form, having significant advantage over capsules because of its tamper proof nature [1]. It is well known that most common methods for tablet manufacturing include dry granulation, wet granulation and direct compression. Direct compression is the simplest and cost effective method to produce tablets. It is economical, less stressful to ingredients in terms of heat and moisture and only few procedures are involved in it [2]. But most of the drugs cannot be directly compressed due to lack of their binding or bonding characteristics into the compact entities. Spherical agglomeration (SA) is a novel particle engineering technique by which crystallization and agglomeration can be carried out simultaneously in one step to transform crystals directly into compacted spherical shape that helps to achieve good flowability and compressibility. The process involves simultaneous crystallization and agglomeration of drugs with or without excipients to obtain directly compressible blends [3,4].

Material and Methods:
Telmisartan (drug) was provided by Healthbiotech India PVT. LTD and PEG 6000, PVP K30, Talc, Magnesium stearate and other chemicals were provided by Sagar Institute of Research Technology & Science-Pharmacy Bhopal.

Method of preparation:
A solution of Telmisartan in chloroform (1.0 g in 13 ml) was prepared. The agglomerates were obtained by adding the above solution drop wise into an aqueous phase water (100 ml) containing dissolved polymer at different concentration in room temperature. The mixture was stirred at 1000 rpm using a controlled speed stirrer for 30 minutes. Agglomerates were prepared using chloroform as a good solvent for Telmisartan and water was used as a bad solvent. The agglomerates were also
formulated using different polymers like PEG 6000 and PVP K30 in 4 different ratios of 1:1, 1:2, 1:3, and 1:4.

The agglomerates formed were then separated by filtration and dried at room temperature.

1. Then Agglomerates were lubricated with 1% w/w talc and 1% w/w magnesium stearate.
2. The lubricated agglomerates were compressed into tablets using a compression machine.

<table>
<thead>
<tr>
<th>Table 1: Formulation of Different Batches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Telmisartan(g)</td>
</tr>
<tr>
<td>PEG 6000 (g)</td>
</tr>
<tr>
<td>PVP K30(g)</td>
</tr>
</tbody>
</table>

Results and Discussion:

Different polymers were tried to prepared agglomerates of Telmisartan, among them PEG-6000 and PVP K30 was found to be most suitable. Formulations F1 to F4 were prepared using PEG-6000 and F5 to F8 with PVP K30 in different concentrations.

In formulations F1 to F4 the flow properties of the agglomerates were found to be excellent as indicated by Carr’s index (≤10 %), Hausner’s ratio (1.0-1.11) and angle of repose (<20). Agglomerates were compressed into tablets and the tablets were evaluated for friability, hardness, disintegration time, thickness, weight variation and in vitro drug release. All the results obtained were conformed to the specifications mentioned in the IP.

In formulations F5 to F8 the flow properties of the agglomerates were also found to be excellent as indicated by Carr’s index (≤10 %), Hausner’s ratio (1.0-1.11) and angle of repose (<20). Agglomerates were compressed into tablets and the tablets were evaluated for friability, hardness, disintegration time, thickness, weight variation and in vitro drug release. All the results obtained were conformed to the specifications mentioned in the IP. The percentage cumulative release of Formulations F5, F6, F7 and F8 was found to be 57.28%, 67.23, 87.23% and 58.78% after 60 minutes respectively. (Formulation F7 was found to give highest cumulative release).

However compared to F1-F4 a moderate improvement in percentage cumulative release was observed. The percentage cumulative release increased with increasing concentrations of PVP-K30 except formulation F8.

Thus agglomerates containing polymers were found to be better flow properties compared to pure drug and also better drug release compared to conventional tablets of Telmisartan.

Conclusion:

The spherical crystallization technique is a new inexpensive technique for reducing time and cost by enabling faster operation, less machinery and fewer personnel because it removes most of the steps.
which are required in granulation technology of tablet manufacturing. It provides advances in
tabletting technology by introduction of number of directly compressible excipients. The spherically
agglomerated crystals can be prepared into tablet form or compounded directly into a pharmaceutical
system without further processing such as granulation. This technique of particle design of drugs has
emerged as one the areas of active research currently of interest in pharmaceutical manufacturing.

Acknowledgement:
First of all, I sincerely acknowledge my gratitude to most essential my beloved Parents and family for
whom even word used to express thanks will be miserable to acknowledge them. I consider myself
fortunate enough to be in right place with person. I take this opportunity to express my deep sense of
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Vijay Nigam for their valuable guidance and support for the project. I am heartily thankful to them for
providing me valuables suggestion and remarks to complete this project.

References:
CBS Publications 293-297.
Nasal delivery of solid lipid nanoparticles for the treatment cerebral malaria

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Abstract: The objective of work is to target solid lipid nanoparticles to brain for treatment of cerebral malaria. Artemether and lumefantrine loaded solid lipid nanoparticles were prepared by a hot homogenization method followed by solvent injection technique. The formulations were characterized for their size, shape, zeta potential and encapsulation efficiency and drug release study. These solid lipid nanoparticles were observed spherical in shape in scanning electron microscopy and optimized size was 140nm (PI < 0.33), with −29mV zeta potential value. The maximum entrapment efficiency was 79% (w/w), and optimized formulation showed 40% release of artemether and 34% release of lumefantrine in 12hrs. In conclusion, intranasal administration of SLNs of artemether could provide better protection against cerebral malaria.

Introduction:
Malaria is an infectious disease caused by the Plasmodium genus of protozoan parasite. During the course of the disease, an initial asymptomatic infection of the liver is followed by parasites invasion into red blood cells, causing clinical symptoms of malaria. The most severe complication of Plasmodium falciparum infection is cerebral malaria, which implies the presence of neurological features, especially impaired consciousness [1], on the other side drug delivery to the brain is made difficult by the presence of the blood brain barrier (BBB), which is formed by tight junctions within the capillary endothelium of the vertebrate brain. The drug delivery into central nervous system through intranasal route has been reported [2-5], recently. In present work we tried to prepare solid lipid nanoparticles for intranasal delivery of drugs. These solid lipid nanoparticles would have great potential to deliver the drug to CNS and could show controlled drug release and site-specific drug targeting.

Material and Methods:
Materials: Artemether and Lumefantrine were procured as gift sample from IPCA laboratory Mumbai, India. Glyceryl monostearate (GMS) was purchased from CDH (India). Lecithin was purchased from Across Chemicals (India). Pluronic F127 was purchased from Hi-media (India). All other reagents used in this study were of analytical grade.
Preparation of solid lipid nanoparticles: SLNs were prepared using the solvent diffusion method. The aqueous phase was prepared by dissolving the surfactant and co-surfactant in double distilled
water. The organic solution was prepared by completely dissolving the drugs and lipid in water-miscible solvent in a water bath at 70 °C, which is above the melting point of the GMS. The resultant organic solution was injected into aqueous phase containing the surfactant, at 1,000 rpm at 61 °C for 1 h. The nanosuspension formed was allowed to cool at room temperature.

Results and Discussion:

Characterization of Solid Lipid Nanoparticles

Size and size distribution: The average particle size of the solid lipid nanoparticles dispersion was determined using Zetasizer (Malvern Instruments, UK). The size of formulations was in the range of 140-300nm.

Zeta potential: The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential of solid lipid nanoparticles dispersion was determined using Zetasizer (Malvern Instruments, UK). The zeta potential value of optimized formulation was −29mV.

Surface morphology: Morphological characterization of solid lipid nanoparticles was confirmed by Scanning electron microscopy. The surface of nanoparticles was smooth in nature and spherical in shape. SEM photograph of SLN is given in figure 1.

Figure 1 SEM photograph of solid lipid nanoparticles.

% Yield of solid nanoparticles: Total amount of SLN obtained were weighed individually for each batch and percentage yield was calculated taking into consideration the weight of drug and polymer. % yield of formulations was in the range of 70%.

Percent drug entrapment efficiency: The entrapment efficiency was determined by analyzing the free drug content in the supernatant obtained after centrifuging the SLN suspension at 1,7000 rpm for 1 h at 0 °C. The % entrapment of optimized formulation was 79%.

In-vitro drug released study: In vitro release studies were performed using modified Franz diffusion cell in phosphate buffer, pH 6.4. The samples were analyzed at different time interval for amount of drug released using HPLC method. Percent cumulative drug release of optimized solid lipid nanoparticles is shown in figure 2. The system showed 40% release of Artemether and 34% release of lumefantrine in 12hrs.
Acknowledgement:
Authors would like to acknowledge UGC for providing grant for research work and IPCA laboratory Mumbai, India for providing drug samples. Authors would also like to acknowledge School of Pharmaceutical Sciences for providing necessary facilities to carry out research work.

Conclusion:
The results of study indicate that solid lipid nanoparticles would be potential carriers systems for drug delivery to brain in treatment of cerebral malaria.

References:
Development and evaluation of Terbinafine Hydrochloride topical polymeric microsphere drug delivery system.

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Abstract: Microsponges are porous beads normally 5-300 μm in diameter, loaded with drugs which releases it onto the skin in a sustained manner. The objective of present study was to develop and evaluate topical polymeric microsphere delivery of Terbinafine HCl for sustain release and its deposition in skin. Microsponges of ethyl cellulose (EC) containing Terbinafine HCl were prepared by quasi emulsion solvent diffusion method. Effect of drug polymer ratio on active drug content, particle size and entrapment efficiency were studied. Optimisation study was carried out by taking internal phase volume, stirring rate, emulsifier concentration as independent variables and their effects on entrapment efficiency, particle size were studied. Morphology of obtained microsponges was revealed by scanning electron microscope. Developed microsponges were found to be porous and spherical. Optimized formulation of microsponge was dispersed in carbopol gel and evaluated for drug content, pH, viscosity, in vitro drug release etc. Release of drug was found to be sustained through microsponge gel as compared to marketed product and pure drug gel. Ex vivo drug deposition study was carried using rat abdominal skin. Drug deposition was found to be satisfactory.

Introduction:
Terbinafine is an allylamine antifungal agent widely utilized in the treatment of infections caused by dermatophytes (Trichophyton, Epidermophyton and Microspora). Microsponges are polymeric delivery systems. It consists of porous microspheres of an inert polymer that can entrap active ingredients and control their delivery rate. Microsponges are true sponge like spherical particle that consist large amount of interconnecting voids within a non-collapsible structure with large porous surface. Microsponge technology allows an even and sustained rate of release, reducing irritation while maintaining efficacy. Microsponge can be prepared with two methods one step process (liquid liquid polymerization method) and two step process (quasi emulsion solvent diffusion method). Most common and feasible method used for preparation of microsphere is quasi emulsion solvent diffusion method [1-5].

Materials and Methods:
Material: Terbinafine HCl was obtained as gift sample from Wockhardt Research Centre, Aurangabad, Ethyl cellulose was obtained as gift sample from Colorcon Asia Pvt. Ltd. Mumbai and
all other ingredients used in this study were of analytical grade and purchased from Research Lab Fine Chemicals Ltd., Mumbai.

**Method:** Terbinafine HCl microsponges were prepared by the quasi emulsion solvent diffusion method. The organic internal phase (DCM) containing Terbinafine HCl and ethyl cellulose was gradually added into distilled water (external phase) containing polyvinyl alcohol (PVA) as emulsifying agent. The mixture was stirred on digital mechanical stirrer for 60 min in order to remove dichloromethane. The formed microsponges were filtered through whatman filter paper, dried at 40°C and weighed.

Ethyl cellulose gave spherical, rigid and required micrometer size microsponge. The effect of different weight ratios of drug to ethyl cellulose (2:1, 1.5:1, 1:1, 1:1.5 and 1:2) on actual drug content (ADC), particle size and entrapment efficiency of microsponges was determined. Further Box Behnken design was adopted to optimize the amount of internal solvent volume (5, 10 and 15 ml), emulsifier concentration (400, 500 and 600 mg) and effect of stirring rate (1200, 1500 and 1800 rpm) as the independent variables affecting the particle size, entrapment efficiency and active drug content.

**Evaluation of TBF microsponges**
1. Drug content and entrapment efficiency
2. Particle size
3. Scanning electron microscopy
4. Powder X-ray diffraction
   - The carbopol gel of prepared microsponges was prepared (TBFMG- Terbinafine microsponge gel). The prepared gel was subjected to following evaluation parameters.
   1. *In-vitro* release studies
   2. Ex-vivo drug deposition study
   3. Antifungal activity
   4. Stability study

**Results and Discussion:**

**Formulation and optimization of TBFMG:** Terbinafine HCl microsponges (TBFMG) was prepared using different polymer ratios (2:1, 1.5:1, 1:1, 1:1.5 and 1:2). These were evaluated for actual drug content (ADC), entrapment efficiency (EE) and particle size. TBFMG3 showed highest ADC, EE and smaller particle size, so 1.5:1 ratio of drug and polymer was selected for optimization study. Effect of independent factors (PVA, DCM, and stirring speed) on EE, particle size and ADC was studied.
Effect of emulsifier (PVA) concentration on entrapment efficiency and particle size: Entrapment efficiency was found to be inversely proportional and particle size was directly proportional to quantity of emulsifier (PVA).

Effect of internal phase volume on particle size: The particle size was found to be inversely proportional to quantity of internal phase (DCM).

Effect of stirring rate on particle size: The particle size was found to be inversely proportional to stirring rate.

PXRD study confirmed formation of microsponges. SEM confirmed spherical nature of microsponges.

Conclusion:
Microsponges produced by quasi emulsion solvent diffusion method were found to be porous, spherical and rigid.

References:
PB-34

RP-HPLC method for simultaneous estimation of Drotaverine Hydrochloride and Mefenamic Acid

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Abstract: A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method is developed and validated for simultaneous determination of drotaverine hydrochloride and mefenamic acid in tablets. The compounds were separated on an ODS analytical column with a mixture of acetonitrile, triethylamine solution (pH 3.0 ± 0.1) and tetrahydrofuran in the ratio 50:40:10 (v/v) as mobile phase at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 220 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The developed and validated method used for analysis of marketed formulation

Introduction:
Drotaverine hydrochloride (DH), 1-[(3,4-Diethoxy phenyl) methylene]-6,7-diethoxy-1,2,3,4-tetrahydro isoquinolene, (Fig. 1) is an analogue of papaverine [1]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain. Mefenamic acid (MA), 2-[(2,3-Dimethyl phenyl)amino] benzoic acid (Fig. 1), is an orally active analgesic, anti-inflammatory drug used to relieve pain [1].

![Chemical structures of analytes.](image)

Figure 1 Chemical structures of analytes.

A combination of DH and MA is used to treat excessive labor pain. Literature survey revealed methods of simultaneous estimation by UV [2-3] & HPLC [4-5].

Materials and Methods:
DH and MA were supplied by Blue-Cross Laboratories Ltd. (Nasik, India), certified to contain 99.62% (w/w) and 99.48% (w/w) respectively on dried basis. Drugs were used as it is without further purification.
Chromatographic system and conditions: Shimadzu (Japan) manufactured HPLC instrument was employed with specifications of a LC-10 AT vp solvent-delivery module, SPD-10A UV-visible detector and a Rheodyne model 7725 I injector. Samples were separated on a Phenomenex C$_8$ column (250 × 4.6 mm i.d., 5 µm particles). The mobile phase was a mixture of acetonitrile (ACN), triethylamine (TEA) solution (pH 3.0 ± 0.1 adjusted with 85% phosphoric acid) and tetrahydrofuran (THF) in the ratio 50:40:10 (v/v/v). Drugs were monitored at 220 nm with of 1 mL min$^{-1}$.

Construction of calibration plots: Individual stock solutions (100 µg mL$^{-1}$) of DH, MA, and IS were prepared by dissolving drugs standards in mobile phase. Calibration standards for each analyte were prepared at concentrations of 16, 32, 48, 64, 96, 112 µg mL$^{-1}$ for DH and 50, 100, 150, 200, 250, 300 and 350 µg mL$^{-1}$ for MA. DH solutions of concentration 16, 64 and 112 µg mL$^{-1}$ and MA solutions of concentration 50, 200 and 350 µg mL$^{-1}$ were prepared as quality-control (QC) samples.

Assay sample preparation: Twenty tablets of Brand I (Dotra-M, Emcure Pharmaceuticals Ltd., Pune) containing 250 mg of MA and 80 mg of DH were weighed and finely powdered. Accurately weighed powder, equivalent to average weight of one tablet was transferred to a 100 mL volumetric flask, dissolved in 80 mL mobile phase, filtered and volume made up to the mark with mobile phase.

Results and Discussion:

Method development and optimization: Column chemistry, solvent selectivity, solvent strength, additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions. Other criteria, viz. time required for analysis, appropriate $k$ range ($1 < k < 10$) for eluted peaks, assay sensitivity and use of the same solvent system for extraction of drug from formulation matrices, were also considered. To ascertain appropriate wavelength for simultaneous determination of DH and MA, solutions of these compounds were scanned by UV–visible spectrophotometer and 220 nm was fixed for estimation. Because the compounds of interest have a polarity difference, two reversed-phase columns of different polarity, C$_8$ & C$_{18}$ Phenomenex were tried. The retention times of the solutes decreased with increasing concentration of organic modifier. It is well known that multiple-component mobile phases result in better separation efficiency than binary mobile phases. A component, tetrahydrofuran was included and use of 50:40:10 (v/v/v) provided satisfactory separation.

Method validation: System suitability was evaluated by replicate ($n=6$) injection of the same standard solution. The number of theoretical plates and the USP tailing factor were within the acceptance criteria of $>2000$ and $\leq 1.5$, respectively, indicating good column efficiency and optimum mobile phase composition. Linearity was tested in the concentration range 16–112 µg mL$^{-1}$ for DH and 50–350 µg mL$^{-1}$ for MA, in the presence of IS (50 µg mL$^{-1}$). The values obtained for LOD were 1.0991 and 10.003 µg mL$^{-1}$ for DH and MA, respectively; the respective LOQs were 3.331 and 30.312 µg mL$^{-1}$. Accuracy and precision data obtained were well within the acceptable limits.
**Tablet assay and content uniformity:** The method is used for assay of the estimation of DH and MA content of Dotra-M tablets. Total HPLC analysis time per sample was 17 min with DH, IS, and MA eluting at 2.47, 11.29, and 15.15 min, respectively (Fig. 2). Assay from six replicate analyses of Dotra-M tablets exhibited label content of 100.25 & 101.24% with RSD of 1.45 and 1.61% for DH and MA, respectively.

![Figure 2 Typical chromatogram for separated drugs.](image)

**Conclusion:**
An isocratic RP-HPLC method has been developed for simultaneous estimation of DH and MA. The method conforms the ICH validation parameters.

**References:**
Synthesis and evaluation of 2-(4-methoxy-2-oxo-1-phenyl/methyl-1, 2-dihydroquinolin-3-yl)-2-methyl-3-(phenyl/substitutedphenyl amino) Thiazolidin-4-one as antibacterial and anticancer agents.

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Department of Pharmaceutical Chemistry, P.E.S’s Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda-Goa, 403401.
E-mail address: vivekasintia@gmail.com

Abstract: A series of 2-(4-methoxy-2-oxo-1-phenyl/methyl-1,2-dihydroquinolin-3-yl)-2-methyl-3-(phenyl/substitutedphenylamino)thiazolidin-4- onederivatives(III-a(1-5)/III-b(1-5)) were synthesized by synthetic routes. Characterized by UV, IR, NMR and Mass spectra. Compounds were evaluated against two Gram positive and two Gram negative strains. Compound III-a2 with R1=C6H5 and R2=Cl showed promising activity with MIC between 0.25-4µg/mL. Ciprofloxacin was used as the reference standard. The compounds were also screened for their anticancer activity against A549-Human lung carcinoma cell line. Compound III-a2 (IC50=<10µg) with R1=C6H5 and R2=Cl, III-a5 (IC50=10µg) with R1=C6H5 and R2=CH3 and III-b2 (IC50=10µg) with R1=CH3 and R2=Cl showed 100 % cell death.

Introduction:
The quinolin-2-one moiety is an important component of numerous natural products and synthetic analogues that exhibit wide variety of biological activities1. Literature reveals Linomide(Pharmacia & Upjohn Inc, Sweden), quinolin-2-one derivative, as a lead molecule for the development of anti-cancer and anti-HIV agents2,3. In the present investigation, we are reporting modification at 3rd and 4th position of the Linomide moiety.

Materials and Methods:
The title compounds were synthesized by the following reaction conditions mentioned in the scheme i.e. Figure 1.
Table 1 List of thiazolidinone derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-a₁</td>
<td>C₆H₅</td>
<td>H</td>
<td>III-b₁</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>III-a₂</td>
<td>C₆H₅</td>
<td>4-Cl</td>
<td>III-b₂</td>
<td>CH₃</td>
<td>4-Cl</td>
</tr>
<tr>
<td>III-a₃</td>
<td>C₆H₅</td>
<td>4-Br</td>
<td>III-b₃</td>
<td>CH₃</td>
<td>4-Br</td>
</tr>
<tr>
<td>III-a₄</td>
<td>C₆H₅</td>
<td>2-F</td>
<td>III-b₄</td>
<td>CH₃</td>
<td>2-F</td>
</tr>
<tr>
<td>III-a₅</td>
<td>C₆H₅</td>
<td>4-CH₃</td>
<td>III-b₅</td>
<td>CH₃</td>
<td>4-CH₃</td>
</tr>
</tbody>
</table>

The compounds were satisfactorily characterized by UV, IR, NMR and Mass spectral data. The spectral data of representative compound is given here: 2-(4-Methoxy-2-oxo-1-phenyl-1,2-dihydroquinolin-3-yl)-2-methyl-3-(phenylamino) thiazolidin-4-one III-a₁:

Yield 82.35% (ethanol); m.p. >300; IR (KBr, cm⁻¹): 3367.71 (N-H); 3061.03 (aromatic C-H); 2972.31, 2931.80, 2852.72 (aliphatic C-H str.); 1668.43 (C=O amide of 4-thiazolidinone); 1612.49 (C=O amide of quinolin-2-one); 1H NMR (DMSO-d₆, δ ppm): 8.1 (s, 1H, N-H); 6.4-7.9 (m, 14H, Ar-H); 3.9 (s, 3H, -OCH₃); 27 (s, 3H, C-CH₃); 2.6 (s, 2H, S-CH₂); 13C NMR (DMSO-d₆, δ ppm): 160.40 (1C, C=O of 4-thiazolidinone); 158.02 (1C, C=O of quinolin-2-one); 156.18 (1C, C-4 of quinolin-2-one); 148.23-121.10 (18C, aromatic carbon); 110.44 (1C, C-3 of quinolin-2-one); 61.08 (1C, OCH₃); 52.30 (1C, CH₂ of 4-thiazolidinone); 28.60 (1C, C-3 of 4-thiazolidinone); 12.70 (1C, CCH₃); \(\lambda_{max}(nm)\): 318.40. Mass spectra with molecular formula C₂₆H₂₃N₃O₃S (m/z) = 457.54 9 [M⁺]

Results and Discussion:

The tables showing the MIC values for antibacterial activity and IC₅₀ values against A549-Human lung cancer cell line.

Table 2 Antibacterial activity by MIC method in µg.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive bacteria</th>
<th>Gram positive bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>III-a₂</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Standard (Ciprofloxacin)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3 Anticancer activity by MIC method in µg

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration µg</th>
<th>O.D at 492nm</th>
<th>% of cell lysis</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-a₂</td>
<td>10</td>
<td>0.747</td>
<td>50%</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.926</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.320</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>III-a₅</td>
<td>10</td>
<td>0.608</td>
<td>50%</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.939</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.020</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>III-b₂</td>
<td>10</td>
<td>0.672</td>
<td>50%</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.815</td>
<td>75%</td>
<td></td>
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<td></td>
<td>30</td>
<td>1.165</td>
<td>100%</td>
<td>30</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----</td>
<td>-------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>Standard (Vincristine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

4-Methoxy-1-phenyl/methyl-3-(5-phenyl/substitutedphenyl-4,5-dihydro-(1H)-pyrazol-3-yl)quinolin-2(1H)-one derivatives were synthesized from 1-substituted-3-acetyl-4-hydroxyquinolin-2-one. These compounds were satisfactorily characterized by UV, IR, NMR and Mass spectral data. All the compounds were screened for their *in-vitro* anticancer and antibacterial activity. The activity data reveals that the newly synthesized compounds were active against all the strains. However compound **III-a2** with $R_1=C_6H_5$ and $R_2=Cl$ showed promising activity against all bacterial strains with a MIC between 0.25-4µg/mL. Compound **III-a2** (IC$_{50} =$<10µg) with $R_1=C_6H_5$ and $R_2=Cl$, **III-a5** (IC$_{50} =$10µg) with $R_1=C_6H_5$ and $R_2=CH_3$ and **III-b2** (IC$_{50} =$10µg) with $R_1=CH_3$ and $R_2=Cl$ showed 100% cell death in case of anticancer activity.

**Acknowledgement:**

We would sincerely thank the Directors, NMR and Mass Centre, SAIF, Punjab University-Chandigarh, for providing the necessary spectra.

**References:**

Synthesis and anticancer evaluation of \( N \)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives.

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Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124001, India.
2Collaborative Drug Discovery Research Group, Faculty of Pharmacy, Campus Puncak Alam, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.
3Brain Research Laboratory, Faculty of Pharmacy, Campus Puncak Alam, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.
E-mail address: naru2000us@yahoo.com

Introduction:
Cancer has been ranked as a major health burden. At present, a wide range of anticancer drugs with different mechanisms of action are used to treat human cancer, either alone or in combination but current chemotherapeutic agents suffer from various drawbacks, such as toxicity to normal cells, bone marrow depression, gastrointestinal disturbances [1]. This undoubtedly underscores the need of developing novel anticancer agents. Pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have considerable chemical and pharmacological importance [2]. Literature report reveals that pyrimidine derivatives are reported to have potent anticancer activities viz. aurora kinase inhibitors, B raf kinase inhibitors [3] and VEGFR2 inhibitors etc. Recently, it was reported that amino group at 2\(^{nd}\) position of pyrimidine nucleus (I) improved the anticancer activity [4]. Nagaraj et al. identified that bromophenyl group directly attached to 4\(^{th}\) position of pyrimidine ring (II) caused the enhancement in the antimicrobial activity of pyrimidine nucleus while Kaspersen et al. identified that bromophenyl group attached to pyrimidine moiety through ethyl amine (III) showed anticancer activity at a much lower extent. Therefore, we hypothesised the direct attachment of bromophenyl group to 4\(^{th}\) position of pyrimidine nucleus to enhance anticancer activity. It was also identified that nitrophenyl group attached to the pyrimidine also has antitumor activity (IV) [5]. Prompted by the facts above and in continuation of our search for novel anticancer agents, in the present study, \( N \)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives (Figure 1) were synthesized and their anticancer potential was evaluated.

![Figure 1 Design of \( N \)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine derivatives based on literature](image-url)
Material and Methods:

General procedure for the synthesis of N-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine derivatives (1-14): p-Bromo acetophenone (0.01 mol) and 3-nitrophenyl benzaldehyde (0.01 mol) were stirred in ethanol (10-20 ml) for 2 h and 10 ml 40% sodium hydroxide solution was added to it after stirring. The mixture was kept overnight at room temperature. The contents were then poured on crushed ice and acidified with dilute hydrochloric acid, which resulted in the precipitation of chalcone (1-(4-bromophenyl)-3-(3-nitrophenyl) prop-2-en-1-one). The crude chalcone was filtered, dried and recrystallized from ethanol. To a mixture of chalcone (0.01 mol) and potassium hydroxide (0.01 mol) in 80 ml absolute ethanol, 40 ml 0.25 M solution of guanidine hydrochloride in ethanol was added. After addition, the mixture was refluxed for 2-6 h; the reaction mixture was cooled at room temperature and quenched with 20 ml of 0.5 M solution of hydrochloric acid in water. The reaction mixture was shaken to ensure mixing and concentrated to obtain solid 4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine. A mixture of aminopyrimidine (0.1 mol) synthesized as above and aromatic aldehyde (0.1 mol) was refluxed in minimum amount of ethanol in presence of small amount of glacial acetic acid for 2-3 h. The mixture was cooled and poured in ice cold water. The solid thus obtained was filtered and recrystallized from ethanol.

**Compound 1:** IR (KBr pellets, cm⁻¹): 581 (C-Br str., C₆H₅Br), 3087 (C-H str., phenyl nucleus), 1581 (C=C str., phenyl nucleus), 1668(C=N str., OCH₃), 1231 (C-O-C str., OCH₃), 1527 (NO₂ asym. str.); ¹HNMR (DMSO, δppm): 6.74-8.37 (m, 11H, ArH), 3.78 (s, 9H, OCH₃), 8.23 (s, 1H, N=CH); Anal. Calculated for C₂₆H₂₁N₄O₅Br: C, 56.84; H, 3.85; N, 10.20; Found: C, 55.76; H, 3.97; N, 10.42

**Evaluation of anticancer activity:** The anticancer activity of synthesized compounds (1-14) was determined against human colon (HCT116) cancer cell line. The cell line was cultured in RPMI 1640 (Sigma) supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin (PAA Laboratories). Cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO₂. Anticancer activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann, but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve from which the concentration of test compounds required to kill 50% of cell population (IC₅₀) was determined.

**Results and Discussion:**

**Chemistry:** The title compounds, N-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives (1-14) were synthesized as outlined in Scheme 1. The physicochemical properties of the synthesized compounds are presented in Table 1.
Scheme 1: Synthetic scheme for the synthesis of \(N\)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives (1-14):

<table>
<thead>
<tr>
<th>Comp.</th>
<th>(X_1)</th>
<th>(X_2)</th>
<th>(X_3)</th>
<th>(X_4)</th>
<th>(X_5)</th>
<th>Comp.</th>
<th>(X_1)</th>
<th>(X_2)</th>
<th>(X_3)</th>
<th>(X_4)</th>
<th>(X_5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>OCH(_3)</td>
<td>OCH(_3)</td>
<td>OCH(_3)</td>
<td>H</td>
<td>8</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>OCH(_3)</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>9</td>
<td>H</td>
<td>CH(_3)</td>
<td>OCH(_3)</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>H</td>
<td>10</td>
<td>H</td>
<td>H</td>
<td>N(CH(_3))(_2)</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>OCH(_3)</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>11</td>
<td>H</td>
<td>H</td>
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<tr>
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<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>13</td>
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<td>7</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>14</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

Anticancer activity: Anticancer screening results (Table 1) indicated compounds 1 and 13 were more active than the standard carboplatin and less active than the reference compounds, terandrine, doxorubicin, camptothecin against HCT 116 cancer cell line (Table 1). The compounds 1 and 13 may be taken as lead compounds for the development of novel anticancer agents.

Structure activity relationship (SAR) studies: The SAR for the anticancer activity of the synthesized pyrimidine derivatives are as follows:

1) The presence of electron donating group (-OCH\(_3\)) in the benzylidene portion of the pyrimidine derivatives improved the anticancer potential of Compound 1, \(N\)-(3,4,5-trimethoxybenzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine.

2) It is important to note a fact here that the high anticancer activity of compound 13, 4-(4-bromophenyl)-6-(3-nitrophenyl)-N-(3-phenylallylidene)pyrimidin-2-amine, which has an unsubstituted phenyl ring attached to sixth position of pyrimidine ring may be attributed to the presence of an additional two carbon units in the benzylidene portion which may help in binding of compound 13 to the target site of HCT116.

Table 1 The physicochemical properties and anticancer activity of synthesized \(N\)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives (1-14)

| Comp. | Mol. Formula | M. Wt. | m.p. (°C) | \(R_y\) Value* | % Yield | HCT 116 (IC\(_{50}\) in |
**Conclusion:**

In the present study, a series of \(N\)-(substituted benzylidene)-4-(4-bromophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine derivatives (1-14) were synthesized and tested for its anticancer potential. Anticancer screening results indicated that compounds 1 and 13 were more active than the standard drug, carboplatin and have the potential to be selected as lead compounds.

**References:**

Development and validation of UV-visible spectroscopic method for estimation of Ferrous Ascorbate alone and in presence of Folic acid

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E-mail address: dipakpatil888@gmail.com

Abstract: The simple, selectiveaccurate, precise method was optimized for determination of ferrous ascorbate alone and in combination with folic acid. Inderivatization reaction 1, 10-phenanthroline forms a complex with ferrous iron showing absorbance maximum at 509.50nm. The proposed method was validated as per ICHQ2 (R1) validation guidelines for accuracy, precision, linearity, and range, specificity, robustness etc. The method was found linear in the range 2.0-10.0 μg/mL (r = 0.9983). The content of tablet was found 98.13±0.86%. The interference due to folic acid was not observed in estimation of ferrous ascorbate.

Introduction:
Ferrous ascorbate indicated in the treatment of iron deficiency anemia [1]. Iron deficiency results in the production of small RBCs, and haemoglobin synthesis is insufficient [2]. It is hygroscopic, fine dark violet powder, which is odorless and has no characteristic taste. Iron is most readily absorbed in the ferrous state. But most of the dietary iron is in the ferric form. Not more than a trace amount of iron is absorbed in the stomach [1]. Iron ascorbate was more stable than either extreme. Iron ascorbate is a labile ferriferro complex [3].
Ferrous ascorbate (Fig. 1.A) is newly introduced salt of ferrous having more bioavailability than other salts of ferrous ascorbate [4]. But till date, no more literature available for estimation of ferrous ascorbate. Therefore in the present investigation, an attempt has been made to develop an accurate, simple and an economic UV-Visible spectrophotometric determination of Ferrous ascorbate alone and in combination with folic acid.

Materials and Methods:
Double beam UV-Visible spectrophotometer with 10mm matched quartz cell (Shimdzu-1700) with UV-Probe software. Ferrous ascorbate pure sample was supplied by Curex Pharmaceuticals (Pvt.) Ltd. Jalgaon as gift samples. All reagents and solvents used were of Analytical Reagent Grade. While 1,
10-phenanthroline solution (0.3% w/v) solution was prepared in double distilled water containing two drop of hydrochloric acid. The standard drug solution (100ppm) was prepared in 0.1M Hydrochloric acid.

**Selection of wavelength and Linearity study:** The 0.4 mL of standard stock solution of ferrous ascorbate (100µg/mL) transferred to flask containing 2.0 mL of sodium acetate to which a quantity of about 1.5 mL of 1, 10-phenanthroline reagent (0.3% w/v) solution was added. The reaction mixture was allowed to developed full color for 15 min and final volume made with double distilled water. The color solution was scanned in the range of 400-800 nm against reagent blank. The wavelength of maximum absorbance was determined. The analyte concentrations between range 2.0-10.0 µg/mL were prepared and measured for absorption at 509.50 nm against blank.

**Optimization of reaction parameters (one variable at a time):** Different experimental parameter such as reagent concentration (1, 10-phenanthroline), volume of reagent, pH of solution, stability of complex with respect to the time were optimized by conventional method to obtain maximum absorption value.

**Estimation of Ferrous ascorbate from a bulk material:** The physical mixtures were analyzed by optimized method.

**Tablet analysis:** An accurately weight quantity of OroferXT equivalent to 100mg of ferrous ascorbate was transfer to 100.0 mL volumetric flask containing 40mL Hydrochloric acid (0.1M). Aliquot portion of standard solution (0.5mL) was transferred to ten different 10mL of volumetric flask. To each flask, added 1.5mL of 0.3% w/v of 1, 10-phenanthroline. The solution was allowed to stand for 15 min and final volume made with double distilled water. The absorbance of these solutions was measured at 509.50 nm against blank.

**Method validation:** The method was validated for parameters as linearity and range, accuracy, precision and specificity of developed method as per ICH Guidelines.

**Results and Discussion:**

The aim of the present work was to develop a UV-Visible spectrophotometric method for determination of ferrous ascorbate alone and in combination with folic acid. In present work 1, 10-phenanthroline used as a derivitizing agent for estimation of ferrous ascorbate. The complex shows maximum absorption at wavelength 509.5 nm. The 0.1 N HCl solution used as solvent to dissolve the ferrous ascorbate as it has solubility at lower pH. For derivative formation, acidic conditions required. The wavelength 509.50 nm was selected for further study. The different experimental parameter such as reagent concentration (1, 10-phenanthroline), volume of reagent, pH of solution, stability of complex with respect to the time were optimized by one variable at a time approach to obtain maximum absorption value which was utilized in experimentation.
Linearity study: The solutions were found to be linear over concentration range 2.0-10.0 µg/mL with r value 0.9983 (Slope = 0.0327, Intercept= 0.0862).

Bulk material analysis: The % amount found for bulk material of ferrous ascorbate was 100.49 ±0.33.

Tablet Analysis: The results of tablet analysis as 98.13 ± 0.86 with % RSD value 0.89.

Method validation:

Accuracy: The average recovery ranged from 100.08 ± 0.69 % confirmed the accuracy of the method.

Precision: The% RSD for Intra-day was 0.21 where as % RSD for Inter-day was 0.77.

Specificity study: The interference study for estimation of ferrous ascorbate in presence of folic acid was performed. The folic acid does not interfere in the estimation of ferrous ascorbate by forming complex with reagent.

Conclusion:

A simple UV-Visible spectroscopic method was developed and validated for estimation of ferrous ascorbate alone and in presence of ascorbic acid. In this, optimization of method for estimation of ferrous ascorbate was done by conventional way as one variable at a time. The proposed method is precise, accurate, and sensitive. Hence, it can be employed for routine quality control of ferrous ascorbate formulations.

References:

Development of validated analytical method for estimation of Ibuprofen and Famotidine in combined dosage form.

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Abstract: A simple, precise, accurate, simultaneous stability indicating RP-HPLC method for the estimation of IBU (Ibuprofen) and FMT (Famotidine) in combined dosage form was developed using Grace RP-C18 (4.6 x 250mm, 5µm) in an gradient mode with mobile phase comprising of Methanol: Water (pH 2.5 using OPA) The flow rate was 0.7 mL/ min and effluent was monitored at 240.0 nm. The retention times were found to be 6.68 min for IBU and 1.76 min for FMT. The assay exhibited a linear dynamic range of 30- 150 µg/mL for IBU and 1- 5 µg/mL for FMT. The calibration curves were linear (r² = 0.994for IBU and r² = 0.997for FMT) over the entire linear range. Mean % recovery was found to be 99.82 %for IBU and 99.91 % for FMT with % RSD was NMT 2 for both estimations which fully agrees with system suitability which is in good agreement with labeled amount of formulation. The % RSD for Intra- Day & Inter-Day Precision was NMT than 2 for both the drugs. The developed method was validated as per ICH guidelines

Introduction:
HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. The parameters for method validation as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantitation, Linearity, Range, Robustness and Ruggedness.

Material and Methods:
Standard samples of IBU&FMT were received as gift samples from the leben laboratories akola (Maharashtra) and taj pharmaceutical mumbai (Maharashtra). The marketed formulation Duexis (Horizon Pharma) was purchased from the local market containing IBU 800 mg and FMT 26.6 mg and all the chemicals used were are of analytical grade.

Optimization of mobile phase and chromatographic conditions: The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by
permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents

**Analysis of marketed formulation:** Equal volume (20.0 μL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured.

**Results and Discussion:**

**Optimization of Mobile Phase and Chromatographic Conditions**

![Optimized Chromatogram of IBU&FMT](image)

**Analysis of standard laboratory mixtures:**

<table>
<thead>
<tr>
<th>Std weight (mg)</th>
<th>Sample weight (mg)</th>
<th>Area of Std</th>
<th>Area of Sample</th>
<th>% Labeled Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU</td>
<td>FMT</td>
<td>IBU</td>
<td>FMT</td>
<td>IBU</td>
</tr>
<tr>
<td>120.0</td>
<td>4</td>
<td>794</td>
<td>5817.66</td>
<td>173.802</td>
</tr>
<tr>
<td></td>
<td></td>
<td>796</td>
<td></td>
<td>128.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>798</td>
<td></td>
<td>125.16</td>
</tr>
</tbody>
</table>

**Analysis of Marketed Formulation**
Figure 4 Chromatogram of Marketed Formulation

Conclusion:
The developed RP-HPLC method was found to be linear over wider concentration range. Therefore the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of IBU and FMT in bulk and pharmaceutical formulations like tablets. This method was also used to check quality of product after different storage condition and when stress degradation is carried out. The developed RP-HPLC method was validated as per the ICH guidelines. The developed RP-HPLC method has a stability indicating nature hence the proposed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

Acknowledgement:
The authors are thankful to The Principal, Government College of Pharmacy, Amravati.

References:
Micropropogation of *Alpinia purpurata* using low cost media for quantification of Rutin

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E-mail address: anamdeo@gmail.com

Abstract: Present study was designed to find simple and cheaper alternatives for micropropogation of *Alpinia purpurata* for determination of rutin. The accumulation of flavonoids was evaluated in organogenic cultures of *A. purpurata* using HPTLC. *A. purpurata* were cultured on MS medium supplemented with low cost alternatives like coconut water, marketed sugar, benzylaminopurine (BA), Kinetin, NAA (Naphthalene-3-acetic acid), IAA (Indole-3-acetic acid) and 2,4-D (2, 4-Dichlorophenoxyacetic acid) at the different concentration range for callus, shoots and root initiation. Combination of low cost additives in MS media gave the best result of *A. purpurata* in 2, 4-D (2 ppm) and kinetin (2ppm) for callus initiation, maximum number (9-11) of shoots were observed in medium with NAA (0.1ppm) and BA (3.0 ppm). Roots initiation was found in IAA at the concentration of 3ppm. The results of HPTLC methods revealed that rutin content in the leaves extract of *A. purpurata* was more in low cost tissue culture grown plant micropropogated with 20% coconut water and 3% marketed sugar. It indicate that low cost media in tissue cultures of *A. purpurata* could be a valuable alternative approach for rutin production.

Introduction:
Secondary metabolites production has some limitations like unpredicted environmental conditions, time consuming, desired quality, and a gap between the demand and supply. Therefore micropropogation through plant tissue culture can be an attractive alternative method. By suitable manipulation of hormones and contents of the medium, it is possible to initiate the developments of roots, shoots and complete plants from callus cultures. [1] Rutin is a naturally occurring bioflavonoid having broad range of physiological activities. Therefore, an attempt was made to compare the rutin content in the low cost tissue culture extract of *A. purpurata* with conventional extracts using HPTLC.

Materials and Methods:
Authenticated Plant material of *A. purpurata* (Vieillard) K. Schumann was collected from the Jawaharlal Nehru TBGRI, Thiruvananthapuram. HiMedia Murashige and Skoog’s (1962) (MS) medium [2] was used. Additives like marketed sugar, coconut water, gelling agent and plant growth regulators were added as required for the experiments. CAMAG LINOMAT 5 instrument was used for HPTLC fingerprinting.
Results and Discussion:

Figure 1 Different stages of callus initiation and shoot proliferation in *Alpinia purpurata*

Figure 2 Determination of concentration of rutin in natural grown and tissue culture grown plant of *Alpinia purpurata*

The cost of tissue culture of banana was reduced by 90% by replacing the tissue culture sucrose grade with a commercial sugar. It is recommended that table sugar be considered as low-cost substitute for potato micro-propagation and it was significantly lowered the production input costs by 34 to 51% when compared with the analytical grade sucrose.[4] Water is one of the major medium components. Distilled water obtained by electrical distillation is costly and required a sophisticated distillation apparatus. However, rain water or tap water can be used as a substitute in banana, ginger and strawberry respectively.[5] The time required for sample analysis in HPTLC is much less compared to HPLC. In HPLC, one sample is injected at a time and after every injection there is a washing period. On the other hand, in HPTLC more than one sample is applied on a plate and quantitated in a single run. According to the results obtained by our study the concentration of rutin was increased in low cost grown plant of *Alpinia purpurata.*
Conclusion:
Plant tissue culture technique with low cost produced healthy planting material throughout the year and also it represents an improvement over traditional methods. The high survival rates of 100% and the achievement of vigorous plantlets were indications that change in the variables does not affect the growth and production of secondary metabolites. The concentration of secondary metabolites was also changed with change in different growth parameters or by different variables. So there is need to try low cost growth regulators, infrastructure, equipments and energy on micropropogation technique.

Acknowledgement:
Authors kindly acknowledge the UGC for granting Major Research Project to Dr. Namdeo and AICTE (QIP) fellowship to Mr. Kale.

References:
Diversified antioxidant, intercellular reactive species scavenging, antiproliferative and molecular docking study of some phytochemicals

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Abstract: In the present study, ten phytochemicals were isolated from the three plants belonging to rosacea family and evaluated for antioxidant, intercellular reactive species scavenging, antiproliferative activities. Among these, chlorogenic acid, gallic acid, ferulic acid and pyrogallol exhibited significant antioxidant activity. Reactive species scavenging and antiproliferative activity were performed on two breast cancer cell lines viz. MCF-7 and MDA-MB-468 and highest scavenging activity were shown by gallic and chlorogenic acid. Gallic acid also showed potent antiproliferative activity. In order to establish in vitro and in silico activity, docking study on estrogen receptor- α and DNA topoisomerase II were also performed.

Introduction: Overproduction of reactive species (RS) through endogenous or exogenous insults can cause oxidative stress. The unregulated or prolonged production of reactive species, damages critical biomolecules and eventually leads to cancer. Oxidative stress can be prevented by the consumption of antioxidants present in fruits and vegetables. In a review it has been reported that, both positive and negative correlation exist between antioxidants and antiproliferative activity[1]. In view of that the present study was performed.

Materials and Methods: 
Chemicals: All chemicals were procured from Sigma Chemicals Co., St. Louis, USA.
Plant: Prunus persica and Prunus domestica was collected from Himachal Pradesh whereas Prunus dulcis was collected from Jammu and Kashmir.
DPPH scavenging activity and Scavenging of superoxide was determined using our previously published method [2] and also antiproliferative activity of compounds on two breast cancer cell lines viz. MCF-7 and MDA-MB-468 was determined using the method published in our previous paper [3].
ABTS scavenging activity: For ABTS assay, the procedure followed the method of Arnao et al. (1999) with some modifications [4].
Scavenging of intercellular ROS: Bes-H2O2-AC was used to determine scavenging of intercellular ROS by the procedure followed the method of Li Yet al [5].
Molecular docking study: Molecular docking study was performed on two PDB; estrogen receptor alpha (PDB ID: 1GWR) and DNA topoisomerase II (PDB ID: 3QX3) through CLC drug design workbench.

Results and Discussion:
Among all compounds chlorogenic acid, gallic acid, pyrogallol, rutin and protocatechuic acid possess highest DPPH scavenging activity which was also confirmed by ABTS and superoxide scavenging activity (Table 1). Further all the compounds were evaluated for intercellular ROS scavenging on breast cancer cell lines and was found that gallic acid and chlorogenic acid reduces the fluorescence in dose dependent manner (Figure 1).

Table 1 IC50 values of different antioxidant activities of isolated compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DPPH</th>
<th>ABTS</th>
<th>Scavenging of superoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>115.90 ± 0.07</td>
<td>0.133 ± 0.003</td>
<td>0.117 ± 0.001</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>118.28 ± 0.67</td>
<td>0.132 ± 0.003</td>
<td>0.116 ± 0.002</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>121.41 ± 0.14</td>
<td>0.167 ± 0.002</td>
<td>0.123 ± 0.002</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>122.66 ± 0.37</td>
<td>0.177 ± 0.001</td>
<td>0.129 ± 0.003</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>444.59 ± 3.75</td>
<td>0.248 ± 0.002</td>
<td>0.123 ± 0.002</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>524.11 ± 1.97</td>
<td>0.312 ± 0.003</td>
<td>0.142 ± 0.003</td>
</tr>
<tr>
<td>p-hydroxybenzoic acid</td>
<td>870.32 ± 1.07</td>
<td>0.435 ± 0.002</td>
<td>0.157 ± 0.002</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>119.00 ± 1.01</td>
<td>0.140 ± 0.002</td>
<td>0.118 ± 0.002</td>
</tr>
<tr>
<td>Ethyl gallate</td>
<td>125.00 ± 2.07</td>
<td>0.188 ± 0.003</td>
<td>0.130 ± 0.001</td>
</tr>
<tr>
<td>Rutin</td>
<td>121.68 ± 0.46</td>
<td>0.170 ± 0.002</td>
<td>0.124 ± 0.003</td>
</tr>
</tbody>
</table>

Gallic acid and pyrogallol showed potent antiproliferative activity as compared to adriamycin and showed cytocidal effect whereas other compounds were cytostatic in nature (Fig. 2). Molecular docking study revealed that all isolated compounds binds to the active site of both estrogen receptor alpha (ERα) and DNA topoisomerase II. Gallic acid showed interactions with Leu 346, Glu 353 and Arg 393 active amino acids of ERα and Asn 525 and Asp 479 of DNA topoisomerase II (Figure 3).
Figure 2 Antiproliferative activity of isolated compounds and standard.

Figure 3 Molecular docking study.

A. Binding site of gallic acid and incorporated ligand in protein estrogen receptor alpha  
B. hydrogen bond interaction of gallic acid with estrogen receptor alpha  
C. binding site of all compounds and incorporated ligand with DNA topoisomerase II  
D. hydrogen bond interaction of gallic acid with DNA topoisomerase II.

Conclusion:  
All isolated compounds possess antioxidant activities. Gallic acid showed most potent intercellular ROS scavenging and antiproliferative activity and also binds to the active site of protein. So it is suggested that gallic acid further studied for their therapeutic potential in treating different chronic diseases.

Acknowledgement:  
We would like to thank DST, New Delhi for providing INSPIRE fellowship to Naveen Dhingra (SRF).

References:
Standardization of three different cultivars of *Lagenaria siceraria* (Molina) Standl fruits using Cucurbitacin-E by RP-HPLC

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**Abstract:** *Lagenaria siceraria* (Molina) Standl is widely used plant with both nutritional and medicinal benefits. Different cultivated varieties of *Lagenaria siceraria* (Molina) Standl fruits are available differing mainly in shape and size thus having different local names. These fruits contain a high amount of cucurbitacin glycosides. The amount of cucurbitacin E present in cucurbitaceae fruits differs within the cultivars due to its genetic variations. Here, a validated high performance liquid chromatography (HPLC) method has been developed in reverse phase C18 column using acetonitrile and water (1% glacial acetic acid) as mobile phase in the ratio of 70:30 (v/v), at the flow rate of 1ml/min and the λ_{max} was set at 254 nm. This method will help to ensure the proper quality of these varieties and will also be beneficial in controlling the toxic effects of cucurbitacin E.

**Introduction:**
*Lagenaria siceraria* (Molina) Standl commonly known as Bottle gourd (“lauki” in Hindi) is an important member of the cucurbitaceae family containing almost all the essential nutrients and phytoconstituents required for living a normal and healthy life [1]. Phytoconstituents such as cucurbitacins E and their glycosides, saponins, essential oils, fixed oils, vitamins have been reported in the plant [2]. Cucurbitacin E and their glycosides are abundant in *L. siceraria* cultivars [3] having different pharmacological activity along with some toxic effects. The fruits of different cultivars of *Lagenaria siceraria* (Molina) Standl have been named according to the shape and size such as long hybrid bottle, short hybrid bottle, shorter hybrid, bottle gourd, water jug variety 1, water jug variety 2, cave man’s club, long handle dipper, tobacco box etc [4]. The present study aims to develop a validated RP-HPLC method to summarise our knowledge on the occurrence of cucurbitacin E in different cultivars of *Lagenaria siceraria* (Molina) Standl.

**Materials and Methods:**
**Chemicals and reagents:** Acetonitrile and glacial acetic acid (HPLC grade) were procured from Merck (Mumbai, India). All other solvents used were of analytical grade, procured from Merck. Cucurbitacin E was purchased from Sigma Aldrich (St. Louis, MO, USA).

**Extraction of plant material:** Fresh fruits of three cultivars of *Lagenaria siceraria* namely long hybrid bottle, short hybrid bottle and shorter hybrid bottle were weighed and individually cut to small
pieces and put into laboratory mixer grinder for making the juice. The juice was then filtered using filter paper for removing the fibres and other insoluble materials present to get the pure juice. The juice obtained from individual cultivars was lyophilised for removing water or moisture from extract which was then used for standardization with suitable marker. The resultant lyophilized juice extract were weighed. The percentage of yield obtained were 15%, 12.8%, 16.2% w/w respectively.

**Preparation of standard and sample solution:** About 10 mg of Cucurbitacin E standard was weighed and taken in 10 ml volumetric flask. Then 5 ml diluent (acetonitrile: water::70 : 30 v/v) was added in the flask, mix thoroughly and sonicated for 5 minutes. The volume was made up to 10 ml with diluents. The sample solutions were prepared by taking 10 mg of extract in 1 ml diluent. The solution was filtered through 0.45 µl syringe filter prior to injection.

**Chromatographic conditions:** HPLC assays were performed using isocratic conditions by the external standard method. Mobile phase composition was optimized to acetonitrile (solvent A) and water (solvent B) in the ratio of 70: 30 (v/v). The pH of the solvent B was adjusted at 3.8 by using 1%(v/v) glacial acetic acid.

**Method validation:** Method validation was executed by linearity, specificity, accuracy and precision, limit of quantification and limit of detection on the basis of International Conference on Harmonization (ICH) guidelines [5].

**Results and Discussion:**

**Preparation of calibration curve of standard Cucurbitacin E:** The calibration curve was found to be linear in the concentration range of 1-100 µg/ml. The correlation co-efficient were found from the calibration curve as >0.99, which confirms that the data is closer to the line of best fit. The regression equation was found to be Y=19111X-54747.

**Figure 1** RP-HPLC Chromatogram of cucurbitacin E Standard and *Lagenariasiceraria* (Molina) Standl(long hybrid bottle) extract

**Quantification of cucurbitacin E in three different cultivars of Lagenaria siceraria (Molina) Standl fruits:** The content of cucurbitacin E found in different cultivars of *Lagenaria siceraria* (Molina) Standl fruits are as follows: long hybrid bottle-1.9%w/w, short hybrid bottle-0.11% w/w and shorter hybrid bottle-0.04% w/w. This has been represented in table 1. The chromatograms of the standard and of three different cultivars have been shown in Figures 1 and 2.
Figure 2 RP-HPLC Chromatogram of *Lagenaria siceraria* (Molina) Standl (Shorter hybrid bottle) and (Short hybrid bottle) extract

Table 1 Percentage yield and cucurbitacin E content in three different cultivars of *Lagenaria siceraria* (Molina) Standl Fruit.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Cultivar of <em>Lagenaria siceraria</em> (Molina) Standl Fruit Extract</th>
<th>% Yield of Extract (w/w)</th>
<th>% Cucurbitacin E Content Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Long hybrid bottle</td>
<td>15</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>Short hybrid bottle</td>
<td>12.8</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>Shorter hybrid bottle</td>
<td>16.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The study showed that the cultivar named Long hybrid bottle has the maximum amount of cucurbitacin E and hence its consumption as medicinal or nutraceutical agent should be tightly regulated.

**Conclusion:**
The developed HPLC method is very accurate, precise and reproducible for quantification of cucurbitacin E with a narrow linear range. It will also be able to give insight on the cucurbitacin content in food stuff. The study will further necessary for quality control of *L. siceraria* which has been used to manage several lifestyle related disorder.

**Acknowledgement:**
The authors are thankful to the Department of Biotechnology, Government of India, New Delhi, India for financial support through Tata Innovation fellowship program to Dr. Pulok K Mukherjee.

**References:**
Evaluations of interaction potential of Marsilea quadrifolia extract through drug metabolizing enzyme inhibition studies.

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E-mail address: amit.kar2@gmail.com

Abstract: The present study was to determine the interaction potential of the standardized extract of Marsilea quadrifolia (MQ) and its active phyto-constituents against CYP2D6 and CYP3A4. Hydro alcoholic extract of MQ was standardized through RP-HPLC, using Quercetin (QU) as marker. CYP-CO complex assay and fluorescence assay were carried out for this study. CYP450-CO assay result stated that the interaction potential was found to be less than the ketoconazole. In the fluorimetric assay, MQ extract showed higher IC$_{50}$ values than their positive inhibitors. It can be concluded that MQ has very less interaction potential with drug metabolizing enzymes and may not produce any toxic effect upon their therapeutic benefits.

Introduction:
Cytochrome P450 (CYPs) is a diverse group of enzyme involved in the oxidative metabolism of drugs and other xenobiotics. Now days, Multi-drug combination therapy is widely used for several diseases and the drug–drug, herb-drug and food-drug interactions are getting more focused in clinical practices [1]. In the ancient practice of Indian Medicine including Ayurveda, several medicinal plants have been used to relieve and treat human diseases [2]. M. quadrifolia (MQ) (Family - Marsileaceae) is well known in India as leafy vegetable. MQ extract also posses’ good antibacterial, anti malarial, anti diabetic, cytotoxic, antipyretic, antioxidant and anti hangover activity [3]. The present study focused on the standardization of MQ extract by RP-HPLC with respect to its phyto- marker (QU). The standardized extract was further screened for their inhibitory effects on drug metabolizing enzyme with respect to its bioactive compound.

Materials and Methods:
Chemical and reagents: Vivid® CYP450 Screening Kit was purchased from Invitrogen Drug Discovery Solutions, USA, Standard quercetin dihydrate (Sigma, Steinheim, Germany). All other chemicals and reagents were of analytical grade procured locally.

Extraction of plant material: Shade dried MQ leaf (1kg) weighted and was extracted with 70% ethanol. The obtained extract was lyophilized to get a dry powder and final yield was found to be 13.58% (w/w).
**Standardization of crude extract through RP-HPLC analysis:** The presence of quercetin marker was determined by using the isocratic condition of Water: Methanol: Acetic acid (40:60:0.5 v/v/v). Calibration curve of QU was prepared in the concentration range of 20-100 μg/mL.

**Herb-Drug interaction study**

**CYP-CO Complex assay:** Cytochrome P450-Carbon monoxide complex (CYP450-CO) assay was performed with pooled rat liver microsome (RLM) in 96 well microplate. Spectral property was employed for the specific estimation of CYP450 content and possible interaction [3]. Ketoconazole used as positive control.

**Fluorimetric assay:** Fluorogenic assay was performed in black 96-well microplates (NUNC, Roskilde, Denmark); [4] with Vivid® CYP450 Screening Kit. This assay was based on the formation of flurogenic product from enzyme-substrate complex. The spectral data were analyzed. Percentage of inhibition and IC₅₀ was calculated as formula described earlier [5]

**Statistical Analysis:** Experimental data were expressed as mean ± S.E.M. The statistical significance was calculated using GraphPad InStat Version 5.0. Dunnett's multiple comparison tests.

**Results and Discussion:**

**RP-HPLC analysis of MQ extract and QU:** Calibration curve of QU represents a linear relationship between peak area and concentration (20-100 μg/mL). Coefficient of determinants (r²) of was found to be 0.99 and the relationship between peak areas (y) and concentrations in μg/ml (x) was Y = 8.946821e-009X-0.317557. Retention time of QU was found to be 12.1 min. The amount of QU present in extract of MQ extract was found to be 3.79 % (w/w).

**Herb-Drug interaction study**

**CYP-CO complex assay:** The results showed that positive inhibitor ketoconazole showed much more inhibition potential than MQ extract and QU. Observed result revealed that the interaction potential of MQ extract was more rather than its individually bioactive molecule QU. Percentage inhibition of the test substances compared to the positive control has been depicted in Fig. 1

**Fluorimetric assay:** All samples were assayed in triplicate and IC₅₀ values were calculated. The study showed that MQ extract has higher IC₅₀ value than the slandered inhibitor ketokonazole (6.71±1.00) and lower IC₅₀ than the quercetin for both the isozymes. The results indicated that (Table 1) the test substances have very less interaction potential with co-administered drug.
Figure 1 Percentage inhibitions of *M. quadrifolia* extract and quercetin.

Table 1 IC$_{50}$ (µg/ml) value of the MQ and QU on the metabolism mediated by CYP3A4 and CYP2D6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent</th>
<th>IC$_{50}$ value (µg/ml)</th>
<th>CYP3A4</th>
<th>CYP2D6</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Marsilea quadrifolia</em></td>
<td>DMSO</td>
<td>294.33 ± 4.66</td>
<td>189.67 ± 6.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>286.85 ± 3.89</td>
<td>178.98 ± 8.76</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>DMSO</td>
<td>335.17 ± 2.78</td>
<td>232.96 ± 9.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>333.59 ± 4.85</td>
<td>227.71 ± 7.43</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>DMSO</td>
<td>9.52 ± 4.98</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>8.69 ± 3.77</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>DMSO</td>
<td>--</td>
<td>7.63 ± 5.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>--</td>
<td>5.91 ± 6.79</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:
The *in-vitro* study revealed that the test substances produced only minor inhibition of CYP3A4 and 2D6. The outcome revealed that the selected herb and the bioactive compound have less inhibition potential on the tested isozymes compared to their relevant positive inhibitors.

Acknowledgement:
The authors would like to express their gratitude to the State Govt. Fellowship, Govt. of WB for providing financial support to Mr. Amit Kar, SRF, School of Natural Product Studies, Jadavpur University, Kolkata.

References:
Abstract: Lupeol is a triterpenoid reported in Strobilanthus callosus (Family: Acanthaceae) roots. Isolation, semisynthetic modification of lupeol to its various ester derivatives and their immunomodulatory activity studies has been envisaged in this research work. The different ester derivatives of lupeol like benzoate & salicylate have been synthesized which were coded as LBD & LSD. The derivatives were further confirmed by IR, 1H NMR & 13C NMR spectrum. The immunomodulatory activity of various ester derivatives was evaluated using Delayed type hypersensitivity (DTH) model. Cyclophosphamide was used as immunosuppressant. The different derivatives in the doses 25mg/kg and 50mg/kg produced minimum foot pad thickness as compared to control and CP.

Introduction:
Lupeol (LP) was isolated from the roots of S. callosus. The acid chlorides and anhydride on reaction with lupeol which is a triterpenic alcohol in the presence of dry pyridine converted to various ester derivatives coded as LBD (lupeol benzoate), LSD (lupeol salicylate). The structural assignments of the isolated compound were based on their elemental analyses and spectral data (IR, 1H-NMR, MS).

Materials and Methods:
Lupeol was isolated from the root of Strobilanthus callosus (family-Acanthaceae) using petroleum ether (60°-80°C). Cyclophosphamide was used as standard immunosuppressant. Antigenic material - The sheep RBCs (SRBCs) were used as antigenic material.

General procedure for the preparation of different ester derivatives: In a 100 ml round bottom flask placed a mixture of 0.426 g lupeol, 0.210 g of acid chloride (or acid anhydride) and 10 ml dry pyridine. Add small chips of porous porcelain, attach reflux condenser and boil the mixture for few hours at a temperature of 60°-70°C. Check the completion of the reaction using thin layer chromatography until a single spot was obtained. After completion of the reaction, shake the reaction mixture with cold petroleum ether (60-80°C). The excess of acid chloride remains undisolved in cold petroleum ether, collect the cold petroleum ether part and allow it to evaporate. The residue was washed with several times with cold petroleum ether to give the ester derivative.
**Immunomodulatory activity:** All synthesized compound were screened for immunomodulator activity by delayed type hypersensitivity model.

**Preparation of suspension of dose of tested drugs:** 5 ml of 25mg/kg (Lower dose) and 5 ml of 50mg/kg (Higher dose) suspension of ester derivatives were prepared in distilled water using 2% w/v Tween-80 as a suspending agent.

**Statistical analysis:** Data were analyzed using one way analysis of variance followed by Bonferroni multiple comparison test. The values are expressed as mean ± SD

**Results and Discussion:**

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of semisynthetic derivatives of Lupeol on mean foot paw oedema in DTH model.</th>
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</thead>
<tbody>
<tr>
<td><strong>Group No.</strong></td>
<td><strong>Group specification</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Control (TWEEN 80)</td>
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<tr>
<td>II</td>
<td>LP</td>
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<td>III</td>
<td>LBD</td>
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<td>IV</td>
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<td>V</td>
<td>CP 20mg/kg</td>
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<td>VI</td>
<td>LP + CP</td>
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<tr>
<td>VII</td>
<td>LBD + CP</td>
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<tr>
<td>VIII</td>
<td>LSD + CP</td>
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</table>

Statistical method: One way ANOVA followed by Bonferroni multiple comparison test. N = 6; Tabular values represent mean ± SD; *P<0.001 (Comparison of I with IV (H), V). L=Lower, H=Higher. *P<0.001 (Comparison of V with VIII (H) CP: Cyclophosphamide, LBD & LSD: Derivatives of lupeol.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of semisynthetic derivatives on leukocyte and platelet count in DTH model</th>
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<tr>
<td><strong>Group No.</strong></td>
<td><strong>Group specification</strong></td>
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<td>IV</td>
<td>LSD</td>
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</table>
Acute toxicity studies of semisynthetic derivatives of lupeol determined orally in mice: In all the semisynthetic derivatives of lupeol, no mortality was observed up to the oral dose of 75 mg/kg. Beyond 75 mg/kg the derivatives was not studied due to low yield.

Effect of semisynthetic derivatives of LP on Mean Foot pad thickness in DTH model: Animals were treated with cyclophosphamide exhibited maximum potentiation of DTH as observed from an increase in mean foot pad thickness. The derivatives when administered orally at the dose of 25mg/kg and 50 mg/kg suppressed potentiation of DTH response (P<0.01) (Table 1) and animals were treated with CP and receiving the derivatives also significantly suppressed the potentiation induced by CP as compared with animals treated with CP alone (P<0.01). It indicates the derivatives alone and in combination of CP it suppresses or favorably modulates the CP potentiated DTH reaction.

Effect of semisynthetic derivatives of LP on Total WBC Count and Platelet count in DTH model: The CP induced myelosuppression was significantly counteracted by the different derivatives at both dose levels (P<0.01). The results of CP + LP, CP + LBD and CP+LSD groups have shown a marked increase in the Platelet counts respectively as compared with the CP treated group. This clearly indicates that the derivative of lupeol have a strong immunostimulant activity on the suppressed immune system.

Conclusion: In pharmacological screening, the ester derivatives of lupeol were screened for immunomodulatory activity. The derivatives produces minimum foot paw oedema as compared to control and in combination of CP it suppresses or favorably modulates the CP potentiated DTH reaction. The CP induced myelosuppression was significantly counteracted by the derivatives at both dose levels i.e. at 25 mg/kg and 50 mg/kg. The CP induced thrombocytopenia was prominently counteracted by all the derivatives in both lower and higher doses.

References:
Genopreventive potential of *Tinospora cordifolia* against Thioacetamide induced genotoxicity in mouse bone marrow cells

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E-mail address: drnitinnema85@gmail.com

Abstract: Noticeable chromosomal damage or recombination is generally considered an important aspect bringing change in heritable or character which involved in multi-step process of malignancy, where genetic changes play significant role. Inhibitory effects of *Tinospora cordifolia*, was evaluated on the induction of clastogenicity on bone marrow cells induced by Thioacetamide in mice, using Bone Marrow Chromosomal assay. Pre-treatment with methanolic and aqueous extract of Leaves of *Tinospora cordifolia* (100 mg/kg b.wt, p.o) reduced structural chromosomal aberrations scored significantly (P<0.01) along with restoration of enzymatic antioxidants like Catalase, reduced glutathione and malonaldehyde content. In conclusion, it was found that *Tinospora cordifolia* reduces genotoxicity induced by Thioacetamide and thus may decrease the risk of development of secondary tumor. In comparative study methanolic extract was found to be more potent than aqueous extract.

Introduction:
Chromosome aberration has long been recognized as an important biomarker of human exposure to ionizing radiations and genotoxic chemicals. Both structural and numerical aberrations have been associated with problems such as congenital abnormalities in humans. The spontaneous frequency of chromosome aberrations is about 0.6% of live births and chromosome analysis of spontaneous abortions indicates that about 50% of such cases are chromosomally abnormal.

The aim of the present study was to establish genotoxicity database in bone-marrow cells after Thioacetamide induction along with quantification of oxidative stress enzymes in liver, during the process and the role of *Tinospora cordifolia* as a supplement or as an adjuvant to rehabilitation. Our findings also illustrate the involvement of immune system during the same.[1]

Materials and Methods:
Leaves of *Tinospora cordifolia* collected from Jeevan Anand Herbals, Sagar, (M.P.) India and were authenticated by Dr. Pradeep Tiwari, Botanist, Dr. Hari Singh Gour Vishwavidyalaya, with deposition of voucher specimen of the leaves- as Bot./Her./1046 respectively.

The use of animals is as per the Institutional Animal Ethical Committee (IAEC) of Sagar Institute of Pharmaceutical Sciences, Sagar (CPCSEA Registration No. SIPS/EC/2013/19) was followed.

Assessment of oxidative stress: Assessment of oxidative stress in the content of liver for lipid peroxidation (LPO), reduced glutathione (GSH) and catalase (CAT) was done [2, 3].
**Bone Marrow Chromosomal Assay:** Femurs were quickly removed, muscles were cleaned away from the bones and both femurs were flushed or aspirated with normal saline in centrifuge tube. Colchicine (0.025%) was added to the bone marrow cell suspension and incubated for 2 hr. Metaphase plates were prepared by Air Drop Method treated with hypotonic solution of 0.056% KCl and fixed in Cornoy’s fixative stained with 4 % Giemsa and observed under microscope [4,5].

**Results and Discussion:**
The animals administrated with extracts have shown better normal metaphase than the control (P<0.01). The control group revealed more fragmentations and deletions etc as compared to double minutes and Acrocentric Association. But after thioacetamide intoxication the double minutes and Acrocentric Associations also known as Robertsonian Translocation has been increased drastically which are suggestive of genotoxic nature of thioacetamide. When treated with methanolic and aqueous extracts, total aberrations decreased to 10.83% and 15.33% respectively as compared to thioacetamide treatment alone i.e. 19.8% (P<0.01). Thus, there was a drastic improvement in chromosomal protection both the *Tinospora cordifolia* extracts, where methanolic extract exhibited better genoprevention compared to aqueous extract.

<table>
<thead>
<tr>
<th>Treatment Groups (n=6)</th>
<th>LPO (nM/mg)</th>
<th>CAT (U/mg)</th>
<th>GSH (mM/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.39±0.20</td>
<td>36.45±0.68</td>
<td>22.27±2.14</td>
</tr>
<tr>
<td>TAA alone</td>
<td>5.94±0.31##</td>
<td>9.37±0.93##</td>
<td>7.12±0.34##</td>
</tr>
<tr>
<td>Methanolic extract + Thioacetamide</td>
<td>3.68±0.14**</td>
<td>27.43±1.79**</td>
<td>13.56±0.42**</td>
</tr>
<tr>
<td>Aqueous extract + Thioacetamide</td>
<td>4.94±0.21**</td>
<td>18.56±1.23**</td>
<td>9.67±0.61**</td>
</tr>
</tbody>
</table>

## Values represent significant difference when compared with control group, P<0.01; ** Values are significant difference when compared with model group, P<0.01; * Values are significant difference when compared with model group, P<0.05 Data are mean ± SEM; where n=6 mice in each group, LPO-Lipid peroxidation, CAT- Catalase, GSH- Glutathione.

**Conclusion:**
Our present study clearly revealed that *Tinospora cordifolia* preserves the genetic machinery in terms of chromosomal aberrations which is a leading cause for prediction of carcinogenicity. Further study reveals that *Tinospora cordifolia* can be used as an adjuvant during radiotherapy which can bypass the hazardous
Figure 1 Graphical presentation of the effect of extracts of *Tinospora cordifolia* against Thioacetamide induced model in bone marrow chromosomal assay.

** Values are significant difference when compared with control group, P<0.01; *** Values are significant difference when compared with model group, P<0.01; * Values are significant difference when compared with model group, P<0.05 Data are mean ± SEM; where n=6 mice in each group.

effect of radiation during Cancer Chemotherapy at cellular system at low. The compatibility and synergistic action of long term usage of *Tinospora cordifolia* extracts with Cancer Chemotherapeutic agents needs further exploration by modifying protocols to determine whether benefits are further sustained with time along with phytochemical studies. It is also important to further investigate the active entity present in methanolic extract as it has shown better genoprevention.

Acknowledgement:
I would like to express my deepest thanks and sincere appreciation to my guide Retired Prof. M. D. Kharya, former Head of Department, Dr. H.S. Gour, Sagar M.P.

References:
Biochemical and histopathological evidence on the beneficial effects of *Jasminum sambac* in TNBS induced colitis

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Abstract: The present study was designed to study anti ulcerative colitis activity of *Jasminum sambac* on Trinitrobenene sulfonic acid (TNBS) Induced ulcerative colitis (UC) in rats received standardized ethanol extract of *Jasminum Sambac* at 500 mg/kg orally. Also, ulcer index, glutathion (GSH), superoxide dismutases (SOD) and lipid peroxidation (LPO) and histopathological evaluations were measured. The reduction of ulcer index in extract treated animals was found to be statistically significant (P<0.05) with respect to control animals. *Jasminum Sambac* significantly restored the SOD, GSH levels and decreased MDA levels Cellular damages to normal cell structure as observed in histopathological examination at dose of 500 mg/kg. *Jasminum Sambac* possesses promising healing function on colitis via anti-inflammatory, antioxidant, and mucosal healing properties.

Introduction:
Ulcerative colitis and chron’s disease are chronic, relapsing, immunologically mediated disorders that are collectively referred to as inflammatory bowel diseases (IBD) [1]. The ethanolic extract of plants *Jasminum sambac* showed better free radical scavenging activities against different reactive oxygen and nitrogen species. Therefore, considering the important role of polyphenolic compounds in the prevention or reduction of ulcerative colitis induced by different ulcerogenic agents. The aim of the research is to explore the anti ulcerative activity of extracts obtained from leaf of *Jasminum sambac*.

Materials and Methods:

**Materials:**
- Drugs: 2, 4, 6-tri-nitrobenzene sulphonic acid (TNBS), Salphasalazine, Ethanol extract of *Jasminum Sambac*.

**Methods:**
- Animals: Healthy adult rats of Wistar strain (200-250 g) were used for the study.
- Induction of colitis: Rats were anaesthetized lightly with ether following a 24-h fast. Administer TNBS (65 mg/kg in 0.25 ml of 40% ethanol) in 1 minute through an intracolonic catheter with its tip add 8 cm from the canal margin. [3]The control rats receive only 0.25 ml saline. Animals were randomized and divided in four groups (n=6). Group 1: Received 0.25 ml saline. Group 2: Received...
vehicle (0.5% w/v CMC) and TNBS (65mg/0.25 ml) dissolved in 40% (vol/vol) ethanol instilled into the colon (total volume of 0.5 ml per rat) for 14 days. Group 3: Received Sulfasalazine 300mg/kg and TNBS (65mg/0.25 ml) dissolved in 40% (vol/vol) ethanol instilled into the colon (total volume of 0.5 ml per rat) for 14 days. Group 4: Received 500 mg/ kg body weight of ethanolic extract of leaf of *Jasminum sambac* and TNBS (65mg/0.25 ml) dissolved in 40% (vol/vol) ethanol instilled into the colon (total volume of 0.5 ml per rat) for 14 days.

Macroscopic assessment of severity of colitis and ulcer index glutathion (GSH) superoxide dismututases activity, lipoperoxidase activity: Antioxidant activities were expressed as units/mg protein. The calibration curve than plotted using standard units. [2]

**Statistical Analysis:** The results are expressed as the mean ± SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnett’s t-test.

### Results and Discussion:

The histopathology, ulcer index and antioxidant parameter studies marked the significant anti ulcerative activity of *Jasminum sambac*. The percent inhibition of standard group (44.40%), treated group (37.33%) and neg.control group (19.33) which confirms the anti-ulcerative colitis potential of *Jasminum Sambac*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>% Inhibition</th>
<th>SOD (U/mg protein)</th>
<th>LPO (MDA nmol/gm protein)</th>
<th>GSH (nmol/mg proteins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>203.01±19.2</td>
<td>32.01 ±2.7</td>
<td>3.2 ±08</td>
</tr>
<tr>
<td>Negative control</td>
<td>12.17± 0.13</td>
<td>19.33± .25%</td>
<td>97.02 ±7.01</td>
<td>170.05±15.2</td>
<td>0.38±0.03</td>
</tr>
<tr>
<td>Standard</td>
<td>9.53 ± 0.26*</td>
<td>44.40 ± 1.32%*</td>
<td>163.3±16.1**</td>
<td>73±6.7**</td>
<td>2.28±0.06**</td>
</tr>
<tr>
<td>Treatment group</td>
<td>11.36± 0.33*</td>
<td>37.33 ± 0.92%*</td>
<td>132.03±11.3*</td>
<td>121.03±11.2*</td>
<td>1.29±0.2*</td>
</tr>
</tbody>
</table>

Statistical comparison was performed by using one way ANOVA coupled with Dunnett’s test.*P < 0.01 and **P<0.001 when compared with negative control and standard. The results were significant.

In the present study, evaluated the effect of *Jasminum sambac* on the level of inflammatory mediators, oxidative stress parameters, and histopathology and ulcer index in TNBS induced ulcerative colitis model and compared with sulphasalazine.

Ulcer and inflammation of the inner lining of colon is an index to measure activity of drugs and it is employed here to determine the activity of *Jasminum sambac* at the dose level 500 mg/kg/ J.S. administered groups showed marked reduction in colonic ulcer when compared with the negative control group. It found that there was significant weight loss, notice after one day at the day of TNBS
administration but after seven days continued to showed constant weight in rats. The result of the present study also indicates that as the extent of inflammation increase there may be loss of body weight. In this study, treatment of ulcerative rats with *Jasminum sambac* resulted in significant reduction in LPO levels in treated group. In this investigation, the SOD activity was significantly reduced in ulcerative rats compared to the normal rats. Excessive generation of $O_2^-$ could have depleted the SOD. The changes in GSH associated with TNBS induced ulcerative rats. The drug treatment has significantly result altered inflammatory changes in both standard and treated group.

**Conclusion:**
From the above results, it can be inferred that, the ethanolic extracts of leaf showed marked anti ulcerative activity in TNBS induced ulcerative colitis model, the dose of leaf extract was taken as 500 mg/kg that was proved as safe and effective dose required for the activity.

**References:**
Lipid blended polymeric nanovaccines for allergen immunotherapy

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Abstract: This work evaluated the lipid blended polymeric nanovaccines (LPNs) as adjuvant/delivery vehicle in specific immunotherapy using ovalbumin (OVA) as an allergen model. Developed LPNs had negative zeta potential (−42.2. mV) and an average size of 118.5 nm with OVA loading efficiency of ~ 72.3 µg mg\(^{-1}\) of nanoparticles. OVA-sensitized mice model, treatment with OVA loaded LPNs elicited lower levels of IgE (p<0.05), serum histamine and higher survival rate in comparison with alum adsorbed OVA. Symptoms of anaphylactic shock in OVA LPNs-treated mice were weaker comparison to alum adsorbed OVA group. Results demonstrate the valuable application of LPNs in allergen immunotherapy.

Introduction:
Allergy, or hypersensitivity type I, applies to an abnormal reaction against innocuous environmental compounds (allergens) and involves complex interactions between exogenous. So far, the only curative treatment for allergy is specific immunotherapy (SIT), where an immunologic hyporesponsiveness is induced by intradermal injections of gradually increasing quantities of allergen extract. Despite the efficacy of SIT, there are concerns with the treatment including difficulties in standardization of allergen extracts, patient compliance due to frequent injections. One of the strategies to improve vaccines for SIT is to use adjuvants that can modulate the allergen-specific T cell response. For this purpose, nanoparticles based antigen-delivery systems have shown promising results [1].

On the other hand, Lipid blended nanoparticles (LPNs) comprising polymer cores and lipid shells exhibit complementary characteristics of both polymeric nanoparticles and liposomes and found to be superior in vivo cellular delivery efficacy compared to that obtained from polymeric nanoparticles and liposomes [2].

Materials and Methods:
Materials: Poly-lactide-co glycolide (resomer 75:25) PLGA, hydrogenated phosphatidylcholine (Phospholipon 90H) and Aluminium hydroxide gel (Alhydrogel®), were procured as gift samples from Boehringer Ingelheim, Ingelheim, Lipoid GmBH, Germany and Branntag Biosector, Frederikssund, Denmark respectively. The reagents used for sodium dodecyl sulphate–polyacrylamide...
gel electrophoresis (SDS–PAGE) and bicinchoninic acid (BCA) protein assay kit were obtained from Genei (Bangaluru, India). Bovine serum albumin, horseradish peroxidise (HRP) conjugated goat antirnouse IgG, IgG1, IgG2a and IgE antibodies were procured from Sigma (Sigma-Aldrich Pvt. Ltd., St. Louis, USA). Cytokine assay kits were from eBioscience, San Diego, CA. All other chemicals otherwise specified were obtained from Himedia Pvt. Ltd. (Mumbai, India) and of analytical grade.

Methods: Preparation of OVA loaded LPNs: Preformed OVA loaded PLGA nanoparticles (prepared by double Emulsion Solvent Evaporation) are mixed with preformed lipid vesicles, where the lipid vesicles are loaded on the polymeric nanoparticles by electrostatic interactions [3].

Characterization of LPNs: Prepared nanoparticles were extensively characterized for shape, zeta potential, antigen integrity, OVA loading efficiency and in vitro release by transmission electron microscopy, dynamic light scattering, electrophoresis and allergen assay using BCA kit.

Biological evaluation: The humoral and cellular-induced immune responses generated by OVA loaded LPNs were studied by two intradermal immunizations in BALB/c mice. Fifteen days after therapy, animals were challenged with OVA and different signs of anaphylactic shock were evaluated.

Results and Discussion:

Preparation of LPNs: It was possible to obtain homogenous and nanometric typical LPNs formulations of OVA as confirmed by TEM and DLS measurements.

Characterization of LPNs: TEM shows typical LPNs morphology of polymeric core surrounded by lipid shell with nanometric architect (118.5±23.2 nm). High negative zeta potential of OVA loaded LPNs (~42.2 mV) was due to presence of negatively charged phospholipids and OVA at surface of particles at pH 7.4. High antigen association with LPNs (~72.3 µg mg⁻¹ of nanoparticles) was due to incorporation of OVA embedded in polymeric core. SDS–PAGE analysis revealed preservation of structural integrity of allergen in LPNs. Data from in vitro release showed that OVA was very slowly released from nanoparticles up to 12 h. Initially in first two hour, <2% OVA was released and once the phospholipid coating on nanoparticles was dissolves, due to polymer matrix retraction and dissolution in first 12 hour, 13.6% OVA was released in medium.

![Figure 1 A: Serum OVA-specific IgG isotype titers at weeks 2 and 6 (mean±S.D, n=6).](image)

![Figure 1 B: Evaluation of total IgE in sera from BALB/c mice during the sensitization and challenge study. (mean±S.D, n=6).](image)
Biological evaluation

In vivo immune response after two intradermal injections with OVA loaded LPNs resulted in a mixed Th1/Th2-type immune response. In OVA-sensitized mice model, treatment with OVA loaded LPNs elicited lower levels of IgE (p<0.05), serum histamine and higher survival rate in comparison with alum adsorbed OVA. Symptoms of anaphylactic shock in OVA loaded LPNs-treated mice were weaker than the one induced in the alum adsorbed OVA group.

Conclusion:

In summary, present research report demonstrates that OVA loaded LPNs are able to induce a strong T cell specific proliferative response with a cytokine profile suggestive of a Th1 response, prevention of anaphylactic reactions and maintenance of low titers of IgE, without abrogation of Th2-mediated responses. This suggests that LPNs could have possible implications in the future of allergen specific immunotherapy.

Acknowledgements:

Author is thankful to All India Institute of Medical Sciences, New Delhi for providing electron microscopy facilities.

References:

A tribal remedy for controlling oncogenic expression of HPV and cervical cancer by alteration in transcription machinery of cervical cancer cell

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Abstract: Cervical cancer has strong association with HPV infection and is leading cause of cancer associated female deaths in India [1]. The study was undertaken to find the mechanism of anti-cervical cancer activity of *Bryophyllum pinnata* (BP). The HPV transformed cervical cancer cells were treated with BP leaf extract. The study confirmed apoptosis inducing, anti-HPV and anti-cervical cancer activity of herb. The extracts down regulated expression of HPV oncogene and inhibited AP-1 binding to promoter region. Studies indicated shift in composition of AP-1 complex from oncogenic to non-oncogenic form [2]. Thus, the herb can be developed as remedy for HPV and cervical cancers.

Introduction:
The ancient medicinal system remains the source of modern therapeutics. The tribals round the world still have unexplored opulent knowledge of drugs and ways of treating diseases. The tribals of Jhabua district, Madhya Pradesh were observed to use *Bryophyllum pinnata* leaves for treating cancer of cervix. The plant found to contain many bioactive constituents including flavonoids, bufadenolides etc. [3, 4]. The present study was undertaken to scientifically prove evidence based anticancer action of the herb and identify possible molecular mechanism of anti-cervical cancer activity.

Materials and Methods:
Extraction and purification of *B. pinnata* leaf was done by successive solvent extraction method and purified by silica gel column chromatography. The purified extract of *Bryophyllum pinnata* was treated on cervical cancer cells, HeLa (HPV-18). Extract was characterized by HPTLC and NMR. MTT assay, Electrophoretic mobility shift assay, immuno-blotting and northern blotting were performed to check transcription and translation of HPV oncogene [5].

Results and Discussion:
Results showed growth inhibitory activity of purified *B. pinnata* extract, evident by IC50 at 91 μg/ml. The antiHPV activity was evident by inhibition of pivotal host transcription factor AP-1 DNA binding.
and oncogenic c-Fos and cJun expression in HeLa cells. A dose-dependent decrease in the level of HPV18 transcripts was observed.

*B. pinnata* extract induced apoptosis as evident by gradual increase in expression of proapoptotic Bax and suppression of anti-apoptotic Bcl-2 with in 12 h accompanied by cleavage of procaspase-3 and PARP-1 in HeLa cells. The chemical test, HPTLC and NMR of extract indicated presence of compound that resembled Bryophyllin A.

**Figure 1** Anti-cervical cancer effect of *Bryophyllum pinnata* by inhibiting transcription factor AP-1 specific DNA-binding activity by electrophoretic mobility shift assay (EMSA) and its subunit expression by western blotting and densiometric analysis (Fig. A, B)

![Anti-cervical cancer effect of *Bryophyllum pinnata*](image1.png)

(A) AP-1 concentration after treatment of a; crude extract, b; fraction 4, c; control

![Expression of AP-1 constituting proteins](image2.png)

(B) Expression of AP-1 constituting proteins after treatment of a; crude extract, b; fraction 4

**Figure 2** Anti-HPV activity of *Bryophyllum pinnata* on cervical cancer cell HeLa (Fig. A, B)
(A) RNA translation inhibition by

(B) Expression of pro-apoptotic proteins

*B. pinetta* extract

**Conclusion:**
*B. pinnata* leaf found to inhibit problem similar to cervical cancer in tribal area of M.P. It was confirmed at molecular level by treating *B. pinnata* extract on cervical cancer cells. The data thus obtained, strongly support its merit in treatment of cervical cancer. Thus, it can be developed further as a potential anticancer, anti-HPV drug for treatment of HPV infection and cervical cancer.

**Acknowledgement:**
The authors acknowledge Indian Council of Medical Research (ICMR) and Indian Institute of Science (IISc) for infrastructure and financial support for the study.

**References**


Novel DNS-mind power techniques for scoring in exam-DNS-tricks in pharmacognosy

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Abstract: The focus of this project is to motivate UG students to seek admission in M. Pharm Pharmacognosy by developing interest in subject through DNS MPTs and thereby positioning Pharmacognosy at the top of all subjects in all Indian Universities and developing DNS mind power techniques for scoring in exam.

In initial phase one of the simple methods is DNS Clock-based circle model for remembering Biological Source, Chemical Constituents, Tests, Use. If students learn this one page data of DNS Clock-based circle model within 24 hours after learning in class, they can write in exam by visual memory mechanism and score. Till date 51 models/techniques are developed which are proved effective for my students.

The results are amazing, these DNS MPTs helped scholars to grab the Distinctions in my subject and slow learners to pass in annual exam, results are calculated in terms of less failure as compared to other 5 subjects (for 4-5 years) and my subject result was 100% for 4 year.

Introduction:

This project is focused on use of simple tricks to learn Pharmacognostic study of many metabolites like Carbohydrates, glycosides, alkaloids, terpenoids, tannins etc., that are included in semester or annual pattern of all Universities spread over India and abroad. The aim is to develop interest in subject by delivering Guest Lectures in various Pharmacy Institutes in India and abroad that will motivate students and generate interest in them to peruse Ph. D in Natural Products and Pharmacognosy.

As a teacher I have prepared these “DNS Tricks” for my UG students that really helped them to score high in exams like sessional, annual and GPAT. Pharmacognosy is a subject that comprises of Pharmacognostic study of various crude drugs that serves as plant metabolites for treating diseases or disorders like.

A student when selects Pharmacy as carrier and take admission in F. Y. B. Pharm, she/he actually starts his Pharma-journey from first lecture to his successful carrier as PG Research student, Hospital Pharmacist, Medical representative, Pharmacist in medical store, MS studies abroad, Assistant
Professor etc. This project is focused to observe happiness in eyes of students once they score in subject and show gratitude towards Teacher after achieving success.

Materials and Methods:
Discussion amongst Pharmacognosy teachers from all regions of Maharashtra. Various developed formats/models like; DNS Clock-based circle model, DNS Tricks for crude drugs-Wool, tabular form method for botanical name, chemical constituents and uses, layers method for extraction etc. All these models are developed by me over a period of 15 years (since 2000). Selected levels- B. Pharm and M. Pharm first year. For this project syllabus of about top eight Universities in India were considered.

Methods
FY- Students are exposed to topics like, History, tissues, cell, cell division, ecology etc. Similarly SY, TY, final yr, PG students’ study various topics for which 51 novel DNS MPT are developed by me depending on topic. Other developed DNS TRICKS for FY to PG levels are depicted in following table 1.

Table 1 Developed few DNS TRICKS for Pharmacognosy

<table>
<thead>
<tr>
<th>Levels</th>
<th>Developed Dns Models/Mpt</th>
<th>Description</th>
<th>Results (Since 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>DNS trick for all crude drug</td>
<td>Simple tricks like C O T S S Ef, it means color, odor, taste, size and shape and extra features of crude drug.</td>
<td>98 % followers Excellent</td>
</tr>
<tr>
<td>PG, FY</td>
<td>Focused learning</td>
<td>History- Help students to mention at least 3 -6 important Researchers like; Charak, Hippocrates, Sydler, Linnasus.</td>
<td>Avg.stu.remembers 5(Slow learner- 3)</td>
</tr>
<tr>
<td>ALL</td>
<td>Aim scoring</td>
<td>Teacher decides a no. to be scored in exam.</td>
<td>80 % stu. achieves</td>
</tr>
<tr>
<td>TY, Final</td>
<td>DNS Clock-based circle</td>
<td>It is very simple circles enclosing info of imp Pharmacognostic study like biological source, uses.</td>
<td>85 %. Easy for all stu.</td>
</tr>
<tr>
<td>ALL</td>
<td>24 hr. Base</td>
<td>Rewriting one page data (four square) within 24 hours.</td>
<td>86 %. Slow learners</td>
</tr>
<tr>
<td>PG</td>
<td>Visualization</td>
<td>Students can easily write morphology and TS.</td>
<td>All types of stu.</td>
</tr>
<tr>
<td>All</td>
<td>Aesthetic</td>
<td>Crude drugs are given to student to feel its surface and write rough/ smooth/ furrowed/ plain / black circles etc.</td>
<td>All types of stu.</td>
</tr>
</tbody>
</table>

Results and Discussions:
The number of distinctions or good results for subjects taught by me includes; Pharmacognosy-II (100% 2008), Pharmacognosy-II (98% 2009), Pharmacognosy and phytochemistry-I (6 distinctions compared in 2014), Pharmacognosy and Phytochemistry-II (6 distinctions), and less failures when compared with other subjects in FY, TY, SY etc since 2008. For rest of the years since 2000 my
subject result was above 90% except for 4 subjects (Not from Pharmacognosy area). Results obtained are surprising, these techniques not only helped intellectual students to get Distinction in my subject but also helped slow learner students to pass in exam. A type of respect for teacher was seen in eyes of students and smile on face when they informed me about their results. I felt great when PG students from Pharmacology, QA and Pharmaceutics department have shown keen interest in studying Natural product and happy that few UG students has taken admission for M. Pharm in Pharmacognosy.

**Conclusion:**
Appropriate techniques can be utilized by all teachers to make Pharmacognosy interesting and also helping student fraternity to score in exam.

**Acknowledgement:**
I am grateful to Dr. Raj Bapna, the person who is continuously motivating students to score in exam through CSR. Thankful to Rhonda Byrne for writing excellent book The Secret.

**References:**
Abstract: A drug compound can exist in different crystalline forms known as polymorphs. Patent may be granted for a new product or process involving an inventive step and capable of industrial application. The provisions relating to patentability of polymorphs in the Indian Patents Act have been under constant criticism at the international front. The present study was aimed to examine India’s position on patentability of polymorphs, based on (i) comparison of the related provisions of the patent laws in U.S., Europe, China and India (ii) review of the relevant case laws, and (iii) collection & analysis of the empirical data through research questionnaire. The study further aims to propose measures to strengthen the provisions for patenting in India.

Introduction:
Polymorphs are different physical forms or crystal structures of a drug compound. A patent may be granted for a new product or process involving an inventive step and capable of industrial application. The governing law for the grant of patents in India is the Patents Act, 1970[1]. The provisions relating to patentability of polymorphs in the Indian Patents Act have been under constant criticism at the international front. It has been argued that these provisions are more stringent than that of the other countries. Further, it has been questioned that these provisions are inconsistent with the TRIPS Agreement. Apprehensions have also been raised over the possible negative impact of these provisions on the country’s economy. The present study was aimed to examine India’s position on patentability of polymorphs and to propose measures to strengthen the provisions for patenting in India.

Results & Discussion:
Results of the study are provided below.

Patentability of polymorphs in U.S., Europe, China and India
The relevant provisions relating to patentability of polymorphs in U.S., Europe, China and India. In India, specific restrictions on the patentability of polymorphs have been put through Section 3(d). As per this section; to be patentable, a polymorph of known compound must exhibit enhanced therapeutic
efficacy in comparison to the known substance. In U.S., Europe and China although no specific restrictions on the patentability of polymorphs have been put explicitly in their patent laws; however, in practice restrictions in terms of raised standard of inventive step requirements are put during the patent examination process. In these countries, for a polymorph to be considered inventive, it must display a surprising technical effect or unexpected pharmaceutical activity when compared to the prior art.

Review of the relevant case laws

(a) Novartis-Glivec case of India: In 1998, Novartis filed a patent application in the Chennai Patent Office for the β-crystalline form of Imatinib mesylate (Glivec). In 2006, the Controller of Patents, refused to grant Novartis a patent for β-crystalline form of Imatinib mesylate, on the grounds that the said application lacked novelty, was obvious, and was non-patentable under Section 3(d) of the Act. Novartis challenged the above decision first in the High Court and then in the Supreme Court of India. In a landmark judgment on April 01, 2013, the Supreme Court of India upheld the Indian Patent Office’s rejection of the Glivec patent application and put a full stop on the battle of Novartis.

(b) Atorvastatin polymorph case of the European Patent Office (EPO): In 2011, the Boards of Appeal of the EPO handed down the decision T777/08, concerning the inventiveness of a polymorphic form of the drug atorvastatin. The board held the polymorphic form non-patentable on the ground that the claimed polymorph provides only the obvious advantages of a crystalline material over the amorphous form viz. improved filterability and drying. The board ruled that in the absence of any technical prejudice and in the absence of any unexpected property, the mere provision of a crystalline form of a known pharmaceutically active compound cannot be regarded as involving an inventive step.

Collection & analysis of the empirical data through research questionnaire

A questionnaire was formulated that mentions questions/ concerns raised over the current patenting system in India at national and international fronts in the recent time. Responses were collected online as well as in-person. Responses to the questions which are related to the subject area of this paper are summarized below:

Q.1 The main responsible factor for India’s consistently poor annual FDI (Foreign Direct Investment) inflows is its weak national IP environment as compared to the other BRIC (Brazil, Russia and China) and middle-income countries.

Result: Agree (53.85%) Disagree (46.15%)

Q.2 India’s current IP policy is inducing barrier to trade and is an obstacle in the business environment in India for the companies of foreign countries such as U.S. and Europe.
Result: Agree (62.00%) Disagree (38.00%)

Q.3 Article 27 of TRIPS enlists the subject matter that can be excluded from the patent coverage. Section 3 (d) of the Indian Patents Act, which excludes from patentability any new forms of known substances lacking enhanced efficacy, is inconsistent with the TRIPS Agreement because subject matter of section 3(d) is not included in the permissible list of exclusions for patentability mentioned in the Article 27. Furthermore, Section 3 (d) also conflicts with the non-discrimination principle provided by the Article, as this section sets a higher threshold of patentability specifically for the pharmaceutical inventions.

Result: Agree (43.14%) Disagree (56.86%)

Conclusion:
Comparison of the patent law provisions indicates that the criteria of patentability of polymorphs in India are at par with that of the U.S., Europe and China. The Supreme Court judgement on Novartis-Glivec case throws light on the Indian policy of rewarding the inventors on their true intellectual efforts and at the same time preserving the public interest and to make the public available essential commodities such as drugs at affordable prices. The Boards of Appeal of the EPO decision emphasizes on the raised standard of inventive step requirements for polymorph patenting at the EPO. Results of the questionnaire based survey indicate that the Section 3(d) provisions are consistent with the TRIPS agreement, as majority of the respondents (56.86%) disagreed for any such inconsistency. It is suggested that the Indian government should take appropriate measures in its IP policy to make the business environment in the country conducive to the foreign companies (62.00% of the respondents agreed) and to attract more FDI (53.85% of the respondents agreed).

References:
Academic adoption programme: a synergy between academic and industry partnership

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Abstract: It is observed that the changing complexity of the business environment has necessitated the industry and the academia to develop close links to create the synergy. The interface between these two has led to increasing mutual dependence to ensure their better survival in their domains. In light of this understanding academia has designed different modes of industry-academia interface (IAI). The present study titled “Academic Adoption Programme: A Synergy between Academic and Industry Partnerships”. School of Pharmacy and Emerging Sciences has developed a unique module with few Pharmaceutical Industries. By getting understanding in to these insights Academia can further develop the programmes which add more authenticity to the interface.

Introduction:

Today the industry is playing an increasingly important role in activities of academic institutions to incubate the talent they need so the colleges have to provide a unique platform for interaction between Industry-Academia, where in the actors of the interaction understand the latest trends of Industry-Academia interface keeping in view the perceived benefits and accordingly equip themselves with the skills required in a fast-changing global scenario [1].

In order to actualize the concept of Academia-Industry collaboration, Baddi University of Emerging Sciences and Technology, (BUEST), Baddi, identifies the need to encourage and assist the Industry Employees (who are skilled but any how deprived of formal Higher Education) in their academic development and professional enhancement. The idea of Academic-Adoption Programme shall enable the Employees of the industries/organisation who were not able to complete their dream of Higher Education owing to their job engagements. The Programme is a unique design desired to impart formal education in regular mode. This programme is applicable for the employees of the Industry/Organisation for the purpose of Professional Development through upgradation to next stage of formal higher education. This programme also produces Graduates/Post Graduates in Management and Science courses with a blend of specialization, through industry specified and need based majors.

Why this Programme?

- This programme gives the flexibility of earning a Graduation or Post Graduation Degree along
with a job in a **Regular Mode**.

- Since it is a regular mode programme, the constraint of talking week-off for attending classes or attending classes on Holidays on late evenings when other commitments are lined up is not there like other modes of Education such as Distance Mode/Part time mode.

**Advantage at Baddi:**

Located in the Industrial hub of Baddi-Barotiwalla-Nalagarh, BUEST felt a strong need to address these long pending challenges being faced by the employees of these industries. With the **Academic-Adoption Programme**, we have addressed all the above mentioned challenges being faced by the corporate employees for perusing Formal Higher Education without compromising with the Corporate/Personal Liabilities and Academic Quality.

**Methodology:**

This programme will be like the General Conventional Programmes having the same number of courses, credits earned and contact hours spent.

In a General **Conventional Graduation Programme** the spread is like this.

<table>
<thead>
<tr>
<th>Duration: 3 years</th>
<th>Semester: 6</th>
<th>Theory Courses: 36</th>
<th>Practical Courses: 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Courses: 54</td>
<td>Total Credit of the Programme: 144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Contact Hours Spend: 1728</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Academic-Adoption Programme** shall complete all the requisite Credits and Contact Hours of teaching for awarding a degree like a conventional programme in a regular mode without compromising on academic piety of the programme.

**Benefits:**

**Industry:**

I. This is a strong retention model for any industry. Industry can ask the employee to be associated with him/her till the award of degree.

II. This can be used as a tool for evaluation of the appraisal system.

**Employee:**

I. Will ensure the personal growth of the employees through formal higher education in Regular Mode which was not possible till now without compromising on Corporate and Social liabilities.

II. The students can chose majors depending upon the type of Industry and type of job role being handled in the industry which will enhance the growth prospective for employee.

**Academia:**
I. It’s a strong platform of exchange of learning between Academic and Industry. The faculty will learn procedures, practices and best practices which will enrich them with the knowledge to be imparted to the students.

II. More scope of practical learning for the academic people because the students have years of practical experience while performing their job. So it enriches the faculty with their practical understanding.

Society:

I. It will pave the new dimensions of Academia-Industry relationship and bring them closer and together.

II. It will provide opportunity for perusing higher education to the economically weaker section of the society who could not continue higher education due to economical constraints.

Conclusion:

Academia and industry, which for long have been operating in separate domains, are rapidly inching closer to each other to create synergies. The intersecting needs and mutually interdependent relationship requires identifying means of further strengthening academia-industry partnerships for a better mutual growth of Academia and Industry.

References:

An impact of class attendance and academic success amongst pharmacy student

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Abstract: Student absenteeism is a concern in higher education as it results in inadequate learning and poorer academic performance on the part of those missing class. The present study was designed to assess the association of pharmacy students’ personal characteristics with absenteeism and academic performance among the final year students. Participants were divided into “high” and “low” performers based on grade point average. Low performers were significantly more likely than high performers to miss class when the class was held before or after an examination and low performers were significantly more likely to believe that anticipating in class did not benefit them. There was a negative association between the number of hours students’ missed and their performance in specific courses. These findings provide further insight into the reasons for students’ absenteeism in a college or school of pharmacy setting.

Introduction:
The pharmacy curriculum is changing rapidly to meet the industrial knowledge demands; the institutes are being developed to incorporate advances in classroom technology and information access to provide more opportunities for interaction and satisfy the pharmaceutical industry needs. [1]. Several factors such as employment, faculty member behaviors and perceived expectations, increased accessibility to information and advances in technology, and even apathy among students regarding the value of lecture attendance are believed to also impact student performance [2]. The primary objective of this study was to assess various external influences, such as part-time employment, marital and family status, and travel time, and their impact on absenteeism. The secondary objective was to assess the association of academic performance with various student characteristics and reasons for absenteeism.

Methods:
This study was conducted with last semester of final year students of bachelor of pharmacy (B. Pharm) program of different pharmacy colleges of North Maharashtra, in academic year 2014-2015.

Survey Instrument Design
A modified survey instrument based on 2 published studies on student class attendance was developed [3.
4]. The survey instrument was given on the blog www.rcpiper-cognosy.blogspot.in they were allow to fill it online as one online form. This make easy to students as well for analysis. The numbers of absences assessed were initially divided into multiple categories (0 to 8 hours, and more than 8 hours). Cumulative GPA for the academic year 2014-2015 last semester was analyzed as a measure of academic performance and its relationship to student absenteeism was assessed.

**Results:**

The 114 students were responded to this survey. Almost all students were responded for all the questions asked to assess their attitude. Only two students were not responded for certain (Table 1). Among the all respondents 50 were of low performers and 64 of high performers. This separation was done on their last year percentages achieved in university exams. Amongst all the eight were married and rest of were unmarried. Surprisingly the married were found as high performance, similarly the comparison as ratio the females are high performer than males. Almost all the students education expenditures were held by their parents while four out of 114 were doing part time jobs to fulfill their education cost.

A negative association was found when we assessed performance (GPA) with number of hours absent. However, a similar association between absences less than eight hours. Low performers reported having absences due to unforeseen circumstances more frequently than high performers (56.1% vs. 29.0%), respectively; Students reported various reasons for absences (Table 2) Students agreed that technology did not influence absenteeism (for videotaped class lectures and posted class materials on Blackboard, respectively). Student attitudes toward attendance were not associated with performance (Table 2). Students agreed that class attendance was associated with professionalism

**Table 1** Demographics of First- and Second-Year Pharmacy Students Who Participated in a Survey to Identify Factors Associated with Class Attendance, Median (Range)

<table>
<thead>
<tr>
<th>Variable</th>
<th>All respondents No (%) , n=114</th>
<th>Low performers No (%) , n=114</th>
<th>High performers No (%) , n=114</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Material status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>106</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>Married</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Average travel time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30 minutes</td>
<td>80</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>30-60 minutes</td>
<td>20</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>61-120 minutes</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>&gt;120 minutes</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education paid by</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>You / family</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

“Strategic Approaches to Strengthen Academic and Industrial Collaboration” www.ijpsr.com
Table 2 Reasons associated with students’ absenteeism.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All respondent (n =114)</th>
<th>Low performance (n=50)</th>
<th>High performance (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attendance influences my performance</td>
<td>432 (3.8)</td>
<td>284 (4.4)</td>
<td>144 (3.1)</td>
</tr>
<tr>
<td>Attendance has a tight correlation with professionalism</td>
<td>470 (4.1)</td>
<td>282 (4.4)</td>
<td>188 (3.6)</td>
</tr>
<tr>
<td>Class was before or after examination</td>
<td>408 (3.6)</td>
<td>150 (2.5)</td>
<td>210 (4.0)</td>
</tr>
<tr>
<td>Academic day was too long</td>
<td>274 (2.4)</td>
<td>140 (2.2)</td>
<td>134 (2.8)</td>
</tr>
<tr>
<td>Class was early in the morning</td>
<td>360 (3.1)</td>
<td>226 (3.5)</td>
<td>134 (2.6)</td>
</tr>
<tr>
<td>Class handouts were all inclusive, no new information was presented in class</td>
<td>350 (3.1)</td>
<td>188 (3.1)</td>
<td>152 (3.2)</td>
</tr>
<tr>
<td>The class was easy, I didn’t need to attend</td>
<td>350 (3.1)</td>
<td>122 (1.6)</td>
<td>112 (2.2)</td>
</tr>
<tr>
<td>I perceived the class content to be irrelevant to pharmacy</td>
<td>298 (2.1)</td>
<td>164 (2.6)</td>
<td>134 (2.6)</td>
</tr>
<tr>
<td>Class content was redundant</td>
<td>304 (2.7)</td>
<td>192 (3.0)</td>
<td>112 (2.4)</td>
</tr>
<tr>
<td>Taking my own notes is not important to me</td>
<td>284 (2.5)</td>
<td>176 (2.8)</td>
<td>108 (2.1)</td>
</tr>
<tr>
<td>I do not ask questions in class</td>
<td>358 (3.1)</td>
<td>218 (3.4)</td>
<td>140 (2.7)</td>
</tr>
<tr>
<td>I do not feel participating in class benefits me</td>
<td>286 (2.5)</td>
<td>140 (2.2)</td>
<td>190 (3.5)</td>
</tr>
<tr>
<td>Presented materials were posted on Blackboard</td>
<td>424 (3.7)</td>
<td>228 (3.6)</td>
<td>196 (3.8)</td>
</tr>
<tr>
<td>Faculty member read directly from their materials</td>
<td>386 (3.4)</td>
<td>206 (3.4)</td>
<td>180 (3.5)</td>
</tr>
<tr>
<td>Faculty member did not demonstrate the relevance of information to solving real problems</td>
<td>346 (3)</td>
<td>186 (3.0)</td>
<td>160 (3.1)</td>
</tr>
<tr>
<td>My perception of faculty member level of expertise</td>
<td>462 (4.1)</td>
<td>256 (4.0)</td>
<td>206 (4.0)</td>
</tr>
<tr>
<td>class lecture is videotape or recorded that we can use without attending the class</td>
<td>228 (2.4)</td>
<td>148 (2.3)</td>
<td>80 (1.4)</td>
</tr>
<tr>
<td>Faculty member lacked clarity and organization in teaching</td>
<td>178 (2.4)</td>
<td>144(2.5)</td>
<td>124 (2.4)</td>
</tr>
<tr>
<td>Faculty member did not care about class attendance</td>
<td>240 (2.1)</td>
<td>130 (2.0)</td>
<td>110 (2.1)</td>
</tr>
<tr>
<td>Teachers are not creating interest in subject</td>
<td>186 (2.5)</td>
<td>114 (2.3)</td>
<td>142 (2.1)</td>
</tr>
</tbody>
</table>

References:
PA: Pharmaceutics and Pharmaceutical Technology
PB: Medicinal Chemistry, Pharmaceutical Analysis, Quality Assurance and Drug Regulatory Affairs
PC: Pharmacognosy and Phytochemistry, Pharmaceutical Biotechnology
PD: Pharmacology, Toxicology, Clinical Pharmacy, Pharmacoepidemiology and Pharmacovigilance
PE: Pharmaceutical Education, Hospital Pharmacy, Community Pharmacy, Professional Pharmacy, Pharmaceutical Management and Pharma Marketing
Formulation and evaluation of gastro-retentive multiparticulate drug delivery system

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Abstracts: Gastroretentive dosage forms have potential use for use as controlled release drug delivery systems. Multiple units avoid ‘all-or-none’ gastric emptying nature of single unit systems. A controlled release system for lafutidine designed to increase its residence time in stomach was achieved through the preparation of floating micro particles by ionic-gelation method with the help of 3^2 factorial design using sodium alginate and HPMC in varying concentration. The shape and surface morphology of prepared microsphere characterized by SEM the prepared microsphere exhibited prolonged drug release for and remained buoyant for >12h from results of in vivo study.

Introduction:
Multi-particulate drug delivery systems are mainly oral dosage forms consisting of multiplicity of small discrete units, each exhibiting some desired characteristics. Floating systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. Peptic ulcers are crater like lesion or open sores in the lining of the upper gastrointestinal tract. Ulcer that develop in area of the G.I. tract expose to acidic gastric juice are called peptic ulcer. Lafutidine is a newly developed second generation histamine H2-receptor antagonist has a potent and long lasting gastric anti-secretory effect mediated by H2-receptor blockade [1, 2].

Material and Methods:
Lafutidine was obtained as a gift sample from Emcure Pharmaceuticals ltd. Pune; HPMC K100M was obtained as a gift sample from Colorcon Asia Pvt Ltd, Sodium alginate from Loba Chemical Pvt Ltd. First, Sodium alginate (% w/v) in distilled water 50ml, Drug: Polymer ratio, NaHCO₃: Polymer ratio and HPMC K 100M: Polymer ratio stirred for 60 min by mechanical stirrer, onwards sonicated for 30 min. Dropping solution through 22G syringe needle in CaCl₂ solution (% w/v) 70ml. After specific time, curing beads were prepared. Then, these beads were separated by filtration and washed 2 times with distilled water. Air dried for 24 hrs.3^2 full-factorial a design was used to determine the effect of the NaHCO₃: Polymer and Drug: Polymer ratio on the alginate bead properties.

Results and Discussion:
Physiochemical characterization of lafutidine and polymers were carried out successfully. Afterwards,
formulation of lafutidine beds was carried out by inotropic gelation method. Then, evaluation of all batches were carried out, from which were selected for further study by using optimization $3^2$ factorial design software.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Batch code</th>
<th>LAF 1</th>
<th>LAF 2</th>
<th>LAF 3</th>
<th>LAF 4</th>
<th>LAF 5</th>
<th>LAF 6</th>
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<td>26.35</td>
<td>25.28</td>
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<td>38.99</td>
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<td>70.9</td>
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<td>63.42</td>
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</tr>
</tbody>
</table>

Table 1 *In-vitro* % cumulative drug release from different batches of sodium alginate beads.

**Figure 1** A, B: Formulation of lafutidine entrapped alginate beads, C: Porous structure of alginate beads
**Figure 2** Images of Lafutidine beads

![Images of Lafutidine beads](image)

**Figure 3** Scanning Electron Microscopic images of Lafutidine beads

![Scanning Electron Microscopic images of Lafutidine beads](image)

**Figure 4** *In-vitro* % cumulative drug release from different batches of sodium alginate beads

![% cumulative drug release](image)

**Conclusion:**

Multiple unit buoyant beads for the gastroretentive delivery of Lafutidine were successfully developed by ionic-gelation method with the help of $3^2$ factorial design. The *In-vitro* and *In-vivo* results indicate that the beads were potentially useful. The ionic-gelation method was found to be simple, reproducible, easily controllable, economical and consistent process. Additionally, the excipients used for the formulation of this buoyant system were cheap and easily available.

**References:**

Studies in physico-chemical characterizations and in-vitro dissolution behavior of solid dispersions of Reserpine with Polyvinyl Pyrrolidone K 30

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E-mail address: nikonjanapatel@rediffmail.com

Abstract: The objectives of this research were to prepare and characterize Reserpine (RES) solid dispersions (SDs) with PVP K30 to improve solubility and dissolution profile. RES is an indole alkaloid having antipsychotic and antihypertensive effect. The phase solubility profile at 37°C with PVP K30 was classified as A_L-type, indicating the linear increase in solubility of RES with increase in concentration of carrier. \( \Delta G^\circ \) and \( \Delta H^\circ \) values were all negative, indicating the spontaneous nature of RES solubilization, and they decreased with increase in the PVP concentration, demonstrating that the reaction conditions became more favorable as the concentration of PVP increased. The SDs of RES with PVP were prepared at 1:1, 1:2.5, 1:5, and 1:10 ratio by physical mixing, melting, and lyophilization method. Solid state characterization of SDs were carried out by FTIR spectroscopy, DSC, and photomicrography which indicated RES was present as amorphous material and entrapped in polymer matrix. The SDs of RES with PVP K30 exhibited enhanced dissolution rate of RES, and the rate increased with increasing concentration of PVP in SDs. SDs prepared by lyophilization techniques showed highest improvement in solubility and dissolution profile in comparison to melting and physical mixing method. Tablets containing SDs of RES with PVP showed significant improvement in the release profile of RES as compared to tablet containing RES without PVP. The findings of this research work suggested that the drawback of poor dissolution profile of Reserpine could be overcome by preparing its solid dispersions with PVP K30 by lyophilization method.

Introduction:
RES is an indole alkaloid having antipsychotic and antihypertensive effect but water insoluble. Solid dispersions (SDs) of RES with PVP K30 were prepared by different methods to improve solubility and dissolution profile.

Materials and Methods:
Phase solubility study: Phase-solubility studies were performed according to the method reported by Higuchi and Connors, 1965 [1]. Solubility studies were performed in triplicate. The apparent stability constants and thermodynamic parameters derived from phase solubility curve.
Preparation of solid dispersion and physical mixture: Four SDs preparations containing different weight ratios of RES in PVP K30 (1:1, 1:2.5, 1:5, 1:10, and denoted as SDML 1/1, 1/2.5, 1/5, 1/10, respectively) were prepared by the melting method. SDs of RES in PVP K30 containing different weight ratios (1:1, 1:2.5, 1:5, 1:10, and denoted as SDLY 1/1, 1/2.5, 1/5, 1/10, respectively) were prepared by the lyophilization method. Physical mixtures (PMs) having the same weight ratios described above were also prepared which are denoted as SDPM 1/1, 1/2.5, 1/5, 1/10, respectively.

Characterization of solid dispersion and physical mixture: All samples were characterized by methods such as Fourier transformed infrared (FT-IR) spectroscopic analysis, differential scanning calorimetry (DSC) analysis, and photomicrography analysis.

Dissolution studies: Dissolution studies of RES in powder form, its PMs and SDs were carried out using USP dissolution apparatus type II with 500 ml demineralized water containing 0.25 % (w/v) of sodium lauryl sulfate as dissolution medium at 37°C ± 0.5°C and 50 rpm for 4 h. Similarity factor (f2), percent drug dissolved within 30 minutes (DP30 min), time to dissolve 50% drug (t50%) and mean dissolution time (MDT) were determined for comparison between dissolution profiles of different samples.

Results and Discussion:

Phase solubility study: The phase solubility curve of RES in the presence of PVP indicated that the solubility of RES increases with increasing temperature and carrier concentrations. At the highest polymer concentration (10% w/w), the solubility increased 3.6-fold and 4.2-fold at 25°C & 37°C, respectively at 37°C. ΔG° and ΔH° values were all negative for both polymers, indicating the spontaneous nature of RES solubilization.

Characterization of complexes: In the FTIR spectras of SDML 1/10 and SDLY 1/10, the characteristic of PVP were present at almost same position whereas peaks due to RES were absent indicating trapping of RES inside the PVP matrix. DSC scan of SDLY 1/10 and SDML 1/10 showed only one peak at 58°C in due to melting point of PVP. These two samples of SDs showed complete absence of drug peak at 286.13°C. This complete absence of RES peak indicates that RES is present as amorphous or as a solid solution inside the PVP matrix in these two samples. In the photomicrography studies of SDML 1/10 and SDLY 1/10, it was possible to distinguish RES crystals agglomerated on the surface of PVP particles that had lost their original shapes and in this case crystal sizes were smaller.

In-vitro dissolution studies: Dissolution rate of pure RES is very low (DP240 min 39.1%, t50% >>6 h, and MDT value 79.50 at 4 h). SDs of RES with PVP significantly enhanced dissolution rate of RES (55-90%) within 4 h as compared to PM as well as pure RES. PM with PVP also improve the dissolution rate of RES. Highest improvement was obtained in SDs prepared with PVP using
lyophilization techniques. SDLY 1/10 showed lowest MDT value (71.81 min). The release profile of RES from all the samples (i.e. SDs and PMs of PVP) and from pure RES is dissimilar as $f_2$ values for all these comparisons were less than 50. Release of RES from tablets containing SDs with PVP was faster and higher as compared to the conventional tablets prepared using RES without PVP. This confirmed the advantage of improved aqueous solubility of RES in its SD form, which can be formulated as tablets with better dissolution characteristic.

Conclusion:
Solid dispersions of RES with PVP demonstrated a higher dissolution rate than physical mixtures and plain RES. The increased dissolution rate in systems containing PVP was probably the result of increased wettability and dispensability of RES, may be due to surface tension lowering effect of PVP to the medium, resulting in wetting of RES.

Reference:
Preparation and characterization of soy-phytosomes

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2BN Institute of Pharmaceutical Sciences, Udaipur (Raj.), India
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Abstract:
The aim of this research work was to optimize the formulation quantities of soy extract, phospholipid (soy-lecithin) and aprotic solvent (ethanol, ethanol: water mixture) and variables of spray drying process to be used for preparation of soy-phytosomes and incorporated into a topical dosage form.

Ethanol, Methanol, Soy-Lecithin, Di-methyl-sulphoxide (DMSO), n-Hexane, Whatman filter paper-42, De-ionized water were used with Phospholipid complex and spray dry method.

Phytosomes were prepared by spray drying technique using Spray dryer (LU-222 Advanced Labultima, India) with 0.7mm nozzle size and various process parameters were studied during preparation of phytosomes. Two formulations were prepared successfully using soy-extract and phospholipid in ratio of 1:1 and 2:1; with solvent system ethanol: water (70:30) using the optimized spray drying condition.

Introduction:
The bioavailability and absorption of water soluble phytoconstituents is unpredictable due to poor solubility of these constituents in gastrointestinal tract [1]. This problem can be solved by a novel delivery system known as phytosome technology in which water soluble phytoconstituents are allowed to react with phospholipids. For enhanced and improved bioavailability, natural phytoconstituents must have a good equilibrium between hydrophobicity (helps to cross lipid rich cell membranes) and hydrophilicity (help in dissolution in gastro-intestinal fluids). Phospholipids have a dual solubility and acts as an emulsifier. Phytosome acts as a bridge between novel and conventional delivery systems [1-3].

Materials and Methods:
Ethanol, Methanol, Soy-Lecithin, Di-methyl-sulphoxide (DMSO), n-Hexane, Whatman filter paper-42, De-ionized water and other required solvents/chemicals were of good quality.

Formulation of Soy extract-phytosomes (The preparation of phytosomes consists of two stages)

Preparation of soy extract-phospholipid complex [4]

Selection of the appropriate solvent system: Ethanol: Water (70:30), Since ethanol is highly volatile in nature so it needs adding de-ionized water and an ethanol: water in a ratio of 70:30 was
used to check the solubility of SE-PL mixture. The same method was followed at 800 rpm for 30 min. for obtaining homogenous mixture.

**Determination of appropriate ratio of soy extract – phospholipid:** The formulation components (Soy-powder extract, Phospholipid (Soy lecithin), Ethanol: Water (70:30)) were placed in a 100 ml conical flask and the contents were magnetically stirred for 30 minutes followed by Sonication for another 15 min.

**Preparation of soy-phytosomes (by spray drying method)** [5]: Soy-phytosomes were prepared by spray drying method. Aqueous dispersion of SE-PL mixture of 1:1 and 2:1 ratios were spray dried and various process parameters were studied. The mixtures were sonicated up to 15 minutes. Then the mixtures were dried by spray dryer (LU-222 Advanced Labultima, India) with 0.7mm nozzle size. Spray dryer’s parameters were changed and products were collected separately in every run. Process variables that were studied during spray drying method were as follow:

a) Feed flow rate (F.F.R.)
b) Inlet/Outlet (I/O) temperature
c) Aspirator flow rate
d) Air flow rate and pressure.

**Characterization of the prepared soy-phytosomes** [2, 5]: Characterization of prepared soy-phytosomes was performed as follow: 1. Physical appearance, 2. Visualization of Shape, 3. Vesicle size determination and 4. Drug content determination.

**Results and Discussion:**

Phytosomes were prepared by spray drying technique using Lab spray dryer with 0.7mm nozzle and various process parameters were studied during preparation of phytosomes. The prepared soy-phytosomes were characterized based on various physicochemical characteristics as shown in Table 1.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical appearance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Color</td>
<td>Yellow in color</td>
</tr>
<tr>
<td></td>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td></td>
<td>Taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td></td>
<td>Physical state</td>
<td>Powder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1 Preparation</td>
</tr>
<tr>
<td>2</td>
<td>Vesicle size determination</td>
<td>109.5 µm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68 µm</td>
</tr>
<tr>
<td>3</td>
<td>Drug content determination</td>
<td>(96.76±0.47) %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(92.51±0.21) %</td>
</tr>
</tbody>
</table>
Figure 1 Visualization of shape - (A) SE-PL mixture and, (B) Spray dried Phytosomes

Figure 2 Vesicle size determination (A) 1:1 ratio and (B) 2:1 ratio

Conclusion:
Two formulations were prepared successfully using soy-extract and phospholipid in ratio of 1:1 and 2:1; with solvent system ethanol: water (70:30) using the optimized spray drying condition. The prepared soy-phytosomes were characterized based on various physicochemical characteristics.

Acknowledgements
Authors are very thankful to College staff for their constant and perennial support and friends Komal Sharma, Arpana Kumari and Poonam Kumari.

References:
Formulation, development and evaluation of Metformin Hydrochloride tablets

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E-mail id: ritika401@gmail.com

Abstract: Metformin Hydrochloride used in diabetes mellitus, decreases the elevated blood glucose level by decreasing hepatic glucose output and insulin resistance. PVP K90 is used as binder, colloidal silicon dioxide as glidant, CCS and SSG as disintegrates, magnesium stearate as lubricant and MCC as a diluent. Different formulations were prepared by wet granulation method and using different toolings. The tablet was film coated using Titanium dioxide (Tab Coat®) as a film coating agent. The prepared tablets were evaluated for various parameters like hardness test, friability, weight uniformity, drug content uniformity, in-vitro drug release and short term stability studies.

Introduction:
Metformin HCl is an orally administered anti-diabetic drug from the Biguanide class. It is recommended as the first-line drug of choice for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) or type 2 diabetes mellitus. The present study was an attempt to develop a solid dosage form by using suitable binders and disintegrants in a film coated tablet with desired shape to facilitate coating in conventional coating pan and compare it to a reference product [1-5].

Materials and Methods:
Materials: Metformin HCl (IPCA Laboratory Ltd.), PVP K90 (Nanhang Industrial Co. China), Colloidal silicon dioxide (Correl Pharma, India), Microcrystalline cellulose (Patel Industries, Ahmedabad), Magnesium stearate (Amishi drugs & Chemicals, Ahmedabad), Croscarmellose sodium (Signet Chemical Corp.), Sodium starch glycolate (S.D. Fine Chemicals Ltd, India) and TABCOAT® (Colorcon Asia). Brand of reference product used was Obimet* by Abbott Group of Companies.

Methods:
Preformulation Studies: The calibration curve of the drug was prepared. The micromeritic properties of all the formulations were also recorded.

Method: Metformin HCl, MCC and SSG were sifted through sieve#40 and kneaded with previously prepared solution of PVP K90 in water for about 15 min to form granules. The granules were dried in FBD and sifted through sieve#20 and sifted CSD and CCS were added and mixed. Then sifted magnesium stearate was added as lubricant. The blend was compressed using SC and Dc punches. The tablets were coated using solution of TABCOAT® in IPC and MDC.
Table 1 Formulation of Metformin HCL tablets:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F₁</th>
<th>F₂</th>
<th>F₃</th>
<th>F₄</th>
<th>F₅</th>
<th>F₆</th>
<th>F₇</th>
<th>F₈</th>
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</thead>
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<td>Metformin HCL</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
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<tr>
<td>MCC</td>
<td>45</td>
<td>40</td>
<td>35</td>
<td>30</td>
<td>50</td>
<td>50</td>
<td>45</td>
<td>40</td>
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<tr>
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<td>5</td>
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<td>5</td>
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<td>-</td>
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<tr>
<td>SS3</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PVP K₁₅₂₀</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PVP K₁₀₀</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CSD</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CCS</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mag. stearate</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>580</td>
<td>580</td>
<td>580</td>
<td>580</td>
<td>580</td>
<td>580</td>
<td>580</td>
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<td>Shape</td>
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<td>SC</td>
<td>SC</td>
<td>SC</td>
<td>SC</td>
<td>SC</td>
<td>DC</td>
<td>DC</td>
</tr>
<tr>
<td>Punch Size</td>
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<td>16/32</td>
<td>16/32</td>
<td>16/32</td>
<td>16/32</td>
<td>16/32</td>
<td>18/22</td>
<td>18/22</td>
</tr>
</tbody>
</table>

Evaluation of tablets: The tablets were evaluated for hardness, thickness, friability and disintegration time and dissolution profile.

Parameters of dissolution test

Medium: Phosphate buffer pH 6.8 maintained at 37±0.5°C
Volume: 900 ml
Apparatus: 2 (Basket type)
Speed: 100 rpm
Time: 45 minutes

Preparation of phosphate buffer pH 6.8

Phosphate buffer pH 6.8 was prepared according to BP.

Result and Discussions: The calibration curve obtained is shown in Fig. 1. The micromeritic properties of the formulation blends are shown in table 2.

Table 2. Micromeritic properties of formulation.

<table>
<thead>
<tr>
<th>Powder Blend</th>
<th>Bulk Density (g/ml)</th>
<th>Tapped Density(g/ml)</th>
<th>Carr’s index (%)</th>
<th>Hausner’s Ratio</th>
<th>Angle of Repose (°)</th>
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</thead>
<tbody>
<tr>
<td>F₁</td>
<td>0.458</td>
<td>0.551</td>
<td>17.7</td>
<td>1.2</td>
<td>30.5</td>
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<tr>
<td>F₂</td>
<td>0.498</td>
<td>0.532</td>
<td>15.8</td>
<td>1.18</td>
<td>29.1</td>
</tr>
<tr>
<td>F₃</td>
<td>0.517</td>
<td>0.507</td>
<td>13.4</td>
<td>1.15</td>
<td>27.1</td>
</tr>
<tr>
<td>F₄</td>
<td>0.501</td>
<td>0.701</td>
<td>15.60</td>
<td>1.18</td>
<td>29.7</td>
</tr>
<tr>
<td>F₅</td>
<td>0.515</td>
<td>0.609</td>
<td>12.34</td>
<td>1.19</td>
<td>29.0</td>
</tr>
<tr>
<td>F₆</td>
<td>0.526</td>
<td>0.612</td>
<td>14.0</td>
<td>1.15</td>
<td>27.1</td>
</tr>
<tr>
<td>F₇</td>
<td>0.519</td>
<td>0.588</td>
<td>11.7</td>
<td>1.13</td>
<td>27.8</td>
</tr>
<tr>
<td>F₈</td>
<td>0.531</td>
<td>0.387</td>
<td>12.04</td>
<td>1.14</td>
<td>26.0</td>
</tr>
</tbody>
</table>

The results of the evaluation of tablets are given in table no. 3 and their comparative release profile in fig.3 and fig.4.
Table 3 Results of the quality control evaluation parameters.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness (Kg/cm²)</th>
<th>Thickness (mm)</th>
<th>Friability (%)</th>
<th>Disintegration Time</th>
<th>Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁</td>
<td>2.12</td>
<td>5.08</td>
<td>0.64</td>
<td>1 min</td>
<td>92.8</td>
</tr>
<tr>
<td>F₂</td>
<td>2.37</td>
<td>5.99</td>
<td>0.89</td>
<td>1 min 15 sec</td>
<td>97.11</td>
</tr>
<tr>
<td>F₃</td>
<td>2.69</td>
<td>6.01</td>
<td>0.86</td>
<td>1 min 23 sec</td>
<td>92.3</td>
</tr>
<tr>
<td>F₄</td>
<td>2.56</td>
<td>6.02</td>
<td>1.06</td>
<td>1 min 50 sec</td>
<td>95.8</td>
</tr>
<tr>
<td>F₅</td>
<td>2.98</td>
<td>6.05</td>
<td>0.54</td>
<td>3 min 55 sec</td>
<td>97.12</td>
</tr>
<tr>
<td>F₆</td>
<td>3.12</td>
<td>6.05</td>
<td>0.25</td>
<td>3 min 55 sec</td>
<td>97.48</td>
</tr>
<tr>
<td>F₇</td>
<td>3.51</td>
<td>6.04</td>
<td>0.22</td>
<td>3 min 55 sec</td>
<td>98.57</td>
</tr>
<tr>
<td>F₈</td>
<td>3.72</td>
<td>6.05</td>
<td>0.18</td>
<td>3 min 55 sec</td>
<td>98.85</td>
</tr>
<tr>
<td>Reference</td>
<td>5.05</td>
<td>5.08</td>
<td>0.19</td>
<td>4 min 20 sec</td>
<td>98.85</td>
</tr>
</tbody>
</table>

Figure 3 Zero order release for F₃, F₄, F₅ and reference Figure 4: Zero order release for F₆, F₇, F₈ and reference.

Conclusion:
The comparison of all the parameters show that the parameters of the formulation 8 are nearly equivalent to that of the reference, so the F₈ was considered as the final batch. From this study it was concluded that PVP K₉₀ as binder and SSG and croscarmellose as disintegrant are ideal for the given formulation. The effect of different shape was also observed and concluded that deep concave shape is ideal and there are no coating problems with the shape as compared to standard concave shape.

References:
Nasal inserts containing ondansetron hydrochloride based on Chitosan-Gellan Gum Polyelectrolyte Complex: in-vitro, in-vivo studies

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Abstract: The aim of this study was the formulation of ondansetron hydrochloride loaded lyophilized insert for nasal delivery. The nasal insert were prepared by the lyophilisation technique using chitosan-gellan gum polyelectrolyte complex as the polymer matrix. The ondansetron loaded inserts were evaluated with respect to water uptake, bioadhesion, drug release, ex vivo permeation study, and in vivo study. Scanning electron microscopy confirmed the porous sponge like structure. The nasal delivery showed improved bioavailability as compared to oral delivery. In conclusion, the ondansetron containing nasal inserts based on chitosan-gellan gum complex with potential muco-adhesive potential are suitable for nasal delivery.

Introduction:
Nasal mucosa has been considered as a potential site of administration. Nasal insert is the novel solid dosage form, which is prepared by lyophilisation, consists of a sponge like hydrophilic polymer matrix and combines the advantages of a solid, single unit dosage for nasal drug delivery by using carrier systems that hydrate quickly after contact with mucosa [1, 2]. Chitosan is a hydrophilic, biocompatible and biodegradable polymer of low toxicity. The cationic polyelectrolyte nature of chitosan could provide a strong electrostatic interaction with negatively charged materials [3]. Gellan gum is an anionic hetero polysaccharide produced by aerobic fermentation of the bacterium sphingomonas eloda (formerly known as pseudomonas eloda). Because of its ability to form strong clear gels at physiological ion concentration, it can provide a longer contact time for drug transport across the nasal membrane before the formulation is cleared by mucociliary clearance mechanism. These features, along with bioadhesivity, biodegradability, biocompatibility and absence of toxicity of this polymer, attracted widespread interest in gellan gum as an ion responsive gelling agent [4].

Materials and Methods:
Materials: Ondansetron hydrochloride was gift sample from Alkem Laboratories (Mumbai, India). Chitosan (degree of deacetylation ~ 79%) from Sigma Aldrich, India, Gellan gum (Low acyl content) obtained as gift sample from Burzin and Leons, CPKelco division of the Monsanto Company, USA. All other reagents used were of analytical grade.
Method: Chitosan (0.85%w/v) and gellan gum (0.04% w/v) polymeric solutions were prepared separately in 25 ml of acetate buffer solution (pH 5.0) and stirred at room temperature for 24h.
Ondansetron hydrochloride and mannitol (as cryoscopic agent) were added to a mixture of both solutions to produce a viscous dispersion. 0.1ml of the prepared viscous dispersion was placed into blister molds and frozen at -25°C for 1hr. The samples were then freeze dried (0.25 mbar for 24 hrs with increasing shelf temperature -15 to 0°C and final drying for 2 hr at +15°C and 0.01 mbar). The inserts were then stored in desiccators until further use.

**Characterization of Nasal Inserts:** Inserts were characterized for general properties like color, appearance and thickness measured by using a digital vernier caliper (Aerospace Digimatic Vernier Calliper, India).

**Water Uptake studies:** Percent water uptake of insert was calculated using the following equation:

\[
\% \text{ Water uptake (mg/mg)} = \frac{W_w - W_d}{W_d} \times 100
\]

Where, \(W_w\) is Weight of wet insert and \(W_d\) is Weight of dry insert.

**Mucoadhesion test** – Performed by using agar/mucin gel by measuring displacement of inserts. The adhesion potential was inversely related to the displacement of insert [2].

**In-vitro drug release:** In-vitro drug release was carried out USP XXX-NF XXV (Apparatus-1 Basket type) dissolution apparatus, where phosphate buffer pH 6.6 was used as dissolution media maintained at 37°C ± 0.5°C at 50 rpm [5].

**Ex-vivo permeation study:** Ex-vivo permeation study of the lyophilized insert was performed using Franz type diffusion cell using sheep nasal mucosa, [6]. The permeability coefficient was calculated by using the formula:

\[
J_{ss} = \frac{dQ}{dt} C_0 \times A \quad \text{(2)}
\]

**Morphological examination:** The surface morphology of lyophilized inserts were examined morphologically by scanning electron microscope (JSM-6390LV, JEOL, Japan) with 20 kV accelerating voltage. Inserts were cut with a razor blade to expose the inner structure and fixed onto an aluminium specimen stub, dried overnight and sputter coated with gold prior to imaging.

**In-vivo studies:** Bioavailability studies were performed using rabbit as animal models as per approved protocol.

**Results and Discussion:**

**Characterization of nasal insert:** The lyophilized nasal inserts were cream colored with spongy appearance and 0.48±0.05 N hardness. In vitro water uptake of the freeze dried inserts was expressed as % water uptake by use of eqn. 1 was found to be 1511.76±78.22%. The water uptake was an important attribute of studying degree of hydration and gel formation in nasal cavity. The displacement (downwards movement of the insert) was 0.6 cm up to 8 h demonstrating better...
bioadhesive capability of chitosan/gellan gum complex based nasal insert. Chitosan, a cationic polymer, formed a thin film on the agar/mucin gel due to its opposite charge to mucin and agar. A positive mucoadhesion performance of the negatively charged polymer gellan gum may be related to its ability to interact with mucus as result of physical entanglement and secondary bonding (i.e. H-bonding and Van der Waals attraction). The in vitro release study of ondansetron hydrochloride loaded microspheres was carried out using dissolution apparatus. About 96.58% drug release within 8 hr demonstrate slow with negligible burst release effect. This slow drug release could be attributed to higher water uptake (swelling) of these formulations. Greater swelling of the polymers has been reported to result in an increase in the diffusional path length. Thus, the drug has to diffuse through the thick viscous gel layer formed outside the polymeric matrix hence resulting in slower drug release. About 43.46% ondansetron was permeated across cell membrane after 6 hr. The permeability coefficient (P) was also calculated and found to be 0.2691 cm²/hr. SEM shows the morphology of the nasal inserts observed by scanning electron microscopy (SEM). Chitosan/gellan gum complex based inserts having sponge like structure with better porosity as nasal inserts were obtained by freeze-drying which consists of sublimation of the frozen water yielding to the formation of pores or channels in the polymer matrix. The relevant pharmacokinetic parameters are reported in Table 1. These values corresponded to absolute bioavailability values (F-abs) of 125.93 ±2.14 and 38.41 ±2.51% for the nasal inserts and oral solutions respectively.

**Table 1 Results of pharmacokinetic parameters.**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Intravenous solution</th>
<th>Oral solution</th>
<th>Nasal Inserts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>63.90 ± 25.23</td>
<td>32.06 ± 22.30</td>
<td>41.59 ± 12.38</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-360&lt;/sub&gt; (ng/ml* min)</td>
<td>69.14 ± 7.30</td>
<td>26.54 ± 3.03</td>
<td>87.07± 8.22</td>
</tr>
<tr>
<td>F&lt;sub&gt;abs&lt;/sub&gt; (%)</td>
<td>100</td>
<td>38.41 ± 2.51</td>
<td>125.93± 2.14</td>
</tr>
</tbody>
</table>

**Conclusion:**
This study concludes that the lyophilized insert based on chitosan-gellan gum complex to be considered as a promising nasal delivery system for the administration of ondansetron hydrochloride.

**References:**
Development and evaluation of pulsatile release capsules of Divalproex Sodium

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Abstract: Divalproex sodium is used in the treatment of epilepsy and migraine disorder. The objective of the present study was to develop pulsatile release capsules of divalproex sodium to reduce the frequent administration of the drug. The release of the drug was obtained by the cross linking of capsule body with formaldehyde to make it significantly insoluble. For the sustain release of drug HPMC, xanthan gum and eudragit were used with the mixture of drug and diluents in the ratio of 1:1:1. The formulations were evaluated for various physico-chemical tests for empty capsule, weight variation, content uniformity and in-vitro dissolution studies for loaded capsule. The F2 formulation showed the 99.39% of the drug release.

Introduction:
Pulsatile Drug Delivery Systems is the rapid and transient release of a certain amount of drug molecule within a short time-period immediately after a lag time. This pulsatile dosage form i.e. PULSIN CAP which consists of water soluble cap and water insoluble body. The drug formulation is sealed within the capsule body by means of hydrogel plug. When the PULSIN CAP is swallowed the water soluble cap dissolves in the gastric juice and exposed hydrogel plug begins to swell. At predetermined time after ingestion the swollen plug is ejected out and the encapsulated drug formulation is then released into alimentary tract, where it is dissolved in gastro intestinal fluid and then absorbed into blood stream [1].

Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms due to abnormal, excessive or synchronous neuronal activity in the brain.

Divalproex sodium is a stable compound comprised of sodium valproate which is connected to valproic acid with a coordinate bonding. Divalproex has been administrated in various conditions such as seizures, bipolar disorder, and migraine headaches [2].

The present investigation aimed at developing PULSINCAP system containing divalproex sodium and various polymers which give immediate as well as sustained release of the drug from the hard capsules cross linked with formalin.

Materials and Methods:
Divalproex sodium was obtained as gift sample from ROAQ Chemicals Pvt.Ltd. Vadodara. Dicalcium phosphate, HPMC, xanthan gum and eudragit were obtained from SD Fine chem.Ltd.,Mumbai.
Preparation of cross-linked gelatin capsule

**Formaldehyde treatment:** About 100 hard gelatin capsules size ‘0’ were taken. Their body was separated from the cap and placed on a wire mesh kept on a beaker containing 25 ml of 37% formaldehyde solution and 2.5 g of potassium permanganate. And the beaker was kept immediately in a desiccator with tightly closed and sealed. The body of the capsule was made to react with formaldehyde vapours for 12 hrs, then removed and kept on a filter paper for drying for 48 hrs to ensure completion of reaction between gelatin and formaldehyde vapours. Finally the capsules were kept in an open atmosphere, to facilitate removal of residual formaldehyde and rejoined with untreated cap and stored in a polythene bag.

**Solubility tests for formaldehyde treated capsules:** The empty hard gelatin capsule was stirred vigorously in 100 ml of dissolution medium (buffers of different pH) taken in 250 ml beaker, with magnetic stirrer. The time at which the capsule dissolves or forms a soft mass was noted. Further the empty capsules were subjected for various physic chemical test parameter

**Formulation of pulsatile release capsules:** An accurately weighed quantity of divalproex sodium was mixed with the diluents, dicalcium phosphate in the ratio of 1:1 and this physical mixture was stored in polythene bag.

**Filling of capsules:** Hard gelatin capsules size ‘0’ with formaldehyde treated cap and untreated body was taken for filling. The body and cap was separated manually. The physical mixture of drug and diluents equivalent to 125mg of divalproex sodium was accurately weighed and filled into the treated bodies; this forms the second dose of the drug. The polymer was added to the above physical mixture in the ratio of 1:1:1 then pressed with a glass plunger. Similarly the first dose of the drug was also filled and finally the cap was locked into the capsule body and stored in tightly packed container for further studies

**Evaluation:** The weight variation and content uniformity was performed as per the literature [3].

**In-vitro release profile:** Dissolution studies were carried out by using rotating basket method where pH 1.2 buffers was used as dissolution medium for first 2hrs, followed by pH 7.4 buffer for 10 hrs. 5 ml of the sample was withdrawn from dissolution media at an interval of 1 hr and same amount replaced with fresh buffer. The absorbance was measured spectrophotometrically at 210 nm. The results are subjected for the various kinetic parameters [4].

**Stability Studies:** The selected formulations were packed in an air tight polythene bag and stored at 60 °C and 45 °C for a period of 3 and 10 weeks respectively. The samples were withdrawn at the end of 5th, 8th and 10th week in case formulations stored at 45 °C and 1st, 2nd and 3rd week in case formulations stored at 60°C. All the samples were evaluated for the physical changes.

**Results and Discussion:**
The pulsatile release capsules of divalproex sodium were obtained by cross linking of empty capsules by formalin treatment in order to modify the solubility of gelatin capsules. All the empty capsules were of lockable type, odourless, soft and sticky when touched with wet finger. After formalin treatment, there were no significant changes in the capsules except for the stickiness. The body of the capsules was hard and non sticky even when touched with wet hand. The empty capsules were tested for the capsule length ,diameter of the capsule and percentage of moisture content for both treated and untreated capsule was in the range of 21.13,7.26 and16.05. The physical characteristics of the formulation like weight variation were found within the limit of official standards. 99.56% of drug content uniformity was found. The in-vitro release for the different formulations was found to be 98.61%, 99.39% and 96.76% respectively. There was no physiological changes was observed during the stability studies.

![in-vitro release profile](image)

**Figure 1** In-vitro drug release study.

**Conclusion:**

The present work is an attempt to develop pulsatile release capsules of divalproex sodium where the body of the capsule is made insoluble by the treatment of formaldehyde to obtain pulsatile release of the drug. The second dose of the drug was filled into the insoluble body followed by polymer plug and the first dose was filled into the soluble cap. The results of evaluation tests were found to be satisfactory and all the values comply within the pharmacopoeia standards. In-vitro release profiles showed the drug release at the end of 12 hrs. When the in-vitro release data were fitted into kinetic models, it was found to follow Higuchi’s model and drug release was by non-fickian mechanism.

**References:**

Abstract: Majority of the orally administered drugs are absorbed into the systemic circulation via portal blood and undergo first pass metabolism, thus exhibit low oral bioavailability. To overcome this and to enhance the bioavailability, intestinal lymphatic transport of drugs can be exploited. The objective of work was to enhance the oral bioavailability of Atorvastatin (AC), by enabling the lymphatic uptake and increased diffusion through intestinal wall, by virtue of formulating as lipid nanoparticulate system. AC-SLN dispersions were studied for In-vitro drug release by using Franz diffusion cell and found to be < 60% AC release in 24 h study. Optimized AC-SLN dispersion was then converted to solid form by freeze drying technique and formulated it into capsule dosage form. The formulated capsule was studied for In-vitro dissolution study and it shows controlled release over 24 h, which follows Hixon Crowell model. The model signifies that the release was depending on diameter of particle and diffusional path of the drug for its release. The In-vivo study was done on Wistar rats to investigate the improvement in bioavailability. Analysis of rat plasma was done by HPTLC technique. The results were confirms that there is improvement in bioavailability by incorporating the AC in lipid carrier.

Introduction:
Solid lipid nanoparticles recently emerged as a novel approach to drug delivery systems. In theory, solid lipid nanoparticles combine the advantages of lipid emulsion systems and polymeric nanoparticle systems while overcoming the temporal and in vivo stability issues that plague the aforementioned approaches. Utilizing biological lipids is theorized to minimize carrier cytotoxicity, and the solid state of the lipid is theorized to permit more controlled drug release due to increased mass transfer resistance. These early efforts generated significant excitement in solid lipid nanoparticles as a drug delivery system for hydrophobic compounds. Majority of the orally administered drugs are absorbed into the systemic circulation via portal blood and undergo first pass metabolism, thus exhibit low oral bioavailability. To overcome this and to enhance the bioavailability, intestinal lymphatic transport of drugs can be exploited [1-3]. The objective of work was to enhance the oral bioavailability of AC, by enabling the lymphatic uptake and increased diffusion through intestinal wall, by virtue of formulating as lipid nanoparticulate system [4, 5].
Material and Methods:
The sample of atorvasatatin was obtained as a gift sample from Blue Cross, Nashik, India. The other excipients Glyceryl Monostearate, Span 60, tween 80, Soy lecithin, Poloxamer 188, Methanol, Acetonitrile, Chloroform from Loba Chemicals, Mumbai, India, Dialysis membrane 135 (2.4 nm) from Space lab, Nashik, India.

Method for preparation for SLN: Solid Lipid Nanoparticles (SLN) was prepared by pre-emulsion probe sonication method.

Results and Discussion:
Evaluation of solid lipid nanoparticle
The SLNs were evaluated for particle size where the polydispersibility index was found to be greater than 0.3. The shape of SLN by SEM images revealed the size of particle were of spherical morphology. Entrapment efficiency was found to be 85.37%. A high amount of drug could be incorporated in nano particle dispersion. In XPRD study, it was confirmed that AC existed in amorphous state in the AC-SLN because of the disappearance of sharp peak of AC in the diffraction pattern. The diffraction pattern of the GMS showed remarkable difference from those of the SLN, as it showed relative sharp peak than the SLN. It was clear that from AC-SLN, the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity as seen previously. FTIR of AC shows the characteristic peak such as of CO-NH stretch (1651 cm\(^{-1}\)), Free –OH (3646 cm\(^{-1}\)), N-H bend (3082-3609 cm\(^{-1}\)) and C-N (1217 cm\(^{-1}\)). From FTIR study of GMS and AC-SLNs, the characteristic peaks of drug was disappear and were replace by the peak of GMS such as H-OH(3300-3311 cm\(^{-1}\)), Carboxyl –OH (2614-3548 cm\(^{-1}\)), C=OH(1731 cm\(^{-1}\)) and C-H stretch (2849-2915 cm\(^{-1}\)) while remaining peaks also either shifted or replaced in the IR spectra of formulation. This established drug entrapment in lipid matrix. DSC is a highly useful means of characterizing material with respect to crystalline behaviour and physical changes in the formulation. During solidification of GMS-SLN polymorphism of lipid can be takes place. Monoglycerides are known to crystallize mainly in polymorphic forms which transforms from \(\alpha\) to \(\beta\) stable form. United State of Pharmacopoeia describes glyceryl monostearate as consisting of not less than 90% of monoglycerides, chiefly glyceryl monostearate and glyceryl monopalmitate. The bulk material melts between 57.8-63.0°C with the melting point at 61.2°C. Stability study: After one month storage the SLN dispersion at various temperature parameters showed little difference in particle size and entrapment efficiency. There is no change in clarity and phase separation was observed. The average particle size and entrapment efficiency of optimized sample O\(_2\) stored for 1 month was 166.4 nm ± 2.88 shown in Fig.25 and 83.12 % ± 0.5263 respectively. Centrifugation at 3000 rpm for 30 min showed there is no precipitation and the AC-SLN had a good physical stability. Change in particle size and entrapment
efficiency was due to polymorphic transition of the lipid which leads to expulsion of drug from SLN (transformation of higher energy α and β’ modification to the lower energy β modification). This may imply that the transition of dispersed GMS in SLN from β’ form to stable β form might occur extremely slowly. AC-SLN dispersions were studied for In-vitro drug release by using Franz diffusion cell and found to be < 60% AC release in 24 h study. Optimized AC-SLN dispersion was then converted to solid form by freeze drying technique and formulated it into capsule dosage form. The formulated capsule was studied for In-vitro dissolution study and it shows controlled release over 24 h, which follows Hixon Crowell model. The model signifies that the release was depending on diameter of particle and diffusional path of the drug for its release. The In-vivo study was done on Wistar rats to investigate the improvement in bioavailability. Analysis of rat plasma was done by HPTLC technique. The results were confirms that there is improvement in bioavailability by incorporating the AC in lipid carrier. The result of stability study indicates no significant difference in the parameters tested during the stability study.

Conclusion:
AC- SLN dispersion and be successfully formulated by the probe sonication method with high entrapment efficiency and smaller particle size and its evaluation parameters evaluated. In vivo studies show improved bioavailability of AC in lipid carrier.

<table>
<thead>
<tr>
<th>Code</th>
<th>Dispersion composition</th>
<th>Particle size (nm)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_1</td>
<td>Drug: Lipid 1: 8.18, Span 60 1.00, Sonication time 11.36</td>
<td>148.55</td>
<td>82.89</td>
</tr>
<tr>
<td>O_2</td>
<td>Drug: Lipid 1: 8.18, Span 60 1.90, Sonication time 11.36</td>
<td>148.91</td>
<td>83.66</td>
</tr>
<tr>
<td>O_3</td>
<td>Drug: Lipid 1: 8.18, Span 60 2.36, Sonication time 11.36</td>
<td>149.50</td>
<td>84.34</td>
</tr>
<tr>
<td>O_4</td>
<td>Drug: Lipid 1: 8.18, Span 60 2.81, Sonication time 11.36</td>
<td>150.32</td>
<td>84.92</td>
</tr>
</tbody>
</table>

References:
Formulation and evaluation of polyherbal gel for anti-ulcer activity

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Abstract: Smyle gel is a topical preparation used for treatment of mouth ulcer. Gels have better potential as a vehicle to administer drug topically in comparison to ointment. Topical gels are intended for skin application or to certain mucosal surfaces for local action or percutaneous penetration. Overall, the clinical evidences indicate that topical gel is a safe and effective treatment option for use in the management of skin related disease. The present study involves preparation and evaluation of polyherbal gel for the treatment of mouth ulcer.

Introduction:
Smyle gel is used for treatment of mouth ulcer. It relieves inflammation and pain associated with mouth ulcer and heals the damaged mucosal lining of mouth. A gel is a two-component, cross linked three-dimensional network consisting of structural materials intermixed by an adequate but proportionally large amount of liquid. Chemical gels are associated with permanent covalent bonding while physical gels resulted from relatively weaker and reversible secondary intermolecular forces such as hydrogen bonding, electrostatic interactions, dipole-dipole interactions, vanderwaals forces and hydrophobic interactions [1-5].

Materials and Methods:
Collection of Plant Materials: Various raw materials required for preparation of Anti-ulcer smyle gel namely khadir, Tagor, Yashtimadhu, Rasna, Kushtha, Lodhra, Karpoor, Sharkara and Gel base were purchased from Mankarnika Ayurvedic Aushadhalaya, Pune in the month of October 2014 and Authentication was confirmed by Department of Pharmacognosy, Marathwada Mitra Mandal’s College of Pharmacy, Pune.
Method of Preparation: Powdered drug materials used for preparation of gel were passed through sieve number 85. These powders were separately weighed and added into the 250ml of beaker. Then the resulting mass was mixed properly using stirrer. Sufficient amount of glycerin as gel base is added. Sufficient quantity of Sodium carboxy methyl cellulose is added.
Evaluation of Mouth Ulcer Gel:
1) Measurement of pH:
Procedure- 1 gm of gel dissolved in 100 ml water & pH was measured by using Digital pH meter.
2) Spreadability: It indicates the extent of area to which gel readily spread on application to skin or affected part therapeutic potency of a formulation also depend on its spreading value.
Procedure- spreading is expressed in terms of time in seconds taken by slide to slip off from gel which is placed in between the two slides.

It is calculating by formula – \( S = \frac{M \cdot L}{T} \)

\( M = \) Wt of slide, \( L = \) length of slide, \( T = \) Time to separate slide in sec

3) **Drug content:** 1 gram gel is mixed with 100 ml of alcohol. Aliquots of different concentration were prepared by suitable dilution after filtering the stock solution & absorbance was measured. Drug content was calculated by linear regression analysis of calibration curve.

4) **Viscosity:** Measurement of viscosity of prepared gel was done with Brookfield viscometer. At each speed the corresponding dial reading was noted. The viscosity of gel was obtained by multiplication of the dial reading with factor given in Brookfield viscometer catalogues.

**Results and Discussion:**

**Organoleptic Analysis:** Formulation studies of gel produce during the preparation of polyherbal gel shows that an appearance of formulated gel was gritty. Taste and odor of gel is sweet and characteristic. Color of the gel is dark brown. Formulated gel is homogeneous, all particles are evenly distributed

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Organoleptic parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Dark brown</td>
</tr>
<tr>
<td>odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Slight sweet</td>
</tr>
<tr>
<td>Grittiness</td>
<td>Gritty particles present in formulation</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>All particles are homogeneously distributed</td>
</tr>
</tbody>
</table>

**Analytical Parameter:** pH of formulated gel found to be 6.71, while pH of std formulation is 7.11. Spreadability is required for easy application of gel on mucous membrane. Spredability of formulated gel is 8 min, while spreadability of std. formulation is 2 min. Drug content of 1% w/v of alcohol found to be 30mg/ml.

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation of formulated gel</th>
<th>Observation of standard preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.71</td>
<td>7.11</td>
</tr>
<tr>
<td>Spreadability</td>
<td>2 min</td>
<td>8 min</td>
</tr>
<tr>
<td>Drug content</td>
<td>30mg/ml</td>
<td>-</td>
</tr>
</tbody>
</table>

**Conclusion:**

From the present investigation various standardization parameters such as organoleptic standards, analytical parameters and safety evaluation were carried out, it can be concluded that the Anti-ulcer smyle gel contains all good characters of an ideal anti-ulcer gel and is economic. The study shows that the contents of the formulation are of good quality and purity, all these investigations were may be
helpful in authentication of Anti-ulcer smyle gel and its ingredients. The result of present study will also serve as reference monograph in the preparation of drug formulation.

References:
Enhancement of dissolution rate and bioavailability of water insoluble Itraconazole by liquisolid technique

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Abstract: The liquisolid (LS) tablets of Itraconazole were prepared by wet granulation method to treat fungalinfection. The granules exhibit good acceptable flow and compressibility properties. Formulation F4 and F6 showed good dissolution profile and the drug release followed first order kinetics. Based on the results, formulation F4 was subjected to in-vivo pharmacokinetic studies and showed an increase in bioavailability (1.548 folds) compared to marketed tablets.

Introduction:
LS compacts prepared by using different non-volatile solvents which dissolve the poorly soluble drug and gives better bioavailability [1]. LS system is novel technique, capable of enhancing solubility and improving dissolution which in turns increases the bioavailability [2]. LS involves conversion of liquid lipophilic drug or water insoluble solid drug dissolved in non-volatile solvent and this liquid medication can be converted into free-flowing, non-adherent, dry looking and readily compressible powders with the use of carrier and coating materials [1]. Advantages of LS technique are better availability of an orally administered water-insoluble drug is achieved when the drug is in solution form, production cost of LS systems is lower than of soft gelatin capsules, useful for the formulation of oily drugs/liquid drugs [1,3]. Applications LS system can be efficiently used for water insoluble solid drug or liquid lipophilic drugs, sustained release of drugs which are water soluble drugs has been obtained by the use of this technique, solubility and dissolution enhancement, designing of controlled release tablets and in probiotics [4].

Materials and Methods:
Itraconazole (Curex Pharmaceutical, Nepal.), Avicel PH-101, SSG, Glycolate, Silicon Dioxide, Crospovidone, Tween 20 and PEG were procured from (S.D fine chem, Mumbai).

Table 1 Formulation of Itraconazole liquisolid compact tablets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
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<td>SSG</td>
<td>-</td>
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<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Magnesium Ster.</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
Silicon dioxide | 9 | 9 | 9 | 9 | 9 | 9
Total weight | 450 | 450 | 450 | 450 | 450 | 450

Evaluation parameters:

**Hardness and Friability:** For each formulation, the hardness and friability of 6 tablets were determined using the Monsanto hardness tester and the Roche friabilator respectively [2].

**Drug Content:** 10 tablets were weighed from each batch and average weight is calculated. Dissolve in phosphate buffer and solution was filtered and absorbance was measured spectrophotometrically at 263 nm against pH 6.8 phosphate buffer as a blank [3].

**Disintegration time:** The test was carried out on 6 tablets using the disintegration test apparatus and time was measured in seconds [2, 4].

**In-vitro dissolution studies:** The in-vitro dissolution studies were performed using the USP-II (Paddle) dissolution apparatus at 50 rpm. Dissolution media used was phosphate buffer pH 6.8. The sample was withdrawn at 5 min interval and analysed on UV spectrophotometer at 263 nm [3].

**Drug release kinetics:** Investigation of the drug release from tablet was done by fitting the release data with zero order, first order and Higuchi equation. The release mechanism was understood by fitting the data to Korsmeyer Peppa’s model [2].

**Compatibility studies by FTIR and DSC, FTIR studies:** possible interaction between drug and any excipients was conducted using a Thermo Nicolet FTIR spectrophotometer [2].

**Differential scanning calorimetric studies:** Thermal properties of the pure Itraconazole and its physical mixture were analysed by Shimadzu DSC-60, Shimadzu Limited Japan [4].

**Stability Studies:** The optimized formulation was subjected for three month stability study [3].

**Results and Discussion:**

**Compatibility studies using FT-IR and DSC:** Compatibility studies shows that there was no possible interaction between drug and excipients used.

**Hardness** of the tablets was found to vary from 3.67 to 3.82 kg/cm². The % friability was less than 1% for all formulation indicates that tablets formulations are of good mechanical strength to withstand abrasion in handling.

**Drug Content:** The range of uniformity of drug content for all formulation was 95.13±0.91 to
102.5±1.5. Thus all the formulations were found to be complying with the standards given in official pharmacopoeia.

**In-vitro disintegration time:** The average DT for all the formulations were in the range of 110-170 sec. Formulation F4 showed least DT i.e. 110 sec which might be due to presence of crospovidone, which in turn provides amorphization to the tablets and results rapid breakdown of the tablets.

**In-vitro drug release studies:** All the LS formulation was evaluated for their *In-vitro* release and the formulation F4 and F6 shows 98.8% and 98.6% of cumulative drug release.

**Release kinetics:** All formulation showed first order release and mechanism was found to be non-fickian.

**In-vivo pharmacokinetic studies:** Pharmacokinetic studies were carried out on Albino rats. Higher AUC$_{0-t}$ values after test formulation application clearly showed that comparatively higher amount of drug was available to rat body because excipients used in LS formulation increased the solubility as well as dissolution rate of test formulation compared to reference formulation. The relative bioavailability of LS formulation was found to be 1.548 times higher than that of reference formulation i.e. bioavailability increased from 45% to 69.66%.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$T_{max}$ (Hrs)</th>
<th>$C_{max}$ (ng/mL)</th>
<th>AUC$_{0-t}$ (ng.hrs/mL)</th>
<th>$K_E$ (Hrs$^{-1}$)</th>
<th>$t_1/2$ (Hrs)</th>
<th>Relative bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6</td>
<td>4</td>
<td>164.8</td>
<td>1839.13</td>
<td>0.0952</td>
<td>17.42</td>
<td>1.548</td>
</tr>
<tr>
<td>Marketed tablet</td>
<td>3.5</td>
<td>193.42</td>
<td>1187.53</td>
<td>0.0841</td>
<td>18.44</td>
<td>-</td>
</tr>
</tbody>
</table>

**Stability studies:** Stability studies results showed that formulations F4 and F6 do not showed any significant change in physical appearance, friability, drug content and drug release, hence stable.

**Conclusion:**

In this present study an attempt has been made to increase the dissolution rate and bioavailability of water insoluble Itraconazole by liquisolid technique. Results of pharmacokinetics studies showed that bioavailability of tested drug increased from 45% to 69%. By this we can conclude that addition of superdisintegrants and non-volatile solvents enhanced the dissolution rate which in turn increased the bioavailability of Itraconazole by liquisolid technique.

**References:**

An approach to increase the solubility of Glipizide by self emulsification technique

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Abstract: In the present study, self-emulsifying drug delivery system (SEDDS) of Glipizide was developed for the treatment of II-type diabetes. Pseudo-ternary phase diagrams were plotted to check the emulsification range and also to evaluate the effect of Glipizide on the emulsification behaviour of the phases. The prepared formulations were evaluated for robustness to dilution, drug content, in-vitro dissolution and release data was fitted to evaluate the kinetics of drug release. From this study it can be concluded that SEEDS showed significant improvement in terms of the drug release and almost all drug was released within 18 minutes.

Introduction:
SEDDS however have a smaller lipid droplet size (<200 nm) and the dispersion has an optically clear to translucent appearance. Both systems are associated with the generation of large surface area dispersions that provide optimum conditions for the increased absorption of poorly soluble drugs [1]. Potential advantages of SEDDS include protection of sensitive drug substances, selective targeting of drug(s) toward specific absorption window in GIT, Protection of drug(s) from the gut environment, enhanced oral bioavailability enabling reduction in dose, for both liquid and solid dosage forms [2]. According to Reiss, self-emulsification occurs when the entropy change that favours dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional emulsion is a direct function of the energy required to create a new surface between the oil and water phases [2, 3]. Factors affecting SEDDS are drugs which are administered at very high dose are not suitable for SEDDS, unless they exhibit extremely good solubility in at least one of the components of SEDDS, preferably lipophilic phase [4].

Materials and Methods
Glipizide (Micro labs Ltd. India), PEG-200, Tween 80, Oleic acid, NaOH (Fine Chem. Ltd, India).

Table 1 Formulations containing various concentrations of excipients.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug (Glipizide)</th>
<th>Surfactant (Tween 80)</th>
<th>Co-surfactant (PEG 200)</th>
<th>Oil (Oleic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>5</td>
<td>600</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>S2</td>
<td>5</td>
<td>530</td>
<td>270</td>
<td>200</td>
</tr>
<tr>
<td>S3</td>
<td>5</td>
<td>470</td>
<td>230</td>
<td>300</td>
</tr>
<tr>
<td>S4</td>
<td>5</td>
<td>400</td>
<td>200</td>
<td>400</td>
</tr>
</tbody>
</table>
Formulation of SEDDS: The formulations were prepared by dissolving the accurately weighed Glipizide in co-surfactant at 60°C in an isothermal water bath. Oil was then added and mixture was cooled to ambient temperature. Then surfactant was added and the final mixture was mixed by stirring until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hrs, and examined for signs of turbidity or phase separation prior to self-emulsification and particle size studies. Final formulation was filled in hard gelatin capsule and stored in well closed container [2].

Evaluation parameters:

Droplet size analysis: A 10 mg quantity of the SEDDS was placed inside the ring of the internally calibrated microscopic slide (Optical micrometer) and a drop of each non-solvent used above was added for a clearer view. Different particles of the SEDDS from a particular batch were counted manually since they were sizeable enough to be distinguished and the mean value was taken [4].

Robustness to dilution: The emulsions were diluted with 0.1N NaOH and stored for 24 hrs at 37.0±0.5°C and observed for any signs of phase separation and drug precipitation [3].

Zeta potential measurement: Zeta potential was observed with the help of Malvern Zetasizer [3].

Dissolution studies: Dissolution profile was determined using USP paddle method at 37 °C and a rotating speed of 100 rpm. Samples were analysed spectrophotometric analysis at 288 nm [2].

Kinetic Analysis of in-vitro release rates: Drug release kinetics from the various formulations was determined by fitting in-vitro data to various release models like zero order, first order, Higuchi and Korsmeyer and peppas model [2].

Drug content: For drug content estimation 1 ml of the solution was diluted with 10 ml fresh 0.1N NaOH and drug content was determined using UV spectrophotometer at 228 nm [3].

Transmission electron microscopy: Transmission electron microscope was used as a visualizing aid for the observation of morphology of droplets [4].

Stability: Best formulations was subjected to 3 month stability studies, they were kept at two different temperature (2°C– 8°C), room temperature) and observed for any evidences of phase separation, flocculation [2].

Results and Discussion:

FT-IR Studies: The results of FT-IR spectrum showed no interaction between drug and excipients, hence compatible.

In-vitro drug dissolution study: In-vitro release of drug from the formulations containing lesser amount of drug (3 mg) was completed 96.01% within 18 min (figure 4).

Drug content: Percent drug content was found in the range of 93.79-98.82%.
Transmission electron microscopy: From the TEM study the average particle size was found to be within 50 nm and the shape was found to be spherical (figure 3).

Robustness to dilution: Robustness to dilution was studied by diluting the SEDDS 100 and 1000 folds with 0.1N NaOH. The diluted emulsions showed no visible signs of phase separation or drug precipitation after storage for 24 hrs at 37.0°C+0.5°C.

Zeta potential: Formulations are stabilised by a greater zeta potential. The zeta potential of formulation S4 (-35.45 ± 2.8mV) was high when compared to other formulations.

Kinetic studies: All formulation follows first order release kinetics and drug release mechanism was found to be non-Fickian.

Stability study: From the stability studies it was concluded that SEDDS formulation was stable thermally as well as under stress full conditions.

Figure 3: TEM of formulation S4

Figure 4: Percent CDR of Formulations S1, S2,S3, S4

Conclusion:

In this present study an attempt has been made to increase the solubility of Glipizide by self emulsification technique. From the experimental results it can concluded that, the SEDDS formulations prepared met the standard evaluation parameters with a slight deviation within the prescribed limits. In-vitro drug dissolution studies revealed that release of Glipizide from SEDDS was faster than the conventional formulation. From the drug content study it is found that all the formulation contain above 90% of drug. Formulation S4 has released a maximum of 96.01% drug from the formulation. Thus it can be concluded that the self-emulsifying drug delivery system of Glipizide showed a better solubility which in turn increases the bioavailability.

References

Formulation and development of film forming gel of Terbinafine Hydrochloride for prolonged dermal drug delivery

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Abstract: Limited effectiveness and residence time of conventional topical formulations for treatment of fungal infections of skin. A ‘film-forming gel’ which on application forms a thin, transparent film on skin surface. Eudragit RS PO and hydroxypropyl cellulose were used in combination to provide a matrix film that would permit the release of the antifungal agent for a prolonged time. The formulations were prepared using $3^2$ full factorial designs. They were tested for drying time, drug release, antifungal activity, skin irritation and stability studies. The gel was characterized for pH, viscosity, drug content, effective dosage volume and mechanical properties of the film formed after application; bioadhesion and water vapour permeability were also tested. The optimized formulation showed drug release of 99.84% and antifungal activity in terms of efficacy as 99.44%. Such a formulation can be claimed to decrease duration of therapy, will be more accepted by the patients and be a breakthrough in treating fungal infections of the skin.

Introduction:
Approximately 90% of fungal skin infections are caused by ‘dermatophytes’, which are parasitic fungi affecting the skin, hair, nails [1]. Terbinafine is an allylamine antifungal agent widely utilized in the treatment of infections caused by dermatophytes. It is also reported to have good activity in vitro against Cryptococcus, some species of Candida, Penicillium marneffei, Aspergillus, and other filamentous fungi [2]. Topical therapy is an attractive choice for the treatment of the cutaneous infections due to its advantages such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects. Conventional topical formulations are unable to retain the drug over the skin for a prolonged period and hence necessitate longer treatment duration or have to be supplemented by oral therapy [3]. We hypothesized that incorporation of the drug in a film forming gel would facilitate prolonged contact of the drug on the skin and the film formed on drying would improve its skin retention ability, thereby improving the topical treatment of fungal skin infections. This approach does not only sustain the release of drug and enhance percutaneous absorption, but may even allow for drug targeting to the skin or even its substructure, thereby enhancing drug efficacy and improving patient compliance by reducing application frequency. In this study a dermal gel containing terbinafine hydrochloride (TH) was prepared using the film forming polymer, Eudragit RS PO (Eudragit) and gelling agent, Hydroxypropyl cellulose (HPC). HPC also played the role of a secondary film forming polymer. Triethyl citrate (TEC) was used as a plasticizer. It is more efficient...
to use a multi-factorial design than one-factor-at-a-time experimentation since it can give a combination of variables that give better results for the optimization study. Using the response surface analysis technique, we evaluated the effects of two factors: the amount of Eudragit and the amount of HPC on the drug release rate and antifungal activity by utilizing $3^2$ full factorial designs. The optimized formulation was also evaluated for skin irritation in rats.

**Materials and Methods:**

TH was received as gift sample from FDC Ltd., Mumbai, India. Eudragit was received as gift sample from Evonik Degussa India Pvt. Ltd., Mumbai. HPC HF and TEC were purchased from SD Fine Chemicals, Mumbai. Wistar albino rats and were purchased from National Institute of Biosciences, Pune. All other chemicals were of analytical grade and were obtained commercially.

**Preparation of dermal gel:** The polymeric solutions of Eudragit RS PO and Hydroxypropyl cellulose were prepared in ethanol using dispersion method. Eudragit RS PO was sprinkled over 10 mL of ethanol containing triethyl citrate (7.5 % w/w of Eudragit RS PO). Hydroxypropyl cellulose was sprinkled over 10 mL of ethanol separately. Both solutions were allowed to swell for 24 hours to produce clear solutions. The polymeric solutions were mixed properly with continuous stirring. Accurately weighed quantity (0.25g) of the TH was dissolved in 5 mL ethanol. The drug solution and polymeric dispersion were mixed properly with continuous stirring and volume was made up to the mark using ethanol.

**Results and Discussion:**

**In-vitro drug release study:** Drug release was found to be sustained at intermediate levels of hydrophobic polymer, Eudragit and hydrophilic polymer, HPC. Of the nine formulations, maximum release was found to be for formulation F5 after 24 hours shows 99.84% of the drug in the formulation was available for antifungal activity.

![In vitro drug release](image-url)
Antifungal activity: The standard value of TH against Candida albicans for zone of inhibition is 24 mm. F5 formulation showed a zone of inhibition of 23.87 mm and 99.44% efficacy [4].

Skin irritation study:
Skin irritation study on rats showed that after application of the optimized formulation there was no evidence of irritation (erythema and oedema). Hence, the optimized formulation F5 was found to be safe [5].

![Figure 2 Results of pharmacological study.](image)

Conclusion:
Film forming gel of TH was prepared using Eudragit RS PO and hydroxypropyl cellulose. The desirable goals could be achieved by systematic formulation approach. Antifungal study showed that developed film forming gel can reduce the fungal burden and thus, is more effective as compared to commercial product. This novel dosage form will improve both the accuracy and the positioning of a delivered dose.

References:
Formulation and evaluation of orodispersible tablets of Dimenhydrinate by using Co-processed superdisintegrants

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Abstract: Dimenhydrinate is an antiemetic drug. Orodispersible tablets were prepared by direct compression method using co-processed superdisintegrants. Angle of repose and Carr’s index indicated that powder mixtures showed good to acceptable flow properties. All formulations containing co-processed superdisintegrant showed short disintegration time (38.23-17.67 s) and maximum water absorption ratio 73.39%-91.35%. Formulation F7 containing crospovidone: guar gum in 1:3 ratio showed highest percentage of drug release (98.89%), which is due to high degree of swelling caused by guar gum and rapid hydration by crospovidone. In conclusion, co-processed superdisintegrants were superior over individual super disintegrating agent.

Introduction:
Even though there are many routes of drug administration, oral route of drug administration is most popular route. Among all solid oral dosage forms; tablet is most popular because of self-medication, compactness and ease of manufacturing. The main problem of the tablet in relation with patients is dysphagia. To overcome these problems, scientists have developed an innovative technique known as orodispensible tablet Orodispersible tablet disintegrate within a minute which results easy swallowing of tablets [1]. Dimenhydrinate is a salt of diphenhydramine and chlorotheophyllinate. Dimenhydramine is an antihistaminic drug that is antagonistic at the H1 receptor in order to prevent or suppress nausea and vomiting [2]. Co-processing method is new and novel concept, where more than one excipient interact each other to mask the undesirable properties of an individual excipient. Co-processed excipients so formed are superior in many properties such as improved flow properties, compressibility and better dissolution profile and reduced lubricant sensitivity.

Materials and Methods:
Drugs and chemicals: Dimenhydrinate was obtained as gift sample from S.S Pharma, Mumbai. Crospovidone, Guar gum, were procured from S.D fine chem. limited, Mumbai.
Preparation of Dimenhydrinate orodispersible tablets: The co-processed superdisintegrants were prepared by solvent evaporation method where crospovidone and guar gum were mixed in with 10-15 ml of chloroform in different ratio. The solutions were stirred thoroughly till almost all chloroform evaporated. Each formulation contains 50mg of pure drug. Mannitol was used as diluent, aspartame as
sweetening agent, magnesium stearate as well as talc as lubricants. The calculated quantity of drug and excipients were weighed and passed through sieve # 60, mixed together and compressed into tablet using single punch tablet machine, by direct compression method (Table 1).

Table 1 Composition of Dimenhydrinate FDTs prepared by direct compression method.

<table>
<thead>
<tr>
<th>Ingredients (mg)</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0</td>
</tr>
<tr>
<td>Dimenhydrinate</td>
<td>50</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>5</td>
</tr>
<tr>
<td>Guar gum</td>
<td>-</td>
</tr>
<tr>
<td>CPS</td>
<td>-</td>
</tr>
<tr>
<td>MCC</td>
<td>10</td>
</tr>
<tr>
<td>Aspartame</td>
<td>1.5</td>
</tr>
<tr>
<td>Menthol</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2</td>
</tr>
<tr>
<td>Talc</td>
<td>1</td>
</tr>
<tr>
<td>Mannitol up to</td>
<td>150</td>
</tr>
</tbody>
</table>

Pre-compression evaluation parameters: Before compression, powder mixtures were evaluated for angle of repose, bulk density, true density, compressibility index (CI) and Hausner ratio [1, 2].

Post compression parameters of prepared tablets: After compression, prepared tablets were subjected for various tests such weight variation, thickness, hardness, friability, wetting time, water absorption ratio, drug content, disintegrating time, drug release kinetics and stability study.

In-vitro drug release study: In-vitro dissolution studies carried out using USP type II paddle type dissolution apparatus (Lab India). The dissolution medium used was pH 6.8 phosphate buffer (900ml) maintained 37±5°C with a paddle rotation speed at 50 rpm. Absorbance was measured at 278 nm using UV-Visible spectrophotometer-1800 (Shimadzu, Japan) and drug concentration was calculated.

Results and Discussion:
Compatibility studies: FTIR spectroscopy showed that no significant interaction between the drug and excipients, hence compatible.

Angle of repose: Formulations containing co-processed superdisintegrants showed lower values (23.89° to 28.27°) of angle of repose compared to control formulation (38.42°), hence all formulations showed good to acceptable flow properties.

Carr’s index: Carr’s index (23.29 to 15.48) showed all the formulations exhibits acceptable to good flow ability.

Haunser ratio: Haunser showed that powders with low inter-particle friction had ratios below 1.25.

Post-Compression Evaluation: Hardness of the tablets was found to vary from 2.67 to 3.52 kg/cm² compared to 2.15 kg/cm² of control tablets. Thickness of tables for all formulation was approximately
3.34 mm. The % friability was less than 1% for all formulation indicates that tablets formulations are of good mechanical strength to withstand abrasion in handling. Water absorption ratio and wetting time were found in the range of 73.39-91.35% and 38.23-17.67s respectively compared to 3.54s min (wetting time) of control formulation. Formulation containing crospovidone: guar gum (1:3) ratio showed highest water absorption ratio 91.35% and lowest wetting time. This may be due to rapid water absorption and swelling capacity of superdisintegrant, which results in rapid breakdown of tablets. FormulaF7 showed lowest disintegration time (17.37s), might be due to higher capillary action of crospovidone and swelling ability of guar gum.

**In-vitro dissolution study:** The results obtained from the dissolution study are dissipated in Figure 1. Formulae F0 showed minimum cumulative % drug release (65.21%) over a period 30 min because of absence of superdisintegrants. The amount of drug release from these preparations was comparatively higher than control formulation. Formulation F3, F4, F5, F6 and F7 prepared by using co-processed superdisintegrant was found to release 96.16%, 96.87%, 97.32%, 98.31%, 98.89% respectively, at the end of 30 min. In all formulations the cumulative % drug release was closer to 100% within 30 min which might be due to rapid breakdown of particles of co-processed superdisintegrants and porous structure of the tablets. The formulation F7 showed maximum percentage of drug release (98.89%) which may due to presence of higher concentration of guar gum along with optimum level of crospovidone compared to other formulations.

**Figure 1** Dissolution profile of Dimenhydrinate orodispersible tablets (Batch F0 to F7)

**Conclusion:**

In conclusion, co-processed superdisintegrants were superior over individual super disintegrating agent, hence formulation containing CPS disintegrate faster; thereby enhance the absorption leading to increased bioavailability of Dimenhydrinate, hence gives quick relief from emesis.

**References:**


Formulation and evaluation of lipospheres as delivery system for topical delivery in treatment of acne

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Abstract: The aim of present research work was to formulate and evaluate lipospheres as potential delivery system for topical acne treatment. Lipospheres loaded with tretinoin were prepared by solvent evaporation technique using glyceryl monostearate and cetyl alcohol. These lipospheres were characterized by SEM, TEM for surface morphology, zeta potential for particle size, DSC for interaction study, in-vitro drug release study, entrapment efficiency and drug loading capacity. Results revealed that lipospheres system were able to entrap 84% tretinoin and found to be superior compared to the marketed product. The stability study was carried out at different accelerated and non-accelerated conditions.

Introduction:
Acne is a condition that affects 80% of the human population. Various conventional dosage forms like cream, lotion, gel, solution and suspension commonly used in topical treatment of acne. Topical treatment of skin diseases is very attractive, since systemic load of active pharmaceutical ingredients (API) and thus also systemic side effects are reduced as compared to parenteral or oral drug administration. Novel drug delivery systems have been used to reduce the side effect of drugs commonly used in the topical treatment of acne.

Lipospheres are a class of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds. They consist of water-dispersible solid microparticles of particles between 0.2–100 μm in diameter and composed of a solid hydrophobic fat core stabilized by one monolayer of phospholipid molecules embedded in their surface which is a potential group of penetration enhancers. The objective of this work is to prepare and characterize tretinoin loaded lipospheres for improved topical treatment of acne.

Materials and Methods:
1. Selection of lipid
Solubility of drug in various solvents and lipids were carried out to select the best lipid component for preparation of lipid based delivery system. Analysis of the drug was carried out on UV Spectrophotometer.

2. Preparation of Lipospheres
Lipospheres of tretinoin were prepared by solvent evaporation technique. Accurately weighed amounts of the drug, lipid core and phospholipids were dissolved in ethyl acetate. The organic solvent was slowly evaporated under reduced pressure at 50–60°C using a rotary evaporator. The resulting solid was mixed with 10 ml PBS at 50–60°C with continuous mixing till the formation of a homogenous dispersion. The temperature was then reduced to 10°C with continuous rotation at 150 rpm for 5 min. The system was sonicated for 15 min in a bath type sonicator then cooled down to 20°C with continued shaking for another 5 min. Table 1 highlights the role of different excipients used in lipospheres. Figure 1 enlists the steps for preparation of lipospheres.

**Results and Discussion:**

The size of lipospheres was in the range of 3385 μm to 7280 μm. Entrapment efficiency of lipospheres with glyceryl monostearate as core material and stirring rate was changed for each formulation. The formulation which contains 400 mg of glyceryl monostearate and prepared by 1000 rpm stirring rate possessed highest entrapment efficiency. Figure 2 shows the TEM image of lipospheres. Also, when amount of core material and stirring speed was increased then the % entrapment efficiency also increased. This is based on the fact that as the ratio of drug to the lipid matrix increases, space also increases and more space available for the drug entrapment. Table 2 covers the characterization parameters of various lipospheres containing formulations.

<table>
<thead>
<tr>
<th>Table 1 Ingredients used in preparation of lipospheres</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
</tr>
<tr>
<td>Core material</td>
</tr>
<tr>
<td>Coat material</td>
</tr>
<tr>
<td>Surfactant</td>
</tr>
<tr>
<td>Organic solvent</td>
</tr>
<tr>
<td>Buffering agent</td>
</tr>
<tr>
<td>Drug</td>
</tr>
</tbody>
</table>

| Table: 2 Percentage yield and Entrapment efficiency of different formulation of Lipospheres |
|---|---|---|---|---|
| Formulation Code Drug | Percentage Yield (%) | Entrapment Efficiency (%) | Drug content | Vesicle size(μm) |
| F1 | 81.1 | 83.2 | 5.17 | 3383.82 |
| F2 | 83.6 | 85.9 | 5.28 | 3489.98 |
| F3 | 86.4 | 88.7 | 5.20 | 5960.51 |
| F4 | 72.8 | 75.1 | 5.23 | 5291.61 |
| F5 | 75.1 | 77.6 | 5.18 | 5507.67 |
| F6 | 73.7 | 76.3 | 5.12 | 5553.84 |
| F7 | 62.6 | 65.8 | 5.15 | 6586.70 |
| F8 | 65.3 | 67.3 | 5.17 | 5723.00 |
| F9 | 69.6 | 71.4 | 5.11 | 5255.38 |
Conclusion:
From the overall results obtained, it can be concluded that lipospheres could be an interesting carrier for tretinoin in skin disease treatment i.e. acne, when appropriate formulations are used. In fact, tretinoin dermal delivery was found to be affected by several factors including vesicle composition, morphology and size. Further in-vivo studies have to be carried out in order to obtain more information on vesicle–skin interactions.

References:
**Design and evaluation of Dorzolamide ocular inserts for Glaucoma**

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**Abstract:** The aim of present study was to develop and evaluate novel ophthalmic ocular insert of dorzolamide. Ocuserts were prepared by solvent casting method using polyvinyl alcohol (PVA) and propylene glycol (PG) as a plasticizer at various proportions. The prepared ocular inserts were evaluated for drug content, weight uniformity, folding endurance, thickness and in vivo studies. Optimization of ocular inserts was carried out by using $3^2$ factorial designs. In vivo study was performed by using rabbit as animal model. Comparative study with marketed formulation shows significant reduction in IOP for 7 hours.

**Introduction:**
Topical application of drugs to the eye is the well-established route of administration for the treatment of various eye diseases like dryness, conjunctiva, glaucoma, macular degeneration. The protective mechanisms of the eye such as blinking, baseline and reflex lachrymation, and drainage decrease the bioavailability of drug and also help to remove the installed formulation rapidly. Glaucoma is a condition that is characterized by increased intraocular pressure (IOP) which causes damage to optic nerve and result into irreversible loss of vision if not treated in early stage. Normal IOP is 15-20 mm Hg, when it get exceed to 22 mm Hg it results into glaucoma. It is the second most leading cause of world blindness which may affect around 80 million people in 2020.

**Materials and Methods**

**Materials:** Dorzolamide is obtained from FDC Mumbai, Polyvinyl Alcohol (PVA) of LR grade was obtained from Loba chemicals Pvt.ltd, Mumbai, Propylene glycol (PG) from Research lab.

**Method:** Solvent casting method was used to prepare ocular inserts. The required quantity of polymer is dissolved in 10.ml of distilled water and stirred for 2hr then weighed quantity of Dorzolamide was added and stirred for 2hr on magnetic stirrer to get a uniform dispersion. After complete mixing casting solution was poured on petridish and allowed it to dry.

**Characterization of optimized ocular inserts**
A. Thickness  
B. Folding Endurance test  
C. Weight Variation  
D. Drug content uniformity  
E. In vitro drug diffusion study
F. Sterility testing as per I.P. 2014
G. In vivo drug release studies
H. Accelerated stability studies as per ICH guidelines

Results and Discussion:
Thickness- The inserts thickness was found in between 0.03mm to 0.05mm. Thus it indicates that the prepared films were uniform in thickness.

Folding Endurance- The folding endurance of the films was found to be from 210 to 330. Thus from the results obtained it was observed that all film formulation were having considerable flexibility.

Weight Variation- The weight of drug loaded PVA films tested was found to be from 14.30mg to 16.20mg indicating uniform weight distribution.

Drug Content Uniformity- Drug content of dorzolamide films was found to be in between 97.5-99.3%. The results indicated that drug was uniformly dispersed as per official specification.

In vitro drug diffusion study- The optimized batch F8 is evaluated for in vitro drug release studies and it was observed that 98.6% drug released for 7hrs.

Sterility testing as per IP 2014- The presence of microorganisms was checked after ocular inserts were sterilized by UV radiation. Optimized batch F8 passes the sterility test as per IP 2014.

In vivo drug release studies- In vivo studies of prepared formulation were performed on albino rabbits. It was found that 25% decrease in IOP takes place in case of ocular insert while marketed formulation shows 18% decrease in IOP. Developed ocular inserts were found to be safe for ocular administration and capable of residing in the precorneal area of eye for extended period of time.

Accelerated stability studies as per ICH guidelines- Stability study was carried out on optimized ocular inserts formulation for three months. The results obtained concluded that Ocuserts were chemically and physically stable at accelerated conditions of temperature 45°C and 75% relative humidity.

Table 1 Physiochemical evaluation of ocular inserts.

<table>
<thead>
<tr>
<th>Different Batches</th>
<th>Evaluation Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness(mm)</td>
</tr>
<tr>
<td>F1</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>F2</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>F3</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>F4</td>
<td>0.04 ± 0.005</td>
</tr>
<tr>
<td>F5</td>
<td>0.05 ± 0.005</td>
</tr>
<tr>
<td>F6</td>
<td>0.04 ± 0.005</td>
</tr>
<tr>
<td>F7</td>
<td>0.04 ± 0.005</td>
</tr>
<tr>
<td>F8</td>
<td>0.05 ± 0.005</td>
</tr>
<tr>
<td>F9</td>
<td>0.04 ± 0.005</td>
</tr>
</tbody>
</table>

Conclusion:
The results obtained after characterization of Dorzolamide complies with reported standards. Dorzolamide ocular insert was successfully formulated by using PVA as polymer and PG as plasticizer.

Appearance, Thickness and Folding endurance of prepared ocular insert was found to be satisfactory. The optimized batch F8 showed desirable drug release at 7h (98.60%), Thickness (0.05mm) and folding endurance (330).

Optimized ocular insert passes all safety tests used for evaluation of ophthalmic formulation as per regulatory guidelines.

Stability study performed over a period of three month showed that optimized formulation is stable. Hence prepared formulation may be the potential alternate for conventional ophthalmic drug delivery system use for glaucoma treatment.

**Acknowledgements:**
Authors are thankful to Principal Dr. K. G. Bothara for providing excellent facilities for this research work.

**References:**
Formulation development and evaluation of HPMC-TSG based buccal patch of Eletriptan Hydrobromide.

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Abstract: Mucoadhesive buccal patch were developed using Tamarind seed gum (TSG) as mucoadhesive polymer for systemic delivery of Eletriptan Hydrobromide. 3² factorial designs was applied to study effect of independent variables viz. concentrations of TSG and HPMC K4-M, on dependent variables like folding endurance, swelling index, in vitro drug release. Ex vivo diffusion studies were carried out using Franz diffusion cell. The optimised batch shows 99.61% drug release for 5h and no buccal mucosal damage observed in histopathological studies. In conclusion, TSG can be used as potential drug release modifier and mucoadhesive polymer for successful formulation of Eletriptan HBr buccal patches.

Introduction:
Among the transmucosal routes, buccal route has prominent advantages such as faster uptake of drug into the systemic circulation and enhanced bioavailability of therapeutic agents, leading to rapid onset of action. In addition, buccal drug delivery system avoids first pass metabolism by directing absorption through the venous system that drains from the cheek [1]. Buccal cavity provides easy accessibility for self-medication and hence it is well accepted by patients [2]. Various buccal adhesive delivery devices have been developed such as, buccal tablet, buccal films, buccal wafers, buccal gels and ointments [2]. Amongst various buccal dosage forms, buccal patches provide more flexibility, as they can be very easily administered and removed from the application site, terminating the input of drug whenever desired [3].

Eletriptan Hydrobromide is a selective 5-HT (1B/1D) receptor agonist used in the treatment of migraine. Although, it is absorbed well after oral administration, it is extensively metabolized hepatically resulting in oral bioavailability 40%. The recommended dose of Eletriptan HBr is 10–20 mg. In this study Tamarind Seed Gum (TSG), a glycosaminoglycan polysaccharide extracted from the kernels of seeds of Tamarindus indica Linn. Family Fabaceae was used for mucoadhesion, film former and penetration enhancer in the dosage form [4].

Materials and Methods:
Materials: Eletriptan Hydrobromide was obtained from USV Pvt. Ltd. (Mumbai), Tamarind Seed Gum Powder from Altrafine Gum (Ahmadabad), HPMC K-4 M was purchased from S. D. Fine
(Mumbai), Polyvinyl Alcohol from Loba Chem. Pvt. Ltd. (Mumbai), Propylene Glycol from Research Lab Fine Chem. Industries (Mumbai).

**Method:** The Bilayered Patches of Eletriptan Hydrobromide was prepared using solvent casting method using TSG as a mucoadhesive polymer, HPMC K-4M as a film former and PG was used as a plasticizer. PVA was incorporated in the formulation to increase the tensile strength.

**Characterization of buccal patch:** The prepared buccal patch were evaluated for Folding endurance [1, 2], Tensile strength [1], *Ex vivo* mucoadhesive time [5], % Swelling index [1, 2, 5], *In vitro* drug release studies [4], *Ex vivo* mucoadhesive strength [2], Buccal mucosa sensitivity test [2].

**Results and Discussion:**

Folding endurance was obtained in between 260 to 308; reveals stable patch. Tensile strength of formulation found to be ranging from 8.00 to 17.98. The values showed that the mechanical strength of patches was enough to bear stress during transport and administration of patches. *Ex vivo* mucoadhesion time of the formulations (Table 1) dependent on swelling index of the patches; that is *ex vivo* mucoadhesion time was directly proportional to the swelling index. The swelling index of the patches increased with the increasing concentration of TSG. % swelling index was given in table 1, the combination of HPMC K-4 M with TSG increases swelling index of TSG. That is significant decrease in swelling index was obtained at same level of TSG with increase in HPMC K-4 M concentration. The % drug release at 5 h was found to be in between 89.63% to 99.61% (Table 1) indicates there is significant decrease in % drug release at 5h was obtained at same level of HPMC K-4 M with increase in TSG concentration. *Ex vivo* mucoadhesive strength of B1-B9 found between 11.98-34.08 kg/mm². Mucoadhesion force increased with an increase in concentration of TSG and HPMC K-4 M. The optimized formulation B4 containing 0.1% TSG and 2.5 % HPMC K-4 M was subjected to buccal mucosa sensitivity test. The histopathological evaluation of sections showed that cellular membrane was intact and there was no damage to the epithelial layer.

**Table 1 Physicochemical evaluations parameter of Eletriptan buccal patch**

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Folding endurance*</th>
<th>Tensile strength* (N/mm²)</th>
<th><em>Ex vivo</em> mucoadhesive time (min)</th>
<th>Swelling index (%)</th>
<th>Mucoadhesive strength* (Kg/mm²)</th>
<th>% drug release at 5 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>260 ± 1.51</td>
<td>8.00 ±0.50</td>
<td>245</td>
<td>345</td>
<td>18.20</td>
<td>88.86</td>
</tr>
<tr>
<td>B2</td>
<td>270 ± 1.00</td>
<td>11.15±0.02</td>
<td>285</td>
<td>335</td>
<td>14.15</td>
<td>90.88</td>
</tr>
<tr>
<td>B3</td>
<td>275 ± 1.22</td>
<td>12.13±0.04</td>
<td>300</td>
<td>289</td>
<td>11.98</td>
<td>89.63</td>
</tr>
<tr>
<td>B4</td>
<td>285 ± 1.52</td>
<td>10.34±0.21</td>
<td>300</td>
<td>349</td>
<td>25.03</td>
<td>99.61</td>
</tr>
<tr>
<td>B5</td>
<td>295 ± 1.52</td>
<td>12.13±0.17</td>
<td>280</td>
<td>334</td>
<td>21.03</td>
<td>96.18</td>
</tr>
<tr>
<td>B6</td>
<td>308 ± 2.32</td>
<td>15.78±0.40</td>
<td>286</td>
<td>320</td>
<td>20.75</td>
<td>97.38</td>
</tr>
<tr>
<td>B7</td>
<td>295 ± 1.52</td>
<td>14.89±0.37</td>
<td>295</td>
<td>370</td>
<td>33.90</td>
<td>98.8</td>
</tr>
<tr>
<td>B8</td>
<td>300 ± 2.08</td>
<td>17.12±0.22</td>
<td>290</td>
<td>365</td>
<td>34.08</td>
<td>95.94</td>
</tr>
<tr>
<td>B9</td>
<td>305 ± 2.51</td>
<td>17.98±0.50</td>
<td>287</td>
<td>345</td>
<td>33.05</td>
<td>96.88</td>
</tr>
</tbody>
</table>
*All values are mean ± SD, n=3

**Conclusion:**
The results obtained indicating TSG has potential to modify drug release rate and shows good mucoadhesion. Hence this natural polysaccharide which is economic and abundantly available can be used as a potential release modifier and mucoadhesive polymer for successful formulation of Eletriptan HBR bilayered mucoadhesive buccal patches, which may prevent hepatic metabolism to a large possible extent.

**Acknowledgement:**
Authors are thankful to USV Ltd., Mumbai, India for providing Eletriptan HBr as a gift sample.

**References**
Novel approach to development of ethosomal gel containing Lornoxicam

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Abstract: In this present investigation, an approach has been made to develop ethosomes containing Lornoxicam, which was incorporated into a gel base for transdermal delivery. The ethosomal formulations were developed by using phospholipids and different concentration of ethanol (20% to 40%) and all prepared formulations were optimized and characterized. The sonicated ethosomal formulation containing phospholipids (2%) and ethanol (30%) (ET3) showed the highest entrapment efficiency (93.96%) with small particle size (100±3.9nm). Formulation ET3 was selected for further skin permeation studies and the result showed that the formulation (ET3) exhibited higher percentage of drug permeation i.e.73.18%.

Introduction:
Transdermal route is such route, which is having several advantages over conventional routes such as it avoids of first pass metabolism, predictable, extended activity, reduces side effects, utilizes short half-life drugs, improves pharmacological, physiological response and also avoids the fluctuation in the drug levels plasma, inter and intra patient variations, and most importantly, it provides patient convince in term of application [1].

Ethosomes are referred as “soft vesicles” and these novel vesicular carriers are utilized for the transdermal delivery. The size of ethosomes vesicles can be modulated i.e. nanometres to microns. Ethosomes are the modified forms of liposome, composed of phospholipids, ethanol and water in different ratio. They have ability to penetrate and deliver the drug through the skin which in term drug reaches systemically. Ethanol fluidizes both ethosomal lipids and bilayers of the stratum corneum intercellular lipid. The soft, malleable vesicles, they penetrate the disorganized lipid bilayers [2, 3].

Materials and Methods:
Phospholipid was dispersed in water; heat the solution at 40°C in a water bath until a colloidal solution was obtained. In a separate vessel ethanol and propylene glycol are mixed together and heated up to 40°C. Once both mixtures reach the temperature, the organic phase was added to the aqueous phase. The drug was dissolved in water or ethanol depending on its properties. The size of the ethosomal vesicle can be decreased to the desire extent by using probe sonication or extrusion method[3].

Preparation of sonicated Lornoxicam Ethosomes: Ethosomes prepared by the using above
procedure were subjected to sonication using ultrasonic bath sonicator for an hour with a cycle of 10 min.[4].

**Incorporation into Gel:** Carbopol 0.75% w/w was soaked in minimum amount of water for 24 hours. Ethosomal, suspensions 20 ml containing Lornoxicam (100mg) was added to the swollen carbopol under stirring. The gel was incorporated with continuous stirring at 700 rpm in a closed vessel and maintained at temperature 30ºC until homogeneous gel was achieved. The pH was then adjusted to neutral (pH=7) using (TEM) triethanolamine.

Table 1 Composition of different un-sonicated (US) and sonicated (S) ethosomal formulations

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>ET₁(US)</td>
<td>2.0</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>0.4</td>
<td>Q.S</td>
</tr>
<tr>
<td>ET₂(US)</td>
<td>2.0</td>
<td>30</td>
<td>-</td>
<td>20</td>
<td>0.4</td>
<td>Q.S</td>
</tr>
<tr>
<td>ET₃(S)</td>
<td>2.0</td>
<td>30</td>
<td>-</td>
<td>20</td>
<td>0.4</td>
<td>Q.S</td>
</tr>
</tbody>
</table>

**Evaluation:**

**Visualization:** The initial characterization and visualization of the lamellar character of the ethosomal vesicles was performed by using transmission electron microscopy (TEM).

**Vesicle size and zeta potential:** Particle size of vesicle can be analysed by dynamic light scattering (DLS). The charge of the ethosomal vesicle is an important parameter that can influence vesicular properties such as stability and skin-vesicle interactions. Zeta potential was determined by using computerized inspection system. The size of the vesicles can be characterized by optical microscopy with an eyepiece micrometer which is calibrated with a stage micrometer.

**Entrapment efficiency:** Separation of un-entrapped and evaluation of entrapment efficiency can be measured by ultracentrifugation.

\[
\text{Entrapment efficiency} = \frac{(T-C)}{T} \times 100
\]

Where ‘T’ is total amount of drug and ‘C’ is the amount of drug detected only in the supernatant liquid.

**In-vitro drug release:** A suitable size of previously soaked cellophane membrane was mounted in between donor and receptor compartment of the Franz diffusion cells. The receiver cell contains 15 ml phosphate buffer solution (PBS) of pH 6.8. The receiver compartment was constantly stirred by magnetic stirrer at 100 rpm and a temperature of 37 ± 1°C was maintained throughout the experimental period. Lornoxicam ethosomal gel equivalent to 10 mg was applied homogenously in the donor compartments; 1ml samples were withdrawn from receiver at predetermined time intervals over 24 hours and immediately replenished with an equal volume of fresh buffer. Samples were analysed for drug content spectrophotometrically using spectrometer. The anti-inflammatory activity
was performed as per the literature [4].

Results and Discussion:
FT-IR studies revealed that there was no any possible interaction between the drug and excipients used; hence the prepared formulation was compatible. The vesicle size analysis was done and the formulation ET$_2$ having average diameter of 3.53µm and ET$_3$ of 0.251 µm. The Lornoxicam ethosomal gel has shown maximum anti-inflammatory activity of 64.2% at 4 hours. The data of drug released from the formulations were subjected to various kinetic models. The prepared formulations showed first order kinetics and follows non-fickian mechanism.

![Figure 1](comparison_of_in-vitro_skin_permeation_of_drug_from_various_formulations.png)

Conclusion:
The Lornoxicam ethosomes has been prepared by an optimized technique and results indicated that the optimized formulation showed up to 12 hours drug release. It has been also observed that the ethosomal system showed higher skin permeation of drug. The optimized formulation was subjected to the short term stability studies and the obtained results showed no significant changes in the with respect to the initial observations, hence stable.

References:
Abstract: Minoxidil loaded solid-lipid nanoparticles (SLN’s) based hydrogel system was developed for topical application on to the scalp with respect to avoid the problems associated with the Minoxidil topical solution. The objective of this work was to prepare and evaluate hydrogel system bearing minoxidil loaded solid-lipid nanoparticles. SLN’s were prepared by microemulsion technique through homogenization of two different phases i.e. organic phase and aqueous phase at 65°C using homogenization speed of 5000-6000 rpm. The prepared formulations were optimized using different production parameters like stirring speed and stirring time. The characteristics of final optimized SLN’s formulation were investigated. The optimized SLN’s formulation was assimilate in the hydrogel formulations and was evaluated for in-vitro diffusion studies. The results concluded that minoxidil bearing SLN’s based hydrogel could be a new and viable alternative to the marketed product.

Introduction:
Androgenic alopecia (AGA) is a genetically determined pattern of baldness in which scalp follicles show signs of improved levels and activity of scalp due to the conversion of dihydrotestosterone (DHT) by an isoenzyme 5α-reductase in the presence of testosterone (T). Minoxidil, a pyrimidine derivative (2, 4- diamino-6-piperidino-pyrimidine-pyrimidine- oxide), is most commonly used for the treatment of Androgenic alopecia. Minoxidil prolongs anlagen phage, promotes growth and enlarges the hair follicles in telogen phase [1].
Minoxidil is poorly soluble in water and in most of the organic solvents, by reason of this it has been formulated in an ethanol based preparations [2]. These formulations associated with certain disadvantages and/ or problems including reverting minoxidil to an insoluble crystalline form due to evaporation of ethanol on application onto the scalp, burning sensation, irritation to the skin, dry hair and scalp, dandruff and contact dermatitis [3].
Solid-lipid nanoparticles (SLN’s) are one among the nano-carrier, having a particle range in between 10-1000 nm can effectively go through quite a lot of anatomical barriers. The objective of this study is to prepare and evaluate minoxidil loaded SLN’s based hydrogel to avoid the problems associated with existing minoxidil solution.
Materials and Methods:
Minoxidil was received as gift sample by Zyga Pharma Pvt Ltd Indore; cholesterol, Soy lecithin and Pleuronic F-68 were procured from Loba Chemie Pvt. Ltd. Mumbai, High Media Mumbai and Sigma life science, USA, respectively. Carbopol 934 was obtained from Central Drug House Pvt. Ltd. New Delhi. All other ingredients used were of analytical grade.

Preparation of solid lipid nanoparticles (SLN’s): Maloxicam bearing solid lipid nanoparticles was prepared by microemulsion technique through homogenization of two different phases i.e. organic phase (containing drug, soya lecithin and lipid) and aqueous phase (containing pluronic F68 and sodium glycocholate). The organic mixture was subjected to sonication for a certain period of time with bath sonicator (PCI, Mumbai) to get a clear organic phase and added to this phase into aqueous phase, which consisted dissolved pluronic F68 and sodium glycocholate, at 65 °C by a different homogenization speed of 5000- 6000 rpm. The resulting emulsion was stored at 2-8°C. Compositions of different SLN’s formulations are presented in table 1.

Table 1 Composition of different solid lipid nanoparticle formulations.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug (mg)</th>
<th>Cholesterol (%W/V)</th>
<th>Soy lecithin (% w/v)</th>
<th>Pleuronic F-68 (% W/V)</th>
<th>Sod.Glycocholate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN1</td>
<td>200</td>
<td>2.0</td>
<td>0.5</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>SLN2</td>
<td>200</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>SLN3</td>
<td>200</td>
<td>2.0</td>
<td>1.5</td>
<td>1.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Optimization of production parameters and Characterization of SLN’s: The optimization of SLN’s formulations was done using different production parameters like stirring speed and stirring time and three different formulations were prepared on the basis of optimized parameters. The prepared formulation was characterized for its physical stability, particle size and shape. Physical stability (parameters like change in appearance, Ostwald ripening and settling behaviour were observed) of prepared SLN’s was studied at room temperature for two weeks. The ostwald ripening was observed by photomicroscope (radical instrument) at magnification 1x1000, 4x4000. The size and shape of optimized SLN’s was determined using scanning electron microscope (JSM 6301F, JEOL, Japan), respectively. Three hydrogel formulation containing prepared SLN’s (hydrogel formulation HFA, HFB and HFC having SLN’s: carbopol 934 ratio is 1:1, 1:2, 1:3, respectively) was formulated by dispersing carbopol 934 (1 % w/v) in water with constant stirring for 2 h. Thereafter propylene glycol, propyl paraben, and ethanol were added into it. Now to this mixture, prepared SLN’s formulation was added with mild stirring. Then the remaining quantity of water was added to produce clear hydrogel solution. The prepared hydrogel systems were characterized for their pH, spreadability, viscosity, drug content and In-vitro diffusion study.
In-vitro diffusion study was carried out in the modified Franz diffusion cell in which cellophane membrane was mounted in-between the donor and receiver compartments. The sample was collected at predetermined interval and immediately replaced with fresh medium to maintain the sink condition. The drug released was assessed by U.V. spectroscopy (UV-1800; Shimadzu) at 288 nm. The release mechanism of prepared hydrogel was analyzed the obtained data were fitted to zero order, first order, Higuchi, and Korsmeyer-Peppas model.

Results and Discussion:
Optimization of SLN’s formulations was done using different production parameters like stirring speed and stirring time. The premix suspension with different concentration of soy lecithin was stirred at 5000 rpm and 6000 rpm at different time interval i.e. 30 minutes, 45 minutes, and 60 minutes. At stirring rate of 5000 rpm for 30 minutes, the observed particles are larger crystals with aggregation. On increasing the stirring rate and stirring time, smaller particles with heterogeneous distribution were obtained. At stirring rate of 6000 rpm for 1 hour, the having the particle size in between 300 nm to 400 nm were obtained and this optimized formulation was used for further study.

The observation of physical stability study indicated that small crystal growth was noted in formulations SLN1 and SLN3 but no crystal growth was observed in formulation SLN2 at the predetermined intervals. Size and shape of formulation SLN2 was observed using scanning electron microscopy (SEM). SEM photomicrograph showed that the prepared smaller, uniform and having narrow size distribution range.

All the characterization parameters of prepared hydrogel systems were found within a limit. The cumulative percent drug release from formulations HFA, HFB and HFC was found to be 63.11±0.02, 59.19±0.22 and 52.79±0.81, respectively at the end of 6th hours and the release kinetics showed that the formulation follows zero order release model.

Conclusions:
In this study, minoxidil bearing solid lipid particles based hydrogel system was prepared. The SLN’s were found to have smaller, uniform and having narrow size distribution range. This preparation was incorporated into hydrogel system. On the basis of different characterization parameters, a controlled release topical formulation of minoxidil was prepared. The results concluded that minoxidil bearing SLN’s based hydrogel could be a new and viable alternative to the marketed product.

References:
Formulation of Docetaxel nanoemulsion: *In-vitro* characterization and cell proliferation study

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**Abstract:** The aim of present study was to improve aqueous solubility and *in vitro* dissolution rate of docetaxel. Docetaxel nanoemulsion was prepared using Sefsol 218 as internal phase, tween 20 as emulsifier and water as an external phase. Pseudoternary phase diagrams were constructed on the basis of solubility data of docetaxel to determine the nanoemulsion region. The formulated nanoemulsion was characterized by thermodynamic stability, morphology, droplet size, zeta potential, viscosity and *in vitro* cell proliferation studies. The *in vitro* dissolution studies and the cell proliferative assay (IC$_{50}$) confirmed the efficacy of optimized nanoemulsion over the available injectable formulation of docetaxel.

**Introduction:**  
Docetaxel, a potential anticancer agent against solid tumors has been restricted from oral use due to poor water solubility as well as Pgp efflux property. The present study was aimed to improve aqueous solubility, *in vitro* dissolution rate and *in vitro* activity of docetaxel through development of (o/w) nanoemulsion consisting of Sefsol 218 as internal phase with Tween® 20 as emulsifier and water as an external phase.

**Materials and Methods:**  
**Material:** Docetaxel was generously gifted by Cipla laboratories, Mumbai. Sefsol was purchased from Nikko Chemicals, Japan, All other oils and solvents were procured from Research Lab Fine Chem. Industries, Mumbai.

**Preparation of Docetaxel nanoemulsion:** In first step surfactant and co-surfactant mixture (Smix) was made homogenous with continuous stirring at 550 rpm (Remi motors, India) The second step involved the addition of an oil phase containing docetaxel to the surfactant mixture in an appropriate ratio with vigorous stirring. The aqueous phase was added in a drop wise manner at a rate of 1ml/min to the oil and Smix under vigorous stirring. Nine formulations (F1-F9) were prepared with varying % of Sefsol, Smix and water.

**Characterization of Docetaxel nanoemulsion:**
Based on thermodynamic stability studies, dispersibility test and % transmittance formulations F1, F2, F4, F5 were selected for further characterization.

**Particle size and zeta potential:** The particle size and poly dispersibility Index (PDI) of the formulations were investigated by photon cross correlation spectroscopy (PCCS). The PDI was calculated using the following formula. \[ \text{PDI} = \frac{X_{90} - X_{10}}{X_{50}} \]

Where, \( X_{50} \) is mean particle size, \( X_{90} \) and \( X_{10} \) are the size of nanoparticles below 10% and 90% respectively of the sample population. Zeta potential was determined using Zeta meter (Delsa Nano C, USA). The formulation was optimized on the basis of refractive index, particle size, Scanning electron microscopy and viscosity studies.

**In-vitro drug release:** The in-vitro release of optimized nanoemulsion (F1) was compared with the marketed i.v formulation Taxotere® and docetaxel coarse suspension in gradually changing buffer solution; pH 1.2 for first 2 h followed by 0.2 M pH 6.8 for the rest of the study. The formulations were placed in the activated dialysis bags and samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically at 229 nm [1].

**In-vitro cell line assay in breast cancer cell lines:** Cytotoxicity of docetaxel i.v. marketed formulation Taxotere® and the formulated nanoemulsion of docetaxel on MCF-7 were determined by MTT assay [2]. The cytotoxic effect of docetaxel nanoemulsion was expressed as percentage of cell viability relative to the untreated control cells (% control) defined as:

\[
\frac{\text{Optical density of treated cells at 560 nm}}{\text{Optical density of control cells at 560 nm}} \times 100
\]

**Stability of the nanoemulsion:** The optimized nanoemulsion was subjected to stability studies at 40°C and 75% RH for 3 month and samples were evaluated for physicochemical parameters like particle size, microscopic appearance and drug content at 1 month interval.

**Results and Discussion:**

F1 formulation showed minimum globule size 26.71 nm with PDI 0.29. The release of docetaxel from formulation F1 (91.20 ± 4.20%) was much higher as compared to that of marketed formulation Taxotere®(58.35± 2.91%) and coarse suspension (7.50 ± 1.35%) of docetaxel. Therefore, it can be apprehended that the nano sized oil droplets of F1 favored transit through the dialysis membrane resulting in better drug release from the nanoemulsion [3-5]. The in vitro cell line assay (IC_{50}) indicates that F1 efficiently inhibited the proliferation of MCF-7 cells in a dose dependent manner as shown in figure 1. Stability studies confirmed chemical and physical stability of optimized nanoemulsion.

**Conclusion:**

The optimized nanoemulsion formulation was successfully formulated using Sefsol 218, Tween 20 and Ethanol (Smix), and distilled water. The mean globule size of the optimized nanoemulsion was
found to be 26.71 nm and PDI 0.29. The zeta potential of formulation was found to be -3.59 mV due to the presence of non-ionic surfactants. The optimized nanoemulsion showed promising in-vitro dissolution owing to its lipidic nanoform. The in vitro dissolution studies and cell line assay of optimized nanoemulsion confirm the efficacy of docetaxel nanoemulsion.

![Figure 1](image1.png)

**Figure 1** Antiproliferative effect of (a) Docetaxel nanoemulsion (F1), (b) Docetaxel i.v. marketed formulation on MCF-7 breast cancer cell lines.

**Acknowledgement:**
The authors are thankful to Cipla laboratories, Mumbai for providing docetaxel. We are grateful to National toxicology centre, Pune for technical guidance for cell line assay study.

**References:**
Formulation, development and evaluation of effervescent tablet of Esomeprazol and Aceclofenac

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Sagar Institute of research technology & Science Pharmacy Bhopal
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Abstract: The Present research work is to develop effervescent tablet of esomeprazole and aceclofenac. Effervescent tablet is a tablet intended to be dissolved or dispersed in water before administration. Esomeprazole is a proton pump inhibitor which reduces acid secretion and treat ulcers while Aceclofenac is a potent anti-inflammatory drug. Six formulation were prepared. The final blend of the drug and excipients were evaluated for powder flow properties, bulk, density, tapped density, compressibility index and hausners ratio. All the formulations were evaluated for thickness, weight variation, disintegration time, hardness, friability, drug content. The study reveals that that the F4 showed the best effervescence time. In vitro dissolution studies indicate that formulation F4 shows more than 95.96% drug release at the end of 5 min.

Introduction:
Esomeprazole is a proton pump inhibitor which reduces acid secretion through inhibition of the H+/K+ ATPase in gastric parietal cells. By inhibiting the functioning of this transporter, the drug prevents formation of gastric acid. Aceclofenac is a potent anti-inflammatory drug. It is used in treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and acute gout disease. It is poorly water soluble drug. The present work is aimed to treat both pain and gastric ulcers [1-3].

Materials and Methods:
Materials
Esomeprazole and Aceclofenac were provided by Torrent Pharmaceutical Pvt. Ltd, and other chemicals were provided by SIRTS-Pharmacy Bhopal.
Preparation
Wet granulation method: Accurately measured quantities of drug and excipients were taken. IPA was used as granulating agent in case of wet granulation process. Mixture of drug and excipients were taken for granulation. Granules formed through wet granulation were dried in oven till the moisture content was below 1%. The tablets were punched using Rotary compression machine (Jaugur) of 25 mm punch.
Optimization: To optimize the formulation we took six formulations F-1, F-2, F-3, F-4, F-5 and F-6. In each formulation we changed concentration of the ingredients.
Table 1 Optimized formula for Aceclo-esomeprazole effervescent tablets 500mg.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceclofenac</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Esmaprazole</td>
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<td>40</td>
<td>40</td>
<td>40</td>
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<td>40</td>
</tr>
<tr>
<td>Citric acid</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>40</td>
<td>50</td>
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<tr>
<td>Ascorbic acid</td>
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<td>70</td>
<td>70</td>
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<tr>
<td>Sodium bicarbonate</td>
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<td>170</td>
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<tr>
<td>Sodium carbonate</td>
<td>40</td>
<td>50</td>
<td>40</td>
<td>60</td>
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</tr>
<tr>
<td>PEG-6000</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>PVP-K-30</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Results & Discussion:

The preformulation study of esomeprazole and aceclofenac was performed. In this aceclofenac the esomeprazole and aceclofenac powder was examined for its organoleptic properties and it was observed that both drugs were white crystalline powder. Esomeprazole was found soluble in methanol, sparingly soluble in ethanol. The melting point of esomeprazole was found to be 153-155 and the melting point of aceclofenac was found to be 149-153. The peak of the graphs of UV, IR report was resemble like Indian Pharmacopeia standard. Hence on the basis of preformulation study the drug sample of esomeprazole and aceclofenac was found to be pure and authenticated and the sample can be use for the further preparation and evaluation.

In-vitro drug release studies:

In-vitro drug release studies were carried out by using USP XXIII dissolution apparatus II (Paddle type) [Electro lab (TDT-06T) Tablet Dissolution Tester] at 50 rpm. The drug release profile was studied in 900 ml of hydrochloric acid buffer at pH 6.8 by maintaining at 37±0.5°C. Aliquots of 10 ml of a dissolution medium were withdrawn at specific time intervals (1, 2, 3, 4 and 5 min), filtered and the amount of drug released was determined spectrophotometrically at 290 nm.

Figure 1 Shows drug release of esomeprazole aceclofenac study.

Conclusion:
The present study was an attempt to develop and evaluate an effervescent tablet in combination with aceclofenac and esomeprazole. Aceclofenac is an effective analgesic and anti-inflammatory agent with a good tolerability profile. Through its analgesic and anti-inflammatory properties, aceclofenac provides symptomatic relief in a variety of painful conditions. Esomeprazole reduces the production of digestive acids and treatment of gastric ulcers. The formulated tablet met the pharmacopoeial requirement of uniformity of weight, the entire tablet conformed to the requirement of assay as per IP, hardness, friability, and thickness and weight variation and content uniformity were within acceptable limit.

References:
Synthesis, characterization and evaluation of mannosylated 5-FU nanoparticles for enhanced activity in skin cancer

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Abstract: The objective of the present investigation was to develop and examine the prospective of surface engineered polymeric nanoparticles as cargoes for site specific delivery of 5-fluorouracil (5-FU). 5-FU-loaded nanoparticles, decorated with mannose were prepared and characterized by, particle size analysis, FTIR, DSC, TEM, X-ray and NMR studies. The nanoparticulate formulation was evaluated for in vitro release. Cytotoxicity studies were performed on J774 cell lines (Macrophage cells) using MTT cell proliferation assay. The results brought us to derive the conclusion that the conjugation of MA offers targetability to the formulation and enhanced selectivity by cancerous tissues in vitro.

Introduction:
Macrophages have been crucially involved in various immune responses occurring in skin cancer. Keratinocytes and dermal macrophages are believed to be significant in peeling off the extracellular matrix by the activation of metalloproteinase enzymes. Keratinocyte macrophages cells express mannose receptor (MR, CD206 or MRC1) having higher affinity for mannose. Moreover, keratinocyte and dermal macrophage cells express macrophage mannose-binding receptor which is having affinity towards mannose [1]. These receptors are chemically transmembraneous glycoprotein in nature and belong to the C-type lectin. Mannosylation technique facilitates the process of internalization of any therapeutic moiety tailored with carbohydrate-binding proteins (lectins). This decoration provides advantages like specific cellular recognition for site specific delivery of drug.

Materials and Methods:
Experimental: 5-FU was obtained as a gift sample from Glenmarck, India, Poly (lactide-co-glycolide) copolymer (PLGA) (Mw~17 000–22000, 50:50) was obtained with thanks from Resomer, Evoniks, Germany, Folate, N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC), DCC and N-hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich, USA and D(+)-Mannose was purchased from Himedia. Phosphate buffered saline—PBS (pH5.5 and pH 5.6) used for drug release. All other reagents used were of analytical grade.

Synthesis of 5-FU-NPs and its surface modification: Nanoprecipitation technique was used to prepare the polymeric nanoparticles of 5-FU and EDC-NHS conjugation chemistry was employed to
alter the surface of prepared nanoparticles by attaching them with mannose [2]. Then the prepared mannosylated nanoparticles were evaluated for Particle size, zeta potential, FTIR, XRD, DSC, NMR studies. The release of 5-FUercetin from the MA-5-FU-NPs were prepared by DCC-NHS activation method was determined at two pH conditions, i.e; 5.5 to trigger the release of macrophages in the endosomal compartment and pH 5.6 to match the physiology conditions of the skin.

**MTT Assay:** Cells were seeded in 96 well plates at a density of 1x10^5 cells/ well. There J774 cell lines were subjected to DMSO and DMSO containing MA-5-FU-NPs at concentrations from 0-75 µm for both the cell lines, taking as control and test sample respectively.

**Results and Discussion:**

In order to find the best optimized formulation with minimum particle size and maximum entrapment efficiency; the different batches of MA-5-FU-NPs were characterized and evaluated [3]. Particle size distributions [(mean diameter (nm)], zeta potential, poly dispersity index of MA-5-FU-NPs were determined by dynamic light scattering using Malvern Zetasizer, ZS nano 90, Malvern Instruments, USA (Table 1). The results of the study showed that the mannosylated nanoparticles of 5-FU seems to be appreciable in comparison to non-mannosylated 5-FU nanoparticles in terms of cellular viability, stability and targetability. The synthesis of mannosylated nanoparticles were confirmed by FTIR, XRD, DSC and NMR studies was able to show an improved in vitro profile.

**Table 1** Summarizes the physiochemical characteristic of the prepared nanoformulation before and after incorporation of mannose.

<table>
<thead>
<tr>
<th>PD Ratio (w/w)</th>
<th>Entrapment Efficiency (%)</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
<th>Mannose (mg/mg polymer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain 5-FU-NPs</td>
<td>59.6 ± 4.2</td>
<td>100.4±10.5</td>
<td>0.208±0.23</td>
<td>-22.98±2.2</td>
<td>--</td>
</tr>
<tr>
<td>MA-5-FU-NPs nanoparticle</td>
<td>57.89 ± 3.20</td>
<td>102.39 ±7.64</td>
<td>0.324±0.16</td>
<td>-27.43±1.65</td>
<td>2. 54 ± 0.01</td>
</tr>
</tbody>
</table>

**Conclusion:**

The study presented here opens new window in the field of targeting of anticancer drugs for NMSC through MMR. The model drug taken here was 5-FU, but these mannosylated nanocargoes could also serve as a carrier for phytoactives too. Through the findings of present research, we purview that the limitations of targeted drug delivery system could be minimizied by using mannosylated nanocargoes for skin cancer.

**Acknowledgement:**

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Delhi, India, and University Grant Commission (UGC) New Delhi, Under MRP Scheme Major Research project, F. No 39-170/2010 (SR), for financial assistance. One of the authors extend their gratitude towards the head of the cosmetic lab, University Institute of Pharmacy, Pt., Ravishankar Shukla University, Raipur (C.G.) for providing facilities to carry out research work.

References:
Abstract: Multiple emulsions have been proposed to have numerous uses including their use for enhancement of bioavailability or as a prolonged drug delivery system. But the inherent instability of this system needs to be overcome before they find potential application in pharmaceuticals. Multiple emulsions are often stabilized using a combination of hydrophilic and hydrophobic surfactants. The ratio of these surfactants is important in achieving stable multiple emulsions. Clotrimazole was selected as a model drug to study the potential of multiple emulsions to improve bioavailability with the hypothesis that improvement of drug release profile will reflect the enhancement of bioavailability of the drug. The objective of this study was to prepare multiple emulsion of clotrimazole by two step emulsification using different nonionic surfactants, Tweens & Spans, and evaluate for stability, percentage drug entrapment, in vitro drug release. The study concluded that stable multiple emulsions with high entrapment efficiency can be prepared by two step emulsification method using Spans40, 60, 80 as primary emulsifier and Tween80 as secondary emulsifier.

Introduction:
Multiple emulsions are complex liquid description systems in which the droplets of the one dispersed liquid are further dispersed in another liquid. The inner dispersed globule/droplet in the multiple emulsions is separated from the outer liquid phase by a layer of another phase. There are mainly two types of multiple emulsions W/O/W and O/W/O emulsions. Clotrimazole is an antifungal medication commonly used in the treatment of fungal infections (of both humans and other animals) such as vaginal yeast infections, oral thrush, and ringworm. It is also used to treat athlete's foot and jock itch [1-4].

Materials and Methods:
Materials: Clotrimazole drug was arranged from Zee laboratory, konta sahib. (H.P) Span 40 purchased from Hymedia laboratories. Span 60, span 80 and Tween 80 from Merch laboratories. Heavy paraffin oil from Rankem.
Method of Preparation: Multiple emulsions were prepared by two step emulsification process:
1) Preparation of primary emulsification; b) Secondary emulsification.
Primary emulsification: 10 ml of distilled water containing 25 mg of drug was gradually added to 14 ml of oil phase containing primary emulsifier (Span40, Span60, and Span 80)
and 25mg of drug with continuous stirring at 5000 rpm for 5 minutes. It gives the primary emulsion.

**Secondary emulsification:**

20 ml of viscous primary emulsion was emulsified further with an external aqueous phase containing secondary\emulsifier (Tween80) and 50 mg drug with continuous stirring at 1000 rpm for 10 min. All the formulations were prepared by following the same procedure. Effect of primary emulsifier was observed by evaluating several formulations.

**Results and Discussion:**

**Preformulation Study:**

The drug preformulation studies were carried out on different parameter like physical appearance, solubility profile, melting point estimation, partition coefficient, calibration graph potting, FTIR, DSC studies to find out that the various physiochemical data collection. Physical Appearance testing of drug sample was performed for determination of Color, Odour, State and Nature that was white, odourless, solid and amorphous respectively. Solubility study of drug was performed in various organic and inorganic solvent. After performing solubility studies was found that drug was freely soluble in ethanol & methanol and insoluble in water. Melting point estimation of drug sample was performed by capillary tube method and result was 139-142°C. Partition coefficient studies are carried out (in n-octanol/Distilled water) to find out extent of drug transfer in the aqueous and the other non-aqueous layer. After study Partition coefficient of drug was 0.57 that give the indication of drug are highly participated in non-aqueous layer. Calibration curve was plotted in best soluble solvent. clotrimazole solution was scanned in the U.V. range of 200-400 nm using Systronic UV Visible spectrophotometer. Regression Coefficient value was found R²=0.993 and Regressed line equation y=0.002x+0.001 Concentration range and calibration graph was show given figure.

Freshly prepared primary emulsion was creamy white in color. There was no change in color at different storage conditions. This shows that primary emulsion was stable at different storage conditions up to 21 and 28 days. Primary emulsion, no phase separation was observed in any of samples. This indicates that primary emulsion was stable at all storage conditions for 28 days. For the multiple emulsions, no phase separation was seen in the samples kept at all storage conditions, except slight phase separation beginning on the 21\textsuperscript{st} day. The in-vitro dissolution studies have shown that F3 formulation has higher release profile as compare to other formulation. As the concentration of span20, 60, & 80 and Tween 20, 60, & 80 increases, the release profile of the formulation was improved. Hence it is concluded that the multiple emulsions are useful for the improvement of dissolution rate and thereby oral bioavailability of poorly water soluble drug clotrimazole.
Conclusion:
The present study was an attempt to formulation and evaluation of multiple emulsion of using clotrimazole. The objective of this work is to development and analysis of multiple emulsion of clotrimazole for oral drug delivery. Clotrimazole is an antifungal medication, works to kill individual candida or fungal cells by altering the permeability of the fungal cell wall. It binds to phospholipids in the cell membrane and inhibits the biosynthesis of ergosterol and other sterols required for cell membrane production. This leads to the cell's death via loss of intracellular elements. Multiple emulsions are usually stable employing a combination of deliquescent and hydrophobic surfactants. The quantitative relation of those surfactants is vital in achieving stable multiple emulsions. The formulated multiple emulsion met the all the pharmacopoeial requirement of different parameter like physical appearance, solubility profile, melting point estimation, partition coefficient, calibration graph potting, FTIR, DSC etc.

References
Formulation and evaluation of dispersible tablet of Piroxicam by using different superdisintegrants

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Abstract: Piroxicam is a potent anti-inflammatory drug. In the present research 6 formulations (F1 to F6) were prepared by using two different superdisintegrants namely cross carmelllose sodium and gelatinized starch by sublimation method. The drug and excipients final blend and were evaluated for the properties like; compressibility index, powder flow properties, bulk density, tapped density, and hausners ratio. Thickness, weight variation, disintegration time, hardness, friability, drug content was evaluated for all the formulations prepared. The study reveals that that the F4 showed the lowest disintegration time. In vitro dissolution studies indicate that formulation F4 prepared by sublimation of camphor shows 99.96% drug at the end of 5 min.

Introduction:
Piroxicam NSAID’S one of most potent anti-inflammatory drug of this class. It is indicated in the treatment of different inflammatory disease like (osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and acute gout). Half-life of Piroxicam is about 45hrs. Piroxicam may cause bioavailability problems due to its poor solubility in water when administered orally and dissolution rates in biological fluids. [1-3] the present work was aim to prepared to increase the dissolution rate of piroxicam using different disintegrants like crosscarmellose sodium and gelatinized starch.

Material and Methods:
Material: Piroxicam were received as gift samples from Torrent Pharmaceuticals Pvt. Ltd, crosscarmellose sodium, gelatinized starch and other chemicals were taken from Sagar Institute of Research Technology & Science - Pharmacy Bhopal.

Preparation of orodispersible tablets by sublimation method: The basic principle involved in preparing orodispsirable tablets by sublimation technique is inert solid solid ingredients camphor were added to other tablet expients and the blend was compressed into tablet. Removal of volatile material by sublimation generated a porous structure. By using different disintegrates 6 formulation were prepared and compressed tablets then subjected to sublimation at 60c for 1 hour in hot air oven.

Table 1 Formulation of Piroxicam ODTs.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>20</td>
</tr>
</tbody>
</table>
### Results:

**Pre formulation Study:** Study of piroxicam was examined for its organoleptic properties and it was observed that Piroxicam was white odor less crystalline powder, hygroscopic. Piroxicam was found soluble in ethanol, 6.8 phosphate buffers, sparingly soluble in water. The melting point of piroxicam was 198°C. The partition coefficient of piroxicam was found 0.4069 which shows it is lipophilic in nature. The peak of the graphs UV, IR reported was resembled like Indian pharmacopeia standard. Hence on the basis of pre formulation study the drug sample of piroxicam was found to be pure and authenticated and the sample can be used for the further the preparation and evolution.

**In-vitro release:** For the release studies were carried out using USP tablet dissolution test apparatus paddle method at 37±0.5 °C, at pH-6.8 taking 900 ml of dissolution medium. The paddle rotation speed was set at 50 rpm. After 30-300 sec interval Aliquots of 5 ml were withdrawn and analyzed spectrophotometrically (λ 336 nm). The Figure 1 shows in-vitro dissolution profile of piroxicam, indicates faster and maximum drug release from formulation F6. The sublimation of camphor from final tablets showed release of 99.96% drug at the end of 5 min for F6 formulation.

![Figure 1 Cumulative release of Piroxicam ODTs.](image)

**Accelerated stability studies:** Twenty tablets of optimized formulation were placed in Petridis. Which was kept in desiccators containing calcium chloride (desiccant) at room temperature for one day? Then the tablets were weighted and placed in humidity chamber, which was maintained at 30°C 25°C 40°C 50°C and 75% RH for one month.

**Conclusion:**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Camphor</td>
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<tr>
<td>Microcrystalline Cellulose</td>
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<tr>
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<tr>
<td>Lactose</td>
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<tr>
<td>Cross Carmellose Sodium</td>
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<td>15</td>
<td>20</td>
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</tr>
<tr>
<td>Gelatinized Starch</td>
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<td>10</td>
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<tr>
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</tr>
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<td>Talc</td>
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</tr>
</tbody>
</table>
Piroxicam dispersible tablets using super-disintegrates which would release the drug rapidly with predetermined rate. Piroxicam facilitates wicking action of super-disintegrates bringing about faster disintegration due to its porous structure; responsible for faster water uptake of drug. The in-vitro dissolution profile indicated faster and maximum drug release from formulation. The final tablets showed release of 99.96% drug at the end of 5 min by sublimation of camphor from prepared formulations.

References:

Formulation and evaluation of sustained release floating-mucoadhesive tablet of Cimetidine Hydrochloride

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Abstract: The purpose of the present study to develop an optimized gastric floating drug delivery system (GFDDS) to enhance the gastric residence time after oral administration, controlling the release of drug especially useful to satisfy control plasma level as well as improving drug bioavailability. The drug is a histamine H2-receptor antagonist which inhibits stomach acid production and used in treatment of peptic ulcer disease (PUD) and gastro esophageal reflux disease (GERD). It is also used alongside fexofenadine and other antihistamines for the treatment of skin conditions such as hives. The proposed work is envisaged to carry out the preformulation, optimization, development of in-situ orifice forming floating tablet of cimetidine and its evaluation. Floating muco adhesive tablet of cimetidine is the one which suit the concept of better patient compliance, delayed release, more efficacies and enough bioavailability to show required pharmacological action and less gastrointestinal side effects. The sustained release floating-muchoadhesive tablet system was successfully developed and evaluated.

Introduction:
The design of oral control drug delivery systems (OCDDS) should be primarily aimed to achieve more predictable and increased bioavailability Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting gastric emptying rate for prolonged period of time [1-3].

Materials and Methods:
Materials: Cimetidine (drug) was provided by Herb Edge Health Care Pvt. Ltd., Ujjain HPMC, Carbopol, Citric acid, Talc, Magnesium stearate, Aerosil-200 and other chemicals were provided by Sagar Institute of Research Technology & Science-Pharmacy Bhopal.
Method of Preparation:
Granulation Techniques
Dry Granulation: The active constituent along with diluents with a part of lubricant is blended, the powdered material contains a considerable amount of air, as this air to expel and a fairly dense piece is formed. As the slugging method have been used, large tablets are made as slugs because fine
powders flow better into large cavities, these compressed slugs are comminuted through the desirable mesh screen either by hand or for large quantities through the mill, after this the granulation is blended gently with lubricants and then compressed to form tablets.

**Wet Granulation:** As this method is used to prepare granules by binding the powders together with an adhesive instead of compaction. This technique employs a solution suspension or slurry containing a binder which is usually added to the powder mixture and the binder may also be incorporated into the dry powder mix and the liquid may be added by itself. After the granulating liquid has been added mixing continues until a uniform dispersion is attained and all the binder has been activated, after sufficient blending, then the wet mass is made to undergo wet screening by passing through a hammer mill or granulator equipped with screens having large perforations.

**Results and Discussion:**

**Pre formulation Study:** The pre formulation study of Cimetidine was performed. In this Cimetidine Hydrochloride was found white to pale yellow crystalline powder, Odour-Odourless, Taste-Bitter. The melting point was found to be 136-142°C. Cimetidine HCL is soluble in water and insoluble in ethanol the pH of 1% aqueous solution of Cimetidine Hydrochloride was found 4.5-6.0.which was reported 5.10 & 7.3. Thus it complies with USP standard limits indicating the purity of sample. The value of log P for the partition coefficient of Cimeitidine Hydrochloride was found to be 8.2. The value of log P indicates highly soluble property of drug in water & alcohol. The result of the calibration curve of Cimetidine Hydrochloride is as follows.

**In vitro release:**

![Figure 1 In-vitro release profile.](image)

**Evaluation of Floating Tablets:** The values of pre-compression parameters evaluated for batches SR-I to SR-VIII was tabulated. And they were within the limits. The value of angle of repose for both batches varies between the range 24°17' to 28°15', and the value of compressibility index varies between the range of 17.60 to 20.95 and 25.32 to31.77 also the value of bulk density, tapped density, hasuner’s ratio of SR-I to SR-VIII varies between the range 0.356 to 0.385, 0.506 to 0.569, and 1.15 to 1.37.

**Characterization:** As we know that the size and shape of the tablet can also influence the choice of tablet as per selection of machine is to be done.
**Tablet thickness**: Tablet thickness is an important characteristic in reproducing appearance and also in counting by using filling equipment.

**Tablet hardness**: Hardness of the tablet of each formulation was determined using Monsato Hardness tester. It was measured in kg/cm².

**Weight variation**: Initially twenty (20) tablets were taken and their weight was determined individually and collectively on a digital weighing balance having sensitivity to the four places after decimal. The average weight of one tablet was determined from the collective weight.

Formulation development & evaluation parameters have been performed in satisfactory data. Title of this study will be done for prolonged the bioavailability of the dosage form.

**Conclusion**:

This study discusses the preparation of floating tablets of Cimetidine. The effervescent-based floating drug delivery was a promising approach to achieve in vitro buoyancy. The addition of gel-forming polymer HPMC K4 M, HPMC K15 M, HPMC K5, HPMC K100, carbopol 934P and gas-generating agent sodium bicarbonate was essential to achieve in vitro buoyancy. Addition of citric acid, to achieve buoyancy under the elevated pH of the stomach, caused an enhancement in drug release. The type of polymer affects the drug release rate and the mechanism. Polymer swelling is crucial in determining the drug release rate and is also important for flotation.

**References**:


Colon targeted liquid filled hard gelatin capsule of Ibuprofen using band sealing technology

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Abstract: The goal of lipoid drug delivery system was to improve poor aqueous solubility, less content uniformity, to overcome poor tableting properties, its high coalescence, and low flow ability of drug Ibuprofen (IBF) to avoid processibility problems. Thus the research work was to develop a self emulsifying liquid formulation compatible in hard capsule shells, filled and finally coated to achieve colon targeting. This innovative approach will improve bioavailability, eliminate the upper gastric track bleeding caused by Ibuprofen and will also avoid the process problems encountered during tableting of large doses of ibuprofen.

Introduction:
Ibuprofen is NSAID widely used in treatment of generalised acute pain, arthritis, headache, fever and gout. Immediate release drug delivery systems of Ibuprofen are largely formulated as compressed tablets. The liquid formulation of Ibuprofen in hard capsule will deliver the solubilised Ibuprofen. Manufacturing of such a delivery system will ease the steps at industrial level. Colon targeted Ibuprofen will be beneficial for patients suffering from rheumatoid arthritis to relive acute early morning pain for chronotherapy. To keep pace with the changing trends, many companies have introduced liquid filling and band sealing technology. This technology enables the use of hard capsules as a parallel dosage form to soft gelatin capsule for filling liquid/semi-solid formulations. This not only makes the encapsulation process easy and cost effective, but also eliminates several manufacturing and stability concerns associated with soft gelatin capsules. It is considered that this technology can make a significant contribution to the development of efficacious pharmaceutical products by providing the flexibility to rapidly develop and test in house formulations when only small quantities of drug substance is available. The process can be scaled-up and also kept in-house in a similar way to the operations of tableting or powder/pellet filling of hard gelatin capsules [1-3].

Material and Methods
Material: Ibuprofen was procured from Flamingo pharmaceutical limited, Mumbai, India. Capmul PG 8, Transcutol P, was obtained from Gateffose Mumbai, India. Tween 80, PG, PEG 400, glycerol were purchased from Loba Chemie, Mumbai. Eudragit S 100 was procured from Evonik Degussa India. Empty hard gelatine capsule shells were obtained as a gift sample from ACG Associated Capsules Pvt. Ltd., All other chemical and reagents used were of analytical grade.
**Method:** Aqueous titration method was used to prepare self-emulsifying liquid formulation of ibuprofen. Pseudo ternary phase diagram were constructed in the presence of drug to contain optimum concentration of oil, surfactant and co-surfactant. SEDDS forms fine o/w emulsion with only gentle agitation upon its introduced into aqueous media. After filling the formulation in hard gelatine capsules finally capsules were coated with polymer Eudragit S 100 and stored in tightly closed container.

**Result and Discussion:**

The preformulation studies included characterization of pure drug, solubility and compatibility studies of pure drug Ibuprofen with different excipients and capsule shell compatibility studies. The formulation was evaluated for liquid fill material for density, viscosity, pH, DSC and studies, XRD. The non coated capsule dosage form was evaluated as per IP. The capsules were found to disintegrate at 4.25 minutes. The drug content was found 100.3%. The formulation was also compared with various available marketed solid oral dosage forms of Drug Ibuprofen. These included softgels (Advil) and tablets (Brufen). They were compared based on dissolution profiles as per official monograph of Drug Ibuprofen tablets in USP 2014 and IP 2014. These capsules were subjected for further studies. Coating trials were taken using the polymer Eudragit S 100. Colon targeting was achieved at 11.75% weight gain. The final dosage form was evaluated according to IP. The developed formulation was also studied for stability as per ICH guidelines. The formulation were tested till 3 months and found stable at room temperature as well as at accelerated conditions.

![Figure 1](image)

**Figure 1** Dissolution profile of coated hard capsule.

**Conclusion:** Band seal technology helps the liquid to encapsulate in hard gelatine capsules to avoid leakage. The capsule coated using Eudragit S 100 were stable throughout process and found to have colon targeted release.
Acknowledgement:

The authors are thankful to ACG Associated Capsules Pvt. Ltd., Mumbai, for providing with all the necessary facilities required to complete the research work.

References:

Formulation and evaluation of submicron emulsion for enhancing the bioavailability of Fluroquinolones

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Abstract: Various types of novel drug delivery systems are now available for the treatment of ocular infection. Like microspheres, nanoparticles, nanoemulsion, collagen Shields, ocsuerts. Ocular infection like-conjunctivitis endothermalitis, bacterial keratitis treated by conventional eye drops, but poor absorption through cornea observed due to precorneal loss factor and bioavailability of drug is very less, to avoid this problem submicron emulsion use to entrap drugs in oil phase of o/w type of emulsion. It has convenience of drop but will localize and maintain drug activity at its site of action. This results improved bioavailability of drugs.

Introduction:
The term submicron emulsions is applied to emulsions that possess a dispersed phase mean droplet diameter under 1 um and also referred to as mini-emulsion, ultrafine emulsions, nano-emulsions [1]. Phase behaviour studies have shown that the size of the droplets is governed by the surfactant phase structure (lamellar) at the inversion point induced by either temperature or composition [2-4]. Main three components of Submicron emulsions are as follows: oil, surfactant and co-surfactant. Submicron emulsion can be given by variety of route- parenteral, topical, ophthalmic, nasal delivery, as a vehicle in cosmetics. As compare to conventional ocular dosage form submicron emulsion gives better retention time in to the eye. Long duration of drug for a particular amount of drug, reduces the dosage frequency, patient compliance.
Materials and Methods:
All chemicals were of analytical grade. Sparfloxacin, Pluronic F-68, Soya lecithin, Soya oil.

Methods [5-6]
Preformulation studies: organoleptic properties, Solubility by equilibrium solubility method, melting point by melting point apparatus, identification of drug done by FT-IR Spectroscopy, pH by pH meter, \( \lambda_{\text{max}} \) by UV-Vis spectrophotometer.

Submicron emulsion preparation: Prepared by mixing of soya oil, drug and soya lecithin with stirring in high shear mixer and water phase constitutes water and poloxamer. Oil phase is added to water phase drop wise with continuous stirring until both the phases mixed completely. This emulsion is subjected to sonication for 10 min.

Table 1 Final composition of Submicron emulsion.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Quantity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sparfloxacin</td>
<td>0.3</td>
</tr>
<tr>
<td>2.</td>
<td>Lecithin</td>
<td>0.125</td>
</tr>
<tr>
<td>3.</td>
<td>Soya oil</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>Poloxamer</td>
<td>0.25</td>
</tr>
<tr>
<td>5.</td>
<td>Water</td>
<td>100</td>
</tr>
</tbody>
</table>

Drug and excipient compatibility studies
FT-IR spectra analysis: The main purpose of compatibility studies was to find any interaction between drug and excipients present and compatibility between drug and excipients.

Levels of investigations: IR spectrum (1) = Sparfloxacin, IR spectrum (2) = Sparfloxacin+ Soya lecithin, IR spectrum (3) = Sparfloxacin+ Poloxamer

Evaluation of Submicron Emulsion
Particle Size: Droplets size and size distribution of emulsion system were determined using Malvern Mastersizer 2000 laser diffraction particle analyzer (Malvern instruments).

pH of the Emulsion: Determined by pH meter and it was found to be 6.2.

Viscosity of submicron emulsion: The viscosity of submicron emulsion of Sparfloxacin was determined by Brook field viscometer. Viscosity of submicron emulsion was found to be 3.2±0.5 cps.

Drug Entrapment Efficiency: The Sparfloxacin loaded emulsions was centrifuged at 18,000xg and at 4°C for 15 min at ultracentrifuge in order to separate free drug from the entrapped drug. After centrifugation the supernatant was collected and was analyzed by UV visible spectrophotometer at 289nm for the free drug or unentrapped drug (A1) concentration to determine the encapsulation efficiency from total amount of drug (A2).

\[
\text{EE}\% = \frac{(A2 - A1/A2) \times 100}{A2}
\]
In-vitro dissolution study: In-vitro release was determined using dialysis tubing. The content of Sparfloxacin from withdrawn sample was measured after dilution using UV visible spectrophotometer.

Stability assessment studies: Stability studies can be done on different parameters such as the drug content, pH and droplet size distribution were monitored over periods of 90 days stored at 4°C and 37°C. The creaming and the phase separation were assessed visually at given time intervals. To evaluate its mechanical and physical resistance, the emulsion was subjected to an accelerated mechanical stress and its globule size distribution was measured before and after shaking at 100 strokes per min over 48 h at room temperature [5]

Result and Discussion

The appearance of Sparfloxacin was visually observed and it was found that it complies with the standard limit. Solubility of drug was found in 0.1 N sodium hydroxide and dilute acetic acid. The melting point of Sparfloxacin was found to be 265°C. The pH of the drug was found to be 5.9 which resembles with standard limits for ocular use. The average particle size was found to be 193.1 nm which is under comfort zone for ocular route. The drug content in the submicron emulsion was found to be 62.4%. Viscosity and pH of final formulation was found to be 3.2 cp and 7.2 respectively which is good indication for ocular route. The data obtained from dissolution studies of submicron which was satisfactory, kinetic study done by zero order release, first order release and Higuchi equation.

Conclusion

From this study it is concluded that the submicron emulsion have increased bioavailability into the eye. In this way submicron emulsion proved to be an effective dosage form for the treatment of ocular diseases and improving the bioavailability of Sparfloxacin as compare to other dosage form.

References

Comparative study on Pioglitazone HCl liquisolid compacts and solid dispersion tablets

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Abstract: In this study, different formulations of pioglitazone HCl liquisolid compacts with Avicel 101, Aerosil and solid dispersions were prepared with PEG4000 & PEG6000. Liquisolid compacts and solid dispersion tablets were evaluated for drug content, friability, hardness, disintegration time, and dissolution rates. The results showed that the liquisolid compacts exhibited higher drug dissolution rates than solid dispersion tablets. This is probably due to an increase in wetting properties and surface area of drug particles available for dissolution. The results of this work suggested that liquisolid technique is a useful technique to enhance the solubility and dissolution rate of poorly water-soluble drugs.

Introduction:
Pioglitazone HCl is a thiazolidinedione antidiabetic drug that has been used in treatment of Diabetes type 2. It reduces the hyperglycemia, hyperinsulinemia and hyper-triglyceridermia [1]. It is a BCS class-II drug; In fact its solubility and dissolution rate are key factors in its bioavailability. There are several methods for enhancing dissolution rate of poorly water-soluble drugs like solid dispersions and liquisolid technology. A liquisolid system refers to formulations formed by conversion of liquid drug, drug suspension or drug solution in non-volatile solvents into dry, non-adherent, free-flowing and compressible powder mixtures by blending the suspension or solution with selected carriers and coating materials. Solid dispersion is defined as the dispersion of one or more active ingredients in an inert hydrophilic carrier or matrix at solid state prepared by the fusion, solvent evaporation or solvent–fusion method [2-5].

Materials and Methods:
Pioglitazone HCl, (Dr. Reddys Lab, Hyd.) Avicel PH 101, Aerosil, Sodium starch Glycolate (Yarrow Chemicals, Mumbai) Propylene glycol, PEG 4000, PEG 6000, Magnesium stearate etc. (Desai chemicals, Vizag)

Methods:
Pioglitazone HCL was dispersed in Propylene glycol then a binary mixture of carrier-coating materials (microcrystalline cellulose or lactose, as the carrier powder and silica as the coating material) was added to the obtained liquid medication under continuous mixing in a mortar. Finally, 5% (w/w) of sodium starch glycolate as the disintegrant and 1% Mg, stearate as lubricant were mixed
with the mixture for a period of 10 mm (as per formulation Table-1). The final mixture was compressed into tablets.

Results and Discussions:

<table>
<thead>
<tr>
<th>Liquisolid system</th>
<th>Conc. of Drug in PG</th>
<th>Powder excipient ratio</th>
<th>Liquid load factor $L_l$</th>
<th>liquid medication</th>
<th>Carrier material (MCC)</th>
<th>Coating material Aerosil</th>
<th>Sod. Starch Glycolate (5%)</th>
<th>Mg. Stearate (1%)</th>
<th>Unit dose weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS-1</td>
<td>15</td>
<td>10</td>
<td>0.307</td>
<td>0.1</td>
<td>0.325</td>
<td>0.032</td>
<td>0.029</td>
<td>0.0058</td>
<td>0.4918</td>
</tr>
<tr>
<td>LS-2</td>
<td>15</td>
<td>15</td>
<td>0.264</td>
<td>0.1</td>
<td>0.378</td>
<td>0.025</td>
<td>0.032</td>
<td>0.0064</td>
<td>0.5414</td>
</tr>
<tr>
<td>LS-3</td>
<td>15</td>
<td>20</td>
<td>0.239</td>
<td>0.1</td>
<td>0.418</td>
<td>0.020</td>
<td>0.034</td>
<td>0.0068</td>
<td>0.5788</td>
</tr>
<tr>
<td>LS-4</td>
<td>30</td>
<td>10</td>
<td>0.297</td>
<td>0.05</td>
<td>0.168</td>
<td>0.016</td>
<td>0.018</td>
<td>0.0036</td>
<td>0.2556</td>
</tr>
<tr>
<td>LS-5</td>
<td>30</td>
<td>15</td>
<td>0.252</td>
<td>0.05</td>
<td>0.198</td>
<td>0.013</td>
<td>0.020</td>
<td>0.0040</td>
<td>0.2850</td>
</tr>
<tr>
<td>LS-6</td>
<td>30</td>
<td>20</td>
<td>0.230</td>
<td>0.05</td>
<td>0.217</td>
<td>0.010</td>
<td>0.022</td>
<td>0.0044</td>
<td>0.3034</td>
</tr>
</tbody>
</table>

Pioglitazone HCl solid dispersions were prepared by solvent evaporation method using PEG4000 & PEG6000 in different ratios (1:1, 1:2 &1:3) (as per formulation Table-2)

<table>
<thead>
<tr>
<th>Solid dispersions</th>
<th>Drug: carrier ratio</th>
<th>Carrier</th>
<th>Avice pH 101</th>
<th>Sod. Starch Glycolate (5%)</th>
<th>Mg. Stearate (1%)</th>
<th>Total weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>1 : 1</td>
<td>PEG4000</td>
<td>168</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>SD2</td>
<td>1 : 2</td>
<td>PEG4000</td>
<td>163</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>SD3</td>
<td>1 : 3</td>
<td>PEG4000</td>
<td>158</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>SD4</td>
<td>1 : 1</td>
<td>PEG6000</td>
<td>167</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>SD5</td>
<td>1 : 2</td>
<td>PEG6000</td>
<td>156</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>SD6</td>
<td>1 : 3</td>
<td>PEG6000</td>
<td>151</td>
<td>10</td>
<td>2</td>
<td>200</td>
</tr>
</tbody>
</table>

All the tablets were evaluated for Post compression parameters and in vitro dissolution studies.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness</th>
<th>Friability (%)</th>
<th>Weight variation</th>
<th>Disintegration time(Sec)</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS1</td>
<td>4</td>
<td>0.14</td>
<td>491.7±0.15</td>
<td>36</td>
<td>98.89±1.4</td>
</tr>
<tr>
<td>LS2</td>
<td>3</td>
<td>0.24</td>
<td>541.9±0.51</td>
<td>40</td>
<td>97.2±1.7</td>
</tr>
<tr>
<td>LS3</td>
<td>3</td>
<td>0.32</td>
<td>578.4±0.4</td>
<td>43</td>
<td>96.12±2.2</td>
</tr>
<tr>
<td>LS4</td>
<td>4</td>
<td>0.42</td>
<td>255.2±0.25</td>
<td>45</td>
<td>97.26±1.9</td>
</tr>
<tr>
<td>LS5</td>
<td>3</td>
<td>0.46</td>
<td>284.3±0.76</td>
<td>48</td>
<td>97.64±1.2</td>
</tr>
<tr>
<td>LS6</td>
<td>4</td>
<td>0.13</td>
<td>303.5±0.5</td>
<td>42</td>
<td>98.54±2.6</td>
</tr>
<tr>
<td>SD1</td>
<td>3</td>
<td>0.09</td>
<td>205±0.25</td>
<td>50</td>
<td>88.8±1.9</td>
</tr>
<tr>
<td>SD2</td>
<td>3</td>
<td>0.06</td>
<td>203±0.15</td>
<td>48</td>
<td>85.6±1.4</td>
</tr>
<tr>
<td>SD3</td>
<td>3</td>
<td>0.1</td>
<td>198±0.19</td>
<td>54</td>
<td>78.8±2.9</td>
</tr>
<tr>
<td>SD4</td>
<td>3.5</td>
<td>0.12</td>
<td>195±0.23</td>
<td>52</td>
<td>86.2±1.9</td>
</tr>
<tr>
<td>SD5</td>
<td>3</td>
<td>0.09</td>
<td>203±0.5</td>
<td>43</td>
<td>83.3±2.1</td>
</tr>
<tr>
<td>SD6</td>
<td>3</td>
<td>0.1</td>
<td>206±0.3</td>
<td>46</td>
<td>77.2±1.6</td>
</tr>
</tbody>
</table>

All the liquisolid compacts exhibited greater drug release. LS1 released 70.12% and 99.9% in 10min and 20min respectively. All the liquisolid compacts released more than 85% within 30 minutes. As the R value increases dissolution rate decreases in both drug concentration. Among the solid dispersion tablets, tablets containing drug and PEG6000 (1:1) exhibited greater dissolution rate.
A

**Figure A, B** Dissolution Profile of Pioglitazone HCl liquisolid compacts and solid dispersion tablet respectively.

The drug release was 31.48% and 48.84% in 10min and 20min respectively. Among the solid dispersion tablets, tablets containing drug and PEG4000 (1:1) exhibited greater dissolution rate. The drug release was 28.58% and 41.89% in 10min and 20min respectively. As the concentration of the polymer increased drug release was decreased. Among the solid dispersion tablets, tablets with PEG4000 exhibited greater dissolution rate. Among the liquisolid compacts and solid dispersion tablets, liquisolid compacts exhibited greater dissolution rate. From all the dissolution profiles and dissolution rate release of the drug, LS1 was selected as the optimized formulation.

**Conclusion:**

Pioglitazone HCl exhibits high permeability through biological membranes, but its absorption after oral administration is limited by its low dissolution rate due to its very low aqueous solubility. The aim of this study was to improve the dissolution rate of Pioglitazone HCl and thereby increase solubility. Hence, liquisolid technique and solid dispersion by solvent evaporation technique were chosen to enhance the dissolution rate of Pioglitazone HCl. The wettability of the compacts by the dissolution media is one of the proposed mechanisms to explain the enhanced dissolution rate of Pioglitazone HCl. Liquisolid formulations showed better dissolution rate than solid dispersion tablets.

**References:**

[5] ACTOS-pioglitazonehydrochloride tablet,
Preparation and evaluation of fast dissolving tablets of Diclofenac Sodium using natural superdisintegrants

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Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam, India
E-mail address: smedapati@yahoo.com

Abstract: The aim of present study is to formulate a tablet disintegrate and dissolve rapidly by using natural super disintegrates like Fenugreek seed mucilage (FSM) and Hibiscus leaf mucilage (HLM). These mucilage were extracted and tested for their disintegrate activity by using Diclofenac Sodium (DS) as model drug. DS is among the most extensively used NSAIDs. The fast dissolving tablets of FSM & HLM at different concentrations were prepared by direct compression and evaluated for all post compression parameters & in vitro disintegration, dissolution studies. These natural disintegrants showed excellent disintegration time, enhance dissolution rate. It was concluded that FSM (6%) & HLM (9%) can be used as super disintegrates.

Introduction:
The main objective of the research is to extract the mucilage of Fenugreek seeds & Hibiscus leaves and use of this mucilage as superdisintegrants [1]. Superdisintegrants swells up to ten folds within 30 seconds when contact with water and break up the tablet quickly. So these are widely used in fast dissolving tablets. A fast dissolving drug delivery system can be defined as a dosage form for oral administration, which when placed in mouth, rapidly disintegrates or dissolves and can be swallowed in the form of liquid [2]. Conventional dosage form is very popular in pharmaceutical industries because of its easy transportation and low manufacturing cost. Fast Dissolving Tablet disintegrates and/or dissolves rapidly in the saliva without the need of water. Some tablets are designed to dissolve in saliva remarkably fast, within a few seconds, and are true fast-dissolving tablets [3, 4]. Diclofenac sodium is a synthetic, non-steroidal anti-inflammatory & analgesic compound [5]. The mechanism responsible for its anti-inflammatory / antipyretic / analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). Diclofenac may also be a unique member of the NSAIDs. It is well absorbed orally and shows 100% bioavailability, more than 99% Protein bound, metabolized and excreted both in urine and biles, and plasma t 1/2 is 1.2-2 h.

Materials and Methods:
Diclofenac sodium (Vera Labs, Vizianagaram) Fenu greek seeds, Hibiscus leaves, (local market) MCC, Sodium saccharine, Mannitol, magnesium stearate and Talc etc. (Desai chemicals, Vizag).

Methods: Extraction of mucilage from Fenugreek seed powder and dried leaves of Hibiscus. Preparation of fast dissolving tablets of FSM & HLM at different concentrations was compressed by
direct compression by using following formulation table. In-vitro dissolution rate study was done by using USP Type II apparatus which was rotated at 75 rpm. Phosphate buffer pH 6.8 (900 ml) was taken as dissolution medium. Temperature of the dissolution medium was maintained at 37±0.5°C. Aliquots of dissolution medium were withdrawn at specific time interval and it was filtered. Absorbance of filtered solution was determined by Spectrophotometer (Elico-UV Double beam spectrophotometer) at 283 nm.

**Results and Discussions:**

**Table 1 Composition of formulations.**

<table>
<thead>
<tr>
<th>Ingredient (mg)</th>
<th>HF1</th>
<th>HF2</th>
<th>HF3</th>
<th>FF1</th>
<th>FF2</th>
<th>FF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>FSM</td>
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<td>10</td>
<td>14</td>
<td>-</td>
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<tr>
<td>HLM</td>
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<td>-</td>
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<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Mannitol</td>
<td>124</td>
<td>118</td>
<td>112</td>
<td>124</td>
<td>118</td>
<td>112</td>
</tr>
<tr>
<td>Peppermint flavor</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Saccharine sodium</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Mg. stearate</td>
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<tr>
<td>Talc</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
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</tr>
</tbody>
</table>

**Table 2. Results of post compression parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HF1</th>
<th>HF2</th>
<th>HF3</th>
<th>FF1</th>
<th>FF2</th>
<th>FF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight variation (%)</td>
<td>2</td>
<td>2.4</td>
<td>2</td>
<td>2.2</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.7</td>
<td>0.64</td>
<td>0.6</td>
<td>0.72</td>
<td>0.7</td>
<td>0.68</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td>4.2</td>
<td>4.2</td>
<td>4.8</td>
<td>4.8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Disintegration time</td>
<td>122±4</td>
<td>108±5</td>
<td>98±4</td>
<td>162±3</td>
<td>146±4</td>
<td>112±4</td>
</tr>
</tbody>
</table>

The post compression parameters like weight variation, friability, hardness, disintegration time were found to be satisfactory. The disintegration time of FDT with HLM & FSM slowly decreased. Among all FDTs HF3 formulation showed less disintegration time and between FDT with HLM showed less disintegration time than FDT with FSM at all respective proportions. This is might be due to the mucilages has gum property apart from disintegration activity.

**Table 3 In vitro dissolution studies.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HF1</th>
<th>HF2</th>
<th>HF3</th>
<th>FF1</th>
<th>FF2</th>
<th>FF3</th>
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<tbody>
<tr>
<td>2</td>
<td>48.6</td>
<td>54.6</td>
<td>62.6</td>
<td>42.2</td>
<td>52.2</td>
<td>59.6</td>
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<tr>
<td>5</td>
<td>57.5</td>
<td>61.9</td>
<td>78.2</td>
<td>53.2</td>
<td>60.6</td>
<td>70.2</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>73.1</td>
<td>88.8</td>
<td>67.5</td>
<td>69.9</td>
<td>81.2</td>
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<tr>
<td>12</td>
<td>82.5</td>
<td>89.9</td>
<td>97.2</td>
<td>78.4</td>
<td>85.5</td>
<td>92.2</td>
</tr>
<tr>
<td>16</td>
<td>92</td>
<td>96.9</td>
<td>99.9</td>
<td>87.6</td>
<td>92</td>
<td>98.8</td>
</tr>
<tr>
<td>20</td>
<td>98.5</td>
<td>99.9</td>
<td>98.2</td>
<td>94.2</td>
<td>98.2</td>
<td>99.9</td>
</tr>
</tbody>
</table>
In *in-vitro* dissolution studies 97.2% of drug released in HF3 formulation at 12th minute and 92.2% of drug released in FF3 formulation at 12th minute. All the formulations with HLM & FSM showed more than 94% of drug released at 20 minutes.

**Conclusion:**

From the present study it may be concluded that fast dissolving tablet of Diclofenac Sodium can be formulated by direct compression method by using natural super disintegrate Hibiscus leave mucilage and Fenugreek seed mucilage. After observation of disintegration time and dissolution studies FDT with HLM showed better disintegrate properties than FDT with FSM. So both Hibiscus leave mucilage and Fenugreek seed mucilage can be used as superdisintegrants. The proposed fast dissolving formulations possess ideal and reproducible characteristics of disintegration time and enhanced dissolution and thus give better patient compliance.

**Acknowledgement:**

The authors gratefully acknowledge Dr. Lavu Rathaiah, chairman of Vignan Group of institutions A. P. and T.S, India for providing the necessary facilities to carry out the research work.

**References:**

Formulation and evaluation of mucoadhesive buccal tablets of Sumatriptan Succinate

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Abstract: Mucoadhesive buccal tablets of sumatriptan succinate were prepared with an objective of enhanced bioavailability using chitosan in combination with sodium alginate and carbopol 934P by direct compression method. The tablets were evaluated for all physical parameters & results were in acceptable range of pharmacopoeial specification. The tablets were studied for surface pH, swelling index, in vitro drug release, ex vivo residence time, mucoadhesion, ex vivo permeation. The surface pH of the tablet was in the range of salivary pH & ex vivo residence time indicated good adhesive capacity of tablet. The buccal tablet showed good swelling up to 7 hr maintaining the integrity of polymers. The in vitro release of sumatriptan succinate was prolonged up to 8 h. The in vitro release obeyed zero order kinetic with mechanism of release was erosion followed by non fickian diffusion. All the tablets showed good mucoadhesive strength of 4.86 to 11.88 gm and the ex vivo permeation revealed that chitosan enhanced the flux and permeability coefficient of sumatriptan succinate.

Introduction:
Bioadhesive drug delivery systems are the delivery systems, which utilized the property of bioadhesion of certain polymers, which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended period of time [1]. The term bioadhesion is used to define the attachment of synthetic or natural macromolecules to a biological substrate. When the substrate is mucosal epithelium, a bioadhesive system adheres; this phenomenon is referred as mucoadhesion [2].

Sumatriptan is a selective serotonin agonist drug with good vasoconstrictor properties. Chemically it is [3-[2(Dimethylamino) ethyl]-1H-indol-5-yl]-N methylmethane sulphonamide hydrogen butanediocate. It is used in the treatment of migraine. Sumatriptan is rapidly but incompletely absorbed following oral administration and undergoes first pass metabolism resulting in a low absolute bioavailability of 14%. The biological half life of sumatriptan succinate is about 2.5 hours. The physicochemical properties of sumatriptan succinate, its suitable half-life (2.5 h) and low molecular weight (295.13) make it suitable candidate for administration by buccal route [3-5].

Materials and Methods:
Sumatriptan succinate was gifted by Sun Pharmaceuticals Industries Ltd, Silvassa. Chitosan was gifted by (Colorcon Asia Pvt. Limited, Verna, India), HPMC K4M was gifted by (Watson Pharma...
Ltd, Thane) and sodium alginate was gifted by (S.D. Fine chemicals, Mumbai). All other materials were of analytical or pharmacopoeial grade and used as received.

**Results and Discussion:**
Before designing various formulations the drug-polymer-excipients compatibility studies were conducted by FTIR spectroscopy and the results are presented in fig.1. The IR study reveals that sumatriptan succinate was in the free form and no drug-polymer and polymer-polymer interactions took place during formulation development. All the prepared mucoadhesive buccal tablets of sumatriptan succinate were evaluated for thickness, hardness, friability, weight variation, uniformity of drug content, surface pH determination and represented in Table.

**Table 1** Evaluation parameters of mucoadhesive buccal tablets of sumatriptan succinate

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Hardness (kg/cm²)</th>
<th>Thickness (mm)</th>
<th>Weight variation (mg)</th>
<th>Friability (%)</th>
<th>Drug content (%)</th>
<th>Surface pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4.8±0.02</td>
<td>2.80±0.00</td>
<td>149.6±0.99</td>
<td>0.79±0.01</td>
<td>100.09±0.56</td>
<td>6.96±0.09</td>
</tr>
<tr>
<td>F2</td>
<td>4.6±0.07</td>
<td>2.83±0.06</td>
<td>148.8±0.99</td>
<td>0.67±0.01</td>
<td>102.73±0.46</td>
<td>7.01±0.17</td>
</tr>
<tr>
<td>F3</td>
<td>4.3±0.05</td>
<td>2.87±0.06</td>
<td>149.8±0.38</td>
<td>0.57±0.01</td>
<td>98.75±0.88</td>
<td>6.95±0.79</td>
</tr>
<tr>
<td>F4</td>
<td>5.7±0.06</td>
<td>2.86±0.06</td>
<td>150.7±0.99</td>
<td>0.55±0.00</td>
<td>99.70±0.34</td>
<td>6.89±0.17</td>
</tr>
<tr>
<td>F5</td>
<td>5.4±0.03</td>
<td>2.87±0.06</td>
<td>149.8±0.38</td>
<td>0.51±0.01</td>
<td>97.95±0.38</td>
<td>6.94±0.12</td>
</tr>
<tr>
<td>F6</td>
<td>5.0±0.02</td>
<td>2.90±0.00</td>
<td>150.1±0.99</td>
<td>0.87±0.03</td>
<td>98.75±0.88</td>
<td>6.98±0.11</td>
</tr>
<tr>
<td>F7</td>
<td>5.9±0.04</td>
<td>2.96±0.02</td>
<td>250.6±0.99</td>
<td>0.52±0.07</td>
<td>99.22±0.29</td>
<td>6.89±0.17</td>
</tr>
<tr>
<td>F8</td>
<td>5.7±0.06</td>
<td>2.97±0.06</td>
<td>251.0±0.40</td>
<td>0.56±0.06</td>
<td>99.68±0.34</td>
<td>7.02±0.11</td>
</tr>
<tr>
<td>F9</td>
<td>5.4±0.03</td>
<td>3.00±0.06</td>
<td>249.8±0.38</td>
<td>0.40±0.04</td>
<td>98.16±0.27</td>
<td>6.88±0.11</td>
</tr>
</tbody>
</table>

**Conclusion**
The results of the present study indicate that mucoadhesive buccal tablets of sumatriptan succinate can be successfully prepared by direct compression method using chitosan, HPMC K4M and sodium alginate as mucoadhesive polymers. Hence, the mucoadhesive buccal tablets of sumatriptan succinate can be prepared with enhanced bioavailability and prolonged therapeutic effect for the better
management of migraine. The study conducted so far reveals a promising result suggesting scope for pharmacodynamic and pharmacokinetic evaluation.

**Table 2** Regression analysis of the *in vitro* release data according to various release kinetic models

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order $r^2$</th>
<th>First order $r^2$</th>
<th>Higuchi $r^2$</th>
<th>Korsmeyer-Peppas $r^2$</th>
<th>Hixon-Crowell $n$</th>
<th>Erosion $r^2$</th>
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</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.996</td>
<td>-0.725</td>
<td>0.998</td>
<td>0.998</td>
<td>0.910</td>
<td>-0.910</td>
</tr>
<tr>
<td>F2</td>
<td>0.982</td>
<td>-0.817</td>
<td>0.995</td>
<td>0.994</td>
<td>0.987</td>
<td>-0.987</td>
</tr>
<tr>
<td>F3</td>
<td>0.995</td>
<td>-0.788</td>
<td>0.998</td>
<td>0.998</td>
<td>0.975</td>
<td>-0.975</td>
</tr>
<tr>
<td>F4</td>
<td>0.996</td>
<td>-0.914</td>
<td>0.992</td>
<td>0.995</td>
<td>0.996</td>
<td>-0.996</td>
</tr>
<tr>
<td>F5</td>
<td>0.996</td>
<td>-0.925</td>
<td>0.990</td>
<td>0.991</td>
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<tr>
<td>F6</td>
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<td>0.998</td>
<td>0.994</td>
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<tr>
<td>F8</td>
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<td>0.995</td>
<td>0.992</td>
<td>0.998</td>
<td>-0.998</td>
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<tr>
<td>F9</td>
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<td>0.937</td>
<td>0.993</td>
<td>0.997</td>
<td>-0.997</td>
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</table>

**References:**


Formulation & evaluation of osmotic controlled porosity tablet of anti-diabetic drug

Jagdale S K, Ratnaparakhi M P, Pare J K
Marathwada Mitra Mandal’s College of Pharmacy, Thergaon (Kalewadi), Pune-33
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Abstract: Extended release controlled porosity osmotic pump formulations of model drug glipizide were developed using osmotic agent, pore former and coating agent. The effect of different formulation variables like level of osmotic agent, effect of pH, effect of agitation on in vitro release were studied. Drug release was found to be affected by the level of osmotic agent. Glipizide release from controlled porosity osmotic pump was directly proportional to the pore former. Drug release from the developed formulations was independent of pH and agitational intensity and was dependent on osmotic pressure of the release media.

Introduction:
With the conventional dosage forms it is difficult to achieve and maintain the concentration of the administered drug within therapeutic range, leading to fluctuations in the plasma drug levels. However, significant stride has been made in the development of drug delivery devices that can precisely control the rate of drug release for an extended period of time. In the recent years, pharmaceutical research has led to the development/invention of several novel controlled drug delivery systems of which oral controlled drug delivery system has received greater attention since it is the most popular route of drug administration. Osmotically controlled oral drug delivery systems utilize osmotic pressure as the energy source for the controlled delivery of drugs. Osmotically controlled drug delivery offers advantages like pH and gastric motility independent drug delivery, delivery of drugs by a zero order. Therefore, it is possible to achieve and sustain a drug plasma concentration within the therapeutic window of drugs, which reduces the side effects and frequency of administration considerably.

Glipizide is one such poorly soluble oral hypoglycemic agent belonging to class 2 of biopharmaceutical classification system and is one of the most commonly prescribed drugs for the treatment of patients with type II diabetes mellitus. It is practically water insoluble, but its absolute bioavailability is close to 1 and its dissolution is considered to be a rate determining step (i.e., an effective factor) in its absorption from the gastrointestinal tract. It also has a relatively short elimination half-life of 2-4 h, there by requiring twice daily dosing in large number of patients, which often leads to non-compliance. Therefore present work is aimed towards development of glipizide osmotic tablet using a wicking agent and osmotic agent [1-4].

Materials and Methods
Materials: Glipizide, Mannitol, Fructose, Lactose, Poly vinyl pyrroial K-30, Cellulose Acetate
Poly ethylene glycol – 400, Potassium chloride

Methods: Tablets were prepared by wet granulation method and coating was carried out by using R and D coater.

Results and Discussion:

Table 1 Composition of formulation.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
<th>F-5</th>
<th>F-6</th>
<th>F-7</th>
<th>F-8</th>
<th>F-9</th>
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<td>10</td>
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<tr>
<td>Dextrose</td>
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<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>25</td>
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<tr>
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<td>50</td>
<td>30</td>
<td>50</td>
<td>-</td>
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<td>PVP K-30</td>
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<td>Mg. Sterate</td>
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<td>2</td>
<td>2</td>
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<td>2</td>
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</tr>
</tbody>
</table>

All the tablets were evaluated for pre compression and post compression evaluation parameters.
The in vitro release profile shows that release from F1, F2, F3, F4, F5 and F6 were highly variable.
The F1, F2 shows higher drug release as compare to F3, F4, F5 and F6. It is due to different osmotic agent and their concentration. The F1 and F2 containing mannitol and fructose having higher osmotic pressure which gives higher drug release. The F3 and F4 containing mannitol and dextrose having low osmotic pressure which gives low drug release as compared to fructose. The F5 and F6 containing mannitol and lactose having lower osmotic pressure which gives lower drug release as compared to fructose and dextrose. So it can be concluded that if the osmotic pressure is higher than the drug release is also higher. The F7 and F9 containing mannitol, fructose, dextrose and mannitol, fructose, lactose respectively. They have higher osmotic pressure which gives higher drug release.
The F8 and F19 containing mannitol, dextrose, lactose and mannitol, fructose, dextrose lactose having low osmotic pressure which gives low drug release.

The effect of pH change method and the effect of agitation rate was also studied. Results of in-vitro drug release kinetics study suggests that, formulations F-1, F-4 to F-9 follows zero order kinetics with $r^2$ value in range of 0.98 to 0.99 and fitting the release data to korsmeyers equation release exponent n ranged from 0.62 to 0.88 this indicated that the nature of drug release from the osmotic tablets followed Anomalous non-Fickian diffusion mechanism ($0.45 < n < 0.89$).

Acknowledgement:
The authors are thankful to Marathwaa Mitra Mandal’s College of Pharmacy, Kalewadi Pune (MH) for providing necessary support to carry out research work.

References:
Colloidal gold nanoparticles enhance antioxidant and antiradical effects of a preferential Cyclooxygenase-2 inhibitor.

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Abstract: Oxidative stress and decreased antioxidant status are the hallmarks in patients suffering from rheumatoid arthritis (RA). Colloidal gold nanoparticles (AuNPs) synthesized using green chemistry approach can enhance the therapeutic efficacy of a preferential cyclooxygenase-2 inhibitor (COX-2) with potential suppressive effects on free-radical mediated damage. AuNPs were successfully characterized for morphometrical parameters. TEM and AFM confirmed the production of AuNPs. Free radical scavenging activity of AuNPs capped with COX-2 inhibitor in all assays was higher than the free drug and was found to increase in a dose dependent manner. GNP based NDDS of COX-2 inhibitors can enhance its therapeutic efficacy in the management of RA.

Introduction:
Defective antioxidant status contributes to the pathology of RA [1]. AuNPs are widely used because of their biocompatibility, simple synthesis, facile surface modification and tunable properties. Moreover, AuNPs are potential antioxidants. AuNPs inhibit the receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast formation, which results in bone and cartilage erosion by quenching reactive oxygen species (ROS) [2]. Since osteoclast and ROS are the main contributors to the pathogenesis of RA, AuNPs have immense potential as novel RA therapeutics. The present study aims to synthesize AUNPs with green chemistry approach as a carrier for COX-2 inhibitor and evaluate its potential antioxidant effects in various antiradical and antioxidant assays in vitro.

Material and Methods:
Drug was a kind gift by Zest Pharma., Indore, Gold (III) chloride trihydrate (HAuCl₃.3H₂O), DPPH, thio barbituric acid, ethylene diamine tetraacetic acid (EDTA) and deoxyribose was purchased from Himedia. Laboratories, Mumbai. Trichloroacetic acid and ascorbic acid were procured from Sisco Laboratories, Mumbai. Riboflavin was purchased from Qualigens Fine Chemicals, Mumbai. Hydroxylamine hydrochloride and naphthyl ethylene diamine dihydro chloride was purchased from Sigma Aldrich, USA. All other chemicals used were of analytical grade.

Methods: AUNPs were synthesized using green chemistry approach. In a typical synthesis, 40 μl of aqueous CS extract was added to 5 x 10⁻³ M solution of Gold (III) chloride trihydrate at room temperature and constant stirring at 800 rpm on a magnetic stirrer (1 MLH, Remi, India). Appearance of light red colour indicated the redox change due to the presence of polyphenolic compounds present
in CS extract. Further drug solution and stabilizer (2.5% v/v) was added to the gold chloride solution. The reaction was stirred for 180 min and kept overnight in dark for complete reduction of gold ions and attachment of drug to the nano gold surface.

The reduction of AuCl₄⁻ ions was monitored by measuring UV-Vis spectra in the range 400-800nm. A 3² full factorial design was adopted to optimize critical formulation and process factors based on their effect on Critical Quality Attributes (CQAs). The CQAs for AuNPs include particle size and encapsulation efficiency. Particle size and zeta potential measurements in solution were determined with Nano particle Tracking Analysis using Nano sight NS500 (Malvern, UK).

The surface morphology was investigated by TEM and AFM. The structural characterization and crystalline nature of AuNPs was studied by X-Ray diffraction studies.

The *in vitro* antioxidant and antiradical assays were conducted to evaluate the antioxidant status of drug and drug capped AuNPs according to previously reported methods [3].

**Results and Discussion:**

Drug capped AuNPs were successfully developed using 3² factorial designs. The particle size and zeta potential of the optimized batch was 43nm and -21.8mV respectively. The EE was found to be 92.08%. UV-Vis spectra showed distinct surface plasmon resonance of drug capped nano particles, confirming AuNPs formation in the nano dimension. The synthesized particles were largely spherical in shape with a smooth morphology. Selected area electron diffraction (SAED) pattern revealed diffraction patterns of crystalline gold structure. Drug capped AuNPs showed free radical scavenging potential in all the *in vitro* models studies. It was observed that free radicals were scavenged by drug capped AuNPs in a concentrate ion dependent manner up to the given concentration (50-150µg/ml) in all the models. On a comparative basis, drug capped AuNPs showed superior antioxidant activity as pure drug.
Figure 1 TEM image of drug capped AuNPs

Conclusion:
AuNPs synthesized using green chemistry approach ameliorates the antioxidant status of the COX-2 inhibitor.

Acknowledgement:
We would like to thank Zest Pharma for the gift sample of the drug. We also acknowledge Croda, India for the gift sample of the stabilizer.

References:
Development and characterization of COX-2 inhibitor encapsulated niosomes for enhanced transdermal delivery

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Abstract: The aim of this current research is to study a novel carrier, niosome, for its enhanced transdermal delivery of COX-2 inhibitor. Niosomes are self-assembled vesicles composed primarily of synthetic surfactants; cholesterol & edge activator prepared by Organic solvent injection technique and characterized by particle size, entrapment efficiency, transmission electron microscopy (TEM), in-vitro dissolution and in-vivo study. Vesicle size, Zeta potential, and % EE of the optimized niosomal formulation was found to be 79.46 ± 55 nm, -39.6 ± 7.43 mv and 88.8 ± 4.5% respectively. Experimental work concludes niosomal formulation is safe and effective carrier for TDDS.

Introduction:
Recent researchers found that vesicular structures [1] such as liposomes, niosomes, transferosomes, etc acting as best carriers for the administration of drugs across the skin. Liposomes exhibit certain disadvantages as, their components such as phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and the purity of natural phospholipids is variable. Apart from these disadvantages, researchers proved that the liposomes are incapable of penetrating into the deeper layers of the skin [2]. Transferosomes also have different disadvantages like expensive, tedious manufacturing processes and chemical instability [3]. Niosomes are globular submicroscopic structures and are prepared using nonionic surfactants such as Tweens, Spans etc. [4]. The present study aims to develop drug loaded niosome and its evaluation for enhanced transdermal drug delivery.

Materials and Methods:
Materials: Commercial grade COX-2 inhibitor was a gift sample obtained from Ramdev chemicals, Mumbai. Span-60, Cholesterol, Sodium deoxycholate (SDC) purchased from S.D Fine Chemicals, India. Wistar rats were obtained from Bharat Serum and Vaccines Pvt. Ltd. (Mumbai, India).

Methods:
Formulation of niosomes: The surfactants, lipid and drug were first dissolved in a suitable organic solvent. The prepared organic phase was then added drop wise into the aqueous phase. Surfactant: cholesterol ratio of 1:1 along with drug and Sodium deoxycholate were dissolved in chloroform:
methanol in the ratio of 2:1. Thus the dissolved organic solution containing drug were injected drop wise through 24 gauge needle into preheated PBS PH 7.4, which was magnetically stirred (Mechanical stirrer, Remi., Mumbai) and maintained at 65 °C. Stirring was continued until all chloroform & methanol evaporated to get drug loaded niosome. Vaporization of chloroform & methanol leads to the formation of single layered vesicles [5], these were further size reduced by ultrasound cavitations using probe sonicator (Oscar, Japan) to form small unilamellar vesicles. Shape and morphology of the Niosomes was investigated using transmission electron microscopy. Entrapment efficiency of Niosomes was determined by ultracentrifugation method. The vesicle size and distribution were determined by dynamic light scattering method using Malvern zetasizer (Malvern Instruments Ltd.). Measurements were carried out at an angle of 90° at 25°C. The in vitro release of tenoxicam from the niosomes was examined under sink conditions. One Milliliter samples of niosomes were placed in dialysis bags and suspended in 1000 ml of phosphate buffer saline PBS, pH 7.4 at 37 °C under gentle magnetic stirring at 100 rpm. The anti-inflammatory activity of the gel was carried out by carrageenan induced paw edema method. Ex-vivo skin permeation study was done with porcine ear skin using the niosomal suspension and niosomal gel.

**Results and Discussion:**

The niosomes were spherical in shape. In the present study the observed percentage entrapment efficiency for all batches were in the range of 77%-91%. The mean vesicle size and zeta potential of drug loaded niosomes of the different batches ranged between 79-190 nm and -39.6 ± 7.43 mv respectively. The polydispersivity index (PDI) was in the range of 0.207 – 0.341 for drug loaded niosomes. Release profile of plain COX-2 inhibitor was a mere 20% as compared to ~74% of drug loaded niosomes which results in sustained release effects. *In-vivo* studies showed better anti-inflammatory activity as compared to the marketed formulation. The *ex-vivo* study showed transdermal flux of niosomal gel to be 43.09± 0.106µg/cm²/h which was 2.9 fold higher than that obtained after application of plain drug gel whose flux was 15.05±0.09 µg/cm²/h).
Conclusion:
The COX-2 Inhibitor encapsulated niosomes enhances the transdermal skin permeability due to its better penetration as compared to hydroethanolic drug solution and plain drug solution.

Acknowledgement: We would like to thank Ramdev chemicals, Mumbai for the gift sample of the drug.

References:
Formulation development and \textit{ex-vivo} evaluation of polymeric (PLGA) nanoparticles of \textit{Ketoconazole} enriched gel for the treatment of \textit{Candida albicans} skin infection.

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Abstract: In present study ketoconazole loaded PLGA [Poly (lactic-co-glycolic acid)] nanoparticles were prepared for topical delivery. The nanoparticles were optimized using $3^2$ full factorial designs to evaluate the effects of process and preparation variables. Nanoparticles were prepared by the nanoprecipitation method and characterized for particles size and entrapment efficiency. The nanoparticles prepared as per design, possessed entrapment efficiency in the range of 58-84 \%. Particle size and morphology analysis revealed that the nanoparticles were found in the size range of 122-435 nm in average diameter and exhibited good sphericity. Zeta potential analysis showed value of -18mV. The nano suspension was suitably gelled and characterized. The efficacy of the nanoparticles loaded gel was confirmed using \textit{ex-vivo} drug performance.

Introduction:  
The topical drug application is less prone to severe systemic side-effects than systemic application. Starting with the liposomes, various types of nanosized and micro-sized drug carriers have been developed to increase the notoriously low penetration of active agents into the skin, which limits not only the topical therapy of skin disease but also transdermal therapy [1] Nano particles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure. These properties facilitate the administration of antifungal drugs, thereby overcoming some of the limitations in traditional antifungal therapeutics. In recent years, encapsulation of antifungal drugs in nano particle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the antifungal drugs [2] Nano particulate drug delivery is multi particulate drug delivery and used for improved bioavailability and target to specific sites. Biodegradable polymeric nanoparticles are of interest for extended drug delivery system and drug targeting [3] Some of the most widely used polymers in nanoparticles formulations are poly (lactic acid), poly (glycolic acid), poly (lactide-co-glycolide) (PLGA) which having a good biodegradable biocompatibility and resorbability through natural pathways. In oral and parenteral application biodegradable polymeric nanoparticles based on PLGA have shown a better advantage over liposome by increasing their stability, but using PLGA in dermal application their potential appears to be unexplored [4]
Materials and Methods:
Ketoconazole was obtained as a gift sample from Ajanta Pharma (Mumbai, India), PLGA (50:50) from gift sample from Evonik Degussa (Mumbai, India), Poloxamer 407 from Ajanta Pharma. (Mumbai, India), Carbopol 940 Oxford Laboratories (Mumbai, India). All solvent used were analytical grade. Nanoprecipitation technique is used for preparation of nanoparticles.

Results and Discussion:
The results of a $3^2$ full factorial design revealed that the concentration of PLGA ($X_1$) and Poloxamer 407 ($X_2$) significantly affected the dependent variables such as, entrapment efficiency and particle size of nanoparticles. The polynomial equation based optimization model was generated and validated. The accuracy of the model was established on the basis of magnitude of errors and $R^2$ values. The nanoparticles of the optimum batch (KPN5) exhibited mean particle size of 122 nm and 84% entrapment efficiency. An appropriate balancing between the levels of the polymer (PLGA) and Poloxamer 407 was imperative to acquire maximum drug entrapment efficiency and adequate particle size. Hence it could be established that among the prepared formulations, KPN5 was the optimum formulation. In vitro data obtained for PLGA-ted nano particles of ketoconazole showed excellent particle size, excellent drug entrapment good buoyancy and drug release. All the nine batches of drug loaded PLGA nanoparticles were converted in to hydrogel by using optimized Carbopol 940 (0.6%) as a gelling agent. The skin diffusion and skin deposition study (Franz diffusion cell Orchid) of ketoconazole loaded PLGA nano particles hydrogel was carried out using human cadaver skin membrane. The formulation G5 shows highest skin deposition (92.20%), showing that high concentration of drug is available at the site of action.

In vitro drug performance of ketoconazole PLGA nanoparticles loaded gel was compared with marketed gel. It was observed that drug loaded G5 hydrogel exhibited greater zone of inhibition of Candida albicans (13mm), as compared to the pure drug (10mm), it can be concluded that formulation G5 has greater efficacy in the treatment of candidacies. Stability data of optimized batches of ketoconazole loaded PLGA nanoparticles revealed that there were no changes observed in the appearance, drug content which showed that the ketoconazole loaded PLGA nanoparticles gel formulation is stable at 35ºC/65% RH. Overall, topical drug delivery system for ketoconazole has been successfully developed.

Conclusion:
From the present study it can be concluded that preparation of PLGA nanoparticles using nanoprecipitation method proved to be a sound approach to obtain stable PLGA nanoparticles of ketoconazole. Components such as concentration of polymer and surfactant concentration have a profound effect in terms of particle size and entrapment efficiency. Optimized PLGA nanoparticles
based Carbopol 940 gel formulation had the desired properties with respect to their pH, viscosity, drug content, and spreadability. *In-vitro* skin permeation, skin deposition indicated that the effect of drug was prolonged by prepared optimized PLGA nanoparticles. PLGA nanoparticles proved the potential for topical delivery of antifungal drugs over the conventional formulations.

**References:**


Characterization and *in-vitro* cytotoxicity activity of pegylated liposomes encapsulating a COX-2 inhibitor optimized using quality by design approach

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**Abstract:** The aim of the study was to formulate and characterize conventional and PEGylated liposomes of a preferential COX-2 inhibitor. The formulation was prepared by adhering to the Quality by Design approach and was optimized for parameters such as particle size and entrapment efficiency. The PEGylated liposomes had high percent entrapment efficiency in the range of 72.8%±0.73 to 91.85%±0.44 and a particle size range of 94nm±61nm to 171nm±73nm. The optimized formulation was characterized for parameters such as, particle size, percent entrapment efficiency, zeta potential, *in vitro* release and *In vitro* cytotoxic activity. The MPL liposomal formulations were found to be unilamellar using small angle neutron scattering analysis (SANS). SANS and the transmission electron microscopy revealed the spherical shape of the liposomes. The drug entrapment was confirmed by Fourier transform infrared spectroscopy and powder X-Ray diffraction studies. The cytotoxicity assay carried out in HT-29 cell lines showed that the PEGylated liposomal formulations had higher cytotoxicity than the conventional liposomes after 48 hrs of incubation.

**Introduction:**

The advancement in the field of molecular biology has been burgeoning, leading to the discovery of newer molecular targets for colorectal cancer (CRC). A target that is gaining a lot of importance in this newly emerging field is cyclooxygenase-2 (COX-2). A non-steroidal anti-inflammatory preferential COX-2 inhibitor drug MX, has demonstrated inhibitory actions against COX-2 and antitumor effects in several human tumor cell lines. Chronic use of MLX has its side effects such as, ulceration in stomach, cardiovascular thrombotic events, and acute renal failure. Thus a vehicle for the safe delivery of MX is necessary. Liposomes are self-assembling phospholipid bilayer structures which can encapsulate both hydrophilic as well as hydrophobic drugs. PEG enhances the association of the liposomes with the cancer cell. This leads to better homing of the liposomes inside the cancer cells [1].

**Materials and Methods:**

MX was obtained as a gift sample from Ramdev Chemicals (Mumbai). Phospholipids HSPC and PEG anchored phospholipids MPEG 2000 DSPE were obtained as a gift sample from Lipoid GmbH.
(Ludwigshafen, Germany). Cholesterol was obtained as gift sample from Sun Pharmaceuticals (Gujarat). All the other chemicals and solvents were of analytical grade.

**Method:** The conventional liposomes (CLs) were prepared using the Bangham thin film hydration technique with slight modifications. To fabricate PEGylated liposomes (MPLs), MPEG 2000 DSPE was added as the additional lipid along with the other lipids, and the same procedure was followed.

**Results and Discussion:**

Plackett-Burman design for screening and Box-Behnken design for optimization: The factors showing high risk were screened using Plackett-Burman design. Box-Behnken design was used to optimize the formulation based on the data obtained from the Plackett Burman design [2].

Particle size and Zetapotential: The PS fell between the range 94nm±61nm to 171nm±73nm. The PEGylated liposomes had a zeta potential of -35mV whereas the conventional liposomes had a zeta potential of -41mV.

Entrapment efficiency: The entrapment efficiency of all the formulations was in between 72.8%±0.73 to 91.85%±0.44.

Transmission Electron Microscopy (TEM): The formation of the nanocarrier i.e. liposomes and the morphology of the same was confirmed by TEM. The PS of the liposomes was in agreement with the PS analysis data of being less than 200nm.

Fourier Transform Infrared (FT-IR): The prominent peak of MX, 3292cm⁻¹ is not seen in the IR spectra of MPL. This is probably due to the entrapment of MX in the lipids.

Powder X-Ray Diffraction (PXRD): It can be observed that, the sharp diffraction peaks of MLX could not be detected in diffractogram of MPL which indicate that MX was entrapped within the lipid based vesicles and stabilized in amorphous form.

Small-Angle Neutron Scattering (SANS) study: The SANS analysis proves that both the blank liposomes and drug loaded MPLs are unilamellar and that they are spherical in shape.

**In vitro drug release**

![In-vitro release graph of MPL, CL and free MX.](image-url)
In-vitro cytotoxicity

![In vitro cytotoxicity of free MX, MPL, CL and PL on HT-29 cell line](image)

**Figure 2** *In vitro* cytotoxicity of free MX, MPL, CL and PL on HT-29 cell line

**Conclusion**

The most significant finding of surface modification carried out in this study is that PEGylated liposomes with high content of MX could be successfully developed by using the QbD principles. The cytotoxicity of the PEGylated liposomes was much higher as compared to free MLX as well as conventional liposomes. This was essentially due to the presence of PEG corona which enhanced the cellular association.

**References**


Design and evaluation of solid dispersion of poorly water soluble drugs: Application of novel mixed solvency concept.

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Abstract: The increased solubility and dissolution rate of poorly water soluble drug facilitated by mixed solvency is presented. Drug and developed solid dispersion were characterized by FTIR, DSC, PXRD and SEM. Desired solubility of drug achieved in a developed solid dispersion ASD3 (more than 80 fold) as compared to the solubility in distilled water. Analytical results may support intermolecular hydrogen or electrostatic bonding between drug and mixed solvent system. No significant changes in crystal form were observed by PXRD. In vitro dissolution rate of aceclofenac from solid dispersion was significantly higher. Mixed solvency concept may reduce the individual concentration of solubilizers and so reduce their toxicity associated with them.

Introduction:
Aqueous solubility of poor water soluble drug is an important factor that influences their dissolution rate and bioavailability after oral administration. Highly water-soluble carrier promotes rapid influx of hydration media into the solid dispersion matrix, as result there is increase in wetting effect. Solubilizers like urea, Sodium citrate, niconinamide, sugar derivetives, Maheshwari [1-2] Ghanemet al. [3] Dabbagh and Taghipour [4] takes advantages of this effect to prepare solid dispersion. In present investigation solid dispersion was prepared by mixed solvency concept using water as solvent system, this technique preclude the use of organic solvent like dimethylsulfoxide, acetone, methanol, ethanol, chloroform, dichloromethane and hence this approach is free from concerns of residual solvent toxicity and pollution.

Materials and Methods:
Aceclofenac (ACF) was obtained as gift sample from IPCA Laboratories, Ratlam, India. Mannitol (MAN) and D-glucosamine (DGA) were purchased from CDH Labs, Delhi. Urea (UR) and Sodium citrate (SC) were obtained from Merck Chemicals Limited, Mumbai, India. All other chemicals and solvents used were of analytical/HPLC grade.

Preparation of solid dispersion: Solid dispersions of drug with mixed solid solvent like UR, DGA, MAN and SC at their optimum combinations ratio (fixed concentration of 40%) at 1:8, 1:10 and 1:12 (multi component system) were prepared by the freeze drying method. Accurately weighed mixed solid solvent were taken in beaker and dissolve in minimum quantity of warm distilled water using
high-speed magnetic stirrer. Once the solubilizers get dissolved, add weighed amount of drug in that mixed solvent. After complete dissolution of drug, the mixed solvent blend was transferred to glass vial and lyophilized in a freeze dryer at a temperature of −70ºC and vacuum of 100 mTorr. The freeze dried mass was then sifted through 60 mesh sieve and stored in air-tight containers

**Preparation of physical mixture:** For comparative studies, the physical mixtures prepared were having the same composition of the solid dispersions; however, they were prepared by simple trituration in porcelain mortar.

**Evaluation of developed solid dispersion:** Drug content: Solid dispersions equivalent to 10 mg of ACF were weighed accurately and dissolved in 100 ml of distilled water. The stock solutions were diluted and analyzed by UV-Visible Spectrophotometry at 274 nm.

**Determination of saturated solubility:** Solubility of as received bulk drug, physical mixture and developed solid dispersion were determined using shake flask method.

**FTIR spectroscopy:** FTIR spectra were obtained by means of a FTIR spectrophotometer (IR Prestige 21, Shimadzu, Japan). Then measurements were attempted over the range of 400–4000 cm\(^{-1}\) at resolution of 1 cm.

**Differential scanning calorimetry analysis:** Thermal characteristics of the pure materials, PM and SD were determined by a differential scanning calorimeter (Jade DSC, Perkin Elmer, Japan).

**Scanning electron microscopy (SEM):** The surface morphologies of the drug, PM and SD were carried out using a scanning electron microscope.

**Powder X-ray diffraction analysis (PXRD):** PXRD measurement for identification of crystalline phase of drug, PM and SDs were carried out using Bruker X-ray diffractometer (Bruker, Germany) over an angular range of 0–40º 2θ.

**In vitro dissolution studies:** In vitro dissolution study of bulk drug sample, physical mixture and solid dispersion were performed in a paddle type dissolution rate test apparatus (USP Type II). Calculations for amounts of drugs released were done using respective regression equations in distilled water (linear in the range of 4-20 μg/mL, \(R^2 = 0.999\)).

**Results and Discussion:**

Drug content for all SSDs were in the range of 98.74–100.09%. The maximum solubility was observed in ASD3 with enhancement ratio of more than 80. The solubility of ACF was greater in solid dispersions than in physical mixtures. No significant difference in the FTIR spectra of pure drug, physical mixture, and solid dispersion were observed. All major peaks of ACF were observed at 3317.56 cm\(^{-1}\), 1770.65, 1716.65 cm\(^{-1}\). Same peaks were retained in physical mixtures and solid dispersion. DSC curves obtained for pure ACF exhibited endothermic peaks at 153.07ºC corresponding to its melting points. The physical mixtures and solid dispersions which exhibited two endothermic peak somewhat broader and displayed a shifting of peaks towards lower temperature.
than that of the single component and this indicates mutual dissolution of carriers and absence of sharp melting peak of ACF in physical mixture and solid dispersion indicates its complete homogeneous dispersion within the mixed solvent. SEM micrographs of as received ACF revealed crystalline nature of drug, whereas the characteristic ACF crystals adhered to carrier’s surface, were clearly detectable in all PM. Solid dispersions appeared in the form of irregular matrix particles/flacks in which the original morphology of drug disappeared and tiny matrix particle of irregular size were present. The results could be attributed to complete molecular dispersion of drug in hydrophilic solid solvent. Therefore, the reduced particle size, increased surface area, and the close contact between the hydrophilic carriers and ACF might be responsible for increased solubility and dissolution. The XRD pattern of prepared solid dispersions exhibited a decreased in intensity of peaks but increased in number of peaks compared to the plain ACF indicating that the formulation remains crystalline in its solid dispersion form due the crystalline nature of solubilizers used. Solid dispersion formulations ASD3 showed higher rates of dissolution than pure drug and corresponding physical mixtures. The pure drug showed up to 54% dissolution over 60 minutes, but its solid dispersions prepared (1:12 w/w) showed dissolution of greater than 76 % over 10 min. SDs formulations showed better results of about 94-98% within 60 min of dissolution study. The dissolution rate of ACF from physical mixtures (1:12) with all carriers was up to 89% higher than that of pure ACF (54%) within 60 min.

**Conclusion:**

The mixture of different solid solvents may be more effective in solubilizing an aromatic molecule than a single agent at similar total concentrations. Mixed solvency concept may reduce the individual concentration of solubilizers and so reduce their toxicity associated with them. The synergistic enhancement in solubility and dissolution of ACF is clear indication of its fruitful approach in future for other poorly water soluble drugs in which low bioavailability is major concern.

**Acknowledgement**

The authors would like to acknowledge Ipca Laboratories, Ratlam, India for providing gift sample of aceclofenac and also gratefully acknowledge the School of pharmacy DAVV, Indore for providing necessary facilities.

**References**

Formulation, development and evaluation of microballoons of Ranitidine HCL

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Abstract: The present work ranitidine loaded microballoons of a drug management of GERD to formulate sustained release gastro retentive microballoons of ranitidine HCL with improving its bioavailability. Ranitidine is a histamine H$_2$-receptor antagonist that inhibits stomach acid production. It is commonly used in treatment of peptic ulcer disease and gastro esophageal reflux disease. These subunits are filled into a capsule or compressed into a tablet these gaining interest rapidly in the pharmaceutical industry due to their many advantages the most important being improved patient compliance in pediatric and geriatric population because of their easy of administration.

Introduction:
Ranitidine is a histamine H2-receptor antagonist that inhibits stomach acid production. It is commonly used in treatment of peptic ulcer disease and gastro esophageal reflux disease. Ranitidine is a competitive, reversible inhibitor of the action of histamine at the histamine H$_2$-receptors found in gastric parietal cells. This result in decreased gastric acid secretion and gastric volume, and reduced hydrogen ion concentration. The objective of the work was to design multiple unit dosage form as microballoons of a drug management of hyperacidity. Microballoons offers numerous advantages for releasing one of the drugs or part of the same drug immediately while remaining drug or parts of the same can be sustained release. These are useful where drug-excipients and drug-drug interactions are predictable with single type dosage form [1-3].

Materials and Methods:

Chemical and working equipment used: All chemicals and solvents provided by SIRTS-Pharmacy Bhopal were of AR-grade purity. Ranitidine HCl was obtained as gift sample from Torrent Pharmaceutical Private limited, Ahamadabad. All reactions are carried out at laboratory Condition. Melting points were determined with capillary MP Apparatus; FT-IR spectra were recorded on a Bruker Germany. UV - spectrophotometer were recorded on systronic, Mechanical stirrer, melting point apparatus and separating funnel were recorded on remi India private limited Mumbai.

Preformulation studies: Preformulation studies for the selected drug Ranitidine HCL include test for identification (examination of melting point determination, IR spectroscopy, and determination of absorption maxima), solubility studies and determination of partition coefficient.

Drug Identification test: Melting point determination: A small quantity of powder was placed into a fusion tube. The tube was placed in the melting point determining apparatus. The
temperature of the apparatus was gradually increased and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

**IR Spectra of ranitidine**: Infrared spectrum of any compound given information about the functional group present in particular compound. An Infrared spectrum of drug was taken using KBr pellet method. Various peaks in IR spectrum were interpreted for presence of different group in the structure of drug. The IR spectrum was recorded on Shimadzu 8300 FTIR spectrophotometer Japan.

**Solubility study**: The quantitative solubility of drug was determined and it was found that drug freely soluble in ethanol and methanol, soluble in water and slightly soluble in chloroform. And this result indicated that the drug is insoluble in ether.

**Partition coefficient**: For the determination of partition coefficient 25 μg/ml solution of pure drug in n-octanol was prepared. Then the mixture of n-octanol and water were mixed in ratio of 1:1. Then this mixture was mixed properly for 30 minutes. Further the mixture was allowed to stand for one hour. After this the mixture was centrifuged at 5000 rpm at 25°C. Mixture was than separated and the absorbance of individual phases of water and octanol were measured by ultraviolet-spectroscopy.

**Procedure**: Inner phase, Eudragit RS 100 was dissolved in 3 mL of methanol and triethylcitrate (TEC) was added at an amount of 20% of the polymer in order to facilitate the plasticity. The drug was then added to the solution and dissolved under ultrasonication at 35°C. And the outer phase PVA dissolved in 200 ml of water in a separate container. The inner phase was poured into the PVA solution in 200 mL of water (outer phase). The resultant mixture was stirred for 60 min, and filtered to separate the microballoons. The microballoons were washed with distilled water and dried at 40°C.

**Results and Discussion**: 

**Preparation of calibration curve**: The absorbance value of standard concentration of 5-35 μg/mL were plotted (table 1 & figure 1) and linearity was observed with an r² = 0.9979 for ranitidine HCl at 290 nm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.114</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.210</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.318</td>
</tr>
</tbody>
</table>

**Standard Calibration Curve of Ranitidine HCl**
Figure 1 Standard curve of drug in methanol

Conclusion: Suspension polymerization reaction conditions used conventionally to prepare microballoons were observed to be compatible with ranitidine. The entrapment efficiency and the drug release profile depend on cross-linking densities of microballoons. Quasi-emulsion solvent diffusion is now a days the preferred method to prepare porous micro particles. Eudragit RS100 microballoons containing ranitidine were successfully prepared by this method as the drug were found incompatible with reaction conditions of liquid-liquid suspension polymerization.

References
Design of orally disintegrating polymer based system for treatment of Xerostomia

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Abstract: Xerostomia is a condition very common in patients due to lack of formation of saliva and can have negative effects on dental health. Its treatment depends on the cause and the degree of damage to the salivary glands. There are medicated liquid, spray and gel formulations available in the market for the treatment. The present study aims at designing orally disintegrating polymer based system and study the influence of superdisintegrants on wetting time and disintegration time in vitro and in vivo, for improved patient compliance and convenience. Use of novel excipients helped in optimizing the formulations with enhanced palatability.

Introduction:
Xerostomia, a condition wherein the functioning of salivary gland is diminished resulting in dry state of mouth. Many medications marketed focus on temporary hydration or enhancing secretion of Saliva. Thus our study is based on using a long term acting polymer as a novel approach to treat the symptom and relieve the patient from ailment for prolonged period of time [1-3].

Materials and Methods:
Polymer X, Sodium Croscarmellose, Novel Diluent, Sweetener, Flavorant, Lubricant

Method: Following were the variables for selection of hydrating polymers: Viscosity at different concentrations, Moisture content, Water holding capacity, Water retention capacity. Uniform blend of all the powder mixture was compressed into tablet of 12 mm size and 500 mg weight as orally disintegrating tablets with hardness 2 – 2.5 kg/sq.cm. The tablets were evaluated for standard tablet parameters such as appearance, hardness, weight variation, friability along with specific tests for orally disintegrating tablets viz., disintegration time, dispersion time, wetting time, water absorption time.

Results and Discussion:
Polymer X gave satisfactory results for viscosity at a wide range of concentrations, moisture content, water holding capacity and water retention capacity, amongst the other screened polymers. Croscarmellose sodium proved better than the other superdisintegrants. Optimization of formulations was done with respect to dispersion of the system in the oral cavity to achieve the desired organoleptic property.
Table 1 Evaluation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White, Smooth surface</td>
</tr>
<tr>
<td>Hardness</td>
<td>2-2.5 kg/cm²</td>
</tr>
<tr>
<td>Friability</td>
<td>0.7%</td>
</tr>
<tr>
<td>In vitro dispersion time</td>
<td>60 secs</td>
</tr>
<tr>
<td>Disintegration time:</td>
<td></td>
</tr>
<tr>
<td>a. in vitro</td>
<td>a. 50-60 secs</td>
</tr>
<tr>
<td>b. in vivo</td>
<td>b. 40-55 secs</td>
</tr>
<tr>
<td>Friability</td>
<td>0.7%</td>
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<tr>
<td>In vitro dispersion time</td>
<td>60 secs</td>
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<tr>
<td>Disintegration time:</td>
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<tr>
<td>a. in vitro</td>
<td>a. 50-60 secs</td>
</tr>
<tr>
<td>b. in vivo</td>
<td>b. 40-55 secs</td>
</tr>
<tr>
<td>Friability</td>
<td>0.7%</td>
</tr>
<tr>
<td>Wetting time</td>
<td>100-125 secs</td>
</tr>
<tr>
<td>Water absorption ratio</td>
<td>105.45</td>
</tr>
<tr>
<td>Taste</td>
<td>Sweet and Acidulating</td>
</tr>
<tr>
<td>Palatability and acceptability</td>
<td>Palatable and well accepted by the volunteers</td>
</tr>
</tbody>
</table>

Figure 1 In vitro Dispersion Test: 50-60secs.

Conclusion:
Concentration of Polymer X showed desired effect in presence of higher concentration of superdisintegrates resulting in rapid disintegration of the tablets. Optimized formulations showed better mouth feel due to flavorant and sweetener making palatable orodispersible tablets for targeted release in the mouth. Use of novel excipients helped in optimizing the formulations with enhanced palatability.

Acknowledgement:
Gangwal Chemicals Pvt. Ltd. for providing samples of Polymers, Superdisintegrants and diluents.
References:
Studies on different techniques in water dispersible system for treatment of dry mouth syndrome

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Abstract: Dry mouth syndrome is a condition where the salivary glands do not produce saliva, a side effect of some drugs and as a symptom of Sjögren’s Syndrome, an endocrine condition such as diabetes mellitus. The present work aims at formulating water dispersible solid dosage system as a salivary substitute and comparing the techniques of preparation for better treatment and patient compliance. Optimization of formulations was done with respect to dispersion of the system in water to achieve the desired organoleptic properties. Reproducibility of the trials ensured achievement of desired clarity of dispersion, viscosity and organoleptic properties.

Introduction:
Dry Mouth Syndrome is a common side effect of medications mainly seen in elderly people because they tend to take number of medications and also in dehydration, radiotherapy involving salivary glands. The main objective of our study is to compare techniques of preparation of water dispersible solid dosage system to enhance the salivary secretion in the mouth leading to hydration for prolonged effect to the patients [1-2].

Materials and Methods:
Novel hydrating Polymer A, Sodium Croscarmellose, Sweetener, Flavourant, Lubricant

Method:
Following were the variables for selection of hydrating polymers:
1. Viscosity at different concentrations
2. Moisture content
3. Water holding capacity
4. Water retention capacity
Uniform blend of all the powder mixture was compressed into tablet of size 12mm and weight 500mg with hardness 2 – 2.5 kg/sq.cm. Study of comparison of three techniques for preparation of water dispersible system viz., effervescent and non-effervescent using direct compression was carried out. The tablets were evaluated for standard tablet parameters such as appearance, hardness, weight variation, Friability along with specific tests for water dispersible system.
Results and Discussion:
Polymer A gave satisfactory results for viscosity at 0.5% and 1%, moisture content, water holding capacity and water retention capacity, amongst the screened polymers. Croscarmellose sodium proved better than the other superdisintegrants. Technique of combination of effervescent agents and superdisintegrant gave faster dispersibility of tablets with excellent hydration of polymer in the mouth for prolonged period of time.

Table 1. Evaluation parameters for polymer.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Water Holding Capacity</th>
<th>Water Retention Capacity</th>
<th>concentrations</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrating Polymer A</td>
<td>20.12</td>
<td>50.9%</td>
<td>0.5% 1%</td>
<td>0.0310 poise 0.3432 poise</td>
</tr>
<tr>
<td>Carbopol</td>
<td>20.17</td>
<td>7.76%</td>
<td>0.5% 1%</td>
<td>0.0614 poise 0.3643 poise</td>
</tr>
<tr>
<td>HPMC</td>
<td>20.13</td>
<td>3.95%</td>
<td>3% 5%</td>
<td>0.1625 poise 0.2964 poise</td>
</tr>
</tbody>
</table>

Before Dispersion                           After Dispersion

Figure 1 Dispersion of prepared solid dosage formulation.

Conclusion:
Concentration of Polymer A showed desired effect in presence of higher concentration of superdisintegrand and effervescent agents resulting in rapid disintegration of the tablets in the water medium. Optimized formulations showed better mouth feel and palatability. Optimization of formulations was done with respect to dispersion of the system in water to achieve the desired organoleptic properties. Reproducibility of the trials ensured achievement of desired clarity of dispersion, viscosity and organoleptic properties.
Acknowledgement:
Authors are thankful to Gangwal Chemicals Pvt. Ltd. for providing the samples of Polymers, superdisintegrants and diluents.

References:
Effect of different surfactants on particle size & drug entrapment in κ- Carrageenan nanogels

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Abstract: κ- Carrageenan hydrogel nanoparticles, so called nanogels, were formulated by reverse emulsification method. Effect of various surfactants in designing of nanogels was evaluated for particle size, zeta potential and entrapment efficiency of model drug. Nanosized particles were formed due to the formation of reverse micelles, which depends on the temperature solubility profile of surfactant. From the results it was found that ionic surfactants react with charged κC to alter the size and drug entrapment. These altered nano sized hydrogels particles may be promising candidate for drug delivery system.

Introduction:
A fascinating approach to improve aqueous solubility involves use of hydrogel nanoparticles (nanogels) employing hydrophilic polymers. Advantageous small dimension, hydrophilicity and biocompatibility makes it strong candidate for drug delivery. Natural polysaccharide k-carrageenan was studied for the formulation of nanogels. The work objectives are to develop the nanogel of k-carrageenan containing poor water soluble drug.

Materials and Methods:
k-Carrageenan, GELCARIN GP812 (USP/NF) was a gift provided by Marine colloids, other chemicals used were of analytical grade. Cefdinir Monohydrate was used as a model drug and was kind gift obtained from Lupin Laboratories, Mandideep India.
Method of preparation: k -Carrageenan Nanogels were prepared by reverse emulsification method as reported by Silva et al.(2011) with slight modifications [1]. Surfactant was dissolved in 10 ml of organic phase of cyclohexane: n-butanol mixture (7.7:2.3). The aqueous phase was k-carrageenan in 0.1M KCl solution (4% w/v). Cefdinir was added to aqueous phase (1mg/ml). Temperature of both phase were maintained at 75°C. Aqueous phase was added to the organic phase so as to keep molar ratio of water and surfactant 25, at 75°C and mixture was sonicated for 2 minutes by probe sonicator, mixture was gradually cooled to room temperature which induces thermal gelation of the nanogels. The particles were collected by centrifugation. Surfactants were removed by extraction with ethanol followed by centrifugation, until the surfactant was removed. Finally the nanogel was lyophilized. Similar method was used for different type of surfactants CTAB (cationic surfactant), SLS (anionic surfactant), Span-80 (Non-ionic, oil soluble) and Tween-80 (nonionic, water soluble).
Nanogel Characterization: Particle size analysis and zeta potential measurement: The average hydrodynamic diameter and surface charge of nanogels was determined by dynamic light scattering (DLS) by Zetasizer Nanoseries instrument from Malvern Instruments Model DTS Ver. 5.03. (n=3)

Drug entrapment efficiency
The drug content of κC nanogel was determined by adding lyophilized nanogel equivalent to 0.5 mg of drug to 10.0 ml water, followed by heating up to 80ºC. After heating, solution was centrifuged and analyzed by UV. (n = 3)

Results and Discussion:
In present work reveres micro-emulsification combines with thermally induced gelation, in this approach initially water-in-oil (w/o) emulsion was prepared by using an aqueous phase by dispersing in a continuous organic phase at 75°C. As the temperature reduces; due to change in solubility of surfactant; reverse micelles will form which are responsible for nano sized particles.

![Figure 1 Particle size of formulation](image1.png)

![Figure 2 Zeta potential of nanogels](image2.png)

Z avg. of nanogels prepared by ionic surfactant was significantly smaller (p< 0.05) as compare to nanogels prepared by nonionic surfactant. The reason could be possible ionic interaction between polymer and surfactant. When aqueous phase was added it was surrounded by organic surfactant phase. κC posses negative sulfate group on his surface. SLS also has sulfate group which possibly, repel each other. This repulsion may be responsible for small particle size of κC nanogel. In the same way CTAB possibly increases cross linking in nanogel particle, responsible for small size. Another important reason may be the solubility of the surfactant in aqueous phase. Nanosized particles are due to the formation of reverse micelles, which depends on the temperature solubility profile of surfactant.

On comparing both the ionic surfactants i.e. CTAB & SLS, polydisparity index (0.75) suggests that interaction between κC and SLS may not be consistent; there for size uniformity was not observed in the particle size. The zeta potential of the nanogels was found to be negative consistently with negatively charged κC nanogels dispersed in solution [2]. Cefdinir was added as model drug in aqueous phase during formulation. Entrapment efficiency in all the nanogels was found to be less than 40 %, possibly because of loss of drug during emulsification and washing. Ionic surfactant gave not
only more ordered structure to particle but also rigidity to particle. Drug entrapment of nanogel prepared by CTAB was more as compared to nanogels prepared by SLS. The reason behind this may be negative effects of SLS on the cross-linking efficiency of kappa-carrageenan with KCl. This effect should lead to significant leakage of the entrapped cefdinir and therefore reduce drug entrapment.

Figure 3 % Drug entrapment efficiency

While cross linking of polymer structure was higher in nanogel prepared by CTAB, this is because surfactant did not interfere with the formation of double helices between κC chains due to the presence of potassium ions.

Conclusion:
On the basis of above results it can be concluded that characteristics of κC nenogels can be altered by using different surfactants. These altered nano sized hydrogels particles may be promising candidate for drug delivery system.

Acknowledgement:
Authors are thankful to Marine colloids (USA), Lupin Ltd. (Mandideep) for providing gift samples. Also to the Management, VNS Group of Institutions, Bhopal, for providing basic facilities for research.

References:
Design, development and evaluation of Prochlorperazine films for buccal delivery

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Abstract: The buccal region offers an attractive site of administration of drugs for systemic use. A buccal drug delivery system was developed for Prochlorperazine, a dopamine D₂ receptor antagonist with antiemetic property using HPMC, ethyl cellulose, and PVP. The prepared patches were characterized by means of film thickness uniformity, folding endurance, weight uniformity, content uniformity, swelling behaviour, percentage moisture loss, tensile strength, percentage elongation, surface pH, in-vitro studies, ex-vivo mucoadhesion. In vitro release studies of drug-loaded patches in phosphate buffer solution (pH, 6.8) exhibited drug release in the range of 35.64 to 72.33% in 30 min. Good correlation among in vitro release and in vivo studies was observed. Short-term stability study on the films revealed no significant changes in drug content and release studies after 4 weeks. The findings suggest that the present prochlorperazine maleate containing buccal film could be potentially useful to control the emesis induced by anti-cancer agents or opioid analgesics in patients who limit the oral intake.

Introduction:
Approximately 70% of patients with advanced cancer complain of pain and about half of them have severe symptom that require medication with strong opioid analgesics [1, 2]. Nausea and vomiting is known to be elicited in 30–50% of strong opioid analgesic users. Dopamine D₂ receptor antagonists such as prochlorperazine are effective in suppressing opioid analgesic-induced nausea and vomiting. The buccal route and buccal dosage forms have the advantage of allowing excellent accessibility, reasonable patient acceptance and compliance avoids first pass metabolism and involves relatively robust mucosa and utilize the property of bioadhesion of certain water soluble polymers which become adhesive on hydration [3]. Hence, Prochlorperazine maleate containing buccal film were developed which can be a potential dosage form to control emesis induced by Anti-cancer agents.

Materials and Methods:
Prochlorperazine Maleate was received from Nicholas Piramal, Mumbai, India as a gift sample. HPMC, Ethyl cellulose, PVP were purchased from SD fine-chemicals limited, Mumbai. Glycerin, Tween 80 was purchased from Loba chemie, Mumbai.

Preparation of Buccal Mucoadhesive Films: Buccoadhesive films were prepared by solvent casting method using polymer HPMC (15cps and 47cps) along with the drug PCZ and a suitable solvent. The composition of the patches is given in table below.
**Evaluation:** For weight uniformity 1 sq.cm. film of each formulation was taken and weighed individually on a digital balance. The surface pH was measured using universal pH paper. The thickness of the film was measured using digital vernier caliper. The number of times of film could be folded at the same place without breaking gave the value of the folding endurance. Percentage moisture loss was calculated using the following formula, Percentage Moisture Loss = ((Initial weight – Final weight)/ Initial weight) x 100. A Brookfield viscometer (LVDV-E model) attached to the helipath spindle number 18 was used to measure viscosity. Swelling index of the buccoadhesive film was measured at respective time points of 5, 10, 20, 30, 45 and 60 minutes. Drug Content Uniformity was determined using phosphate buffer pH 6.6. The solutions were filtered and absorbance was measured in UV-spectrophotometer at 256 nm. Tensile strength of the films was determined using universal strength testing machine. The percentage elongation was calculated by equation, [4] Percentage elongation = (Increase in length/ Original length) x 100. The release of PCZ from the patches into phosphate buffer pH 6.6 at 37 ± 0.5 °C was performed using a special modified Levy method [5]. The samples assayed spectrophotometrically at λmax 256 nm.

**Table 1 Composition of formulations.**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prochlorperazine maleate (mg)</td>
<td>I 150 150 150 150 150 150</td>
</tr>
<tr>
<td>HPMC, 15cps (mg)</td>
<td>250 200 200 200 200 200</td>
</tr>
<tr>
<td>HPMC, 47cps (mg)</td>
<td>* 250 * 200 * 200 *</td>
</tr>
<tr>
<td>Ethyl cellulose (mg)</td>
<td>* * 50 * 50 * *</td>
</tr>
<tr>
<td>PVP (mg)</td>
<td>* * * 50 50</td>
</tr>
<tr>
<td>Ethanol (ml)</td>
<td>8 8 8 8 8 8</td>
</tr>
</tbody>
</table>

**Results and Discussion:**
All the drug-loaded films had almost uniform thickness ranging from 0.2018 to 0.2272 mm and the films weight ranged from 14.6833 to 22.8500 mg. The surface pH of all prochlorperazine maleate patches was within ± 0.3 units of the neutral pH. Films did not show any cracks even after folding for more than 300 times. Folding endurance did not vary when the comparison was made between plain films and drug-loaded films.

**Swelling studies** The Study shows patch V and I containing HPMC (15 cps) have more pronounced swelling. Swelling in terms of weight, Patch IV shows least swelling, possibly due to the presence of ethyl cellulose in formulation.

**Mechanical Properties of Films:** The tensile strengths of drug loaded patches were higher than blank patches as PCZ is slightly soluble and is strengthened by the bonding of polymer chains. The tensile strengths of drug loaded patches are in the order of IV > II > III > I > VI > V. While the order of percentage elongation for the blank and drug loaded films in the Figure 4 follows the order as IV > III > II > I > VI > V. HPMC chains produce effective cross-linking.
**Percentage moisture loss:** Percentage moisture loss is least in patches III and IV as these contain water insoluble polymer ethyl cellulose. However, patches I and II exhibited highest loss due to presence of water soluble polymer HPMC.

**Content uniformity of prochlorperazine maleate patches:** All the formulations showed more than 80% of the drug loading indicating much of the drug is not lost.

**Viscosity:** Viscosity of film IV was high because of ethyl cellulose.

**In Vitro Release Studies:** Figure 5 shows the cumulative drug release profiles of PCZ films. It is apparent from the graph that the release of prochlorperazine maleate decreased when the viscosity of HPMC is increased. Ethylcellulose retarded the release rate of drug from HPMC patches (patches III and IV) while the PVP in the films increased the drug release rate from HPMC films.

![Figure 5: In-vitro Release Studies.](image)

**Conclusion:**
This study demonstrated that prochlorperazine maleate could be successfully delivered in buccal films. These films met various criteria revealing excellent stability and dissolution profile. The films exhibited satisfactory characteristics regarding to integrity, flexibility, dispersion of drug, and other quality control parameters. The release kinetics indicated zero order release from all the patches. Hence the development of bioadhesive buccal formulations for prochlorperazine may be a promising one as the dose of prochlorperazine may be decreased and hence side effects may be reduced.

**References:**
Formulation and evaluation of press coated tablets for pulsatile drug delivery of Ketoprofen

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Abstract: The aim of study was to develop press coated tablet for pulsatile drug delivery of Ketoprofen. This system delivers the drug at such a time when it could be most needful to patient of rheumatoid arthritis. The press coated tablet was formulated by Ketoprofen as inner core and outer shell by using different ratios of polymers. Factors influencing on the lag time such as the particle size, viscosity of ethyl cellulose, outer coating weight and the paddle rpm also studied. The surface morphology of the tablet was examined by a Scanning Electron Microscopy.

Introduction:
Pulsatile drug delivery system (PDDS) delivers the drug at specific time as per the pathophysiological need of the disease, resulting in improved patient therapeutic efficacy and compliance [1, 2].

Materials and Methods:
Materials: Ketoprofen received as a gift sample from Shreya Pharmaceuticals, Aurangabad.

Table 1 Composition of press coated tablet

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Variable Specification</th>
<th>Composition (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC</td>
</tr>
<tr>
<td>F1</td>
<td>Ethocel 10 cP</td>
<td>300</td>
</tr>
<tr>
<td>F2</td>
<td>Ethocel 45 cP</td>
<td>300</td>
</tr>
<tr>
<td>F3</td>
<td>Ethocel 100 cP</td>
<td>300</td>
</tr>
</tbody>
</table>

Evaluation of Ethocel Viscosity

<p>| | | | |</p>
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<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>F4</td>
<td>20%</td>
<td>240</td>
<td>60</td>
</tr>
<tr>
<td>F5</td>
<td>30%</td>
<td>210</td>
<td>90</td>
</tr>
<tr>
<td>F6</td>
<td>40%</td>
<td>180</td>
<td>120</td>
</tr>
<tr>
<td>F7</td>
<td>10%</td>
<td>270</td>
<td>---</td>
</tr>
<tr>
<td>F8</td>
<td>20%</td>
<td>240</td>
<td>---</td>
</tr>
<tr>
<td>F9</td>
<td>30%</td>
<td>210</td>
<td>---</td>
</tr>
</tbody>
</table>

Evaluation of Natural Polymer Concentration

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F10</td>
<td>20%</td>
<td>500</td>
<td>100</td>
</tr>
</tbody>
</table>

Evaluation of Coating Weight

- Precompressional parameters, Characterization of core and press coated tablet
- Dissolution study of core and press coated tablet
• Determination of different Factors affecting on lag time
• Surface morphology study and Stability study

**Results and Discussion:** Solubility study of the drug

**Table 2** Solubility data of ketoprofen in various buffers

<table>
<thead>
<tr>
<th>Media</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 pH</td>
<td>0.0069859</td>
</tr>
<tr>
<td>7.4 pH</td>
<td>10.94277</td>
</tr>
<tr>
<td>6.8 pH</td>
<td>8.731293</td>
</tr>
</tbody>
</table>

**Drug excipients compatibility study:** Not observed any interaction between Drug & polymers

**Pre compression parameters of coating powder blend and core tablet powder blend:** The results indicates that powder blend has passable flow property with good compressibility and suitable for direct compression method.

**Characterization of core and press coated tablet:** Weight variation was found to be within IP limit. Drug content was observed within the range 99 - 102%.

**Effect of hydrophilic polymers concentration on lag time:** Amount of husk and sodium alginate increased in formulation leads to faster swelling and erosion which is responsible for breakdown of outer coating.

**Effect of outer coating weight on lag time:** Formulations F4 and F10 containing different outer coating weight 300 and 600 mg respectively. The result indicates that coating weight proportional to the lag time.

**Effect of EC viscosity on lag time:** As increases in the viscosity of EC, lag time of formulations decreases. It was due to the viscosity of EC proportional to the % porosity of coating.

**Surface morphology study:** The morphological evaluation of press coated tablet was performed by scanning electron microscopy. Small pores or fractures were found on coating surface which may be due to hydrophilic polymer used in combination with EC.
Figure 1  Scanning electron photomicrographs of cross section of press coated tablet (A), press coated tablet of formulation F4 (B), press coated tablet of formulation F7 (C).

Conclusions:
Pulsatile drug delivery system is the time and site specific drug delivery system, where there is rapid and transient release of certain amount of drug within a short time period immediately after predetermined off-release period. The lag time and time-controlled release behavior of ketoprofen from press-coated tablets could be modulated by changing the particle sizes of EC powders in outer coating, viscosity of EC, paddle rpm, coating weight and natural polymer concentration. Formulations F4 and F7 compression coated tablets achieve a burst release after 6 hrs lag time which is applicable pulsatile drug delivery of ketoprofen for rheumatoid arthritis.

Acknowledgement:
The authors are thankful to HSBPVT’s, GOI, College of Pharmacy, Kashti for permitting to carry out the research work in the laboratory. The authors are also thankful to Shreya Pharmaceuticals, Aurangabad for providing the gift samples.

References:
Formulation development and characterization of orodispersible tablets of *Mukta bhasma*

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Abstract: Mouth dissolving tablets of *Mukta bhasma* were prepared. Orodispersible tablets were prepared by direct compression method. The orodispersible tablets were investigated for weight variation, drug content, percentage friability, water absorption ratio, disintegration time, presence of nano crystals and stability. A $3^2$ full factorial design was used to study the joint influence of two formulation variables: amount of superdisintegrant and diluent. Multiple linear regression analysis indicated that, tablets should be prepared using an optimum concentration of diluent and a higher percentage of superdisintegrant for faster disintegration. The Effects of formulation processing variables were correlated with the results obtained using systematic formulation approach.

Introduction:
Traditionally, various metals and minerals are triturated and calcinated repeatedly with herbal juices to make them assimilable for biological delivery and these herbo-metallic preparations are well known as *bhasma* (ash). *Bhasmas* are considered as potent formulation and are prescribed in low doses. It has been reported by many researchers that the potent activity of the bhasma may be attributed to presence of nano crystals in the bhasma preparations. But bhasma are poorly flowing, powder preparations and suffer from practical drawbacks such as difficulties in accurate dosing, difficulty in handling and dispensing. This may result is noncompliance with prescription and ineffective therapy [1]. Thus attempts are being made to design such dosage form for oral administration, which can overcome these drawbacks and improve patient compliance without distorting the nano crystal content of bhasma preparations [2, 3].

Materials and Methods
Materials: *Mukta bhasma* was procured from reputed Ayurvedic drug manufacturer. Superdisintegrants Ac-Di-Sol, crospovidone and sodium glycolate, Avicel pH 102 (Microcrystalline cellulose), directly compressible mannitol (Pearlitol SD200) and lactose were received as gift sample from Signet Chemicals, Mumbai. All reagents and solvents were of analytical grade.

Preparation of orodispersible tablets (preliminary trials and $3^2$ factorial designs): The preliminary trials were performed by using 2% superdisintegrants (Ac-Di-Sol, crospovidone and sodium glycolate). A $3^2$ randomized full factorial design (Table 1 a) was used in the present study. Independent variables were identified as the amount of diluent ($X_1$) and the amount of...
superdisintegrant ($X_2$). The disintegration time of tablets was selected as dependent variable. Orodispersible tablets (OD) of *Mukta bhasma* were prepared by direct compression method. All the designed formulations were prepared as a batch of 100 tablets. A control formulation (A0, without super-disintegrant) and checkpoint batches COD1 (0.6, -0.6) and COD2 (0.4, -0.9) were prepared.

**Evaluation of tablets:** Tablets were evaluated for weight variation and content uniformity test. Transmission electron microscope (TEM) (Tecnai-G2, FEI and Hillsboro, USA) was used to study nanosized particles in dispersed phase after disintegration. Interferograms of the active ingredient and its formulations were studied using Perkin-Elmer FTIR series (model 1615) spectrophotometer to identify any drug-carrier interactions. The promising formulation (OD7) was studied for short-term stability parameters by storing the tablets (in rubber stoppered vials) at 40º/ 75% RH for 3 w. At intervals of one week, the tablets were inspected visually for any physical changes, changes in drug content and disintegration time.

**Results and Discussion:**

| Table 1 (a) Factorial design formulations of Mukta Bhasma tablets |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **Ingredients (mg)** | **OD 1** | **OD 2** | **OD 3** | **OD 4** | **OD 5** | **OD 6** | **OD 7** | **OD 8** | **OD 9** |
| *Mukta Bhasma* | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Ac-Di-Sol | 2.5 | 2.5 | 2.5 | 5 | 5 | 5 | 7.5 | 7.5 | 7.5 |
| Lactose | 15 | 30 | 45 | 15 | 30 | 45 | 15 | 30 | 45 |
| Avicel pH 102 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Pearlitol SD 200 | 47.5 | 32.5 | 17.5 | 45 | 30 | 15 | 42.5 | 27.5 | 12.5 |
| Mag. stearate | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Purified Talc | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Total Weight** | **150** | **150** | **150** | **150** | **150** | **150** | **150** | **150** | **150** |

| Table 1 (b) Evaluation of orodispersible tablets containing Mukta Bhasma |
|-----------------------------|-----------|
| **Evaluation parameters for powder blend** | **Table 1 (c) Evaluation parameters for prepared tablet** |
| **Bulk Density (gm/cm^3)** | 0.514 | 0.538 | 0.573 | 0.482 | 0.525 | 0.561 | 0.469 | 0.491 | 0.536 |
| **Tapped Density (gm/cm^3)** | 0.553 | 0.574 | 0.623 | 0.531 | 0.568 | 0.610 | 0.511 | 0.524 | 0.582 |
| **Angle of repose (º)** | 21.03 | 21.57 | 22.04 | 22.13 | 22.91 | 23.67 | 24.37 | 24.89 | 25.43 |

| **Weight variation** | 150.34 ± 2.44 | 151.02 ± 3.25 | 149.89 ± 2.53 | 151.11 ± 2.85 | 151.20 ± 1.45 | 150.82 ± 2.27 | 149.71 ± 3.65 | 150.49 ± 2.12 | 151.20 ± 2.89 |
| **Drug content (%)** | 98.11 ± 1.5 | 96.68 ± 1.4 | 101.38 ± 1.38 | 97.24 ± 1.49 | 99.76 ± 1.72 | 98.55 ± 2.03 | 97.94 ± 2.15 | 98.47 ± 1.08 | 98.22 ± 1.19 |
| **Friability (%)** | 0.23 ± 0.017 | 0.169 ± 0.019 | 0.13 ± 0.014 | 0.26 ± 0.014 | 0.21 ± 0.014 | 0.205 ± 0.013 | 0.39 ± 0.018 | 0.25 ± 0.018 | 0.22 ± 0.015 |
| **Hardness(kg/cm^3)** | 2.45 | 2.77 | 2.98 | 2.34 | 2.55 | 2.68 | 2.10 | 2.28 | 2.52 |
| **Wetting time (Sec)** | 73 ± 1.3 | 86 ± 2.4 | 97 ± 4.29 | 31 ± 4.44 | 36 ± 3.29 | 56 ± 3.72 | 21 ± 4.67 | 31 ± 1.92 | 37 ± 3.12 |
| **Disintegration time (Sec)** | 84 ± 1.5 | 95 ± 3.6 | 108 ± 5.37 | 42 ± 3.54 | 49 ± 1.33 | 68 ± 2.43 | 32 ± 3.65 | 40 ± 2.08 | 48 ± 1.11 |
The superdisintegrants serve as the basic approach used in the development of the fast-dissolving tablet. Preliminary screening studies indicated the candidature of batch containing Ac-Di-Sol for further studies. Direct compression method was selected to prevent agglomerations or distortion of the nano crystals present in Mukta bhasma. The prepared powder blend was free flowing (angle of repose value <30°) and had desired compressibility index. Tablets obtained (OD1 to OD9) were acceptable as per IP specifications (Table 1 c). The nano crystals are detected in all the batches of tablets (Fig. 1). The batches OD1 to OD9 shows different wetting time and disintegration time. Disintegration time was found to be a little longer than the arbitrarily chosen value of less than 50 seconds for batch OD1-OD3 and OD6. Multiple linear regression analysis concluded that, on increasing the concentration of Ac-di-sol, a decrease in disintegration time is observed. It is obvious as presence of higher percentage of superdisintegrant, wicking is facilitated. But on increasing the concentration of lactose, an increase in disintegration time is observed due to decreased water uptake. Acceptable friability values were found in the whole of the contour area. The disintegration time of checkpoint batches COD1 (0.6, -0.6) are in close agreement with predicted values. Thus, we can conclude that the statistical model is mathematically valid. The IR spectrum confirmed no interaction of drug with the components of the formulation. Stability studies indicated that no significant change in disintegration time. Appearance of the tablets, and percentage friability were under acceptable limits.

**Conclusion:**

The results of a 32 full factorial design indicated that the amount of lactose and Ac-di-sol significantly affect disintegration time and percentage friability, the dependent variables. Systematic formulation approach may be useful in reaching an optimum point in the shortest time with minimum efforts. An effective alternative approach may be the use of lactose when compared with the use of more expensive adjuvant in the formulation of orodispensible tablets.

**Acknowledgement:** Authors are thankful to SICART, Vallabh Vidyanagar (Gujarat) for analytical facilities.

**References:**


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Abstract: Niosomes are non-ionic surfactant vesicles which act as drug carrier system for topical delivery. In the present study, we carried preparation and evaluation of proniosomal gel of karanj oil (Derris Indica family - Fabaceae). Karanj oil is traditionally used for the treatment of eczema, leprosy and some common skin infections. The proniosomal gel was prepared by slurry method using span 40, soya lecithin and cholesterol, using karanj oil as the active pharmaceutical ingredients. Alcohol and phosphate buffer pH 7.4 were used in formulation as pharmaceutical excipients. The resulting formulations were evaluated and observed by transmission electron microscopy. Evaluation of formulation confirmed the optimum result like uniform size distribution (150nm-330nm), drug entrapment (70-75%) and antimicrobial property. It can be concluded that it is visible to prepare an acceptable proniosomal gel of karanj oil for therapeutic application.

Introduction

Derris indica, a plant belonging to family Fabaceae is an erect perennial tree. The tree is frequently found in pastures, waste lands, cultivated lands, roadsides, lawns and in planted forests. In India and Nepal it is found throughout the hotter parts and also worldwide. The plant is used for the treatment of many diseases such as skin infection, leprosy, and wound healing in India. In India seeds were used for skin ailments. Today the oil is used as a liniment for rheumatism traditionally it has been used antibacterial. Leaves are active against micrococcus. Fresh leaf extract is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea & leprosy. Fresh leaf extract as well as oils are used as antiseptic. According to ayurvedic medicine the root and bark has anthelmintic activity, used in the diseases of eye, skin & vagina, itch, piles, splenomegaly tumors [1]. Gels can resist the physiological stress caused by skin flexion, mucociliary movement, adapting to the shape of the applied area and for controlling drug release. To attain optimal drug action, functional molecules should be transported by a carrier to the site of action and released to perform their task [2]. But plant active and extracts having some own problems. (Low absorption and bioavailability, Poor stability, Poor lipid solubility, Gastric degradation, Poor pharmacokinetic, Pharmacodynamic) Thus, there is a need to develop a drug and delivery system. Hence we are proposed to prepare proniosomal gel for effective delivery of karanj oil.

Materials and Methods:
Drug and chemicals: Soya lecithin, karanj oil was purchased from Himedia labs. Agar sodium hydroxide, sodium chloride, agar, cholesterol, span 40, 60, 80 potassium dihydrogen phosphates and toluene were purchased from loba chemie Pvt. Ltd. Mumbai India.

Preparation of proniosomes of karanj oil: Proniosomal gel was prepared by slurry method.

Size distribution Particle size range and vesicle distribution was evaluated by labomed optical microscope. Average particle size was determined along with standard deviation.

Percentage drug entrapment (PDE): Proniosomal gel 25mg was dissolved in 80^0 C distilled water and wait for few minutes then solution was centrifuged 18000 rpm for 40 minutes at 5^0 C. The supernatant was studied spectrophotometrically at 520nm. The percentage entrapment efficiency was calculated using formula

\[ \text{PDE} = \left[ 1 - \frac{\text{unentrapped drug}}{\text{total drug}} \right] \times 100 \]

Where; PDE percentage drug entrapment

Morphology Dissolved proniosomal gel was also determined for transmission electron microscope.

Microbiological activity of formulation: The antibacterial activity of formulations were evaluated by agar well diffusion method [3, 4] All the microbial culture were adjusted to 0.5 McFarland standards visually comparable to a microbial suspension of approximately 1.5 × 108 cfu/ml. 20ml of Mueller Hinton agar media was poured into each Petri plate and plates were swabbed with 100µl inoculate of the test microorganisms and kept for 15 minutes for absorption. All the plates were incubated at 370C for 24 hours. Antibacterial activity of formulation was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hiantibiotic zone scale). DMSO as solvent was used as a negative control where as ciprofloxacin was used as positive control. The experiments were performed in triplicates.

Stability study: For stability testing three batches were prepared and kept in a vial closed with lid and stored at

a) Room temperature (b) 40^0C-80^0C (c) 45^0C ± 2^0C.

b) The sample was evaluated for the ability of vesicle to retain the drug. The sample was visually verified from time point during 3 month; various factors like aggregation, flocculation and loss of solvent were observed.
Results and Discussion:
We designed several formulations and prepared them using standard methods. These formulations were evaluated as per the physical characteristics shown in table 2 Transmission electron microscope was used to evaluate morphology of niosomes derived from niosomal dispersion of formulation code D1 and D2 of karanj oil. Niosomes prepared from proniosomal gel were uniform in shape. Particle size analysis of niosome preparation showed that the size distribution of niosome based on karanj oil were approximately the same the average particle size range of karanj oil were between 150-330nm.

Table 3 Average particle size of niosomes.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Particle size D1(nm)</th>
<th>Particle size D2(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>153.43</td>
<td>163.31</td>
</tr>
<tr>
<td>2</td>
<td>163.25</td>
<td>218.52</td>
</tr>
<tr>
<td>3</td>
<td>168.27</td>
<td>267.90</td>
</tr>
<tr>
<td>4</td>
<td>237.51</td>
<td>323.23</td>
</tr>
<tr>
<td>5</td>
<td>190.25</td>
<td>310.24</td>
</tr>
<tr>
<td>6</td>
<td>176.18</td>
<td>299.53</td>
</tr>
<tr>
<td>Mean</td>
<td>181.49</td>
<td>263.79</td>
</tr>
</tbody>
</table>

For niosomal derived proniosomal gel based on karanj oil percentage drug entrapment efficiency of karanj oil formulation D1 was found to be 85% while the entrapment efficiency of D2 was approximately 87%. D1 shows higher entrapment due to less amount of lecithin used therefore thinner film on surface. In microbial study 6 formulations of karanj oil were evaluated against gram-positive and gram-negative bacteria. Broad spectrum antibacterial agent like ciprofloxacin was used as standard for comparison to the all formulation. Based on this finding, it may be suggested that the compounds used and oil may be used as source for formulating antimicrobial agents as they are active against both gram-negative and positive bacteria.

Conclusion: Based on the study we concluded that proniosomal gel of karanj oil is acceptable for therapeutic use and effective against some microorganisms.

Acknowledgments: The authors are thankful to Director, University Institute of Pharmacy, Pt. Ravi Shankar Shukla University, Raipur, Chhattisgarh, India, for providing necessary infrastructural facilities.

References:
Drug resin based gastro-retentive microspheres for effective treatment of *H. pylori*

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Abstract: It was attempted to develop and characterize a drug resin complex based multiparticulate delivery system of Amoxicillin trihydrate (AMT) for the effective treatment of colonization of *H. pylori*. Mucoadhesive microspheres of ethyl cellulose and chitosan with floating ability were developed using solvent evaporation approach. Formulations were characterized for micromeritic properties, % drug entrapment, % yield, surface morphology, % mucoadhesion, *in vitro* buoyancy behavior and *in vitro* drug release in simulated gastric fluid. The optimized microspheres shows 79.65±2.13% drug entrapment, 78.60±3.3% buoyancy, 78.25±0.14% mucoadhesion and floating lag time of >20 min with a mean particle size of 106.49±4.45 μm. During the *in vitro* release studies a sustained delivery of AMT was observed over a period of 24 h which was superior (54.65±0.95% within 8 h) to the drug release from the formulation without drug resin complex (80.15±0.45% within 8 h) and marketed formulation (98.63±0.95% within 2.5 h). The superior sustainability of the developed microspheres may attribute to the combination of floating and mucoadhesive properties which successfully triumph over the problems associated with conventional therapy.

Introduction:
The prevalence of *Helicobacter Pylori* (*H. pylori*) is subjected to the age of individuals, as the occurrence rate in individuals over 60 years is 60% in comparison to 10% for younger ones [1]. The present attempt endeavors to develop a sustained release gastro-retentive multiparticulate system of amoxicillin trihydrate by utilizing an ion exchange resin, for the management of *H. pylori* infection. For the accomplishment of this objective, some ion exchange resins were selected and tested for the binding efficiency with amoxicillin as well as for gastric retention capability. The drug resin complex was prepared and finally encapsulated with rate controlling semi-permeable polymeric membrane to achieve more sustainable drug release.

Materials and Methods
Materials: Amoxicillin trihydrate was gifted by M/s Aurobindo Pharma Ltd., Ahmedabad, India. Cholestyramine resin (Tulsion 412) was obtained as a gift sample from Thermex Ltd., Pune, India. Chitosan (degree of deacetylation 85% and molecular weight 65 kDa) was received as gift sample from Central Institute of Fisheries Technology, Cochin, India. Ethyl cellulose was purchased from Hi-Media, Mumbai, India. All other chemicals used were of analytical reagent grade.
Methods: Cholestyramine resin was purified and activated and were further used for complex formation with drug [2]. Ethyl cellulose and chitosan based mucoadhesive microspheres with floating ability were developed using solvent evaporation approach [3]. IR Spectroscopy of drug, resin, ethylcellulose, chitosan, DRC and formulation was performed on a FTIR spectrophotometer. Formulations were characterized for micromeritic properties, % drug entrapment, % yield, surface morphology, % mucoadhesion, in vitro buoyancy behavior and in vitro drug release in simulated gastric fluid. Differences in in vitro drug release of Amoxicillin trihydrate from Cholestyramine resin based microspheres formulations, non resinate microspheres formulation, drug resin complex and marketed formulation of Amoxicillin trihydrate (E-MOX) were statistically analyzed by one way analysis of variance (ANOVA) with post test (Dunnett’s multiple comparison test).

Results and Discussion
Amoxicillin loading involved replacement of Cl⁻ of cholestyramine resin with COO⁻ of amoxicillin [4]. Cholestyramine is a strong anionic exchange resin which means that it can exchange its chloride anion with anionic drug and form cholestyramine-amoxicillin complex and hydrochloride.
Drug resin complex (DRC2) is irregular in size and shape with rough surface. The scanning electron microscope images of prepared formulation shows a large population of microspheres which are spherical in shape. The optimized microspheres shows 79.65±2.13% drug entrapment, 78.60±3.3% buoyancy, 78.25±0.14% mucoadhesion and floating lag time of >20 min with a mean particle size of 106.49±4.45 μm. During the in vitro release studies a sustained delivery of AMT was observed over a period of 24 h which was superior (54.65±0.95% within 8 h) to the drug release from the formulation without drug resin complex (80.15±0.45% within 8 h) and marketed formulation (98.63±0.95% within 2.5 h).

When the data of E-MOX (marketed product) was compared with DRC2, microspheres only containing drug not resin (NRMS) and formulation containing DRC2 by one-way ANOVA (Dunnett’s multiple comparison) test, the in vitro release in SGF (pH 1.2) from formulations were found to be significant (P<0.01) except DRC2 (P>0.05). When the data of NRMS (Non-Resinate Microspheres) was compared with DRC2, E-MOX and formulations containing DRC2 by one-way ANOVA (Dunnett’s multiple comparison) test, the in vitro release in SGF (pH 1.2) were found to be less significant (P<0.05) in MS-5, MS-4 and very significant (P<0.01) in DRC2.

The release of the drug was fitted with both Reichenberg’s model and Bhaskar’s model as indicated by a correlation coefficient >0.95. The release of the drug was also fitted with Ritger-Peppas model as indicated by a correlation coefficient >0.95. The values of n (Ritger-Peppas) for MS formulations and DRC2 were found to be between 0.45 and 0.85 which an indication of both diffusion controlled and swelling controlled transport mechanism (anomalous/non-Fickian transport).
Figure 1 Comparison of *in vitro* drug release profile of prepared formulations and marketed product.

NRMS = Non-Resinate Microspheres; E-MOX = Marketed formulation of Amoxicillin trihydrate,
DRC2 = Drug resin complex; MS = Microsphere formulations; Values are mean ± S.D. (n=3)

**Conclusion:**

The method of preparation of microspheres was found to be simple, reproducible and provides good yield. Prepared formulation showed better controlled release behavior when compared to marketed product of Amoxicillin trihydrate. Such developed formulation could be subjected to future *in vivo* studies in order to design a viable formulation for better treatment of *H. pylori* infection.

**Acknowledgement:**

The research was supported by research grant under research promotional scheme (RPS) (F. no. 20/AICTE/RIFD/RPS (Policy-III) 13/2012-13) from All India Council of Technical Education (AICTE), New Delhi, India.

**References:**

Formulation and evaluation of sustained release matrix tablets

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Abstract: The aim of the present research work was to formulate and evaluate the sustained release matrix tablets of Lornoxicam. Sustained release matrix tablets were prepared by wet granulation method using Xanthan gum as polymers. The different formulation taking xanthan gum in different concentration were prepared (F1, F2, F3), this formulation were characterized for hardness, thickness, diameter, friability, weight variation and drug entrapment. On the basis of results obtained F3 was found to be better, F3 was further subjected to dissolution study. Formulation showed 99.15±0.24 this up to 24 hr and found to sustained release behavior.

Introduction:
Lornoxicam is a non-steroidal anti-inflammatory drug of the oxicam classes with analgesic, anti-inflammatory and antipyretic property. Anti-inflammatory and analgesic activity of drug is related to its inhibitory action on prostaglandin and thromboxane synthesis through the inhibition of both COX-1 and COX-2[1]. Sustained release (SR) system is a type of modified drug delivery system that can be used as an alternative to conventional drug delivery system. These systems sustain the release of drug and maintain the plasma drug concentration in therapeutic window [2]. Lornoxicam has shorter half-life (3-5 hours), which necessitates frequent dosing in 2-3 divided doses daily. This necessitates dosage form that can reduce the dosing frequency with better patient compliance [3]. Tablets of this drug could be a better option that may avoid the complications associates with conventional system.

Materials and Methods:
Lornoxicam was an obtained gift sample from Lupin Pharmaceutical Ltd (Goa), Xanthan gum, Magnesium stearate, Talc were purchased from Loba Chem. Pvt. Ltd, (India).

Preparation of SR Matrix Tablets: Preparation of SR matrix tablets of Lornoxicam were prepared by wet granulation method using xanthan gum as polymer and different batches were prepared (F1-F3). Required quantities of all ingredients were weighed individually and powders passed through mesh 60# sieve and mixed for 5 min. Isopropyl alcohol was added drop wise till damp mass for the granulation was obtained. The wet mass was passed through sieve 22# and granules were prepared. The prepared granules were dried at 60°C in a oven. The dried granules were passed through sieve 24# and then blended with talc and magnesium stearate. Finally granules were compressed on multiple station tablet punch machine and the weight of tablet was adjusted to 200 mg containing 8 mg drug. Different optimized post- compression parameters like F1, F2 and F3 were selected and
characterized for hardness, thickness, diameter, friability, weight variation and drug entrapment (Table 1). The in vitro dissolution studies were carried out on best formulation using USP apparatus type II (paddle type). The dissolution medium used was 900 ml of 0.1 N HCl for 2 hr and the phosphate buffer pH 6.8 for the rest of period as dissolution medium, maintained at 37±5°C. The paddle was adjusted at 50 rpm [4]. The dissolution profile of F3 is shown in Fig.1.

Table 1 Optimization of post-compression parameters of the different SR matrix tablets.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Formulation Code</th>
<th>Hardness (Kg/cm²)</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
<th>Friability (%)</th>
<th>Weight variation (mg)</th>
<th>% Drug entrapment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>5.33±0.4</td>
<td>2.15±0.0</td>
<td>9.18±0.1</td>
<td>0.24</td>
<td>203.85±3.2</td>
<td>97.14±0.3</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>5.00±0.6</td>
<td>2.15±0.0</td>
<td>9.15±0.0</td>
<td>0.24</td>
<td>203.25±3.4</td>
<td>97.93±0.4</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>6.12±0.4</td>
<td>2.15±0.0</td>
<td>9.10±0.0</td>
<td>0.33</td>
<td>203.85±3.1</td>
<td>99.29±3</td>
</tr>
</tbody>
</table>

Figure 1 Dissolution profile of optimized batch F3

Results and Discussion:
SR matrix tablet were prepared by wet granulation technique using xanthan gum as polymer. Different formulation (F1, F2, F3) were prepared by varying concentration of xanthan gum. Results obtained for hence parameters were 5.00±0.66 to 6.12±0.44 (kg/cm²), 2.15±0.001 to 2.15±0.013 (mm), 9.10±0.06 to 9.18±0.13 (mm), 0.24 to 0.33 (%), 203.25±3.4 to 203.85±3.25 (mg), and 97.14±0.31 to 99.29±35 (%) respectively.
The dissolution study was done on optimized F3 formulation. 99.15±0.24 release was observed up to 24 hr with F3 formulation.

Conclusion:
SR matrix tablets of lornoxicam using xanthan gum was prepared successfuly in vitro drug release was observed up to 24 hr that shows the sustained release behavior of the formulation.
References:


Formulation and evaluation of mouth dissolving tablet of Enalapril Maleate

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Abstract: The aim of the present study was to formulate and evaluate FDT of Enalapril maleate, prepared with using superdisintegrant cross carmellose sodium and using other excipients like mannitol, microcrystalline cellulose, magnesium sterate and talc by direct compression technique on the basis of pre-compression and post-compression parameters. FDT of Enalapril maleate was formulated to achieve quick dissolution rate and to improve efficacy by minimizing the disintegration time and by providing fast release.

Introduction:
A FDT is a tablet that dissolves or disintegrates in the oral cavity without requirement of water as well as chewing. The active ingredient is then swallowed by the patient’s saliva along with the soluble and insoluble excipients [1]. Enalapril maleate is rapidly metabolized by liver esterases to enalaprilat following oral administration. Enalapril maleate itself has little pharmacologic activity. Enalapril maleate lowers the blood pressure by antagonizing the effect of the RAAS. This is nowadays very popular dosage form as typically no water is required for the administration. The bioavailability of some drugs may be enhance due to absorption of drug in oral cavity, also due to pre gastric absorption of saliva containing dispersed drugs that pass down in to the stomach [2].

Materials and Methods:
Enalapril maleate was obtained as a gift sample from Medibest Pharmaceutical Ltd. N – 31, sidco industrial estate, Hosur – 635126 (Tamil Nadu) sodium starch glycolate, microcrystalline cellulose, magnesium stearate, mannitol and talc were obtained from Loba chem. Pvt. Ltd. (S.K. traders) 48, Jaora compound Indore - 452001 (M.P.) India. All the other chemicals/reagents used were of analytical grade.

Preparation of FDT: Preparation of FDT was done by direct compression with enlapril maleate as API and using superdisintegrants sodium starch glycolate and excipients such as mannitol, microcrystalline cellulose, magnesium stearate and talc. Various combinations (F1, F2 and F3) in different ratio were used to choose correct formulation and to formulate FDT [3]. Different optimized post-compression parameters like weight variation, thickness, hardness, diameter, friability, water absorption ratio, wetting time, in-vitro disintegration time and in-vitro dispersion time and drug content were determined (Table 1). The in-vitro dissolution studies were carried out on best formulation using USP apparatus type II (paddle type).The dissolution medium used was volume (900
ml) of pH 6.8 phosphate buffers for 1 hr and the dissolution medium, maintained at temperature of 37±0.5°C was maintained. The paddle was adjusted at 50 rpm. Samples were collected at the interval of every 5 minutes, filtered and suitably diluted with pH 6.8 phosphate buffers [4]. Finally drug concentration was determined using UV spectrophotometer at 210 nm. The dissolution profile of (F1, F2 and F3) is shown in (Figure 1).

Table 1 Different parameters of FDT

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Weight variation</td>
<td>207.2±6.8</td>
<td>212.9±7.7</td>
<td>204.3±7.2</td>
</tr>
<tr>
<td>2.</td>
<td>Hardness (kg/cm²)</td>
<td>2.43±0.03</td>
<td>3.33±0.03</td>
<td>3.40±0.03</td>
</tr>
<tr>
<td>3.</td>
<td>Thickness (mm)</td>
<td>3.25±0.006</td>
<td>3.37±0.003</td>
<td>3.48±0.006</td>
</tr>
<tr>
<td>4.</td>
<td>Friability (%)</td>
<td>0.33</td>
<td>0.49</td>
<td>0.59</td>
</tr>
<tr>
<td>5.</td>
<td>Wetting time (second)</td>
<td>18.04±1.47</td>
<td>11.68±1.21</td>
<td>12.17±1.26</td>
</tr>
<tr>
<td>6.</td>
<td>Water absorption time (second)</td>
<td>83.24±1.18</td>
<td>86.28±0.62</td>
<td>92.18±2.06</td>
</tr>
<tr>
<td>7.</td>
<td>In-vitro disintegration time (second)</td>
<td>16.04±1.97</td>
<td>13.68±1.68</td>
<td>14.47±1.49</td>
</tr>
<tr>
<td>8.</td>
<td>In-vitro dispersion time (second)</td>
<td>103.94±2.17</td>
<td>88.86±3.62</td>
<td>95.83±2.82</td>
</tr>
<tr>
<td>9.</td>
<td>Drug content (%)</td>
<td>98.36±1.27</td>
<td>99.48±0.78</td>
<td>98.76±1.18</td>
</tr>
</tbody>
</table>

![Dissolution profile of F1, F2 and F3 formulation](image)

**Figure 1** Dissolution profile of optimized formulation F1, F2 and F3

Results and Discussion:

Preparation of FDT was carried out by direct compression technique. Different batches were prepared (F1, F2 and F3) and optimization on basis of different post-compression parameters for optimum batch F1 was found to be - 98.36±1.27 (drug content %), 16.04±1.97 (in-vitro disintegration time), 83.24±1.18 (water absorption ratio), 18.04±1.47 (wetting time), 0.33 (Friability %), 3.25±0.006 (thickness mm), 2.43±0.03, (hardness kg/cm²) and 207.2±6.8 (weight variation %). Therefore, formulation F1 was selected as an optimized formulation and the drug content for this formulation was carried out and the drug content of all the three drug viz. enalapril maleate was found to be optimum in accordance with official monograph.
The dissolution study was carried out for F1, F2 and F3. The superdisintegrants used for preparing FDT are soluble in the medium. It was assumed that the drug release data obtained in dissolution for optimized formulation was treated accordingly to zero order equation by plotting cumulative % of drug release against time in minutes. Therefore formulation was selected as optimized formulation which showed the release of drug found to be 86.5±0.30 up to 24 hr. So, F1 was considered as the best formulation.

Conclusion:
FDT of enalapril maleate was prepared successfully by direct compression technique using superdisintegrating agent that rapidly dissolve in oral cavity within 16.04 second without need of drinking water, had a pleasant mouth feel with improved patient compliance, particularly for those who have difficulty in swallowing like paediatric and geriatric patients. Formulation F1 containing sodium starch glycolate 15% with appropriate amount of other excipients was considered to be the optimized formulation with drug content 98.36%. FDT of this drug improved efficacy by minimizing the disintegration time and by providing fast release. In-vitro drug release showed fast behaviour of F1 formulation. All the physical and chemical parameters were found to be in acceptable range and fulfil the entire requirement for FDT.

References
Formulation development and evaluation of microbeads of Clonidine

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Abstract: Clonidine a centrally acting sympatholytic and Imidazoline-derivative hypotensive agent selective α2-adrenergic agonist. It stimulates alpha2-adrenergic receptors in the brainstem to decrease sympathetic nervous system outflow. It is also administered epidurally to treat pain. The objective of the work was to design multiple unit dosage form as microbeads of a drug meant for management of anxiety and hypertensive disorder. Microbeads offers numerous advantages for releasing one of the drugs or part of the same drug immediately while remaining drug or parts of the same can be sustained release. Microbeads of Clonidine obtained utilizing orifice ionic gelation technique using HPMC and sodium alginate as a polymer with various ratios. Prepared beads were evaluated for drug entrapment, drug content, in-vitro release, release kinetic and stability study. Particle size of optimized beads was determined by SEM.

Introduction:
Oral drug delivery is the most desirable and preferred method of administering therapeutic agents for their systemic effects. In addition, the oral medication is generally considered as the first avenue investigated in the discovery and development of new drug entities and pharmaceutical formulations, mainly because of patient acceptance, convenience, and cost effective manufacturing process. For many drug substances conventional immediate release formulations provide clinically effective therapy while maintaining the required balance of pharmacokinetic and pharmacodynamic profiles with acceptable level of safety to the patient1. Multiple unit dosage forms such as microspheres or micro beads have gained in popularity as oral drug delivery systems because of more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation and elimination of unwanted intestinal retention of polymeric material, when compared to non-disintegrating single unit dosage form. Micro beads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects.

Materials and Methods:
Clonidine was received as a gift sample from Unichem Private Limited, Mumbai (India). Sodium Alginate, Calcium Chloride, HPMC and chitosan were obtained from SIRTS- pharmacy.
Methods:

**Preparation of Clonidine loaded micro beads:** In 50 ml of sodium alginate solution, 200 mg of Clonidine was dispersed uniformly. Bubble free dispersion was dropped through a syringe with a needle into 100ml aqueous calcium chloride solution and stirred 500rpm. After stirring for 30minutes, the mirobeads were separated by filtration, washed with distilled water and finely dried at 70°C for 6h in an oven.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Sodium Alginate % (w/v)</th>
<th>Calcium Chloride % (w/v)</th>
<th>HPMC % (w/v)</th>
<th>Chitosan % (w/v)</th>
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</thead>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>F4</td>
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<td>0</td>
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<tr>
<td>F5</td>
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<td>1</td>
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<tr>
<td>F6</td>
<td>2</td>
<td>5</td>
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</table>

**Results and Discussion:**

*In-vitro* Release Profile Study of Formulated microbeads

<table>
<thead>
<tr>
<th>S. No</th>
<th>Time</th>
<th>% Drug Release</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>F1</td>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>7</td>
<td>10</td>
<td>98.659</td>
</tr>
</tbody>
</table>

**Micromeritics studies:** The results of the density of bulkiness and density of tapping were mentioned in table. Bulkiness values were lies in 0.297 to 0.542 g/cm3 and density of tapping values lies in 0.508 to 0.654 g/cm3 i.e. less than 1.2, indicates good packing. The values of Average particle size and angle of repose were lies in between 291.46 ±8.3 to 432.62 ±7.3, and 250-12’ to 300-20’, respectively indicates acceptable particle size, flow property and also good packing ability

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“Strategic Approaches to Strengthen Academic and Industrial Collaboration”

www.ijpsr.com
Figure 1 In-vitro Release Study of Formulated Microbeads of Clonidine

References:
Design and evaluation of controlled release gastroretentive drug delivery system of Bisoprolol

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E-mail address: Mohammedgulzar1@gmail.com

Abstract: The main objective of the dosage form is to improve the gastric residence time, which in turn increases the bioavailability of the drugs. Bisoprolol fumarate is one of cardio selective beta blocker used in the management of hypertension. Floating dosage form which increase the gastric residence time, helps in preventing the degradation of drugs in colon. The optimum formulations were obtained using constrains on duration of buoyancy, $T_{0.5}$ and diffusion coefficient. In this work an attempt was made to formulate the floating dosage form of bisoprolol fumarate. The optimum formulations are obtained from the optimisation process and the evaluation of these formulations showed promising results.

Introduction:
Oral administration is the most convenient and preferred means of drug delivery to the systemic circulation. This method is widely accepted due to its ease of administration and patient acceptance. An ideal drug delivery system is that which possesses two main properties i.e., Spatial placement targeting a drug to specific organ or tissue and Temporal delivery controlling the rate of drug delivery to the targeted tissue. This led to the development of systems such as Sustained and controlled release drug delivery systems. The modulation of gastrointestinal transit time was reported to be one of the methods to formulate the drugs into suitable dosage form with good therapeutic effects [1]. The modulation of gastrointestinal transit time can be achieved using gastric retentive drug delivery devices or systems. Gastric retentive drug delivery system are primarily controlled release drug delivery systems, which gets retained in the stomach for longer period of time, thus helping in absorption of drug for the intended duration of time [2]. Gastric retentive drug delivery devices can be useful for the spatial and temporal delivery of many drugs like, Drugs that act locally in the stomach e.g. antibiotics for bacterially based ulcers, etc. Drugs that are absorbed primarily in the stomach e.g. Albuterol, drugs that are poorly soluble in alkaline pH, drugs that have a narrow window for absorption i.e. drugs that are absorbed mainly from the proximal small intestine e.g. Riboflavin, Levodopa [1, 2].

Bisoprolol is one of cardio selective beta blockers used in the management of hypertension. The maintenance of a constant plasma level of a cardiovascular drug is important for the desired therapeutic response. The formulation is based on factorial design. A gastric retentive drug delivery system of the drug is thought to be better in achievement of good therapeutic response. Instead of normal trial and error method for the search of best formulation,
adaptation of optimisation technique was thought. To study the effect of various factors like drug to polymer ratio, polymer to polymer ratio and polymer grade on the response parameters like duration of buoyancy, \( T_{0.5} \) and release rate kinetics [3, 4].

**Materials and Methods:**
Bisoprolol Fumarate was procured from Yarrow Chem Products, Mumbai. HPMC 4,000, HPMC 10,000 and sodium carboxy methyl cellulose were procured from SD fine chemical, Mumbai, India. All other reagents used were analytical grade.

**Preparation of the dosage form:** The drug and excipients were passed through an 80-mesh screen prior to the preparation of the dosage form. The powders were weighed separately and mixed by geometric dilution. The capsules were hand filled with the total powder content in the capsule weighting constant 100 mg (1). The runs are formulated in a random order to avoid any bias.

**Table 1** Formulation design.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
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</thead>
<tbody>
<tr>
<td>Bisoprolol Fumarate</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HPMC 4,000 c.p.s</td>
<td>-</td>
<td>-</td>
<td>17.5</td>
<td>-</td>
<td>45</td>
<td>-</td>
<td>85</td>
<td>-</td>
<td>50</td>
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</tr>
<tr>
<td>HPMC 10,000 c.p.s</td>
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<td>10</td>
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<td>Sodium CMC</td>
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<td>Lactose</td>
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<td>70</td>
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<td>-</td>
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<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

**Evaluation:** The drug polymer compatibility study and assay for drug content was performed as per the literature [3].

**Duration of buoyancy:** Duration of the buoyancy was determined by USP XXII dissolution vessels. The dosage form was placed under a 10 mess stainless screen in 900ml of simulated gastric fluid (pH 1.2). The paddle was rotated at 50 rpm. The time taken by the dosage form to sink under side of the stainless steel mesh to the bottom of the vessel was noted. The measurement was done by visual observation [4].

**Dissolution study:** The dissolution studies were performed using a USP XII paddle apparatus at a rotational speed of 50 rpm. The temperature was maintained at 37±1°C. 900ml of simulated gastric fluid was used as dissolution media (pH 1.2). About 9 ml of the dissolution media was withdrawn at 0.25hrs, 0.5hrs and at intervals of every hour for 8hrs and same amount is replaced with fresh gastric fluid. The samples withdrawn were analysed for the amount of drug release by UV-spectrophotometric method at 222 nm. The results are subjected for the various kinetic parameters [4].

**Stability studies:** To confirm the stability, best formulations were subjected to short term accelerated stability studies. The study was carried out by keeping the optimised formulation at various temperatures for period of 90 days.

**Results and Discussion:**
FT-IR studies revealed no chemical interaction between drug and excipients, hence compatible. The formulation has shown the 100% of drug content, the buoyancy test has been conducted and it has shown the maximum amount of drug release. The correlation coefficient for the standard curve in ethanol and in stimulated gastric fluid was found to be 0.9993 and 0.9998 respectively. The release rate data to the Korsmeyer and peppas release rate profile (K) ranges from 0.2198 to 0.5222. The diffusion coefficient (n) ranges from 0.536 to 0.7891 and the T_{0.5} ranges from 0.938 to 3.6672 hours, with a correlation coefficient of 0.9843 to 0.9964. The stability study has been conducted for 90 days and there were no changes in the physiochemical properties of the prepared formulation, hence stable.

![Graph](image.png)

**Figure 1** In-vitro dissolution studies

**Conclusion:**

The gastric retentive drug delivery devices can be used for controlled drug delivery systems containing drugs, which are degraded in the colon. Optimisation technique can be used in the pharmaceutical field to obtain desired formulation in minimum numbers of trial and time.

In conclusion, by the application of optimisation technique, optimised formulation can be obtained with minimum expenditure of time and money and the gastric retentive time can be increased for a drug by formulating it in a floating dosage form.

**References:**

**Formulation and evaluation of oral liquid formulation for the treatment of thyroid conditions**

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KCT's R G Sapkal Institute of pharmacy, Anjaneri, Nashik, India  
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**Abstract:** Levothyroxine is a naturally occurring thyroid hormone which is widely prescribed for the treatment of underactive thyroid conditions. As solid dosage form of levothyroxine has a complex stability profile and has been reported to be sensitive to some common excipients, light, temperature, moisture, pH and environmental oxygen. Levothyroxine Oral Liquid formulation is very effective than solid oral dosage form. Preformulation studies were carried out, the oral liquid formulation was developed using the stabilizing agent. The formulation was evaluated for appearance, pH, specific gravity and stability. The physical and chemical parameters were found to be satisfactory.

**Introduction:**

An underactive thyroid (Hypothyroidism) is a condition in which the thyroid glands of an individual are not completely active and do not produce required amount of hormones. It shows signs such as tiredness, weight gain and depression. This condition may be caused due to the immune system attacking the thyroid gland and damaging it. The line of treatment depends upon taking daily hormone replacement tablets called levothyroxine to raise the thyroxin levels in the body. If this condition is not treated properly it may lead to complications such as heart disease, goitre, pregnancy problems and a very rare life threatening condition called myxoedema coma. As a solid dosage form, levothyroxine has a complex stability profile and has been reported to be sensitive to sensitive to some common excipients, light, temperature, moisture, pH and environmental oxygen [1-3].

**Materials and Methods:**

During Pre formulation study stress testing of levothyroxine was performed. Categories of the excipients which were predicted for the master formula of the formulation were, API, Sugars, Preservative, Antioxidants, Viscosity modifier, Alkalizer, Flavouring agent and Solvent. After this the degradation pathway of the drug was determined. After the primary evaluation following method was adopted for the formulation of oral liquid formulation of levothyroxine, first alkalizing property of various alkalizer agents was investigated to determine or choose the best candidate for the formulation. Then this agent was mixed in some quantity of warm water, then sugar was added to the sorbitol solution and temperature was maintained. Then a slurry of magnesium oxide was prepared and to it levothyroxine was added, simultaneously with this a solution of xanthine gum in glycerine was prepared and was kept for half an hour. After this all the solutions were mixed and cooled to
room temperature. After cooling flavouring agent was added to the formulation and volume was made with the solvent. After formulation was prepared various evaluation parameters were selected for assessing the formulation. Those parameters include appearance of the formulation, pH at two levels at initial level and after the estimated time gap, specific gravity of the formulation, assay of the drug or api used in formulation, and most important stability study of the formulation.

Results and Discussion:
After performing the evaluation of available solid dosage form of levothyroxine it was observed that the formulation has a wide range of stability problems associated with some basic environmental parameters. To avoid such stability problems an oral liquid formulation of Levothyroxine was developed. For this formulation some Preformulation studies were carried out and those studies showed that liquid formulation is suitable for levothyroxine. The degradation pathway of levothyroxine is shown in figure no.1.

After Preformulation studies all the excipients were finalised for the formulation and the formulation was prepared as per the methodology adopted for the formulation.

![Degradation pathway](image-url)

**Figure 1** Degradation pathway
**Conclusion:**
Based on the IHE studies and the evaluation parameters, we can formulate an oral liquid dosage form for the treatment of under treated thyroid conditions using levothyroxine.

**References:**
Formulation and characterization of Ivermectin loaded solid lipid nanocarriers for enhanced solubility and better therapeutic activity

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E-mail address: dr.chanchaldeep@gmail.com

Abstract: The present investigation aims to develop and examine the efficacy of Ivermectin loaded solid lipid nanocarriers against human lymphatic filariid Brugia malayi for antifilarial activity. The drug is very poorly soluble in water and has narrow therapeutic index. Homogenization and Ultrasonification method was used to prepare SLN of Ivermectin and further analysed for particle size, drug loading, in vitro release and antifilarial activity. The SLN exhibited a good particle size at a range of 130-150nm, with a drug loading of 33.89% ± 0.12. The in vitro release study showed a sustained effect for 48 hours and also exhibited significant antifilarial activity.

Introduction:
Filaria is a very common tropical disease remained unfocused due to vulnererable nature of organism, the complex anatomy of lymphatic tissues, improper drug outreach, high doses leading to undesired side effects and poor patient compliance [1, 2]. Therefore, an attempt has been made to develop a nanocarrier of antifilarial drug Ivermectin to overcome the problems of poor solubility and higher toxicity.

Materials and Methods:
Poloxamer188 from Sigma, India, Soya lecithin was obtained as a gift sample from Lipoid, Germany, Ivermectin was purchased from Sigma Chemicals Co., USA. Culture media, Luria Broth was also purchased from Sigma Chemicals Co., USA. The solvents (chloroform and methanol) were purchased from Ranbaxy, India.

Preparation of Ivermectin loaded SLN: Homogenization and Ultrasonification method was used to prepare SLN of Ivermectin and further analysed for particle size, zeta potential, FTIR, XRD, and DSC studies. The release of the drug from the SLN was determined and found to be significant for a duration of 48 hours (P=0.05). In order to access the sustainability and bioavailability of drug the pharmacokinetic parameters like peak plasma concentration and area under the curve were observed on Wistar rats.

Results:
In order to find the best optimized formulation with minimum particle size and maximum loading efficiency; the different batches of Ivermectin loaded SLN were characterized and evaluated [3, 4].
Particle size distributions [(mean diameter (nm)], zeta potential, poly dispersity index of Ivermectin loaded SLN were determined by dynamic light scattering using Malvern Zetasizer, ZS nano 90, Malvern Instruments, USA (Table 1). The study showed that the Homogenization and Ultrasonification method yielded uniform spherical solid lipid nanospheres at range of 130-150nm (Figure 1), with a drug loading of 33.89±0.12. The FTIR, XRD, and DSC studies also confirmed the compatibility of the drug with the polymer and copolymer and proved its amorphous nature inside the SLN. The in vitro release study showed a sustained effect for 48 hours with 30% burst release in initial two hours followed by slow sustained release approx. 70% till 48 hours and also exhibited significant antifilarial activity against human lymphatic filarial Brugia malayi in vitro (P=0.05). The pharmacokinetic study also showed that the peak plasma concentration was found to be (57.20 ± 1.58 ng/ml), area under the concentration curve (265 ± 34.2 ng d/ml) and extended mean residence time (22.74 ± 7.5 days) for Ivermectin loaded SLN. The result of the study shows that solubility of Ivermectin is increased with enhanced therapeutic effect.

Table 1 Summarizes the physiochemical characteristic of the prepared Ivermectin loaded SLN and Blank SLN

<table>
<thead>
<tr>
<th>PD Ratio (w/w)</th>
<th>Drug loading (%)</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank SLN</td>
<td>--</td>
<td>125.4±10.5</td>
<td>0.28±0.23</td>
<td>-25.98±2.2</td>
</tr>
<tr>
<td>IV-SLN</td>
<td>33.89 ± 0.12</td>
<td>130.39±7.64</td>
<td>0.34±0.16</td>
<td>-22.43±1.65</td>
</tr>
</tbody>
</table>

Figure 1: Showing TEM of Ivermectin loaded SLN

Conclusion:
The study presented here aims in providing new insight for developing more advanced drug delivery system with the help of nanotechnology for quick diagnosis, control, and elimination of this tropically neglected disease.
Acknowledgements:
The author acknowledges the Chhattisgarh Council of Science and Technology (CGCOST), Raipur, Chhattisgarh for financial assistance and providing facilities to carry out research work.

References:
Exploration of solid dispersion technique for enhancement of solubility

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Abstract: The purpose of present work was to enhance the solubility and dissolution rate of Valsartan drug by solid dispersion (SD) technique using mannitol as carrier. Solid dispersion was prepared by solvent evaporation method. Taking various ratios of drug and carrier as 1:1, 1:1.5, 1:2, 1:2.5 and 1:3, 1:3.5, prepared SD was subjected to analysis of various parameter like % practical yield, % drug content, solubility study (mg/ml) and bulk characterization. Results showed enhanced solubility by SD technique.

Introduction:
Valsartan is marketed in the form of conventional tablet and capsule only; but both oral solid dosage forms have limited solubility and dissolution profile. To overcome this problem manufacturer incorporated high amount of disintegrate (10-80%) which is hazardous to patient and also inflate overall cost of product [1]. The poor stability and low oral bioavailability (BA) with poor aqueous solubility warrants the administration of large dosage of valsartan to maintain desired therapeutic concentration in blood. Poor aqueous solubility can be improved by applying SD technique which will not only improve the solubility but also can improve stability and bioavailability [2]. Hence the main objective of the present work was to improve solubility by means of SD technique. SD can deliver the drug in both solubilize form and in a predictable manner which is independent of pH in gastrointestinal tract [3]. By preparing its SD we can reduce amount of disintegrate in formulation in contrast to present dosage form. Thus it reduces overall cost of the product and prevents the patient form exposure of such a high concentration of disintegrate. SD can also improve flow property by which drug can be compressed in tablet form or it can be suitably dispensed in capsule form [4].

Materials and Methods:
Valsartan was obtained as gift sample from Aurobindo Pharma Ltd., Hyderabad. Mannitol, Methanol, Sodium starch glycolate (SSG), microcrystalline cellulose (MCC), Talc, Magnesium stearate were purchased from Loba chem., Mumbai. All reagents and solvents were of analytical grade and supplied without need to purification.

Preparation of SD: Different SD formulations of valsartan were prepared by solvent evaporation method [5]. Valsartan SD were prepared by using mannitol as carrier in proportions viz. drug: carrier (1:1, 1:1.5, 1:2, 1:2.5 and 1:3, 1:3.5). Finally, dispersions were passed through sieve number 80# and
were stored in air tight containers till further use. Prepared SD formulations were optimized on the basis of different parameters like % practical yield, drug content, solubility studies, and bulk characterization (Table 1).

**Table 1** Different parameter of SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td><strong>% Practical yield</strong></td>
<td>98.42%</td>
</tr>
<tr>
<td><strong>% Drug content</strong></td>
<td>97.93±0.02</td>
</tr>
<tr>
<td><strong>Solubility study (mg/ml)</strong></td>
<td>0.0186</td>
</tr>
<tr>
<td><strong>Angle of repose (°C)</strong></td>
<td>29.78°±0.22</td>
</tr>
<tr>
<td><strong>Bulk density (gm/ml)</strong></td>
<td>0.358±0.008</td>
</tr>
<tr>
<td><strong>Tapped density (gm/ml)</strong></td>
<td>0.414±0.01</td>
</tr>
<tr>
<td><strong>Carr’s index (%)</strong></td>
<td>13.86±0.12</td>
</tr>
<tr>
<td><strong>Hausner’s ratio</strong></td>
<td>1.23±0.002</td>
</tr>
</tbody>
</table>

**Results and Discussion:**

Preparation of SD was carried out by solvent evaporation method and different batches were selected and optimized on the basis of % practical yield, drug content, solubility, and bulk property. The practical yield of formulation SD. Maximum yield was found to be 98.42%, percent drug content of formulations SD. Maximum % drug content was found to be 98.62%, solubility data of pure drug was found to be 0.0089 mg/ml. After solvent evaporation method was determined. Maximum solubility was found to be 0.0269 mg/ml. It was observed that as the concentration of carrier is increased the solubility was increased, the SD prepared with solvent evaporation method displayed greater solubility than pure drug. Optimized batches of SD prepared were found to be fine free flowing powders. Angle of repose, bulk density, tapped density, carr’s index and Hausner’s ratio was calculated and were found to be maximum 26.82°, 0.379 gm/ml, 0.415 gm/ml, 6.26%, 1.08. All the above value showed that the good powder possess good compressibility and optimum flow rate.

**Conclusion:**

To enhance dissolution rate and oral bioavailability of poorly soluble drugs remains one of the most challenging aspects of drug development. In present work SD technique was successfully employed to improve the solubility of valsartan.

**References**

Controlled delivery of Quercetin via nanospheres for mitigating oxidative stress in Rheumatoid Arthritis

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E-mail address: shikha_ceutics@yahoo.co.in

Abstract: The present work aims development of drug delivery system for controlled release of quercetin via nanospheres for the management of rheumatoid arthritis. Quercetin proved to be effective in the management of arthritis. Bioavailability of quercetin is an alarm for such treatment. Polycaprolactone polymer has been used for the preparation of quercetin loaded nanospheres using the solvent evaporation method. The physio-chemical characterisation of polycaprolactone-loaded quercetin nanospheres was carried out to obtain in order about particle size distribution, drug loading efficiency, morphology, and release trends in phosphate-buffered saline at pH 7.4 and 37°C. Quercetin-loaded polycaprolactone nanospheres were found to be biocompatible as evidenced from in vitro and in vivo studies using a Wistar rats. Quercetin release from optimized formulations showed biphasic nature due to initial burst effect followed by a controlled release. These results advised that optimised quercetin-loaded polycaprolactone nanospheres as workable strategy for controlled release of quercetin in the joint cavity for more than 30 days by intra-articular injection to treat rheumatoid arthritis.

Introduction:
RA is characterized by chronic inflammation of the synovial cells lining the joints, abnormal proliferation of cells and by irreversible destruction of the cartilage, tendons and juxta-articular bone that comprise joint structure. Quercetin has many pharmacological effects, such as expanding coronary arteries, lowering blood pressure, anti-inflammation, anti-allergy, anti-platelet aggregation, antitumor, etc [1]. The other pathological change of RA is synovial pannus formation. In India, the rheumatoid arthritis represents a significant health problem. QU inhibits the activity of VEGF, bFGF, MMP-2 and other cytokines, inhibits angiogenesis in multiple links, and inhibits the formation of synovial pannus to certain extent. Thus, QU plays a certain role in relieving RA inflammation and preventing pannus formation. Thus we aimed to developed, a controlled drug delivery system of quercetin which reduce inflammation and reduces patient stress [2, 3].

Materials and Methods:
Quercetin (Sigma aldrich). All other reagents and solvents were of analytical grade. Formulation of Nanospheres [Emulsion solvent diffusion method]. Characterization for Particle size, Drug
entrapment efficiency, in vitro drug release, in vivo characterization (Anti-inflammatory studies, Antiarthritic studies) performed.

Characterization

Morphology and Particle size analysis: Microspheres were characterized by optical microscopy, SEM.

In-vitro release studies: In-vitro release studies were conducted in triplicate and mean values and standard deviations were calculated.

In-vivo characterization: The biocompatibility to the synovium was evaluated using male Wistar rats by injecting 0.1mL of optimised quercetin-loaded PCL nanospheres up to 7–10 mg suspension prepared using saline into the left knee joint, whereas 0.1mL saline was injected into the right knee joint as control.

Results and Discussion:

Characterization of nanospheres: The attractive characteristics of the prepared microspheres are high entrapment efficiency, small particle size and controlled drug release.

In-vitro release studies: In-vitro release data showed that hydrogel showed a typical biphasic release pattern with burst release followed by sustained release. The burst release is mainly due to quercetin actives entrapped on the surface.

In-vivo studies: Significant positive differences (p < 0.01) between treated and control groups were observed at different aspects of diabetic wound healing process. Inflammation and the swelling started to reduce after 6 h and the left knee joints were found to be normal by 8th hour. Histological examinations of rat synovium taken on the 3rd day shows the absence of inflammation and proliferation.

Table 1 Reduction in paw volume

<table>
<thead>
<tr>
<th></th>
<th>2 hr</th>
<th>4 hr</th>
<th>8 hr</th>
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<tbody>
<tr>
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<td>4±234</td>
<td>7±567</td>
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<td>FM 2</td>
<td>3±456</td>
<td>7±123</td>
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<td>FM 4</td>
<td>3±682</td>
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</tr>
<tr>
<td>FM 6</td>
<td>4±467</td>
<td>9±234</td>
<td>18±567</td>
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</table>

Table 2 Reduction in paw Thickness

<table>
<thead>
<tr>
<th></th>
<th>5 days</th>
<th>10 days</th>
<th>15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyrrhizin</td>
<td>5±246</td>
<td>7±376</td>
<td>9±376</td>
</tr>
<tr>
<td>FM 2</td>
<td>6±456</td>
<td>9±236</td>
<td>11±456</td>
</tr>
<tr>
<td>FM 4</td>
<td>7±352</td>
<td>10±654</td>
<td>16±682</td>
</tr>
<tr>
<td>FM 6</td>
<td>8±277</td>
<td>17±277</td>
<td>21±467</td>
</tr>
</tbody>
</table>
Conclusion:
Nanospheres were prepared successfully. Formulations show satisfactory buoyancy, percentage release and entrapment efficiency. Drug release follows erosion with diffusion controlled drug release except few followed burst release. Thus, it is concluded from research are effective for treatment of rheumatoid arthritis. In current scenario traditional medicine combined with novel system appear to be widely accepted in future.

Acknowledgement:
The presenter is thankful to Director, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G), India for this study and UGC-BSR for financial assistance relating to this work.

References:
Formulation and evaluation of medicated Jelly Candy

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E-mail address: adityatanwar6692@gmail.com

Abstract: The goal of the present work is to formulate and evaluate the Medicated Jelly Candy for Motion sickness. The requirement of water is the main problem with solid dosage form, especially tablet. So here we present attractive, tasty and easy to handle medicated jelly candy for treating the motion sickness. In present work formulations were prepared by varying composition of different constituents and the best suitable formula was F2 which has following values: drug pH 6.81±0.02, Spreadibility 6.40±0.51s, viscosity 5852±47cps, % Weight variation 2.62±0.31, % Drug Content 98.67±0.42, drug release 80%, along with stability over period of 3 months.

Introduction:
However, tremendous advancement in drug delivery system, oral route remains the preferred route for the administration of therapeutic agents as low cost of treatment and easy in administration leads to patient compliance and preferred for pediatrics and geriatrics. But major problem associated are stability, dosage wastage, dose dumping and difficulty of administration in children etc. So convenience in administration and patient compliance are gaining significant importance in the design of dosage forms. Medicated jelly today is gaining consideration as a “vehicle” or a “delivery system” to administer active principles that can improve health and nutrition. But it’s potential as an “Alternative drug delivery system” has not been yet fully discovered and exploited. So the present investigation is focused to develop the elegant, acceptable, stable oral medicated jelly, using suitable combination of excipients [1, 2].

Materials and Methods:
Materials: The drug Meclizine di hydrochloride was gifted by the Zest Pharmaceutical, Indore (M.P.) India. Pectin, gelatine and guar gum were purchase from Loba Chemie Pvt. Ltd., Mumbai India. Sodium benzoate, citric acid, glycerine and sucrose purchased from Merk Specialties Pvt. Ltd.
Method: The blank jelly candies were prepared by using gelatine, pectin and guargum, and various combinations of these jellying agents. In the procedure, after weighing, gelling agent glycerine and water were mixed together and heated to form molten mass. Sucrose was powdered and passed through sieve number 100. Powdered sucrose was dissolved in molten mass, and temperature was controlled up to 90°C. Colouring, flavouring agents and preservative were added respectively. Then molten mass was poured in to the jelly candy moulds to get solidified. Meclizine loaded jellies were prepared using various gelling agents like gelatine, pectin, gelatine-pectin combination and guar gum...
in various concentrations. 5% of ethanol was used to dissolve meclizine by changing formulation variable parameter [3].

Results:

Table 1 Formula of medicated Jelly Candy.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>F2</th>
<th>F8</th>
<th>F16</th>
<th>F19</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gelatine</td>
<td>6%</td>
<td>-</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>2</td>
<td>Pectin</td>
<td>-</td>
<td>1.2%</td>
<td>0.9%</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Sucrose</td>
<td>4.5 g</td>
<td>4.5 g</td>
<td>4.5 g</td>
<td>4.5 g</td>
</tr>
<tr>
<td>5</td>
<td>Glycerine</td>
<td>9 mL</td>
<td>9 mL</td>
<td>9 mL</td>
<td>9 mL</td>
</tr>
<tr>
<td>6</td>
<td>Water</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>7</td>
<td>Sodium Benzoate</td>
<td>0.05 gm</td>
<td>0.05 gm</td>
<td>0.05 gm</td>
<td>0.05 gm</td>
</tr>
<tr>
<td>8</td>
<td>Colouring Agent</td>
<td>qs</td>
<td>0.05 gm</td>
<td>qs</td>
<td>qs</td>
</tr>
<tr>
<td>9</td>
<td>Flavouring Agent</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
</tr>
<tr>
<td>10</td>
<td>Drug</td>
<td>25 mg</td>
<td>25 mg</td>
<td>25 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>11</td>
<td>Ethanol</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>10</td>
<td>Temperature</td>
<td>90°C</td>
<td>90°C</td>
<td>90°C</td>
<td>90°C</td>
</tr>
</tbody>
</table>

Table 2 Evaluation of medicated Jelly Candy.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Wt variation</th>
<th>% Drug Content</th>
<th>pH</th>
<th>Viscosity (CPS)</th>
<th>Spreadibility (S)</th>
<th>Syneresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>2.62±0.31</td>
<td>98.67±0.42</td>
<td>6.81±0.02</td>
<td>5852±47</td>
<td>6.40±0.51</td>
<td>-</td>
</tr>
<tr>
<td>F8</td>
<td>1.81±0.44</td>
<td>99.78±0.51</td>
<td>6.62±0.01</td>
<td>4845±53</td>
<td>5.02±0.23</td>
<td>-</td>
</tr>
<tr>
<td>F16</td>
<td>2.69±0.25</td>
<td>97.61±0.72</td>
<td>6.75±0.05</td>
<td>9987±41</td>
<td>12.01±0.11</td>
<td>-</td>
</tr>
<tr>
<td>F19</td>
<td>3.01±0.12</td>
<td>98.01±0.15</td>
<td>6.95±0.04</td>
<td>6852±31</td>
<td>7.08±0.32</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3 In-Vitro studies of medicated Jelly Candy F2.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>Absorbance</th>
<th>Concentration µg/ml(x)</th>
<th>X*5</th>
<th>X*900</th>
<th>Cr in µg</th>
<th>Cr in mg</th>
<th>% cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1487</td>
<td>8.85321</td>
<td>14.42661</td>
<td>2596.789</td>
<td>2596.789</td>
<td>2.5967</td>
<td>10.387</td>
</tr>
<tr>
<td>20</td>
<td>0.3045</td>
<td>6.458716</td>
<td>32.29358</td>
<td>5812.844</td>
<td>5827.27</td>
<td>5.8272</td>
<td>23.309</td>
</tr>
<tr>
<td>30</td>
<td>0.4689</td>
<td>10.22936</td>
<td>51.14679</td>
<td>9206.422</td>
<td>9253.14</td>
<td>9.2531</td>
<td>37.012</td>
</tr>
<tr>
<td>40</td>
<td>0.7405</td>
<td>16.45872</td>
<td>82.29358</td>
<td>14812.84</td>
<td>14910.71</td>
<td>14.910</td>
<td>59.642</td>
</tr>
<tr>
<td>50</td>
<td>0.9041</td>
<td>20.21101</td>
<td>101.055</td>
<td>18189.93</td>
<td>18320.06</td>
<td>18.320</td>
<td>73.280</td>
</tr>
<tr>
<td>60</td>
<td>0.9851</td>
<td>22.06881</td>
<td>110.344</td>
<td>19861.93</td>
<td>20143.14</td>
<td>20.143</td>
<td>80.572</td>
</tr>
</tbody>
</table>

Table 4 Stability studies of medicated Jelly Candy.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature (°C)</th>
<th>Viscosity (CPS)</th>
<th>pH</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>8±1</td>
<td>6.82±0.05</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>5852±47</td>
<td>6.81±0.02</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>40±2</td>
<td>6.72±0.04</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>F8</td>
<td>8±1</td>
<td>6.60±0.02</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>4845±53</td>
<td>6.62±0.01</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>40±2</td>
<td>6.58±0.01</td>
<td>No change</td>
<td>decrease</td>
</tr>
</tbody>
</table>
Conclusion:
The present research was aimed to formulate and evaluate the medicated jelly candy for motion sickness. Formulation F2 which was formulated only with gelatin was found to have the best drug release properties and consistency among all other formulation. Gelatin was found to be most suitable candidate for jelly candy preparation in comparison to pectin. Gelatin formulation was having better consistency, viscosity, spreadibility, pH stability, and homogeneity and in-vitro drug release. Sodium benzoate is used as the preservative and the citric acid is used as the preservative too with the acidifier effect. This both the candidate enhances the stability of the medicated jelly candy and gives the better formulation. Glycerin was added to the formulation to give the additive effect along with the sucrose. Process variable studies were also carried out. In this study gelatin concentration was kept constant and heating temperature was kept variable. In this study processing temperature was also optimized. So from the above studies it was concluded that jelly candy is a better form of formulation in case of motion sickness as it is palatable easy to administered along with all age group patient compliance.

Acknowledgments:
We gratefully acknowledge all the faculty members of College of Pharmacy IPS Academy for their support and motivation.

References:
Formulation and evaluation of microspheric tablets of Artemether and Lumefantrine

Sheetal Mane, Dinesh Kumar Jain
College of Pharmacy, IPS Academy, Indore-452012 (M.P.), India
E-mail address: manesheetal25@gmail.com

Abstract: The objective of the present study was to fabricate and evaluate a combination of Artemether and Lumefantrine as tablets and to make Artemether in sustained form so as to prolong its elimination time. Artemether was formulated in form of microspheres and was then formed into the tablet along with the Lumefantrine. Artemether microspheres were prepared and compressed into compressible tablet by direct compression process using the compressible excipients along with Lumefantrine, and was further evaluated for various parameters such as hardness, thickness, weight variation, friability, drug content, in vitro drug release and stability.

Introduction:
Malaria is a major health problem with at least 300 to 500 million people diagnosed with the illness every year [1]. Due to the high resistance of Plasmodium falciparum, there has been the urgent need for drug combination therapy. Artemisinin- based combination treatments have the most potent and rapid onset of anti-parasitic activity and are active against all Plasmodium species that infect humans. Artemether-Lumefantrine is one such drug combination. Artemether has a rapid onset of action and is rapidly eliminated from the plasma. Lumefantrine is cleared more slowly and has a longer elimination half life. The rationale behind this combination is that artemether initially provides rapid symptomatic relief by reducing the number of parasites present before Lumeфанtrine eliminates any residual parasites [2-4].

Materials and Methods:
Materials: Artemether, Lumefantrine, Ethyl Cellulose, Polyvinyl alcohol (PVA), Distilled water, MCC, Sodium Starch Glycolate, Croscarmellose Sodium, Magnesium Stearate, Colloidal Silicon Dioxide.

Methods: Artemether microspheres were obtained by solvent evaporation technique. Polymer was dissolved in dichloromethane and then the drug was added to the above solution. This solution was injected into the PVA solution maintained at variable speed using mechanical stirrer. Stirring was continued until all the dichloromethane evaporated. The formed microspheres were collected by filtration and washed with n-Hexane and dried. Tablets of Artemether microspheres and Lumefantrine were prepared by direct compression technique. The corresponding amount of Artemether microspheres equivalent to 20 mg drug, Lumefantrine, MCC, superdisintegrants, magnesium stearate
and colloidal silicon dioxide were accurately weighed and blended and allowed for direct compression into tablets weighing 300mg using a tablet punching machine with 8 mm flat faced punches.

**Results:**

<table>
<thead>
<tr>
<th>FC</th>
<th>ART microsphere eq. to</th>
<th>LUM</th>
<th>MCC</th>
<th>SSG</th>
<th>CCS</th>
<th>Mag. Stearate</th>
<th>Colloidal Silicon Dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1</td>
<td>20</td>
<td>120</td>
<td>101</td>
<td>9</td>
<td>-</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>MT2</td>
<td>20</td>
<td>120</td>
<td>98</td>
<td>12</td>
<td>-</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>MT3</td>
<td>20</td>
<td>120</td>
<td>95</td>
<td>15</td>
<td>-</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>MT4</td>
<td>20</td>
<td>120</td>
<td>91</td>
<td>-</td>
<td>9</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>MT5</td>
<td>20</td>
<td>120</td>
<td>88</td>
<td>-</td>
<td>12</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>MT6</td>
<td>20</td>
<td>120</td>
<td>85</td>
<td>-</td>
<td>15</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

FC= Formulation codes, MT= Codes for microspheric tablets, ART= Artemether, LUM= Lumefantrine, MCC= Microcrystalline cellulose, SSG= Sodium starch glycolate, CCS= Croscarmellose sodium

**Table 2 Evaluation parameters of microspheres.**

<table>
<thead>
<tr>
<th>FC</th>
<th>Percentage Yield</th>
<th>Entrapment Efficiency (%)</th>
<th>Particle Size (µm)</th>
<th>Cumulative Release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>86</td>
<td>67.42</td>
<td>19.10</td>
<td>61.95</td>
</tr>
<tr>
<td>M2</td>
<td>88.33</td>
<td>67.81</td>
<td>21.00</td>
<td>69.01</td>
</tr>
<tr>
<td>M3</td>
<td>91.73</td>
<td>52.81</td>
<td>14.80</td>
<td>76.72</td>
</tr>
<tr>
<td>M4</td>
<td>84.06</td>
<td>68.39</td>
<td>23.80</td>
<td>76.45</td>
</tr>
<tr>
<td>M5</td>
<td>71.88</td>
<td>74.42</td>
<td>26.10</td>
<td>71.02</td>
</tr>
<tr>
<td>M6</td>
<td>90.11</td>
<td>67.07</td>
<td>18.95</td>
<td>79.48</td>
</tr>
</tbody>
</table>

FC= Formulation codes, M= Codes for microspheres

**Table 3 Evaluation parameters of tablets**

<table>
<thead>
<tr>
<th>FC</th>
<th>Hardness (kg/cm²)</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
<th>Friability (%)</th>
<th>Weight variation (mg)</th>
<th>Drug Content (%)</th>
<th>Drug Release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1</td>
<td>4.67</td>
<td>4.86</td>
<td>9.06</td>
<td>0.80</td>
<td>302.00</td>
<td>98.13</td>
<td>63.55</td>
</tr>
<tr>
<td>MT2</td>
<td>5.33</td>
<td>4.88</td>
<td>9.06</td>
<td>0.85</td>
<td>300.90</td>
<td>98.62</td>
<td>72.84</td>
</tr>
<tr>
<td>MT3</td>
<td>6.00</td>
<td>4.83</td>
<td>9.07</td>
<td>0.92</td>
<td>303.24</td>
<td>97.94</td>
<td>79.73</td>
</tr>
<tr>
<td>MT4</td>
<td>5.00</td>
<td>4.85</td>
<td>9.05</td>
<td>0.85</td>
<td>301.00</td>
<td>98.64</td>
<td>80.02</td>
</tr>
<tr>
<td>MT5</td>
<td>5.33</td>
<td>4.87</td>
<td>9.08</td>
<td>0.86</td>
<td>302.89</td>
<td>99.33</td>
<td>70.13</td>
</tr>
<tr>
<td>MT6</td>
<td>4.67</td>
<td>4.88</td>
<td>9.06</td>
<td>0.85</td>
<td>301.75</td>
<td>98.77</td>
<td>81.03</td>
</tr>
</tbody>
</table>

FC= Formulation codes, MT= Codes for microspheric tablets

**Figure 1 Cumulative release of microspheres**
Discussion:
It depicts that the release of artemether either from microspheres or from the tableted microspheres was found to be relatively near about same. M6 formulation showed better sustained effect over a period of 10 hours in comparison to other formulations.

Conclusion:
The release from tablets was slightly greater than the release from the untableted microspheres which may be due to a slight rupture or fracture of microspheres on tableting. The microspheres retained their sustained release properties.

References:
Development and evaluation of self emulsifying drug delivery system for a suitable antibacterial agent

Praveen Kumar Sharma, Mousumi Kar, Dinesh Kumar Jain
College of Pharmacy, IPS Academy, Indore, Madhya Pradesh, 452012
E-mail address: sharmapraveen138@gmail.com

Abstract: Self-emulsifying drug delivery system (SEDDS) was developed to overcome problems associated with the delivery of the anti bacterial drug Cefpodoxime Proxetil (CFP). Solubility of CFP in oil phase and surfactants was determined to identify components of SEDDS. The optimized globule size was 299.7 nm and the highest entrapment efficiency was found to be 101.24 and the percentage drug release was about 90%. The antibacterial property of the prepared formulation was found to be comparable with the marketed preparation. Hence, it is expected that the formulation of SEDDS for the antibacterial drug will be helpful in increasing the efficacy of the drug by improving the rate and extent of absorption.

Introduction:
SEDDS are defined as isotropic mixture of lipid, surfactant, co-surfactant and drug substance that rapidly form a microemulsion upon mixing with water [1]. A nano droplet size distribution is often seen with a droplet size typically less than 50 nm. SEDDS offer an improvement in both rate and extent of absorption by increasing the solubilisation of lipophilic drug [2] & promote the wide distribution of the drug through the GIT thereby minimizing irritation [3].

Materials and Methods:
Materials: Cefpodoxime proxetil, Tween and Span 80, Castor oil, Olive oil and Liquid paraffin.
Methods: Preparation of Cefpodoxime Proxetil SEDDS was done by mixing method. The drug was dissolved in oil and then Tween and Span were added slowly with gentle stirring until homogeneous mixture was formed. This mixture was then stored under ambient conditions until use [4].

Results and Discussion:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Castor oil (%)</th>
<th>Olive oil (%)</th>
<th>Liquid paraffin (%)</th>
<th>Tween (%)</th>
<th>Span (%)</th>
<th>Drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>500</td>
</tr>
<tr>
<td>F2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>F3</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>15</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>F4</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>14</td>
<td>14</td>
<td>200</td>
</tr>
<tr>
<td>F5</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>14</td>
<td>200</td>
</tr>
<tr>
<td>F6</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>15</td>
<td>200</td>
</tr>
<tr>
<td>F7</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>13</td>
<td>200</td>
</tr>
<tr>
<td>F8</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>14</td>
<td>6</td>
<td>200</td>
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<tr>
<td>F9</td>
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<td>10</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>F10</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>12</td>
<td>8</td>
<td>200</td>
</tr>
</tbody>
</table>
Figure 1  Emulsification Time

Figure 2  Size Distributions

F7 shows better emulsification time of five second as compared with other formulations that exhibited emulsification time ranging from 12.5 sec & the size distributions remained in the range of 299.7 to 353.2 nm values for all batches.

Figure 3  Zeta Potential

The zeta potential was present for all the formulations in the range of -5.78 mV to -8.99 mV.

Figure 4  Percentage entrapment efficiency

Figure 5  Ternary phase diagram

The % entrapment of the formulations was good indicating no significant dug loss during formulation.
Figure 6 In-vitro dissolution studies.

At 75 rpm, after 6 hr F7 shows higher release as compared to other formulations, (≈90%). The antibacterial studies for three best formulations were done (F6, F7 and F8) were done and the results obtained were satisfactory.

Figure 7 TEM analysis

Conclusion:

From the above investigation, it was concluded that the SEDDS could be successfully formulated to improve the solubility of Cefpodoxime Proxetil using suitable surfactant and co surfactant. The formulation F7 showed promising improvement in the dissolution characteristics and thus there is possibility of enhancement in the bioavailability of Cefpodoxime Proxetil.

References:

Formulation & optimization of sustained release Glimepiride nanoparticles

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Abstract: Glimepiride is practically insoluble in water as a result it shows erratic oral absorption. The purpose of this investigation was to increase & sustain the dissolution for Glimepiride by formulating nanosuspension using Eudragit RL100 polymer. A $3^2$ randomized full factorial design was used to optimize the influence of polymer and sonication time. The obtained result showed that nanoparticles increased the extent of dissolution and retarded the release rate. The drug to polymer ratio was controlling factor for release of the drug and extent of sonication controlled the particle size.

Introduction:
Poor aqueous solubility is the major hurdle for formulation development. Nanoparticulate drug delivery systems have been utilized for the enhancement of solubility and dissolution rate of poorly soluble drugs. Glimepiride is practically insoluble in water as a result it shows erratic oral absorption and has half life of 3-5 hrs. In present study Glimepiride nanoparticles were prepared with Eudragit RL 100 by nanoprecipitation method. The amount of polymer and the process parameter were optimized by using $3^2$ full factorial designs [1-3].

Materials & Methods:
Glimepride was received as gift samples from Amsal Chem Pvt. Ltd. Mumbai. Eudragit RL100 was purchased from Research Lab. Poloxamer 407 was purchased from sigma Aldrich and Methanol from Lobachemie, Mumbai; all excipient were of AR grade and used as received.

Preparation of Nanoparticles: Glimepiride loaded EudragitRL100 nanoparticles were prepared by dissolving drug & Eudragit RL100 in 20ml methanol separately; both solutions were then mixed at room temperature. The prepared organic solution was injected into the aqueous phase containing 1% w/v poloxamer 407 kept over magnetic stirrer & sonicated for predetermined time interval, the organic solvent was evaporated using rotary evaporator.

Results and Discussion:
Glimepiride loaded Eudragit RL100 Nanoparticles were prepared by nanoprecipitation method. The prepared formulations were subjected for evaluation parameters such as entrapment efficiency, particle size, zeta potential drug content, PDI, in- vitro dissolutions study, Infrared spectroscopy, DSC and X-ray diffraction.
### Table 1 Results of evaluation parameters.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Variables</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug: Polymer ratio (% W/W)</td>
<td>Sonication Time (Min)</td>
</tr>
<tr>
<td>GNS1</td>
<td>1:2</td>
<td>30</td>
</tr>
<tr>
<td>GNS2</td>
<td>1:2</td>
<td>45</td>
</tr>
<tr>
<td>GNS3</td>
<td>1:2</td>
<td>60</td>
</tr>
<tr>
<td>GNS4</td>
<td>1:4</td>
<td>30</td>
</tr>
<tr>
<td>GNS5</td>
<td>1:4</td>
<td>45</td>
</tr>
<tr>
<td>GNS6</td>
<td>1:4</td>
<td>60</td>
</tr>
<tr>
<td>GNS7</td>
<td>1:6</td>
<td>30</td>
</tr>
<tr>
<td>GNS8</td>
<td>1:6</td>
<td>45</td>
</tr>
<tr>
<td>GNS9</td>
<td>1:6</td>
<td>60</td>
</tr>
</tbody>
</table>

Entrapment efficiency of the formulations increased as the amount of polymer increased which can be attributed to more amount of polymer for encapsulation of the drug. It was found that the drug release was in the range of 75-101%. As the polymer concentration increased the release was retarded. The release showed initial burst resale attributed to unbounded drug on the walls of nanoparticles. DSC study And XRD study revealed that the drug polymer complex converted the drug to partial amorphous form which increased the solubility of the complex and dissolution of the drug

![XRD Patterns of optimized batch GNS5.](image1)

![DSC study](image2)
**Conclusion:**
The present study showed the suitability Eudragit RL100 as a carrier for the preparation of Glimepiride Nanoparticles. The significant effects of the interaction and polynomial variables on the investigated characteristics of Glimepiride nanoparticles were verified using \(3^2\) randomized full factorial designs.

**References:**
Abstract: The objective of the present investigation was to formulate, evaluate and optimize the mouth dissolving film of Escitalopram oxalate to achieve fast release and quick disintegration of the drug for depressant and anxiety patients. In the present study, HPMC E15 was used as film forming polymer that showed rapidly disintegration time of film in saliva fluid. These formulations were evaluated for the parameters like drug excipient compatibility study, uniformity of weight, thickness, tensile strength, content uniformity, folding endurance, in-vitro drug release and accelerated stability studies. On the basis of preliminary results, the amount of HPMC E 15 (X₁) and the amount of Propylene glycol (X₂) were chosen as independent variables in $3^2$ full factorial design, while Disintegration time (DT), Tensile strength (TS) and Cumulative % drug release after 10 min (%CDR) were taken as dependent variables. Multiple linear regression analysis was performed using Demo version of Design Expert. Check point batch was prepared to validate the evolved model. In the present study, the following constraints were arbitrarily used for the selection of an optimized batch: DT < 42 sec, TS between 1.0 to 1.2 kg/cm², and %CDR > 98. Batches F₁, F₂, F₄ and F₇ met the selection criteria. Batch F₁ was selected as an optimized batch. Skin permeation study of the Batch F₁ exhibited 95.43 % of drug permeation in 10 min. The optimized batch F₁ was found to be stable in the stability evaluation.

Introduction:
Oral route of administration is one of the most convenient and preferred routes of administration. Various bio-adhesive mucosal dosage forms have been developed, which includes adhesive tablets, gels, ointments, patches and more recently used of polymeric film for buccal delivery also known as Mouth Dissolving Film. It consists of a very thin film, that employs a water-dissolving polymer which allows the dosage form to quickly hydrate, adhere and dissolve to release the drug when place on the tongue or the oral cavity without need of water [1-4].

Materials and Methods:
Materials and Reagents: Escitalopram oxalate was received as a generous gift from Mepro Pharmaceutical Pvt. Ltd., Wadhwan, Gujarat. All other materials and chemicals used were of either pharmaceutical or analytical grade.
Formulation of mouth dissolving film containing Escitalopram oxalate: Mouth dissolving film of Escitalopram oxalate was prepared using hydrophilic polymers by solvent casting method. The resultant viscous solution was casted in petridish and it was dried. The film was carefully removed from the petridish by forceps and cut into 3 cm x 2 cm in size. Each film contained 5 mg of Escitalopram oxalate. The sample was stored in a desiccator maintained at a temperature of 30°±1°C and relative humidity 60±5% [5].

Preliminary screening of mouth dissolving polymers: Preliminary study of different polymers was carried out to check its effect on release profile of mouth dissolving formulation.

Optimization of variables using full factorial design: A $3^2$ full factorial design was used in the present study. On the basis of preliminary results, the amount of HPMC E 15 ($X_1$) and the amount of Propylene glycol ($X_2$) were chosen as independent variables in $3^2$ full factorial design, while Disintegration time (DT), Tensile strength (TS) and Cumulative % drug release after 10 min (%CDR) were taken as dependent variables. Multiple linear regression analysis and contour plots were performed using Demo version of Design Expert. The experimental runs and measured responses of $3^2$ factorial design batches of Escitalopram oxalate were depleted in Table 1.

Evaluation of parameters of mouth dissolving film: Thickness, Tensile Strength, % Elongation, Folding endurance and Drugcontent of the formulations were measured as described by Kalyan S et.al, Yellangi SK et.al, Juluru N et.al, Ciluzzo F et.al, and Chauhan S et.al, respectively.

Results and Discussion:

Preliminary study: The results of preliminary study revealed that HPMC E5 or PVP K30 alone was not sufficient to achieve the desired release profile. Hence, further trials were carried out using combination of HPMC E15 and propylene glycol in order to understand their effect and to optimize concentration of both for desired release profile.

Full factorial design batches: On the basis of preliminary study, the amount of HPMC E15 and propylene glycol were used as independent variables in a $3^2$ full factorial design. The Disintegration Time (DT), Tensile strength (TS) and Cumulative % drug release after 10 min (%CDR) were taken as dependent variables as $Y_1$, $Y_2$ and $Y_3$, respectively (Table 1). Regression analysis was carried using MS Excel and the full and reduced models for response variables were used to draw response surface plots. The mathematical model was validated by formulating check point batch.

Table 1 Runs and measured responses of $3^2$ factorial design of mouth dissolving film

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>(Amount of HPMC E15) $X_1$</th>
<th>(Amount of PG) $X_2$</th>
<th>Disintegration time (sec) $Y_1$</th>
<th>Tensile Strength (kg/cm$^2$) $Y_2$</th>
<th>%CDR $Y_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
<td>40.21</td>
<td>1.0</td>
<td>99.58</td>
</tr>
<tr>
<td>F2</td>
<td>0</td>
<td>-1</td>
<td>44.37</td>
<td>1.1</td>
<td>98.15</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>-1</td>
<td>45.31</td>
<td>1.2</td>
<td>95.43</td>
</tr>
<tr>
<td>F4</td>
<td>-1</td>
<td>0</td>
<td>43.82</td>
<td>1.1</td>
<td>98.12</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
<td>47.72</td>
<td>1.3</td>
<td>97.78</td>
</tr>
<tr>
<td>F6</td>
<td>1</td>
<td>0</td>
<td>50.31</td>
<td>1.4</td>
<td>93.23</td>
</tr>
</tbody>
</table>
Factors and the levels in the design

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of HPMC E15 (X1), mg</td>
<td>Low (-1)</td>
</tr>
<tr>
<td></td>
<td>Medium (0)</td>
</tr>
<tr>
<td></td>
<td>High (1)</td>
</tr>
<tr>
<td>Amount of Propylene glycol (X2), ml</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
</tr>
</tbody>
</table>

**Selection of optimized batch in factorial design study:** In the present study, the following constraints were arbitrarily used for the selection of an optimized batch: DT < 42 sec, TS between 1.0 to 1.2 kg/cm², and % CDR > 98. Batches F₁, F₂, F₄ and F₇ met the selection criteria. Batch F₁ showed lowest disintegrating time (40 sec) and more % cumulative drug release (99.58) after 10 min, hence Batch F₁ was selected as an optimized batch. The optimized formulation was subjected to accelerated stability study. The optimized batch F₁ was found to be stable in the stability evaluation.

**Conclusion:**

The mouth dissolving film of Escitalopram oxalate was prepared to achieve quick disintegration and fast release. The formulations were evaluated for the parameters like uniformity of weight, thickness, tensile strength, content uniformity, folding endurance, *in-vitro* drug release and accelerated stability studies. On the basis of preliminary results, the amount of HPMC E15 (X₁) and the amount of Propylene glycol (X₂) were selected as independent variables in 3² full factorial design, while Disintegration time (DT), Tensile strength (TS) and Cumulative % drug release after 10 min (%CDR) were taken as dependent variables. Multiple linear regression analysis was performed using Demo version of Design Expert. Check point batch was prepared to validate the evolved model. Batch F₁ was selected as an optimized batch which was found to be stable in the stability evaluation.

**Acknowledgements:**

We sincerely thank to Mepro Pharmaceutical Pvt. Ltd., Surendranagar, Gujarat, India for providing us a gift sample of Escitalopram oxalate. We are also thankful to Nootan Pharmacy College, Visnagar, Gujarat, India for providing the facilities of FTIR and DSC.

**References:**

Formulation, development and evaluation of Lansoprazole microspheres

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Abstract: The micro particulates delivery system are considered and accepted as a reliable means to deliver the drug to the target site with specificity and to maintain the desired volume at the site of interest without untoward effects. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 μm. In the present study an attempt has been made to reduce the dosing frequency and side effects associated with using dosage form as LPZ. In this study reveal that achievements of complete release of drug with Co-operation of excipients and various formulation batches were prepared using the different ratios of LPZ : HPMC K15M: EC in order to achieve better release pattern matching with the help of comparative study of Lansec 30 marketed formulation.

Introduction:
The research these days is focused on improving the bioavailability of drugs and making these drug formulations does their therapeutic best. To achieve the therapeutic effect conventional dosage forms have to be administered as repeated doses. The multiple dosing would give rise to a number of problems, which can be solving by sustained release drug formulations. Wide ranges of possible method are available for the development of controlled release dosage form, micro-encapsulation and microsphere being one of them. The concepts of micro-encapsulation have been developed to an unimaginable extent since its introduction for manufacturing carbonless copy paper and later photosensitive paper. [1-5]
The objectives of oral sustained release formulations are:
- To attain blood concentration of the drug rapidly, this will elicit the desired.
- To maintain the plasma concentration at constant level for a desired period of time.
- To reduce the doses administrated as compared to conventional dosage form.
- To give a uniform biological response and reduce the incidence of side effects.
- Therapeutics effects.

Materials and Methods:
Lansoprazole, ethyl cellulose, ethanol, dichloromethane, HPMC, tween 80, light liquid paraffin, petroleum ether.

Method: The polymer ethyl cellulose and hydroxyl propyl methyl cellulose was dissolved in the mixture of ethanol and dichloromethane (1:1). The drug Lansoprazole was dispersed in step 1 for 10
minutes under stirring at 200 rpm. The resulting dispersion of step 2 was poured slowly under stirring into liquid paraffin (dispersion medium) containing 0.01% of Tween 80 and the stirring speed was maintained at 600 rpm with gentle heating by means of heating plate at about 45°C-50°C and stirring was continued for 2 to 5 hrs and allow to evaporate dichloromethane and ethanol completely. After evaporation of dichloromethane and ethanol, the microspheres formed were collected by filtration under vacuum, and then washed 3 to 4 times with petroleum ether. After step 4 the microspheres are washed 2 times with distilled water and dried at room temperature for 24 hrs. A mechanical stirrer (Mechanical stirrer, REMI, India) with a blade of 3.5 cm diameter were used.

**Result and Discussion:**

![Dissolution Profile of Stability Batch F8-1](image)

**Figure 1 In-vitro** drug release profile of stability for batch F8-1 after 3 months.

![Dissolution Profile of Stability Batch F8-2](image)

**Figure 2 In-vitro** drug release profile of stability for batch F8-2 after 3 months.

![Dissolution Profile of Stability Batch F8-3](image)

**Figure 3 In-vitro** drug release profile of stability for batch F8-3 after 3 months.

**References**


Designing of novel ophthalmic formulation containing permeation enhancers for the treatment of eye infections

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Abstract: Ocular drug delivery has remained as one of the most taxing for pharmaceutical scientists. The unique structure of the eye restricts the entry of drug molecules at the required site of action. Conventional drug delivery systems; suffer with the problems such as poor corneal permeability, naso-lacrimal drainage, systemic absorption and blurred vision. The present study performed to see the effect of permeation enhancers in Ciprofloxacin eye drop. The developed formulation is a viable alternative to conventional eye drops for treatment of eye infections. The bioavailability of ciprofloxacin can be improved by addition of penetration enhancers.

Introduction:
Ocular drug delivery has remained as one of the most fascinating area for Pharmaceutical scientists. The unique structure of the eye restricts the entry of drug molecules at the required site of action [1]. In the earlier period, drug delivery to the eye has been limited to topical application [2]. There is only 3 to 4% ocular bioavailability after topical administration with traditional eye drops. Conventional aqueous eye drops not suitable for lipophilic drugs (40–60% of new chemical entities) [3]. The present study was performed to study the effect of permeation enhancers in Ciprofloxacin eye drop.

Materials and Methods:
Pure culture of S. aureus, E.coli was obtained from Mata Gujri College of Professional Studies; Indore M.P. was used for antimicrobial activity. Ciprofloxacin, Polyethylene Glycol, EDTA, Sodium chloride, Nutrient broth and other chemicals of analytical grade were used. CIPLOX eye drop was used as Standard to compare the formulations.

Methods: $2^2$ factorial designs for formulation development of eye drop were used. The optimized formulations were tested for sterility using Streak plate method.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin Hydrochloride</td>
<td>0.3%w/v</td>
<td>0.3%w/v</td>
<td>0.3%w/v</td>
<td>0.3%w/v</td>
<td>0.3%w/v</td>
</tr>
<tr>
<td>EDTA</td>
<td>30mg</td>
<td>20mg</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>PG</td>
<td>---</td>
<td>---</td>
<td>0.3ml</td>
<td>0.1ml</td>
<td>---</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>20mg</td>
<td>20mg</td>
<td>20mg</td>
<td>20mg</td>
<td>20mg</td>
</tr>
<tr>
<td>Distilled water (upto 100 ml)</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
</tbody>
</table>
Results and Discussion:
The results were similar to the reported data in official compendia hence the procured drug sample were considered as pure and used for the further studies.

Table 2 Tests to evaluate optimized formulations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Methods</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calibration method of ciprofloxacin in Saline Phosphate Buffer (pH-7.4) at 272 nm.</td>
<td>Linear plot, $R^2 = 0.997$</td>
<td>Determine the strength of optimized formulation.</td>
</tr>
<tr>
<td>2</td>
<td>Solubility analysis</td>
<td>Soluble in PG, EDTA</td>
<td>Clear, no precipitation seen</td>
</tr>
<tr>
<td>3</td>
<td>pH determination</td>
<td>Stable at 7.4</td>
<td>Maximum activity</td>
</tr>
<tr>
<td>4</td>
<td>Sterility test (Streak plate method)</td>
<td>No growth seen</td>
<td>Pure and free from contamination</td>
</tr>
<tr>
<td>5</td>
<td>Zone of inhibition (Agar Diffusion Method)</td>
<td>F3 formulation is highly effective in S. aureus and E. Coli.</td>
<td>Compare with standard eye drop.</td>
</tr>
</tbody>
</table>

Conclusion:
The developed formulation is a viable alternative to conventional eye drops. Also ease and decreased frequency of administration result in better patient compliance. The optimized formula (F3) showed higher zone of inhibition among all formulations when compared with Standard eye drop solution (CIPLOX). Hence, it can be concluded that the ophthalmic formulation containing permeation enhancer (PG) can be employed to improve the bioavailability of a highly soluble drug showing first-pass metabolism. However, further studies in higher animals and humans need to be performed before this formulation can be commercially exploited.

Figure 1 Graphical representation of bacterial growth in S. aureus
Figure 2 Graphical representation of bacterial growth in *E. Coli*

Acknowledgement:
I am highly acknowledged the Dr. Mamta Shrivastav, Principal and HOD Dr. Sadhana Nighojkar (MGCPS, Indore) for providing us a microbial culture for antimicrobial testing.

References:
Determination of targeting potential of Galactose anchored liposomes in the delivery of Artemether

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Abstract: The present study was aimed to determine targeting efficacy of nanocarrier system for delivery of anti malarial agent to hepatic parenchymal cells. Galactose anchored liposomes were chosen for present research and prepared by rotary evaporation method. Prepared formulations were characterized for vesicle size and shape, in vitro drug release, hepatic cellular accumulation and in vivo plasma and tissue distribution. These were also examined for any possible harmful changes in blood cells and liver enzymes. Results of in vitro drug release indicate that Galactose conjugated liposomes show extended release of artemether as compared to other formulations. Results from in vivo studies confirmed the suitability of galactose conjugated liposomes for targeted and extended delivery of artemether.

Introduction:
In present study targeting potential of artemether to hepatocytes was determined using galactose modified liposomes as carrier system.

Materials and Methods:
Artemether was obtained from Ipca laboratories, Ratlam, India as gift sample. DSPE was purchased from Sigma Eldrich. Cholestrol and other chemicals were purchased from Hi-Media Labs, Mumbai.

Liposome Preparation and Optimization:
Liposomes were prepared and optimized following the method described by Garg et al. with slight modification. Galactose conjugation to the lipid was done by method previously reported by Wang et al [1, 2].

Drug entrapment:
Entrapment efficiency was determined by using sephadex minicolumn centrifugation method.

Characterization of Liposomes
Vesicle Size: Vesicle morphology and size were determined by scanning electron microscopy and transmission electron microscopy, respectively [3].

Drug release studies:
Drug release from liposomes was determined by dialysis bag diffusion technique.

Hematological studies:
The animals were divided into four groups having eighteen rats in each group. First group was kept as control. Hematological parameters, i.e. white blood corpuscles (WBC), red blood corpuscles (RBC), hemoglobin (Hb) and platelet count were determined by using NOVA Cell Counter. Samples were also estimated for Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT).

**Plasma and tissue distribution study:**
Albino rats (Sprague–Dawley strain) of either sex of uniform body weight (100 ± 20 g) were used for the determination of plasma and tissue distribution of drug. The rats were divided into four groups of eighteen animals each. Among these groups one group was taken as control and to first group PBS (pH 7.4) was administered. Formulations PArT (Plain Artemether solution), ULArT (uncoated liposomes) and GLArT (Galactose conjugated liposomes) were injected to second, third and fourth group. After definite intervals tissues were excised homogenized, lysed and analyzed for drug concentration.

**Results and Discussion:**

*Preparation and Optimization of Liposomes and Galactose conjugation*
Liposomes were prepared by rotary evaporation method and optimized for establishing a ratio of Lipid and Cholesterol. Ratio 7:3 was found to be optimum on the basis of increased drug entrapment, optimum vesicle size etc.

*Vesicle size and shape:*
Results of Scanning electron microscopy show spherical shape of vesicles.

*In Vitro Drug release studies:*
Percent cumulative drug release from uncoated liposomal formulation was found to be higher as compared to galactose conjugated formulation. It can be concluded that galactose conjugated liposomal formulation have much extended release potential. Reason may be coating of galactose which provide an additional barrier to drug for diffusing out from formulation.

*Hematological studies:*
Haematological studies reveal any changes in number of blood cells and enzyme levels on administration of any formulation.

*Plasma and tissue distribution study:*
Upon administration of ULArT drug concentrations in Liver, Spleen and Lymph nodes were found to be increased due to higher RES (Reticulo-endothelial system) uptake of Liposomal formulation in MPS (Mononuclear Phagocytic system) rich organs. Whether in case of Formulation GLArT drug concentrations in these organs found to be further increased, but in liver this increase was higher as compared to spleen and lymph nodes. It may be due to asialoglycoprotein receptor mediated accumulation of drug which was proved earlier by hepatic cellular accumulation.
Figure 1 Percent cumulative drug release from liposomal formulations

Figure 2 Tissue and plasma distribution of Galactose conjugated liposomal formulation of Artemether

References:

PA-73

Statistical optimization and validation of multiparticulate drug delivery system for an anti bacterial agent

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Abstract: The multi-particulate drug delivery system as microspheres, for the antibacterial drug Cefaclor was prepared using 3-factor 3-level Box–Behnken design. The screened independent variables were stirring speed, temperature, polymer concentration, and the dependent variables were particle size, entrapment efficiency and drug release. Experimental responses of a different formulations resulted in mean formulation diameters ranging from 394 to 588 nm and entrapment efficiency up to 83%. Drug release from microsphere in the defined pH was found to be about 71%, with significant anti bacterial activity on *S aureus* and *E coli*. Hence, statistical optimization could be considered as a potent tool for the design of dosage forms.

Introduction:
Microspheres are free flowing solid particle made up of biodegradable and non-biodegradable material, ideally having a particle size in micron range. Or they may be defined as “Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles”. Cefaclor is a second generation cephalosporin antibacterial agent available in the market in strength of 250-500 mg/tablet. It is well absorbed from the gastrointestinal tract. The plasma half-life of 0.5 to 1 hour has been reported.

Optimization techniques are used in industrial planning, allocation, scheduling, decision-making, and provides both of understanding and an ability to explore and defend ranges for formulation and processing factors. This design was developed by Box and Behnken technique which provides three levels for each factor and consists of a particular subset of the factorial combinations from the 3k factorial design [1-4].

Materials and Methods:
Cefaclor, liquid paraffin, ethylcelullose, span80, acetonitrile, dichloromethane, n-hexane

Method: The microspheres were fabricated using multiple emulsion-solvent evaporation method. Ethylcellulose was dissolved in solvent mixture consisting of DCM: acetonitrile: water (3:3:3). Weighed quantity of drug Cefaclor was dissolved in polymer solution. The primary emulsion obtained was then poured into liquid paraffin-span80 mixture maintained at variable temperatures and stirred for 2 h at variable rpm. Finally, suspension was filtered and washed with n-hexane and then used as such for further analysis.
Table 1 Formulation codes and results for the evaluation parameters.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation code</th>
<th>Stirring speed (rpm)</th>
<th>Polymer concentration (mg)</th>
<th>Temperature °C</th>
<th>Dichloro methane (ml)</th>
<th>Acetonitrile (ml)</th>
<th>Distilled water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>700.00</td>
<td>375.00</td>
<td>30.00</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>700.00</td>
<td>500.00</td>
<td>35.00</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>700.00</td>
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<td>35.00</td>
<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>700.00</td>
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<td>3</td>
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<td>5</td>
<td>F5</td>
<td>950.00</td>
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<td>8</td>
<td>F8</td>
<td>950.00</td>
<td>500.00</td>
<td>40.00</td>
<td>3</td>
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<td>9</td>
<td>F9</td>
<td>950.00</td>
<td>250.00</td>
<td>40.00</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>F10</td>
<td>1200.00</td>
<td>375.00</td>
<td>30.00</td>
<td>3</td>
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<td>375.00</td>
<td>40.00</td>
<td>3</td>
<td>3</td>
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</tr>
</tbody>
</table>

Results and Discussion:
The entrapment of the formulations varied with polymer concentrations, rpm and volume of the aqueous media added, as the drug Cefaclor shows water solubility.

Figure 1 Percentage yield (%) of microspheres

Figure 2 % Entrapment efficiency of microspheres

Figure 3: Optimized graph between particle sizes, % entrapment efficiency, % drug release.
The SEM microphotographs of the formulations show uniform and perfectly spherical particles. The presence of drug particles on the surface is not there. The F2 formulation shows higher release as compared to other formulations, which may be due to the better entrapment of the drug in the formulation.

Conclusions:
The yield, entrapment efficiency, size distribution and the drug release characteristics of the formulation F2 were found to be good and well within the acceptable range. The anti bacterial studies also show that the optimized formulations can exhibit significant results comparable to the marketed product. Thus, with the use of the optimization software, the designing of formulation with few trials could be achieved.

References:
Development and in vitro evaluation of liquisolid tablets of Lornoxicam

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E-mail address: shailpharma@gmail.com

Abstract: The present study deals with the development of liquisolid tablets of lornoxicam with an aim to increase its flowability, solubility and dissolution rate. Liquisolid compacts were prepared as per the reported mathematical model employing PEG 400, Avicel PH 112 and Aerosil 200 as solvent, carrier and coating materials respectively. The flow properties of the drug improved significantly after formulating it into the liquisolid compact as revealed by the angle of repose (22.24±0.98 - 30.52±0.98°), Carr’s index (7.43±0.98 - 15.45±0.14%) and Hausner’s ratio (1.08±0.43 - 1.18±0.48) values. The prepared tablets as characterized for post-compressional parameters revealed the uniformity within the different batches for thickness (0.24±0.37 - 1.04±0.37mm), friability (0.32±0.03 - 0.60±0.03%), average weight variation (107.68±0.30 - 430.21±0.43mg), drug content (98.49±0.05 - 99.92±0.05%), and disintegration time (1.54 - 4.45min). In vitro drug release studies showed significant improvement in the dissolution of lornoxicam in its liquisolid form compared to the pure form. The dissolution studies revealed that as the carrier: coat ratio (R value) increases, a proportionate increase in the drug release was observed. However, increasing the drug concentrations from 10-40% in the liquisolid systems resulted in reduced drug release. DSC and XRD studies suggested the conversion of crystalline form of drug into amorphous form supporting the enhanced solubility and dissolution of lornoxicam from the liquisolid systems. Overall, the developed liquisolid tablets showed promising in-vitro results of increased solubility and dissolution rate of the lornoxicam.

Introduction:
Lornoxicam, a potent non-steroidal anti-inflammatory drug (NSAID) exhibits poor aqueous solubility leading reduced bioavailability [1]. The drug also possesses poor flow properties thereby making the direct compression of tabletting a difficult task. A “liquisolid system” refers to formulations by conversion of liquid drugs, drug suspensions or drug solutions in non-volatile solvents into dry, non-adherent, free-flowing and compactible powder mixtures by blending the suspension or solution with selected carriers and coating materials. Liquisolid compacts possess acceptable flowability and compressibility properties [2].

Materials and Methods:
Lornoxicam was obtained as gift sample from Micro labs, Goa and Avicel PH 112 from LibrawPharma, New Delhi. Aerosil 200 was obtained from Hi-Media Laboratories Pvt. Ltd., Nasik. Sodium Starch Glycolate, Magnesium Stearate, Propylene Glycol, Polyethylene Glycol 400, Polyethylene Glycol 600, Sodium hydroxide were from S. D. Fine-Chem. Ltd., Mumbai.

**Preparation of liquisolid compacts:** Various liquisolid compacts were prepared by initially dissolving the known quantity of lornoxicam in the nonvolatile vehicle PEG 400. To this, a binary mixture of carrier (Avicel PH 112) and coating material (Aerosil-200) prepared at a ratio of 10:1, 20:1, 30:1 and 40:1 was added. From the calculated Φ-value, the liquid load factor (Lf) was calculated. Depending upon the drug concentration in liquid medication, different liquid load factors were employed in liquisolid preparations. Finally, sodium starch glycolate as a super-disintegrant and magnesium stearate and talc were added to the above powder blend and mixed. The final powder blend was subjected to compression [3] by using 10 station rotary compression machines (Rimek mini press, Karnavathi Engineering Ltd, Gujarat).

**Results and Discussion:**

![Figure 1](image)

*Figure 1 In vitro release of lornoxicam from liquisolid tablets*

The flow properties of the drug improved significantly after formulating it into the liquisolid compacts. The post-compressional parameters of prepared tablets revealed the uniformity and maintenance of standards within the different batches. *In vitro* drug release studies showed significant improvement in the dissolution of lornoxicam in its liquisolid form compared to a commercial product. The effect of formulation parameters, such as drug concentration and carrier to coat ratio, on enhancing drug dissolution was also explored. FT-IR, DSC and XRD techniques were employed for
the physical characterization of drug in the liquisolid systems. The drug release from the optimized liquisolid compacts (F8) followed first order release kinetics.

References:
Formulation and evaluation of poly herbal antidiabetic capsule madhuneel

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School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Rajasthan)
E-mail address: shrivastavapharma@gmail.com, satyaendracognosy@gmail.com

Abstract: Plants are very useful to mankind. Many of them are used exclusively for medicinal purposes. According to the World Health Organization (WHO), “a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis.” Such plants are in great demand by pharmaceutical companies for their active ingredients. The present study deals with the in-house formulation of herbal antidiabetic capsule Madhuneel. Various evaluation parameters of capsules were evaluated and were compared with standard drug madhuneel

Introduction:
Diabetes mellitus is one of the most common disorders affecting almost 6% of the world population and the dynamics of the diabetes are changing rapidly in low- to middle-income countries [1]. Development of an adverse event is one of the complications in the treatment of any systemic disorder; hence, many of the research institutes and pharmaceutical companies are involved in drug development to find the molecules with good therapeutic potential and less adverse events.

Materials and Methods:
Selection, collection and authentication of plant material: The herbs viz., Gymnea sylvestre (Leaves), Eugenia jamboloma (seeds), Aegle marmelos (leaves), Azadirachta indica (leaves), Cinamomum zeylanicum (leaves), Sphaeranthus indicus (flower), Momordica charantia (fruits), Trivang bhasma, Shilajeet were collected in the months Jan 2014 to August 2014 from the in and around local areas of Indore District.
Preparation of formulation (PHF: Capsule) by wet granulation method: Each 50 mg of herbal capsule contained the extracts of Gymnea sylvestre (Leaves), Eugenia jamboloma (seeds), Aegle marmelos (leaves), Azadirachta indica (leaves), Cinamomum zeylanicum (leaves), Sphaeranthus indicus (flower), Momordica charantia (fruits), Trivang bhasma, Shilajeet and excipients—quantity sufficient (q.s.) Further, Scanning and determination of maximum wavelength (λmax)
Evaluation Parameters [2-4]: The evaluation parameters that were observed as per standard methods are organoleptic characters, moisture content, pH, weight variation, disintegration time, drug content and drug release were evaluated. Stability studies were performed as per the guidelines.
Results and Discussion:

**Scanning for $\lambda_{\text{max}}$:** For UV scanning for $\lambda_{\text{max}}$, about 100 mg of formulated and standard drug was weighed, powdered and transferred to a 100ml volumetric flask containing water solution and was shaken to dissolve and volume was made to 100 ml. Then 10 ml of this solution was diluted to 100ml to obtain a solution of 100µg/ml and further diluted to obtain 10, 20, 30, 40 and 50 µg/ml and scanned for $\lambda_{\text{max}}$ determination. From the curve, peaks for the drugs were determined. Maximum absorption was shown to be 222 for both formulated and standard drug.

**Standard curve of drug and formulated drug:** Standard calibration curve of standard and formulated drug were determined by plotting graph between absorbance v/s concentrations on double beam U.V. spectrophotometer using $\lambda_{\text{max}}$ at 222 nm, it follows the Beer’s law. Straight line was obtained after plotting absorbance on X axis and concentration on Y axis. The line of equation was $Y= 0.012X+0.3$ & $Y= 0.010X+0.430$ respectively for standard and formulated drug. The $R^2$ value found was 0.981 & 0.956 respectively. The results were shown in table.

**Pre-Formulation Studies:** The standard and formulated drug were blended and various pre-formulation studies such as bulk density, tapped density, carr’s index, hausner’s ration and angle of repose were recorded. The data were presented in table. It was found from the present investigation that all the studied parameters were within the limit. (Table 1)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Standard</th>
<th>Formulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bulk Density</td>
<td>0.615</td>
<td>0.649</td>
</tr>
<tr>
<td>2.</td>
<td>Tapped Density</td>
<td>0.549</td>
<td>0.583</td>
</tr>
<tr>
<td>3.</td>
<td>Carr’s Index</td>
<td>12.02</td>
<td>11.32</td>
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<tr>
<td>4.</td>
<td>Hausner’s Ratio</td>
<td>0.892</td>
<td>0.898</td>
</tr>
<tr>
<td>5.</td>
<td>Angle of Repose</td>
<td>21.45</td>
<td>23.62</td>
</tr>
</tbody>
</table>

**Evaluation:**

The formulated capsules were visualised for any visual defects. The organoleptic properties such as color, odor and taste for both i.e. standard and formulated poly herbal capsules were noted down and presented in table 2. Various evaluation parameters of capsules were mentioned in table. The stability studies at three temperature and RH were recorded and mentioned in table 3. The data obtained from dissolution studies of different batches was analyzed using different mathematical model for the determination of release kinetics.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Evaluation Parameters</th>
<th>Standard</th>
<th>Formulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nature</td>
<td>Powder granules</td>
<td>Powder granules</td>
</tr>
<tr>
<td>2.</td>
<td>Size of capsule</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Color of Cap</td>
<td>Light Brown</td>
<td>Light Brown</td>
</tr>
<tr>
<td>4.</td>
<td>Color of body</td>
<td>Dark Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>5.</td>
<td>Moisture Content</td>
<td>0.93% w/w</td>
<td>0.96% w/w</td>
</tr>
</tbody>
</table>
Table 3: Evaluation parameters of at 25°C ± 2°C/ 60% ± 5% RH

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Evaluation Parameters</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>1.</td>
<td>Moisture Content</td>
<td>0.93</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>7.0</td>
</tr>
<tr>
<td>3.</td>
<td>Average Weight</td>
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<tr>
<td>4.</td>
<td>Weight Variations</td>
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<td>5.</td>
<td>Disintegration Time</td>
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<td>6.</td>
<td>Drug Content</td>
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</tr>
<tr>
<td>7.</td>
<td>In-Vitro drug release</td>
<td>97.98</td>
</tr>
</tbody>
</table>

References

Formulation and in vitro evaluation of gastro retentive bilayer floating tablet of Famotidine Hydrochloride

Rashmi Dahima¹, Mithlesh Sahare², Devashish Rathore¹
¹School of Pharmacy, Devi Ahilya Vishwa Vishwavidyalaya, Indore- 452001, Madhya Pradesh, India
²School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal-462036, Madhya Pradesh, India
E-mail address: dahimarashmi@rediffmail.com

Abstract: The present study aims to formulate bilayered tablets of famotidine hydrochloride with a fast release layer using sodium starch glycolate, cross povidone and a sustaining floating layer using polymers like HPMC K100M and HPMC K15M by effervescent approach. The release characteristics were studied on the basis of gel forming polymer, methocel with different concentration of citric acid and sodium bicarbonate. The in vitro buoyancy and floatability were found to be optimum in combination of sodium bicarbonate, citric acid and methocel at concentration of 13 mg, 6 mg and 90 mg respectively. The drug release from floating tablets was found to be 93.87% for F1 with methocel K15M. The drug release was sustained for a period of 20-24 hours. When compared different grades of methocel (K100M and K15M), the methocel K15M (low viscosity grade) provided better-sustained release characteristics with excellent in vitro buoyancy. The IR study reveals that there is no any possible interaction between drug and excipients used for such formulation. The result indicated the coupling of swelling and diffusion mechanism so called as Fickian diffusion of famotidine from floating tablets.

Introduction

With many drugs, the basic goal of therapy is to achieve a steady state blood or tissue level that is therapeutically effective and nontoxic for an extended period of time. Famotidine is histamine H2 - receptor antagonist. The low bioavailability (40-45%) and short biological half- life (2.5-4.0 hours) of famotidine following oral administration favors development of a sustained release formulation. The multilayered tablet concept has been long utilized to develop sustained release formulations. One layer releases the drug rapidly while other layer sustains the release for an extended period of time. As both rate and extent of drug absorption are important as far as optimum bioavailability of drug is concerned, here this rapidly released drug will cross minimum effective concentration (beginning of therapeutic response) rapidly while other layer will sustain the therapeutic effect and will maintain drug plasma level at steady state [1-3].

Materials and Methods:

Materials: Famotidine was received as a gift sample from Aristo Pharmaceuticals Ltd, Bhopal, India.
Methocel K100M and methocel K15M were received as gift samples from Colorcon Asia Pvt. Ltd., Goa, India. All other ingredients were of laboratory grade.

**Preparation and characterization of bilayer tablets:** The bilayer tablets of famotidine hydrochloride were prepared by the wet granulation method. The drug and polymers for both fast release and sustaining layer were passed through a respective sieve before their use in the formulation.

**Formulation of the fast release layer:** The granules were prepared by kneading the intragranular disintegrating agent and excipients and adding water as a binder. Granules screened with an 18 mesh sieve were dried and mixed with excipients. Disintegrating agent could also be added to the granules as extra granular disintegrates, and then mixed with talc and magnesium stearate. The blends was compressed into tablets using flat punch of diameter 8.00 mm Rimek 12 station mini press tablet machine.

**Formulation of the sustained release layer:** The granules were prepared with a solution of PVP K 30 in sufficient alcohol. They were then passed through sieve no 40. Drying (45°C) was done using conventional hot air oven. Drying of the granules was stopped after proper drying. Then dried granules were sized from mesh 40/60 and lubricated with purified talc and magnesium stearate. The blend was compressed into tablet flat punch of diameter 8.00 mm Rimek 12 station mini press tablet machine.

**Table 1** Optimization of fast release layer (All the ingredient in mg)

<table>
<thead>
<tr>
<th>BATCH CODE</th>
<th>INGREDIENT</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
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<tbody>
<tr>
<td></td>
<td>FAMOTIDINE</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Povidone</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SSG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>6</td>
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<tr>
<td></td>
<td>DCP</td>
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<td>83</td>
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<td>81</td>
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<tr>
<td></td>
<td>TALC</td>
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<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>MG STEARATE</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

**Table 2** Sustained release layer

<table>
<thead>
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<th>Batch code Ingredients*</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
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</thead>
<tbody>
<tr>
<td>Famotidine</td>
<td>30</td>
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<tr>
<td>HPMC K 15 M</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HPMC K 100 M</td>
<td>-</td>
<td>40</td>
<td>30</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Sodium bi carbonate</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>13</td>
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<tr>
<td>Citric acid</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3.75</td>
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</tr>
<tr>
<td>Lactose</td>
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<td>20</td>
<td>24</td>
<td>24</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Floating lag time (Seconds)</td>
<td>42</td>
<td>44</td>
<td>39</td>
<td>60</td>
<td>51</td>
<td>44</td>
<td>52</td>
<td>70</td>
</tr>
</tbody>
</table>

*All the ingredient are in mg

**Compression of bilayer tablet** – The quantity of granules for the sustained release layer was
compressed lightly using a single punch tableting machine equipped with 8 mm round, flat punches. The required quantity of fast release layer was placed over this compressed layer and compressed again to obtain bilayer floating tablet with hardness in the range of 4-5 kg cm\(^{-2}\).

**Results and Discussion:**

Angle of repose was found to be in the range of 31.23 to 34.02 with granules containing methocel K100M and 32.16 with methocel K15M. Hausner ratio ranged from 1.1 to 1.2 for granules of different formulations. The result reveals good flow properties of prepared granules. As per FT-IR spectrum, major frequencies of functional groups of pure drug remain intact in granules containing polymers; hence, there is no major interaction between the drug and polymers used in the study. With decreasing citric acid concentration, the floating lag time was found to be increasing and tablets floated for longer period of time. Short floating lag time and long duration of floating time were observed with tablets prepared from methocel K15M or low concentration of polymer as compared with formulations containing high-viscosity grade methocel K100M or high concentration of polymer. Reduction in methocel level in the formulations F4, F5, and F9, F10 prolonged the floating lag time and shortened the total floating time.

The *in vitro* buoyancy and floatability were found to be optimum in combination of sodium bicarbonate, citric acid and methocel at concentration of 13 mg, 6 mg and 90 mg respectively. Citric acid is directly proportional to drug release and inversely proportional to floating time, probably due to of excess carbon dioxide, disturbing the monolithic tablet. The citric acid level in the formulations greatly influenced the drug release, irrespective of methocel grade. The drug release from floating tablets was found to be 93.87% for F1 with methocel K15M. The drug release for formulations containing methocel K100M (F2 to F8) varied between 84.41 to 94.83%. The data from release studies were fitted in different models viz. zero order, first order and Korsemeyer’s equation. Slope values (0.5<n<1.0) of 0.37 in case of Korsemeyer’s plot indicates the coupling of swelling and diffusion mechanism so called as Fickian diffusion of famotidine from floating tablets.

**Conclusion:**

This system can be useful for pharmaceuticals following chronopharmacology and having limited physiological stability and absorption window in upper part of GI tract. However, further clinical studies are needed to explore potential of system for antibiotics.

**References:**

Formulation and evaluation of gel containing liposome entrapped with Etodolac

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Abstract: The objective of the present work is to formulate liposomal gel of etodolac and to study drug release properties \textit{in vitro}. Liposome composed of Egg phosphatidyl choline and cholesterol, with etodolac entrapped, was prepared by thin film hydration method. Liposomal gel formulations were prepared by incorporation of liposome into a structured vehicle (HPMC). The percentage encapsulation of liposome was found to be more than 70%. Liposomal entrapped gel formulations provide the prolonged drug release rate. Release kinetics of the drug formulation was found to be diffusion controlled, while liposome acts as local depot.

Introduction:
During the past few decades, more interest has been shown in developing new techniques to modulate the drug permeation through the skin. The synthetic and natural phospholipids were used for the preparation of the liposome. Liposome may act as a solubilization reservoir, for the controlled release of active compound, as permeability enhancer for the modulation of systemic absorption of drug through the skin. Liposomes have become a valuable experimental and commercially important drug delivery system, having biodegradability, biocompatibility, low toxicity and ability to entrap both lipophilic and hydrophilic drugs [1, 2].

Materials and Methods:
Egg phosphatidylcholine was purchased from Sigma Chemical Co. Etodolac was obtained as a gift sample from Mylan lab Nasik. Butylated hydroxyl toluene, cholesterol, ethanol, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose and chloroform were obtained from CDH India. All the above chemicals and solvents used are of analytical grade.

Liposome preparation: Multilamellar liposomes were prepared by thin film hydration method. Accurately weighed quantities of egg phosphatidyl choline (PC), cholesterol (such that the ratio between PC and C is 3:1) and etodolac were dissolved in chloroform-ethanol mixture. Butyl hydroxyl toluene, equivalent to 2% of the total lipids as an antioxidant was added in the organic phase in the round bottom flask. Reduced pressure was applied to evaporate the chloroform-ethanol mixture. After evaporation of organic solvents, hydration of the thin film was carried out using normal saline solution. Then the etodolac loaded liposomes (vesicles) were collected [3, 4].

Formulation of HPMC Gel: 1gm, 1.5 gm, 2gm of HPMC were weighed accurately and dissolved in the 100ml of distilled water and followed by continuous stirring until HPMC completely dissolved
in it, Then it is kept overnight to form the 1%, 1.5%, 2% HPMC gel respectively.

**Figure 1** Microscopic image of liposomes of Etodolac

**Table 1** Evaluation parameters of liposomal entrapped gel

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Formulation Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH</td>
<td>F1</td>
</tr>
<tr>
<td>2.</td>
<td>Spreadability (g.cm/sec)</td>
<td>F2</td>
</tr>
<tr>
<td>3.</td>
<td>Viscosity (cps)</td>
<td>F3</td>
</tr>
<tr>
<td>4.</td>
<td>Entrapment efficiency (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1.</td>
<td>pH</td>
<td>5.5±0.34</td>
</tr>
<tr>
<td>2.</td>
<td>Spreadability (g.cm/sec)</td>
<td>21.02±0.4</td>
</tr>
<tr>
<td>3.</td>
<td>Viscosity (cps)</td>
<td>120±0.04</td>
</tr>
<tr>
<td>4.</td>
<td>Entrapment efficiency (%)</td>
<td>80.8±0.12</td>
</tr>
</tbody>
</table>

**Figure 2** *In vitro* drug release of prepared liposomal gel

**Result and Discussion:**
Liposome based novel drug delivery system has been employed to provide sustained release of the medication for the topical delivery of etodolac. The enhanced retention on the skin provides better potential of the delivery system as compared to the conventional drug delivery system. Liposome based gel formulation of etodolac will significantly provide the better role in the treatment of arthritis and joints related pain effectively.

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Formulation and characterization of pulsatile delivery of Valsartan core in cup tablets

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Abstract: To design and characterize an oral, pulsatile drug delivery system of valsartan which is a chronopharmaceutical approach for the better treatment of nocturnal hypertension. Core tablet was prepared and compressed the tablet with cellulose acetate propionate polymer which is used as impermeable polymer and which acts as cup in which core is placed over it. Then final step is compressional coating with different grades of sodium CMC, HPMC K4M and Sodium alginate. Drug release after 7 h was found to be 96.92%, 96.1% and 98.3% for formulations F4, F5 and F6 respectively. F6 formulation showed burst release at particular lag time so the release profiles of the formulation are desirable. On the basis of in vitro release studies, effective lag time and swelling index, F6 was selected as an optimized formulation for designing “core in cup” device.

Introduction:
Present study attempts to design and evaluate a chrono modulated drug delivery system of valsartan, angiotensin II receptor antagonist for the treatment of hypertension using a novel technique “Core in cup tablet” device [1]. Valsartan belongs to a class of angiotensin-II receptor antagonist and because of its vasodilation action it is used as a form of chemotherapy for cardiovascular diseases. The half-life (4-5 h.) of the necessities 2-3 times daily dosage, which may reduce patient compliance. Thus, this study attempts to design and evaluate a chronomodulated pulsatile drug delivery system of valsartan for the treatment of cardiovascular diseases occurring in early hours.

Materials and Methods:
Valsartan (Vera labs), Cross carmellose sodium, MCC, HPMC K4M (Yarrow Chemicals), Sodium alginate, Magnesium stearate and Talc from Desai Chemicals.

Methods: Compatibility of valsartan with the respective polymers HPMCK4M, Sodium CMC, Sodium alginate, MCC by Infrared Absorption Spectral Analysis (FTIR). Preparation of core tablets: The core tablet of valsartan was prepared by direct compression technique using cross carmellose sodium as disintegrate and microcrystalline cellulose as diluent to obtain a fast disintegrate tablet as shown in Table 1. The mixture was lubricated with magnesium stearate and compressed in to tablets using Minipress tablet compression machine.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Valsartan</th>
<th>MCC</th>
<th>CCS</th>
<th>Mg. stearate</th>
<th>Talc</th>
<th>Total weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity(mg/tablet)</td>
<td>80</td>
<td>12.5</td>
<td>5.5</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

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The core tablet was compressed into a tablet with cellulose acetate propionate polymer which acts as a cup in which the core is placed over it. Then the final step is compressional coating with different grades of sodium CMC, HPMC K4M, and sodium alginate as shown in Table no. 2. Polymers are used as swellable polymer for achieving suitable lag time [2].

**Table 2:** Formulation for core in cup tablet

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan core</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>CAP</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>HPMCK4M</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sodium CMC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sodium alginate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
</tbody>
</table>

Evaluation of “Core in cup tablet” devices:
The entire prepared core in cup tablets was evaluated for hardness, friability, weight variation, lag time. Drug release studies of coated tablets were carried out using a LAB INDIA DISSO 8000 apparatus (Apparatus 2, 100 rpm, 37 °C) for 2 h in 0.1 M HCl (900 ml), pH 5.5 phosphate buffer (900 ml) for 1 h, pH 6.8 phosphate buffer (900 ml) for 2 hrs and pH 7.4 phosphate buffer (900 ml). Samples were analyzed using UV spectrophotometer at 249 nm. The cumulative amount of Valsartan release from the formulated tablets at different time intervals were fitted to zero order kinetics, first order kinetics, Higuchi model and Korsmeyer –Peppas model to characterize mechanism of drug release.

**Results and Discussion:**

Drug excipient compatibility Studies: From the IR study and physical observation it can be concluded that there is no significant Drug-Excipient interaction. So we can conclude that drug and other excipients are compatible with each other.

Post compressional parameters for core tablets: Disintegration time of core tablet is less than 1.4 min which showed fast release of drug from the core tablet when it comes in contact with the dissolution medium [3]. Optimization enabled formulation of valsartan tablets coated with different concentrations of sodium CMC, HPMC K4M, Sodium alginate with the desired release profiles formulations (F1, F2, F3) and (F7, F8, F9) released the drug within 3.5 h and 2.5 h respectively. These formulations were failed to retard the release of the drug within 4 h of administration due to low viscous nature of different concentrations of Sodium CMC, HPMC and sodium alginate. F6 formulation showed burst release at particular lag time (5 h) so the release profile is desirable.

**In-vitro dissolution profiles of valsartan pulsatile release tablet by using core in cup device:**
**Dissolution studies Valsartan “core in cup” pulsatile release tablets:** Drug release from all the formulations prepared with Sodium CMC, HPMC K4 M and sodium alginate were shown in figure 1. The results revealed that the formulations with HPMC K4 M had a desirable lag time and also released the drug completely within 7 h compared to formulations with that of sodium CMC and sodium alginate. Among the formulations F4, F5, F6 with HPMC K4M, F6 showed best lag time (5 h) and released around 97.5% drug release at 7th hour which is desirable for hypertension medication. Further increase in concentration of HPMC K4M would probably increase the lag time and retard the release further more which is not necessary [4]. After fitting different mathematical models to the dissolution studies of valsartan core in cup tablets, R² values showed first order release with diffusion mechanism and drug release pattern showed non fickian diffusion pattern.

**Figure 1** Results of % drug release study.

**Conclusion:**
From the *in-vitro* release studies of device, it was observed that with all formulations, there was absolutely no drug release in simulated gastric fluid (acidic pH 1.2) for 2 hours. Small amount of drug release was observed in pH 5.5 phosphate buffer for 1 hr and in simulated intestinal fluid (pH 6.8 phosphate buffer) for 2 h [5]. Increase in the polymer content (% coating) resulted in a reduction in release of valsartan. The obtained results showed the capability of the system in delaying drug release for a programmable period of time and the possibility of exploiting such delay to attain colon targeting.

**References:**
Study of effect of porogen on release and anti microbial characteristics of Cefpodoxime Proxetil from porous microspheres

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Abstract: Ethyl cellulose and porogen based super porous microspheres were prepared by solvent diffusion method. Cefpodoxime Proxetil was chosen as the drug, along with different polymer/porogen ratios. The formulated microspheres were evaluated for percentage yield, percentage entrapment efficiency, particle size, size distribution, surface morphology, drug release rates and release mechanism. The type and concentrations of drug/polymer/porogen affected the properties of microspheres. The presence of porogen enhanced the release kinetics as evident from morphology, percentage yield and entrapment, release kinetics and antimicrobial studies.

Introduction:
Porous microspheres are modified form of microspheres which have external pores on the surface or internal pores in the core (usually inter connective), and active substances can be dissolved or dispersed on the surface or in the core of the microspheres [1]. Porosity play important role in determining the capacity efficiency and release kinetics [2].The proposed work aims to develop and evaluate super porous microspheres containing drug using a suitable polymer and porogens which offer large specific surface area, low density and porous microspheres having excellent absorption capacity, and drug release kinetics. The formulations will enhance the dissolution and release kinetics, absorption and availability of the drug which is BCS class IV.

Materials and Methods:
Materials: Cefpodoxime Proxetil, Ethyl cellulose, NaCl, KCl, sugar, sucrose, mannitol, gelatine, glucose, camphor, PVA and chloroform.
Method: Super porous microspheres of Cefpodoxime Proxetil were obtained by solvent diffusion technique. The polymer was dissolved in chloroform and the drug along with porogen was added to it [3]. The polymer drug solution so obtained was injected into the PVA solution which was maintained at variable speeds and porogens using mechanical stirrer. The stirring was continued and the formed microspheres were collected by filtration, washed with n-Hexane and dried [4].

Results and discussion:
It was observed that the change in porogen concentration affected the percentage yield as evident from the increase in yield from 51% (F14) to 80% (F10). With the increase in the porogen
concentration, the entrapment of the drug also increased. The release rates of the formulation were greatly affected by the type of porogen that was added. Addition of sodium chloride as the porogen exhibited zero order release. Since, the drug belongs to Class IV in BCS classification, an enhancement of dissolution by addition of porogen will markedly increase and improve the release behaviour and bioavailability.

Table 1 Physicochemical properties of super porous microspheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Porogen concentration (mg)</th>
<th>Change of concentration of porogen (mg)</th>
<th>Polymer concentration (mg)</th>
<th>% yield</th>
<th>% EE</th>
<th>% CDR</th>
<th>Particle size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>40</td>
<td>40</td>
<td>40.00</td>
<td>67.0</td>
<td>78.0</td>
<td>76.54</td>
<td>8</td>
</tr>
<tr>
<td>F2</td>
<td>40</td>
<td>40</td>
<td>40.00</td>
<td>74.6</td>
<td>61.0</td>
<td>59.03</td>
<td>13</td>
</tr>
<tr>
<td>F3</td>
<td>40</td>
<td>40</td>
<td>40.00</td>
<td>55.6</td>
<td>52.25</td>
<td>66.30</td>
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</tr>
<tr>
<td>F4</td>
<td>40</td>
<td>40</td>
<td>40.00</td>
<td>57.0</td>
<td>65.25</td>
<td>61.08</td>
<td>13</td>
</tr>
<tr>
<td>F5</td>
<td>40</td>
<td>40</td>
<td>40.00</td>
<td>61.7</td>
<td>69.25</td>
<td>62.85</td>
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</tr>
<tr>
<td>F6</td>
<td>25</td>
<td>25</td>
<td>50.00</td>
<td>75.0</td>
<td>71.87</td>
<td>69.89</td>
<td>23</td>
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<tr>
<td>F7</td>
<td>25</td>
<td>25</td>
<td>50.00</td>
<td>60.3</td>
<td>40.70</td>
<td>64.23</td>
<td>23</td>
</tr>
<tr>
<td>F8</td>
<td>25</td>
<td>25</td>
<td>50.00</td>
<td>52.9</td>
<td>51.20</td>
<td>59.91</td>
<td>13</td>
</tr>
<tr>
<td>F9</td>
<td>33.3</td>
<td>-</td>
<td>33.33</td>
<td>70.6</td>
<td>79.01</td>
<td>72.43</td>
<td>08</td>
</tr>
<tr>
<td>F10</td>
<td>35.71</td>
<td>-</td>
<td>28.27</td>
<td>80.2</td>
<td>67.80</td>
<td>74.90</td>
<td>13</td>
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<tr>
<td>F11</td>
<td>38.46</td>
<td>-</td>
<td>23.07</td>
<td>73.5</td>
<td>54.52</td>
<td>77.84</td>
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</tr>
<tr>
<td>F12</td>
<td>31.25</td>
<td>-</td>
<td>37.50</td>
<td>69.3</td>
<td>67.00</td>
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<tr>
<td>F13</td>
<td>33.33</td>
<td>33.33</td>
<td>16.66</td>
<td>57.9</td>
<td>42.26</td>
<td>76.40</td>
<td>08</td>
</tr>
<tr>
<td>F14</td>
<td>40.00</td>
<td>40.00</td>
<td>40.00</td>
<td>51.0</td>
<td>70.64</td>
<td>67.89</td>
<td>08</td>
</tr>
</tbody>
</table>

%= Percentage, CDR= Cumulative Drug Release, E= Entrapment efficiency

Figure 1 SEM Analysis At 45x Magnification

Figure 2 SEM Analysis at 1000x magnification

Figure 3 Percentage yield of formulations

Figure 4 Particle size of formulations
Figure 5 Effect of porogen concentration on % EE

Figure 6 Effect of porogen concentration on % CDR

Figure 7: In-vitro release of formulations

Figure 8 In-vitro release of formulations

Figure 9 Antibacterial studies of formulations

Figure 10 Antibacterial studies of formulations

The antimicrobial analysis was done using the agar well diffusion and MIC (minimum inhibitory concentration) value was determined. In the present study MIC value of the optimized formulation was equivalent to marketed formulation.

Conclusion:
From the results, it can be concluded that presence of porogen alters the percentage yield, entrapment efficiency, cumulative drug release and other process variables and has a pronounced effect on the release mechanism of the formulations.

References:
Development and PKPD evaluation of floating multi-particulate drug delivery system of an antidiabetic agent

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Abstract: Gastro retentive systems significantly prolong the mean residence time of drug/dosage form in the gastric area and are specially suited for drugs having absorption window in the upper GI tract such as metformin hydrochloride. Hence, microspheres were prepared utilizing Eudragits by emulsification-solvent evaporation and evaluated in vitro and in vivo. In vitro results indicated an initial burst release followed by sustained action and were validated with the anti- hyperglycemic effect of formulations in normal and hyperglycemic male albino mice. The blood glucose level at the lower side could be controlled for over 8hrs with significant changes in the pharmacokinetics of the drug.

Introduction:
An ideal controlled release drug delivery system will deliver the drug at a predesigned rate so that the drug stays in the optimal concentrations in blood plasma to exhibit its pharmacological action. Thus, the delivery mechanism should control the rate of release constant, but this also depends on the changing concentration gradients or additives leaching to porosity, etc, and becomes a function of time [1]. The replication and optimization of the formulation for the water soluble anti-diabetic drug was carried out for the selection of polymer and the methodology to be used for the process having good reproducibility, entrapment and stability of drug molecule [2, 3].

Material and Methods:

Materials: Metformin hydrochloride, liquid paraffin, Span 80, acetone, methanol, acetonitrile, dichloromethane, alloxan mono hydrate etc.

Method: The drug was dispersed in the polymer solution, added to liquid paraffin containing 0.1% antifoaming agent and stirred for complete solvent evaporation. The experimental parameters were varied and batches prepared.

Characterization of microspheres: The formulations were evaluated for size, shape, surface morphology using optical microscope and SEM, incorporation efficiency, in vitro buoyancy and dissolution. Experimental results were expressed as mean± SD. Release kinetics was determined considering different models and the selection was based on the comparison of the relevant correlation coefficients and linearity tests.
In-vivo evaluation was conducted on normal, healthy male albino mice using a crossover randomized block design (n= 5). The plasma glucose levels were measured following oral administration of the microspheres equivalent to dose of the drug. The pharmacokinetic parameters were analysed in healthy male rabbits after oral administration.

**Results and Discussion:**
Acrylate polymers were used for the formulation and the rpm used for stirring, concentration of polymer and the quantity of emulgent affected the particle size. With the increase in the emulgent concentration, the stabilization of the interfacial area takes place, causing reduction in size. The percent yield of the microspheres gradually increased with the increase in the polymer concentration from 74.8± 0.8 to 90.4± 0.5% s was the percentage entrapment (MED1 70.0± 1.2 to MED4 87.1± 0.8%). Eudragit membranes are insoluble but swellable at all physiological pH. The difference in lipophilicity and permeability of the ERL and ERS results in controlled release.

**Table 1 Properties of formulations**

<table>
<thead>
<tr>
<th>Codes</th>
<th>% Yield ±S.D</th>
<th>COV</th>
<th>% Entrapment ±S.D</th>
<th>COV</th>
<th>Buoyancy percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MED1</td>
<td>74.8± 0.8</td>
<td>1.1</td>
<td>70.0±1.2</td>
<td>1.7</td>
<td>78.0± 0.8</td>
</tr>
<tr>
<td>MED2</td>
<td>77.4± 0.5</td>
<td>0.7</td>
<td>82.0±0.7</td>
<td>0.8</td>
<td>80.1± 0.5</td>
</tr>
<tr>
<td>MED3</td>
<td>80.4± 0.5</td>
<td>0.6</td>
<td>84.1±0.5</td>
<td>0.6</td>
<td>81.7± 1.1</td>
</tr>
<tr>
<td>MED4</td>
<td>83.8± 0.8</td>
<td>0.9</td>
<td>87.1±0.8</td>
<td>1.0</td>
<td>72.8± 0.7</td>
</tr>
<tr>
<td>MED5</td>
<td>90.4± 0.5</td>
<td>0.6</td>
<td>86.8±0.8</td>
<td>0.9</td>
<td>70.2± 0.5</td>
</tr>
</tbody>
</table>

MED = formulation code, S.D = Standard deviation, COV = Coefficient of variance

**Figure 1** SEM of formulation  
**Figure 2** Size distribution of formulations

The degree of swelling is highly related to the permeability of solutes through the membrane. As the concentration of polymer increased, the release from the microspheres decreased. The drug release of metformin hydrochloride was proportional to square root of time, thus indicating diffusion controlled release mechanism.

The formulation showed a reduction in plasma glucose level of about 28 % at 4.5 hrs and maintained
the effect for 10 hrs in hyperglycemia induced mice while in normal mice maximum reduction of about 39% at the 6th hour was observed. The mean plasma value obtained was of 21% followed by decrease in the plasma glucose level started which was maintained below 20% for about 9 hours. Significant IVIVC was observed with values nearing to one. Microspheres showed a faster drug release reaching maximum at about 3.5 hrs with concentration of about 0.425 ± 0.10 μg/ml. The drug release decreased slowly after about 12 hrs followed by a faster decline after 15 hrs.

Concentration

**Figure 3** In vitro release characteristics

**Figure 4** Higuchi release kinetics of formulations

**Figure 5** Reduction (%) in plasma glucose level

**Figure 6** Average plasma concentration values

**Conclusion:**

The floating microspheres prepared utilizing Eudragit RL-RS as polymers by solvent evaporation method was successful in sustaining the release of the highly water soluble antidiabetic drug for over 12 hrs. The *in vivo* results suggest that the formulated microspheres could maintain the hypoglycemic effect of the drug in the mice for well over 10 hrs. Hence, the formulations can be well utilized for the prolonged anti-hyperglycemic activity of the drug.

**Acknowledgement:**

The author wants to thank Sun Pharmaceuticals, Baroda, Gujarat for the generous supply of drug and the polymers for the research work.

**References:**


Formulation development and characterization of floating microballoons of Metformin Hydrochloride for gastroretentive drug delivery

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Abstract: In the present work sustained release gastroretentive microballoons of metformin hydrochloride were prepared. Microballoons of metformin hydrochloride were prepared by solvent evaporation diffusion process using different mixtures of hydroxyl propyl methylcellulose and ethyl cellulose polymers. The microballoons were characterized for, surface morphology, particle size, floating behavior, incorporation efficiency and in vitro drug release. The mean particle size of the prepared microballoons was found in the range of 34.2 ± 4.7 to 95.7 ± 2.2 µm. Incorporation efficiency of over 83.8 ± 0.8 % were found for the optimized formulation of microballoons. Most of formulations were buoyant with maximum buoyancy of 81.4 ± 2.0 % for > 12 h, indicating good floating property of microballoons. Microballoons are found to be suitable delivery system for sustained release drug delivery of metformin hydrochloride with improved bioavailability.

Introduction:
Oral administration is considered as the most convenient and preferred route of drug delivery for the systemic circulation. To develop a suitable oral drug delivery systems, it is required to optimize the release rate of the drug as well as the residence time of the system within the gastrointestinal tract [1]. Several approaches have been used now days to retain the dosage forms in the gastric region in order to increase the gastric residence time (GRT) of the drug. These include those that utilize mucoadhesive, high density, floating, magnetic, unfoldable, superporous hydrogel and extendible or swellable systems [2]. In the present work metformin hydrochloride was used. This drug is poorly absorbed from the lower gastrointestinal tract and has short elimination half life of 1.5-1.6 h. The aim of the present study was to develop floating gastroretentive microballoons of metformin drug in order to extend its retention in the upper GIT, which may also result in enhanced absorption of the drug and thereby improved its bioavailability.

Materials and Methods:
Metformin hydrochloride was received as a gift from Sohan Pharmaceuticals Ltd, Pune. Ethyl cellulose, Hydroxypropyl methylcellulose and Tween 80 were purchased from Loba Chem Pvt. Ltd., Mumbai. Ethanol was obtained by S.D. Fine Chem Ltd, Mumbai. Dichloromethane was purchased from CDH Lab, New Delhi. All chemicals/reagents which were used are of analytical grade. Microballoons were prepared by solvent evaporation technique [3]. Metformin hydrochloride and
HPMC and EC were used in different ratios. The polymers which were used were dissolved in a mixture of dichloromethane and alcohol at the room temperature. The resulting solution was poured in distilled water, maintained at different temperatures and then stirred at varying speed for 30 min to evaporate the solvent. The microballoons which were formed were filtered, washed with solvent and dried.

Results and Discussion:
Size and shape of micro balloons:
The mean particle size of the formulations was in the range of 34.2 ± 4.5 to 95.7 ± 2.5 µm. The average particle size and thickness of wall of microballoons increased with increase in the concentration of the polymer. The results also revealed that the larger the particle size, the longer the floating time of the microballoons. Scanning electron microscopy revealed pores on the microballoons.

![Figure 1 Scanning electron micrograph (SEM) of microballoons](image)

Yield and drug loading (DL): The yield of microballoons was > 72 % for all the formulations while drug loading was in the range of 105.3 ± 1.4 to 125.6 ± 1.5 µg/mg.

Incorporation efficiency (IE): Incorporation efficiency was found to be in the range of 73.5 ± 1.1 to 83.8 ± 0.8, while more than 71 % of the microballoons remained floating at the end of 12 h of the study.

In-vivo floating test: The floating test of the microballoons was carried with the help of dissolution test apparatus method specified in the USP XX. The microballoons were spread over the simulated gastric fluid which was agited by a paddle rotated at 100 rpm. After agitation for a previously determined interval, the microballoons that were found to be floating and the ones that settled to the bottom of the flask were recovered separately.
In-vitro drug release: The results of the in-vitro study indicate that the proportion of polymers in the formulation was the major factor governing the drug release from the prepared microballoons. The results revealed that as the polymer concentration increases in the microballoons, the drug release was decreases. Cumulative drug released by the microballoons after 12 h was in the range of 60.4 ± 1.3 to 96.5 ± 1.2.

Conclusion:
Metformin hydrochloride microballoons with good floatation sustained release characteristics in simulated gastric fluid in-vitro has been successfully developed using the solvent evaporation and diffusion method. In-vivo studies are, however, required to establish the suitability of the formulation method used.

Acknowledgement:
The authors are very grateful to IPS Academy, College of Pharmacy, Indore, for providing all the necessary facilities for carrying out this study. The authors also wish to thank Indian Institute of Technology (IIT), New Delhi, India, for assistance with SEM facilities.

References:
Formulation & development of Aceclofenac and Omeprazole sustained release microspheres

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Abstract: The purpose of this research was to develop and formulate combination of two drug Aceclofenac and Omeprazole in the form of sustained release microspheres having pH sensitivity property and microspheres were prepared by solvent-evaporation method using different drug polymer ratios (1:1 to 1:3), stirring speeds (500-1000rpm), to prevent the side effect of Aceclofenac the stomach and small intestine for this proton pump inhibitor Omeprazole used. The prepared microspheres were characterized by percentage yield, particle size, entrapment efficiency, micromeritics properties, FTIR, in-vitro release behaviour, etc.

Introduction:
The main objective of any drug delivery system is not only to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and maintain the desired drug concentration too. Oral ingestion has become convenient and commonly employed route of drug delivery because of its ease. Most of the Drugs those are eliminated quickly from the blood circulation have easily absorbed from the GIT and having a short half-life. To overcome the problem oral controlled drug delivery systems have been developed having slow release into the GIT and maintain a constant drug concentration in the serum for longer period of time. As incomplete release of the drug and a shorter residence time of dosage forms in the upper gastrointestinal tract as site for absorption of many drugs, will lead to lower bioavailability [1]. Microspheres are small particles (in 1-1000 micrometer size range) used as carriers of drugs and other therapeutic agents. They describe a monolithic spherical structure with drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersed particle [2-5].

Material and Methods:
Materials: Aceclofenac and Omeprazole (drug) was provided by Ranbaxy Pvt. Ltd Gurgaon and Biotrans Chennai respectively, Eudragit S100, HPMC K4M, Span 80, Acetone other chemicals were provided by Sagar Institute of Research Technology & Science-Pharmacy Bhopal.
Method of preparation: Microspheres were prepared by solvent evaporation technique, Using Eudragit S100 as polymer. Polymer was dissolved in desired quantity of acetone, the HPMC K4M and drug dispersed with the polymer solution. After that the dispersed content was placed drop wise in mineral oil containing span80 maintained at 40°C while stirring at 800 rpm. After that the solvent,
acetone was removed by continuous stirring at room temperature for three hours to produce spherical microspheres. Prepared microspheres were than separated from mineral oil by filtration through whatmann filter paper, the microspheres were collected and washed for consecutive three times with n-hexane and dried using vacuum filtration. Product was then air-dried to obtain microspheres.

**Table 1** Formulations of different batches.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Batch code</th>
<th>Eudragit(mg)</th>
<th>Ethanol(ml)</th>
<th>Hpmc k4M(w/v)</th>
<th>Mineral oil (ml)</th>
<th>Span 80(w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AC1</td>
<td>100</td>
<td>35</td>
<td>0.5</td>
<td>35</td>
<td>1%</td>
</tr>
<tr>
<td>2</td>
<td>AC2</td>
<td>150</td>
<td>35</td>
<td>0.5</td>
<td>35</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>AC3</td>
<td>200</td>
<td>35</td>
<td>1.0</td>
<td>40</td>
<td>2%</td>
</tr>
<tr>
<td>4</td>
<td>AC4</td>
<td>250</td>
<td>35</td>
<td>1.0</td>
<td>40</td>
<td>2%</td>
</tr>
<tr>
<td>5</td>
<td>AC5</td>
<td>300</td>
<td>40</td>
<td>1.5</td>
<td>40</td>
<td>2%</td>
</tr>
<tr>
<td>6</td>
<td>AC6</td>
<td>350</td>
<td>40</td>
<td>1.5</td>
<td>40</td>
<td>2%</td>
</tr>
<tr>
<td>7</td>
<td>AC7</td>
<td>400</td>
<td>40</td>
<td>1.5</td>
<td>40</td>
<td>2%</td>
</tr>
</tbody>
</table>

**Results:**

Microspheres of Aceclofenac and Omeprazole combination with Eudragit-S 100 polymer in various ratios were formulated by solvent evaporation method. Addition of HPMC K4M to the continuous phase agglomeration of particles were reduced which showed larger in the initial trials. It was seen that as the ratio of drug to the polymer increased the production yield also increased and was found to be 72 – 84%. Due to increase in polymer concentration which leads to increase in viscosity of the phase the particle size of the formulation also found to be increased and the mean diameter of the microspheres was 91 – 109%.

Entrapment efficiency was in the range from 73 to 83%. It was observed that as the polymer ratio increases, entrapment efficiency also increases. The in-vitro drug release was carried out using Phosphate buffer pH 6.8 up to 6 hours. The cumulative percentages of release for all the formulations were found to be 58 to 67%. The effects of polymer on release rate of the formulations were found to be decreasing with increasing the concentration of polymer. The burst effect of in-vitro may be due to the adherence of the drug particles at the surface of the microspheres.

**Conclusion:**

Microspheres are the carriers which make the drug more stable at acidic environment and protect its metabolism and some modifications in the dosage form make it a career for sustained release. The release of NSAID in stomach causes several implications in which one of the major is erosion of gastric mucosa and ulceration. To prevent this some proton pump inhibitors can also be given with aceclofenac. The present system is designed in a manner to deliver both the drugs in a system which deliver the drug in alkaline pH and prevent its degradation in acidic pH i.e. in stomach, thus minimizing their side effects and also make it a system for sustained release which increases patient compliance.
The present experiment has the following outcomes,

- Site specific and prolong delivery of selected drug
- Minimize dose frequency
- Reduced side effects
- Combination therapy thus reduces dose administration

References:

Preparation and characterization of Olanzapine loaded PLGA microspheres by solvent evaporation method.

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Abstract: The aim of this work was to prepare olanzapine loaded enzymatically synthesized PLGA microspheres to achieve sustained release olanzapine. Olanzapine loaded PLGA microspheres (batch M1 and batch M2) were prepared by solvent-evaporation method using DCM/polyvinyl alcohol system. The resultant microspheres were evaluated for drug loading, encapsulation efficiency, average particle size and particle size distribution, in-vitro drug release and SEM studies. The encapsulation efficiency of microspheres were 24.1% and 9.95%, drug loading was 5.51% and 2.51%, particle size distribution was 60.54µm and 113.15µm respectively for batch M1 and M2. SEM analysis showed microspheres were rigid with varied surface roughness. In-vitro release profile showed the controlled release till 32 days. The olanzapine loaded PLGA microspheres prepared under optimized conditions showed good sustained release characteristics and can be used for drug delivery system.

Introduction:
There are so many approaches for delivering a therapeutic substance or drug to the target site in a controlled or sustained release fashion. One of such approach is using polymeric microspheres as carriers for drug. The microspheres are free flowing powders consisting of proteins or synthetic polymers contains drug in their pockets or networks, which are biodegradable in nature and ideally, having a particle size not more than 200 µm. These carriers received much attention not only for the targeting of the drugs to the particular site but also for the prolonged release. [1-2]
Microspheres of biodegradable or non-biodegradable polymers have been investigated for sustained release depending on the final application. In case of non-biodegradable drug carriers, the carrier remaining after drug is completely released possesses the possibility of carrier toxicity over a long period of time. Biodegradable carriers that degrade in the body to non-toxic degradation products do not pose the problem of carrier toxicity. Biodegradable carrier matrices can be designed to deliver the therapeutic agent for periods ranging from a few days to a few years.
In the present study, an attempt has been made to prepare olanzapine microspheres prepared by solvent evaporation method using enzyme (Lipase) catalysed PLGA as a polymer and polyvinyl alcohol as a cross linking agent. PLGA is a biodegradable polymer with great potential for pharmaceutical applications owing to its biocompatibility, non-toxicity properties. In vivo, they completely degrade into non-toxic degradation products.
Materials and Methods:
Olanzapine was a gift sample provided by research guide, enzymatic PLGA was synthesized in laboratory, PVA was purchased from Loba Chemicals. All other chemicals used in experiment were of analytical grade.

Preparation of Microspheres:
Olanzapine loaded microspheres were prepared by a solvent evaporation method. Polymer PLGA 50:50, PLGA 75:25 and was dissolved in Dichloromethane (organic phase or phase1). Olanzapine drug was dissolved in the same polymer solution in order to obtain a drug polymer solution. This drug polymer solution was added drop wise to external aqueous phase (phase 2) containing surfactant polyvinyl alcohol stirred by using high speed homogenizer operating at constant speed of 4000-5000 rpm for about 15 minutes and 2000 rpm for the next 45 minutes. The solution was flushed with nitrogen for the complete removal of the organic solvent (DCM). The prepared microspheres were collected by centrifugation. The collected microspheres were washed several times with water to remove the free drug. The wet microspheres were lyophilized to get dried solid microspheres in free flowing powder form. The formulated microspheres were analysed for drug loading, drug entrapment and encapsulation efficiency, particle size analysis and particle size distribution, Scanning electron microscopy. The microspheres were also studied for the in vitro drug release.

Results and Discussion:
Particle size and Particle size distribution:
The various characteristics of microspheres prepared are shown in table 1

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Batch</th>
<th>PLGA</th>
<th>Theoretical drug loading</th>
<th>Actual drug loading</th>
<th>Encapsulation Efficiency</th>
<th>D10%</th>
<th>D50%</th>
<th>D90%</th>
<th>SPAN = (D90% - D10%)/(D50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>M1</td>
<td>50:50</td>
<td>20%</td>
<td>5.5%</td>
<td>24.1%</td>
<td>9.65</td>
<td>24.93</td>
<td>60.54</td>
<td>2.04</td>
</tr>
<tr>
<td>2.</td>
<td>M2</td>
<td>75:25</td>
<td>20%</td>
<td>2.51%</td>
<td>9.95%</td>
<td>12.95</td>
<td>42.77</td>
<td>113.15</td>
<td>2.34</td>
</tr>
</tbody>
</table>

Scanning electron microscopy studies:
Figure 1 and 2 shows the SEM images of olanzapine microspheres batch M1 and M2 respectively. SEM studies revealed that the microspheres were rigid, porous with varied surface roughness.

In-vitro release studies:
Table 1.2 shows the data in-vitro release profile of microspheres prepared from synthesized poly lactide-co-glycolides.
Table 2: \textit{In-vitro} release profile of microspheres

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Batch</th>
<th>% Drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>1.</td>
<td>M1</td>
<td>19.92</td>
</tr>
<tr>
<td>2.</td>
<td>M2</td>
<td>24.85</td>
</tr>
</tbody>
</table>

As shown in table 2, the microspheres prepared from the synthesized poly lactide-co-glycolides had released almost 70\% drug release on the 7\textsuperscript{th} day but controlled the release till 32 days.

**Conclusion:**

The olanzapine loaded PLGA microspheres prepared under optimized conditions showed good sustained release characteristics and can be used for drug delivery system.

**Acknowledgement:**

Authors wish to thank UGC-DAE-CSIR for carrying out SEM analysis and providing us data.

**References:**

Comparative study of sodium alginate and gellan gum based in situ gel formulations of Nizatidine

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Abstract: The purpose of the study was to select the best gelling agent amongst Sodium alginate and Gellan gum. The In-Situ gels of Nizatidine are the dosage form which are used for the treatment of gastric ulcer disease. The gelling agent used for the formulation are Sodium alginate (1.0%) w/v and Gellan gum (1.5%) w/v are used. The Results of in-vitro studies showed that best gelling agent is Sodium alginate at 1% with sodium citrate, Calcium Carbonate and Sodium citrate.

Introduction:

Oral administration is the most convenient and preferred means of any delivery to the systemic circulation. Oral controlled release dosage forms becoming an interesting topic of research for the past few decades due to their considerable therapeutic advantages to Oral controlled release drug delivery have recently been of increasing interest in Pharmaceutical field to achieve improved therapeutic advantages such as ease of dosing administration, patient compliance and flexibility in formulation[1-3].

This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semisolid dosage forms. In- situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered [4].

Material and Methods

Preparation of in-situ gelling solution Sodium alginate and Gellan gum solutions of 1.0 and 1.5% (w/v) concentrations were prepared by adding the alginate to ultra-pure water containing sodium citrate 0.25% (w/v) and 0.075% (w/v) calcium chloride and heated up to 60 °C while stirring. Nizatidine was then dissolved in 10 ml of 0.1N hydrochloride acid solution of pH 1.2 and added in the resulting solution. The 0.1N sodium hydroxidesolution was used for the neutralization. A 1% (w/v) control solution was prepared by dissolving 0.6% (w/v) Nizatidine in aqueous solution of sodium
alginate. Nizatidine 1% (w/v) solution was prepared in ultra-pure water. **Evaluations:** The optimized formulation was subjected to different evaluation parameters listed below:

**Physical evaluation:** The solutions were evaluated physically for appearance and taste.

**Physical appearance and pH:** The pH of each of sodium alginate and gellan gum based in-situ solutions of Nizatidine was measured using a calibrated digital pH meter at room temperature in triplicate.

**Determination of Viscosity:** Viscosity of the samples was determined using *Brookfield Digital Viscometer.* The formulation (100 ml) was taken in a beaker and maintained at room temperature. For determination of viscosity was used. Viscosities were determined at different shear rates from 0 to 100 rpm.

**In-vitro gelation study:** The gelation cells are used for gelling studies fabricated locally using Teflon. The cells were holding 10 ml of simulated gastric fluid as gelation solution (0.1 N HCl, pH 1.2). Within the cells located at the bottom is a transparent plastic cup to hold the gel sample in place after its formation. 2 ml of the formulation was placed into the cavity of the cup using a micropipette, and 6 mL of the gelation solution was added slowly and the rate of gelation was detected by visual examination.

**In-vitro floating ability:** The *in-vitro* floating study was carried out using 0.1N HCl of pH 1.2. The temperature of the medium was kept at 37± 0.5°C.10 ml formulation was introduced into the dissolution vessel without disturbance in medium. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on surface of the dissolution medium (duration of floating) were noted.

**Determination of Drug Content:** Accurately, 10 ml of *in-situ gel* was measured and transferred to 100 ml of volumetric flask. To this 50-70 ml of 0.1N HCl was added and shaken on mechanical shaker for 30 min, followed by sonication for 15 min. Complete dispersion of contents were ensured, visually and filtered using 0.45 membrane filter. From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1N HCl. Contents of Nizatidine were determined spectrophotometrically.

**In-vitro release studies:** The drug release studies was carried out in USP XXVI dissolution test apparatus using basket apparatus at 37 ± 0.5°C at 50 rpm using 900 ml of pH 1.2 buffer as a dissolution medium (n=6). *In-situ* gel equivalent to 20 mg of Nizatidine (13.5 ml) was used for test. 5 ml of aliquot was withdrawn at predetermined time intervals of 5, 10, 15, 20, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min. The contents were filtered using 0.45 μ nylon filters and analyzed spectrophotometrically. Same volume of dissolution fluid maintained at 37 ± 0.5°C was replaced immediately.

**Table 1 and 2:** Comparison of *in vitro* Evaluation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Viscosity (cp)</th>
<th>Gelling time (in sec)</th>
<th>Floating time (in hours)</th>
<th>In-vitro Gelation Studies</th>
<th>Drug Content</th>
</tr>
</thead>
</table>

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**Conclusion:**

This study reports the development and characterization of a novel in-situ delivery system. A sustained release of the drug, Nizatidine is achievable from the gel vehicles over a period of at least 8 hrs. so we may conclude that sodium alginate may be a useful oral sustained release vehicle to improve patient compliance and bioavailability and which may be most useful for pediatrics and geriatrics patients. It was concluded that Sodium alginate is important for in-situ gel behavior along with calcium carbonate, and sodium citrate is vital for controlling and extending the release from formulations. Lastly, formulations containing higher and moderate amount of sodium alginate were considered as optimized formulation. Where as the concentration of the Gellan gum required for gelling is higher that increase the cost so it is batter using sodium alginate as gelling agent.

**References:**


Formulation development of sustained release solid dispersion of poorly water soluble drug, Piroxicam, employing novel application of mixed solvency concept and their evaluations

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Abstract: Piroxicam is in class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs). Piroxicam is orally administered drug. It is practically insoluble in water. In this present study an attempt was made to reduce the concentration of organic solvents used in the preparation of solid dispersion by the help of mixed solvency concept. The present investigation is an attempt to show that solids can also be wisely used to act as solvent precluding the use of organic solvents. The main objective of the present study is to demonstrate the solvent action of solid. In the present study, a eutectic liquid of menthol-thymol (1:1) was used as a solvent to formulate the solid dispersion. And solid dispersion was evaluated for powder x-ray diffraction, differential scanning calorimetric studies, scanning electron microscopy, and dissolution studies.

Introduction:
More recently, the concept of solid dispersion has been explored using insoluble carrier materials. These sustained release solid dispersion system may be useful for enhancing bioavailability and suitable for sustained release formulations [1]. The sustained solid dispersion offer various potential advantages for drugs having poor bioavailability and can be delivered efficiently there by maximizing their bioavailability and sustained action [2]. Solid dispersion can be prepared by various techniques that include mixed solvency concept also [3].

Materials and Methods:
The piroxicam bulk drug sample was a generous gift by Shreya life Sciences Pvt. Ltd., Aurangabad. All other chemicals and solvents used were of analutical grade. A Shimadzu- 1700 UV visible spectrophotometer with 1 cm matched quartz cells was used for spectrophotometric analysis.

Preparation of solid dispersion by mixed solvency concept: For preparation of solid dispersion in 1: 4 ratio, accurately weighed 6 gm of ethyl cellulose was dispersed in 20 ml of eutectic mixture (1:1 ratio of menthol- thymol) in a petri dish. Then, it was heated over heating mantle to dissolve the ethyl cellulose in eutectic mixture resulting into a solution. After that 1.5 gm of piroxicam was dissolved in the above solution and the temperature was maintained in the range of 55-60°C so as to facilitate dissolution. The resultant dispersion was spread on several glass petri plates and these petri plates were kept on hot plate maintained at 150°C so that remaining eutectic mixture could also be evaporated easily and a constant weight with no further weight loss could be obtained. After complete
drying solid dispersion was crushed using a glass pestle mortar and passed through sieve # 40 and was finally stored in a glass bottle. Same procedure was utilized to prepare solid dispersion in the ratio of 1:3, 1:5 and 1:6 using appropriate quantity of eutectic mixture.

**Evaluation of Solid Dispersion:**

**Drug content uniformity:** By shake flask method

**Dissolution Rate Studies:** Solid dispersion equivalent to 50 mg of piroxicam were tested in dissolution rate studies using USP XXIV (type II) dissolution test apparatus (Model TDT6P, Electrolab Mumbai, India) with paddle to rotate at 50 rpm.

**X-Ray Diffraction:** X-ray diffraction pattern were obtained using RU-H3R, Horizontal Rotaflex rotating anode X-ray generator instrument, Rigaku (Rigaku International Corporation, Tokyo).

**Differential Scanning Calorimetry:** In order to obtain the DSC thermograms, a thermal analysis instrument Pyris DSC 6000 (Perkin Elmer) was employed. Heating at a rate of 10°C/ min with was done to the reference in the temperature range of 25-350°C.

**Scanning Electron Microscopy:** S.E.M. photographs of piroxicam and its solid dispersion were obtained using a scanning electron microscopic model JEOL JSM 5600 with accelerating voltage from 0.5 to 30 KV.

**Results and Discussion:**

**Drug content uniformity:** The result of drug content analysis for formulated solid dispersion in 1:3 ratio was 24.95%, in 1:4 ratio was 20.01% and in 1:5 ratio was 16.04%.

**Dissolution studies:** From the result of dissolution studies, it is evident that solid dispersions of ratio 1:3 to 1:5 are showing sustained release of drug. Since there was no improvement in dissolution rate when drug: polymer ratio was increased. So, to minimize the concentration of polymer and other excipients, 1:3 ratio was considered to be optimum ratio and used for further studies.

**Figure 1** Comparative account of dissolution profiles of solid dispersions containing different drug: polymer ratio

**X-Ray Diffraction:** From XRD studies it is observed that there is increase in intensities of the characteristic peak in case of solid dispersion. This indicates that the dissolution rate in case of solid dispersion was enhanced.
dispersion has been decreased more resulting in the sustained release action.

**Differential Scanning Calorimetry:** The DSC curve of the crystalline form of pure drug (piroxicam) exhibits a sharp endothermic peak at 202.82°C, attributing to melting. However, the solid dispersion and physical mixture of ethyl cellulose resulted in complete suppression of the drug peak (fig.4), suggesting homogeneous dissolution of the drug in polymer and also shows that there is no interaction between drug and polymer.

**Scanning Electron Microscopy:** SEM photographs of pure piroxicam shows characteristic shaped structures, indicating the crystallinity of piroxicam. In case of SDs it was difficult to distinguish the presence of piroxicam crystals. Piroxicam crystals appeared to be incorporated into the polymer.

**Conclusion:**
The aim of the present research study was to explore the possibility of employing mixed solvency technique in the formulation of poorly water soluble drug. The main objective of this study is to show that solids can be employed to function as solvent. In future, the solids may be properly utilized for solvent action giving the alternate sources for solvents giving eco friendly methods excluding the use of toxic organic solvents. In the present study, practically water insoluble drug, piroxicam was tried to be solubilized by employing the combination of physiologically compatible menthol: thymol mixture in the form of their eutectic mixture to make its sustained release formulations. In the present study liquid solvents were not used purposely. From all the above studies, it was concluded that the approach of mixed solvency is novel, safe and user friendly. It also eliminates the problem of toxicity associated with the use of toxic organic solvents. So, it may be employed in the dosage form development of drugs where sustained release is desired. By study of suitable drug: polymer ratio, the dissolution pattern may be improved.

**References:**
Development and characterization of fast dissolving tablets by optimization using $3^2$ factorial design

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Abstract: The fast dissolving Tablets of Promethazine Thecolate were prepared by direct compression technique, using $3^2$ full factorial designs. This research work aimed to study and to develop a unique drug delivery system for immediate release of drugs which can dissolve readily when placed in the oral cavity. The superdisintegrants (Ac-Di-Sol, Sodium starch glycolate and Crospovidone) in varying concentration (1-4% w/w) were used to develop the tablets. Total 12 formulations were prepared and evaluated for pre-compression and post compression characteristics. The optimization of the batches was carried out using $3^2$ full factorial design and results of polynomial equation were analysed using ANOVA and regression analysis. Then another batches using effervescent technology were prepared. By the use of desirability approach final optimized formulation was prepared.

Introduction:

With the invent of a novel type of delivery systems for oral administration, various technologies have emerged with the fast onset of action including, Fast Dissolving Tablet (FDT) that disintegrates and dissolves rapidly in saliva without need of water. As soon as the drug goes into solution, the absorption becomes quicker and so is the onset of clinical effects. The retention of administered oral doses of an anti-emetic drug and its immediate absorption during anti-emetic therapy is affected by regular emesis, a process controlled by vomiting centre in lateral reticular formation of the medulla receiving inputs from the chemoreceptor trigger zone and other neural sites. So, presence of oral dose is therefore, mandatory for absorption to prevent emesis. Normally, emesis is preceded with nausea and in this condition it is not easy to administer drug with a glass of water; hence it is beneficial to administer such drugs as fast dissolving tablets. [1-3].

Materials and Methods:

Method of Preparation: The superdisintegrants (Ac-Di-Sol, SSG and CP) in different concentration (1-4% w/w) were used to develop the tablets. All the excipients were passed through sieve no. 60 and then were co-grounded in a pestle motor3-5.

Post compression characterization: Friability, Wetting Time, In vitro Dispersion time and Disintegration

Table 1 Formulation by varying superdisintegrants

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
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<tr>
<td>SSG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>3</td>
<td>4</td>
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<td>2</td>
</tr>
<tr>
<td>Avicel PH102</td>
<td>48</td>
<td>47</td>
<td>46</td>
<td>48</td>
<td>47</td>
<td>46</td>
<td>48</td>
<td>47</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
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<td>20</td>
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<td>Talc</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DT</td>
<td>98</td>
<td>84</td>
<td>63</td>
<td>51</td>
<td>87</td>
<td>76</td>
<td>68</td>
<td>57</td>
<td>52</td>
<td>59</td>
<td>48</td>
<td>39</td>
</tr>
<tr>
<td>% Friability</td>
<td>0.6</td>
<td>0.62</td>
<td>0.69</td>
<td>0.69</td>
<td>0.60</td>
<td>0.60</td>
<td>0.72</td>
<td>0.74</td>
<td>0.77</td>
<td>0.54</td>
<td>0.67</td>
<td>0.71</td>
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<tr>
<td>Coded values</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2 $3^2$ Full factorial design layouts (Lyophilized)

<table>
<thead>
<tr>
<th>Batch Codes</th>
<th>Variable Levels in Coded Form</th>
<th>Disintegration Time</th>
<th>% Friability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
<td>DT (s)</td>
</tr>
<tr>
<td>FDT1</td>
<td>-1</td>
<td>-1</td>
<td>52</td>
</tr>
<tr>
<td>FDT2</td>
<td>-1</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>FDT3</td>
<td>-1</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>FDT4</td>
<td>0</td>
<td>-1</td>
<td>50</td>
</tr>
<tr>
<td>FDT5</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>FDT6</td>
<td>0</td>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>FDT7</td>
<td>1</td>
<td>-1</td>
<td>48</td>
</tr>
<tr>
<td>FDT8</td>
<td>1</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>FDT9</td>
<td>1</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>OPT</td>
<td>0.75</td>
<td>0.72</td>
<td>30</td>
</tr>
</tbody>
</table>

Coded values

<table>
<thead>
<tr>
<th>Actual Values (mg)</th>
<th>$X_1$</th>
<th>$X_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Preparation of fast dissolving tablets factorial design batches: The initial material was passed through a no. 100 screen before mixing. Promethazine Thecolate, SSG, crospovidone, microcrystalline cellulose and lactose were mixed using a mortar and pestle. The blends were lubricated with 2% w/w talc and 2% w/w magnesium stearate. The blends were compressed into tablets using a single-punch tablet machine (Cadmach, Ahmedabad, India). The composition of the factorial design batches is shown in Table 2 respectively.
X indicates SSG (mg); X₂, Crospovidone (mg); DT, disintegration time; and F, friability. PCP used as check point and optimized batch. (n=6)

Figure 1 Response Surface for Optimized Formulation

Table 3 Development of Optimized Formulation (PMT)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>OPT 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promethazine Thecolate</td>
<td>6</td>
</tr>
<tr>
<td>Sodium Starch Glycolate</td>
<td>2.75</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>2.72</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>20</td>
</tr>
<tr>
<td>Avicel PH 102</td>
<td>44.53</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20</td>
</tr>
<tr>
<td>Talc</td>
<td>2.00</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Evaluation

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (mg)</td>
<td>100.024±2.120</td>
</tr>
<tr>
<td>Hardness (kg/cm²)</td>
<td>3.5±0.135</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.499±0.028</td>
</tr>
<tr>
<td>Wetting time (s)</td>
<td>25±1.98</td>
</tr>
<tr>
<td>Disintegration time (s)</td>
<td>31±2.01</td>
</tr>
<tr>
<td>Drug Content (%)</td>
<td>99.35±2.325</td>
</tr>
</tbody>
</table>

Results and Discussion:

The fast dissolving tablets of Promethazine Thecolate were successfully prepared by direct compression and effervescent technology. Total twelve formulations were prepared and optimized using 3² full factorial designs; that after the ANOVA was applied and the formulations showed the significant model. Using the desirability approach the final optimized formulation was prepared with the targeted results.

References:

Formulation development of topical solutions of poorly water soluble drugs, Gatifloxacin and Indomethacin, employing novel application of mixed solvency concept and their evaluations

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Abstract: Based on the large number of experiments on solubilization of poorly water-soluble drugs, the author is of the opinion that hydrotropy is another type of cosolvency and all water soluble substances whether solids, liquids or gases have solubilizing property therefore, an aqueous solution containing small quantities of several water-soluble excipients giving a concentrated solution may act as a solvent system for some poorly water-soluble drugs. This is an example of the concept of mixed solvency. This same concept has been explore to formulate the topical solutions of poorly water soluble drugs, Gatifloxacin, Indomethacin and topical solutions were evaluated for its chemical stability, thin layer chromatography, pH determination, viscosity, freeze thaw testing.

Introduction:
The main objective of present study was to explore the mixed solvency concept in the preparation of the topical solution of poorly water soluble drugs- gatifloxacin and indomethacin. The aim was to make water a strong solvent for the poorly water soluble drugs, gatifloxacin and indomethacin for the preparation of the topical solution of drug using mixed solvency concept by the use of safe solubilizers like niacinamide, sodium benzoate, PEG 4000, PEG 400, caffeine and glycerin. The procured samples of gatifloxacin and indomethacin were characterized by melting point, IR spectroscopy, UV characterization, and DSC study. Pre-formulation studies were performed which included solubility studies. There was significant increase in the aqueous solubility of both the drugs using mixed solvency blends.

There was no interference in UV spectrophotometric analysis of gatifloxacin and indomethacin due to the presence of different excipients.

Topical solutions of gatifloxacin and indomethacin were prepared individually, and stability studies were performed. The stability studies were carried out for two months at room temperature, 30°C and 40°C. The results of stability studies of gatifloxacin and indomethacin topical solution gave the satisfactory results. For the evaluation different studies were performed like thin layer chromatography, freeze thaw study, viscosity, pH and satisfactory result were found. The concept of Mixed Solvency [1-5] was successfully employed in formulating the topical solution of poorly soluble model drugs, gatifloxacin and indomethacin. In this study, there was no involvement
of organic solvent to prepared topical solution. Main drawbacks of organic solvents include higher costs and the toxicity due to residual solvent. The present study illustrates the novel application of mixed solvency concept to solve the problems of above mentioned drawbacks associated with organic solvents. Pharmaceutical companies may be benefited by this concept, not only to manufacture topical solution but also to develop other pharmaceutical formulations.

Materials and Methods:

Drug characterization of Gatifloxacin and Indomethacin: The maximum wavelength of gatifloxacin and indomethacin were found to be 330 and 320 nm respectively. Melting point of gatifloxacin and indomethacin were found to be 180-185°C and 155-160°C respectively. The FTIR spectrum of drug sample of gatifloxacin and indomethacin had shown identical peaks as reported in references sample of gatifloxacin and indomethacin. The DSC curve of the crystalline form of gatifloxacin and indomethacin showed a sharp endothermic peak at 185°C and 161.8°C respectively demonstrated a melting peak corresponding to its melting point.

Pre-formulation studies of Gatifloxacin and Indomethacin: Calibration curve of gatifloxacin and indomethacin gave the regression value $R^2=0.999$. The pre-formulation studies showed that there was no interference in UV spectrophometric analysis of gatifloxacin and indomethacin in presence of excipients. The solubility of gatifloxacin and indomethacin drug sample in de-mineralized water was found to be 0.380g/100ml and 0.105g/100 ml respectively. There was no physical incompatibility between drugs (gatifloxacin, indomethacin) and the solubilizers.

Formulation development of Gatifloxacin and Indomethacin:

Solubility determination of gatifloxacin and indomethacin in various aqueous solutions of solubilizers (blends):

Maximum increase in solubility of gatifloxacin was observed in Blend H (20% SB + 10% NM + 10% C + 10% PEG 400) and in Blend N (15% SB + 10% NM + 10% GN + 5% PEG 4000) so these were selected to be used in topical formulations of gatifloxacin. For indomethacin maximum increase in solubility of indomethacin was observed in Blend Q (20%SB + 10%NM + 10%C + 10%PEG 400) and in Blend T (20%SB+10%NM+ 15%C+10% PEG 400) so these were selected to be used in topical formulation of indomethacin.

Stability study:
The stability studies were carried out for two months at room temperature, 30°C and 40°C. The results of stability studies of topical solutions of gatifloxacin and indomethacin gave the satisfactory results.
References

Microspponge delivery system of Propyl Paraben for sustained preservation of creams and ointments: Optimization, characterization and antimicrobial evaluation

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Abstract: Propyl paraben, which is lipophilic and an indispensable preservative in creams and ointments is notorious for its skin irritating side effects. If entrapped in a high efficiency delivery system like microsponges, they can be used in safer concentrations, for a prolonged period of time. The microsponges of propyl paraben were prepared by the quasi emulsion solvent diffusion method. These microsponge were then characterized for particle size, production yield and encapsulation efficiency. In-vitro release studies, revealed that the release of propyl paraben from the microsponges to the cream and finally into the receptor cell fluid followed zero order diffusion. These formulations were also subjected to microbiological evaluation. The findings confirmed that microsponge loaded propyl paraben were far superior in their antibacterial effect as they could control the release of propyl paraben and prolonged its preservative action.

Introduction:
Microsponges are polymeric delivery systems composed of porous microspheres possessing a large surface area [1]. These porous microspheres can entrap active ingredients in very large quantities and control their delivery rate since they are made up of interconnecting voids within a non collapsible structure [2]. Parabens are a very important class of anti fungal preservatives and unavoidable in the recipe of creams as they protect the product from spoiling and turning stale and useless for consumers, but the literature is full of their side effects [3]. The objective of the present work was to entrap propyl paraben in a delivery system like Microspponge Drug Delivery System (MDDS), which has a high payload. The aim of the present study was to ensure the prolonged release of the active constituent in a time release manner without coming in direct contact with the skin, thereby avoiding situations like skin irritancy, allergy, contact dermatitis, toxicity, etc.

Materials and Methods:
Research grade ethyl cellulose, polyvinyl alcohol, ethanol, triethyl citrate, white beeswax, borax, mineral oil, propyl paraben, etc., purchased from Loba Chemie, Pvt. Ltd. Mumbai, were used.

Method of preparation of microsponges: The inner phase was prepared by dissolving ethyl cellulose polymer in ethanol, to which the propyl paraben (preservative) was added. Then outer phase was prepared by dissolving PVA in distilled water. The inner phase was then poured into the outer phase under continuous agitation, at room temperature and was continuously stirred for about 4
hours at 1000 rpm, filtered and dried in an oven at 40°C for 24 hours [4]. The microsponges were prepared by $3^2$ factorial design and F7 batch was found to be the best batch based on particle size, entrapment efficiency and drug release rate data. The shape and surface characteristics of microsponges were analyzed using Scanning Electron Microscope [5].

![SEM images of propyl paraben microsponges of the optimized batch (F7)](image)

**Figure 1** SEM images of propyl paraben microsponges of the optimized batch (F7)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Ethyl cellulose</th>
<th>PVA</th>
<th>Weight of excipients &amp; propyl paraben (mg)</th>
<th>Weight of microsponges (mg)</th>
<th>Particle size (µm)</th>
<th>Production yield (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>300</td>
<td>250</td>
<td>590</td>
<td>490</td>
<td>59.23</td>
<td>83.0</td>
<td>64.95</td>
</tr>
<tr>
<td>B4</td>
<td>600</td>
<td>250</td>
<td>890</td>
<td>785</td>
<td>54.72</td>
<td>88.2</td>
<td>70.28</td>
</tr>
<tr>
<td>B7</td>
<td>900</td>
<td>250</td>
<td>1190</td>
<td>1070</td>
<td>42.62</td>
<td>89.9</td>
<td>78.28</td>
</tr>
<tr>
<td>B9</td>
<td>900</td>
<td>1000</td>
<td>1940</td>
<td>1035</td>
<td>69.11</td>
<td>53.3</td>
<td>62.53</td>
</tr>
</tbody>
</table>

Beeswax - borax type of cold cream was prepared in which a calculated quantity of propyl paraben loaded microsponges were incorporated. *In vitro* diffusion study of propyl paraben loaded microsponges in cream were carried out. The samples withdrawn were analyzed spectrophotometrically at 258 nm.

For antimicrobial preservative testing *Agar Well Diffusion* method was used and slants of microorganisms *Staphylococcus aureus* and *Pseudomonas auregonosa* were collected from Department of Microbiology, M.D University, and Rohtak. Then three wells were bored on agar media plate with a sterile cork borer. In first well microsponges loaded propyl paraben (batch 7) were placed. In the second well plain cold cream was introduced and in the third well cold cream having microsponges loaded with propyl paraben was introduced. Then these plates were incubated for 24 hours at 37°C. After incubation, diameter of zone of inhibition was measured to test the preservative efficacy.

**Results and Discussion:**

Particle size, percentage yield, encapsulation efficiency of optimized batches of microsponges is shown in table 1. Result of compatibility studies showed no interactions between drug and excipients.
which was confirmed by FTIR and DSC studies also. SEM images of propyl paraben loaded microsponges were in micro range and porous.

Conclusion:
Diameter of zone of inhibition of cold cream containing microsponges loaded propyl paraben was greater than that of cold cream with plain propyl paraben. The results were encouraging and indicated that preservatives in microsponge drug delivery system are good alternatives as they can prolong the release of propyl paraben and sustain the antibacterial activity.

Table 2 Measurement of Diameter of Zone of Inhibition (24 hrs).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Formulations</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas auregenosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propyl-Paraben Microsponge</td>
<td>21mm</td>
<td>24mm</td>
<td></td>
</tr>
<tr>
<td>Cold cream containing Microsponge</td>
<td>11mm</td>
<td>13mm</td>
<td></td>
</tr>
<tr>
<td>Plain cold cream</td>
<td>09mm</td>
<td>10mm</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 Zone of Inhibition of Propyl Paraben against *Staphylococcus aureus* (a) and *Pseudomonas auregenosa* (b)

Acknowledgment:
Authors wish to thank Head, Dept. of Pharmaceutical Sciences and Head Dept. of Microbiology, M.D University, Rohtak, for providing facilities to carry out the experiments.

References:
Formulation and evaluation of herbal remineralizing tooth powder to cure dental carries

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E-mail address: rvsheovey@gmail.com

Abstract: Maintenance of health of the teeth and gums well is very important for having good general health. Denitrifies are the preparations used for cleaning the surface of teeth and keep them shiny and to preserve the health of teeth and gums. The study deals with formulation of remineralizing tooth powder which consist of Bentonite, Baking soda, Ground Myrrh gum, Saccharine, Cinnamon and Ground clove. The evaluation parameter includes physical parameters like Color, Odor, pH, Consistency, Feel, Foaming character, Flow property, Angle of Repose, Bulk density, Tapped Density, Carr’s Index, Stability studies. The evaluation of formulations shows golden brown color, free flowing property, good acceptable odour, as desired in dentifrices with pH range of 7 which is near to natural pH. The results of present study indicate that all other parameters were within the limits.

Introduction:
Although dental caries is one of the most preventable diseases known to man, caries experience is on the rise. [1] In the US, for instance, the National Institute of Dental and Craniofacial research reports that dental restorations are needed in almost four out of every five children by the age of 17. Thus, despite widespread water fluoridation, installation of protective dental sealants and increased understanding of caries management, additional measures may be required to improve caries prevention. The primary function of tooth powder is the cleaning of the accessible surfaces of the teeth. [2] Freshen breath, help heal gums, rid teeth of bacteria, and reduce the amount of inflammation in the mouth as well as remineralise the teeth. [3]

Material and Methods:
Collection, processing and authentification: All drugs bentonite, Baking Soda, Ground Myrrh gum, Sachharine cvinnamon and Ground Clove are collected from the local market of Indore, M.P. The material was dried under shade. Powdered it and used for the formulation of Herbal tooth powder.

Method of preparation of tooth powder.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bentonite</td>
<td>88.8gm</td>
</tr>
<tr>
<td>Baking soda</td>
<td>3gm</td>
</tr>
<tr>
<td>Ground Myrrh gum</td>
<td>4gm</td>
</tr>
<tr>
<td>Saccharine</td>
<td>0.2gm</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>2gm</td>
</tr>
<tr>
<td>Ground clove</td>
<td>2gm</td>
</tr>
</tbody>
</table>
The all natural materials was used in the present study i.e., saccharine, bentonite, baking soda, myrrh gum powder, ground cloves, ground cinnamon was purchased from local market of Indore and was kept for drying. The ingredients was weighed accurately and then ground into fine powder. The grounded ingredients were sieved separately by using sieve # 120. Further the mixed powder is again passed through sieve # 120 so as to break the lumps and to get a fine powder. [2] Then the prepared tooth powder was packed into a self-sealable polyethylene bag, labeled and used for further studies.

**Standardizations / Evaluation parameters**

**Determination of Physical Parameter**

**Physical parameters** All formulations were evaluated for physical parameters like Color, Odor, pH, Consistency and Feel.

**Volatile matters and moisture:** A specific amount of the product required to be taken in a dish and drying was done till constant weight. Loss of weight will indicate percentage of moisture and volatile matters 2, 16, 18.

**Foaming character:** This test was specially required for foam forming tooth powders. Specific amount of product can be mixed with specific amount of water to be shaken. The foam thus formed was studied for its nature, stability, washability1, 2.

**Flow property:** Flow property is determined by Angle of repose in Funnel method.

\[ \tan \alpha = \frac{h}{r} \]

where, \( h \) = Height, \( r \) = Radius

The angle to the horizontal cannot exceed a certain value this known as ‘angle of repose

**Bulk density:** Bulk density was determined by Tapped and untapped volume of the powder. Firstly accurately weigh 100 gm of the tooth powder and fill in the 100 ml measuring cylinder. Then note down the volume of the measuring cylinder fill with powder. Then put the value in the given formula:

\[ \text{Tapped density} = \frac{\text{Mass of powder}}{\text{Bulk volume}} \]

**Tapped Density** Firstly accurately weigh 100gm of the powder and fill in to the 200ml measuring cylinder. Then note down the volume of the measuring cylinder fill with powder. Then tapped the measuring cylinder for 1000 time & note measuring cylinder reading.

Then put the value in given formula: Tapped Density= Mass of powder / Tapped volume

**Carrs Index**= Tapped Density / Pored Density *100/ Tapped density

\[ \tan(\alpha) = \frac{\text{Height}}{\text{Radias}} \]

\[ \tan(\alpha) = 0.43 \]

**Stability studies:** The prepared formulations are subjected to stability studies by storing at different
temperature conditions for the period of one month. All the formulations will be packed in glass vials separately and stored at different temperature conditions viz., room temperature, 35ºC and 40ºC and will be evaluated for physical parameters like Color, Odor, pH, Consistency and feel. [2]

Results and Discussion:

Physical parameters: The results for physical parameters were showed in the table 2. The prepared formulations showed colors like Golden Brown. The formulations showed free flowing. The prepared formulations were having good acceptable odor which is desirable as cosmetic formulations. The pH all formulations lie in the range of 7 which is near to neutral.

Table 1 Results for Physical parameters.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Physical Parameter</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Golden Brown</td>
</tr>
<tr>
<td>2</td>
<td>Odor</td>
<td>Acceptable</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Feel</td>
<td>Acceptable</td>
</tr>
<tr>
<td>5</td>
<td>Consistency</td>
<td>Flowing powder</td>
</tr>
</tbody>
</table>

Evaluation of herbal tooth powder: Moisture content: Loss of weight will indicate percentage of moisture and volatile matters.

Foaming character and Bulk density

The foaming character and bulk density of 1% and 2% solution of formulation 1 and formulation 2 were shown in Table no.3 and 4.

Flow property: Flow properties determined by angle of repose were found to be 47.2 θ} 0.2292, 47.37 θ} 0.2719 of formulation 1 and formulation 2 respectively (Table no.)

Table 2 Physical Parameters

<table>
<thead>
<tr>
<th>S. No</th>
<th>Powder Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bulk density</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>Tapped density</td>
<td>1.176</td>
</tr>
<tr>
<td>3</td>
<td>Carr’s index</td>
<td>31.97 (good)</td>
</tr>
<tr>
<td>4</td>
<td>Haustners ratio</td>
<td>147(poor)</td>
</tr>
<tr>
<td>5</td>
<td>Angle of repose</td>
<td>27 Degrees</td>
</tr>
</tbody>
</table>

References:


Preparation and evaluation of preconjugated gastro-retentive microspheres of Sitafloxacin for effective treatment of Helicobacter pylori

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Abstract: The objective of the present investigation was to prepare and evaluate the mucoadhesive microsphere for the treatment of mucosal ulcer consisting of Eudragit S100 biocompatible polymer and phosphotidylethanolamine (PE) coating on surface of Eudragit S100 microsphere to permit target specific delivery of drugs to H. Pylori. The microsphere were formed with the help of emulsion solvent diffusion method and characterized for various parameters. The microspheres were then conjugated with PE. Conjugated microspheres were further characterized for mucoadhesiveness to gastric mucosae and PE conjugation efficiency.

Introduction:
Helicobacter pylori is a spiral-shaped, highly motile organism with a unipolar flagellum found within and beneath the mucous layer of the stomach and often attached to the gastric mucosae. H. pylori enter into the stomach and attach itself to the lining of the stomach to establish an environment to grow. Resistance of Helicobacter pylori to the standard therapeutic antimicrobials clarithromycin, metronidazole, amoxicillin and tetracycline has been demonstrated. Recently, novel quinolones, including garenoxacin (GRNX) and sitafloxacin (STFX), that are more potent against grampositive bacteria than LVX and GAT are have become available .Among the quinolones tested against H. pylori, STFX was the most active (MIC for 90% of the strains tested [MIC90], 0.008 mg/liter . To overcome all the constraints in H. pylori treatment, an attempt is made to develop PE Conjugated Eudragit S100 microspheres of Sitafloxacin to deliver the antimicrobial agent to the target cells.

Materials and Methods:
Sitafloxacin hydrate (STFX) was obtained from M/s poultry India, Hyderabad. Eudragit S100 was obtained as a gift sample from M/s S. Zhaveri and Company Mumbai., Phosphotidylethanolamine (PE) was supplied as a gift sample by M/s Lipoid GmBH (Germony). N-Hydroxysuccinimide (NHS) was supplied as a gift sample from Shivam Enterprises, Pune. 1-Ethyl-3, 3-(dimethylaminopropyl) carbodiimide (EDC) was procured from HiMedia labs. Dichloromethane, ethanol and all other chemicals used were of analytical reagent grade.

Preparation of Microspheres
Microspheres were prepared by emulsion solvent diffusion method reported by Kawashima et al. [1].

Characterization of Microspheres
Entrapment efficiency of STFX Eudragit microspheres was determined by method suggested by Whitehead et al. [2]. Micromeritic properties such as particle size, true density, tapped density, compressibility index and flow properties were determined by the methods reported by Jain S.K. et al. [3]. Percentage buoyancy was calculated by a method suggested by Jain et al. [4]. The external and internal morphology of the microspheres was studied by scanning electron microscopy.

**Dissolution Study**

The release rate of sitafloxacin from microspheres was determined using a USP type-II paddle type dissolution apparatus and samples were analysed using a UV double-beam spectrophotometer at 295 nm. Folin–Ciocalteu reagent used to determine the amount of PE bound to the Eudragit microspheres. Everted sac experiment was carried out by method used by Jain S.K.et.al. [4] To determine percentage of mucoadhesion using rat stomach for selected microspheres (STFX 3b) and PESTFX and percentage of binding was calculated using formula suggested by Jain et al. [4]

**Conjugation of Phosphotidylethanolamine (PE) to Eudragit Microspheres**

PE coupling was carried out using carbodiimide technique reported by Olde Damink et al. [5]

**Statistical Analysis**

*In vitro* drug release of STFX from STFX3b and PESTFX were statistically treated by one-way analysis of variance (ANOVA), followed by Dunnett’s test where *P*<0.05 was considered statistically significant.

**Results and Discussion:**

Microspheres prepared by emulsion solvent diffusion method and optimized as 1 : 1, 1 : 1.5 and 1 : 2 (drug : polymer), keeping different stirring speeds viz. 100, 300, 500 rpm (total nine formulations). The SEM images of STFX3b and PESTFX microsphere were spherical shape with a smooth uniform surface. Percentage yield, drug entrapment efficiency (DEE), buoyancy (%), of the prepared formulations was found in the range 70.1 ±7–79.26±1.4, 62.23±1.6–71.92±2.5%, and 42.3±1.2–84.76±1.5 respectively for the developed formulations. The average particle size of microspheres was found to be in the size range 112.1±1–189.3±2µm. The size of PE conjugated formulation (235.32 ± 1.2µm) was higher compared to non-conjugated microspheres (184.43±.02µm), Optimum speed was found to be 300 rpm as Below 300 rpm, shear unstable emulsion droplets were formed and above 500 rpm larger droplets were formed and aggregated. The apparent density, tapped density, Compressibility index, per cent porosity, angle of repose was found between .55±1.1-.75±2.3 g cm–3, .71±.01-.95±.24 g cm–3. 15.57±2.97-22.12±.56%, 24.49±3-35.52±3%, 23.87±83-28.54±75(θ) respectively, suggesting good flow characteristics of the microspheres. PE conjugation efficiency of PESTFX was found to be 78.26± 0.98 and maximum mucoadhesion (83.71 ± 1.039%) was shown by PESTFX microspheres.
Table 1 Evaluation parameters of Sitafloxacin Microspheres (Values are expressed as mean ± SEM)

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particulate Size (µm)</th>
<th>Tapped density g cm⁻³</th>
<th>Apparent density g cm⁻³</th>
<th>True density g cm⁻³</th>
<th>Porosity (%)</th>
<th>Compressibility index (%)</th>
<th>Angle of repose (°)</th>
<th>Yield (%)</th>
<th>Drug entrapment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STFX1A</td>
<td>112.1±1</td>
<td>71±0.1</td>
<td>55±1.1</td>
<td>72±0.1</td>
<td>35.5±3</td>
<td>22.12±0.56</td>
<td>26.12±1</td>
<td>70.1±7</td>
<td>62.23±1.6</td>
</tr>
<tr>
<td>STFX1B</td>
<td>109.1±2.5</td>
<td>76±0.2</td>
<td>57±2.3</td>
<td>75±0.2</td>
<td>33.43±0.8</td>
<td>21.13±0.98</td>
<td>27.14±1</td>
<td>71.2±2</td>
<td>64.24±1.8</td>
</tr>
<tr>
<td>STFX1C</td>
<td>107.1±3</td>
<td>79±0.2</td>
<td>60±3.4</td>
<td>78±0.5</td>
<td>31.45±0.9</td>
<td>19.23±0.48</td>
<td>28.54±0.75</td>
<td>77.8±1</td>
<td>65.36±1.9</td>
</tr>
<tr>
<td>STFX2A</td>
<td>135.1±1</td>
<td>76±0.4</td>
<td>57±3.6</td>
<td>79±0.9</td>
<td>33.32±0.1</td>
<td>18.24±0.38</td>
<td>26.76±1</td>
<td>71.2±7</td>
<td>63.46±2.1</td>
</tr>
<tr>
<td>STFX2B</td>
<td>131.2±7</td>
<td>81±0.4</td>
<td>61±8.7</td>
<td>80±5.8</td>
<td>29.54±0.2</td>
<td>17.35±0.92</td>
<td>25.34±1</td>
<td>75.28±2</td>
<td>65.38±1.5</td>
</tr>
<tr>
<td>STFX2C</td>
<td>123.2±5</td>
<td>85±0.9</td>
<td>65±9.3</td>
<td>81±4.8</td>
<td>28.65±0.1</td>
<td>16.45±1.45</td>
<td>24.35±1</td>
<td>77.12±1</td>
<td>67.79±2.4</td>
</tr>
<tr>
<td>STFX3A</td>
<td>189.3±2</td>
<td>84±1.7</td>
<td>62±0.1</td>
<td>85±3.7</td>
<td>29.34±0.9</td>
<td>16.36±2.65</td>
<td>23.67±1</td>
<td>72.37±3</td>
<td>67.48±3.1</td>
</tr>
<tr>
<td>STFX3B</td>
<td>184.4±2</td>
<td>89±3.4</td>
<td>68±1.4</td>
<td>91±0.6</td>
<td>27.54±0.7</td>
<td>15.57±2.97</td>
<td>23.87±1</td>
<td>79.26±1</td>
<td>71.92±2.5</td>
</tr>
<tr>
<td>STFX3C</td>
<td>175.2±1</td>
<td>95±2.4</td>
<td>75±2.3</td>
<td>95±3.9</td>
<td>24.49±0.3</td>
<td>17.56±0.82</td>
<td>25.56±1</td>
<td>75.11±3</td>
<td>66.14±1.5</td>
</tr>
</tbody>
</table>

Figure 1 Comparative cumulative % drug release profile of STFX3B (Series 1) and PESTFX (series 2) in simulated gastric fluid (pH 1.2) at 37°C.

Conclusion:
PE conjugated Eudragit microspheres of Sitafloxacin were successfully prepared with significantly enhanced the mucoadhesiveness and controlled release. Such developed formulations could be subjected to in vivo studies in future for complete eradication of H. pylori.

References
Synergistic effect of drug-cyclodextrin vesicular carriers looms for controlled targeting of nevirapine for HIV management.

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Abstract: Effective Formulation designing for drugs has long been a major defy, for the reason that drug effectiveness can be sternly limited by instability or poor solubility in the vehicle. Cyclodextrin (CD) have reported to tailored transdermal drug permeation of many compound by complexation and accelerate drug release by enhancing the proportion of diffusible substance. The Nevirapine and Nevirapine-CD complex loaded elastic liposomal formulation were prepared and characterized for shape, size, entrapment efficiency, and ex-vivo skin permeation and deposition study. Nevirapine-hydroxypropyl-β-CD inclusion complex and encapsulates this complex in elastic liposomes novel formulation strategy of dual carrier approach on skin targeting of Nevirapine.

Introduction:
The novel drug delivery approaches including Highly Active Anti-Retroviral Therapy (HAART) have augmented the life duration of the HIV/AIDS patient. Nevirapine was the first Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) approved by the FDA, is considered a breakthrough in HIV treatment. [1-4] A range of novel strategies are currently being developed for efficient delivery of ARV drugs. Efficient delivery could be achieved by encapsulating the drug or by attaching it with a carrier system. [2] To fulfill the need of long-term treatment with anti-HIV agents controlled drug delivery systems are preferred. Cyclodextrin have reported to modified transdermal drug penetration of many compound by complexation and accelerate drug release by enhancing the proportion of diffusible substance. [1-4]

Our aim was to explore the possible applicability of cyclodextrin as a novel carrier to improve the solubility and dissolution rates of poorly soluble drug NVP through solid dispersion technology. The elastic liposome will directly target the lymph node and spleen through the skin, which are virus reservoirs. Encapsulation of drug in the form of cyclodextrin-drug complex in vesicular formulation elastic liposome has been explored as new approach for amalgamation the relative advantages of the two types of carrier into a single system.[2, 3][Figure 1].

Material and Methods:
Saturated soy lecithin (PC; Phospholipon 90H) Lipoid GmbH, Germany, Nevirapine (Mylan Ltd) Span 60 and Tween 80 (Croda) Hydroxypropyl β-cyclodextrin (HP-β-CD) (Himedia), Cholesterol (Oxford reagent), Polycarbonate membrane (Merck),

**Characterization and Evaluation:** The solid inclusion complex of nevirapine was prepared by freezing method as reported by Jain et al. where inclusion complex is hydroxypropyl β-cyclodextrin (HP-β-CD) and drug is nevirapine, in a drug: carrier ratio at 1:1 and 1:3 w/w. The elastic liposomes and niosomes were prepared by thin film hydration and sonication method as described by Jain et al. [3] ratios as per shown in Table: 1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation code</th>
<th>PC (mg)</th>
<th>Span 60 (mg)</th>
<th>Tween 80 (mg)</th>
<th>Cholesterol (mg)</th>
<th>Drug-CD (mg)</th>
<th>Vesicle shape</th>
<th>Entrapment Efficiency (%)</th>
<th>Zeta potential mV</th>
<th>Particle size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EL-1</td>
<td>75</td>
<td>15</td>
<td>10</td>
<td>-</td>
<td>14.56</td>
<td>Oval</td>
<td>96.84±2.75</td>
<td>-21±0.64</td>
<td>2.94±0.08</td>
</tr>
<tr>
<td>2</td>
<td>EL-2</td>
<td>75</td>
<td>10</td>
<td>15</td>
<td>-</td>
<td>14.56</td>
<td>Oval</td>
<td>82.43±6.87</td>
<td>-18±0.73</td>
<td>2.89±0.87</td>
</tr>
<tr>
<td>3</td>
<td>EL-3</td>
<td>85</td>
<td>15</td>
<td>10</td>
<td>-</td>
<td>14.56</td>
<td>Spherical</td>
<td>95.09±1.87</td>
<td>-16±0.67</td>
<td>3.87±0.78</td>
</tr>
<tr>
<td>4</td>
<td>EL-4</td>
<td>85</td>
<td>10</td>
<td>15</td>
<td>-</td>
<td>14.56</td>
<td>Spherical</td>
<td>81.62±4.98</td>
<td>-17±0.87</td>
<td>3.98±0.56</td>
</tr>
<tr>
<td>5</td>
<td>NIO</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>14.56</td>
<td>Rings oval</td>
<td>74.98±6.98</td>
<td>-99±0.93</td>
<td>3.92±0.79</td>
</tr>
</tbody>
</table>

*EL- elastic liposomes, NIO- Niosomes, CD- Cyclodextrin, PC- Phospholipid, b- particle size before sonication

**Characterizations of Elastic Liposomal and niosomes Formulation**

The drug and drug-HP-β-CD complex loaded elastic liposomal and niosome formulation were prepared and characterized for shape, size, entrapment efficiency, ex-vivo skin permeation. [3]

**Invitro release study**

All animal experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee (IAEC) of Smriti College of Pharmaceutical Education. The in vitro skin permeation of nevirapine from elastic liposomal formulations, niosomes, and drug-HP-β-CD complex was studied using Franz glass diffusion cell maintained at 37±1°C under non-occlusive conditions.

**Results and Discussion:**

The DSC thermogram of Nevirapine exhibited a typical anhydrous crystalline substance showing a sharp endothermic peak corresponding to melting point of the drug (t onset = 245.54°C, t peak = 249.14°C, H = 241.0175 J/g). *(Figure 2).* The Drug-HP-β-CD solid dispersion was used for drug loading in liposomal and niosomes formulation.

**Elastic Liposomal and Niosomes Formulations**

The elastic liposome formulations obtained using phospholipid, Span 60 and Tween 80 concentrations have similar physicochemical properties (ie, shape, size, entrapment efficiency). Formulations were initially characterized for particle size, shape and zeta potential, and entrapment as can be seen from **Table 1.** The shape of liposomal and niosomes were examined shown in **Figure 3.** The size of
liposome and niosomes were in average of range of the 2-4µm (Table 1) But increasing the concentration of Tween 80 decrease the entrapment of drug shown as in EL-2 and EL4 were 82.43±6.87 and 81.62±4.98% respectively. The EL-3 and EL-4 formulation where phospholipid content were increased and it shown increase in zeta potential and increase in size of vesicles.

**In vitro release study**

The *in vitro* release behaviour of the nevirapine-HP-β-CD loaded elastic liposomes, nevirapine-HP-β-CD niosomes and nevirapine-HP-β-CD solution for 12 hours is summarized in the cumulative percentage release shown in (Figure 4). All formulation were analysed through rat skin for *in vitro* release study. The all Elastic Liposomal have shown more than 75% release in 12 hours, whereas niosomes and nevirapine-HP-β-CD solution have shown less than 60% release in 12 hours. The EL-1 and EL-3 was superior release profile form all other formulation with 93.35±1.43% and 92.35±1.65% respectively. EL-2 and EL-4 also had shown 79.35±1.97% and 77.35±2.53% respectively.

**Conclusion:**

Results reveal that Elastic liposomal is better than niosomes and drug-CD solution. EL-1 and EL-3 were superior in elastic liposomes in case of entrapment, release profile, and stability. Nevirapine-HP-β-CD with low log P value is hydrophilic and nevirapine-HP-β-CD Elastic liposomal has high log P values and after release it will have low log P values.

**References:**

Localized delivery of an anticancer drug via poloxamer based in situ injectable hydrogel

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Abstract: In the present study, we have investigated the significance of the PEGylated anticancer loaded drug onto poloxamer based thermo responsive injectable hydrogel to understand the role of delivery system. In our previous study, the prepared conjugates efficiently enhanced the solubility of the melphalan and significantly reduced the hemolytic effect due to the presence of the PEG chains. The prepared conjugates (MLPEG 5000 and 2000) were loaded to the thermo sensitive Poloxamer 407 (P407) gel to produce an injectable hydrogel (MPX). Presence of NaCl salt in turn tightened the PEO chains and remarkably reduced the drug’s initial burst from the delivery system as only 43% of drug released during 2 hours from MPX-CG hydrogel. Prepared hydrogels were administered to Wistar rats via subcutaneous and intramuscular routes, to confirm the depot formation.

Introduction:
Over the past few decades, hydrogels have been an issue of intensive research. Hydrogels are supposed to retain large amount of water among its polymeric network [1]. Poloxamer, a copolymer of poly (oxyethylene)–poly (oxypropylene)–poly (oxyethylene), is the choice of polymer for the formulation of thermo sensitive hydrogels. Melphalan (p-[bis (chloro-2-ethyl) amino-Lphenylalanine) is an alkylation agent, familiarized in the late 1950s with a wide spectrum of antitumor activity. In the present we have loaded the PEGylated conjugates into a thermo sensitive P407 based gelling system for injectable in situ forming hydrogel. In addition, administration of the prepared hydrogel via subcutaneous and intramuscular routes in rats, confirms the good syringeability, depot formation, and biocompatibility.

Materials and Methods:

Gel Preparation
The PEGylation of the melphalan with two grades of linear methoxy poly (ethylene glycol) (M-PEG), viz. M-PEG 2000 and 5000 to form a PEGylated melphalan conjugate (MLPEG) according to our previous work (Ajazuddin et al, 2012) [2]. The concentration of 25% (w/w) was preferred for the P407 gelling system. The cold method as described by Schmolka, 1972 was referred for the preparation of the gels [3].

In-vitro release studies: Drug release study performed using a diffusion system, to evaluate the efficiency of an injectable delivery system [4]. Franz type cell (Vertical) having a fixed volume
receptor chamber with controlled temperature and port to sample stirred receptor fluid was used for the experiment.

**In-vivo animal tests**

For the *in vivo* animal test, 25% (w/w) of P407 was loaded with the PEGylated melphalan to produce (MPX-CG) and (MPX-7.4) solutions. From this formulation, 0.5 ml of solution was injected subcutaneously. About 0.2 ml injected via intramuscular route into the dorsal muscle pouch of adult female Wistar rat (200 g) using a 26G needle.

**Results:**

**Gel Preparation**

The rheological studies of the formulation performed with and without presence of the melphalan conjugates. All formulations remain to be viscous liquid at low temperature (4°C) and transforms to a semisolid gel at 20°C, exhibiting a reverse transition (liquid state) below the sol-gel transition temperature (Figure 1). No substantial difference noticed between the gel-sol transition temperature estimated by flow and oscillatory measurements thereof. Presence of melphalan conjugate does not alter the gelation temperature (Table 1). P407 (25% w/w) was considered as the base for the hydrogel and was kept constant throughout the study.

**Table 1** Gel-sol transition temperatures of P407 (25%), representing different formulations. Each value is expressed as mean ± standard deviation (SD) (n=3).

<table>
<thead>
<tr>
<th></th>
<th>Gelation temperature ± SD (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow</td>
</tr>
<tr>
<td>Control gel</td>
<td>12.6 ± 0.2</td>
</tr>
<tr>
<td>pH 7.4 gel</td>
<td>12.7 ± 0.1</td>
</tr>
<tr>
<td>Control gel + melphalan conjugate (MPX-CG)</td>
<td>13 ± 0.9</td>
</tr>
<tr>
<td>pH 7.4 gel + melphalan conjugate (MPX-7.4)</td>
<td>12.9 ± 0.7</td>
</tr>
</tbody>
</table>

**Figure 1** Showing sol-gel phase transitions of P407 at critical temperatures.

**In-vitro release studies:** The purpose behind the present study is also to underline the presence of the salt (NaCl) on the release behavior of the drug from the P407 hydrogel. To maintain the pH both in donor and the receptor compartment’s medium, no buffer were used. Sterile water free from any
additive was used as receptor phase. Initial burst release was reduced in case of the MPX-CG gel, as 43% of the drug was released, when compared to the MPX-7.4 gel, the initial burst release was found to be 55% within 2 hrs.

**In-vivo animal tests:** In-vivo experiments confirm the possibilities of muscular injections of the formulations made by using P407 and *in situ* gelation feasibilities. Depots from outside the skin can be seen just after the administration of the hydrogel, which assures that the formulation has gone through the sol-gel transition (Figure 3).

![Figure 3](image_url)

**Figure 3** (a) Depot formation of the hydrogel formulations viz. MPX-7.4 and MPX-CG, (b) Depot formation of the formulations when administered subcutaneously, (c) Depot formation of the formulations when administered intramuscularly, (d) Recovery of the hydrogels after the administration of the hydrogels.

**Conclusion:**

The present investigation is the extension of our previously reported study on the solubility enhancement of the poorly soluble melphalan. The solubility of the melphalan conjugate was remarkably increased after the PEGylation with mPEG 5000. We have therefore, studied the administration of the PEGylated melphalan through the parenteral delivery in the form of hydrogel using thermo responsive polymer P407 (25% w/w). Addition of NaCl salt abridged the initial burst release of the melphalan, meanwhile the host cytotoxicity was minimized due to the formation of the depot both in case of the subcutaneous and intramuscular routes. In addition, MPX-CG owing to the presence of the NaCl salt, possess good mechanical strength and the depots formation showed good diffusion and the degradation of the hydrogel as well.

**References:**

Formulation and development of β-Lactam antibiotics suspension for oral delivery

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E-mail address: itsmeamitalex@gmail.com

Abstract: In the present study, we have investigated the significance of the xanthan gum and aerosil 200 in the dry suspension or powder for oral suspension to understand the role of excipients which affect the formation using QbD approach. In this study we have taken various concentrations of xanthan gum and aerosil 200 in the formulation trials. We found that F3 formulation is similar to reference listed product because the xanthan gum and aerosil 200 used as per IIG limit and optimized concentration of the formulations. When we increased the amount of xanthan gum the dissolution profile of the formulation was slow, it do not gives the 75 % drug release within the time limits as per FDA.

Introduction:
Quality by design is an emerging concept which provides pharmaceutical manufacturer with increased self-regulated flexibility while maintaining tight quality standards and real time release of the product. The concept of QbD was mentioned in the ICH Q8 guidance, which states that “quality cannot be tested into products, i.e. quality should be built in by design”. ICH Q8 (pharmaceutical development) that mention the definition of “QbD as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management” [1-3].

Materials and Methods:
Formulation Trials: The goal of this study was to select the Xanthan gum, Colloidal silicon dioxide (Aerosil 200) concentration. This study also sought to establish the robustness of the proposed formulation. A full factorial Design of Experiments (DOE) using MINITAB software, to study the impact of these three formulation factors on the response variables listed in following table. DOE was performed on 200mg/5ml strength as it the RLD strength.

Manufacturing Procedure
Step 1: - Co-sift API, xanthan gum, sodium benzoate, aerosol, flavor-1 and 2 with approximately third parts of total amount of sucrose 250-75 (from part 1)through #40 mesh.
Step 2: - Sift step -1 material with approximately half of sucrose 250-75 (from part 1).
Step 3: - Sift step -2 material with remaining qty of sucrose 250-75 (from part 1)
Step 4: - Blending for 10 minutes, unloading from blender.
Step 5: Sift Sucrose 250-75 (part-2) through #40 mesh.
Step 6: Co-sift step 4 material with step 5 material through #20 mesh.
Step 7: Add step 6 material into blender, mixing for 20 minutes.

**Characterization of Dry Suspension:** Suspensions are evaluated by determining their physical stability. Two useful parameters for the evaluation of suspensions are sedimentation volume and degree of flocculation. The determination of sedimentation volume provides a qualitative means of evaluation. A quantitative knowledge is obtained by determining the degree of flocculation.

**Stability Studies:** Optimized formulation is treated with the Accelerated stability study at 40 °C ± 2 °C /75 % RH ± 5%, 1M, 2M, 3M and 6M as per ICH, after a specific time interval sample will withdrawal and evaluated the various physiochemical parameters.

**Results:**

**Evaluation**

All prepared dry powder formulations were coded as F1, F2, F3, F4 and F5 by using various concentrations of xanthan gum and colloidal silicon dioxide. Base formula for further study was optimized for their stability at room temperature, physiochemical parameters such as pH, Bulk Density, Tapped Density, Carr’s Index, Hausner’s Ratio and Angle of Repose as show in below Table.

### Table 1 Ratio and angle of repose.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Density</td>
<td>0.770 gm/ml</td>
<td>0.784 gm/ml</td>
<td>0.847 gm/ml</td>
<td>0.735 gm/ml</td>
<td>0.724 gm/ml</td>
</tr>
<tr>
<td>Tapped Density</td>
<td>0.90 gm/ml</td>
<td>0.912 gm/ml</td>
<td>0.943 gm/ml</td>
<td>0.909 gm/ml</td>
<td>0.909 gm/ml</td>
</tr>
<tr>
<td>Hausner’s Ratio</td>
<td>1.180</td>
<td>1.164</td>
<td>1.113</td>
<td>1.230</td>
<td>1.254</td>
</tr>
<tr>
<td>Angle of Repose</td>
<td>36.5°</td>
<td>33.02°</td>
<td>32.56°</td>
<td>33.464°</td>
<td>34.372°</td>
</tr>
</tbody>
</table>

**Sedimentation Rate:**

**Table 2 Sedimentation volume data of formulation trials.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (Days)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>3rd</td>
<td>0.95</td>
<td>0.98</td>
<td>1</td>
<td>0.96</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>7th</td>
<td>0.89</td>
<td>0.91</td>
<td>0.98</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>10th</td>
<td>0.83</td>
<td>0.84</td>
<td>0.92</td>
<td>0.84</td>
<td>0.96</td>
</tr>
<tr>
<td>5.</td>
<td>14th</td>
<td>0.75</td>
<td>0.79</td>
<td>0.89</td>
<td>0.74</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Drug Release Profile or Dissolution Studies:**

**Dissolution profile of Formulation trail (F3):**

- Apparatus: USP II (paddle), 50 rpm
- Media: pH 7.2 phosphate buffer
- Volume: 900 ml
- Time points: 10, 15, 20, 30, 45, 60 minutes
Table 3: Drug release profile data of formulation trail (F3):

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (min)</th>
<th>% Cumulative drug Release</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>93</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>103</td>
<td>102</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>103</td>
<td>102</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>105</td>
<td>103</td>
</tr>
</tbody>
</table>

Stability Studies: We performed the stability studies of the optimized batch in varies condition and evaluated various parameters for concluded the stable formulation.

Table 4 Stability data of optimized formulation after 3 Months.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical characteristics</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Assay</td>
<td>103.2 %</td>
<td>102.8 %</td>
<td>102.7 %</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>4.2</td>
<td>4.3</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>2.32</td>
<td>2.33</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td>220.3 cp</td>
<td>220.2 cp</td>
<td>220.2 cp</td>
<td></td>
</tr>
<tr>
<td>Dissolution</td>
<td>105</td>
<td>105</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>F2 Value</td>
<td>68</td>
<td>65</td>
<td>62</td>
<td>67</td>
</tr>
</tbody>
</table>

Conclusion:
The optimized trials F3 was very close in vitro drug release profile compared to the reference product. The f2 value of Trial F3 in pH 7.2 phosphate buffer was 62. This was because the xanthan gum and aerosil 200 used as per IIG limit and optimized concentration of the formulations. When we increased the amount of xanthan gum the dissolution profile of the formulation was slow, it do not gives the 75 % drug release within the time limits as per FDA. Hence, by just increasing the release of drug by giving a uniform blending to the dried mixer of drug and excipients, we could achieve the target dissolution profile similar to the reference product.

References:
Solubility enhancement and preparation of Quercetin loaded (POLY-D-L-LACTIDE) (PLA) nanoparticles

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Abstract: The very foremost limitation of quercetin is its poor water solubility. The quercetin is chemically unstable in aqueous medium, which may possibly involve the attack of hydroxyl ions on the C-ring of quercetin. This limitations of quercetin result in poor permeability, Instability and extensive first Pass metabolism before reaching the systemic circulation. Even though, water soluble derivative of QU (quercetin) has been synthesized but its bioavailability is only 20%. The other major limitation of QU in clinical application is the administration of high dose (50 mg/kg) due to its poor bioavailability. In the present study, Nanoprecipitation method is chosen because of its ease, simplicity and lower energy consumption. Nanoprecipitation method is also known as solvent displacement method and as well as antisolvent precipitation method. Since quercetin was successfully encapsulated in PLAN (poly-d-l-lactide nanoparticle), this formulation becomes the best candidate for targeted drug delivery. More over QU-PLAN (F3) showed sustained release of drug quercetin on performing In-Vitro drug release analysis.

Introduction:
A biodegradable polymeric nanoparticle PLA is widely used for encapsulation of many therapeutic drugs. PLA has the wide range of properties such as biodegradability, biocompatibility, high hydrophobicity, strong mechanical strength and slow drug release. Advantages using biodegradable polymeric nanoparticles formulation include reduced systemic side effects, targeted and controlled drug release and high capability to cross various physiological barriers. Thus, for nanoencapsulation of quercetin, PLA has been employed as ideal nano-carriers. Quercetin molecule has been successfully encapsulated in to biopolymer PLA nanoparticles. In this study, an efficient method is used for preparation of PLA nanoparticles (PLAN) [1-3].

Materials and Methods:
Standard curve and lambda max determination of the drug quercetin: U.V Spectrophotometer was used to determine the lambda max and standard curve for quercetin. Quercetin has maximum absorbance at 371 nm and molar absorbivity of 1.3733 x 104 1/mol x cm. Beers law was obeyed in the concentration range of 2-10 ug/ml per ml. The objective of the study was to develop rapid, accurate and specific spectrophotometric method for the estimation of quercetin. This method was
developed using methanol. The lambda max of quercetin was found to be 371 nm and the beers law was obeyed in concentration range of 2-10 µg/ml. A Shimatzu 1800 U.V spectrophotometer with 1cm matched cuvettes were calibrated and used.

**In-vitro release studies:** The in vitro release profile of the quercetin from the QU-PLAN was studied by using dialysis technique. In the donor compartment nano-suspension containing the known concentration of drug was placed and in the receptor compartment buffer was placed and constantly agitated using a magnetic stirrer at 37 °C. Samples were withdrawn from the receptor compartment for estimation of released drug and replaced with the same volume of buffer. The values were reported as mean value ±SD.

**Results:**

**Synthesis and encapsulation of Quercetin in PLA Nanoparticles:** Quercetin molecule is hydrophobic in nature. The antioxidant molecule quercetin has several beneficial effects in pharmaceutical field, the encapsulation of this molecule is necessary. Thus to entrap this quercetin molecule, the biodegradable polymer PLA was found to be the best used for nanoparticles synthesis. The nanoprecipitation was the method used for the preparation of QU-PLAN (Quercetin loaded poly-d-l-lactide nanoparticle). This method was specifically used to produce small and low polydisperse NPs (nanoparticles) population. This is one of the best and the easiest methods for producing NPs population. In this process the particle formation is spontaneous; it is due to polymer precipitated in the aqueous environment. The Marangoni effect explains the process of nanoprecipitation; solvent flow, diffusion and surface tension at the interface of organic solvent and the aqueous phase cause turbulences, which leads to the formation of small droplets containing the polymer. Later, as the solvent diffuses out form the droplets, the polymer precipitates. Finally the organic solvent is evaporated with the help of rotary evaporator. This method yields very good encapsulation of functional quercetin.

**Table 1** Mean particle size and zeta potential values of various formulations of QU- PLAN

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Average particle size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>220±30</td>
<td>-21.5±2.2</td>
</tr>
<tr>
<td>F2</td>
<td>235±24</td>
<td>-22.4±1.8</td>
</tr>
<tr>
<td>F3</td>
<td>242±20</td>
<td>-22.5±1.5</td>
</tr>
<tr>
<td>F4</td>
<td>250±18</td>
<td>-22.5±1.4</td>
</tr>
<tr>
<td>PLAN</td>
<td>185±10</td>
<td>-20.5±1.0</td>
</tr>
</tbody>
</table>

The functional performance of NPs based delivery systems depends on the physicochemical properties of the NPs, such as size and charge. Therefore we measured the mean particle diameter and zeta-potential of the polymeric NPs produced in this study relatively, with an increase in drug concentration, the size of the formulated QU-PLAN increased proportionally. Molecular weight,
concentrations of polymer, surfactant and the drug used, are the major factors that affect the final size of the particles. In this experiment, the higher molecular weight of PLA was used for encapsulating the drug. Apparently the higher molecular weight of the PLA used in this study increased the viscosity of the internal phase, leading to a decreased net shear stress, thus producing larger NPs with increasing in drug concentration.

![Figure 1 Percentage of drug released from various formulations of QU-PLAN (F1-F4)](image)

**Conclusion:**

Nanoprecipitation method proved successful in encapsulating the drug quercetin in PLAN. Based on its morphology, EE (encapsulation efficiency) and drug release studies, the formulation (QU-PLAN) F3 (Quercetin 75 mg: PLA 200 mg) was found to be the best formulation among the various formulations of QU-PLAN (quercetin loaded poly-d-l-lactide nanoparticles). The morphology of both the PLAN (poly-d-l-lactide nanoparticles) and QU-PLAN (quercetin loaded poly-d-l-lactide nanoparticles) showed spherical shaped particles. The optimized formulation F3 showed 99.7% of drug release. The scavenging activity of QU-PLAN showed better scavenging effects when compared to the free quercetin. Antioxidant activity assay revealed that the functional activity of quercetin was retained even after nano-encapsulation. The spherical shape, small size, high entrapment efficiency and sustained slow drug release makes QU-PLAN (quercetin loaded poly-d-l-lactide nanoparticles) a suitable dosage form for treatment against diseases. More over the major limitation i.e. aqueous solubility of poorly water soluble drug quercetin also enhanced. So it can be concluded that the preparation of (quercetin loaded poly-d-l-lactide nanoparticles) QU-PLAN by nanoprecipitation method broadly enhanced its solubility in aqueousmedium. Since quercetin was successfully encapsulated in PLAN (poly-d-l-lactide nanoparticle), this formulation becomes the best candidate for targeted drug delivery. More over QU-PLAN (F3) showed sustained release of drug quercetin on performing In-Vitro drug release analysis.

**References:**

Formulation and evaluation of Acyclovir loaded lipid emulsions for ophthalmic delivery

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Abstract: Lipid emulsions improve the solubility of lipophilic drugs and enhance the intraocular penetration of drugs for the ophthalmic use. In present study acyclovir (ACV) in used as bioactive which is a potent antiviral drug at the same time practically insoluble in water. We have formulated forty different formulations of lipid emulsion by varying the ratio of sunflower oil, tween 80, carbopol 934 and polaxamer out of them four formulation were optimized on the basis of primary stability studies. These four optimized formulation were selected for further studies. The total drug content of the optimized LEs were found 95.5±0.1%, 84.6±1.2%, 85.3±0.53% and 60.7±0.25% and entrapment efficiencies were found 93.5±0.2%, 86.7±0.5%, 89.6±0.2% and 81.6±0.4% respectively for F1, F2, F3 and F4 formulations. The in vitro drug release data were treated to Higuchi, first order and zero order plots and were found to be linear indication zero order release kinetic. Zeta potentials were determined using Malvern zetasizer for F1, F2, F3 and F4 showed values of −35.8, −32.6, −27.9 and −40.1 mV, respectively indicates stability for lipid emulsions. Among these formulations F1 formulation showed better physical and chemical stability might be due to the formation of reverse micelles of surfactants (and/or co-surfactants), which entrapped ACV inside the micelles surrounded by hydrophilic heads of surfactant. F1 formulation shows no sign of eye irritation and hence we can conclude that a novel low-irritant antiviral ophthalmic formulation of acyclovir emulsion was designed and characterize for better physical and chemical stability of Acyclovir ophthalmic emulsion.

Introduction:
Acyclovir is a potent antiviral agent. It is a synthetic nucleoside analogue active against herpes viruses. Acyclovir exerts its antiviral activity by competitive inhibiting of viral DNA through selective binding to HSV-thymidine kinase with about 200-fold greater affinity than for mammalian enzyme.
Lipid emulsions (LEs) are potential drug carrier, for lipophilic and amphiphilic drugs with many favorable properties; they are biocompatible, biodegradable, stable and easy to prepare and handle. For the treatment of different extra and intra-ocular an etiological conditions such as glaucoma, uveitis, keratitis, dry eye syndromes, cytomegalovirus retinitis, acute retinal necrosis, proliferative vitreoretinopathy, macular degenerative disease, etc. many lipophilic and poorly water soluble drugs have become available in recent years [1]. Thus, the objective of the present study was to develop an
Acyclovir based Lipid Emulsions by varying the ratio of sunflower oil, tween 80, carbopol 934 and poloxamer for Ophthalmic Delivery (Figure 1).

Materials and Methods:
Compatibility Test:
Differential scanning calorimetric of Acyclovir: Perkin Elmer differential scanning Calorimeter (DSC-8500 with Hyper DSC) filled with a thermal analyst system was used for the analysis.
Fourier transform infrared spectroscopy: The primary Drug excipients compatibility studies were done by Fourier Transform Infra-Red spectroscopy (FTIR).

Pre formulation Study
Solubility of Acyclovir in different oils: The solubility of acyclovir in various oils like sunflower oil, soya bean oil, castor oil, oleic acid

Characterization of prepared lipid emulsions [2]
Droplet size analysis: The diameter of the dispersed phase oil droplets in the emulsions were analyzed using a dynamic light scattering particle size analyzer (HPPS, Malvern Instruments, Worcestershire, UK) without dilution at 25 °C.
Zeta potential of lipid emulsion: The charge on lipid emulsion droplets (z potential) is measure by using a Zeta Sizer 3 (Malvern Instruments, Malvern, U.K.). The electrolyte solution use for the dilution consists of double distilled water with a conductivity of 50 mS/cm adjusted by NaCl (0.5 M).

Emulsion rheology: The emulsions were studied for three categories on the basis of both rheological properties and visual appearance that is stable; mesostable and unstable

Determination of total drug content: The absorbance of solution was determined at 266 nm by UV spectroscopy (Simadzu 1800)

In vitro Drug release studies of LE formulations: The drug release was studied by dialysis method using Cellophanemembrane (DM 60 (molecular weight cut off of 12,000–14,000), Hi-media, Mumbai, India).

Stability of Acyclovir ophthalmic lipid emulsions
Effect of centrifugal stress on stability: The optimized LEs, 1mL of each were filled in centrifuge tubes and the emulsions were kept for centrifugation at 3000 rpm for 1 hour and phase separation value was noted for further conclusions.
Effect of autoclaving on the stability of lipid emulsions: Prepared emulsions were filled into vials and subjected to autoclaving at 121 °C for 15 min.

In-vivo study
Eye irritation: The test substance was placed in the conjunctiva sac of one eye of each animal (rabbit) after gently pulling the lower lid away from the eyeball.

Results and Discussion:
The total drug content of the optimized LEs were found 95.5±0.1%, 84.6±1.2%, 85.3±0.53% and 60.7±0.25% and entrapment efficiencies were found 93.5±0.2%, 86.7±0.5%, 89.6±0.2% and 81.6±0.4% respectively for F1, F2, F3 and F4 formulations. The in vitro drug release data were treated to Higuchi, first order and zero order plots and were found to be linear indication zero order release kinetic. Zeta potentials were determined using Malvern zetasizer for F1, F2, F3 and F4 showed values of −35.8, −32.6, -27.9 and −40.1 mV, respectively indicates stability for lipid emulsions. Among these formulations.

![Drug release study](image)

**Figure 1** In-vitro Release profile

**Conclusion:**
In this study, a novel low-irritant antiviral ophthalmic formulation of acyclovir emulsion was developed and prepared with the elevation of oil content, the physical and chemical stability of Acyclovir ophthalmic emulsion was further found to be improved.

**References:**
Preparation and evaluation of Chitosan based thermo responsive \textit{in situ} gelling system for the delivery of Amphotericin B

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E-mail address: itsmemitalex@gmail.com

Abstract: In the present study, we have investigated a comprehensive overview of theory of liposomal Amphotericin B incorporated chitosan based in situ gel and various methods employed to characterize the formulation as well as evaluate them. The additives used in the preparation of liposomal Amphotericin B were inert in nature and they do not interact with drug and polymer during preparation and storage. The determination of zeta potential showed that the formulation will possess high degree of stability. Also the determination of drug entrapment showed fair results. Then the development of chitosan based in situ gel was done in which liposomal Amphotericin B was incorporated. The evaluation showed that in comparison to pure drug incorporated in situ gel, liposomal Amphotericin B incorporated in situ gel was more effectively forming gel and the gelation time was also quick. The determination of viscosity also showed that the viscosity was more in 37°C and comparatively low in storage temperature i.e. 8°C. The \textit{in vitro} drug release studies showed that the pure Amphotericin B incorporated in situ gel gave less release as compared to the liposomal Amphotericin B incorporated \textit{in situ} gel.

Introduction:
Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems. Amongst the extensive research has been carried in designing of polymeric drug delivery systems. In the past few years, increasing number of in situ gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported [1-3].

Materials and Methods:
Preparation of liposome: Accurately weighed 125 mg cholesterol and 250 mg phospholipid was taken in a round bottom flask. In the same round bottom flask, 4.5ml of chloroform and 4.5ml of methanol was added and properly mixed. Now accurately weighed 50mg Amphotericin B was added to the above mixture. This mixture was shaken manually till the complete solvent evaporation. Later, the film formed inside the round bottom flask was hydrated with 25ml of 7.4pH phosphate buffer.
There were other two batches of liposomes prepared in which the amount of cholesterol was varied as mentioned; 100mg cholesterol and 200 mg cholesterol.

### Table 1 Composition of Amphotericin B loaded liposomes

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Cholesterol</th>
<th>Phospholipid</th>
<th>Amphotericin B</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100mg</td>
<td>250mg</td>
<td>50mg</td>
<td>4.5ml</td>
<td>4.5ml</td>
</tr>
<tr>
<td>F2</td>
<td>125mg</td>
<td>250mg</td>
<td>50mg</td>
<td>4.5ml</td>
<td>4.5ml</td>
</tr>
<tr>
<td>F3</td>
<td>200mg</td>
<td>250mg</td>
<td>50mg</td>
<td>4.5ml</td>
<td>4.5ml</td>
</tr>
</tbody>
</table>

**Characterization of Amphotericin B loaded liposomes**

**Zeta Potential:** Zeta potential of the liposomes prepared from hand shaking method was determined using Zeta Sizer 300HSA.

**Percent drug entrapment:** PDE was determined by mini-column centrifugation method.

**Preparation of liposomal Amphotericin B loaded in situ gel:** In three batches of liposomal dispersion 20ml, 6%, 8% and 10% chitosan was added and kept overnight under refrigeration. Next day, the chitosan was dissolved completely and a solution was formed. There were other three batches in which pure drug along with 20ml phosphate buffer was used and to this, 6%, 8% and 10% was added and kept overnight under refrigeration.

**Evaluation of In-situ gel:**

**Determination of Drug Content:**

1 ml of each formulation of in situ gel was dissolved in methanol and the volume was made to 100 ml. Uniformity of the drug content was evaluated by measuring the absorbance at 379nm in UV spectrophotometer (Shimadzu-1800) after suitable dilution. Drug content was also evaluated for pure drug loaded in situ gel.

**In vitro drug release studies:** In vitro drug release study was carried out using open (diffusion) tube apparatus. The semi-permeable cellophane membrane, presoaked overnight in the freshly prepared phosphate buffer (7.4), was tied to one end of an open tube, acted as donor compartment. 1 ml of in situ gel was placed inside the donor compartment in contact with the cellophane membrane. The tube was vertically held by a stand and suspended in 100 ml of simulated phosphate buffer maintained at 37±1°C touching the surface of receptor medium. The receptor medium was stirred at 100 rpm using magnetic stirrer. The aliquots of 3 ml were withdrawn at regular intervals and replaced by an equal volume of warm receptor medium every time. The amount of pilocarpine released was analyzed spectrophotometrically at 215.5 nm (Shimadzu UV-1800, Japan).

**Result:**

**Zeta Potential:** The value of zeta potential of F1, F2 and F3 was found to be -40mV, -30mV and -10mV respectively. Therefore, this suggests the stability of 125mg added Amb liposome the formulation.
Percent drug entrapment: The percent drug entrapment in liposomes was determined by minicolumn method and found to be 70%, 86%, and 68% for F1, F2 and F3 respectively. The drug entrapment in case of this 125mg cholesterol added AmB liposome was high and therefore, this was optimized for further study.

Determination of Drug Content: The Amphotericin B loaded in situ gel was subjected to determine the drug content and % drug content was found to be 92.04% and 71.01% in case of liposomal AmB in situ gel and pure drug loaded in situ gel respectively.

In-vitro release profile:

![Figure 1](image-url)  
**Figure 1** In-vitro Drug release Profile of AmB incorporated gel

Conclusion:
Then the development of chitosan based in situ gel was done in which liposomal Amphotericin B was incorporated. The evaluation showed that in comparison to pure drug incorporated in situ gel, liposomal Amphotericin B incorporated in situ gel was more effectively forming gel and the gelation time was also quick, also the best formulation among liposomal Amphotericin B incorporated in situ gel was G2 which formed gel at 36°C. The overall studies showed that G2 formulation was the best of all.

References:
Formulation and evaluation of Cyclodextrin based solid lipid nanoparticles loaded with alkylating agent for oral delivery in treatment of Ovarian Cancer

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Abstract: The objective of present work was to prepare cyclodextrin based nanoparticles for enhancement of oral bioavailability of poorly water soluble drug altretamine. The binary inclusion system was prepared by freeze drying method. Solid lipid nanoparticles of complexed drug were prepared by hot homogenization ultrasonication method. The results revealed formation of inclusion system in 1:1 M ratio with A_L type solubility. The average size of nanoparticles was 103.57 ±2.53 nm. In-vitro release profile showed initial burst release followed by a slow sustained release pattern. The relative bioavailability of SLNs was found to be 2.75 folds higher than ALT solution.

Introduction:
Ovarian cancer is one of the leading causes of deaths worldwide. It is the fifth most gynecological malignancies in women. Most of the cytotoxic alkylating chemotherapeutic agents are used either alone or in combination for treatment of ovarian cancer. However, these are associated with several factors like poor solubility, short half lives, low bioavailability, hepatic first pass metabolism, side effects, toxic and adverse effects and many more. The objective of present research work was to prepare inclusion complex of altretamine with Epi-β-CD for enhancement of solubility and oral bioavailability; thereby evaluating its in-vitro and in-vivo efficacy.

Materials and Methods:
Phase solubility study: Solubility of drug in water and cyclodextrins was determined by phase solubility study. This was performed according to Higuchi and Connors method.
Preparation and Characterization of Inclusion complex: The inclusion complex of guest (altretamine) with Epi-β-CD was prepared by freeze drying method. The complexed drug was loaded into SLNs. SLNs were prepared by hot-homogenization-ultrasonication method.
Characterization of solid lipid nanoparticles: The prepared SLNs were characterized for following parameters Mean particle size, polydispersity index and zeta potential, Transmission election microscope (TEM) examination, Entrapment efficiency & Drug loading capacity, In-vitro drug release studies
Stability studies: The stability studies were carried out with the prepared cyclodextrin complexed SLN formulation. For this, 10 ml of the dispersion was taken into glass vials and stored at 4 and 25°C for 3 month.
**In-vivo study:** Albino rats of either sex were weighed (250–350 g) and divided into three groups comprising six animals in each. All the animals of group I was given an oral dose of ALT solution in PBS pH 7.4; group II was administered orally with an equivalent dose of ALT-loaded SLNs formulation; the animals of group III received orally Phosphate buffer saline (PBS) pH 7.4.

**Results and Discussion:**

**Phase solubility study:** Figure 1 shows the phase solubility diagram of altretamine under varying (0-20 mM) concentration of EPI-β-CD. The value of stability constants $K_{1:1}$ as calculated from phase solubility diagram was found to be 8219 M$^{-1}$ for ALT-EPI-β-CD complex.

**Preparation and Characterization of SLN loaded with ALT-CD complex:** The hot-homogenization ultrasonication is the easy method which was utilised for the laboratories production of the SLNs. The homogenisation speed and sonicacion time were optimised to 15,000 rpm for 10 minute and 5 minute at 50W respectively. The mean particle sizes, PDI values and zeta potential of the optimized formulation was found to be 103 nm, 0.03 PDI and -21.7 mV zeta potential respectively. The Entrapment efficiency and Drug Loading percentage was to be 89.16% and 52.74% respectively. In order to investigate the morphology and size of the SLNs, TEM was used. The cumulative percentage drug release of optimized SLNs suspension was found to be 74.21±2.49 % in 24 hour. *In-vitro* release curve shown the initial burst release with the about 40% of drug release first two hours after that released sustained from the SLNs.

**In-vivo pharmacokinetic study:** The percentage blood uptake of ALT at various time intervals was recorded and was found to be highest after 3 hours of administration. The ALT-SLNs formulation has shown highest uptake of the lipid particles, which may be attributed to the small size of the particle and also to the solubilization.

**Conclusion:**

This study demonstrated the usefulness of cyclodextrin complexation and loading into nanocarriers. This also highlights the advantages of both the utilized carriers; which led to an improvement in therapeutic efficacy by enhancing the solubility and bioavailability of altretamine. ALT-Epi-β-CD kneaded complex enhanced the aqueous solubility of alkylating agent. On the basis of results, it is concluded that the cyclodextrin based d nanocarriers served as a potential delivery system with the reduction in associated toxic and side effects.

**Table 1** Characterization parameters of ALT-SLNs

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean Particle Size (nm)</th>
<th>Poly Dispersity Index (PDI)</th>
<th>Zeta Potential (mV)</th>
<th>EE (%)</th>
<th>DL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLNs</td>
<td>103.7 ±2.5</td>
<td>0.03 ±0.12</td>
<td>-21.6 ±0.14</td>
<td>89.72 ±0.71</td>
<td>57.13 ±0.04</td>
</tr>
</tbody>
</table>

“Strategic Approaches to Strengthen Academic and Industrial Collaboration” www.ijpsr.com
Acknowledgement:
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References:
Formulation and evaluation of PLGA nanoparticles of antifungal drug

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Abstract: In present study flutrimazole loaded PLGA [Poly (lactic-co-glycolicacid)] nanoparticles were prepared for topical delivery. The nanoparticles were optimized using 3² full factorial design to evaluate the effects of process and preparation variables. Nanoparticles were prepared by the nanoprecipitation method and characterized for particles size and drug release. The nanoparticles prepared as per design, possessed percent drug release of 89.36 %. Particle size and morphology analysis revealed that the nanoparticles were found in the size range of 500-1000 nm in average diameter and exhibited good sphericity. Zeta potential analysis showed value of -28 to -41mV. Zone of inhibition of Candida albicans, all formulation were in range of 12-18 mm.

Introduction:
The topical drug application is less prone to severe systemic side-effects than systemic application. Starting with the liposomes, various types of nanosized and micronized drug carriers have been developed to increase the notoriously low penetration of active agents into the skin, which limits not only the topical therapy of skin disease but also transdermal therapy [1]. Nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure.

These properties facilitate the administration of antifungal drugs, thereby overcoming some of the limitations in traditional antifungal therapeutics. In recent years, encapsulation of antifungal drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the antifungal drugs [2] Nanoparticulate drug delivery is multiparticulate drug delivery and used for improved bioavailability and target to specific sites. Biodegradable polymeric nanoparticles are of interest for extended drug delivery system and drug targeting [3]

Materials and Methods:
Materials: Flutrimazole was obtained as a gift sample from Ajanta Pharma (Mumbai, India), PLGA (50:50) from gift sample from Evonik Degussa (Mumbai, India), Poloxamer 407 from Ajanta Pharma (Mumbai, India), all solvent used were analytical grade.
Methods: Nanoprecipitation technique is used for preparation of nanoparticles.

The formulation was prepared by nanoprecipitation technique as follows flutrimazole and PLGA were co-dissolved in acetone. The organic phase was added drop wise stirring in double-deionized water (1000 rpm). The solution was kept for 6 h to allow for acetone evaporation. After that, suspended NPs were freeze-dried for 48 h.

Results and Discussion:

The results of a $3^2$ full factorial design revealed that the concentration of PLGA ($X_1$) and Poloxamer 407 ($X_2$) significantly affected the dependent variables such as, percent drug release and particle size of nanoparticles. The polynomial equation based optimization model was generated and validated. The accuracy of the model was established on the basis of magnitude of errors and $R^2$ values.

The nanoparticles of the optimum batch (FPN15) exhibited mean particle size of 729.3 nm and 89% drug release. An appropriate balancing between the levels of the polymer (PLGA) and Poloxamer 407 was imperative to acquire maximum drug release and adequate particle size. Hence it could be established that among the prepared formulations, FPN15 was the optimum formulation.

In-vitro data obtained for PLGA-ted nanoparticles of flutrimazole showed excellent particle size, good buoyancy and drug release. It was found that decrease in particle size and increase in drug release was seen with increase in polaxomer 407 concentrations at constant PLGA concentration. So, high concentration of polaxomer shows high drug release due to its surfactant properties.

It was observed that zone of inhibition of Candida albicans was in the range of 12-18mm. Stability data of optimized batches of flutrimazole loaded PLGA nanoparticles revealed that there were no changes observed in the appearance, drug content which showed that the flutrimazole loaded PLGA nanoparticles gel formulation is stable at 35°C/65% RH. Overall, topical drug delivery system for flutrimazole has been successfully developed.

Conclusion:

From the present study it can be concluded that preparation of PLGA nanoparticles using nanoprecipitation method proved to be a sound approach to obtain stable PLGA nanoparticles of flutrimazole. Components such as concentration of polymer and surfactant concentration have a profound effect in terms of particle size and percent drug release. In-vitro skin permeation, skin deposition indicated that the effect of drug was prolonged by prepared optimized PLGA nanoparticles. PLGA nanoparticles proved the potential for topical delivery of antifungal drugs over the conventional formulations.
Acknowledgment:
The author is grateful to the STES’s, Smt. Kashibai Navale College of Pharmacy, Kondhwa (BK), Pune, Maharashtra, India, for financial support.

References:
Formulation & evaluation of sustained release matrix tablet of Simvastatin using synthetic polymers

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Abstract: In the present research work to formulate sustained release matrix tablet of Simvastatin. This study examined the release of Simvastatin from polymer matrices PVP K-30 and PEG 4000 separately. Simvastatin is a anti-hyperlipidemic drug and short half life ($t_{1/2}$) and usually oral dosage regimen (5 to 40 mg) taken to 4 times a day The tablets were evaluated for physical parameters like thickness, hardness, friability, weight variation, and in vitro release studies. The maximum drug release was found to be 98.59% over a period of 24 hours in PEG 4000 based tablets as compare to PEG 4000 and PVP K-30. All the formulations were stored at 45±2°C, 75 ± 5%RH and subjected to stability studies up to 45 days. It showed that all the formulations are physically and chemically stable.

Introduction:
Historically, oral drug administration has been the predominant route for drug delivery. It is known to be the most popular route of drug administration due to the fact the gastrointestinal physiology offers more flexibility in dosage form design than most other routes. It decreases total cholesterol, LDL cholesterol, triglycerides, and apolipoprotein B, while increasing HDL [1-3].

Materials and Methods:
Simvastatin obtained as gift sample from Arihant Medicines Pvt. Ltd., Mumbai, Maharashtra. PEG 4000, PVP K-30 and Microcrystalline Cellulose were purchased from Himedia laboratory, Mumbai. Magnesium Stearate and other chemical was purchase from Loba Chemical Pvt. Ltd, Mumbai.

Method:
Preparation of Sustained release matrix tablet of Simvastatin: The sustained release matrix tablet was prepared by direct compression technique. First accurately weighed quantity of Simvastatin, PEG 4000, PVP K-30 and Microcrystalline cellulose were taken in mortar and mixed. Sufficient quantity of distilled water was added and prepared a compact mass and it was passed through a sieve # 22 mesh size. The granules were dried at 40°C temperature. Granules were lubricated with talc (1 %) and magnesium Stearate (1 %) and compressed into tablets on a 8-station rotatory punching machine using 11mm concave punches. Each tablet contains 40 mg of Simvastatin.
Table 1 Composition of Sustained Release Matrix Tablet of Simvastatin

<table>
<thead>
<tr>
<th>Ingredients(mg)</th>
<th>F₁</th>
<th>F₂</th>
<th>F₃</th>
<th>F₄</th>
<th>F₅</th>
<th>F₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>PEG 4000</td>
<td>40</td>
<td>80</td>
<td>120</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>PVP K-30</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>40</td>
<td>80</td>
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</tr>
<tr>
<td>MCC</td>
<td>165</td>
<td>125</td>
<td>85</td>
<td>165</td>
<td>125</td>
<td>85</td>
</tr>
<tr>
<td>Talc</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>Mg. Stearate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Total wt.</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Results and Discussion:

Evaluation of Simvastatin Granules and Tablets: The granules prepared for compression of matrix tablets were evaluated for their flow properties. The bulk density was within the range of 1.201±0.03 to 1.652±0.05 gm/cm³. Tapped density ranged between 1.430±0.04 - 1.843±0.12 gm/cm³. Angle of repose was within the range of 23 ±1.75 to 29±1.85. Compressibility index was found to be 14.25±1.64 - 18.37±1.45 and Hausner’s ratio ranged from 1.125±0.04 - 1.214±0.01 for granules of different formulations these values indicate that the prepared granules exhibited good flow properties.

Figure 1 In-vitro drug release of Simvastatin matrix tablet in 1.2 pH HCl buffer
Figure 2. *In-vitro* drug release of Simvastatin matrix tablet in 7.4 pH Phosphate buffer

As increasing the polymer concentration, the drug release was retarded due to presence of thick matrix of polymer. The maximum drug release was found to be 98.59% over a period of 24 hours in PEG 4000 based tablets (F1). Similarly maximum drug release was found to be 97.41% over a period of 24 hours in PVP K-30 based tablets (F4). The in-vitro drug release result showed F1 matrix tablet containing PEG 4000 was best for the further study.

Conclusion:

From the overall investigation, one can conclude that the optimized sustained release matrix tablet of simvastatin using both polymers can meet ideal requirements for matrix tablet. Once daily sustained release matrix tablet of Simvastatin having short half life was found to exert a satisfactory sustained release profile which may provide an improved bioavailability, increased therapeutic efficacy, patient compliance, less side effects and reduced dosage regimen with less toxicity for treatment for many acute and chronic diseases.

Acknowledgement:

The authors are thankful to Sagar institute of research technology & science-pharmacy Bhopal, for providing necessary facilities to carry out the work.

References:

Preparation and evaluation of solid self-emulsifying drug delivery system (SEDDS) of Ramipril

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Abstract: The primary goal of the present work was to prepare solid self-emulsifying drug delivery systems (S-SEDDS) of Ramipril for improving solubility. SEDDS are isotropic mixtures containing oils, surfactants, co-surfactants including drug and have the ability to self-emulsify when introduced to aqueous medium upon gentle agitation. Ramipril is a highly lipophilic anti-hypertensive drug. Reason to choose SEDDS formulation for ramipril is that lipid based formulations may enhance solubility of lipophilic drugs that may further enhance dissolution rate and absorption. After screening several vehicles, Capmul PG8 NF, Gelucire 44/14 and Transcutol P were selected as oil, surfactant and co-surfactant respectively as they showed higher solubility for drug. Nine different liquid SEDDS formulations (L-SEDDS) were prepared containing various ratios of oil: surfactant: co-surfactant. Stable formulations were selected and ternary phase diagram was plotted to identify the emulsification region. Formulation containing 16.5% of Capmul PG8 NF, 68.75% of Gelucire 44/14 and 13.75% of Transcutol P was optimized as it showed least globule size (22.56nm) and high in vitro drug diffusion. Ex vivo studies also proved better diffusion of drug through isolated rat intestine in case of optimized L-SEDDS when compared to that of pure drug. The optimized formulation was loaded on to inert carrier (Sylysia FCP 350) to obtain solid SEDDS (S-SEDDS). Solid state characterization such as XRD, FTIR and SEM results confirmed the transformation of native crystalline nature of drug to amorphous state. Finally, improved in vitro dissolution behavior of Ramipril from S-SEDDS over pure drug was observed.

Materials and Methods:
Initially pre formulation studies were performed to determine the solubility of ramipril. The solubility of ramipril in various vehicles like oils, surfactants and co-surfactants was performed by addition of excess amount of drug to glass vial containing 2 ml of the selected vehicle [1]. These vials were placed in rotary shaker for 48 h till equilibrium was obtained. Samples were centrifuged and supernatant liquid was collected and diluted with methanol. The amount of ramipril was quantified by UV spectrophotometer at 210 nm.

Based on the solubility data obtained from solubility studies of ramipril in different vehicles Capmul PG8 NF was selected as oil phase, Gelucire 44/14 and Transcutol P as surfactants and co-surfactant phases respectively as they showed higher solubility for drug. Finally, liquid SEDDS formulations were prepared using Capmul PG8 NF, Gelucire 44/14 and Transcutol P as oil, surfactant
and co-surfactant respectively. A series of self emulsifying systems were prepared in each set with varying concentrations of oil, surfactant, and co-surfactant. Here nine SEDDS formulations were prepared. These formulations were coded as S1, S2, S3, S4, S5, S6, S7, S8 and S9. In all the formulations, the amount of ramipril was constant (i.e. 2.5mg). Initially, single dose of ramipril was accurately weighed and dissolved in calculated amount of oil, surfactant and co-surfactant in glass vial. The contents were vortexed using cyclomixer and then heated at 40°C (if necessary) until homogenous isotropic mixture was obtained [2]. The obtained SEDDS formulations were stored at room temperature until used. Formulations were tested for emulsifying properties like self-emulsification time. Rate of emulsification was assessed by visual observation. Cloud point temperature for all formulations was in the range. Results proved that SEDDS formulations were stable and resist separation in the GIT temperature. Resulting emulsions obtained from SEDDS formulations were observed for 48 h for any phase separation or drug precipitation. The formulations S3, S6, S8 and S9 were found to be stable as they did not show any drug precipitation or phase separation in the formed emulsions after 48h. 

Ternary phase diagram was constructed using stable formulations and those formulations were selected to perform further studies. Globule size analysis was performed for the stable emulsions obtained from stable formulations were determined by photon correlation spectroscopy [3]. With the decrease of oil proportion globule size was found to be decreased. The globule size of stable SEDDS formulation was found to be in the range in SGF. The value of polydispersity index indicates good uniformity in globule size in case of S3, S6, S8 and S9 formulations.

In vitro drug diffusion studies were carried out by open tube dialysis method using DM 70 dialysis membrane. Formulation S9 showed higher drug diffusion across the membrane compared to other SEDDS formulations. Based on clarity, rate of emulsification, droplet size and percent drug diffused S9 formulation was optimized. Optimized formulation S9 showed emulsification time of 9 seconds and globule size of 22.56 nm respectively.

Ex vivo drug diffusion studies were also performed using isolated male wistar albino rat intestine. It was performed for both pure drug and optimized liquid SEDDS formulation

**Results and Discussion:**

S9. Formulation S9 showed higher drug diffusion through the rat intestine compared to that of pure drug. Optimised liquid SEDDS were converted to solid SEDDS (S-SEDDS) by adsorption technique. Here sylsia FCP 350 was used as inert carrier. Obtained S-SEDDS with sylsia showed good flow properties after testing angle of repose for obtained solid SEDDS solid state characterization was performed. SEM, DSC and PXRD results suggested the transformation of crystalline nature of drug to
amorphous or molecular state. FT-IR spectra showed no evident extra peaks indicating the compatibility of all the excipients with drug without any chemical interactions. *In vitro* dissolution studies were also performed for pure drug as well as S-SEDDS and S-SEDDS of ramipril showed higher percentage of drug release compared to that of pure drug. Finally according to the plan of research work stable self-emulsifying drug delivery system (SEDDS) of ramipril was developed in order to enhance solubility as well as dissolution rate of this highly lipophilic drug. The optimized SEDDS have shown good clarity, spontaneity of emulsification and good stability. Further ramipril loaded solid self emulsifying delivery system(S-SEDDS) was successfully prepared by adsorption of optimized formulation on to sylysia which showed good flow properties and solid state characterization studies suggested that ramipril in solid SEDDS exist in molecular or amorphous state. Prepared S-SEDDS showed significantly higher dissolution efficiency compared to that of pure drug. Ramipril loaded S-SEDDS preserved the self-emulsification performance of the liquid SEDDS. S-SEDDS formulation was encapsulated in hard gelatin capsules. Thus, this S-SEDDS may provide a useful solid dosage form for ramipril drug.

References


A Demand of 21st Century – Oral Insulin

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Abstract: The prevalence of diabetes mellitus has been growing at a rapid pace which has been expected to reach up to 438 million by 2030, indicating 7.8% of total population. Rates of diabetes have increased markedly over the last 50 years in parallel with obesity. The treatment of diabetes involves conventional route of insulin administration via subcutaneous route which is associated with several limitations as well. Subcutaneous insulin treatment does not replicate the normal dynamics of endogenous insulin release, resulting in failure to achieve a lasting glycemic control in diabetic patients. The aim of this study was to develop a formulation of natural polymer based, insulin-loaded nanoparticles for oral delivery of insulin. The nanoparticles were spherical in shape with sizes in the range of 150-200 nm, and induced hypoglycemic effects for several days. Drug entrapment efficiency was found to be greater than 90%. These polymeric nanoparticles fulfill the criteria of nanocarriers to be employed for oral delivery of insulin.

Introduction:
Oral route is the most desirable and preferred over any other routes of drug delivery because of its non-invasive nature. Such medications would be particularly beneficial in managing disease such as diabetes, which requires life-long therapy. This can be attributed to several advantages of oral route of administration such as easy administration and better patient compliance; psychological barriers such as needle anxiety and possible infections can be overcome; easy production methods etc. Injectable insulin is not only inconvenient, but as it is administered into the peripheral blood stream, does not mimic the way natural insulin is processed in the body, it is not effective as it could be. From the pharmacological point of view, oral insulin is more physiologic than injectable since it is absorbed via the gut and therefore passes directly into the portal circulation, i.e. the same system that natural insulin is secreted into from the beta cells of the pancreas. Subcutaneous insulin gets to the liver only after passing through the systemic circulation. Mimicking physiology is usually a good thing. Nanoparticles provide an alternative means of administering polypeptide insulin molecule via oral route. These novel drug delivery formulations protect the insulin molecule against acidic pH and enzymatic degradation in the gastrointestinal tract by encapsulating the molecule within nanoparticle carriers.

Materials and Methods:
The recombinant human insulin was provided as a gift sample by Biocon Ltd., Bangalore. Triton X-
100 was purchased from TriveniInterchemPvt. Ltd., Gujarat. Guar gum polymer and glutaraldehyde were acquired from Burgoyne Burbidges and Co., Mumbai. Span 80 was purchased from LobaChemiePvt. Ltd., Mumbai.

**Preparation of Insulin loaded Guar Gum nanoparticles**

These nanoparticles were prepared by o/w nanoemulsification and in-situ polymer cross-linking method. 500 mg guar gum polymer was weighed accurately, to which 0.1% of Triton X-100 was added and make up the volume up to 100 ml to prepare 0.5% aqueous guar gum solution under constant magnetic stirring. Accurately weighed 50 units of insulin were taken in 10 ml acetone and formed the oil phase and Span 80 was added. This oil phase was then added to previously prepare aqueous phase under constant magnetic stirring. After mutual saturation of the oil phase and the continuous phase, the mixture was rapidly stirred at very high rpm. 1 ml glycerol was added followed by addition of 1 ml 25% glutaraldehyde solution. Nanosuspension was kept overnight. Nanoparticles were obtained after centrifugation at 20,000 rpm at 0°C for 30 minutes. It was then washed with HPLC grade water and centrifuged. The yielded nanoparticles were lyophilized, harvested in microcentrifuge tubes and preserved in vacuum desiccators.

**Characterization of nanoparticles**

**Determination of particle size:** Nanoparticles were placed in a desiccator overnight prior to sample preparation to remove any residual moisture. The sample of powdered nanoparticles was then mounted on aluminum stub and coated with a thin layer of gold. The particle size and morphology was then analyzed at an accelerating voltage of 10 kV and magnification of 20,000.

**Determination of drug entrapment efficiency:** Insulin guar gum nanoparticles were centrifuged at 15,000 rpm for 30 minutes at 15°C and the insulin content in the supernatant was assayed by UV-Visible spectrophotometer. The absorbance of insulin was determined at 270 nm.

**Zeta potential analysis:** The zeta potential measurements were carried out with zeta sizer at 25°C. Samples of free guar gum and insulin-guar gum cross linked complex were measured in folded capillary cells integrated with gold electrodes.

**In-vitro release study:** It was performed under simulated gastric and intestinal environment by using hydrochloric acid solution, pH 1.2 and phosphate buffer saline, pH 7. The concentration of insulin in both the simulation fluids was determined by UV-Vis spectrophotometer at 270 nm.

**Results and Discussion:**

The particle size of nanoparticles was determined by SEM, which ranges between 150 to 200 nm. Drug carriers sizing in sub-micron range have shown a higher potential in oral delivery of insulin as these nanoparticles are able to be carried via the walls of the human intestine. Drug entrapment efficiency of insulin in nanoparticles was determined by UV-Visible spectrophotometer and was found to be greater than 90%. Zeta potentials of guar gum and guar gum-insulin (1:1 w/w) cross
linked complex was determined as 56.8±0.31 mV and 32.97±1.6 mV respectively. This result serves as an evidence for the cross-link formation between guar gum and insulin. All guar gum-insulin cross linked complex displayed a lower positive zeta potential as compared to the free guar gum. *In-vitro* release of insulin shown the retention of insulin in gastric fluids (pH 1.2) for 2 hours followed by intestinal media (pH7.4) for about 4 hours simulating the passage from stomach to intestine. At pH 1.2 less than 10% of insulin could be released, while at higher pH value of 7.4 about 91% insulin released from the nanoparticles.

**Conclusion:**
The aim of this research work was to produce insulin loaded guar gum nanoparticles by emulsification in situ polymer cross-linking method which could potentially be applied in oral delivery applications. The guar gum nanoparticles fulfil the criteria of nanocarriers to be employed for oral delivery of insulin. These result support the feasibility of developing nanoparticle based biodegradable nanoinsulin for human application at prolonged time period.

**References:**
Formulation and evaluation of cream using gigawhite as a skin lightening agent

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Abstract: Cosmetology is one of the best professionally considered skills. Cosmetology is not only to beautify but also to heal the human body. It is very important to keep the body feeling beautiful, relaxed and healthy. “Whiteness” or having white skin is considered an important element in constructing female beauty. The present research deals with better approach towards formulating essential ingredients into creams with Gigawhite as a skin lightening agent. The main aim of research is basically to optimize the approach of skin lightening in a better way. The designed cream then further evaluated under parameters like pH, Dye test, Homogeneity, Spreadability, After feel test and Irritancy test of various formulation. At last, discussions are led with positive results and article concluded with an optimistic approach as to be used as a novel concepts to enlighten the phenomenon of skin lightning.

Introduction:
Skin care formulation that supports the health, texture and integrity of skin, moisturizing, maintaining elasticity of skin by reduction of type I collagen and photo protection etc. This is due to presence of ingredients in skin care formulation, because it helps to reduce the production of free radicals in skin and manage the skin properties for long time. The plant parts used in cosmetic preparation should have varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antikerolytic activity and antibacterial etc [1-3].

Major Usage/Purpose of Skin Lightening Cream:
The formulation is designed to reduce the appearance of hyper pigmentation on the face or body. Major usage deals with:
- Liver spots
- Freckles
- Sun damaged skin
- Melasma and chloasma
- Birthmarks or old scars
- Acne scarring
- Dark underarms
- Uneven skin tone

Significance of using Gigawhite: Gigawhite is safe, effective and natural alternative. It is obtained from plant extracts, Gigawhite, when used in combination with some other ingredients, can work
wonders in treating the skin affected by hyper-pigmentation, controlling the melanin production, bleaching effect on the skin and it reduces sun damaged skin, melasma, chloasma, birthmarks or old scars, acne scarring, and uneven skin tone.

**Materials and Methods:**
The emulsifier specified and other oil soluble components were dissolved in the oil phase and heated to 75° C. The water soluble components were dissolved in the aqueous phase and heated to 75° C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring. At last preservatives and perfumery material is added. Specific ingredients used are mentioned in Table1:

**Evaluation of skin lightening cream:** The formulations are evaluated for various evaluation parameters like pH, dye test, homogeneity, appearance, after feel, removal, irritancy test, viscosity etc.

<table>
<thead>
<tr>
<th>Table 1: Materials Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Gigawhite</td>
</tr>
<tr>
<td>Mometasone Furoate</td>
</tr>
<tr>
<td>Tretinoin</td>
</tr>
<tr>
<td>Cetyl Alcohol</td>
</tr>
<tr>
<td>Propylene Glycol</td>
</tr>
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<td>Glycerine</td>
</tr>
<tr>
<td>Sorbitol</td>
</tr>
<tr>
<td>Water to make</td>
</tr>
<tr>
<td>Preservatives</td>
</tr>
<tr>
<td>Perfume</td>
</tr>
</tbody>
</table>

**Results and Discussion:**
The formulations were evaluated under various parameters like pH and were found in the range of 6.2-6.5, the formulation shows no redness, edema, inflammation and irritation, dye test confirms that all formulations were o/w type emulsion cream. When formulation were kept for long term stability studies, it was found that no change in color, emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was found good feel effect. The cream applied on skin was easily removed by washing with tap water. Among the three formulations F1 was best because in this formulation concentration of Gigawhite is high as compare to other formulations.

**Conclusion:**
From our study it is revealed that gigawhite o/w cream formulation should be useful for treatment of skin lightening. The cream effectively treats various disorders such as dark spots, age spots, blemishes and dark underarms, without generating any side effects.
Acknowledgement:
Authors show their deep regards to Mahakal Institute of Pharmaceutical Studies, Ujjain for providing compatible environment for research work.

References:
Formulation and evaluation of porous solid dispersion of Piroxicam by flash evaporation technique using meltable carrier

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Abstract: In the current study, simultaneous improvement in dissolution profile and content uniformity of potent poorly soluble drug candidate has been successfully attempted using combined action of solid dispersion and capillarity. Piroxicam, a first line drug used in treatment of osteoarthritis exhibits dissolution rate limited absorption. An attempt was made for preparation of porous solid dispersion using flash evaporation technique for enhancement of dissolution rate of piroxicam using meltable and water soluble carrier, PEG 6000. The solid dispersions were characterized by DSC, FTIR and XRD. Content uniformity studies revealed uniform distribution of potent drug. In vitro dissolution studies exhibited significant improvement in dissolution profile of piroxicam.

Introduction:
The enhancement of oral bioavailability of potent poorly soluble drugs is one of the fascinating areas of research and exhibits challenging aspects for research scientists in drug development. Literature reveals numerous techniques for enhancement of dissolution rate of poorly soluble drugs namely, particle size reduction, surfactants, co-solvents, complexation, prodrug approach, drug derivatization, solid dispersions etc [1]. Amongst various approaches, solid dispersion presents a promising potential for enhancement of dissolution rate of poorly soluble drugs [2, 3]. In the present study, an attempt was made to improve the dissolution profile of piroxicam by converting it in to porous form using flash evaporation technique. This not only led to reduction of molecular size but also resulted in formation of porous mass containing numerous capillaries. The porous solid dispersion displayed steep improvement in dissolution rate due to combined action of solid dispersion and porosity. Piroxicam, a widely used non-steroidal drug in rheumatoid arthritis revealed dissolution rate limited absorption resulting in its low bioavailability. The present study was aimed to enhance the dissolution profile of a BCS class II drug piroxicam exhibiting erratic dissolution profile in gastrointestinal fluids [4].

Materials and Methods:
Materials: Piroxicam was supplied as a gift sample by Bangia Pharmaceuticals, Karnal, India. All other reagents used in the current study were of analytical standards.
Methods: A quick fit glass distillation apparatus was modified by connecting the receiver to a vacuum pump along with provision of vacuum gauze for measurement of vacuum. The porous solid
dispersion was prepared by dissolving 500 mg of piroxicam in 6 ml of chloroform. PEG 6000 (2.5 g) was then dissolved in drug solution and heated. The resulting viscous solution was subjected to sudden vacuum at 760 mm Hg to yield porous solid dispersion. The resulting porous solid dispersion was crushed and used for further study. For comparison with porous product, the non porous solid dispersion was prepared by conventional melting method in which PEG 6000 (2.5g) was melted in a beaker in oil bath and 500mg of piroxicam was added with continuous stirring. The resulting hot solution was solidified at room temperature.

**Optimization of parameters:** The effect of parameters namely volatility of solvent, carrier: solvent ratio, vacuum and drug: carrier ratios were studied for optimization of flash evaporation technique.

**Characterization of solid dispersions**

**Differential scanning calorimetry:** DSC was performed using Waters Ltd. DSC Q10V9.0 Build 275 Model. Samples (~5mg) were heated in hermetically sealed aluminium pans from 50° to 220°C under nitrogen flow rate (60 ml/min).

**FTIR analysis:** FTIR spectra were obtained on Thermo Nicolet infrared spectrophotometer using KBr disc method and samples were scanned from 400- 4000 cm⁻¹.

**Powder X-ray diffraction studies:** XRD diffraction patterns were obtained using X’Pert Pro Philips diffractometer with CuKα radiation over [°2 Th.] range from 3.0164° to 49.9874°.

**Content uniformity studies:** Exactly weighed amounts of randomly drawn ten samples of solid dispersions of piroxicam, each containing quantity equivalent to 20 mg of piroxicam were dissolved in 0.01N methanolic HCl and after suitable dilution estimated spectrophotometrically at 334 nm.

**Dissolution rate studies:** *In vitro* dissolution studies were conducted using USP dissolution apparatus II (paddle) in 900 ml of simulated gastric fluid without pepsin (pH 1.2), maintained at 37±0.5°C at a speed of 50 rpm. A quantity equivalent to 20 mg of piroxicam in solid dispersions was added to the dissolution medium. Aliquots of 10 ml of the samples were withdrawn and assayed spectrophotometrically at 334 nm.

**Results and Discussion:**

The following conditions must be maintained to obtain maximum porosity in the product:

- The maximum increase in bulk and porosity occurred at boiling point of solvent and chloroform was found more suitable solvent as compared to higher boiling point solvents (ethanol, methanol).
- A carrier: solvent ratio of 1:2 offered greater increase in bulk and porosity.
- Maximum porosity was obtained at 760 mm Hg since porosity was found directly proportional to vacuum.
- A drug: carrier ratio of 1:5 revealed maximum bulky and porous product.
Characterization of solid dispersions: DSC thermogram of piroxicam revealed a melting endotherm at 203.04°C. The porous and non porous solid dispersion reported absence of melting endotherm suggesting presence of drug at the molecular level in an amorphous form.

FTIR study was employed to study interaction of drug and carrier in solid dispersions. The FTIR spectra of non porous and porous product revealed presence of specific functional groups of piroxicam at 1629,1529, 1435,1350,1149,1065, 773 and 731 cm \(^{-1}\) and of carrier PEG 6000 at 2888 cm \(^{-1}\) in solid dispersions.

XRD diffractogram of piroxicam exhibited crystalline peaks of drug at 8.59°, 14.84°, 17.67° and 27.38°. The XRD patterns of non porous and porous solid dispersion reported absence of crystalline peaks of drug indicating the presence of drug in amorphous form.

Table 1 Content uniformity studies

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Non PSD (mg)</th>
<th>Drug Claimed (%)</th>
<th>PSD (mg)</th>
<th>Drug Claimed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>18.65</td>
<td>93.25</td>
<td>18.80</td>
<td>94.00</td>
</tr>
<tr>
<td>2.</td>
<td>18.95</td>
<td>94.75</td>
<td>18.90</td>
<td>94.50</td>
</tr>
<tr>
<td>3.</td>
<td>18.40</td>
<td>92.00</td>
<td>19.00</td>
<td>95.00</td>
</tr>
<tr>
<td>4.</td>
<td>18.30</td>
<td>91.50</td>
<td>17.90</td>
<td>89.50</td>
</tr>
<tr>
<td>5.</td>
<td>18.10</td>
<td>90.50</td>
<td>18.05</td>
<td>90.25</td>
</tr>
<tr>
<td>6.</td>
<td>17.70</td>
<td>88.50</td>
<td>17.40</td>
<td>87.00</td>
</tr>
<tr>
<td>7.</td>
<td>17.75</td>
<td>88.75</td>
<td>17.50</td>
<td>87.50</td>
</tr>
<tr>
<td>8.</td>
<td>19.05</td>
<td>95.25</td>
<td>18.25</td>
<td>91.25</td>
</tr>
<tr>
<td>9.</td>
<td>17.80</td>
<td>89.00</td>
<td>19.10</td>
<td>95.50</td>
</tr>
<tr>
<td>10.</td>
<td>18.00</td>
<td>90.00</td>
<td>17.60</td>
<td>88.00</td>
</tr>
</tbody>
</table>

Conclusion:
The porous solid dispersion prepared by flash evaporation technique offers superior dissolution profile over non porous solid dispersion and pure drug piroxicam. The present technique offers an advantage of steep reduction in material cost of carrier due to steep increase in bulk and porosity of product.

Acknowledgement:
Thanks are due to Jubilant Organosys Ltd., Noida for providing DSC, FTIR and XRD facilities.

References
Release of Atenolol from hydrophilic matrix tablets containing different grades of Hydroxypropyl Methylcellulose

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Abstract: Sustained release atenolol matrices are used to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose for the treatment of hypertension. The current study examines the relationship between swelling and drug release from the hydrophilic matrices of atenolol matrices prepared using combination of different grades of hydroxypropyl methylcellulose (HPMC), viz, HPMCK4M, HPMCK15M and HPMCK100M. The Degree of Swelling and Percent water uptake were determined for the matrices containing different concentrations and combinations. Swelling studies reveals an inverse relationship between swelling and drug release in the sustained release atenolol matrices.

Introduction:
Atenolol is a β-blocker, prescribed widely in hypertension, angina pectoris, arrhythmias, and myocardial infarction. It has been reported that atenolol undergoes extensive hepatic first-pass metabolism following oral administration and has a short biological half-life.

To reduce the frequency of administration and to improve the patient compliance, a once daily sustained release formulation of atenolol is desirable [1-2].

The aim of the present study was to investigate relationship between swelling and drug release from the sustained release hydrophilic matrices of atenolol prepared using combination of different grades of hydroxypropyl methylcellulose (HPMC), viz. HPMCK4M, HPMCK15M and HPMCK100M [3-5]. The release is mainly determined by the Fickian diffusion which is also confirmed from the n values.

Materials and Methods:
Atenolol was obtained as a gift sample and tablets were prepared by direct compression using HPMCK4M and HPMCK15M polymer combinations. Other excipients used were Magnesium stearate, Talc, MCC and dibasic calcium phosphate.
Nine formulations employed for investigations containing different ratios of HPMC of different grades were prepared by direct compression and coded C1, C2, C3, D1, D2, D3, E1, E2 and E3.

**Matrix swelling and water uptake studies:** Swelling was evaluated by weight. The matrices were placed in 900 ml dissolution medium pH 6.3, at 37°C. The percent water uptake i.e., degree of swelling due to absorbed test liquid, can be estimated at regular time intervals.

**Results and Discussion:**
The percent water uptake and degree of swelling as a function of time is reported. The dissolution parameters of varied formulation with different ratios of polymer combinations obtained during studies are shown in Table 1.

The higher amount of HPMC irrespective of different grades causes a greater degree of swelling. It is also demonstrated that HPMC of higher viscosity grades swells to greater extent and has greater intrinsic water uptake property than that of the lower viscosity grades.

**Table 1** Dissolution parameters of different formulations.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Release at 12 hr</th>
<th>n</th>
<th>Degree of Swelling (%)</th>
<th>Percent of water uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>96.14</td>
<td>0.502</td>
<td>258.33</td>
<td>1291.67</td>
</tr>
<tr>
<td>C2</td>
<td>83.86</td>
<td>0.451</td>
<td>285.83</td>
<td>756.25</td>
</tr>
<tr>
<td>C3</td>
<td>74.5</td>
<td>0.442</td>
<td>336.51</td>
<td>588.89</td>
</tr>
<tr>
<td>D1</td>
<td>103.5</td>
<td>0.548</td>
<td>245.83</td>
<td>1212.50</td>
</tr>
<tr>
<td>D2</td>
<td>102.2</td>
<td>0.545</td>
<td>270.08</td>
<td>725.00</td>
</tr>
<tr>
<td>D3</td>
<td>85.5</td>
<td>0.456</td>
<td>292.86</td>
<td>513.89</td>
</tr>
<tr>
<td>E1</td>
<td>93.6</td>
<td>0.506</td>
<td>259.84</td>
<td>1320.83</td>
</tr>
<tr>
<td>E2</td>
<td>74.6</td>
<td>0.442</td>
<td>316</td>
<td>822.92</td>
</tr>
<tr>
<td>E3</td>
<td>64.1</td>
<td>0.439</td>
<td>357.50</td>
<td>595.83</td>
</tr>
</tbody>
</table>

**Conclusion:**
Swelling studies reveals an inverse relationship between swelling and drug release in the sustained release atenolol matrices. The rational combination of different grades of HPMC can
be used satisfactorily to regulate the release of drug for extended period of time in such matrices.

References:
Particle Design of Meloxicam – Disintegrant Agglomerates for Fast Dissolution and Direct Compression by Crystallo-Co-Agglomeration Technique

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Abstract: The aim of present study was to prepare meloxicam-disintegrant agglomerates with improved flow solubility and by novel crystallo-co-agglomeration (CCA) technique of compression characteristics. Various solvents system comprising of acetone: DCM: water. Acetone-water was used to prepare Meloxicam agglomerates. PEG 6000, HPMC E50 LV and disintegrant crospovidone (CP) in different concentrations were also used as the crystallization medium. FTIR, DSC, XRD, SEM studies and were conducted to characterized agglomerates. Flow, packing, tableting properties and drug release studies were also performed for quality evaluation of formulation. Study concluded that fragmentation which occurred during compression improved compaction properties of the agglomerated crystals.

Introduction:
In area of powder methodology attempts are undertaken to describe primary and secondary particles of pharmaceutical substances for various applications, such as enhancement of solubility, obtaining suitable, improvement in micrometrics and compression parameters and changes in biolavaibility [1-3].

Materials and methods:
Meloxicam was gift sample of Cipla Pvt. Ltd, India and hydroxyl propylmethyl cellulose of Lobachemi Pvt. Ltd. Crospovidone was supplied by Ranbaxy Pharmaceuticals Ltd, India. PEG 6000, acetone, dichloromethane were purchased from aristo chemicals Pvt. Ltd, India. All other chemicals/solvents used were of analytical grade.

Crystallo-co-agglomeration technique:
Meloxicam agglomerates were prepared using a solvent system comprising ratio of different solvents ratio (good solvent, bridging liquid and bad solvent, respectively). In a vessel, mixture of PEG 6000 (6.5% w/w of total solid content) and hydroxyl propyl methyl cellulose was dissolved in distilled water (25 ml) and 1/3 of the total disintegrate was uniformly dispersed in the solution. Acetone (4 ml) at 50°C containing 7.5 mg of meloxicam and the other 2/3 of disintegrate was separately stirred for 20 min. The latter dispersion was added immediately to the dispersion containing dissolved polymer under constant stirring conditions (400 rpm, paddle type agitator with 4 blades) kept at room temperature. The stirring was continued for 20 min and 1 ml bridging liquid dichloromethane was added drop wise to obtain agglomerates, which were then filtered and dried overnight. By changing the concentration of disintegrate and PEG 6000 nine batches were prepared. Different composition of meloxicam agglomerates is shown in Table 1.
Table 1: Pre compression parameters of the meloxicam agglomerates

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Yield (% w/w)</th>
<th>Drug loading (% w/w)</th>
<th>Ratio of disintegrate/drug measured in agglomerates (%)</th>
<th>Solubility in PBS (6.8) (gm.\text{ml})</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>78.27</td>
<td>95.2</td>
<td>16.62</td>
<td>0.23</td>
</tr>
<tr>
<td>M2</td>
<td>79.14</td>
<td>96.8</td>
<td>19.23</td>
<td>0.29</td>
</tr>
<tr>
<td>M3</td>
<td>81.63</td>
<td>97.0</td>
<td>22.41</td>
<td>0.26</td>
</tr>
<tr>
<td>M4</td>
<td>80.71</td>
<td>97.5</td>
<td>14.37</td>
<td>1.82</td>
</tr>
<tr>
<td>M5</td>
<td>80.66</td>
<td>98.8</td>
<td>19.81</td>
<td>1.78</td>
</tr>
<tr>
<td>M6</td>
<td>82.25</td>
<td>98.6</td>
<td>22.86</td>
<td>1.96</td>
</tr>
<tr>
<td>M7</td>
<td>84.07</td>
<td>97.6</td>
<td>16.43</td>
<td>2.63</td>
</tr>
<tr>
<td>M8</td>
<td>79.20</td>
<td>99.2</td>
<td>18.81</td>
<td>2.76</td>
</tr>
<tr>
<td>M9</td>
<td>81.14</td>
<td>98.7</td>
<td>23.46</td>
<td>2.57</td>
</tr>
<tr>
<td>Pure drug</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.021</td>
</tr>
<tr>
<td>Solid dispersion</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Fourier Transform Infrared Spectroscopy (FTIR) studies

Figure 1. FTIR spectra of Formulation

X-ray diffraction of powder (XRDP):

Figure 2. PXRD Spectra of Agglomerates

Scanning electron micrographs (SEM) analysis:
Result and discussion:

The particle size of agglomerates was determined by SEM. Increase in carrier ratio resulted in increased solubility of the drug. This increase in the carrier ratio was directly related to increase in solubility of meloxicam. The particle size of the drug, spray dried drug and solid dispersions was analysed by optical microscopically. There observed a significant particle size reduction in the spray dried meloxicam and different solid dispersions as compared with the pure drug.

Conclusion:

Meloxicam-disintegrant agglomerates were successfully prepared by using of a crystallo-co-agglomeration technique. Study concluded that the micromeritics such as; flowability, packability and compactibility were improved significantly which possessed successful direct tabletting without any problem of capping. Reduction in inter particle friction due to the spherical shape of the tabletted particles may be attributed to the improvement of the flowability and packability.

References

Design and evaluation of buccal film of Carvedilol

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Abstract: The main objective of this study is to prepare a buccal film of Carvedilol and optimize the effect of various polymers to give maximum retentive effect with good drug release profile. Carvedilol having biological half life (2.2 ± 0.3 hours), selected model drug as it reduce both systolic and diastolic Blood Pressure and reduced first pass metabolism, In this study the order of drug release from the different films was PVA > HPMC: PVP > PVA: PVP > Gelatin > HPMC > HEC: PVP > Gelatin: PVP > HEC and glycerin increased the drug release.

Introduction:
Over the last fifteen years the interest is growing to develop a drug delivery system with the use of bioadhesive polymers that will attach to related tissue or to the surface coating of the tissue for targeting various absorptive mucosas such as ocular, nasal, pulmonary, buccal etc [1-5]. The present project is designed to deliver anti-hypertensive drugs by buccal route to overcome problems with aerosol and oral route.

Materials and Methods:

Materials: Carvedilol was obtained as gift sample from Torrent Research Centre Ahemdabad Guajrat, HPMC, PVP, PVA, Gelatin and HEC were obtained gift sample from Dr. Reddy Laboratories Ltd. Hyderabad. All other chemicals were of analytical grade.

Methods: The polymers (2% w/v) were dissolved in casting solvent and Plasticizer (30% w/w of polymers) were incorporated then calculated amount of drug was added with continuous stirring till homogeneous mixture was formed. From this 20 ml of the polymeric solution was poured within a glass bangle (6.0 cm diameter) placed on a mercury substrate in a Petri dish and allowed to dry till a flexible film was formed. For evaporating solvent the rate of evaporation was controlled by inverting the cut funnel over the petri dish. Then these composite patches were cut into 20 mm diameter and wrapped in aluminum foil and stored over fused calcium chloride in desiccators at room temperature until further use.

Results and Discussion:
In the present investigation, total sixteen formulations were formulated and designated as F1-F16 respectively. The prepared Buccal films were evaluated for various physiochemical parameters like
weight variation, thickness, surface pH ,swelling index, drug content uniformity, tensile strength,%
elongation at break, folding endurance, In vitro residence time, In-vitro release study and stability
studies. The weight variation ranged from 54 to 58 mg and thickness was found to be between 0.608
mm to 0.669 m. The tensile strength and % elongation at break gives an indication of strength and
flexibility of the films. Tensile strength of the films was found between 1.02 to 1.34 kg/mm² and %
elongation at break were found to vary between 10.11 to 14.42. Result showed that incorporation of
glycerin as a plasticizer enhanced the Tensile strength and elongation at break i.e. mechanical
properties to the films.
Plasticizer molecules are capable of forming hydrogen bond with polymer molecules thereby
imparting flexibility of the film. Folding endurance of all films was found more than 300, which
showed satisfactory film properties. Surface pH was found between 5.4 to 5.8 hence no mucosal
irritation was expected. Highest residence time was found in HEC + PVP film and lowest in HPMC
films. Swellability of the films was determined at specific time intervals. Maximum swelling showed
in HPMC + Glycerin + films and minimum in PVA + Glycerin films. Addition of PVP in the
formulation showed increases the swelling characteristic of the films. The Drug content uniformity
was determined for all sixteen formulations by UV-Spectrophotometer method. It was found uniform
in all formulations. The result of the drug content varies between 12.289 to 12.549 mg .It was
considered that the drug dispersed uniformly throughout the film. The formulated buccal films were
subjected to in–vitro drug release study for 6 hrs and distinguishable differences were obtained in the
release pattern of Carvedilol films.

**Stability Studies:** Stability studies were conducted for the formulations F5, F6, F10, and F12. The
reasons for selection are, these four formulations have shown best results in in-vitro drug release
studies. The R² value of selected formulations for stability studies are more close to 1 as compare to
other formulations. Stability studies of the prepared buccal films were performed at different
temperatures. The films were analyzed for drug content in each formulation at a time interval of
fifteen days till a period of two months.
The results showed that the drug content decreased up to 99.86 % in two months, from this study it is
summarize that, at room and refrigeration temperature was comparatively more stable then oven
temperature for storage of films.

**Conclusion:**
The result indicated that, the order of drug release from the different films was PVA > HPMC: PVP >
PVA: PVP > Gelatin > HPMC > HEC: PVP > Gelatin: PVP > HEC and glycerin increase the drug
release. The data from in-vitro release study was fitted to various kinetic models to determine the
kinetics of drug release. The main models are zero order, first order, Higuchi equation and Kosymer
peppas Equation to understand the drug release from the Buccal films. Plots of Zero order release was
found to be linear, which shows the linearity of the plot and linear curves were obtained for Higuchi’s plot, suggesting matrix diffusion mechanism of drug release from buccal films. The “n” values of kosymer model show that drug follows Supercase II transport Mechanism while diffusing through matrix.

Acknowledgement:
The authors thank to Torrent Research Centre Ahemdabad Guajrat India for a gift sample of Carvedilol.

References:
Nanotherapy for Topical Application of Leflugel

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Abstract: In the present study leflunomide (LFD) loaded solid lipid nanoparticles were prepared using probe sonication technique. LFD-SLNs was developed using $3^2$ factorial design. Optimized batch shown 113.86nm particle size with 76.82% entrapment efficiency, an initial burst release of drug i.e. 12.05% till 3h followed by sustained release up to 82.48% of at end of 24h. Further it was evaluated for thermal analysis, accelerated stability studies as per ICH (Q1A), in vitro drug release studies, ex vivo permeability studies, in vitro skin occlusivity test and histological examination and pharmacodynamic study.

Introduction:
Rheumatoid arthritis is a chronic disabling condition associated with a significant long-term loss of function and a significant socio-economic impact on individual sufferers and their families, as well as on society as a whole. 1% world population and affects 15% people i.e. over 180 million people in India. Hence there is a need to improved therapeutic strategies and rehabilitative programs to improve the quality of life of patients with RA. In the present study Leflunomide (LFD) was selected as a model drug. It is used as DMRD (Rheumatoid and Psoriatic Arthritis) and mode of action by Pyrimidine synthesis inhibition [1]. Oral formulation of LFD is available in the market. Oral administration of LFD associated with undesired gastrointestinal disturbances, diarrhea, hematologic, liver and lung diseases. The research work aims to eliminate the side effects associated with oral administration of LFD by formulating a topical drug delivery system for prolonged release of LFD and evaluate its pharmacodynamics activity.

Materials and Methods:
Compritol 888 ATO (glyceryl behenate), Gelucire 44/14 was obtained from Gattefosse Pvt., Ltd., (Mumbai, India). Glycerol monostearate (GMS) was obtained from Loba Chemie Pvt. Ltd., (Mumbai, India) and Tween 80 was obtained from S. D. Fine Chemicals, Mumbai, India. Carbopol 974 P was obtained as gift sample from Lubrizol Advanced Material India Pvt. Ltd., Mumbai.

LFD loaded SLNs were prepared by employing simple and reproducible method of ultra sonication. In the preparation the lipid and aqueous phase made separately. In lipid phase, required quantity of GMS was melted at a temperature 5ºC above its melting point, to this drug was added to get clear solution. To form a coarse pre-emulsion 2-4% Tween 80 was slowly added to aqueous phase. To get homogeneous mixture this premulsion was stirred at 1000 rpm for 30 min and maintained at 65ºC.

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The premulsion was cooled down to room temperature and was later subjected to ultrasonication for 30 min. The formed micro emulsion was cooled to room temperature to obtain LFD loaded SLNs [2]. Design of expert was applied in order to obtained nanoparticles of desired physical properties. Developed SLNs were converted into dry powder using lyophilisation technique.

The lyophilized nanoparticles were characterized by performing zeta potential, XRD and SEM. Gel formulation of LFD loaded SLNs (lyophilized) was prepared and evaluated for spreadability, drug content, In vitro drug release, In vitro skin occlusivity, Ex vivo skin permeation, skin compliance study, In-vivo Anti-Inflammatory activity by Rat paw edema method and Hemolytic activity [3-5].

Results and Discussion:
UV and HPLC method was employed for quantitative estimation of LFD. LFD loaded solid lipid nanoparticles shown zeta potential in range of +30 to -30 mv confirmed potential physical stability. Particle size was found in the range of 97.80nm – 384.70nm with PDI ranging from 0.2 – 0.4 with entrapment efficiency in the range of 64.85±1.55 to 81.7±0.64 % 76.82%. In XRD study llyophilized SLN shown deformed peak for LFD with reduced intensity, indicating its presence in amorphous or molecular dispersion state. Occlusion factor (F) for plain and LEF loaded SLN gel was found to be 94.83 and 97.58 respectively, which showed significant prevention of water loss at the end to 48 hrs. In vitro drug release profile of LEF from SLN gel through the dialysis membrane is given in Fig 1. From Ex vivo skin permeability studies, Steady state flux (Jss) and apparent permeability coefficient (Papp) was found to be 292.96 (µg cm-2 h-1) and 34.57 (cm-2 min-1) ×10-3 respectively, which shows significant higher deposition of LFD was found in the skin treated with LFD loaded SLN gel. This loaded amount serves as depot for prolonged release into the deeper tissue over a period of time thus extending its duration of action at the target site. Histopathology was performed to observe the integrity of skin and irritation or toxicity caused to by formulation. There was also no evidence of hemorrhage; necrosis and ulceration in LFD loaded SLN gel treated skin tissue.

Conclusion:

**Figure 1** Comparative drug release for LEF loaded SLN and plain LFD gel

**Figure 2** Histological section of rat skin:
(a) Untreated Skin (b) skin treated with LEF loaded SLN gel
Developed LFD loaded gel (Leflugel) formulation exhibited faster onset of action with prolonged drug release and was stable, non-irritant, and therapeutically effective in the treatment of rheumatoid arthritis. SLN using ultrasonication method proved to be ease, reproducible and economical approach to develop stable SLNs of LFD.

Acknowledgement:
The authors are thankful to Alembic Pharmaceuticals Ltd. (Vadodara, India) for providing Leflunomide.

References:
Design and Development of Ocular In-situ Gel of Acyclovir

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Abstract: The objective of the present work was developing an in-situ gel formulation of Acyclovir to effectively deliver the drug into the eye with sustained and prolonged drug release. This would further enhance the patient compliance by reducing the frequency of drug administration. The poor bioavailability and therapeutic response associated with the conventional ophthalmic solutions can be overcome by the use of in-situ gel forming systems that are instilled as drops and undergo a sol-gel transition in the cul-de-sac of the eye to form the drug reservoir over the corneal surface. The present research work deals with the design and development of ocular in-situ gel of acyclovir by using gellan gum as a phase transition polymer. Formulation containing 0.6% gellan gum and 0.5% PEG 400 was selected as an optimized formulation, as it had satisfactory attributes of gelling property, flow characteristics, and prolonged in-vitro release over a period of 8 hr.

Introduction:
Acyclovir is a Guanosine analogue antiviral drug used in herpes simplex keratitis. Its ophthalmic ointment preparation is only used for HSK. It was, therefore, thought worthwhile to develop an in-situ gel formulation using a suitable phase transition polymer. Transcorneal permeation study of developed acyclovir in-situ gel formulation was carried out using excised goat’s cornea and was compared to that of marketed formulation of acyclovir (Acivir) in order to determine the permeation profile of acyclovir from gel across the corneal membrane. Permeation of acyclovir from in-situ gel formulation was 79.3% while that from marketed eye drops was 65.8% in 7 hr. It concluded that the developed in-situ gel formulation can be viewed as a better alternative to the conventional eye ointment of acyclovir by virtue of its ability to enhance precorneal residence time and consequently ocular bioavailability with lesser frequency of administration [1-2].

Material and Methods:
Selection of Excipients: Gelling capacity of various polymers, i.e., Carbopol (934, 940, and 980), sodium alginate, xanthan gum, and gellan gum in simulated lacrimal fluid was estimated, for the selection of appropriate polymer, having good phase transition property for in-situ gel formation.

Preparation of Gellan Gum Solution: Solution of gellan gum in distilled water was prepared by dispersing the gellan gum in known amount of distilled water heated previously to 50°C. It was
allowed for mixing on the magnetic stirrer for 20 min with heating up to 90°C. The solution was then cooled at room temperature.

**Properties of Gellan Gum Solution:** Gellan gum solution resulted in gel formation with simulated lacrimal fluid, and human tears, which confirmed its ion activated phase transition behaviour.

**Formulation of In-Situ Gelling System:** Boric acid was dissolved in 100 ml of distilled water. Gellan gum was dispersed in a part of above solution and stirred for 20 min at 90°C temperature. After cooling to room temperature, drug and preservative were added and stirred until drug dissolved. Remaining amount of boric acid solution was added into it. The resulting solution was stored in a refrigerator for further use. Crystallization of the drug was observed after 48 hour. To avoid crystallization of drug in freeze thaw conditions, 0.05% of disodium edetate was incorporated in the formulation. It is also reported in literature, that disodium edetate prevents crystallization in freeze thaw conditions. Formulation was terminally sterilized by autoclaving at 121°C temperature and 15 psi pressure for 15 min.

**Evaluation of Developed In-Situ Gelling System:** The final optimized in-situ gel formulation was subjected to the isotonicity testing. Viscosity determinations of the developed Acyclovir in-situ gel formulation was done using Brookfield viscometer (LVT) model. Het-Cam (Hen’s Egg Test or HulnerEmbryogen) Test was performed for the detection of ocular corrosives and irritants. The physical stability, including appearance, colour, and pH of the formulation were studied under various storage conditions.

**Results and Discussion:**
Transcorneal permeation study of developed acyclovir in-situ gel formulation was carried out using excised goat’s cornea and was compared to that of marketed formulation of acyclovir (Acivir) in order to determine the permeation profile of acyclovir from gel across the corneal membrane. Permeation of acyclovir from in-situ gel formulation was 79.3% while that from marketed eye drops was 65.8% in 7 hr.
**Figure 1** Transcorneal drug permeation profile of developed acyclovir in-situ gel formulation and marketed ointment (ACIVIR)

**Conclusion:**
It can be concluded that the developed in-situ gel formulation can be viewed as a better alternative to the conventional eye ointment of acyclovir by virtue of its ability to enhance precorneal residence time and consequently ocular bioavailability with lesser frequency of administration.

**References:**
Designing and evaluating pulsatile drug release for Cimetidine

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2Sinhgad College of Pharmacy, Vadgaon (budruk), Pune-41, India.
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Abstract: The objective of present investigation was to prepare and evaluate a time lagged pulsatile Drug delivery system of cimetidine. The prepared time lagged pulsatile delivery system Consisted of two different types: a core tablet, containing the active ingredient, and an Erodible outer shell. Dry Coating was done by using different concentration of hydroxyl propyl methyl cellulose (HPMC) HPMC K4M and ethyl Cellulose. Developed formulation was evaluated for their physical characteristics, in vitro drug release profile. On the basis of these evaluation parameters time lagged release showed time lagged of 3 hrs, and in 2 hrs its shows 99.54% drug absorption.

Introduction:
Pulsatile systems are gaining a lot of interest as they deliver the drug at the right site of action at the right time and in the right amount, thus providing spatial and temporal delivery and increasing patient compliance. These systems are designed according to the circadian rhythm of the body. Circadian rhythms are self-sustaining, endogenous oscillations that occur with a periodicity of about 24 Hours. Normally, circadian rhythms are synchronized according to internal biologic clocks related to the sleep-wake cycle. Our circadian rhythm is based on sleep-activity cycle and is influenced by our genetic makeup and thereby affects our bodies’ function throughout day and night (24-hour period). Because of maximal acid secretion, peptic ulcer disease pain, and perforation of gastric and duodenal ulcers are more common at night, administration of drugs at bedtime is more effective. Nocturnal administration not only reduces acid secretion more effectively but also promotes ulcer healing and reduces ulcer recurrence. The H2 receptor antagonists are a class of drugs used to block the action of the main aim of the studies described was to develop a time lagged pulsatile drug delivery system using cimetidine. The intention was that the time lagged pulsatile drug delivery formulation administered at night at 8 p.m., which provides treatment for diseases in which symptoms are experienced in the late night at 1 a.m. (i.e. chronopharmacotherapy) as the acid secretion starts from 12 to 2 am. [1-4]

Materials and Methods:
Preparation of powder blend for core tablets and time lagged tablets prepared using direct compression method:
The powder blend of core tablets was prepared using cimetidine, Microcrystalline Cellulose (MCC, Avicel PH-102), Cross-carmellose Sodium (Ac-Di-Sol). All above ingredients were dry blended for 20 min. followed by addition of Magnesium Stearate. The mixture was then further blended for 10 min. Ethylcellulose 80 mg and HPMC K4M 120mg (FI) dry blended at about 10 min. and used as press-coating material to prepare time lagged pulsatile tablets respectively by direct compression method. The tablets were evaluated for thickness, diameter, average weight, hardness, friability and in vitro drug release study.

**Results and Discussion:**

**Table 1 Evaluation of core tablet**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness*</td>
<td>0.102 ± 0.115 cm</td>
</tr>
<tr>
<td>Hardness*</td>
<td>6.50 ± 0.25 kg/cm²</td>
</tr>
<tr>
<td>Average Weight</td>
<td>239.50 mg</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.7415(%)</td>
</tr>
</tbody>
</table>

* Indicates mean ± SD (n=3)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Thickness* (cm)</th>
<th>Average Weight (mg)</th>
<th>Hardness* (kg/cm²)</th>
<th>Friability* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>0.310 ± 0.11</td>
<td>448.67</td>
<td>11.10 ± 1.21</td>
<td>0.398 ± 0.15</td>
</tr>
</tbody>
</table>

Friability of the core as well as the press coat tablet [asses USP/NF limit that is less than 1% loss during friability test

**Table 2 In-vitro evaluation of press-coated tablets (direct compression)**

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Cumulative % Release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>5.080813953</td>
</tr>
<tr>
<td>5</td>
<td>93.48901163</td>
</tr>
<tr>
<td>6</td>
<td>99.52424419</td>
</tr>
</tbody>
</table>

* Indicates mean ± SD (n=3)

Ethyl cellulose = 80mg L-HPC = 120mg. This showed 3 hours lag time, after 3 hours the press coating material got completely eroded and core tablet was exposed with the dissolution medium, within 1hr the core tablet got completely disintegrated and released the drug. The highest percentage (%) release was observed at 6th hour that is 99.52%.
Conclusion:

The main objective in relation to this study was to design and evaluate core tablets using direct compression. From the in vitro evaluation of core tablets it was observed that within the 5 hrs the core tablets get disintegrated and released the drug. By observing the in vitro release pattern of the time lagged pulsatile drug delivery systems it was concluded that time lagged formulations prepared by direct compression method showed good in vitro release patterns. Ethyl cellulose 8% and Low-substituted Hydroxypropylcellulose (L-hpc) 12% as outer barrier layers and which was prepared by direct compression method was suitable to achieve the 5hrs lag time, so it was the best composition of the outer barrier layers and direct compression was the best method. The formulations had greater hardness, friability and showed more lag time.

References

Central composite designed Atorvastatin loaded Chitosan nanoparticles

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Abstract: The aim of present study was to formulate atorvastatin loaded chitosan nanoparticles by ionic gelation method using central composite design. The concentration of chitosan ($x_1$), concentration of sodium tripolyphosphate (NaTPP) ($x_2$), and volume of sodium tripolyphosphate ($x_3$) were selected as independent variables. The prepared nanoparticles were evaluated for particle size, encapsulation efficiency and loading capacity. Particle size of prepared nanoparticles was found to be in range between 35 nm to 1090 nm. The optimized batch was evaluated using differential scanning calorimetry (DSC), fourier-transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). In vitro drug release studies revealed that cumulative percent of drug released was found to be 87.9% over a time period of 8 hr. The mathematical developed in the present study can be further utilized as response surface for cumulative percent of drug release of NPs of atorvastatin.

Introduction:
The major goals in designing nanoparticles as delivery system are to control particles size, surface properties and release of pharmacologically active agents in order to achieve the sustain release of the drug at the therapeutically optimal rate and dose regimen [1]. Chitosan has gained importance in recent years for its use in novel drug delivery systems because of biocompatibility, biodegradability, non-immunogenicity, non-toxicity and low cost [2]. Atorvastatin (ATR) is BCS class II drug used for lowering blood cholesterol levels. [3]. Ionic gelation method is a simple method in which reversible physical cross linking occurs by electrostatic interactions. The aim of present work was to formulate atorvastatin loaded chitosan nanoparticles using central composite design. The effect of concentration of chitosan ($X_1$), concentration of sodium tripolyphosphate ($X_2$), and volume of sodium tripolyphosphate ($X_3$) on the particle size, encapsulation efficiency and in vitro percent (%) cumulative drug release.

Materials and Methods:
Atorvastatin (ATR) was kindly gifted by Farma Glow, India. Chitosan (CN) was purchased from Fluka Chemika (Switzerland). Sodium Tripolyphosphate (NaTPP) was purchased from Thomas and Baker Limited (Mumbai). All other chemicals were of analytical reagent grade and used as received.

Formulation and optimization of Atorvastatin loaded Chitosan Nanoparticles: In the present study, nanoparticles were prepared by ionic gelation method [4] using Central Composite design o
study the effect of independent variables on % cumulative drug release of atorvastatin. The study of three factors at two levels leads to 20 formulation (Table 1) of nanoparticles.

Characterization of Nanoparticles: The particle size and particle size distribution of samples were determined by means of Laser diffractometry, using Zetasizer instrument (Malvern, UK) equipped with Hydro dispersing unit. The ATR encapsulation efficiency and loading capacity was determined directly using freeze-dried nanoparticles. The encapsulation efficiency, particle size and loading capacity of all the formulations are tabulated in Table 1. Fourier-transformed infrared (FTIR) spectroscopy was used to ascertain the structural interactions between drug (ATR) and polymer. The spectra were recorded over the range of 400-4000 cm\(^{-1}\). The DSC studies were carried out at the temperature ranging from 30ºC to 300ºC at a rate of 10ºC/min under nitrogen atmosphere (60 ml/min), with empty pan as reference. The in-vitro release study of atorvastatin loaded chitosan nanoparticles were carried out using USP dissolution apparatus type II at a rotating speed of 75 rpm using dialysis method. The sample withdrawn was estimated by HPLC analysis.

Results and Discussion

TPP is a non-toxic and multivalent anion that forms cross-linkage between the positively charged amino group of chitosan and negatively charged TPP molecules leading to the formation of nanoparticles with a size range of nanoparticles [5]. The batch NP-5 (Table 1) was found to have mean diameter of approximately 35 nm and has maximum encapsulation efficiency and loading capacity. There was no significant difference in the FTIR spectra of ATR and drug loaded NP-5. The FTIR data suggested that molecular interactions that could alter the chemical structure of the drug. The DSC curve of atorvastatin shows endothermic peak at 161.41ºC almost corresponding to its melting point and indicating its crystalline nature. The disappearance of peak in thermogram of NP-5 suggested the conversion of drug to amorphous form. TEM study indicated that the particles are of almost spherical shape with uniform size and diameter. The ATR nanoparticles of batch NP-5 showed 87.5 % drug release in phosphate buffer ph 6.8 over a time period of 8 hr. The model, developed from multiple linear regressions (Design Expert Software version 8.0.1.0), to estimate cumulative percent drug release (Y) can be represented mathematically as:

\[
Y = 85.66 + 0.28 X_1 + 1.18 X_2 - 0.63 X_3 + 0.63 X_1 X_2 - 0.12 X_1 X_3 + 0.40 X_2 X_3 + 1.58 X_1^2 + 1.47 X_2^2 + 0.59 X_3^2
\]

Where, \(Y\) = Cumulative Percent of Drug Release, \(X_1\) = Concentration of chitosan (% w/v), \(X_2\) = Concentration of NaTPP (% w/v), \(X_3\) = Volume of NaTPP (ml); \(X_1\) \(X_2\), \(X_2\) \(X_3\), \(X_1\) \(X_3\) =Coefficients of interaction terms; \(X_1^2\), \(X_2^2\) and \(X_3^2\) =Coefficients of quadratic relationship terms). ANOVA was applied on the cumulative percent drug release to study the fitting and significance of model. The ratio \(F= 3.65\) showed the model is significant. This is only 4.10 % chance that a “model F-value” this large could not occur due to noise.
### Table 1 Formulation and characterization of NPs (batch NPs-1 to NPs-18).

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>$X_1$ (Conc. of chitosan) (% w/v)</th>
<th>$X_2$ (Conc. of NaTPP) (% w/v)</th>
<th>$X_3$ (Vol. of NaTPP) (ml)</th>
<th>Encapsulation efficiency (%)</th>
<th>Loading capacity (%)</th>
<th>Mean diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>-1 (0.5%)</td>
<td>-1 (0.1%)</td>
<td>-1 (14)</td>
<td>50.61</td>
<td>28.11</td>
<td>739</td>
</tr>
<tr>
<td>NP-2</td>
<td>+1 (1.5%)</td>
<td>-1 (0.1%)</td>
<td>-1 (14)</td>
<td>55.10</td>
<td>27.85</td>
<td>118.3</td>
</tr>
<tr>
<td>NP-3</td>
<td>-1 (0.5%)</td>
<td>-1 (0.1%)</td>
<td>+1 (20)</td>
<td>47.35</td>
<td>24.54</td>
<td>869.4</td>
</tr>
<tr>
<td>NP-4</td>
<td>+1 (1.5%)</td>
<td>-1 (0.1%)</td>
<td>+1 (20)</td>
<td>62.32</td>
<td>30.97</td>
<td>295.3</td>
</tr>
<tr>
<td>NP-5</td>
<td>-1 (0.5%)</td>
<td>+1 (0.2%)</td>
<td>-1 (14)</td>
<td>63.83</td>
<td>31.98</td>
<td>34.33</td>
</tr>
<tr>
<td>NP-6</td>
<td>+1 (1.5%)</td>
<td>+1 (0.2%)</td>
<td>-1 (14)</td>
<td>63.49</td>
<td>30.13</td>
<td>147.9</td>
</tr>
<tr>
<td>NP-7</td>
<td>-1 (0.5%)</td>
<td>+1 (0.2%)</td>
<td>+1 (20)</td>
<td>52.67</td>
<td>27.01</td>
<td>255</td>
</tr>
<tr>
<td>NP-8</td>
<td>+1 (1.5%)</td>
<td>+1 (0.2%)</td>
<td>+1 (20)</td>
<td>49.70</td>
<td>24.42</td>
<td>423</td>
</tr>
<tr>
<td>NP-9</td>
<td>-1.682 (0.16%)</td>
<td>0 (0.15%)</td>
<td>0 (17)</td>
<td>51.92</td>
<td>30.28</td>
<td>262.4</td>
</tr>
<tr>
<td>NP-10</td>
<td>+1.682 (1.84%)</td>
<td>0 (0.15%)</td>
<td>0 (17)</td>
<td>50.52</td>
<td>25.21</td>
<td>673.5</td>
</tr>
<tr>
<td>NP-11</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>-1.682 (12)</td>
<td>58.21</td>
<td>31.66</td>
<td>122.4</td>
</tr>
<tr>
<td>NP-12</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>+1.682 (22)</td>
<td>45.13</td>
<td>24.40</td>
<td>738.5</td>
</tr>
<tr>
<td>NP-13</td>
<td>0 (1%)</td>
<td>-1.682 (0.066%)</td>
<td>0 (17)</td>
<td>54.95</td>
<td>30.22</td>
<td>230.7</td>
</tr>
<tr>
<td>NP-14</td>
<td>0 (1%)</td>
<td>+1.682 (0.234%)</td>
<td>0 (17)</td>
<td>57.65</td>
<td>30.09</td>
<td>1099</td>
</tr>
<tr>
<td>NP-15</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>0 (17)</td>
<td>55.82</td>
<td>29.36</td>
<td>480.9</td>
</tr>
<tr>
<td>NP-16</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>0 (17)</td>
<td>53.45</td>
<td>28.74</td>
<td>537.9</td>
</tr>
<tr>
<td>NP-17</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>0 (18)</td>
<td>54.11</td>
<td>28.23</td>
<td>396.9</td>
</tr>
<tr>
<td>NP-18</td>
<td>0 (1%)</td>
<td>0 (0.15%)</td>
<td>0 (17)</td>
<td>52.86</td>
<td>29.07</td>
<td>1030</td>
</tr>
</tbody>
</table>

The value in the bracket indicates real values.

**Conclusion**

The formulated atorvastatin loaded nanoparticles having the size 35 nm showed good encapsulation efficiency and loading capacity. The *In-vitro* release study showed good release characteristic at pH 6.8 (86%) and followed the first order kinetics. Therefore, it can be concluded that atorvastatin loaded chitosan nanoparticles can be an effective carrier for the design of controlled drug delivery of poorly water soluble drugs.

**References:**

Formulation, development and evaluation of osmotic drug delivery system of Glibenclamide

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Abstract: The Present research work is to develop osmotic drug delivery of Glibenclamide. Glibenclamide belongs to a class of anti-diabetic (sulfonylurea). It works by Inhibition of ATP-dependent potassium channels. The proposed work is envisaged to carry out the preformulation, optimization, development of in-situ orifice forming osmotic capsule and evaluation of osmotic capsule. In-situ pore forming osmotic capsule of Glibenclamide is the one which suit the concept of better patient compliance, delayed release, more efficacies and enough bioavailability to show required pharmacological action and less gastrointestinal side effects. The Glibenclamide osmotic drug delivery system was successfully developed and evaluated.

Introduction:
Osmotically controlled oral drug delivery systems (OCODDS) utilize osmotic pressure as the energy source for the controlled delivery of drugs. Drug release from these systems is independent of pH and hydrodynamic conditions of the gastro-intestinal tract (GIT) to a large extent, and release characteristics can be easily adjusted by optimizing the parameters of the delivery system [1-2]. Core contain water soluble osmotically active agent and blended with water soluble or insoluble drug, additives and coating has been carried out which functions as semi permeable membrane. The device delivers a saturated volume equal to the volume of water uptake through the membrane. Initial lag time (per hour) during which delivery rate increases to its maximum value, drug release is zero order, until all solid material is dissolved [3].

Materials and Methods:
Materials: Glibenclamide (drug) was provided by Aristo Pharmaceutical Pvt Ltd, and other chemicals were provided by SIRTS-Pharmacy Bhopal. Hard gelatin capsule were purchased.

Filling of capsule body: For the preparation of osmotic capsule first of all a hard gelatin capsule was taken, then the mixture of Glibenclamide, Lactose, Nacl, Magnesium stearate and SLS was prepared and filled into the body part of capsule than the body cap of capsule is placed.

Coating of capsule body: To make insoluble in water, hard gelatin capsule was treated with 1% ethyl cellulose in ethyl alcohol. The coated capsules were then dried at different temperatures ranging from approximately 25 to 50 °C for 15 min.

Optimization: To optimize the formulation we took six formulation F-1,F-2,F-3,F-4,F-5 and F-6. In each formulation we changed concentration of the ingredients. The concentration of osmogen, sodium
chloride and solubilizing agent, sodium lauryl sulphate were changed.

**Table 1** Formula for osmotic capsule

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ingredients</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glibenclamide</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>NaCl</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>Lactose</td>
<td>40</td>
</tr>
<tr>
<td>4.</td>
<td>Sodium lauryl sulphate</td>
<td>1% w/w</td>
</tr>
<tr>
<td>5.</td>
<td>Magnesium stearate</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 2** Optimization of Glibenclamide osmotic capsule

<table>
<thead>
<tr>
<th>S No.</th>
<th>Ingredients</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
<th>F-5</th>
<th>F-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glibenclamide</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>2</td>
<td>NaCl</td>
<td>1 mg</td>
<td>2 mg</td>
<td>3 mg</td>
<td>1 mg</td>
<td>2 mg</td>
<td>3 mg</td>
</tr>
<tr>
<td>3</td>
<td>Lactose</td>
<td>40 mg</td>
<td>40 mg</td>
<td>40 mg</td>
<td>40 mg</td>
<td>40 mg</td>
<td>40 mg</td>
</tr>
<tr>
<td>4</td>
<td>Sodium lauryl sulphate</td>
<td>1% w/w</td>
<td>1% w/w</td>
<td>1.5% w/w</td>
<td>1.5% w/w</td>
<td>2% w/w</td>
<td>2% w/w</td>
</tr>
<tr>
<td>5</td>
<td>Mg stearate</td>
<td>3 mg</td>
<td>3 mg</td>
<td>3 mg</td>
<td>3 mg</td>
<td>3 mg</td>
<td>3 mg</td>
</tr>
</tbody>
</table>

**Results:**

**Preformulation study:** The preformulation study of Glibenclamide was performed. Glibenclamide was found soluble in methanol, sparingly soluble in ethanol. The melting point of Glibenclamide was found to be 173 to 176°C. The partition coefficient was found 17.23 which show it is lipophilic in nature. The peak of the graphs of UV, IR and DSC report was resemble like Indian Pharmacopeia standard. Hence on the basis of preformulation study the drug sample of Glibenclamide was found to be pure and authenticated and the sample can be use for the further preparation and evaluation.

**In-vitro release:** For the release study during dissolution apparatus USP apparatus was used. In this apparatus 900 ml of solution of phosphate buffer 6.8 pH at 75 rpm and 37±1°C used. The capsules are placed inside the apparatus and release of drug is evaluated. The samples were filtered and suitably diluted to determine the absorbance at 300 nm in UV spectrophotometer. On the basis of characterization parameters formulation F2 is considered optimized and studied for release kinetics. Drug release of osmotic capsule follow Zero order, First order kinetics and Higuchi and Peppas release profile.

![In-vitro drug release from osmotic capsule](image)

**Figure 1** In-vitro drug release from osmotic capsule

**Accelerated Stability Studies:** The microbeads from the selected and optimized batch were studied for stability and kept under the accelerated conditions like raised temperature and moisture up to
period of three months. The results revealed no marked alterations in physical appearance and drug releasing properties.

**Conclusion:**
Glibenclamide release from the developed formulation was directly proportional to the osmogen, pore former and solubilizing agents. Based on the present investigation, it was concluded that desired environmentally independent and controlled drug delivery of like Glibenclamide from oral osmotic pump can be achieved by approximately selecting dispersion, type of membrane and optimizing its thickness, by adjusting the concentration of solubilizing agent and incorporating optimized amount of osmogens. Glibenclamide was used as a marker or model agent. On basis of *in vitro* release profile of Glibenclamide, it was concluded that the osmotic pump containing SLS provided sustained release for 7 hour and remain stable and intact.

**Acknowledgement:**
I take this opportunity to express my deep sense of gratitude and thanks to my guide Dr. Jitendra Banweer (Director, SIRTS-Phamacy) and co-guide Dr. Praveen Tabilani for their valuable guidance and support for the project.

**References:**
Validated HPTLC method for Simultaneous estimation of Nifedipine and Lidocaine in cream dosage form

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Abstract: A simple and sensitive high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous estimation of nifedipine and lidocaine in cream. The separation was achieved on precoated silica gel 60F254 using Cyclohexane : Ethylacetate : Methanol : Triethylamine (5.5:3.0:0.5:0.8, v/v/v/v) as mobile phase and UV detection at 236 nm. The method was validated in terms of linearity, accuracy, precision and sensitivity. The calibration curve was found to be linear between 600-3600 ng/spot for nifedipine and 1500-4000 ng/spot for lidocaine. The proposed method can be successfully applied for routine estimation of nifedipine and lidocaine in cream formulation.

Introduction:
Nifedipine (NFD) chemically, 3,5-dimethyl,2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, is a Ca++ channel blocker used in treatment of hypertension and Lidocaine (LDC) chemically, 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, is a local anaesthetic [1]. NFD is official in IP, BP, USP, JP and EP. Literature survey reveals various chromatographic [2] and spectrophotometric methods for estimation of NFD in single dosage form as well in combination with other drugs. LDC is official in IP [3], BP, USP, and EP. Literature survey reveals chromatographic [4] and spectrophotometric methods for determination of LDC in combination with other drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of NFD and LDC in their combined dosage forms. The present communication describes simple, sensitive, accurate, precise and cost effective HPTLC method for simultaneous estimation of both drugs in their combined cream dosage form.

Materials and Method:
Apparatus: A Camag HPTLC system (Switzerland) with Linomat V automatic sample applicator and Camag TLC Scanner III was used for determination. Chromatograms were automatically obtained by Camag Win-CATS software. A Sartorius CP224S analytical balance (Gottingen, Germany) and an ultrasonic bath (Frontline FS 4, Mumbai, India) were used in the study.
Reagents and Materials: NFD and LDC bulk powders were kindly gifted by Cadila Pharmaceuticals Ltd, Gujarat, India. Methanol, ethylacetate, cyclohexane, triethylamine (AR Grade, S. D. Fine
Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions: An accurately weighed quantity of standard NFD (30 mg) and LDC (50 mg) powders were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to obtain a solution containing 300 μg/ml of NFD and 500 μg/ml of LDC.

Preparation of sample solution: Accurately weighed 10 gm of cream which equivalent to 30 mg NIF and 150 mg of LDC was transferred to 100 ml volumetric flask. It was dissolved in sufficient amount of methanol sonicated for 30 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to 100 ml with methanol to obtain solution containing 300μg/ml of NFD and 1500μg/ml of LDC.

Methodology: Calibration curve were plotted over a concentration range of 600-3600 ng/spot for NFD and 1500-4000 ng/spot for LDC. For this, 2, 3, 6, 8, 10 and 12μl of NFD and 3, 4, 5, 6, 7 and 8μl of LDC from their respective stock solution were spotted in band width 5 mm using Camag 100μl syringe on precoated silica gel aluminium plate 60 F254 using automatic application device. Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber saturated with the mobile phase for 30 min. The plate was removed from the chamber, subsequently dried in a current of air and densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorption mode at 236 nm and operated by win CATS software. Peak areas were recorded for all the peaks. The calibration curves for NFD and LDC were constructed by plotting peak area versus concentration (ng/spot) corresponding to each spot and regression equations were calculated for NFD and LDC.

Method Validation: The method was validated for linearity & range, precision, accuracy (% recovery), specificity, limit of detection and limit of quantification as per the ICH guidelines [5].

Analysis of Drugs in Tablets: From the above prepared sample solution, 2.5 μl solution was spotted and the plate was developed as per the above procedure. The peak area of final sample solution was measured densitometrically at 236 nm for quantitation of NFD and LDC. The amount NFD and LDC present in the sample solutions were determined by fitting the response in to the respective regression line equations for NFD and LDC.

Results and Discussion:
Calibration curve was found to be linear in the concentration range of 600-3600 ng/spot with correlation coefficient ($r^2$) 0.9973 for NFD and 1500-4000 ng/spot with correlation coefficient ($r^2$) 0.9997 for LDC.
The % RSD values for repeatability study for NFD and LDC are found to be 0.46 and 0.32, respectively. Low % RSD values shows the proposed method is repeatable. The % RSD values for reproducibility study for NFD and LDC are found to be 0.65-0.82 % and 0.46-0.79 % as intraday precision and 0.85-1.16 % and 0.90-1.03 % as interday precision respectively. Low % RSD values shows the proposed method is reproducible. LOD and LOQ values for NFD were found to be 60 and 200 ng/spot at 236 nm, respectively. While the, LOD and LOQ values for LDC were found to be 300 ng/spot and 1000 ng/spot at 236 nm, respectively. Low value of LOD & LOQ indicates that the method is sensitive. The mean % recoveries were found to be 100.70 ± 1.39 and 101.06 ± 0.95 for NFD and LDC, respectively. The % recoveries results indicate that the proposed method is accurate. The % assay for NFD and LDC was found to be 99.22±2.96 and 98.77±1.084, respectively. The proposed validated method was successfully applied to determine NFD and LDC in combined cream formulation.

Conclusion:
The method described for the simultaneous estimation of NFD and LDC was found to be sensitive, accurate and precise for routine simultaneous estimation of two drugs in their combined dosage form. The values of standard deviation and % RSD were satisfactorily low and recoveries studies indicate the reproducibility and accuracy of the method. The result of the analysis of the dosage form by this method is reproducible and reliable and is in good agreement with label claim of the drugs. The additive present in the formulation did not interfere in the analysis. So the method can be used for the routine quality control test of these drugs in combined cream formulation.

Acknowledgement:
The authors are greatly thankful to Cadila Pharmaceuticals Ltd., Ahmedabad for providing gift sample of NFD and LDC and Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat Vidyanagar for providing all the facilities to carry out the work.

References:
Development and validation of RP-HPLC method for simultaneous estimation of Doxycycline Monohydrate and Ornidazole in Pharmaceutical dosage form

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Abstract: A sensitive RP-HPLC method has been developed for simultaneous estimation of doxycycline monohydrate (DOX) and ornidazole (ORN) in tablet. The sample was analyzed by reverse phase C18 column (ACE C18, 150 mm x 4.6 mm, 5 µ) as stationary phase; methanol: phosphate buffer pH 6.5 (30:70, v/v) as mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with PDA detector at 286 nm. The retention time for DOX and ORN was found to be 8.97 min and 4.45 min, respectively. The linearity was obtained in the concentration range of 5-35 µg/ml for each DOX and ORN.

Introduction:
Doxycycline (DOX) is chemically 4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide is a well-known antibiotic drug [1]. It is official in Indian Pharmacopoeia [2], British Pharmacopoeia, United State Pharmacopoeia, European Pharmacopoeia and Japanese Pharmacopoeia. Literature survey reveals HPLCand UV spectrophotometry methods for estimation of DOX in single dosage form well in combination with other drugs. Ornidazole (ORN) is chemically 1-chloro-3-(2-methyl-5-nitroimidazol-yl) Propan-2-ol. Ornidazole (ORN) is official in IP. Literature survey reveals few analytical methods for simultaneous estimation of DOX and ORN in combination [4-5]. The present communication describes simple, sensitive, rapid, accurate, precise and specific RP-HPLC method for simultaneous estimation of both drugs in their combined tablet dosage form.

Materials and Method:
Apparatus: RP-HPLC instrument equipped with a UV-visible detector and a photodiode array detector (Shimadzu; LC-2010CHT, Japan), auto sampler, ACE C18 column (150 mm x 4.6 mm, 5 µ), LC-solution software, double beam UV-Visible spectrophotometer (Shimadzu, UV-1800, Japan), analytical balance (Sartorius CP224S, Germany), digital pH meter (LI 712 pH analyser, Elico Ltd., Ahmedabad), ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used in the study.
Reagents and Materials: DOX and ORN standard powder were supplied as a gift samples from Astron Pharmaceutical Ltd., Ahmedabad, India. The tablet containing 100 mg DOX and 500 mg ORN
was purchased from the local pharmacy. HPLC grade methanol, acetonitrile, water (Finar Chemicals Ltd., Mumbai, India), nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England), potassium dihydrogenorthophosphate (Finar Chemicals Ltd., Mumbai, India) were used in the study.

**Preparation of standard stock and working standard solutions:** An accurately weighed standard DOX and ORN (10 mg) powder was transferred to separate 100 ml volumetric flasks, dissolved and diluted up to the mark with methanol to obtain standard stock solutions having concentration 100 µg/ml for each DOX and ORN. An aliquot of standard stock solutions (5 ml) were transferred to separate 10 ml volumetric flasks having concentration 50 µg/ml for each drug.

**Preparation of sample solution:** Twenty tablets were accurately weighed and powdered. Quantity of the powder equivalent to 10 mg DOX & 50 mg ORN was transferred in 100 ml volumetric flask separately and powder was dissolved in 50 ml of methanol with sonication to dissolve drug as completely as possible. The solution (0.5 ml) was transferred to 10 ml volumetric flask and diluted up to mark with methanol.

**Chromatographic condition:** Stationary phase: ACE C18 column (150 mm x 4.6 mm, 5 µm particle size) was used at an ambient temperature, Mobile phase: methanol: phosphate buffer pH 6.5 (30:70, v/v), Flow rate: 1 ml/min, Injection volume: 20 µl, Detection: 286 nm using PDA detector.

**Preparation of calibration curves:** Calibration curves were plotted over the concentration range of 5-35 µg/ml for both DOX and ORN. Accurately measured aliquots of working standard solutions of both DOX and ORN (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml) were transferred to a series of 10 ml of volumetric flasks separately and diluted up to the mark with methanol.

**Method Validation:** The method was validated according to ICH guidelines [3].

**Analysis of DOX and ORN from Tablets:** The response of the sample solution was measured at 286 nm under the optimized chromatographic condition mentioned above for estimation of DOX and ORN. The amounts of DOX and ORN present in sample solution were estimated by applying values of the peak area to the regression equations of the calibration curves of DOX and ORN.

**Results and discussion:**

Calibration range was observed in the concentration range of 5-35 µg/ml for both DOX and ORN. The % RSD values for repeatability study for DOX and ORN are found to be 0.84 and 0.51, respectively reveals method repeatability. The low values of %RSD for intraday (0.69-1.25 % and 0.37-1.17 %) and interday (1.47-1.84 % and 1.31-1.69 %) for DOX and ORN, respectively reveals method reproducibility. LOD and LOQ values for DOX were found to be 0.19 and 0.58 µg/ml, respectively. While the LOD and LOQ values for ORN were found to be 0.17 and 0.51 µg/ml,
respectively reveals method sensitivity. The recovery experiment was performed by standard addition method. The mean recoveries were found to be 100.2 ± 0.30 % and 99.69 ± 0.45 % for DOX and ORN, respectively reveals accuracy of the method. The specificity of the method was ascertained by analysing standard solution and sample solution. The peak purity of standardsolutions containing DOX and ORN were found to be 0.9999 and 0.9999, respectively and the peak purity of sample solution containing DOX and ORN were found to be 0.9996 and 0.9999, respectively reveals specificity of the method. The proposed validated method was successfully applied to determine DOX and ORN in combined tablets.

**Conclusion:**

A simple, sensitive, repeatable and specific RP-HPLC method has been developed for the simultaneous estimation of DOX and ORN. The method was validated for accuracy, precision, linearity, LOD and LOQ. The result of analysis of tablet by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drugs. The method can be used for the routine analysis of the DOX and ORN in combined mixture without any interference of excipients.

**Acknowledgement:**

The authors are greatly thankful to Astron Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of DOX and ORN and S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, GanpatVidyanganarfor providing all the facilities to carry out the work.

**References:**


Development and validation of high performance thin layer chromatography method for simultaneous estimation of Chlorpheniramine Maleate and Guaiphenesin in pharmaceutical dosage form

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Abstract: A simple and sensitive HPTLC method has been developed for simultaneous estimation of chlorpheniramine maleate (CPM) and guaiaphenesin (GPN). This method is based on HPTLC separation of two drugs performed on Precoated silica gel on aluminum plate 60F254 using Methanol: Toluene: Triethylamine (3.8: 5.2: 1.0 v/v/v) as mobile phase and UV detection at 265 nm. The linearity was established over the concentration range of 100-600 ng/spot for CPM and 1250-7500 ng/spot for GPN with correlation coefficient ($r^2$) 0.9981 and 0.9975, respectively. The proposed method has been successfully applied to the estimation of CPM and GPN in their combined dosage form.

Introduction:
Chorpheniramine (CPM) is chemically [3-(4-chlorophenyl)-3-(pyridin-2yl) propyl] dimethylamine, a well-known first generation antihistaminic drug [1]. It is official in Indian Pharmacopoeia [2], British Pharmacopoeia, United State Pharmacopoeia and European Pharmacopoeia. Literature survey reveals UV spectrophotometry [3] and HPLC methods for estimation of CPM in single dosage form as well in combination with other drugs. Guaiaphenesin (GPN) is chemically 3-(2-methoxypheenoxy) propane-1,2-diol, an expectorant [1]. It is official in IP [2], BP, USP, EP and JP. Literature survey reveals HPLC [4] and UV spectrophotometry methods for determination of GPN in single dosage form as well in combination with other drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CPM and GPN in their combined dosage forms. Literature survey does not reveal any analytical methods for simultaneous estimation of CPM and GPN in combination. The present communication describes simple, sensitive, accurate, precise and cost effective HPTLC method for simultaneous estimation of both drugs in their combined tablet dosage form.

Materials and Methods:

Apparatus: A Camag HPTLC system (Switzerland) with Linomat V automatic sample applicator and Camag TLC. Scanner III was used for determination. Chromatograms were automatically obtained by...
Camag Win-CATS software. A Sartorius CP224S analytical balance (Gottingen, Germany) and an ultrasonic bath (Frontline FS 4, Mumbai, India) were used in the study.

**Reagents and Materials**

CPM and GPN bulk powders were kindly gifted by Camper Healthcare, Ganpat University, Gujarat, India. Methanol, Toluene, Triethylamine (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

**Preparation of standard stock solutions**: An accurately weighed quantity of standard CPM (10 mg) and GPN (10 mg) powders were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol give a solution containing 100 μg/ml each of CPM and GPN.

**Preparation of sample solution**: Twenty tablets were accurately weighed and powdered. Quantity of the powder equivalent to 20 mg CPM & 250 mg GPN was transferred in 100 ml volumetric flask and powder was dissolved in 50 ml of methanol with sonication and volume was made up with methanol. The above solution (5 ml) was transferred to 10 ml volumetric flask and diluted up to mark with methanol.

**Methodology**: Calibration curves were plotted over a concentration range of 100-600 ng/spot for CPM and 1250-7500 ng/spot for GPN. For this, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 μl of CPM and 12.5, 25.0, 37.5, 50.0, 62.5, 75.0 μl of GPN from 100 μg/ml stock solution were spotted in band width 5 mm using Camag 100 microliter syringe on precoated silica gel aluminium plate 60 F254 using automatic application device. Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber saturated with the mobile phase Methanol: Toluene: Triethylamine (3.8: 5.2: 1.0 v/v/v) for 30 min. The plate was removed from the chamber, subsequently dried in a current of air and densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorption mode at 265 nm and operated by win CATS software. Peak areas were recorded for all the peaks. The calibration curves for CPM and GPN were constructed by plotting peak area versus concentration (ng/spot) corresponding to each spot and used for determination of CPM and GPN.

**Method validation**: The method was validated according to ICH guidelines.

**Analysis of drugs in tablets**: The peak area of final sample solution was measured densitometrically at 265 nm for quantitation of CPM and GPN. The amount CPM and GPN present in the sample solutions were determined by fitting the response into the respective regression line equation for CPM and GPN.

**Results and Discussion**: Calibration range was observed in the concentration range of 100-600 ng/spot for CPM and 1250-7500 ng/spot for GPN. The % RSD values for repeatability study for CPM and GPN are found to be 0.80.
and 0.78, respectively. Low % RSD values shows the proposed method is repeatable. The % RSD values for reproducibility study for CPM and GPN are found to be 0.41-0.72 and 0.51-0.65 as intraday precision and 0.58-0.97 and 0.81-1.16 as interday precision. Low % RSD values shows the proposed method is reproducible. LOD and LOQ values for CPM were found to be 19.99 and 60.50 ng/spot at 265 nm respectively. While the LOD and LOQ values for GPN were found to be 394.9 ng/spot and 1195.3 ng/spot at 265 nm respectively. Low value of LOD & LOQ indicates that the method is sensitive. The mean recoveries were found to be 100.18 ± 0.65 and 99.99 ± 0.13 for CPM and GPN respectively. The recoveries results indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine CPM and GPN in combined tablets.

**Conclusion:**
The method described for the simultaneous estimation of CPM and GPN was found to be sensitive, accurate and precise for routine simultaneous estimation of two drugs. The values of standard deviation and % RSD were satisfactorily low and recoveries studies indicate the reproducibility and accuracy of the method. The result of the analysis of the dosage form by this method is reproducible and reliable and is in good agreement with label claim of the drugs. The additive present in the formulation did not interfere in the analysis. So the method can be used for the routine analysis of drugs in combined mixture.

**Acknowledgement:**
The authors are greatly thankful to Camper Healthcare, Ganpat University for providing gift sample of CPM and GPN and S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar for providing all the facilities to carry out the work.

**References:**
Synthesis and antimicrobial evaluation of novel fluoroquinolones

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Abstract: Different derivatives of fluoroquinolones were synthesized by combining it with different thiadiazoles. The synthesized compounds were characterized by Infra red spectroscopy, Proton nuclear magnetic resonance and Mass spectral data. The compounds were screened for their antibacterial and antifungal activity. Compounds 7c and 8c have good antibacterial activity. While compounds 7c and 8c are found to be good antifungal among ciprofloxacin and sparfloxacin derivatives.

Introduction:
Fluoroquinolones are important broad-spectrum anti bacterial agents. The literature is clearly elucidating when triazole [1], oxadiazole [2], piperidines [3], nitro aryl thiadiazoles [4] were coupled with fluoroquinolones showed good antimicrobial activity. So in view of that this work is being performed on thiadiazole and fluoroquinolones nucleus, it was thought worthwhile to synthesize some newer derivatives of thia Diazole, couple them with fluoroquinolones respectively and evaluate them for their antimicrobial activity.

Materials and Methods:
Melting points of the newly synthesized compounds were determined by open capillary method and were uncorrected. IR spectra were recorded on Shimadzu IR Affinity-1 FTIR spectrophotometer. \(^1\)H NMR spectra were recorded on Bruker DPX-300 MHz NMR spectrophotometer with CDCl\(_3\) as solvent. The Mass spectra were recorded on a Bruker Daltonics micro TOF-Q II by using (ESI) method.

General procedure for the synthesis of 7a-d and 8a-d
A well stirred mixture of fatty acids 4a-d, concentrated sulphuric acid and thiosemicarbazide was slowly heated to 80-90\(^\circ\)C. The crude product thus precipitated to give compound 5a-d. The compound 5a-d was taken in a two necked round bottomed flask fitted containing dioxane with reflux condenser and dropping funnel contained chloro acetyl chloride in 10mL of dioxane. The crude product precipitated to give the compound 6a-d. The compound 6a-d was heated under reflux at 90\(^\circ\)C with Ciprofloxacin and Sparfloxacin to give 7a-d and 8a-d respectively (Scheme I).

Antimicrobial Activity
All novel synthesized compounds were screened for their in vitro antibacterial activity against two Gram-positive (*Bacillus subtilis* [MTCC 121], *Staphylococcus aureus* [MTCC 7443]), two Gram-negative (*Escherichia coli* [MTCC 118], *Klebsiella pneumonia* [MTCC 7028]) and antifungal activity against *Candida albicans* [MTCC 1637] and *Aspergillus tubingensis* [MTCC 2479] by paper disc diffusion method [5].

**Results and Discussion:**
The data of antimicrobial studies showed that compound 7c and 8c have comparable good antibacterial activity which may be due to six carbon chain attached to thiadiazole which may be increasing its lipophilicity among ciprofloxacin and sparfloxacin derivatives. The data of antifungal activity showed that compound 7c and 8c have good activity.
Conclusión:

It is conclude that if the further clinical studies will be performed and such molecule may lead to give better antimicrobial agent where the dose of fluoroquinolones can be reduced without compromising with the activity of the parent drug.

References:

Development of stability indicating RP-HPLC methods for the determination of Adefovir Dipivoxil in bulk and dosage form

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Abstract: The objective of the present work is to develop a simple, precise, accurate, validated stability indicating, RP-HPLC method for the determination of Adefovir in bulk and dosage form. The HPLC separation was achieved by using mobile phase composition of methanol and phosphate buffer (50: 50, v/v) with flow rate 1 ml/min at 260 nm. The retention time obtained at 3.8 ± 0.02 min. Linearity was found in the concentration range of 20-160 μg/ml. When Adefovir was subjected to different stress conditions; it effectively separate the drug from its degradation products, and was thus considered as good stability-indicating procedures.

Introduction:
Adefovir dipivoxil (ADV) is chemically 9-[2-[Bis ([pivaloyloxy] methoxy] phosphinyl] methoxy] ethyl] adenine [1]. Adefovir dipivoxil is a diester prodrug of the active moiety adefovir, it is an acyclic nucleotide analogue of adenosine monophosphate [2]. The aim of this work is to develop and validate an analytical method for the estimation of adefovir in bulk and pharmaceutical dosage forms and also perform stress degradation studies on the drug as per ICH Guidelines using the developed method [3].

Materials and Methods:
Agilent technologies 1260 LC system with gradient pump connected to DAD UV detector, LC-GC AGN204PO balance was used for all weighing. An Agilent zorbax eclipse C18 column (150 mm×4.6 mm i.d., 5μm) was maintained at 30°C.

Preparation of standard solutions and calibration graphs: Stock solutions of ADV (100μg/mL) was prepared by transferring 10 mg each of ADV standard in separate 100mL volumetric flasks, dissolved in 50mL of methanol and made up to volume using the same. From stock solution, aliquots of 2, 4, 6, 8, 10, 12, 14, 16 mL were transferred into 10 mL volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of ADV in the range 20-160 μg/mL. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.
Application of Proposed Method to tablet Formulation: Twenty capsules were accurately weighed and average weight per tablet was determined. A quantity of powder equivalent to 50mg ADV was transferred into 100 mL volumetric flask containing 25 mL of methanol, volume was made up to the mark with methanol and solution was filtered using 0.45 µm filter (Mill filter, Milford, MA). From filtrate, 5 mL of solution was transferred into 100 mL to obtain the concentration of 100 μg/mL. From second dilution 4 ml was transferred to 10 ml of volumetric flask to obtain the final concentration 40 μg/mL was subjected to propose method and the amount of ADV was determined, Fig 1.

Method validation: Method validation is carried out as per ICH guidelines.

Forced degradation studies: To determine whether the analytical method and assay were stability-indicating, ADV standard drug was stressed under various conditions to conduct forced degradation studies. Degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 0.1N HCl), base hydrolysis (0.1N NaOH), oxidative degradation (10% H2O2), and thermal treatment (heated at 55°C for 2 hrs) to evaluate the ability of the proposed method to separate ADV from its degradation products.

Results and Discussion:
The HPLC separation was achieved on Agilent TC C18 (2) 250 x 4.6 mm, 5 μ column using mobile phase composition of acetonitrile: 0.05 M phosphoric acid (55: 45, v/v). The proposed method was applied for pharmaceutical formulation and % label claim ad ADV recovery was found to be 99.95±0.26%. The low RSD indicated that the method is suitable for routine estimation of ADV in pharmaceutical dosage forms. The reliability and analytical performance of the proposed methods, including linearity, range, precision, accuracy, detection and quantitation limits, were statistically validated. Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ were found to be 0.76 μg and 0.89 μg, respectively. Summary of validation parameter is as shown in Table 1. Fig 2. and Table 2.

Conclusion:
All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of adefovir in bulk and pharmaceutical formulation. The proposed method is also useful for determination of adefovir stability in sample of pharmaceutical dosage forms.

Acknowledgement:
The authors are thankful to PRES’S College of Pharmacy, Chincholi, Nasik for providing necessary facilities.
Table 1 Summary of validation parameter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>20-160 µg/mL</td>
</tr>
<tr>
<td>Regression equation [Y = mX + C]</td>
<td>Y=5361X+18507</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.76 µg</td>
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<tr>
<td>Limit of quantitation</td>
<td>0.89 µg</td>
</tr>
<tr>
<td>% Recovery [ n = 3]</td>
<td>99.87-100.18</td>
</tr>
<tr>
<td>Precision [% RSD]</td>
<td></td>
</tr>
<tr>
<td>Repeatability [n = 6]</td>
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<tr>
<td>Inter-day [n = 3]</td>
<td>0.42-0.88</td>
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<tr>
<td>Intra-day [n = 3]</td>
<td>0.56-1.12</td>
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<tr>
<td>Robustness</td>
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<tr>
<td>Specificity</td>
<td>Specific</td>
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</table>

Table 2 Forced degradation studies

<table>
<thead>
<tr>
<th>Sample exposure condition</th>
<th>Number of degradation products [Rt values]</th>
<th>ADV remained [40µg/ml]</th>
<th>SD</th>
<th>Recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M HCl, 1h,Reflux, 60°C</td>
<td>1 (1.8,3.0)</td>
<td>35.23</td>
<td>4.16</td>
<td>88.07</td>
</tr>
<tr>
<td>0.1 M NaOH, 1h,Reflux, 60°C</td>
<td>2 (1.4,2.6)</td>
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<td>86.32</td>
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<tr>
<td>10%H2O2, 1h,Reflux, 60°C</td>
<td>1(1.9)</td>
<td>38.32</td>
<td>7.62</td>
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<td>Photolight, 2 hr</td>
<td>1(2.3)</td>
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<tr>
<td>Thermal Heat, 2H, 55°C</td>
<td>1(2.5)</td>
<td>38.12</td>
<td>3.26</td>
<td>95.30</td>
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</table>

References:


Synthesis and antimicrobial activity of some
1-(2-(ethylamino)-4-methyl-thiazol-5-yl)-3-phenylprop-2-en-1-one derivatives

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Abstract: A series of 1-(2-(ethylamino)-4-methyl-thiazol-5-yl)-3-phenylprop-2-en-1-one derivatives (Thiazole-based chalcones) was synthesized and evaluated for their anti-bacterial activity against gram positive and gram negative bacteria by cup and plate method. The title compounds were further screened for their anti-fungal activity against Candida albicans and Aspergillus fumigatus. The zone of inhibition was recorded and compared with Cefotaxime for anti-bacterial activity and Ketoconazole for anti-fungal activity respectively. Compounds EP3 and EP2 were found to exhibit highest percentage relative inhibition in anti-bacterial and anti-fungal activities respectively.

Introduction:
Microbial resistance towards the drug creates a very serious problem since last three decades. This demands the urgent need for development of such compounds which are devoid of resistance and effective in low doses too. Thiazoles and chalcones possess broad spectra of biological activities such as antimicrobial, anti-inflammatory, analgesic etc [1]. The presence of reactive α, β-unsaturated keto group in chalcones is found to be responsible for their antimicrobial activity [2]. The present study is therefore aimed to explore the antimicrobial properties exhibited by thiazole based chalcones.

Materials and method:
1-ethylthiourea was dissolved in 50 ml of acetone [4]. 3-chloropentane-2,4-dione, diluted in acetone was added drop wise and mixture was refluxed for 1.5 h [5]. The solid product 1-[(2-ethylamino-4-methyl-thiazol-5-yl)-ethanone] was filtered and recrystallized from ethanol. It was then dissolved in methanol and added dropwise to a cooled solution of corresponding aromatic aldehydes in 10% sodium hydroxide. The reaction mixture was kept under stirring at 0 ºC for 30 min and afterwards at room temperature for several hours (5–12 hr.) until solid started separating out. The solid was filtered under vacuum and recrystallized from dioxane to give the title chalcones (Table 1). The synthesized compounds were characterized using FT-IR, NMR and Mass spectroscopic techniques.

Anti-bacterial and anti-fungal activity assay
S. aureus and E.coli were used as gram-positive and gram-negative strains respectively for determining the anti-bacterial activities of title compounds while C. albicans and A. fumigatus were used as the fungal strains The anti-bacterial and anti-fungal assay was carried out using cup plate method using Sabouraud Dextrose Agar growth medium. After incubation at 37°C for 24 hr., the
diameter of zone of inhibition of each compound was recorded and compared with Cefotaxime for anti-bacterial activity and Ketoconazole for anti-fungal activity respectively.

Table 1. Substituted 1-(2-ethylamino-4-methyl-thiazol-5-yl)-3-phenyl-propenone derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
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<tbody>
<tr>
<td>EP1</td>
<td>3,4-dimethoxy</td>
</tr>
<tr>
<td>EP2</td>
<td>2,5-dimethoxy</td>
</tr>
<tr>
<td>EP3</td>
<td>2,3-dimethoxy</td>
</tr>
<tr>
<td>EP4</td>
<td>4-dimethylamino</td>
</tr>
<tr>
<td>EP5</td>
<td>5-bromo-2-hydroxy</td>
</tr>
<tr>
<td>EP6</td>
<td>3,4,5-trimethoxy</td>
</tr>
<tr>
<td>EP7</td>
<td>4-hydroxy</td>
</tr>
<tr>
<td>EP8</td>
<td>2-fluoro</td>
</tr>
<tr>
<td>EP9</td>
<td>3-ethoxy-4-hydroxy</td>
</tr>
<tr>
<td>EP10</td>
<td>2,4-diCl</td>
</tr>
</tbody>
</table>

Results and discussion:
The synthesis of title chalcones was carried out using Claisen-Schmidt condensation and their identity was confirmed from their IR, $^1$H-NMR and mass spectral analysis data. The synthesized chalcones were assayed for their anti-bacterial activity against gram-positive and gram-negative bacteria with cefotaxime as the standard anti-bacterial drug. The anti-fungal activity of title compounds was determined using ketoconazole as standard. The anti-bacterial and antifungal activities zone of inhibition are presented in Fig. 1. All the synthesized compounds exhibited higher inhibitory activity against gram negative bacteria than gram positive bacteria (with the significance level of $P < 0.05$). All the title compounds were active towards $E$.coli at all the concentrations i.e. 6.25, 12.5, 25, 50, 100 µg/ml. Amongst all these compounds, EP3 showed highest zone of inhibition (Fig. 1) at concentration 12.5 µg/ml compared to standard drug. All the compounds were active towards $S$.aureus at all the concentrations. Amongst all these compounds, EP3 showed highest zone of inhibition at concentration 100 µg/ml compared to standard drug. All the compounds displayed significant inhibitory activity towards $C$. albicans at all the concentrations. Amongst all these compounds, EP2 showed highest inhibitory activity (Fig. 2) at concentration 100 µg/ml as compared to standard drug. The compounds EP1, EP5, EP6, EP 7, EP8, EP9 and EP10 were inactive towards $A$. fumigatus at concentrations 6.25 µg/ml. Except EP4, EP6 and EP10, all the synthesized compounds displayed higher or comparable inhibitory activity at concentration 100 µg/ml as compared to standard drug.
Fig 1. Zone of inhibition (in mm) of synthesized substituted 1-(2-Ethylamino-4-methyl-thiazol-5-yl)-3-phenyl-propenone derivatives against (A) *E.coli* (B) *S.aureus* (C) *C.albicans* (D) *A. fumigatus*

**Conclusion**

All the synthesized compounds exhibit higher inhibitory activity towards gram negative bacteria as compared to gram positive bacteria. In the anti-bacterial assay, EP3 exhibited highest percentage relative inhibition against both gram positive and gram negative bacteria. Compound EP2 was found to have highest percentage relative inhibition against *C.albicans* and *A. fumigatus* in the anti-fungal activity assay.

**Acknowledgments:**

The authors are grateful to the Head, School of Pharmacy, Devi Ahilya Vishwavidyalya and Honorable Vice Chancellor, Devi Ahilya Vishwavidyalya, Indore for providing necessary facilities to carry out the research work. One of the authors (SL) is thankful to AICTE, New Delhi for providing Junior Research Fellowship.

**References:**


Design and docking of 6-Methyl-3-Phenoxy-N-Phenylquinoline-4-Carboxamide derivatives as flap Endonuclease-1 Inhibitors

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Abstract: Flap endonuclease-I (FEN-1) is involved in DNA repair via Base excision repair pathway and considered to be a novel target for the development of anticancer agents. Here, we report the design and docking of 6-methyl-3-phenoxy-N-phenylquinoline-4-carboxamide derivatives as Flap endonuclease Inhibitors. To study the interactions required at active site, FEN-1 enzyme (pdb id: 1UL1) was used. Molecular modeling studies for designed compounds were done using Schrödinger suite (maestro 9.4). These series of compounds showed very good docking score and hence are being taken forward to synthesis.

Introduction:
DNA damage in the cells is known to occur intermittently by various types of mutagens, X-ray irradiation and ultraviolet radiation, spontaneous reactions, and by the influence of endogenous and exogenous agents [1]. DNA repair pathways such as base excision repair (BER) pathway enables the cells to survive the DNA damage. Base excision repair (BER) removes small, non-helix-distorting base lesions such as oxidized bases (e.g. 8-oxoguanine), deaminated bases and damaged alkylated bases (like 3-methyladenine and 7-methylguanine) from the genome during cell cycle. Inhibition of enzymes involved in BER pathway appears to be an effective strategy for anticancer therapeutic development. Various enzymes involved in this pathway are DNA glycosylase, apurinic/ apyrimidinic (AP) endonucleases, DNA polymerases, Flap endonuclease-1 (FEN-1) and DNA ligase [2]. FEN-1 is a new target for anticancer drug development. It removes the 5’ flap generated during long patch BER and also participates in Okazaki fragment maturation, cell proliferation, DNA replication, repair and maintenance of genomic stability [3]. In several cancer cells such as prostate cancer, gastric cancer, neuroblastoma, pancreatic cancer, lung cancer, breast cancer, testicular cancer, uterine cancer and colon cancer over-expression of FEN-1 has been reported [4].

Materials and Method:
Docking study was done using Schrödinger suite (maestro version 9.4). Grid (x,y,z coordinates: 37.31, -62.58, 16.44) of 1UL1 active site was created. Using protein preparation wizard, bond orders were assigned for all atoms. Ionization and metal binding modes were detected using Epik at pH
7.0±2.0. All water molecules were removed. Hydrogens were added and protein was minimized to the extent of 0.3 Å RMSD using Optimized Potentials for Liquid Simulations (OPLS) 2005 force field. All the molecules were drawn using Chemaxon Marvin Sketch and prepared with ligand preparation wizard of Maestro. All possible Ionization states at pH 7.0±2.0 were enumerated using Epik and minimized. Docking of Maestro processed ligand database was performed using Glide XP (Extra Precision)

Results and discussion:

It was observed that active site of protein comprises two metal binding sites M1 and M2. The first metal binding site M1 is surrounded by four amino acid residues Asp34, Asp86, Glu158 and Glu168 while second metal binding site M2 by three residues Asp179, Asp181 and Asp233. Design of molecules interacting with these amino acids of active site was thought to be helpful in inhibiting FEN-1.

Considering the requirement of FEN-1 antagonists, a series of 6-methyl-3-phenoxy-N-substituted-phenylquinoline-4-carboxamide derivatives was considered. 15 derivatives were envisaged. These compounds showed very good docking scores in the range of -6.54 to -9.05 (table 1). The compounds showed hydrogen bond interaction with Ash181, hydrophobic interactions with Asp179, Leu 92, Ile 3, Glu 158 and stacking interaction with Phe 185. Molecular docking study indicated that compounds substituted with electron withdrawing groups have high docking score and interactions with desired amino acids.

Table 1: Docking scores of designed 6-methyl-3-phenoxy-N-substituted-phenylquinoline-4-carboxamide derivatives

<table>
<thead>
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<th>Code</th>
<th>R</th>
<th>Glide Score</th>
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<tbody>
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<td>2a</td>
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<td>-6.54</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>-7.10</td>
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<td>2c</td>
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<td>-6.98</td>
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<tr>
<td>2d</td>
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<table>
<thead>
<tr>
<th>Code</th>
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</thead>
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<td>2j</td>
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<tr>
<td>2l</td>
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</table>
Conclusion:
In this study, we have designed 6-methyl-3-phenoxy-N-substituted-phenylquinoline-4-carboxamide derivatives as inhibitors of flap endonuclease-1. Docking studies indicated that these compounds could be very good inhibitors of FEN-1. These are being synthesized and will be put through in-vitro as well as in-vivo assay in near future.

References:
Design, synthesis and evaluation of 2-Thio-Pyrimidine derivatives as BACE-1 inhibitors

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Abstract: Alzheimer's disease (AD) is a fatal neurodegenerative disorder for which there is no cure. Considering the unmet need to develop agents to treat AD and BACE-1 as the most promising target, 2-thio pyrimidine derivatives were designed using molecular interaction studies, synthesized and evaluated in vitro. The designed compounds revealed desired interactions with BACE-1 active site and therefore were taken further for synthesis. The synthesized compounds were subjected to FRET assay to ascertain in vitro BACE-1 inhibitory potency. Most active compound showed 70.27% inhibition at 10 µM concentration indicating that these compounds possess potential to be developed against AD.

Introduction:
Alzheimer’s disease (AD) is a remarkably, and to date inexplicably, most common neurodegenerative disorder. It usually affects the population of 65 years and older. The population suffering from AD is projected to expand with increase in life expectancy [1]. As per National Institute on Aging, USA, 5.3 million Americans have been reported to suffer from AD in 2015 with 5.1 million being from the age group of 65 and older [2]. Currently available drugs for AD are unable to alter or prevent disease progression and provide symptomatic relief only. As per amyloid cascade hypothesis, accumulation of Aβ42 is main cause of the disease. β-secretase APP Cleaving Enzyme (BACE-1) is universally recognized as the protease which initiates the cleavage of APP at the β site and, as such, catalyses the rate limiting step in the production of Aβ42. Hence, inhibition of BACE-1 is promising strategy to treat AD. The crystal structure of BACE-1 reveals the interaction in active site required for design of new BACE-1 inhibitor [3-4].

Figure 1 Reported 8,8-diphenyl-2,3,4,8-tetrahydroimidazopyrimidin-6-amine showing essential interactions.
Materials and Methods:
Design of 2-thio pyrimidine derivatives: The said derivatives were designed using BACE-1 (pdb id: 2OHP) protein. Interactions of the derivatives with BACE-1 active site were studied using Molegro Virtual Docker (MVD). Toxicity risk assessment and drug score was calculated using OSIRIS property explorer.

Synthesis: The derivatives were synthesized using following scheme.

Reagents and conditions: (a) NaOH, EtOH, rt, 4-6 h (b) NaOH, thiourea, EtOH, 70°C, 2-8 h.

c. In vitro screening: For studying the BACE-1 inhibitory potency of all the synthesized compounds, in vitro enzyme inhibition assay was done at 10μM concentration. For this purpose, BACE-1 inhibition assay kit was purchased from Panvera (Madison, WI, U.S.A). The assay is based on Fluorescence resonance energy transfer (FRET) method.

Results and Discussion:
After ascertaining that the designed compounds have requisite interaction with the BACE-1 active site amino acids, toxicity prediction was done to remove flagged compounds from synthesis. It was observed that most of the compounds were safe as no indication of mutagenicity, tumorogenecity, irritant and reproductive effects was seen. Compound having dimethylamino group showed tumorogenicity as this group has been reported to impart tumorogenicity as well as mutagenicity. The drug scores were moderate to medium.

The compounds were then synthesized and evaluated for in vitro potency. It was observed that meta position on ring A is preferable for the activity where as para substitution decreases the activity. Also, presence of a para substituent on ring B favours the activity. T3 bearing m-benzyloxy on ring A and o,p–dichloro on ring B showed maximum inhibition (70.27%) in the series while T2 with m-nitro on ring A and o,p-dichloro on ring B showed 61.9% inhibition. m,p-di-methoxy on ring A and m-nitro on ring B (T9) also revealed moderate inhibition of 52%. Placing a o,p-di-chloro substitution on ring B was found to be favourable over p-chloro alone. It can be concluded that electron withdrawing or deactivating groups (such as -nitro) or weakly activating group (i.e. benzyloxy) on ring A and electron withdrawing group (i.e. o,p- dichloro) on ring B are essential for BACE-1 inhibition.
Table 1 Structure, toxicity and in-vitro inhibition values for 2-ThioPyrimidine derivatives

<table>
<thead>
<tr>
<th>Code</th>
<th>R₁</th>
<th>R₂</th>
<th>MW</th>
<th>HB</th>
<th>HB</th>
<th>Log P</th>
<th>M</th>
<th>T</th>
<th>I</th>
<th>R</th>
<th>Drug Likelines</th>
<th>Drug score</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>m-NO₂</td>
<td>p-NO₂</td>
<td>339.3</td>
<td>5</td>
<td>1</td>
<td>78.15</td>
<td>2.79</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>-8.30</td>
<td>0.31</td>
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<tr>
<td>T2</td>
<td>m-NO₂</td>
<td>o,p-di-Cl</td>
<td>378.2</td>
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<td>1</td>
<td>68.92</td>
<td>4.07</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
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<tr>
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<td>m-O-Bn</td>
<td>o,p-di-Cl</td>
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<td>1</td>
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<td>-7.25</td>
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<td>2.86</td>
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<td>-9.10</td>
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<tr>
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<td>p-NO₂</td>
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<td>1</td>
<td>72.16</td>
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<td>m,p-di-(OCH₃)</td>
<td>p-Cl</td>
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<td>1</td>
<td>44.24</td>
<td>4.25</td>
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<td>G</td>
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<td>G</td>
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<td>o,p-di-Cl</td>
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<td>1</td>
<td>44.24</td>
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<td>T8</td>
<td>Furan-2-aldehyde</td>
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<td>-6.80</td>
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<tr>
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<td>m-NO₂</td>
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<td>4</td>
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<td>G</td>
<td>G</td>
<td>G</td>
<td>-9.32</td>
<td>0.29</td>
</tr>
</tbody>
</table>

M=Mutagenicity, T=Tumorogenicity, I=Irritant effect, R=Reproductive effect

Conclusion:
In this paper, we report design, synthesis and in vitro BACE-1 inhibitory potency of 2-thio pyrimidine derivatives. The compounds were predicted to be safe and had moderate drug score. Most active compound (T3) showed 70.27% inhibition at 10 µM concentration and hence further modification of the structure to enhance the inhibition potential are required to be done.

Acknowledgement:
We are thankful to University Grants Commission (UGC), New Delhi, India, for the financial support (Ref no.41-1376/2012 SR).

References
SYnthesis of ethyl-2-Chloropyridine-4-carboxylate via regioselective chlorination

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Abstract: Regiospecific chlorination of ethyl isonicotinate-N-oxide to give Ethyl-2-chloropyridine-4-carboxylate was achieved in 90% yield with 99.2% selectivity by treatment with phosphorus oxychloride in the presence of a stoichiometric amount of triethylamine. Other chlorinating agents such as sulfuryl chloride, p-toluene sulfonyl chloride and thionyl chloride, produced 2-chloropyridine derivative also under these conditions, albeit in moderate yield.

Introduction:
The substituted pyridine derivatives including isonicotinic acid are the ligand of choice for new drug development. 2-Chloropyridine derivatives are useful for the synthesis of pharmaceutical compounds and dyes [1]. The two most commonly used Method for the synthesis of 2-chloropyridine are the direct chlorination of pyridine with acetyl hypochlorite[2], which is unsatisfactory in yield, and the chlorination of pyridine-N-oxide using various chlorinating agents [3] such as POCl₃, SO₂Cl₂, phosgene in DMF, trichloroacetyl chloride, benzenesulfonyl chloride or p-toluene sulfonyl chloride. The yields for the reactions using trichloroacetyl chloride and the sulfonyle chlorides were acceptable but the waste disposal problems associated with the organic chlorinating agents and their higher cost over inorganics precluded their use in the preparation of large quantities of 2-chloropyridine derivatives. The reaction between POCl₃ and pyridine N-oxide produces a mixture of 2-chloropyridine and 4-chloropyridine approximately in the ratio of 7:3 in around 70% yield [4]. Recovery of pure 2-chloropyridine is not very effective because of difficult separation. A mechanism involving an addition-elimination sequence was proposed to account for the reaction of pyridine-N-oxide with POCl₃. The oxygen of pyridine-N-oxide is coordinated with phosphorus of POCl₃. This complexation activates the adjacent carbon atom to chloride ion attack. Then elimination of phosphorus moiety from this system leads to overall substitution of hydrogen by chlorine. Considering this reaction mechanism, addition of a stoichiometric amount of base such as triethylamine should promote the elimination of hydrogen at C-2 and phosphorus moiety and result in getting higher yield and selectivity (Scheme).
Scheme

An evaluation of several potential chlorinating agents was undertaken and the results are shown in the Table.

**Table 1: Reaction of Pyridine-N-Oxide with Several Chlorinating Agents**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Base</th>
<th>Temp.(^a) (°C)</th>
<th>Time (hour)</th>
<th>Solvents</th>
<th>Yield(^b) (%)</th>
<th>Selectivity(^c) (%)</th>
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</thead>
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<td>Sulfuryl chloride</td>
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<td>16</td>
<td>CH(_2)Cl(_2)</td>
<td>56</td>
<td>45</td>
</tr>
<tr>
<td>Sulfuryl chloride</td>
<td>Et(_3)N</td>
<td>40</td>
<td>3</td>
<td>CH(_2)Cl(_2)</td>
<td>64</td>
<td>78</td>
</tr>
<tr>
<td>Thionyl chloride</td>
<td>none</td>
<td>80</td>
<td>6</td>
<td>DMF</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>Thionyl chloride</td>
<td>Et(_3)N</td>
<td>80</td>
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<td>DMF</td>
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<td>CH(_2)Cl(_2)</td>
<td>90</td>
<td>99.2</td>
</tr>
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</table>

\(^a\) Temperature; \(^b\) Isolated yields; \(^c\) GC-analyzed selectivity.

**Materials and Method:**

Thin layer chromatography (TLC) was performed on precoated silica gel 60 F\(_{254}\) plates from EM reagents and visualized with 254-nm UV light. \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on a Brucker advance IT 400 NMR spectrophotometer at 400 MHz and 100 MHz, respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and J-values were in Hz. IR spectra were obtained on a Bruker FT/IR-spectrometer. GC analyses were recorded with a Shimadzu-14-D GC system.

**General Procedure for the Reaction of Pyridine-N-Oxide with Chlorinating Agent**

A solution of the chlorinating agent (72mmol) in solvent (32mL) was added drop wise at 10°C to a stirred solution of Ethyl isonicotinate-N-oxide (10 g, 60 mmol) prepared through reported procedure [5] and base (72mmol) in solvents (48–66mL). The reaction mixture was heated for the length of time listed in Table. The mixture was concentrated in vacuo and treated with dichloromethane. The product was analyzed with GC to determine the conversion and selectivity of the reaction.
Preparation of 2-Chloropyridine Reacting Pyridine-N-Oxide and Phosphorus Oxychloride in the Presence of Triethylamine: A solution of phosphorus oxychloride (18.7g, 120mmol) in dichloromethane (50mL) was added dropwise at 10°C to a stirred solution of Ethyl isonicotinate-N-oxide (16.7g, 100mmol) and triethylamine (12.1g, 120mmol) in dichloromethane (76–100mL). The reaction mixture was stirred for 30 minutes at room temperature and then refluxed for 1h. The mixture was poured into water (30mL) and neutralized with 2M NaOH. The organic layer was separated and aqueous layer was extracted with dichloromethane. The combined dichloromethane layer was washed with brine, dried and concentrated at reduced pressure to give colorless liquid (16.68g, 90%). In the GC analysis, the product exhibited 99.2% selectivity to Ethyl-2-chloropyridine-4-carboxylate. B.P. 186–187°C/ 760 torr IR (νmax, KBr) 3053, 1578, 764, 725cm⁻¹; ¹H NMR (CDCl₃) 8.75-8.77(d, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 4.13-4.10 (d, 2H, CH₂), 1.15 (t, 1H, CH₃) 13C NMR (CDCl₃) 166.64, 150.65, 149.70, 146.21, 121.62, 121.04, 61.28, 14.70.

Conclusion:
In summary, selective chlorination of Ethyl isonicotinate-N-oxide to give Ethyl-2-chloropyridine-4-carboxylate can be achieved reacting phosphorus oxychloride with pyridine-N-oxide in the presence of a stoichiometric amount of triethylamine.

References:
Design of novel pharmacophore for Histamine 3 receptor antagonists

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Abstract: Histamine H₃ receptors (H₃R) are involved in regulating of release of various neurotransmitters such as histamine itself, Gamma Amino Butyric Acid (GABA), Acetylcholine (ACh), Norepinephrine (NE), Serotonin (5HT), etc in brain. Being hetero as well as auto- receptor, it has been implicated in sleep-wake disorders, attention-deficient hyperactivity disorder, epilepsy, cognitive impairment and obesity. Therefore, designing of novel antagonists for H₃R could be a new approach towards treatment of these disorders. Here, we discuss generation of novel four point H₃R antagonist pharmacophore based on compounds reported in literature.

Introduction:
Histamine (HA) plays a key role in the functioning of peripheral and central tissue via histamine receptors. Although H₁ receptor (H₁R) and H₂ receptor (H₂R) are widely used for treatment of allergy and ulcer, respectively; H₃ histamine receptors (H₃R) are still being explored for application in various central nervous system (CNS) disorders. Role of H₃R in CNS as presynaptic auto and hetero receptor was recognized in 1983. Activation of H₃R reduces the release and deactivation increases the release of histamine, acetylcholine, norepinephrine, serotonin, and dopamine, thereby controlling all major tasks of brain which are involved in sleep, wakefulness, cognition, transmission and various CNS functions.

Early ligands for H₃R
Early H₃R agonists such as N-Methyl histamine, thioperamide, Imetit, immepip, impentamine, proxyfen were derivatives of histamine with imidazole as nucleus. Antagonist development considered replacement of the imidazole moiety as crucial step. Initial pharmacophore, based on a set of imidazole antagonists, suggested presence of two hydrophobic pockets and four hydrogen binding sites (imidazole ring had two). However, later H₃ antagonist pharmacophore consisting of basic moiety and central core, separated by alkyl spacer was reported (figure 1). The early antagonists suffered from various drawbacks such as non specificity at histamine receptor, low bioavailability and poor brain penetration. Especially imidazole compounds have a tendency to bind with heme of cytochrome P450 enzymes (CYP450). Further, in vitro and in vivo did not correlate well for these compounds. Thus, the initial pharmacophore proposed is not suitable for guiding the new drug development. Moreover, recently reported compounds do not follow the pharmacophore but still show
considerable \( H_3R \) inhibition. There was a need to revisit the earlier pharmacophore, considering these newer \( H_3R \) antagonists.

Materials and Method:
In literature, around 200 compounds have been reported to inhibit \( H_3R \). Structurally diverse compounds were selected for developing the pharmacophore. To generate the pharmacophore model, minimum energy conformation for each structure was generated and common structural features were noted. Finally, ligand based distance mapping of the common features was done. The pharmacophore group distance was calculated after performing molecular mechanics simulations with CHARMM force field parameterization. Energy minimization was performed using CHARMM (ACD 3D viewer).

Results and Discussion:
It was noted that for optimum \( H_3R \) inhibition, following structural features are required:
First basic site to interact with Asp 114
Second basic or hydrophobic moiety that interacts with Glu 206 and stabilizes the compound in active state. The interaction with Glu 206 is important and results in enhanced activity.
The central lipophilic core, usually an aromatic ring to interact with nearby hydrophobic side chains.
A spacer between the first basic site and central core. Optimum length is around 3 to 4 atoms.
A spacer should have a hetero atom, preferably oxygen.
These pharmacophoric requirements for antagonists are shown in figure 2 and the model is given in Figure 3.

**Figure 1** Early pharmacophore proposed

**Figure 2:** Proposed requirements for pharmacophore to inhibit \( H_3R \)

**Figure 3:** Proposed pharmacophore for \( H_3R \) antagonists (BM: first basic moiety, HA: hetero atom, LC:...
Table:1 Few representative compounds with pharmacophoric distances

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<tr>
<th>Comp. name</th>
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<td>6.52</td>
<td>10.41</td>
</tr>
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</table>

Conclusion:
Early pharmacophore reported for H3R ligands considered first basic nitrogen, hetero atom and central core. In the proposed four point pharmacophore model, additional basic or hydrophobic moiety has been added. Various drawbacks such as poor blood–brain barrier (BBB) penetration of early ligands can be eliminated by synthesizing the compounds based on proposed four point pharmacophore.

References:
Novel pharmacophore for design of PAD4 inhibitors

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Abstract: Rheumatoid Arthritis (RA) is an autoimmune disease, characterized by destruction of cartilage and inflammation of synovium. Actual cause for RA is still unknown but citrullination by Peptidyl Arginine Deiminase 4 (PAD4) is considered the major factor because citrullinated proteins act as autoantigens. Since PAD4 acts on arginine residues of the protein, 642 arginine mimetic ligands reported in literature were used for the study. Analyzing binding pattern of the hits in Glide and FRED docking and comparing structural similarity of the hits and PAD4 substrate (BAA), we propose herewith a novel pharmacophore for design and synthesis of PAD4 inhibitors.

Introduction:
Rheumatoid Arthritis (RA) is an autoimmune systemic inflammatory disease, characterized by destruction of cartilage and inflammation of the synovium and substantial disability, if not treated early. As the disease progresses, osteoporosis, damage to joint cartilage, bone displacement and reabsorption occurs [1]. Actual cause for RA is still unknown but it has been hypothesized that environmental factors trigger RA in genetically susceptible individuals. In these, self proteins are recognized by immature dendritic cells as antigen. It has been reported that citrullinated proteins- the catalytic products of Peptidyl Arginine Deiminase 4 (PAD4) can also act as self antigen. Compared to RA synovium, PAD4 expression in Osteoarthritis and normal synovium is far less with undetectable levels of citrullinated proteins. RA patients also show higher levels of Anti Citrullinated Peptide Antibodies [2]. These results indicate that PAD4 is a target for drug development against RA.

Materials and Method:
Protein preparation: Human PAD4 crystal structure 1WDA pdb [2] in complex with benzoyl-L-arginine amide (BAA) was used for docking study.
Virtual database creation: A virtual database of 642 arginine mimetic compounds was drawn from 26 articles reporting inhibitors against Thrombin, Factor Xa, Histamine H2 receptor, RAF kinase and Factor VIIa.
Docking using Glide: Grid-based Ligand Docking with Energetic (Glide) performs flexible docking in reference to ligand [3]. Validation of Glide-SP docking was done using cocrystalised ligand BAA.
After satisfactory validation results, virtual database of ligands were docked to 1WDA using Glide SP (standard precision)

**Docking using FRED:** FRED is a Fast Rigid Exhaustive Docking algorithm developed by Openeye. FRED performs rigid docking of multi conformer file of a molecule [4]. PAD4 pdb structure file prepared by Maestro was selected and active site grid was generated using FRED receptor setup program. Multiconformer database was then docked with receptor grid generated.

**Results and Discussion:**

Virtual database of arginine mimetic compounds were created, standardized and minimized using appropriate force field. PDB structure 1WDA containing bound substrate was mutated from Ala645 to Cys645. Hydrogen were added and their position was optimized based on ligands and water molecules. Ionization pattern of PAD4 at pH around 7 was assigned and visually confirmed in the active site. Conserved water was retained in Glide docking and striped off for FRED docking. As Epik identifies physiological ionization state of molecules using Hammett and Taft based pKa prediction, small number of ionization states was produced compared to pka typer. Number of compounds was increased again when possible stereoisomers were created for undefined chiral centers. The RMSD between docked pose and cocrystallised pose of the substrate BAA form both software was well below 2 Å. Using glide standard precision mode and FRED, docking was performed with virtual database. Various stages of VS are illustrated in Figure 1.

**Figure 1:** Schematic representation of different steps of virtual screening of arginine mimetic compounds against PAD4 (1WDA) using FRED and Glide docking software. Docking results were sorted according to the scores. Top 100 compounds were selected and visually analyzed for their binding modes. Glide docking retrieved hits having binding pattern similar to that of substrate BAA as it appears that glide docking score is highly influenced by number of hydrogen bonding interactions. In FRED Chemgauss3, many top ranked poses were different from the ones produced by Glide because the scoring function takes into account many factors. Since it uses steric
parameters most to rank, poses with less number of hydrogen bonding interaction but good van der waals interaction were ranked at top. After analyzing binding pattern of the hits in Glide and FRED docking and comparing structural similarity of the hits and PAD4 substrate (BAA), pharmacophore and structural variations, given in table 1, are suggested:

**Table 1:** Proposed PAD4 inhibitor structures with possible variations.

<table>
<thead>
<tr>
<th>Pharmacophoric group 1</th>
<th>Pharmacophoric group 2</th>
<th>Pharmacophoric group 3</th>
<th>Non pharmacophoric group 4</th>
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</thead>
<tbody>
<tr>
<td>NH₂-</td>
<td>- CO- NH₂-</td>
<td>-NH₂-CO-</td>
<td>-alkyl</td>
</tr>
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<td>-NH₂-SO₂-</td>
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</table>

**Conclusion:**

In this paper, we report design of a novel pharmacophore for PAD4 inhibitors using structure Based Drug Design approach. By docking reported arginine mimetic inhibitors against PAD4, hits were identified by analyzing binding patterns and docking scores. The structural requirements for PAD4 inhibition were derived by using these hits. The suggested variations are being synthesized in our laboratory and will be tested in vitro for their inhibitory potential.

**Acknowledgement:**

This work is financially supported by the Department of Science and Technology, India (Grant No. SR/FT/CS-100/2009).

**References:**

Enhancement of solubility of poorly water soluble Abacavir Sulphate by Hydrotropic technique

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E mail address: jainruchi02@gmail.com

Abstract: A simple, accurate, novel, safe and precise spectrophotometric method has been developed for the estimation of poorly water-soluble abacavir sulphate in tablet dosage form using 8M urea solution (50%V/V) as hydrotropic solution. AS show maximum absorbances at 284 nm and urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drug were seen. AS follows the Beer’s law in the concentration range of 5-25 μg/ml (r2=0.996). Spectrometric method employs calibration curve method using 284 nm as analytical wavelength for estimation of AS. The mean Percent label a claim of tablets of AS is estimated by the proposed method was found to be 97.52±1.65%. The values of mean percent recoveries were also found ranging from 97.68 to 98.53%. All these values were very close to 100, indicating the accuracy of the proposed analytical method. The recovery of added standards (80%, 100% and 120%) was found at five replicate and five concentrations level. The developed Method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values therefore the method can be used for routine monitoring of AS in industry in the assay of bulk drug and tablets.

Introduction:
Abacavir sulphate (AS), chemically, (1S, cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt), it is a synthetic carbocyclic nucleoside analogue having inhibitory activity against HIV-1.

Objective
There are various techniques have been employed to enhance the aqueous solubility. sodium citrate, urea, Sodium salicylate, sodium benzoate, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility. Jain et al has analyzed various poorly water-soluble drugs using hydrotropic technique. Organic solvents have drawbacks withe their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of that organic solvent.

Literature survey reveals that, few Spectrophotometric and chromatographic Method has been reported for the estimation of AS. Preliminary solubility studies reveals that there were more than 46 fold enhancements in the solubility of as in hydrotropic solution. Therefore, it was thought
worthwhile to employ this hydrotropic solution to estimate the drug from tablets to carry out spectrophotometric estimation.

**Method:**
The proposed work was carried out on a UV-1700 series of shimadzu UV-visible spectrophotometer which possesses a double beam double detector configuration with a 1 cm quartz matched cell. Reference standard of AS was a generous gift from GSK, Mumbai, Commercial market and tablets i.e Abamune (CiplaPharmaceutical Ltd.) was procured from the local drug market. Label claim of AS in tablet is 300 mg.

**Preliminary Solubility Studies:** Solubility of AS was determined in distilled water and mixed hydrotropic solution, of 8 M urea and water (50:50% v/v) at 25±1°C. There was more than 46 fold solubility enhanced in mixed hydrotropic solution, as compare with distilled water. This enhancement of solubility is due to the hydrotropic solubilization phenomenon.

**Preparation of calibration curve:** Accurately weighed 10 mg of the AS drug sample were transferred in to 10 ml volumetric flask and it solublized by 10 ml of mixed hydrotropic solution, containing 8 M urea and water (50:50% v/v). The standard solution (1000 µg/ml) was further diluted with distilled water to obtain 5, 10, 15, 20 and 25 µg/ml. Detection wavelength was selected for AS was 284 nm. Absorbances were noted against distilled water as blank. Calibration curve was plotted between concentration and absorbance.

**Analysis of Tablet Formulation:** Twenty tablets of AS were weighted and ground to a fine powder. An accurately weighted powder sample equivalent to 10 mg of AS was transferred to 10 ml of volumetric flask containing 10 ml of ml of mixed hydrotropic solution, containing 8 M urea and water (50:50% v/v). The flask was warm and sonicated for about 15 min to solublize the drug. The solution was filtered through Whatmann filter paper No. 41. The filtrate was diluted appropriately with distilled water and was analyzed on UV spectrophotometer against distilled water as blank. Drug content of tablet formulation were calculated using calibration curve.

**Validation studies:** The accuracy, precision and other validation parameter were determined as per the ICH guidelines. Finally developed and validated Method were used for estimation of drugs in tablet dosage form.

**Results and Discussion:**
Abacavir sulphate estimated by UV spectrophotometer in that, 8 M urea solution (50:50% v/v) was selected as hydrotropic agent. AS was scanned in spectrum mode and 284 nm was selected as wavelength for estimation. Linearity was found in the range of 5-25 µg/ml (r²=0.996). The values of mean percent recoveries were also found ranging from 97.68 to 98.53%. All these values were very close to 100, indicating the accuracy of the proposed analytical method. The values of standard
deviation, percent coefficient of variation (0.055) and standard error (0.099) were also satisfactorily low. The mean Percent label a claim of tablets of AS is estimated by the proposed method was found to be 97.52±1.65%. Result of precision at different level were found be within acceptable limits (RSD < 2). Presence of hydrotropic agent and other tablet excipients do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

**Conclusion:**
The validated spectrophotometric Method employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine analysis of AS in bulk drug and tablet dosage form instead of processing of extraction using organic solvent separately.

**References:**
Development and validation of UV spectrophotometric area under curve (auc) method for DAPOXETINE HCL and Sildenafil Citrate in pharmaceutical formulation.

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Abstract: Simple, precise and economical UV spectrophotometric methods have been developed for the estimation of Dapoxetine HCl and Sildenafil Citrate in pharmaceutical dosage form. Area under curve was integrated in the wavelength range of 285-305 nm and 275.80-303.20 nm. Calibration curves were plotted. Beer’s law obeyed in the concentration range 10-60μg/ml and with correlation coefficient of 0.9981 for Dapoxetine HCl and for Sildenafil Citrate concentration range 5-30 μg/ml with correlation coefficient 0.9986. Accuracy and precision studies was carried out and results were satisfactory. The proposed methods validated as per ICH analytical method development guidelines. The results of the analysis were validated statistically.

Introduction:
Dapoxetine hydrochloride, a fast-acting serotonin reuptake transporter inhibitor, is generally prescribed for the treatment and management of premature ejaculation and erectile dysfunction in adult male\(^1\). Chemically Dapoxetine ((+)-(S)-N, N-dimethyl-(α)-[2(1- naphthalenoxo) ethyl]-benzenemethanamine hydrochloride), Dapoxetine hydrochloride is a water-soluble powder with a molecular weight of 341.88 and has a pKa of 8.63. Sildenafil Chemically, designated as 1-[[3-(6, 7-dihydro-1-methyl-7-oxo- 3-propyl-1H pyrazolo [4, 3-d] pyrimidin-5-yl) - 4ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate.\(^2\) Sildenafil citrate selectively inhibits the enzyme PDE-5A (phosphodiesterase-5A) that hydrolyzes cGMP. The literature study reveals that several spectrometric and HPLC methods available for Dapoxetine AND Sildenafil Citrate in water as well as in combined tablet formulation \(^3\).

Materials and Methods: Selection of Wavelength Range

![Wavelength range selected for Dapoxetine HCl](image1)

![Wavelength range selected for Sildenafil Citrate](image2)

Figure 1: A. Wavelength range selected for Dapoxetine HCl B. Wavelength range selected for Sildenafil Citrate

Preparation of Calibration Curve:
**Assay of Tablet Formulation:** The tablet powder equivalent to 10 mg were accurately weighed and diluted up to mark with water. The solution was filtered and sonicated for 15 min, further diluted subjected for UV analysis.

**Linearity:** The linearity of the Dapoxetine HCl and Sildenafil Citrate were found to be between 10-60 μg/mL and 5-30μg/mL concentrations. The calibration graphs were obtained and treated by linear regression analysis.

**Precision:** Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 6 times in a day and the average % RSD was determined. Similarly the method was repeated on 6 different days for inter-day precision and average % RSD was determined.

**Accuracy (Recovery study):** The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs.

**Results and Discussion:**

Simple, accurate, linear, precise and fast Area under Curve spectrophotometric method was developed for the routine determination of Dapoxetine HCland Sildenafil Citrate. The generated regression equations were Dapoxetine HCl : 305ʃ285 Ad = y =0.1473x+0.0098 (R2= 0.9981) Where, 305ʃ285 Is Area under Curve (AUC) between 285 to 305 nm, R2 is correlation coefficient. Sildenafil Citrate: 309.20ʃ275.80 Ad = y = 0.1931x + 0.0151(R2= 0.9986)

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*n=6

**Table 2 Results of precision study.**
Table 3 Results of recovery study.

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RSD: Relative standard deviation, *Average of 3 observations

Table 4 Results of validation parameters

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</tr>
<tr>
<td>Correlation coefficient*</td>
<td>0.9981</td>
<td>0.9986</td>
</tr>
<tr>
<td>Inter-day*(Precision) (%RSD)</td>
<td>0.15688</td>
<td>0.147255</td>
</tr>
<tr>
<td>Intra-day*(Precision) (%RSD)</td>
<td>0.07671</td>
<td>0.541442</td>
</tr>
</tbody>
</table>

Conclusion:

The authors are thankful to Sava Healthcare ltd. Chinchwad, Pune Maharashtra, India for providing us gift sample and managements of Jspm’s Jayawantrao Sawant College of Pharmacy and Research, Hadapsar, Savitribai Phule Pune University for providing needed facilities for this work.

References:

Bioanalytical method development and validation for the estimation of Levocetirizine in blood plasma by RP-HPLC

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Abstract: An accurate, precise and reproducible liquid chromatographic assay method was developed and validated for the determination of Levocetirizine in tablet dosage form. The RP-HPLC method was developed for estimation of LEVO in tablet dosage form by isocratically using Acetonitrile: Methanol: Ammonium Acetate Buffer pH 5 (25:55:20 % v/v/v) as mobile phase at a flow rate of 1.0 ml/min, Prontosil C-18 column (4.6 x 250mm, 5μ particle size) column as stationary phase and chromatogram was recorded at 232nm. Linearity was evaluated over the concentration range of 2-10 μg/ml in RP-HPLC method (the value of r2= 0.9999). The developed methods was validated according to ICH guidelines were found to be in good accordance with the prescribed values therefore the RP-HPLC methods can be used for routine monitoring of LEVO in industry in the assay of bulk drug and tablets.

Introduction:
Levocetirizine (as levocetirizine dihydrochloride) (LEVO) chemically 2-(2-{4-[(R)-(4-chlorophenyl) (phenyl) methyl] piperazin-1-yl} ethoxy) acetic acid [1] (Fig. 1), is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine [2]. However, various analytical method has been developed [4,5] but best of our knowledge, there is no reported RP-HPLC method available for estimation of levocitrizine in blood plasma. The aim of the present work was to develop easy, economic, accurate, specific and precise RP-HPLC method for estimation of LEVO drugs and tablet dosage form as well as blood plasma [3].

Materials and Methods:
Instrument: Liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector (YL 9160 detector) connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used.

Reagents and chemicals: Pure sample of LEV was obtained as gift sample from Repress Pharmaceutical pvt. Ltd. Ranipur,(India).
Acetonitrile (HPLC Grade), Methanol (AR Grade), Ammonium acetate (AR Grade) obtained from Merck Chemical Division, Mumbai. Milli-Q was used to prepare water used in RP-HPLC method.

**Selection of mobile phase:** Initially to estimate Levocetirizine number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol: 20 mM Ammonium acetate buffer (pH 5.0) in the ratio of 25:55:20 v/v/v. Flow rate employed for analysis was 1.0 ml/min.

**Linearity range and calibration graph**

**Extraction of drug sample:** Accurately weighed 10 mg of LEV were transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of plasma, then volume was made up to 50 ml with methanol and vortex it to get complete precipitation of plasma protein. Stand it aside for few minute, precipitate of protein settled down then collect the supernatant layer and add 10 ml of Acetonitrile in precipitate to complete removal of drug. Centrifuge the collected supernatant layer at 6000 rpm for 7 min. at 4°C and then filtered by whatmann filter paper (no.41). Concentration of LEV in methanol was 200 µg/ml. (stock-A)

**Preparation of Working Standard Solution for calibration curve:** In HPLC method, the standard solutions were prepared by dilution of aliquots of the standard stock solution (1000 µg/ml) with diluents to reach the linearity range of 2-10µg/ml for LEV. The chromatogram was recorded at 232 nm. The! peak areas were plotted against the corresponding concentrations to obtain the calibration graph.

**Analysis of marketed formulation:** Twenty marketed tablets of Italy- 5 (Healthkind Labs Pvt. Ltd., Chandigarh) were weighed and ground to a fine powder; amount equal to 50 mg of LEV was taken in 50-ml volumetric flask. This was than dissolve in 13 ml of plasma then this was than dissolve in 25 ml of diluents by sonication for about 10 minutes. The volume is made up to the mark by diluents as per RP-HPLC method and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

**Validation Parameters:** The optimized spectrophotometric and chromatographic methods were completely validated according to the procedure described in ICH guidelines Q2 (R1) for validation of analytical methods (Linearity, Accuracy, Precision and Robustness).

**LOD and LOQ:** Detection limit and Quantitation limit of described method were observed as 0.0057 µg/ml and 0.174 µg/ml, based on the SD of response and slope, which meet the requirement of new method.

**Results and Discussions:**
Several attempts were performed in order to get satisfactory resolution of LEV in different mobile phases with various ratios mixture of organic phases and buffers by using C\textsubscript{18} column. Initially the mobile phase used was mixture of water and methanol followed by water and acetonitrile in different ratios. The mobile phase used was acetonitrile-ammonium acetate buffer (pH 5.5) in the ratio (60:40, v/v) by isocratic elution could not give satisfactory resolution. Further Acetonitrile: Methanol: Ammonium Acetate Buffer pH-5 (25:55:20 % v/v/v) mobile phase was used by isocratic elution shown satisfactory and good resolution at a flow rate of 1.0 mL/min. The resolution was found reproducible and satisfactory. LEV follow the linearity in the concentration range of 2-10 µg/ml (r\textsuperscript{2} = 0.9998) and chromatogram has recorded at 232nm. The recovery of added standards (80%, 100% and 120%) was in ranging from 97.79 to 99.88%. The mean percent label claims of tablet dosage by RP-HPLC method were found to be 98.80±0.1238 for LEV. The standard deviation, coefficient of variance and standard error were obtained for LEV was satisfactorily low. Result of precision at different level were found be within acceptable limits (RSD<2).

**Conclusion:**

In conclusion, a simple reproducible RP- HPLC method was developed and validated for the determination of LEV in solid dosage form. The advantage of HPLC method is less time consuming and economical. The results indicate HPLC method is adequate methods to quantify LEV in pure form and its dosage form. There was no interference by excipients in the tablets and the mobile phase is easy to prepare. Since these methods are simple, specific, rapid, precise and accurate, they may be successfully and conveniently adopted for routine quality control analysis of LEV in bulk and pharmaceutical dosage form.

**References:**


**In-silico docking studies of Benzimidazole analogues as BACE-1 inhibitor**

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Principal K.M.Kundnani College of Pharmacy, Cuffe Parade, Mumbai-5, India.  
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**Abstract:** BACE1 is believed to be key target enzyme in pathogenesis of Alzheimer’s disease. It catalyzes the rate limiting step of Aβ peptide production leading to formation of amyloid plaques. Modulation of brain Aβ levels can be achieved by inhibiting BACE1. Molecular docking can be a valuable tool for predicting potential small non-peptide inhibitors and to study their binding modes. *In silico* docking studies were carried out to understand interactions of benzimidazole analogues with BACE1. Docking studies reveal that these analogues exhibit interaction with active site amino acid residues of the enzyme suggesting that some of them may be promising candidates.

**Introduction:**  
The amyloid hypothesis asserts that accumulation of Aβ peptides, catalyzed by BACE1, in brain initiates sequence of events which ultimately lead to Alzheimer’s disease (AD) and dementia [1]. BACE1 (β-site APP cleaving enzyme) is recognized as primary drug target for drug development in AD [2]. *In silico* docking can be a valuable tool for predicting potential inhibitors & their binding interactions with BACE1 [3]. Literature survey indicates that active site of BACE1 has two catalytic residues (Asp32 and Asp228) involved in enzymatic catalysis [4].

**Materials and Method:**

**Materialss:**
GOLD ver 5.2.2 (CCDC, UK): Docking studies.

**Method:**

**Ligand preparation:** Benzimidazole analogues were sketched and cleaned using Sybyl ver 8.1.1. Energy minimization was performed using both steepest descent & conjugate gradient Method with TRIPOS force field to attain energy gradient of 0.0001kcal/mol/Å.

**Protein preparation:** The X-ray crystallographic structure of inhibitor/BACE1 complex was retrieved from Protein Data Bank (PDB code: 1m4h) [5]. One monomeric unit of 1m4h was used to define the active site comprising of catalytically important Asp32-Asp228 dyad. The inhibitor OM00-3 was extracted from the receptor along with unwanted waters. Hydrogens to all atoms were added. The active site was defined as a sphere with a radius of 6 Å from the bound ligand and the net charge
of -1 was set with protonated Asp32 and deprotonated Asp228. The side chains were minimized using AMBER7FF99 force field to a gradient of 0.01 kcal/mol/Å.

**Docking:** Docking was carried out for 20 genetic algorithms (GA) runs with 1,00,000 operations. The docking protocol was validated by reproduction of the binding pose of OM00-3 in 1m4h. The same protocol was followed for the benzimidazole analogues to determine binding interactions & evaluated using Gold Score.

**Results and Discussion:**
The binding pose of OM00-3 inhibitor in the active site on validation of docking protocol showed rmsd of 1.5 kcal/mol. With the same protocol, interactions were studied between ligands and the amino acid residues of BACE1. Benzimidazole analogues showed hydrogen bond interactions with amino acid residues within BACE1 active site such as Gly34, Thr72, Gly230, Arg235, Asp32, Asp228, Thr231 and Gln73. The general structure has been depicted in Figure 1.

**Figure 1:** Benzimidazole scaffold

\[ \begin{align*}
R^1, R^2, R^3, R^4, R^5: &\text{ -NO}_2, \text{ OH, OCH}_3, \text{ NH}_2
\end{align*} \]

Amongst the analogues, benz11 exhibited maximum interactions with amino acid residues within BACE1 active site. This ligand interacted with the catalytic dyad which, as reported in literature plays a crucial role in interactions of inhibitor with receptor.

**Conclusion:**
The *in silico* docking results indicate that benzimidazole analogues exhibit interactions with amino acid residues of BACE1 active site. It may be helpful to determine their associated binding modes. Four analogues additionally depicted interactions with the catalytic dyad (Asp32 and Asp228) of BACE1. Amongst them, benz11 exhibited maximum interactions within BACE1 active site. These analogues might be promising candidates for Alzheimer’s disease and can be considered for further studies.

**References:**


QbD approach to analytical RP-HPLC method development and its validation of Tadalafil HCl

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Abstract: A QbD based simple, selective, precise, and stability-indicating HPLC method has been established and validated for tadalafil hydrochloride. The method optimization was accomplished using Design Expert. Separation was achieved on a C18 BDS column using ammonium acetate (pH=4.0): acetonitrile: methanol (40:40:20 v/v) as mobile phase in isocratic elution. Statistical analysis proved the method is repeatable, selective, and accurate for estimation of tadalafil hydrochloride. In force degradation tests the drugs were susceptible to acid, basic hydrolysis and oxidation. Because the method could effectively separate the drug from their degradation products, it can be used as stability-indicating method.

Introduction:
The chemical designation of tadalafil hydrochloride is pyrazino-[1’,2’:1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-(6R,12aR). It is used for the treatment of erectile dysfunction, and is a potent, reversible, competitive inhibitor of phosphodiesterase 5. Inhibition of PDE5 in the corpus cavernosum of the penis increases intracellular cGMP levels, thereby facilitating relaxation of smooth muscle leading to penile erection.
The QbD based method development helped in generating a design space and operation space with knowledge of all method performance characteristics and limitation and successful method robustness within the operating space. The main intention of the work is to build up a stability indicating HPLC for determination of tadalafil hydrochloride.

Materials and Methods:
Tadalafil hydrochloride was supplied by Ipca Laboratories, India as a gift sample.
List of Chemicals: All chemicals used were HPLC Grade by MERCK
List of Instruments: HPLC Jasco PU-2089 plus Quaternary Gradient HPLC pump with ChromNAV integrator, pH meter Lab India PHAN, Balance (Mettler Toledo)

Results and Discussion:
QbD approach was used and several factors were screened by Box- Behnken Design to obtain good resolution and better separation of degradation products. Combinations obtained from software were
performed and results were observed. Lastly, the C18 BDS Column and mobile phase consisting of 0.01M ammonium acetate in water adjust pH 3.10 ± 0.05 with glacial acetic acid, acetonitrile and methanol (40:40:20) v/v was selected which gives a sharp and well defined peak shape. It was found that the solvent system has very good resolution for separation of drug and degradation products.

Analytical method validation:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (r)^2</td>
<td>0.998</td>
</tr>
<tr>
<td>LOD</td>
<td>200 ng/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>600 ng/ml</td>
</tr>
<tr>
<td>Accuracy</td>
<td>% RSD for Assay is within 2%</td>
</tr>
<tr>
<td>Precision</td>
<td>% RSD for Assay is within 2%</td>
</tr>
<tr>
<td>Specificity</td>
<td>Method is specific</td>
</tr>
<tr>
<td>System suitability</td>
<td>% RSD is NMT 2, HETP are more than 2000,</td>
</tr>
<tr>
<td></td>
<td>Tailing factor lies between 0.8-2.0</td>
</tr>
</tbody>
</table>

Stability indicating assay method

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis</td>
<td>10.6 %</td>
</tr>
<tr>
<td>Base Hydrolysis</td>
<td>4.83 %</td>
</tr>
<tr>
<td>Oxidation</td>
<td>28.69 %</td>
</tr>
<tr>
<td>Thermal</td>
<td>2.0 %</td>
</tr>
<tr>
<td>Heat</td>
<td>1.8 %</td>
</tr>
</tbody>
</table>

Optimized chromatographic conditions were validated for standard solution. The linearity range was found to be with regression value (r)^2 = 0.998. The Limit of Detection (LOD) was 200 ng/ml and Limit of Quantification (LOQ) was 600 ng per spot. The precision of the method for the standard solution shows Relative Standard Deviation (RSD) for intraday and for interday were within limits.

Conclusion:
The developed HPLC method is simple, precise, sensitive, fast and stability indicating. Stress conditions indicate that the drug is susceptible to acid, base hydrolysis and oxidation. All the degradation products were fully resolved, this indicate specificity of the method. Thus the method can be employed for monitoring the stability of tadalafil hydrochloride in bulk drug.

Acknowledgement:
The author is thankful to Ipca Laboratories and other those who helped in successful completion of work.

References:

Bioanalytical method development and validation of Everolimus

Shebaz Shoukat Shaikh, Pradeep N. Agrawal, Sagar C. Chaudhari
Department of Pharmaceutical Analysis, K M Kundnani College of Pharmacy, Mumbai
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Abstract: A simple liquid chromatography with tandem mass spectrometry (LC/MS MS) method has been develop and validated in human whole blood. The method employed Protein Precipitation followed by Solid Phase Extraction. Samples containing Everolimus were chromatographed on Chromolith Performance RP-18e (100x4.6mm) at a temperature of 35°C. The isocratic mobile phase composition was a mixture of Methanol: Buffer solution (95:5), which was pumped at flow rate of 1.200 ml / min. The developed LC/MS-MS method was found to be selective, sensitive, accurate and linear for the analysis of Everolimus in human whole blood.

Introduction:
Everolimus is an immunosuppressant. Chemically it is Dihydroxy-12-[(2R)-1-[(1S,3R,4R)-4(2hydroxyet hoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxo-4-azatricyclo[30.3.1.0]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone. It is currently used to prevent rejection of organ transplants and treatment of renal cell cancer and other tumours. Several methods for the determination of Everolimus in biological matrices have been reported including LC-MS/MS method with Electrospray ionization. The reported mass spectrometry methods suffered from several disadvantages, such as time-consuming sample preparation, large plasma volume, and long retention time [1, 2]. The aim of present work is to develop and validate a chromatographic method for Quantitation of Everolimus using LC-MS/MS from human whole blood.

Materials and Methods:
Particulars of reference standards: Everolimus (Analyte), Internal Standard (Everolimus-d4), Biological matrix (Whole blood from human volunteers), Anticoagulant (K2 –EDTA), Mobile phase (Buffer (10 mM ammonium formate): methanol (5:95v/v))

Reagents and solvents: Ammonium Formate (HPLC Grade) and Ammonium acetate (HPLC Grade) was purchased from Fluka. Methanol (HPLC Grade) and Acetonitrile (HPLC Grade) was purchased from JT Baker. Water (HPLC Grade) was purchased from Milli-Q/Merck.

Instruments and Equipments

<table>
<thead>
<tr>
<th>Name</th>
<th>Specification (Model / Brand)</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS/MS</td>
<td>API 5500</td>
<td>MDS SCIEX</td>
</tr>
<tr>
<td>Pump</td>
<td>LC-20AD Prominence</td>
<td>Shimadzu</td>
</tr>
</tbody>
</table>
Sample Preparation: The spiked plasma was retrieved from the deep freezer and thawed in a water bath at room temperature. The thawed samples were vortexed to ensure complete mixing of the contents. Protein Precipitation was carried out by pipetting out 0.300mL of spiked blood sample to this add 25μL of internal standard stock solution except in plasma blank sample. This mixture is precipitating out by using 1.200mL of 0.2M Zinc Sulphate: Methanol (15.85 V/V) in each tube and vortex for 30 seconds. In SPE Phenomenex strata-X HLB SPE cartridge was used. SPE cartridge of 1cc was conditioned on a Speed-disk pressure processor using 1 ml Methanol followed by 1ml Milli-Q water at a constant positive pressure. The prepared plasma samples of calibration curve samples and quality control samples were loaded and then washed with 1ml of Milli-Q water. Then sample were eluted out by using mobile phase i.e. methanol: buffer (95:5 %v/v). The samples were analysed on LC/MS/MS.

Results and Discussion

Optimization Parameters:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>LC-MS/MS API 5500 coupled with APCI ion source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Chromolith Performance RP-18e (100x 4.6mm)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Methanol : Buffer Solution (95:5)</td>
</tr>
<tr>
<td>Rinising Solution</td>
<td>Methanol: Milli-Q water(80:20)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.200mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>Positive ion mode</td>
</tr>
<tr>
<td>Column Oven Temperature</td>
<td>350 C</td>
</tr>
<tr>
<td>Sample Cooler Temperature</td>
<td>40 C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>15.00μL</td>
</tr>
<tr>
<td>Retention Time</td>
<td>Everolimus-3.35 minutes, Everolimus D4 – 3.34 minute</td>
</tr>
</tbody>
</table>

State File Information (API 5500 in Positive Ion Mode)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebuliser Gas</td>
<td>10</td>
</tr>
<tr>
<td>Curtain Gas</td>
<td>20</td>
</tr>
<tr>
<td>Collisionally Activated Dissociation (CAD)</td>
<td>7</td>
</tr>
<tr>
<td>Ion Spray Voltage</td>
<td>4500 Volts</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>400°C</td>
</tr>
</tbody>
</table>

The LCMS/MS method development for quantification of drug Everolimus from human blood has been validated for Limit of Detection (LOD), Limit of Quantification (LLOQ), Linearity & Range, Accuracy, Precision and Specificity. The LLOQ of the method developed for quantification of drug
from calibrator was found to be 503.5 ng/ml and was found to be linear over the range of 0.201 ng/ml to 20.079 ng/ml with regression value of 0.9983. The interday precision and accuracy at LLOQ was found to be 6.14% and 93.39% respectively. Intraday precision and accuracy was found to be 3.70% and 96.43% respectively.

**Conclusion:**
The developed LC-MS/MS method is simple, precise, sensitive, and fast. Thus the method can be employed for Quantitation of Everolimus using LC-MS/MS from human whole blood HPLC.

**Acknowledgement:**
The author is thankful to guide and all those who helped in successful completion of work.

**References:**
PB-20

Docking studies of anti-cancer agents on estrogen receptor from *Oroxylum indicum*

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Abstract: Cancer is a disease caused by an uncontrolled division of abnormal cells in a part of the body. The cells responsible for cancer are known as malignant cells. In this study, the main receptor which was targeted was the Estrogenic Receptor (3-ERT) which has a major role to play in the pathophysiology of Breast Cancer. The docking studies were performed for the various phytochemical constituents which have been obtained from *Oroxylum indicum*. They were compared with some standard anticancer compounds and a comparative study done.

Introduction:
Docking studies were carried out to determine the docking scores of the various anticancer components. The computational studies were carried out in Maestro 8.5, Lead IT and Autodock 4.2. Docking was carried and the docking scores were compared using the above mentioned softwares. The various anticancer agents which were obtained from the leaves of *Oroxylum indicum* were then compared for their affinity towards binding to the Estrogenic Receptor (3 -ERT) using the docking scores.

Materials and Methods:
The computational studies were carried out in Maestro 8.5, Lead IT and Autodock 4.2. All computational studies were carried out using in a machine running on a 2.8 GHz Intel Core 2 Duo processor with 1GB RAM and 160 GB Hard Disk with Linux / Windows XP as the Operating System.

Maestro
**Protein Preparation:** The preprocessing was done. Bond orders were assigned, addition of hydrogen and deletion of waters was performed. Then the heterostates of the ligands, if present, were generated. H-bonding was optimized. OPLS2001 was used for energy minimization.

**Ligand Preparation:** The ligand database includes various phytoconstituents of *Oroxylum indicum*. The structures were drawn in Maestro using the “Build tool” bar, and were saved in .mae format. By using the Ligprep utility of Glide, these structures were geometrically optimized.

**Molecular Docking:** Glide was used for docking phytochemicals into the active site of the estrogen receptor 3ERT. The molecules were docked using standard precision. Residues interaction scores...
were taken within 12 Å range. During the docking process, initially complete systematic search of the conformational, orientational and positional space of the docked ligand was done followed by eliminating unwanted conformations using scoring, finally energy optimization.

**Lead It**

**Protein preparation:** 3ERT Protein was downloaded from www.rcsb.in and the active site for interaction was selected. Particular chains having the receptor were selected as receptor components. The reference ligand was then selected, if present, else active site was specified by selecting specific amino acids. All the chemical ambiguities, which were crystallographically unresolved, were resolved and receptor was confirmed.

**Ligand Preparation:** In case of Lead IT, the ligands prepared in Maestro 8.5 were used for docking. They were saved in .sdf format and used for docking studies.

**Molecular Docking:** Docking was performed using default parameters using hybrid approach, followed by visualization using Pose View.

**Autodock**

In Autodock, protein prepared in Maestro saved in .pdb format was converted to Autodock compatible atom type using OpenBabel. Ligands were prepared in Maestro and saved in .pdb format. These were converted into Autodock compatible atom type using OpenBabel. Grid parameter file (.gpf) and drug parameter file (.dpf) were generated using MGL Tools- 1.4.6. Receptor grids were generated by picking up an atom in active site using 60 x 60 x 60 points in X, Y and Z directions with a grid spacing of 0.375 Å. Later docking was done using Autodock 4.2 with the following parameters. Runs: 50, population size: 150, number of evaluations: 2500000 and generations: 27000.

**Results and Discussion:**

Docking studies were carried for various phytoconstituents from *Oroxylum indicum*. The docking was performed on Maestro, LeadIT and Autodock 4.2 software.

**Table 1** Docking scores of standard compounds on 3ERT using different software.

<table>
<thead>
<tr>
<th>Standard compounds</th>
<th>Maestro</th>
<th>Lead IT</th>
<th>Autodock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Docking Scores</td>
<td>Free binding energy</td>
<td>Estimated inhibition constant</td>
</tr>
<tr>
<td>4-hydroxy tamoxifen</td>
<td>-11.28</td>
<td>-34.94</td>
<td>-4.18</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>-6.10</td>
<td>-17.67</td>
<td>-7.38</td>
</tr>
<tr>
<td>Ursolic Acid</td>
<td>-2.46</td>
<td>ND</td>
<td>-4.04</td>
</tr>
<tr>
<td>Kaemferol</td>
<td>-7.64</td>
<td>-19.94</td>
<td>-6.48</td>
</tr>
</tbody>
</table>
Conclusion:
The Protein-Ligand Interaction is the most important part of the Docking Studies. A successful interaction is depicted with a high docking score. In the above study, the Estrogenic Receptor(3 ERT) was taken and a comparison was made between the docking scores of standard compounds like 4-hydroxy Tamoxifen and Doxorubicin and the compounds obtained from the plant species *Oroxylum indicum*. A fairly good docking score was obtained with Kaemferol, Quercetin, β-sitosterol and Ellagic acid. Thus we can conclude that the components which were obtained from the leaves of *Oroxylum indicum* was found to possess good Anti-Cancer property by exhibiting a fairly good affinity for the Estrogenic 3-ERT receptor.

References:
Development and validation of HPTLC method for quantitative estimation of Asenapine Maleate in pharmaceutical formulations, equilibrium solubility and ex-vivo diffusion studies

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2 Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar - 388 121, Anand, India
E-mail address: rbp.arcp@gmail.com

Abstract: New high performance thin layer chromatography (HPTLC) has been developed and validated for quantitation of Asenapine maleate (ASP) in both marketed tablets and in-house developed formulations as well as for equilibrium solubility study and ex vivo diffusion study. The Asenapine maleate was separated well from degradation products using HPTLC plate; precoated with silica gel G 60F254 on aluminium sheet as a stationary phase and methanol as a mobile phase. The method produced compact band for the drug (Rf = 0.43 ± 0.02). The HPTLC method was successfully validated in accordance with ICH guideline.

Introduction:
Asenapine maleate (ASP) belongs to class atypical antipsychotic with potent dopamine, serotonin and adrenergic antagonist properties used for treating schizophrenia and acute mania concomitant to bipolar disorder [1]. ASP is marketed as 5 mg and 10 mg sublingual tablets dosage form.
Different analytical procedures have been developed for quantitative determination of ASP in pharmaceutical dosage form as well as in biological fluids. Literature survey has shown that no HPTLC method has been reported for the estimation of ASP. The current study elaborates a novel and simple HPTLC method for quantitative determination of ASP in both, marketed tablets and in house developed formulations, for determining equilibrium solubility of ASP in various excipients as well as its ex vivo diffusion study through sheep nasal mucosa from the formulations developed in house.

Materials and Methods:

Chemicals and reagents: Pure samples of ASP were gifted by Sun Pharmaceutical Industries Ltd. Sikkim (India).

Instruments: HPTLC aluminum plates (10 x 10 cm) coated with 0.2 mm silica gel 60 F254 (Merck, Germany), TLC scanner 3 densitometer (Camag, Muttenz, Switzerland), Linomat IV with 100 µL syringe (Camag, Muttenz, Switzerland), The HPTLC system (CAMAG, Muttenz, Switzerland), twin-trough chamber for 10 cm x 10 cm plates (CAMAG, Muttenz, Switzerland).
Preparation of ASPM standard solution: Standard solutions of ASP (100 µg/mL) was prepared in methanol.

HPTLC method development: As suggested by the International Conference on Harmonization, different stress test conditions (alkali, acid, thermal, photolytic and humidity) were used to degrade ASP. The samples produced from this study were utilized to develop a stability indicating HPTLC method. The developed HPTLC method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [2].

Application of developed HPTLC method: Developed HPTLC method was applied for quantitative estimation of Asenapine maleate in both marketed tablets and in-house developed formulations (solution, microemulsion, nanoemulsion and mucoadhesive nanoemulsion) as well as for equilibrium solubility study and ex vivo diffusion study of developed formulation through natural membrane.

Results and Discussion:
The Asenapine maleate was separated well from degradation products using HPTLC plate; precoated with silica gel G 60F254 on aluminium sheet as a stationary phase and methanol as a mobile phase. Using densitometric analysis, Asenapine maleate was quantified at 235nm. The method produced compact band for the drug (R_f = 0.43 ± 0.02). The HPTLC method was validated and statistical analysis of the data confirmed the method to be specific, linear, accurate, precise, reproducible, and selective for Asenapine maleate’s analysis.

![Chromatogram of standard solution containing 1800 ng/band asenapine maleate using proposed HPTLC.](image)

Conclusion:
A new HPTLC method has been developed and validated successfully in accordance with ICH guidelines for quantitative estimation of ASP. The method was successfully applied for the estimation of the equilibrium solubility of ASP in various excipients as well as its ex vivo diffusion study through sheep nasal mucosa from the formulations developed in house.
Acknowledgement:
Science and Engineering Research Board, Department of Science and Technology, Government of India, New Delhi is gratefully acknowledged for financial support (F.No. SR/FT/LS-135/2012). The author(s) confirm and assure that content of this article has no conflicts of interest.

References:
HPTLC method for quantitation of Lurasidone Hydrochloride in nanoemulsion, microemulsion, for equilibrium solubility and ex-vivo diffusion studies.

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Abstract: A new high performance thin layer chromatography (HPTLC) has been successfully developed and validated for quantitation of Lurasidone hydrochloride in pharmaceutical formulations. The Lurasidone hydrochloride (LURA) was well separated from sample matrix and degradation product using HPTLC plate; precoated with silica gel G 60F254 on aluminium sheet as a stationary phase and mixture of hexane: ethyl acetate (6:4 v/v) as a mobile phase. The method was found to give compact band for the drug (Rf = 0.47 ± 0.01). The HPTLC method was successfully validated in accordance with ICH guideline.

Introduction:
Lurasidone hydrochloride (LURA) was approved in USA and Canada in 2010 and 2012 respectively, as an immediate release oral tablet (40, 80, 120 and 160 mg/tablet) for the treatment of schizophrenia [1].
Different analytical methods have been developed for quantitative estimation of LURA in pharmaceutical dosage form and in biological fluids. The new reliable HPTLC method was developed that can be used for proposed analytical task. The present work describes a new and simple HPTLC method for quantitative estimation of LURA in in-house developed formulations, equilibrium solubility and ex vivo diffusion studies.

Materials and Methods:
Chemicals and Reagents: Pure samples of Lurasidone hydrochloride were gifted by Astron Research Ltd. (Ahmedabad, India).
Instrumentation: The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of a Linomat 5 autosprayer, a twin-trough chamber for 10 × 10 cm plates, a derivatization chamber, and a plate heater. Precoated silica gel 60F254 HPTLC plates (10 × 10 cm, layer thickness 0.2 mm; E. Merck KGaA, Darmstadt, Germany) were used as the stationary phase. Densitometric analysis was carried out using a CAMAG TLC Scanner 3 with winCATS software.
HPTLC method: As suggested by the International Conference on Harmonization, different stress test conditions (alkali, acid, thermal, photolytic and humidity) were used to degrade ASP. The samples
produced from this study were utilized to develop a stability indicating HPTLC method. The developed HPTLC method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [2].

**Application of developed HPTLC method**

Developed HPTLC method was applied for quantitative estimation of LURA in both marketed tablets and in-house developed formulations (solution, microemulsion, nanoemulsion and mucoadhesive nanoemulsion) as well as for equilibrium solubility study and ex vivo diffusion study of developed formulation through natural membrane.

**Results and Discussion:**

The LURA was well separated from sample matrix and degradation product using HPTLC plate; precoated with silica gel G 60F\textsubscript{254} on aluminium sheet as a stationary phase and mixture of hexane: ethyl acetate (6:4 v/v) as a mobile phase. LURA was quantified by densitometric analysis at 323 nm. The method was found to give compact bands for the drug (R\textsubscript{f} = 0.47 ± 0.01). Linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100–600 ng. Statistical analysis of the data showed that the method is specific, precise, accurate, reproducible, and selective for the analysis of LURA.

**Figure 1** Chromatogram of standard solution containing 400 ng/band Lurasidone hydrochloride using proposed HPTLC.

**Conclusion:**

A new HPTLC method has been developed for quantification of LURAH. The method was successfully validated in accordance with ICH guidelines, and statistical analysis proved that the method is sensitive, specific, and repeatable. It can be conveniently used for routine analysis of LURA as a bulk drug and in in-house developed formulations (solution, nanoemulsion and microemulsion; with or without mucoadhesive polymer) without any interference from excipients. The method was successfully applied for the estimation of the equilibrium solubility of LURAH in various excipients and ex vivo diffusion study.
Acknowledgement:
Science and Engineering Research Board, Department of Science and Technology, Government of India, New Delhi is gratefully acknowledged for financial support (F.No. SR/FT/LS-135/2012). The author(s) confirm that this article content has no conflicts of interest.

References
Development and validation of UV spectroscopic simultaneous equation method for Rosuvastatin Calcium and Fenofibrate in bulk and combine dosage form

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Abstract: A simple, rapid and accurate UV method has been established and validated for the simultaneous determination of Rosuvastatin and Fenofibrate in tablets. The reproducibility, repeatability and accuracy of the method were found to be good which is evidenced by low values of standard deviation and %RSD. Linearity range was found to be in between 2-12 µg/ml for Rosuvastatin and 4-24 µg/ml for Fenofibrate at 244.2nm and 290.6nm respectively. Correlation coefficient was found to be 0.998 and 0.999 respectively. Hence, this method can be successfully applied for the estimation of Rosuvastatin and Fenofibrate in pharmaceutical formulation.

Introduction:
Rosuvastatin chemically (figure 1) Bis [(E)-7-[4-(4-flurophenyl)-6-isopropyl-2 [methyl (methylsulfonyl)amino]pyrimidine-5yl](3R,5S)-3,5-dihydroxy hept-6-enoic acid]calcium. Rosuvastatin is inhibitor of HMG-CoA. It primarily act in liver it decreases the cholesterol level. FenofibrateChemically (figure 2) Propane-2-Yl-2-[4[4Chlorophenyl) Carbonyl] Phenory]-2-methyl propanoate.It activate peroxisome proliferator activated receptor α (PPARα). Through which it increases lipolysis. There are few method reported for the estimation of fenofibrate in pharmaceutical dosage form. Both drugs are official in Indian Pharmacopoeia 2007, United State Pharmacopoeia 2009. In the literature survey revealed that RP-HPLC, HPTLC and UV-Spectrophometric method were reported for estimation of Rosuvastatin and Fenofibrate. In the present research work we had develop a simple, accurate, precise, reproducible method for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in combined dosage form for routine analysis.

Materials and Methods:

Materials:
Drug Sample- Rosuvastatincalcium, gift sample obtained from Lupin pharmaceutical private Ltd and Fenofibrate- Gift sample obtained from GlenmarkPharmaceutical Pvt Ltd.
Chemicals: NaOH of Qualigenlab (AR) Gradewhich is purches from local market and Distilled water
Instrument: A double beam Schimadzu UV-Visible Spectrophometer-2450, and ultra sonicator is used.

Method development: Selection of wavelength: The UV-Spectrum of Rosuvastatin and fenofibrate was obtained separately by scanning the samples in the wavelength range 200-400 against the blank
solution. Both the drugs shows maximum absorbance at the wavelength range 244.2 nm for Rosuvastatin Calcium and Fenofibrate shown in figure 1.

![Over lain spectra of Rosuvastatin calcium (25µg/ml) and Fenofibrate (25µg/ml).](image)

**Preparation of stock solution of Rosuvastatin calcium and Fenofibrate**

Standard stock solution of 100 µg/ml was prepared. Over lain spectra were obtained (Fig.2) in range 2-4 µg/ml and 4-24 µg/ml at the wavelength 244-2 nm and 290.6 nm

**Preparation of standard mixture:** Standard mix of Rosuvastatin calcium and Fenofibrate is was prepared (conc. Ratio 1:2 concentration range 20 µg/ml of Rosuvastatin and 40 µg/ml Fenofibrate). The absorbances of sample recorded at 244.2 nm and 290.6 nm. Result is shown in **Table 1**.

<table>
<thead>
<tr>
<th>% Mean Amt. Estimated</th>
<th>S.D</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>FENO</td>
<td>ROS</td>
</tr>
<tr>
<td>101.14</td>
<td>100.98</td>
<td>0.00986</td>
</tr>
</tbody>
</table>

**Table 1 Statistical evaluation of standard mixture**

**Tablet analysis**

**Brand Name: Rozavel FLS:** Twenty tablet weighed accurately and powdered. Powder equivalent to 10 mg of fenofibrate was transferred into 100 ml volumetric flask, spiking of standard was done by adding 4 mg of Rosuvastatin dissolved it into 0.1 N NaOH by sonication for 30 min and make up the volume up to the mark. Further dilutions made by conc.10µ/ml of Fenofibrate and 5.2 µg/ml of Rosuvastatin Calcium and Result of tablet analysis are given in **Table 2**.

**Table 2 Tablet Analysis.**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Name of Drug</th>
<th>Amt. calculated(µg/ml)</th>
<th>% Amt. Found (n=6)</th>
<th>%R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ROZ</td>
<td>5.08</td>
<td>98</td>
<td>1.185</td>
</tr>
<tr>
<td>2</td>
<td>FENO</td>
<td>10.01</td>
<td>101</td>
<td>0.923</td>
</tr>
</tbody>
</table>

**Method validation:** Proposed method was validated as per ICH guideline by various parameters such as, precision, Specificity, robustness, ruggedness summary shown in **Table 3**.

**Table 3 Summary of validation**
Conclusion:
A linear, precise, accurate analytical has been developed for Rosuvastatin and Fenofibrate. The method was very simple and liable. Results were within limit.

Acknowledgment:
The authors are thankful to MGV’S College of Pharmacy Panchavati, Nashik for providing all facility.

References:
Validated stability-indicating HPTLC method for Cefixime and Azithromycin with preparative isolation, identification and characterization of potential degradation products

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Abstract: Stability indicating HPTLC method for simultaneous estimation of Cefixime Trihydrate (CEFI) and Azithromycin dihydrate (AZI) was developed. Both drugs were subjected to different stress conditions recommended by International Conference on Harmonization (ICH) guideline Q1A (R2). Forced degradation was carried out for hydrolytic, oxidative, photolytic and thermal degradation conditions. Cefixime was susceptible for degradation under all stress conditions showing major four degradation products (CI-IV). However, azithromycin showed only one prominent degradation product (A-I) under acid hydrolysis. The method was validated for linearity, precision, accuracy, specificity, robustness and has been successfully applied in the analysis of drug in tablet dosage form.

Introduction:
Analysis of stability test samples in the pharmaceutical industry should be done by using stability-indicating assay Method so as to elucidate the inherent stability characteristics of the active substance developed. Extensive literature survey reveals that few analytical Method have been reported for the estimation of either cefixime (CEFI) or azithromycin (AZI) individually or in combination with other drugs, which includes Method like derivative spectroscopy [1], thin-layer chromatography (TLC)–densitometry [2], reversed phase high-performance liquid chromatography (RP-HPLC) [3], and high-performance thin-layer chromatography (HPTLC) [4,5]. However, there is only one reported method for the simultaneous estimation of CEFI and AZI in the presence of degradation products to the best of our knowledge. Hence, it was aimed to develop an alternative stability indicating HPTLC method for simultaneous estimation of Cefixime and Azithromycin in presence of degradation products and to isolate (Preparative HPTLC), Identify and Characterize potential degradants by LC-MS-MS.

MaterialsS and Method:
1) Materials: CEFI and AZI were received as gift samples from Biocon Ltd., India, certified to contain 99.7% and 99.6% purity, respectively. The drugs were used without further purification. All chemicals and reagents used were of analytical grade (Ranbaxy Fine Chemicals Limited, New Delhi, India).
2) **Method:** The samples were spotted in the form of bands of width 8 mm with a CAMAG microliter syringe on precoated silica gel aluminum plate 60 F254 (20 cm × 10 cm, 200 μm thickness, E. Merck, Germany) using a CAMAG Linomat V (Switzerland) sample applicator. A constant application rate of 100 nLs⁻¹ was employed, and space between two bands was 14 mm. The solvent system consisted of Ethyl acetate: Methanol: Acetone: Toluene: Ammonia (1:5:7:0:5:0.5v/v). Chromatogram was developed in a CAMAG twin trough chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimized to 20 min. The length of chromatogram run was 8 cm. Subsequent to the development, the TLC plates were dried in a current of air. Densitometric analysis was performed on a CAMAG TLC Scanner III in the absorbance mode at 235 nm for CEFI and degradation products while Spraying with Sulfuric acid : ethanol (1:4v/v) followed by heating at 100°C for 5 min for AZI and its degradation product. Both the drugs were subjected separately for hydrolytic (acid, base, neutral), oxidative, thermal and photolytic degradation. For Preparative TLC, Acid degradation sample (400 μl) was applied as 180 mm band on 20 x 10 cm plate. Plate was developed in mobile phase. Drug and degradation product bands were marked; plates were cut carefully to separate bands. Individual bands were cut into strips and sonicated with methanol for extraction of degradation products. Methanol fractions were concentrated and evaporated to obtain solid residue which was subjected to mass fragmentation studies.

**Results and Discussion:**

The HPTLC procedure was optimized with a view to develop a stability-indicating assay method to quantify CEFI and AZI from bulk and marketed formulation. Mobile phase consisting of methanol–chloroform (1:7 v/v) gave a sharp and well defined peak at Rf values of 0.64 and 0.27 for CEFI and AZI. Both the drugs were found to be susceptible for acid, alkali as well as neutral hydrolytic and photolytic conditions. AZI was more prone to oxidative degradation than CEFI whereas CEFI was more susceptible to thermal degradation than AZI. In all the degradation conditions, 5-30% degradation was achieved. The peak purity of CEFI and AZI was assessed by comparing the spectra at peak start, peak apex, and peak end positions. The chromatograms of the samples treated with acid, alkali, neutral, hydrogen peroxide, dry heat, and UV light showed well-separated spots of pure CEFI and AZI as well as some additional peaks at different Rf values. The spots of the degraded products were well-resolved from the drug spot. The measurement of the peak area at three different concentration levels showed low values of the relative standard deviation (% RSD) for inter- and intra-day variation, which suggested an excellent precision of the proposed method. The proposed method when used for extraction and subsequent estimation of CEFI and AZI from tablets after spiking with 80, 100, and 120% of additional drug afforded recovery of 98.5–100.1% and 99.1–101.0% of CEFI and AZI, respectively.
Table 1 Summary of degradation behavior of Cefixime and Azithromycin

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Exposure condition</th>
<th>% Drug Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of stressor, heat, duration</td>
<td>CEFI</td>
</tr>
<tr>
<td>Acid Hydrolysis</td>
<td>0.5 N HCl, RT, 30min</td>
<td>15.65</td>
</tr>
<tr>
<td>Base Hydrolysis</td>
<td>0.5 N NaOH, RT, 30min</td>
<td>27.56</td>
</tr>
<tr>
<td>Neutral Hydrolysis</td>
<td>H₂O</td>
<td>21.39</td>
</tr>
<tr>
<td>Oxidation</td>
<td>H₂O₂, 30%, 1hr</td>
<td>21.92</td>
</tr>
<tr>
<td>Photolysis</td>
<td>Fluorescent light 1.2 million lux hours and uv light 200 Whm²</td>
<td>13.87</td>
</tr>
<tr>
<td>Thermal</td>
<td>100°C, 1hr</td>
<td>200°C, 2hrs</td>
</tr>
</tbody>
</table>

Four degradation products of CEFI and One degradation product of AZI was isolated by preparative HPTLC and subjected to MS-MS studies for characterization. Based on fragmentation pattern obtained, structures of DP-I [chemical formula: C₉₁H₁₄N₅O₅S₂], DP-II [Chemical Formula: C₉H₁₀N₂O₃S] and DP-III [Chemical Formula: C₁₃H₁₈N₅O₅S₂], DP IV [Chemical Formula: C₅H₇N₃O₂S] and A-I [Chemical Formula: C₃₀H₅₇NO₉] were confirmed.

Conclusion:
The developed HPTLC technique is precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the proposed method separates the drug from its degradation products, it can be employed as a stability-indicating one.

Acknowledgments:
The authors are sincerely thankful to Maxim Pharmaceuticals (Pune, India) and Aristo Pharmaceuticals (Mumbai, India) for supplying gift samples of pure drugs and to the Anchrom Enterprises (Mumbai, India) for providing facilities to carry out the work.

References:
PB-25

Greener and rapid access to some bioactive 4-Thiazolidinones and 2-Azetidinones

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Abstract: A series of suitably substituted 4-Thiazolidinone and 2-Azetidinone have been synthesized under microwave irradiation and conventional heating. The microwave assisted reaction was remarkably successful in higher yield at lesser reaction time compared to conventional heating. Spectral data (IR, $^1$H NMR) confirmed the structures of the synthesized compounds. All compounds were screened for their In vitro antibacterial and antifungal activity. The results indicated that the synthesized compounds have moderate to good activity with reference to their respective standards.

Introduction-
Heterocyclic compounds are proven backbone for discovery of many bioactive molecules. Among all heterocyclic compounds, nitrogen and sulphur containing 4-thiazolidinone and 2-azetidinone are well known for their broad spectrum of biological activities [1-2]. Synthesis of heterocyclic compounds remains a challenge due to several facts such as use of hazardous chemicals, production of waste or byproducts, higher reaction time along with environmental and health related issues. So, there is wide demand to obtain molecules using some sustainable and alternate route of synthesis, known as Green chemistry [3]. The best option to accelerate synthetic processes is the use of microwave irradiation. The use of Microwave-Assisted Organic Synthesis (MAOS) [4,5] in the synthetic chemistry reduces reaction time and increases product yield. The procedures which have been described for the targeted compounds i.e. derivatives of 4-thiazolidinone and 2-azetidinone require longer reaction time with lesser product yield. Keeping this in view, the present work aims at the synthesis of 4-thiazolidinone and 2-azetidinone analogues using both conventional and microwave (greener) method. Thus, this project describes the comparison between conventional and greener methods for efficient synthesis of target molecules. Furthermore, the compounds were evaluated for antibacterial and antifungal activities to check their biological potential.

Materials and Methods:
Melting points were determined by open capillary method on a ‘Veego VMP-I’ apparatus and are uncorrected. Purity of the compounds was checked on silica gel G F$_{254}$ plates of size 3X8 cm and visualized by UV. The IR spectra were recorded in the 4000-400 cm$^{-1}$ range in SHIMADZU IR INFINITY by placing sample directly on probe. $^1$H NMR were recorded on BRUKER AVANCE II (400 MHz) and Varian Mercury (400 MHz) spectrometer in CDCl3 or DMSO as solvent using
trimethylsilane (TMS) as internal reference standard and values are expressed in δ ppm. Microwave synthesis was carried out using Catalyst Microwave oven-CATA-2R, at power level ranging from 1 to H at 85 to 850 watts. Spectral analyses were recorded at Sophisticated Analytical Instrument Facility (SAIF), Tata Institute of Fundamental Research, Mumbai and Central Drug Research Institute, Lucknow.

Results and discussion-
According to scheme of synthesis, attempts were made to synthesize series of suitable substituted 4-Thiazolidinone and 2-Azetidinone. These compounds were synthesized using two methods, conventional (thermal) and microwave assisted organic synthesis.

% Yield and purity: It was noted that conventional methods used to synthesize these compounds requires longer reaction time with lesser product yield. Therefore microwave assisted synthesis was applied for synthesis of targeted compounds. % Yields were increased compared to conventional process from 35-55 to 55-75%.

Reaction time: Reaction time was also reduced from hours to minutes. It was observed that conventional process requires 6-12 hours to synthesize these compounds whereas microwave methods gave products in 5-30 minutes.
Biological evaluation: In the present study, all derivatives of 4-Thiazolidinone and 2-Azetidinone were screened for antibacterial activity against two pathogenic bacteria like *E.coli* (Gram negative) and *B. subtilis* (Gram positive) and for antifungal activity against *C. albicans* and *A. niger*. Table 1 shows MIC values of all compounds for antibacterial and antifungal activity.

Table 1 MIC values for antibacterial and antifungal activity of targeted compounds

<table>
<thead>
<tr>
<th>Compound code</th>
<th>MIC (μg/mL)</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E.coli</em></td>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>8a</td>
<td>300</td>
<td>400</td>
<td>0.4</td>
</tr>
<tr>
<td>8b</td>
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<td>9a</td>
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<tr>
<td>Fluconazole</td>
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<td>16</td>
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</table>

Conclusion:
Some novel 4-thiazolidinone and 2-azetidinone derivatives have been synthesized. Spectroscopic analysis confirmed the proposed structures of these compounds. These compounds were further evaluated for antimicrobial activities. The results of antimicrobial studies revealed that the compounds possess excellent antifungal activity and moderate-good antibacterial activity against fungal and bacterial strains.

References:
2,5-Disubstituted-1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles of biological importance

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Abstract: For the last few decades, heterocyclic chemistry has assumed immense importance in drug
discovery research. Among them, azole heterocycles are of prime importance. Hence in this work we
have synthesized some Schiff’s bases bearing 1,3,4-thiadiazole scaffold. We have also reported fewer
2,5-disubstituted-1,3,4-oxadiazole derivatives by reacting various 5-(substituted)-1,3,4-oxadizole-
thiols with N-(2-aminophenyl)-4-chlorobutanamide. Structures were confirmed by spectroscopic
methods and purity was established by chromatographic methods. All ten compounds were evaluated
for their anti-microbial activity against pathogenic bacteria like E. coli and B. subtilis and anti-fungal
activity against C. albicans and A. niger using appropriate methods. They were found to be better
antifungal agents than anti-bacterial agents. Compound 1d was found to have maximum anti-fungal
activity with MIC of 0.4 µg/ml, which was better than the standard used. Compound 6b was found to
be moderate anti-bacterial agent and good antifungal agent.

Introduction:
Heterocyclic compounds are proven backbone for discovery of many bioactive molecules. Among all
heterocyclic compounds, nitrogen and sulphur containing Oxadiazole and thiadiazole are well known
for their broad spectrum of biological activities[1-4]. In the present work, we have reported some
Schiff’s bases of 5- substituted -1,3,4 -thiadiazole-2-amines (Scheme 1) and 2,5 -disubstituted
derivatives of 1,3,4-oxadiazole(Scheme 2) and have evaluated them for their anti-bacterial, anti-
fungal activities and anti-tubercular activities.

Material and Methods:
Melting points were determined by open capillary method on a ‘Veego VMP-I’ apparatus and are
uncorrected. Purity of the compounds was checked on silica gel G F254 plates of size 3X8 cm and
visualized by UV. The IR spectra were recorded in the 4000-400 cm-1 range in SHIMADZU IR
INFINITY by placing sample directly on probe. 1H NMR were recorded on BRUKER AVANCE II
(400 MHz) and Varian Mercury (400 MHz) spectrometer in CDCl3 or DMSO as solvent using
trimethylsilylame (TMS) as internal reference standard and values are expressed in δ ppm.

Scheme 1: Synthesis of Schiff’s bases of 2-Amino-5-(3-hydroxyphenyl)-1,3,4-thiadiazole5:
Step 1: Synthesis of 5-(3-hydroxyphenyl)-1,3,4-Thiadiazole-2-amine (Compound 1.1) - To a solution of 5g (0.036 moles) of 3-hydroxybenzoic acid in 50ml ethanol, 4.4g (0.048 moles, 1.3 equiv.) of Thiosemicarbazide was added under stirring. 3.24 ml (0.018 moles, 0.5 equiv) of concentrated sulphuric acid was added dropwise to this mixture and the mixture was refluxed for 15-16 hours. The product was precipitated and filtered off and recrystallized with suitable solvent.

Step 2: General method for synthesis of Schiff’s bases of 5-(3-hydroxyphenyl)-1,3,4-Thiadiazole-2-amine (Compounds 1a-1f) - To a solution of 1.5g (1 equiv) of 5-(3-hydroxyphenyl)-1,3,4-Thiadiazole-2-amine (compound 1.1) in 30 ml methanol, 1.1 equiv. of aldehyde was added. 0.5 equiv. of H₂SO₄ was added drop wise and this reaction mixture was refluxed for 12-18 hrs. The reaction mixture was cooled to room temperature and then poured in cold water to obtain Schiff’s bases and were recrystallized from a suitable solvent.

Scheme 2: Synthesis of 2,5-disubstituted-1,3,4-oxadiazole derivatives:

Step 1: Synthesis of esters of substituted Benzoic Acids (Compounds 2a, 2c, 2d) - To a solution of 5g of substituted benzoic acid (4-hydroxy, 4-chloro or 4-amino) in 50ml ethanol, conc. H₂SO₄ (0.6 equiv.) was added slowly, and this mixture was refluxed. The reaction mixture was poured into a beaker and 50% NaHCO₃ solution was used to neutralise it to pH 7 to get a precipitate of the ester. For ethyl ester of 4-amino benzoic acid, the reaction mixture was basified to obtain a precipitate of the ester. In case of ethyl ester of 4-chloro benzoic acid (liquid), chloroform was used to separate the ester globules from the aqueous solution and was then evaporated to obtain the liquid ester.

Step 2: Synthesis of Hydrazide of Benzoic Acids (Compound 3a-3d) - To a solution of 4g (4ml) of ester in 40ml ethanol, hydrazine hydrate (1.5 equiv.) was added and the reaction mixture was refluxed. The reaction mixture was poured in ice-cold water to obtain precipitate of the corresponding hydrazide and werepurified by recrystallization using suitable solvents.

Results and discussion-
According to scheme of synthesis, attempts were made to synthesize series of suitable substituted 1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles in sufficient yields and purity.

Biological Evaluation-
In the present study, all derivatives of 1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles were screened for antibacterial activity against two pathogenic bacteria like E.coli (Gram negative) and B. subtilis (Gram positive) and for antifungal activity against C. albicans and A. niger. Table 1 shows MIC values of all compounds for antibacterial and antifungal activity.

Compounds 1a–1f were found to have remarkable antifungal activity. However they showed less antibacterial activity. Compound 1d exhibited maximum antifungal activity with MIC value of 0.4.
$\mu g/ml$. It could be regarded to exhibit better Anti-fungal activity as compared to standard used during testing.

Compounds 6a – 6d show moderate antibacterial activity. Anti-fungal activity was found to be good for compound 6a with MICs of 3.12$\mu g/ml$ (C.albicans) 1.6$\mu g/ml$ (A.niger) which was comparable activity to the standards used for anti-fungal testing. Compound 6b with MIC value of 0.4$\mu g/ml$ showed better activity than standard Fluconazole against C.albicans.

### Table 1- MIC values for antibacterial and antifungal activity of targeted compounds

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Minimum Inhibitory Concentration ($\mu g/ml$)</th>
<th>Compound Code</th>
<th>Minimum Inhibitory Concentration ($\mu g/ml$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>E.coli</strong></td>
<td><strong>B.subtilis</strong></td>
<td><strong>C.albicans</strong></td>
</tr>
<tr>
<td>1a</td>
<td>100</td>
<td>12.5</td>
<td>0.8</td>
</tr>
<tr>
<td>1b</td>
<td>100</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>1c</td>
<td>100</td>
<td>50</td>
<td>12.5</td>
</tr>
<tr>
<td>1d</td>
<td>100</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>1e</td>
<td>25</td>
<td>50</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Conclusion:**

Some novel 1,3,4-oxadiazoles and 1,3,4-thiadiazoles derivatives have been synthesized. Spectroscopic analysis confirmed the proposed structures of these compounds. These compounds were further evaluated for antimicrobial activities. The results of antimicrobial studies revealed that the compounds possess excellent antifungal activity and moderate-good antibacterial activity against fungal and bacterial strains.

**References:**


Mild oxidation facilitated conversion of piperine to piperonal and subsequent conversion into a few heteroaromatic derivatives for their in vitro biological studies

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Abstract: Piperine upon oxidation by cold KmnO₄ yielded Piperonal, which via Claisen Schimidt condensation with Acetophenone gave rise to a corresponding Chalcone, which upon treatment with substituted Phenyl hydrazine, Urea, Thiourea and o-Phenylene diamine yielded corresponding Pyrazole, Oxazine, Thiazine and 1, 5 – Benzodiazepine derivatives. The purity of the synthesized compounds were ascertained by Thin Layer Chromatography, while synthetic method of the target compounds were established by their characterization data through IR, NMR and Mass and CHN studies. The synthesized compounds were evaluated for their in-vitro Anti-Oxidant and Anti-Inflammatory activities.

Introduction:
Black pepper (Piper nigrum) is considered the king of spices throughout the world due to its pungent principle piperine.¹ Piper nigrum is known to have diverse biological activities.² On the other hand Heterocycles are present in a wide variety of drugs, most vitamins, many natural products, biomolecules, and biologically active compounds.³ Several synthetic analogues of natural piperine have also been reported⁴ to possess diverse biological activities. In view of these findings in the present study, it has been speculated to carry out the oxidation facilitated conversion of Piperine to Piperonal and subsequent conversion into a few heteroaromatic derivatives as per Scheme – 1, for their in-vitro biological studies.

Materials and Method:
Materials: Black pepper was obtained from local farmers and shade dried. All the chemicals used for the synthesis of desired compounds were of synthetic grade and all the chemicals used for the characterization of the target compounds were of analytical grade with sufficiently high purity, and procured from reputed suppliers like Merck, Loba Chemie, etc.

Method: General synthetic procedure: Piperine was isolated, purified and characterized through Thin Layer Chromatography and hydrolysed to Piperic acid as per reported method⁴, which was converted to Piperonal via Mild Oxidation with cold KMnO₄ under Nitrogen atmosphere as per reported method⁵ to give Compound-1 which upon reaction with acetophenone, as per reported
method\textsuperscript{6} gave Compound - 2((2E)-3-(1, 3-benzodioxol-5-yl)-1-phenylprop-2-en-1-one), which were further used for the synthesis of Compound 3a-fas per reported method\textsuperscript{7}.

1. **Scheme – 1**

\begin{center}
\includegraphics[width=\textwidth]{scheme1.png}
\end{center}

**In-vitro antioxidant Study (Free Radical Scavenging Activity)**\textsuperscript{8}:
The free radical scavenging activity of the synthesis compound was measured by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) using the reported method and the percent DPPH scavenging effect was calculated using the following equation and results are described in **Table 1**:

**In-vitro Anti-inflammatory Activity Study (Inhibition of Protein Denaturation)**\textsuperscript{9}: 
\textit{in-vitro} Anti-inflammatory Activity Study was carried out by reported method and the percentage inhibition of protein denaturation was calculated as follows and represented in **Table 2**:

\begin{table}[h!]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Compound} & \textbf{In-vitro antioxidant activity (Absorbance / % scavenging)} & \\
& \textbf{100µg/ml} & \textbf{250µg/ml} \\
\hline
4a & 0.132/51 & 0.136/49 \\
4b & 0.185/31 & 0.134/50 \\
4c & 0.241/11 & 0.239/11 \\
4d & 0.152/43 & 0.069/74 \\
4e & 0.248/8.4 & 0.110/59 \\
4f & 0.122/54.4 & 0.106/60 \\
Gallic Acid & 0.022/91.8 & 0.016/97 \\
Control & 0.271/00 & 0.271/00 \\
\hline
\end{tabular}
\end{table}

**Conclusion:**
In the present research it has been attempted to synthesize some heterocyclic analogues of Piperonal and report their in-vitro Antioxidant and Anti-Inflammatory activities. Among the Synthesized Compounds 3d (Thiazone analogue) and compound 3f (1, 5-Benzodiazepine analogue) were found to possess potent antioxidant activity as compared to standard drug Gallic Acid. On the other hand compounds 3a and 3b (Pyrazole analogues) were found to possess potent anti-Inflammatory activity as compared to standard drug Citrizine.

Table 2: In-vitro Anti-inflammatory Activity Study (Inhibition of Protein Denaturation):

<table>
<thead>
<tr>
<th>Compound</th>
<th>In-Vitro Anti-inflammatory activity Study (Absorbance / % Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100µg/ml</td>
</tr>
<tr>
<td>4a</td>
<td>0.032/84.85</td>
</tr>
<tr>
<td>4b</td>
<td>0.033/69.7</td>
</tr>
<tr>
<td>4c</td>
<td>0.065/24.2</td>
</tr>
<tr>
<td>4d</td>
<td>0.053/33.40</td>
</tr>
<tr>
<td>4e</td>
<td>0.041/39.3</td>
</tr>
<tr>
<td>4f</td>
<td>0.044/48.49</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.037/33.30</td>
</tr>
<tr>
<td>Control</td>
<td>0.033/0</td>
</tr>
</tbody>
</table>

Acknowledgement:
The authors are grateful to the authorities of GRY Institute of Pharmacy, Vidya Vihar, Borawan, Khargone, India for providing the necessary facility to carry out this research work. We are also thankful to Dr. Gopkumar A and Dr. Sridevi G. GRY Institute of Pharmacy, Vidya Vihar, Borawan, for valuable suggestions.

References:
Studies in synthesis and antiproliferative activity of some new chalcones possibly acting as VEGFR-2 inhibitors

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Abstract: Chalcones are open chain flavonoids. They have been reported to act as inhibitors of EGFR and VEGFR. In our previous work, chalcones showed inhibitory activity on EGFR so we decided to explore other kinase receptor VEGFR. VEGFR-2 has been reported to be involved in cancers like Breast Cancer, Gliomas and others so, the aim of our present work was to synthesize chalcones and test them for antiproliferative activity & docking. Accordingly, 10 compounds were synthesised and tested on MCF-7 cell lines & docking studies were also carried out on VEGFR-2.

Introduction:
Angiogenesis involves the generation and development of new blood vessels from pre-existing vasculature. It is critical for the growth and metastasis of solid tumours. Angiogenesis is regulated by several growth factors and their receptors including Vascular Endothelial Growth Factors (VEGF) and their Receptors (VEGFRs). VEGF expression is markedly increased in the carcinomas. VEGFR-2 is of paramount value because it is primarily expressed on the endothelial cells and therefore contributes majorly. Chalcones, 1, 3-diphenylpropenones, are structurally related to flavones. These molecules possess most of the important structural elements of flavones and can adopt both open and closed conformations, thus offering a better flexibility for receptor binding. Various chalcones inhibit different steps of carcinogenesis from the very early stages, including tumour initiation, through promotion, progression, angiogenesis, and invasion, to the very late stages leading to metastasis. Chalcones derivatives are reported to act as inhibitors of EGFR and VEGFR. Taking this into consideration, we synthesized some new chalcones, tested them for antiproliferative activity and studied their possible orientation in the VEGFR-2 by docking.

Materials and Method:
SYNTHESIS OF CHALCONE ANALOGS:
Docking studies: Computational processes are applied to predict the biological activities of a large set of compounds before actually attempting to synthesize them. It helps in prioritizing synthesis and saves on time and monetary aspects at the same time helps in rationalizing the drug discovery process. In case of VEGFR-2, 33 different crystal structures of kinase domain of VEGFR-2 bound to chemically diverse inhibitors are reported. For the purpose of studies in this case we have selected 3EWH based on cross docking studies. Docking was carried out using GLIDE.

Results and Discussion:

Experimental procedure for Sulforhodamine B Assay:
Standard protocol for procedure was followed.\(^3\)

The results obtained for anti-cancer activity for cell lines MCF-7 are shown in table format:

<table>
<thead>
<tr>
<th>compounds</th>
<th>(R_1)</th>
<th>(R_2)</th>
<th>10-7M</th>
<th>10-6M</th>
<th>10-5M</th>
<th>10-4M</th>
<th>GI50</th>
<th>Dock score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>H</td>
<td>3-NO(_2)</td>
<td>99.5</td>
<td>92.8</td>
<td>85.5</td>
<td>50.1</td>
<td>&gt;100</td>
<td>-9.77</td>
</tr>
<tr>
<td>B</td>
<td>H</td>
<td>4-NO(_2)</td>
<td>90.6</td>
<td>88.8</td>
<td>51.3</td>
<td>21.5</td>
<td>50.3</td>
<td>-9.5</td>
</tr>
<tr>
<td>C</td>
<td>H</td>
<td>4-Cl</td>
<td>99.3</td>
<td>84.1</td>
<td>78.5</td>
<td>44.5</td>
<td>87.3</td>
<td>-8.35</td>
</tr>
<tr>
<td>D</td>
<td>H</td>
<td>2-Cl</td>
<td>98.7</td>
<td>97.4</td>
<td>94.8</td>
<td>51.6</td>
<td>35.4</td>
<td>-8.89</td>
</tr>
<tr>
<td>E</td>
<td>H</td>
<td>3-NO(_2)</td>
<td>95.8</td>
<td>86.7</td>
<td>45.6</td>
<td>-4.7</td>
<td>&gt;100</td>
<td>-9.09</td>
</tr>
<tr>
<td>F</td>
<td>Cl</td>
<td>4-NO(_2)</td>
<td>100.6</td>
<td>103.8</td>
<td>94.7</td>
<td>34.2</td>
<td>76.7</td>
<td>-8.12</td>
</tr>
<tr>
<td>G</td>
<td>Cl</td>
<td>3,4-OCH(_3)</td>
<td>98.8</td>
<td>89.3</td>
<td>84.5</td>
<td>46.5</td>
<td>&gt;80</td>
<td>-8.48</td>
</tr>
<tr>
<td>H</td>
<td>Cl</td>
<td>4-Cl</td>
<td>103.1</td>
<td>99.8</td>
<td>96.1</td>
<td>-18.9</td>
<td>44.1</td>
<td>-8.23</td>
</tr>
<tr>
<td>I</td>
<td>Cl</td>
<td>3-OCH(_3)</td>
<td>103.7</td>
<td>95.8</td>
<td>89.7</td>
<td>27.5</td>
<td>68.4</td>
<td>-8.83</td>
</tr>
<tr>
<td>J</td>
<td>Cl</td>
<td>4-OCH(_3)</td>
<td>97.2</td>
<td>94.7</td>
<td>93.6</td>
<td>46.5</td>
<td>&gt;80</td>
<td>-8.65</td>
</tr>
<tr>
<td>ADR</td>
<td>Cl</td>
<td></td>
<td>55.1</td>
<td>31.8</td>
<td>-12.2</td>
<td>-44.2</td>
<td>&lt;10</td>
<td>-</td>
</tr>
</tbody>
</table>

ADR - ADRIAMYCIN

INTERACTIONS OF D. \([R_1=H, R_2=Cl]\)
Conclusion:
As indicated in docking results and compound D. 1-(3-benzamidophenyl)-3-(2-chlorophenyl)-prop-2-en-1-one shows interactions with ASP 1046 & LYS 868. The antiproliferative activity on MCF-7 shows that D. 1-(3-benzamidophenyl)-3-(2-chlorophenyl)-prop-2-en-1-one shows highest activity as GI50 value. Thus, it can be concluded that this compound may be a potential inhibitor for VEGFR-2.

Reference:
“Solid as solvent” - novel spectrophotometric analytical method for Gatifloxacin tablets using solids (eutectic liquid of Phenol and Niacinamide) as solubilizing agents (mixed solvency concept)

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Abstract: The main objective of the present study is to demonstrate the solvent action of solids. In the present study, a eutectic liquid (PNM 2510) obtained by triturating phenol crystals and niacinamide powder in 25:10 ratio on weight basis was employed to extract (dissolve) gatifloxacin from fine powder of tablets. Dilution was made with distilled water to carry out spectrophotometric estimation at 333 nm without the help of organic solvent. The solubility of gatifloxacin in PNM 2510 was more than 68 mg/ml (of PNM 2510) as compare to diistilled water (1.31mg/ml). Proposed spectrophotometric analytical method is novel, free from toxicity of organic solvent and accurate. Recovery studies and statistical data proved the accuracy, reproducibility and precision of the proposed method.

Introduction:
Majority of drugs show the problem of poor solubility, whether in the case of their analytical estimations or in the field of liquid dosage forms in the form of solutions. Commonly used organic solvents for spectrophotometric analysis of water insoluble drugs include methanol, ethanol, chloroform, benzene, dichloromethane, dimethyl formamide, acetonitrile. The main drawbacks of organic solvents include high cost, toxicity and pollution. The researchers are doing much work to give eco-friendly solutions for this challenge. Maheshwari has given a nice concept, known as mixed-solvency concept. By application of this concept, innumerable solvent systems can be developed. Maheshwari is of the opinion that each substance possesses solubilizing power. He has given several eco-friendly Method in the area of drug estimations and formulations precluding the use of toxic organic solvents. The solubility of a large number of poorly soluble drugs has been enhanced by mixed solvency concept.

Materials and Method:
Gatifloxacin bulk drug sample was a generous gift by M/S Ranbaxy Lab. Ltd. Dewas (India). Commercial tablets of gatifloxacin were procured from local market. All other chemicals used were of
analytical grade. A Shimadzu-1700 UV visible spectrophotometer with 1 cm matched silica cells was used for spectrophotometric analysis.

**Preparation of eutectic liquid:** Phenol and niacinamide were triturated in 25:10 ratio on weight basis to obtain a eutectic liquid (PNM 2510).

**Calibration curve:** 50 mg of drug was added in 500 ml vol. flask containing 10 ml of PNM 2510, and then flask was shaken to dissolve the drug. Then, the volume was made up to 500 ml with distilled water. This stock solution was suitably diluted with DW to produce standard solutions of 20-100 µg/ml. The absorbances of these standard solutions were noted at 333 nm against respective reagent blank.

**Preliminary solubility studies:** Preliminary solubility studies were performed in distilled water and in PNM 2510. The solubility of drug in distilled water was found to be 1.31 mg/ml and solubility in PNM 2510 was found to be more than 68 mg/ml.

**Proposed method of analysis:** Twenty tablets of tablet formulation I were weighed and crushed to get a fine powder. Tablet powder equivalent to 50 of gatifloxacin was transferred to a 500 ml volumetric flask containing 10 ml of PNM 2510, the flask was brisk shaken for 10 min to solubilize the drug from the tablet powder. Then, volume was made up to 500 ml with DW and the flask was shaken for 5 min to homogenize the contents. Filtration was carried out to remove the water insoluble tablet excipients. Then, the absorbance of the filtrate was noted at 333 nm against the reagent blank. The drug content was calculated using the calibration curve. Same procedure was repeated for tablet formulation II.

**Recovery studies:** To perform the recovery studies, standard gatifloxacin drug was added (15 mg and 30 mg, separately) to the pre-analyzed tablet powder equivalent to 50 mg gatifloxacin and the drug content was determined by the proposed method.

**Results and Discussion:**

The solubility of gatifloxacin in distilled water at room temperature was found to be 1.31 mg/ml. The solubility of gatifloxacin in PNM 2510 was more than 68 mg/ml (of PNM 2510). The percent drug estimated in tablet formulation I and II were 98.71±1.171 and 99.88±1.748, respectively. The values are very close to 100.0, indicating the accuracy of the proposed analytical method. Small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error further validated the method. Accuracy, reproducibility and precision of proposed method were further confirmed by percent recovery value. The mean percent recovery values ranged from 98.47±1.088 to 100.53±0.888 which are again very close to 100.0, indicating the accuracy of the proposed method. Proposed analytical technique is further supported by significantly small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error.
Conclusion:
In the present study, a eutectic liquid obtained by triturating phenol crystals and niacinamide powder in 25:10 ratio on weight basis was employed to extract (dissolve) gatifloxacin drug from fine powder of its tablets. Dilution was made with distilled water to carry out spectrophotometric estimation at 333 nm without the help of organic solvent. Proposed method is novel, economic, eco-friendly, rapid, free from toxicity of organic solvent, accurate and reproducible. Recovery studies and statistical data proved the accuracy, reproducibility and precision of the proposed method. The presence of tablet excipients, phenol and niacinamide did not interfere in the spectrophotometric estimation at 333 nm. Phenol and niacinamide do not interfere above 300 nm.

Acknowledgements:
The authors would like to acknowledge Ranbaxy Lab. Ltd. Dewas, India, for providing gift sample of Gatifloxacin and also acknowledge the School of pharmacy DAVV, Indore and Department of Pharmacy, SGSITS, Park Road, Indore (India) for providing necessary facilities.

References
Development of stability indicating assay method for simultaneous estimation of Phenylephrine HCl, Dextromethorphan HBr and Cetrizine HCl in syrup

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E mail address: sunitaverma.bltr@gmail.com

Abstract: The aim of present study was to developed a stability-indicating RP-HPLC method for the determination of Phenylephrine HCl, Dextromethorphan HBr and Cetrizine HCl in bulk and it’s marketed formulation. The method was developed using X'terra C₁₈ (250 x 4.6 mm, 5µ) as stationary phase with a mobile phase consisting of Acetonitrile: Methanol: Ammonium sulphate buffer (30:15:55% v/v/v) pH-3.4, at flow rate 1.0 mL/min. The detection of analytes was carried out at 272 and 230 nm. The stability of the drugs and formulation were evaluated by exposing to various stress conditions like acidic, alkaline, peroxide, thermal and UV light. The drugs were estimated in presence of its degradation products (if any) without interference. The % recovery by the proposed method was found equal to 100% .The method was validated as per ICH guidelines and the results were found to be satisfactory. Hence the method can be adopted for routine analysis of drug in its syrup formulation.

Introduction:
Phenylephrine HCl (PPH) is a selective α₁ adrenergic receptor agonist used as a decongestant, while Dextramethorphan HBr (DEX) as a antitussive and Cetrizine (CET) as antiallergic agent.. The numbers of analytical method were located in literature on selected drugs under study. Meyyanathan N² reported development and validation of dissolution study of sustained release dextromethorphan hydrobromide tablets. Thakkar K³ reported UV Spectrophotometric method for estimation of dextromethorphan in bulk and syrup formulation by area under curve method. Reddy P⁴ reported development and validation of a ultra performance liquid chromatographic method for assay of cetirizine dihydrochloride. Patel B⁵ reported simultaneous determination of phenylephrine hydrochloride, paracetamol, chlorpheniramine maleate and dextromethorphan hydrobromide in pharmaceutical preparations. No method was found in literature for determination of selected drugs in their combined dosage form. Hence, objective of the present study was to develop a stability indicating assay method for the estimation of these drugs.

Materials and Method:
Preparation of Mix stock standard solution: 75.0 mg PPH, 150.0 mg DEX and 50.0 mg CET were transferred to a 50.0 mL volumetric flask and diluted up to mark with mobile phase. (Conc. 1500µg/mL of PPH , 3000µg/mL of DEX and 1000µg/mL of CET)
Preparation of Working mix standard solution: A 5.0 mL of mix stock standard solution was diluted to 50.0 mL in a volumetric flask with mobile phase. (Conc. 150µg/mL of PPH, 300µg/mL of DEX and 100µg/mL of CET)

Preparation of Buffer solution- Ammonium Sulphate solution (pH-3.4): Accurately weighed quantities about 460 mg of Ammonium Sulphate and 1000 mg of Octane 1-sulphonic acid sodium salt were dissolved in 1000.0 mL of double distill water and pH was adjusted to 3.4 with 10% glacial acetic acid.

HPLC method was developed on Jasco LC-Net II/ADC using X'terra C18 (250 x 4.6 mm, 5µ) stationary phase with a mobile phase containing Acetonitrile: Methanol: Ammonium sulphate buffer (30:15:55%v/v/v) of pH 3.4. The reasonable retention time achieved using flow rate 1.0 mL/min at detection wavelengths 272, 230 nm. AR and GR grade chemicals used throughout the experimentation. The HPLC chromatogram of Std. PPH, DEX and CET using optimized chromatographic conditions is shown in Fig. 1.

Forced Degradation Study
This study was carried out via: i) Solution state analysis   ii) Solid state analysis

Preparation of stress sample solutions
Accurately weighed quantities of 5.0 mL of syrup equivalent to 7.5 mg of PPH (15.0 mg DEX, 5.0 mg CET) were transferred to series of 50.0 mL volumetric flasks. To each flask 5.0 mL of reagent (0.1N HCl/0.1N NaOH/ 3% H2O2) was added and kept at RT for a period of 24 h. After 24 h, the content of each flask was diluted with mobile phase, sonicated for 15 min and samples were filtered separately. In solid state analysis sample exposed to heat, humidity and UV light. After specified period stress samples were diluted, filtered and injected to system.

Results and Discussion:
The stability of the drugs was carried out for solution state stability and solid state stability .The results of solution state analysis indicate that in sample PPH, DEX and CET were degraded to about 4.55%, 8.08% and 4.67% in acid, 4.44%, 6.88% and 4.07% in alkali, 2.34%, 6.37% and 2.66% in peroxide when compared to unexposed sample. The results of solid state stability after 48 h indicate
that in sample PPH, DEX and CET were degraded to 1.83%, 3.88% and 3.63% when exposed to humidity, 1.7%, 4.28% and 2.83% when exposed to thermal, 3.99%, 6.43% and 3.45% when exposed to UV radiation as compared to unexposed sample.

Validation of Proposed Method

Validation parameters such as system suitability, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied. Summary of validation parameters is given in Table 1.

Table 1: Summary of validation parameters

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Accuracy Mean</th>
<th>%RSD</th>
<th>Precision Mean</th>
<th>%RSD</th>
<th>Intra-day study Mean</th>
<th>%RSD</th>
<th>Inter-day study Mean</th>
<th>%RSD</th>
<th>Robustness Mean</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPH</td>
<td>99.74</td>
<td>0.94</td>
<td>99.65</td>
<td>0.51</td>
<td>99.93</td>
<td>0.42</td>
<td>98.27</td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>99.66</td>
<td>0.85</td>
<td>99.53</td>
<td>0.55</td>
<td>99.76</td>
<td>0.40</td>
<td>98.60</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CET</td>
<td>99.78</td>
<td>0.41</td>
<td>99.47</td>
<td>0.39</td>
<td>99.76</td>
<td>0.46</td>
<td>99.05</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

The method is precise, accurate, rapid, reasonably specific and rugged. The developed method was found to be superior with respect to resolution of drug from its degradation products under applied stress conditions. Hence, method may be adopted for routine assay of selected drugs free of interferences from its degradation products in bulk and marketed formulations.

**References:**


Modelling, virtual screening and molecular docking to identify novel NS5B polymerase inhibitors as anti-HCV compounds

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Abstract: Prompted by the role of NS5B in the growth of hepatitis C virus we have attempted to develop structure based pharmacophore model with 2HBA, 2HBD and 2HY features was generated using 3D crystal structure of NS5B. The developed model were used for virtual screening of NCI databases and out of 255 compounds, two most potent hits were docked into the active site of NS5B and all the docked conformations showed interactions with ARG158, 386, 394, ASP318, 319, ASN411, 316, LYS141, SER367, GLY449, CYS366, GLN446 and TYR448 amino acids located at NS5B active site. In summary through our stepwise computational approaches we have identified novel structurally diverse NS5B inhibitors.

Introduction:
Hepatitis C virus (HCV) is a common pathogen with an estimated 3% of the world’s population (over 170 million people. At present, neither a vaccine against HCV nor an effective therapy with an acceptable broad spectrum of action against all genotypes of HCV is available. [1] The current standard of care (SoC) for HCV-infected patients consists of a combined therapy of pegylated interferon-a (pegIFNa) and ribavirin (RBV) for 24 or 48 weeks, depending on the HCV genotype. [2] Computational Method are playing increasingly larger and more important role in drug discovery and development and are believed to offer means of improved efficiency for the industry. [3] In this context, we have made an effort to identify novel, structurally diverse and druggable non-nucleoside HCV NS5B allosteric thumb pocket inhibitors through pharmacophore based virtual screening and molecular docking approach.

Materials and Method:
Structure based 3D pharmacophore generation:- Structure based pharmacophore is a powerful tool to interpret the intermolecular interactions between protein and its ligand. Once the pharmacophore model is identified, it can be used as a powerful tool for the discovery and development of new hits. The 3D structure of HCV-NS5B Polymerase Thumb pocket obtained from PDB entry (3G86) complexed with thiazine was used for structure based pharmacophore generation. Particularly, functional features like hydrogen bond donors, hydrogen bond acceptors and lipophilic groups were identified in active site and the complementary features were placed within the binding site in chemically reasonable position. After number of iterations the final hypothesis was selected which
contained six features: two hydrogen bond donors, two hydrogen bond acceptors and two hydrophobic groups (with additional 10 excluded volumes) describing the interactions between the protein and ligand.[4] The structure driven pharmacophore was validated by mapping of NM-283, ABT333, HCV796, PSI7977, R1479 which are known NS5B polymerase inhibitors.

**Virtual screening of chemical compound database**: - The developed structure based pharmacophore was used to screen NCI database. Molecular Docking: - The hits obtained from structure based pharmacophore were analyzed for their ability to interact with NS5B active site amino acids. Molecular Docking studies were carried out using the program CDOCKER which is a molecular dynamics simulated annealing based algorithm and available as extension of DS V.2.0. The structure of all the hits and known NS5B inhibitors (NM-283, ABT333, HCV796, PSI7977, and R1479) were docked into active site. Finally all the possible interaction modes for different alignments were analyzed on the basis of CDOCKER interaction energies. [4]

**Results and Discussion:**

Structure based 3D pharmacophore generation: - The pharmacophore generated from three-dimensional structure of the NS5B polymerase thumb pocket (PDB code 3G86) was validated by mapping of NM-283, ABT333, HCV796, PSI7977, and R1479. All the known NS5B inhibitors showed four features mapping with good fit value testifying the accuracy and strength of the model. Herein we analyzed the high fit value 2.506 and four features mapping of NM-283 which showed good predictions of model. In case of NM283 oxygen of (2R, 3S, 4S)-3, 4-dimethoxy tetrahydrofuran-2-yl) methanol mapped onto HBA1. Nitrogen of (3R, 4S)-3, 4-dimethoxy tetrahydrofuran mapped onto HBD1. 1 methyl pyrimidine -2 (1H)-one mapped onto HY1. Nitrogen of 4-amino 1 methyl pyrimidine-2(1H) one mapped onto HBD2. It showed that binding of pharmacophore with receptor is very strong and model is also Structure driven pharmacophore based virtual screening: - In quest to identify novel, potential lead compounds structure driven pharmacophore based virtual screening was performed using NCI chemical compounds database. 255 hits were retrieved which were sorted on the basis of their fit value and Lipinski’s violation. After filtration five potential compounds namely NSC675004, NSC305724, NSC140715, NSC277284, NSC715977, were selected with fit value ranging from 2.78-1.26 respectively and zero Lipinski’s violation. Out of five NSC675004 exhibited full feature mapping with fit value 2.78 and appeared to be most active compound.

Molecular docking studies: - The 3D structure of NS5B polymerase thumb pocket complexed with thiazine was obtained from PDB (3G86) and used for docking. Potential hits identified using structure based pharmacophore and reference compounds NM283 which are known NS5B polymerase inhibitor were docked into active site of thumb pocket using CDOCKER software implemented in DS
(Discovery studio). NSC 675004 showed a high CDOCKER interaction energy of 48.192 respectively.

It was observed that in case of NSC675004 as shown in Figure 2 that oxygen of ethane 1,2 diol showed hydrogen bond interaction with ARG158,ASN411,ARG386, another oxygen of ethane 1,2 diol showed hydrogen bond interaction with TYR415,ASP318,GLY557.Oxygen of methyl acetate showed hydrogen bond interaction with GLN446,TYR448 similarly oxygen of methyl format showed hydrogen bond interaction with CYS366,LYS141.

![Molecular interaction of NSC675004 with active site amino acids of NS5B polymerase](image)

**Figure 2**: Molecular interaction of NSC675004 with active site amino acids of NS5B polymerase thumb pocket.

**Conclusion:**

With the help of modern days *In-silico* protocols we have developed universal pharmacophores through structure based molecular modelling. Briefly, in present work we have identified potent, structurally diverse, druggable and novel NS5B inhibitors, which could be raised into anti-HCV drugs.

**Acknowledgment:**

Computational resources were provided by Banasthali University and the authors thanks Vice Chancellor for providing all the necessary facility.

**References:**

Isolation, purification and identification of unknown degradation products in Rabeprazole Sodium

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Abstract: In this work, we tried to isolate, purify and identify the unknown degradation related impurities formed in Rabeprazole Sodium tablets during the stability study at 40˚C/75RH for 2 months. During experiment it was observed that the impurities are related to drug substance and are present above the threshold. So, it became necessary to identify and separate it to avoid any side effects and toxicity which can produce after its administration. The degradation samples were analysed by HPLC and LC-MS. The unknown impurities formed were isolated by preparative HPLC method. Five known impurities along with two unknown impurities in Rabeprazole sodium were detected by simple HPLC using column Inertsil ODS-3V, with mobile phase of 0.01 M KH₂PO₄ WITH 6.0 PH adjusted with KOH and Acetonitrile (60:35). LC-MS used to determine molecular weight of impurities. The isolated degradants were identified by UV, MS and NMR studies.

Introduction:
Rabeprazole is an anti-ulcer drug which inhibits the proton pump (H⁺/K⁺-AT-Pase) of coating gastric cells and reduces gastric acid secretion. Degradation product may define as, “A molecule resulting from a change in the drug substance brought about by time”. Rabeprazole tablet formulation was not stable at 40˚C and 75% RH humidity during preliminary study. For characterization of trace level of impurity isolation is necessary thus, acute need exist of preparative HPLC which is the most powerful and versatile method for isolation and purification of impurities. Characterization of impurity is necessary in order to establish the biological safety and toxicological data.

Material and Methods:

Instruments: HPLC equipped with quaternary pump, Auto sampler and DAD detector. The primary goal of good separation method is the resolution of all impurity. Modern HPLC has many applications include separation, identification purification and quantification of various compounds. In which solute partition in mobile phase and finally get traveled to the detector. The elution in HPLC depends upon no of theoretical plate and height equivalent to mechanical plates. Preparative liquid chromatography (PLC) with binary pump Auto sampler, Rheodyne injector, DAD detector.
LC/MS with Ion trap mass spectrometer: LC-MS is superior and advance analytical tool for the identification and characterization of the impurities in the APIs or a drug product. A combination of this technique is finding increase the use in the analytical and structural organic chemistry. HPLC resolved the mixture of compound into its individual components, while MS an excellent technique for identification of compounds.

**Development of HPLC method:** The mobile phase was selected on trail and error basis such that all impurity peaks were well resolved and eluted out having good peak shape and base to base separation.

**Development of LC-MS method:** It is observed that in trial-3 all impurity peaks were well resolved and eluted out within 75 min with good peak shape and base to base separation. Selection suitable chromatographic parameter using suitable gradient system.

**Optimized mass spectrometric parameter:** Using above conditions the stress samples were analysed and the sample kept at 40°C and 75%RH were analysed by LCMS method to determine the molecular weight of the unknown impurities.

**Development of preparative HPLC method:** Before development of preparative HPLC method was carry out the 1, 2, 3- trial. It is observed that in trial-3 all impurity peaks were well resolved and eluted out within 80 min with good peak shape and base to base separation from main peak of Rabeprazole.

Then system suitability study was carry out by trial-3 parameter. Using above conditions the sample kept at 40°C and 75%RH were used for isolation of unknown impurities. The isolated impurities were re inject to confirm the identity by rechecking the retention time of the fraction collected.

**Purification of impurity:** The purification of impurity-1 is carry out by preparative HPLC and impurity-2 was collected by solvent extraction technique respectively. Purified impurity-1 and impurity-2 was solidified using vacuum rotary evaporator.

**Result and Discussion:**

It was observed that various stressed sample two impurities generated i.e. Impurity 1 and Impurity 2 found in different conditions for example Impurity 1 generated during acid hydrolysis where as Impurity 2 generated during oxidative hydrolysis only. Out of the total 5 impurities are known impurities of Rabeprazole sodium but the stability conditions shows the presence of seven impurities out of which two were unknown. The retention time of two unknown impurities were found to be 4.563 min and 51.924 min respectively.

The same stress sample was subjected to LCMS study using the above mentioned chromatographic conditions. The retention time of the unknown impurity 1 was found to be 10.6 and impurity 2 was found to be 53.0 min. The molecular weights of the same were found to be 270.0 and 651.3.

The Mass spectra reveals that the molecular weight of both impurities and of the Rabeprazole standard solution found similar to each other (i.e. 270 and 651.3) The samples were then subjected to
preparative HPLC for their isolation, the isolated impurities were re injected to correlate the retention time of impurity 1 and 2 using HPLC and LCMS method. The isolated fractions were then purified by solvent extraction technique and rechecked for their retention time and molecular weight in LC-MS. The retention time and molecular weight were found to be similar before the isolation and purification process. Finally the identification of the isolated and dried fraction of impurities were subjected to UV, PNMR and CMNR studies. Impurity 1 was completely characterized but the NMR data of Impurity 2 could not be generated as it was undergoing degradation during the recording process.

The λmax of impurity 1 and 2 were found to be 290.0 nm and 260 nm in their solvent of respective solubility. The NMR data of Impurity 1 is given in the table;

<table>
<thead>
<tr>
<th>POSITION</th>
<th>1H</th>
<th>IMPURITY 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4</td>
<td>2H</td>
<td>7.30 (m) (126.47 Hz)</td>
</tr>
<tr>
<td>2,3</td>
<td>2H</td>
<td>7.55 (m) (117.50 Hz)</td>
</tr>
<tr>
<td>6</td>
<td>1H</td>
<td>7.90 (d.6.0) (142.58 Hz)</td>
</tr>
<tr>
<td>7</td>
<td>1H</td>
<td>6.50 (d.6.0) (11747 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>3H</td>
<td>2.0380 (s) (14.11 Hz)</td>
</tr>
</tbody>
</table>

Conclusion:
Two unknown degradation related impurities of Rabeprazole sodium formulation were generated during accelerated stability condition at 40°C/RH-two month. Impurity-1 polar and impurity-2 non polar in nature, it was isolated by preparative HPLC and identified by LC-MS using electrospray ionization technique. The impurity-1 was characterized which conforms the structure of impurity-1 and structure of impurity-2 could not be performed because it undergoes degradation.

References:
Rapid densitometric determination of *cassia fistula* in polyherbal oil formulations

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2 School of Pharmacy, Devi Ahilya University, Indore (Madhya Pradesh), India.
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Abstract: Chrysophanol is a therapeutically important constituent of the seeds of *Cassia fistula*; it can be used as reliable marker for estimation of *Cassia fistula* in oil macerates. Present study reports a simple, selective, rapid and sensitive planer chromatographic method to estimate quantitatively the chrysophanol in polyherbal oil formulations. Separation of chrysophanol ($R_f=0.28$) from methanol extract of oil formulations was achieved using hexane: ethyl acetate (9.5: 0.5 v/v) as mobile phase. Quantitation was achieved at 290 nm over the concentration range of 50–300 ng/band. The method was validated following standard protocol. Poly-herbal oil formulations were analyzed with reasonable accuracy and no matrix interference was observed.

Introduction:
Dried powdered seed pulp of *Cassia fistula* L (Amaltas) is an essential component of many polyherbal oil formulations in Chinese and Indian traditional systems of medicine. Chrysophanol (1, 8-dihydroxy-3 methylanthracenedione, 1), an anthraquinone derivative was identified as major antibacterial and antifungal metabolite in seeds of *Cassia fistula*. It can be used as a reliable marker for quantitative estimation of *Cassia fistula* in oil macerates of seeds of *Cassia fistula* [1, 2]. Quality and safety may be ensured using Standardization of traditional formulations. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Modern chromatographic Method of identification and quantification of active principles are most widely used for marker based standardization of poly herbal formulation [3]. But there is no report on estimation of chrysophanol in complex poly herbal oil formulations. A simple and rapid High performance thin layer chromatography (HPTLC) method for determination of chrysophanol in medicinal oil formulations is highly desired. The method is validated as per ICH guideline [4].

Materials and Method:
Materials: Analytical grade solvents were obtained from Merck (Mumbai, India). Reference Chrysophanol was purchased from Sigma-Aldrich (Germany). Ayurvedic polyherbal oil formulations ‘Kustharaksasa oil’ and ‘Kandughna Oil’ which contains *Cassia fistula* seeds as one of the component were selected for studies. Samples of oil formulations in triplicate, manufactured by various Ayurvedic drug formularies were collected in Indore, India.
Method: (a) Preparation of standard solutions: A Standard stock solution of 1mg/ml of chrysophanol was prepared in methanol keeping the purity of reference chrysophanol into consideration. Whatman filter paper (No. 1) was used for filtration. Working solutions of analyte were prepared by appropriate dilutions of the stock solution with methanol.

(b)Chromatographic conditions: CAMAG HPTLC system (Switzerland) with a Linomat 5 sample applicator was used for the analysis. The analysis was performed in air-conditioned room maintained at 22°C and 55 % humidity. Five microliters of the standard solutions were spotted as bands of 6mm width by using the auto sampler fitted with a 100µl Hamilton syringe. The HPTLC aluminum plates 60F254 plates were developed using hexane: ethyl acetate (9.5: 0.5 v/v) in a CAMAG twin—trough chamber presaturated with 30 ml mobile phase. Band of chrysophanol were scanned from 200 to 400 nm so as to record their UV spectrum. Densitograms were recorded at the wavelength of maximum absorption of chrysophanol (290nm). The concentration of reference compound was plotted against peak area to obtain calibration curve over the concentration range of 50–300 ng/band for chrysophanol.

(c) HPTLC analysis of polyherbal oil formulations: Oil formulations (100 g) were homogenized at 2000rpm with methanol (1:5, w/v) for 20 min and the supernatants were collected. The residues were resuspended in methanol and the extraction was repeated five more times under the same conditions. The supernatants were collected and concentrated using vacuum at ≤45 ºC and made up to a known volume with methanol. The extracts were filtered through 0.45µm filter and HPTLC was performed under the optimized conditions. For the confirmation of identity and purity of bands, the bands corresponding to chrysophanol in the sample extract was overlaid with the absorption spectra of reference chrysophanol at the start, middle and end positions of the bands. The amounts of chrysophanol in formulations were quantified using calibration curves plotted with the reference compounds. ICH guidelines were used for validation.

Results and Discussion:
The literature revealed that methanol is preferred for extraction of chrysophanol from seeds of Cassia fistula. The same was used for extraction of chrysophanol from oil formulations since oils are immiscible in this solvent system. Multiple extractions were carried out to ensure complete extraction. The mobile phase of hexane: ethyl acetate (9.5: 0.5 v/v) was found to produce good resolution for chrysophanol in oil formulations with Rf of 0.28 at λmax 290nm. The calibration curve was linear within the concentration range of 50-300ng/band with a correlation coefficient of 0.9997. Recovery was found to be in range of 97%-102% at three different concentration levels. The repeatability of sample application and peak area measurement by the HPTLC method, expressed as % relative standard deviation (%RSD), was 0.97 and 0.73 respectively. Intra-day and inter-day variations
(Intermediate precisions) are less than 2% RSD. LOD and LOQ value was found to be 5ng/band and 15ng/band respectively. During robustness studies the %RSD was found to be less than 2 in each case. The proposed validated method was successfully applied in quantitative determination of chrysophanol in polyherbal oil formulation (Table 1). The study revealed variation in chrysophanol content in different brands. It stresses the need of standardization of raw Materials used and uniformity in method of manufacturing to be followed by different manufacturers.

Table 1: Chrysophanol content found in various oil formulations

<table>
<thead>
<tr>
<th>Name of polyherbal oil</th>
<th>Sample</th>
<th>HPTLC analysis* (mg/100gm oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kustharaksasa Taila</td>
<td>1</td>
<td>0.0189±0.0002</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0136±0.0002</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0153±0.0002</td>
</tr>
<tr>
<td>Kandughna Taila</td>
<td>1</td>
<td>0.0677±0.0002</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0730±0.0003</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0643±0.0003</td>
</tr>
</tbody>
</table>

*Mean ± Relative standard deviation , (n=3)

Conclusion:
A method for quantification of *Cassia fistula* using chrysophanol as analytical marker in polyherbal oil formulations was developed. This method does not require separation of unsaponifiable matter; oil extract can be directly used for analysis. Also, the low solvent consumption leads to environmentally friendly method. The method was found to be sensitive, simple, specific, precise, and accurate. It can be used for routine quality control of polyherbal oil formulations containing *Cassia fistula*.

Acknowledgement:
Authors are thankful to SICART, Vallabh Vidyamagar (Gujarat) for analytical facilities.

References:
Synthesis and structural activity relationship of β-carboline derivatives as an antitumor agents

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Abstract: The potential of β-carboline compounds as anticancer agents and our work have stimulated studies into their design, synthesis and structure–activity-relationship (SAR) with an aim to the improvement of their antitumor potential. Hence an introspective research is under taken to find out the newer synthetic antitumor drugs with improved activity and less toxicity. We performed the QSAR studies, on a novel series of β-carboline derivatives containing carbohydrazide skeleton as anticancer agents. The best QSAR model has the characters of large F, low P value, r² and q² values close to 1, as well as P< 0.001. We developed four good models. Among these models, model 4 is best model.

Introduction:
In the present scenario, it has been reported to be of high mortality rate of human population due to cancer within all age groups [1]. With the background poverty status, most of the people depend on newer synthetic drugs with lesser toxicity. Major progress in chemotherapy requires new and useful antitumor agents to eradicate the entire range of cancer diseases in the world over. New compounds can be developed by a wide variety of approaches, ranging from empirical screening to rational design: (a) from natural products, (b) by screening of synthetic compounds prepared for other purposes, and (c) by semi synthesis or total synthesis using new biological concepts and structure-activity relationships of potentially interesting known compounds. The potential of β-carboline compounds as anticancer agents and previous work have stimulated studies into their design, synthesis and structure–activity-relationship (SAR). SAR studies on a variety of synthetic β-carboline derivatives have demonstrated that the introduction of appropriated substituents into position-1, -2, -3 and -9 of the β-carboline skeleton resulted in more potent antitumor derivatives, with reduced toxicity.[2]

Materials and Method:
For this study we have selected a series of 20 compounds which is available in literature and on the basis of these literatures we synthesized different compound. For the synthesis of these compounds and performing the QSAR studies of synthesized compound below mentioned scheme was performed.
For the molecular structure generation and calculation of descriptors Chem office 7.0 is used and VALSTAT is used for the statistical analysis.

**Results and Discussion:**

Based on QSAR model, few compounds are designed. Synthesis of designed compounds has been performed as showed in scheme 1. After completion of synthesis, physiochemical characterization of synthesized compounds has been performed, and the result of best model (model 4) is as follow.

QSAR models have been developed by using multiple linear regression analysis. The best QSAR model has the characters of large F, low P value, r2 and q2 values close to 1, as well as P< 0.001. We developed four good models. Among these four models, model 4 is best model. Model 4: BA =
Conclusion:
QSAR studies were performed on the β-carboline derivatives containing carbohydrazide skeleton. For the anti-tumor activity, QSAR models were developed. The best model 4 revealed that which type of physiochemical properties predominantly affect the inhibition of enzyme. Based on QSAR study and developed best QSAR model (model 4), the twenty compounds were designed and their biological activity was calculated with the help of best model.

Acknowledgement:
Authors are thankful to SAIL, RGPV, Bhopal (MP) for spectral analysis. It is a profound pleasure to express my gratitude towards VNS Group of Institutions for there continues support and encouragement.

References:
Synthesis, characterization and evaluation of some BenzodiazepinoQuinolin-2-one derivatives as possible hypnotic agents

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Abstract: A series of 2, 4-disubstituted-1, 5-benzodiazepine derivatives [V-a (1-6)/V-b (1-6)] were synthesized by appropriate synthetic route. All the synthesized compounds were satisfactorily confirmed by IR, NMR and Mass spectral data and were evaluated for their hypnotic activity based on the potentiation of barbiturate induced sleeping time in mice. Among all the synthesized compounds [V-a2], [V-a3] and [V-b6] potentiated the phenobarbitone sleeping time in mice very significantly, thus these compounds exhibited excellent hypnotic activity using diazepam as standard reference.

Introduction:
Insomnia is defined as a sleep disorder with an experience of inadequate or poor-quality sleep.\(^1\)\(^-\)\(^2\) It is the most common disorder affecting millions of people as their primary or co-morbid condition.\(^3\) As per the WHO estimates around 70 million people are suffering from this disorder throughout the globe. The current clinical approach employs different benzodiazepine derivatives that are used as hypnotic agents. Quinolin-2(1H)-one are known for varied biological activity and number of clinically used drugs belong to this class e.g. Carteolol for treatment of glaucoma, Rebamipide for antacid, Aripiprazole as an anti schizophrenic agent etc. Realising importance of quinolin-2(1H)-one, we thought of synthesizing derivatives with substituted 1,5-benzodiazepine moiety at 3\(^{rd}\) position of quinolin-2(1H)-one framework.

Materials and method:

![Chemical structures and reaction scheme]

Fig:1 Compounds were synthesized following reaction conditions maintained in the reaction scheme

Results and discussion:
All the synthesized compounds were satisfactorily characterized by IR, NMR and Mass spectral data. The physical data of 3-(2-(4-substitutedphenyl/phenyl)-1H-benzo[b][1,5]diazepin-4-yl)-4-methoxy-1-phenyl/methyquinolin-2(1H)-one [V-a(1-6)/V-b(1-6):

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment</th>
<th>Sleep Latency (minutes)</th>
<th>Duration of sleep (minutes)</th>
<th>Muscle relaxation time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (vehicle)</td>
<td>67.17 ± 1.621</td>
<td>27.67 ± 1.687</td>
<td>69.17 ± 6.204</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam</td>
<td>37.17 ± 1.873</td>
<td>121.80 ± 3.816</td>
<td>143.50 ± 9.677</td>
</tr>
<tr>
<td>4</td>
<td>V-a2</td>
<td>36.50 ± 1.522</td>
<td>104.80 ± 6.276</td>
<td>176.70 ± 2.692</td>
</tr>
<tr>
<td>5</td>
<td>V-a3</td>
<td>26.67 ± 2.629</td>
<td>110.00 ± 6.028</td>
<td>169.20 ± 3.754</td>
</tr>
<tr>
<td>14</td>
<td>V-b6</td>
<td>38.67 ± 2.060</td>
<td>110.70 ± 2.140</td>
<td>168.30 ± 1.542</td>
</tr>
</tbody>
</table>

Conclusion:
Twelve derivatives of 2,4 disubstituted 1,5-benzodiazepine {3-(2-(4-substitutedphenyl/phenyl)-1H-benzo[b][1,5)diazepin-4-yl)-4-methoxy-1-phenyl/methyquinolin-2(1H)-one} [V-a(1-6)/V-b(1-6)
were synthesized and confirmed by IR NMR and Mass spectral data and were evaluated for their hypnotic activity. Out of the 12 compounds evaluated, compound with R=C\textsubscript{6}H\textsubscript{5}, R\textsubscript{1}=Cl [V-a2]; R=C\textsubscript{6}H\textsubscript{5}, R\textsubscript{1}=OCH\textsubscript{3}; (V-a3) and R=CH\textsubscript{3}, R\textsubscript{1}=Br [V-b6] potentiated phenobarbitone induced sleeping time in mice significantly. These compounds showed significant suppression in sleep latency and increase in duration in sleep thus showed excellent hypnotic activity using diazepam as reference standard.

Acknowledgement:
My sincere thanks to the Directors, NMR and Mass Centre SAIF, Panjab University Chandigarh for providing the necessary spectra.

Reference:
Synthesis of oxime derivative as a possible antidote for organophosphorus poisoning

Emmie de Abreu, Prasad Ramakant Tari, Shivlingrao Mamle Desai
Department of Pharmaceutical Chemistry, P.E.S’s Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda-Goa, 403401.
Email address: emmie.deabreu@gmail.com

Abstract: Organophosphate (OP) compounds exert inhibition on cholinesterase (ChE) activity by irreversibly binding to the catalytic site of an enzyme. Oximes are compounds generally used to reverse the ChE inhibition caused by OP agents. Various oximes and oxazoles having quinolin-2(1H)-one were synthesized by condensation and cyclisation of chalcones with NH2OH.HCl in presence of pyridine respectively. Synthesized compound were tested for in vitro reactivation of methyl parathion inhibited acetylcholinesterase (AChE) enzyme using pralidoxime and obidoxime as standard reference following Ellman et al method.

Introduction: Organophosphates are chemical agents used mainly in agriculture (pesticides), as nerve agents and therapeutic agents(ecothiopate). They are readily available “over the counter”. Exposure to organophosphates in an attempt to commit suicide is a key problem, particularly in the developing countries.WHO estimates indicates that each year, one million accidental poisonings and two million suicide attempts involving pesticides occur worldwide. In India, the rate of poisoning as suicidal method is about 40% and is also a common cause of poisoning due to chronic exposure by farmers. Although atropine remains the mainstay of therapy, the main adverse-effect of atropine is anticholinergic delirium. Clinical application of monopyridinium and bispyridinium oximes are well known eg. pralidoxime ,trimidoxime available in the market, but these too have many side effects. In view of these we thought of a benzo-fused pyridine ring system (quinolin-2(1H)-ones), oximes and oxazoles.

Materials and Method: Figure 1: Scheme
The starting material used in the synthesis was prepared following the literature. The title compounds were synthesized as mentioned in the scheme of the reaction in Figure 1.

**Table 1: Physical and chemical data**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>Molecular formula</th>
<th>M.W.</th>
<th>M.P. °C</th>
<th>Yield</th>
<th>Rᵣ Value</th>
<th>λ max (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>-C₆H₅</td>
<td>-</td>
<td>C₁₈H₁₈N₂O₃</td>
<td>308.26</td>
<td>240</td>
<td>53.84</td>
<td>0.878</td>
<td>280.5</td>
</tr>
<tr>
<td>5a</td>
<td>-C₆H₅</td>
<td>-C₆H₅</td>
<td>C₂₄H₁₆N₂O₂</td>
<td>364.39</td>
<td>154-56</td>
<td>54.05</td>
<td>0.692</td>
<td>238</td>
</tr>
<tr>
<td>7g</td>
<td>-CH₃</td>
<td>3-NO₂</td>
<td>C₂₀H₁₇N₃O₅</td>
<td>379.36</td>
<td>176-78</td>
<td>57.6</td>
<td>0.70</td>
<td>226.7</td>
</tr>
</tbody>
</table>

Spectral data of representative compound has been given herewith:

3-(1E)-N-hydroxyethanimidoyl]-4-methoxy-1-phenylquinolin-2(1H)-one (3a): IR (KBr, cm⁻¹): 3290.56 (-OH oxime); 3062.92, 3008.95 (aromatic -C-H); 2951.09, 2854.65 (aliphatic -C-H str.); 1681.93 (-C=N); 1637.56 (-C=O amide); 1107.14 (-C-O-C).¹H NMR (CDCl₃, δ ppm): 8.1-6.7 (m, 9H, Ar-H); 4.0 (s, 3H, -OCH₃); 2.6 (s, 3H, C-CH₃); 1.2 (s, 1H, oxime).¹³C NMR (CDCl₃, δ ppm): 167.81, 158.76, 141.53, 137.03, 132.02, 130.37, 130.18, 129.26, 129.16, 123.05, 123.03, 117.27, 108.95, 61.48, 10.75. EI-MS (m/e, M⁺): 308.

**In Vitro Reactivation of Methyl Parathion inhibited AChE**:

Reactivation of Methyl Parathion inhibited AChE was performed by Ellman et al method and percentage reactivation were calculated by using following formula:

\[ \text{Percentage reactivation} = \left( \frac{E_r - E_i}{E_o - E_i} \right) \times 100 \]

where

- \(E_o\) is the control enzyme activity at ‘0’ min (without inhibitor or oxime)
- \(E_i\) is inhibited enzyme activity (without oxime)
- \(E_r\) is the activity of reactivated enzyme after incubation with the oxime test compounds.

**Results and Discussion:**

We have tested the potential of reactivation of newly synthesized oximes and oxazoles (Table 2) against methyl parathion inhibited AChE and compared with the currently available oximes (obidoxime and pralidoxime). The mechanism by which the oxime exerts AChE reactivation property is based on the chemical principle that oxime reactivation occurs by the nucleophilic attack of oximate anions on the OP–AChE conjugates. Among the synthesized compounds 7f and 7g showed good reactivation.

**Table 2: Percentage reactivation**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage reactivation (%) 0-60 min (10⁻¹ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>7f</td>
<td>8.56</td>
</tr>
</tbody>
</table>
Conclusion:
Reactivation of methyl parathion inhibited acetylcholinesterase was performed by Ellman et al. method. All the synthesized compounds were found to be good reactivator of inhibited enzyme. Compounds having nitro substitution at 3rd and 4th position gave good activity. Among the synthesized compound, compound with R=CH$_3$, R$_1$= 4-NO$_2$(7f) and R=CH$_3$, R$_1$= 3-NO$_2$(7g) showed good reactivation of 57.46% and 46.94% respectively after 60 minutes of incubation with concentration of 10$^{-3}$ M.

Acknowledgement:
A special thanks to Director, NMR and MASS center, Panjab University Chandigarh for providing necessary spectra and analysis on time.

References:

<table>
<thead>
<tr>
<th></th>
<th>7g</th>
<th>Pralidoxime</th>
<th>Obidoxime</th>
</tr>
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<tbody>
<tr>
<td>4.89</td>
<td>18.09</td>
<td>27.38</td>
<td>34.47</td>
</tr>
<tr>
<td>45.72</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>71.64</td>
<td>82.64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Synthesis of 2-Mercaptopyrimidinylquinolin-2-one derivatives as antibacterial and anti-tuberculular agents

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Abstract: Twelve derivatives {III-a (1-6)/III-b (1-6)} of 2-mercaptopyrimidinylquinolin-2(1H)-one derivatives were synthesized and satisfactorily characterized by UV, IR, NMR and Mass spectral data and elemental analysis. The synthesized compounds were evaluated for their antibacterial and anti-tuberculular activity. For antibacterial activity, derivatives were tested against four bacterial strains two gram positive (Staphylococcus aureus and Bacillus subtilis) and two gram negative (Pseudomonas aeruginosa and Escherichia coli). Among all the compounds, compound III-a2 and III-a6 was found to be most potent against Bacillus subtilis at minimum inhibitory concentration of 0.2µg/ml as compared to the standard drug Ciprofloxacin (2µg/ml).

Introduction:
Bacteria can cause disease by producing harmful substances (toxins), invading tissues, or both. In the United States, bacterial infections are a leading cause of death in children and the elderly. The development of antibacterial resistance is associated with increase in mortality, morbidity, length of hospitalisation, and cost of healthcare. Bacterial diseases like Tuberculosis have also taken a large toll over humanity. According to the WHO in the year 2011, 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people have died from TB (990 000 among HIV negative people and 430 000 HIV-associated TB deaths. This bacterium primarily infects the lung macrophages leading to pathogenesis of the disease. Quinolones have shown to be center of considerable scientific and clinical interest since 1960’s and also shown to possess ideal antibiotic properties like high potency, a broad spectrum of activity and good bioavailability. Several research groups reported Quinolin-2(1H)-one to have anti-HIV, anti-cancer, anti-viral, anti-angiogenic, anti-bacterial activity etc. In view of the above facts, we report the synthesis and in-vitro activities of 2-mercaptopyrimidinylquinolin-2(1H)-one derivatives 1,2.

Materials and Method:
Synthesis of 3-acetyl-4-hydroxy-1-phenyl/methylquinolin-2(1H)-one was carried out by following literature
The title compounds were synthesized by the following reaction conditions mentioned in Figure 1.
The compounds were satisfactorily characterized by UV, IR, NMR and Mass spectral data. The spectral data of representative compound is given here: 3-[6-(4-Chlorophenyl)-2-mercaptopyrimidin-4-yl]-4-methoxy-1-phenylquinolin-2(1H)-one (III-a2):

Yield = 61%, m.p. 152-4ºC, RF value 0.88, λmax 219.70nm.

IR (KBr, cm⁻¹): IR (KBr, cm⁻¹): 3091.89, 3064.89, 3041.74 (aromatic -C-H); 2951.09 (aliphatic -C-H str.); 1641.42 (-C=N); 1610.56 (-C=O amide); 1091.71 (-C-O-C ether); 759.95 (-C-Cl)¹H NMR (DMSO-d⁶, δ ppm): 15.11 (s, 1H, S-H), 7.21-8.36 (m, 14H, Ar-H); 3.51 (s, 3H, O-CH₃)

¹³C NMR (DMSO-d⁶, δ ppm): 198.17; 187.07; 176.47; 165.83; 154.48; 148.14; 139.56; 138.18; 136.38; 135.36; 133.75; 131.59; 129.13; 128.08; 128.01; 126.16; 125.79; 125.31; 120.26; 115.98; 111.40; 30.05 LC-MS: 472 [M⁺⁺]

Elemental (CHN) analysis calculated for C₂₆H₁₈ClN₃O₂S: C, 66.16; H, 3.84; N, 8.90 (Found: C, 66.18; H, 3.89; N, 8.92)

**Biological activity:**³ Antibacterial activity

The antibacterial activity was determined by Minimum inhibitory concentration (MIC).

**Table 1:** shows the antibacterial activity data of the title compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>Minimum Inhibitory concentrations in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. subtilus</td>
</tr>
<tr>
<td>III-a2</td>
<td>C₆H₅</td>
<td>4-Cl</td>
<td>0.2</td>
</tr>
<tr>
<td>III-a6</td>
<td>C₆H₅</td>
<td>4-Br</td>
<td>0.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Anti-tubercular activity

The antitubercular activity of derivatives were assessed against H₃7Rv of *Mycobacterium tuberculosis* (Vaccine strain) using microplateAlamar Blue assay (MABA) (Lourenco et al., 2007).

“Strategic Approaches to Strengthen Academic and Industrial Collaboration”  www.ijpsr.com
Pyrazinamide, streptomycin and ciprofloxacin were used as standard drugs with MIC of 3.125µg/ml, 6.25µg/ml and 3.125µg/ml respectively. Most of the compounds showed MIC at 50µg/ml which was found to be much higher as compared to standards.

**Conclusion:**
The pharmacological studies showed that all the twelve 3-(2-mercapto-6-phenyl/substitutedphenylpyrimidin-4-yl)-4-methoxy-1-phenyl/methylquinolin-2(1H)-one{III-a(1-6)/III-b(1-6)} showed significant antibacterial and antitubercular activity. *Bacillus subtilis* was found to be the most sensitive strain. Two compounds, compound (III-a2) and (III-a6) were found to show better antibacterial activity then the standard drug ciprofloxacin. This probably is due to the electronic effect of the halo substitution at the para position of aryl ring. With respect to antitubercular activity the compounds showed MIC at 50µg/ml which was found to be much higher as compared to the standards.

**Acknowledgement:**
We would sincerely like to thank the Directors, NMR and Mass Center, SAIF Punjab University-Chandigarh, for providing the necessary spectra.

**References:**
Synthesis and evaluation of 1, 6- bis - ((substituted)) - pyridin-2-one derivatives as possible cholinesterase reactivators

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Department of Pharmaceutical Chemistry, P.E.S.’s Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi- Goa.
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Abstract: The Pyridin-2-one system structure is found in many natural products, antibiotic substances and cyclic tricarbonylmethane compounds\(^{(1)}\). The Schiff’s base moiety has displayed affinities for various physiological enzymes \(^{(2)}\). Schiff’s bases of 2-pyridones were synthesized in a series of three steps. All the synthesized compounds were characterized by FTIR spectroscopy, and compounds of interest were characterized by \(\text{\text{\text{\text{\text{\text{$^1$}}H and $^{13}$C)}}}}\) - NMR spectroscopy and LC-MS spectroscopy. All the 1, 6- bis ((Substituted) eneamino) -2-oxo-1, 2, 3, 4- tetrahydropyridine-3,5-dicarboxylic acid (III b) compounds were evaluated \text{in vitro} for cholinesterase reactivation by Ellman assay method. Compound IIIb2 showed good levels of enzyme reactivation in comparison to the standard reactivator Pralidoxime.

Introduction:
Organophosphorous compounds (OPC) have been used as pesticides and developed as warfare agents. Exposure to even small amounts of an organophosphorous compound could be fatal. The mechanism of OPC poisoning involves phosphorylation of the serine hydroxyl group at the active site of Acetyl cholinesterase (AChE), causing inactivation of this essential enzyme which has an important role in neurotransmission, leading to accumulation of acetylcholine at cholinergic receptor sites, producing continuous stimulation of cholinergic fibres throughout the central and peripheral nervous systems. \(^{(3)}\) Acetyl cholinesterase reactivators are a group of drugs originally developed as antidotes for the treatment of nerve agent organophosphorous compounds poisoning. To overcome the disadvantages encountered in present day therapies to combat organophosphorous poisoning and exploit the properties of the molecule under study, we thought of pursuing the 2-pyridone system with a different approach of employing schiff’s base moiety instead of conventional oxime moiety, the rationale being an isosteric replacement of \((-\text{N-OH})\) with \((-\text{N=CH})\).

Materials and Method:
The reaction was shown in scheme with reagents and condition.
Results and Discussion:

2, 3-bis ((E)-(4-Methoxybenzylidene) amino)-4-oxo-3-azaspiro (5.5) undec-1-ene-1, 5-dicarboxylic acid (III b10):

M.p.241-2440 C. IR peaks (KBr, cm\(^{-1}\)): 1150.29, 1249.87(OCH\(_3\)); 1501.26(CH=N); 1602.87(cyclic amide); 1699.47(C=O); 2837-2999.31(alkyl-CH); 3118.90-3072.60(aromatic-CH); broad 3431.36(OH); 2500-3500 hump, COOH). \(^1\)HNMR peaks (DMSO-d\(_6\), δ ppm): 1.522-1.5458(t, 4H, CH\(_2\)); 1.5881-1.6617(q, 2H, CH\(_2\)); 1.7122-1.7032(t, 4H, CH\(_2\)); 3.208(s, 1H, CH); 3.821(s, 6H, OCH\(_3\)); 6.8059-7.9158(m, 8H, Ar); 8.4226(s, 1H, CH=N-N); 8.8785 (s, 1H, CH=N); 11.2929(s, 1H, COOH); 11.5725(S, 1H, COOH). \(^13\)CNMR peaks(DMSO-d\(_6\), δ ppm): 20.49(1C, -C-ring); 21.1,21.28(4C, CH\(_2\)); 27.77(2C, CH\(_2\)); 36.99, 37.15(4C, CH\(_2\)); 38.84-40.09(DMSO d\(_6\) ); 42.43(1C, C=C);127.78-131.94(12C, Ar); 143.63-143.95(1C, CH=N); 162.60(1C, C=O); 171.26(1C, COOH); 176.03 (1C, COOH). LC-MS (M\(^+\)) peak: 519.

**In vitro Reactivation of Organophosphorous inhibited enzyme:**

\[
\% \text{ Reactivation} = \frac{E_r - E_i}{E_0 - E_i} \times 100
\]

(E \(_i\) is enzyme inhibition at 412nm, 
E \(_r\) is optical density of derivatives)
Table: % Reactivation of acetylcholinesterase at six time intervals.

<table>
<thead>
<tr>
<th>Sample</th>
<th>O min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>50 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIb1</td>
<td>18.55</td>
<td>14.36</td>
<td>10.21</td>
<td>15.66</td>
<td>17.16</td>
<td>16.55</td>
<td>18.58</td>
</tr>
<tr>
<td>IIIb2</td>
<td>76.81</td>
<td>45.79</td>
<td>11.10</td>
<td>19.63</td>
<td>17.31</td>
<td>17.95</td>
<td>18.58</td>
</tr>
<tr>
<td>IIIb3</td>
<td>54.07</td>
<td>15.38</td>
<td>17.88</td>
<td>20.77</td>
<td>21.69</td>
<td>22.89</td>
<td>15.98</td>
</tr>
<tr>
<td>IIIb4</td>
<td>50.90</td>
<td>22.93</td>
<td>14.20</td>
<td>12.24</td>
<td>12.94</td>
<td>13.51</td>
<td>24.89</td>
</tr>
<tr>
<td>IIIb10</td>
<td>52.04</td>
<td>22.10</td>
<td>16.77</td>
<td>14.87</td>
<td>15.76</td>
<td>16.80</td>
<td>23.24</td>
</tr>
<tr>
<td>Pralidoxime</td>
<td>253.53</td>
<td>40.45</td>
<td>11.22</td>
<td>10.91</td>
<td>12.30</td>
<td>13.32</td>
<td>20.23</td>
</tr>
</tbody>
</table>

In this method Acetylcholine is cleaved by AChE to form thiocholine, which in turn reacts with 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) to give yellow 5-thio-2-benzoate anion, whose optical density is measured at 412nm. Compound IIIb2 showed good levels of enzyme reactivation comparison to the standard Pralidoxime, however there was substantial decrease of reactivation with progression in time.

Conclusion:
1, 6- bis ((Substituted) eneamino) -2-oxo-1, 2, 3, 4- tetrahydropyridine-3, 5-dicarboxylic acid (III b) were synthesized in a series of three steps. All the synthesized compounds were characterized by FTIR spectroscopy, and compounds of interest were characterized by (1H and 13C) – NMR spectroscopy and LC-MS spectroscopy. All the 1, 6- bis ((Substituted) eneamino) -2-oxo-1, 2, 3, 4- tetrahydropyridine-3,5-dicarboxylic acid (III b) compounds were evaluated in vitro for cholinesterase reactivation by Ellman assay method. Compound IIIb2 showed good levels of enzyme reactivation in comparison to the standard reactivator Pralidoxime.

Acknowledgement:
We would sincerely thank the Directors, NMR and Mass Center, SAIF, Punjab University-Chandigarh, for providing the necessary spectra.

References:
QSAR study of indole Glyoxamide derivatives as GP120-CD4 inhibitors

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School of Pharmacy, Devi Ahilya Vishwavidyalaya, Khandwa Road, Indore (M.P) 452 001 India
Chodhary Dilip Singh Kanya Mahavidyalaya, Bhind (M.P) 477001, India
E-mail address: mukeshcsharma@yahoo.com

Abstract: A forty eight compounds series of gp120-CD4 inhibitors of Indole glyoxamide were subjected to quantitative structure-activity relationship studies to obtained predictive good model. The QSAR model exhibited good statistical values for the training set (r² = 0.8263, q² = 0.7287, F test =33.474) and test set (pred_r² = 0.7992). Multiple linear regression method was then used to linearly correlate the selected descriptors with inhibitory activities. Several validation technique including leave-one-out and leave-group-out cross-validation, Y-randomization method were used to evaluate the internal capability of the derived models. Hence if we substitute electronegative group and polar groups at R1 region it will contribute in enhancing the activity.

Introduction:
Acquired immune deficiency syndrome is the last and pernicious step of human immunodeficiency virus-1 infection. [1] It is related to lentiviridae family of retroviruses [2], and three major enzymes reverse transcriptase, protease, and integrase [3]. HIV-1 entry preceding cellular infection with viral attachment to the host cell via interactions between the viral gp120 and CD4 which is the primary receptor for HIV-1. Quantitative structure- activity relationships studies play a key role in predicting the biological activity of new compound and provide information that is useful for molecule designing and medicinal chemistry [4]. QSAR is an area of computational research were a virtual model is build to predict quantities such as binding affinity or toxic potential of existing or theoretical molecules. The primary goal of this work was to develop a new and validated QSAR model, and then investigating the molecular structural requirements for improving the biological activities based on the derived models.

Materials and Method:
A total of 48 Indole glyoxamide derivatives along with their activities were taken from the literatures Meanwell et al. [5]. The EC₅₀ values were converted to PIC₅₀ by using the formula PIC₅₀ = -log 1/EC₅₀. The molecules were divided into two subsets using multiple linear regression method in which resulted in generation of the training set contained 35 compounds and the test set contained 13 compounds. The two-dimensional structures were sketched in Chem Draw Ultra 7.0 software. They
were converted to three dimensional by VLifeMDS software [6] and energy minimized using the MMFF batch minimization utility with parameters. The reducing data removed that could lead to low predictive of the QSAR results. 2D descriptors with the same values for the compounds were removed.

**Results and Discussion:**
To validate this assumption we performed QSAR studies on 48 Indole glyoxamide derivatives reported as HIV-1 attachment inhibitors. QSAR studies were performed in V-Life MDS Version 3.5 software.

\[
pIC_{50} = 0.0363 \times \text{SsClE-index} + 0.0185 \times \text{StCHE-index} + (\pm 0.0518 \times \text{AveragePotential})
\]

\[
N = 48, r^2 = 0.8263, q^2 = 0.7287, F\ test = 33.474, \text{pred}_r^2 = 0.7992
\]

The simulation reached a cross-validated \( r^2 \) of 0.8263, \( q^2 \) of 0.7287 and yielded a predictive \( r^2 \) (\( p^2 \)) of 0.7992. The SsClE-index descriptor signifies the number of chlorine connected with one single bond in a molecule. Its contribution was 28.412 % in enhancing the biological activity. R1 substitution site was originally occupied by highly electronegative halogens like Cl, which are polarizable groups enhancing the activity of presence of electronegative atoms on the biological activity.

\[
\text{StCHE-index} \text{ descriptor signifies number of } \text{–CH} \text{ atoms connected with one triple bond and corresponds to the heavily substituted R1 site. Its contribution was 31.476 % in enhancing the biological activity of Indole glyoxamide. It suggests that increase of } \text{–CH} \text{ bonds on that site is favourable for the predictive activity. It agreed with the results that compounds 15 and 16 substituted}
\]

**Fig.1:** Graphs of observed vs. predicted activity of QSAR model

StCHE-index descriptor signifies number of –CH atoms connected with one triple bond and corresponds to the heavily substituted R1 site. Its contribution was 31.476 % in enhancing the biological activity of Indole glyoxamide. It suggests that increase of –CH bonds on that site is favourable for the predictive activity. It agreed with the results that compounds 15 and 16 substituted
with –CH group at the R1-position of ring. The next average-potential descriptor signifies vanderwaals surface area of the molecule emphasizing to the R1 position in the derivatives for better activity. It will be enhancing the activity value with the polar groups in the active site. Fig. 1 shows the fit plot of experimental versus predicted pIC₅₀ values for the training as well as the test sets by the best QSAR model.

**Conclusion**

Accuracy of molecular conformation for Quantitative Structure Activity Relationship (QSAR) studies is a most important criteria and the most favorable bioactive conformer selection is a tough task. The good model was obtained having r² and q² value of 0.82 and 0.72 for training and test sets respectively suggesting the stability and robustness of model. The predictive ability of the model was manifested in the good correlation between actual and predicted pIC₅₀ values for the test molecules. The study will serve as a useful guideline for designing the novel compounds with significant HIV-1 gp120 attachment inhibitors activity.

**Acknowledgement:**

Author is thankful to V-Life ltd. for providing a trial version of the QSAR software.

**References:**


Estimation of Gedunin from Chitosan-coated liposome by developed and validated UV spectroscopy method

Anil Kumar Sahu and Vishal Jain
University institute of pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.)
E mail address: anil2484@gmail.com

Abstract: The aim of this study was to prepared chitosan-coated liposomes containing Gedunin for oral delivery and a new, simple, reproducible and cost effective ultraviolet-visible (UV) spectrophotometric method was developed and validated for the estimation of Gedunin in bulk and pharmaceutical carrier liposomes. The liposomes were prepared by conventional thin film hydration method. The average vesicle size, zeta potential and % drug entrapment efficiency was found to be 241±11.53 nm, 38±5.29 mv and 89.73±0.60% respectively. Gedunin was estimated at 220 nm in methanol. Linearity range was found to be 5-25 µg/ml. the analytical method was tested and validated for various parameters according to International Conference of Harmonization [ICH Q2 (R1)] guidelines. The results obtained through experiments indicated that the analytical method is accurate, precise and reproducible. The detection and quantification limits were found to be 0.2063 µg/ml and 0.6250 µg/ml respectively. Hence, the proposed method was successfully applied for the determination of Gedunin in bulk, pharmaceutical carriers/formulations and dissolution studies.

Introduction:

The present research work deals with the preparation of Gedunin loaded liposome by thin film hydration technique in order to overcome the limitation of pure Gedunin and the validation of the developed UV- spectroscopy analytical method for the assay of Gedunin from its carrier (liposome). Hence, the analytical method can be used for routine quality control analysis, dissolution study and stability testing.

Materials and Method:

Materials: Gedunin (GDN), cholesterol, soya lecithin, chitosan, chloroform, methanol and all other chemicals and solvents are used of analytical grade.

Gedunin (GDN) loaded liposome were prepared by conventional thin film hydration method as described previously.[1] Briefly the required amount drug and other excipients were dissolved in organic solution and dried in a rotary evaporator [IKA RV10 Digital Rotary Evaporator, Germany]. The obtaining dried lipid film was rehydrated with phosphate buffered saline solution (PBS; 10 mM, pH 7.4) and incubated at 60°C for 1h with repeated vortexing. The resulting mixture contained MLVs (Multi Lamellar Vesicles) suspension which was further sonicated with probe sonicator [Frontline...
Sonicator, Mumbai, India] for 30 min at room temperature in order to produce LUVs (Large Unilamellar Vesicles). The chitosan-coated liposomes were obtained by adding drop wise 0.1% chitosan solution to equal volume of liposomal suspension with gentle vortexing for 2 hours at room temperature. [2]

**Method validation:** According to ICH guidelines analytical method was validated for several parameters like specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. [3]

**Results and Discussion:**

The liposome was prepared by conventional thin film hydration method. The average vesicle size, zeta potential and % drug entrapment efficiency was found to be 241±11.53 nm, 38±5.29 mv and 89.73±0.60% respectively. Gedunin was estimated at 220 nm in methanol. Linearity range was found to be 5-25 µg/ml. the analytical method was tested and validated for various parameters according to International Conference of Harmonization [ICH Q2 (R1)] guidelines. The results obtained through experiments indicated that the analytical method is accurate, precise and reproducible. The detection and quantification limits were found to be 0.2063µg/ml and 0.6250µg/ml respectively. Validation summary of the proposed method was shown in table no. 1.

<table>
<thead>
<tr>
<th>Table 1: Summary of the proposed method</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum wavelength (λ_{max})</td>
<td>220 nm</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.032x + 0.007</td>
</tr>
<tr>
<td>Regression coefficient (r^2)</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope (SE)</td>
<td>0.032 (3.3 x 10^{-4})</td>
</tr>
<tr>
<td>95% confidence limit of slope</td>
<td>0.0324, 0.031</td>
</tr>
<tr>
<td>Intercept (SE)</td>
<td>0.007 (1.1 x 10^{-5})</td>
</tr>
<tr>
<td>95% confidence limit of intercept</td>
<td>0.0103, 0.0057</td>
</tr>
<tr>
<td>Standard error of estimate</td>
<td>0.1131</td>
</tr>
<tr>
<td>Calculated F-value (Critical F-value)</td>
<td>3252.13 (5.19)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Calculated F-value (Critical F-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>1.04 (9.27)</td>
</tr>
<tr>
<td>Linearity</td>
<td>0.04 (2.44)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>5-25 µg/ml</td>
</tr>
<tr>
<td>Precision (Intra-day)</td>
<td>100.97 ± 1.3485</td>
</tr>
<tr>
<td>Precision (Inter-day)</td>
<td>%RSD NMT 1.0840</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.2063 µg/ml</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>0.6250 µg/ml</td>
</tr>
<tr>
<td>Robustness</td>
<td>220-219 nm</td>
</tr>
<tr>
<td></td>
<td>2.42 (4.30)</td>
</tr>
<tr>
<td></td>
<td>220-221 nm</td>
</tr>
<tr>
<td></td>
<td>2.13 (4.30)</td>
</tr>
</tbody>
</table>

* Standard Error, **Relative Standard Deviation, ***Not More Than

**Conclusion:**
In summary, a simple and cost effective UV- spectrophotometric method was developed, validated and applied for the determination of Gedunin in bulk, pharmaceutical carriers/formulations and for dissolution studies of formulations. The developed method was validated as per ICH guidelines and was found to be selective, rapid, accurate, precise, reproducible and robust.

**Acknowledgement:**

The authors are thankful to Director, University Institute of Pharmacy, Pt Ravishankar Shukla University Raipur, (C. G.) for providing necessary infrastructural facilities. One of the authors is thankful to UGC-BSR for JRF and extends her gratitude towards the supervisor for guidance and support.

**References:**


Synthesis, characterization, and pharmacological evaluation of some novel Hydrazone derivatives derived from 3-((4-formyl-2-methoxyphenoxy) methyl) Benzonitrile

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Abstract: Hydrazones are important classes of biologically active compounds found in many synthetic products. The present article reports that a new series of novel hydrazone derivatives (4a-e) were synthesized by condensation of 3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile (3) with different benzohydrazides and confirmed by spectral studies like IR, 1H NMR, and MS. Then evaluated for anti-inflammatory, analgesic, and antibacterial activities. Among all the compounds 4a, 4d, and 4e are having more anti inflammatory activity. 4d, 4e, and 4b are having the good analgesic activity. 4a and 4e are having the excellent antibacterial activity.

Introduction:
Hydrazones constitute biologically active drug molecules. Hydrazone derivatives are containing highly reactive azomethine group (CONH- N=CH) and thus useful in new drug development [1]. Recently, a lot of biologically important hydrazone derivatives with a number of functional groups have been synthesized from aromatic and aliphatic compounds [2]. Many studies have confirmed that hydrazone derivatives exhibit a wide spectrum of biological activities including antiinflammatory activity-analgesic, anti-bacterial, antifungal, anticancer, anti-oxidant, antidepressant, anti-tubercular, cytotoxicity, antiplatelet, anticonvulsant, antimicrobial, antihypertensive activities. Encouraged by the biological applications of hydrazones, the present research work is aspired to describe the synthesis, characterization and anti-inflammatory, analgesic, and antibacterial activities of novel hydrazone derivatives 4a-e derived from Vanillin.

Materials and Method:
General experimental procedure for the preparation of novel hydrazone derivatives 4a-e:
The hydrazone derivatives (4a-e) were synthesized by addition of benzohydrazide derivatives (a-e) to the solution of ethanol containing the 3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile (3), and refluxed for 1h. The reaction mixture is cooled to room temperature and filtered the precipitated solids
and washed with pet-ether, to obtain the pure compounds 4a–4e. The yield of the product varied from 89 – 97%. New compounds were confirmed by spectral studies like IR, 1H NMR, and MS.

(E)-N’-(4-(3-cyanobenzyloxy)-3-methoxybenzylidene)-4-methoxy benzohydrazide (4a): White solid; Yield: 96%; mp 198-206 °C; FT-IR (KBr): $\nu_{\text{max}}$ 3435, 3232, 2939, 2838, 2230, 1644, 1605, 1576, 1542, 1507, 1462, 1417, 1377, 1295, 1269, 1256, 1232, 1178, 1139, 1052, 1030, 1010, 971, 899, 839, 810, 793, 760, 690, 627, 537 cm$^{-1}$; 1H NMR (400 MHz, DMSO-d$_6$): $\delta$ 3.83 (s, 3H), 3.85 (s, 3H), 5.21 (s, 2H), 7.20-7.05 (m, 4H), 7.37 (s, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.92-7.80 (m, 5H), 8.38 (s, 1H), 11.64 (s, 1H); MS (ESI) m/z, 416 (M+1).

All the synthesized compounds were tested for in vivo anti-inflammatory activity [3] in Adult Wistar rats by paw edema method. Analgesic activity [4] was carried in Adult Wistar mice by using Eddy’s hot plate method. In vitro antibacterial activity [5] was done by the disk diffusion technique.

**Results and Discussion:**

The newly synthesized hydrazone derivatives 4a–e described in this paper were prepared according to the synthetic Scheme 1. All the aliphatic and aromatic protons were observed at expected regions. The 1H NMR data for the derivatives 4a – e are in agreement with the assigned structures. The mass spectra of compounds showed (M+1) peaks, in agreement with their molecular formula.

**Anti-inflammatory activity:** The newly prepared hydrazone derivatives 4a-e were screened for anti-inflammatory activity at concentration 10 mg/kg. Among all tested compounds, 4a, 4d, and 4e exhibited maximum activity, while compounds 4b and 4c showed moderate activity when compared with standard indomethacin, anti inflammatory agent. In general, it is observed from Table 1. As all the tested compounds emerged as active against inflammation, it indicates that this basic moiety can be a promising scaffold for anti inflammatory drugs.

**Analgesic activity:** The newly prepared chalcone derivatives 4a-e were screened for analgesic activity by using hot plate method. Among all tested compounds, 4d, 4e, and 4b showed maximum activity, while compounds 4a, and 4c showed moderate activity when comparable with disease control. In general, it is observed from Table 2. As all the tested compounds emerged as active against analgesia, it indicates that this basic moiety can be a promising nucleus for analgesic drugs.

**Anti- Bacterial Activity:** The anti-bacterial activity of 4a-e was determined by the disc diffusion method with Gentamycin (100&200µg/mL) as the reference standard. The synthesized compounds were screened against two Gram positive bacterial strains viz., *Escherichia .coli*, and *Bacillus subtilis*. The outcome of the results are presented in the Table-3, it is evident from the results that, compounds 4a, 4d, and 4e exhibited high activity against the *E.Coli* bacteria, the rest of the compounds were found to be moderately active 4b and
The compound 4a exhibited high activity against the *B. subtilis* bacteria, the rest of the compounds (4b, 4c, 4d and 4e) were found to be moderately active against the *E.Coli*.

Table 1: Anti inflammatory activity of synthesized compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>Volume of paw edema (ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 1 hr</td>
<td>After 2hr</td>
</tr>
<tr>
<td>Control</td>
<td>0.63 ±0.021</td>
<td>0.70 ±0.036</td>
</tr>
<tr>
<td>Standard</td>
<td>0.55 ±0.034***</td>
<td>0.41 ±0.016***</td>
</tr>
<tr>
<td>4a 4-Methoxy</td>
<td>0.46 ±0.033***</td>
<td>0.40 ±0.044***</td>
</tr>
<tr>
<td>4b 4-Bromo</td>
<td>0.48 ±0.040***</td>
<td>0.38 ±0.060***</td>
</tr>
<tr>
<td>4c 2-Bromo</td>
<td>0.48 ±0.040***</td>
<td>0.36 ±0.055***</td>
</tr>
<tr>
<td>4d 4-Chloro</td>
<td>0.46 ±0.033***</td>
<td>0.38 ±0.016***</td>
</tr>
<tr>
<td>4e 2,4-Dichloro</td>
<td>0.53 ±0.033***</td>
<td>0.43 ±0.021***</td>
</tr>
</tbody>
</table>

All the synthesized compounds were confirmed by spectral analysis. The newly synthesized compounds were screened for antiinflammatory and analgesic activities by *in vivo* Method. Anti-inflammatory activity was found to be more for compound 4a. The rest of the compounds were found to have anti-inflammatory activity. All the compounds exhibited analgesic activity and more activity was found in 4d. The compounds were subjected to their antibacterial activity by *in vitro* method, and 4a and 4e were found to be more effective against gram positive bacteria.

Acknowledgement:
The authors gratefully thank Dr. B. Ram, Director, Green Evolution Laboratories Wangapally Village, Nalgonda District and Telangana State, India. for his helpful suggestions and supporting the work.

References:
Anticonvulsant screening of Quinazolin-4(3h)-one fused chalcone derivatives

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Abstract: A series of novel 3-(4-(3-(phenyl acryloyl)phenyl)-2-phenylquinazoline-4(3H)-one derivatives were synthesized and evaluated for their anticonvulsant, activity. Anticonvulsant activities of compounds were screened by using MES induced seizures and subcutaneous pentylenetetrazole (scPTZ) induced seizure models in wistar rats of either sex. The structures of the compound have been confirmed by spectral analysis. Among the tested compounds 3-(4-(3-(4-hydroxy phenyl)acryloyl)phenyl)-2-phenylquinazoline-4(3H)-one (4f) has shown significant activity against tonic seizure by the MES model and clonic seizure by PTZ-induced seizure model.

Introduction:
Epilepsy is a common neurological condition, affecting 0.5-1% of the population worldwide (45-100 million people). It is a family of neurologic disorders, if not treated is often accompanied by neurobiologic, cognitive, psychological, and behavioral changes that may heighten susceptibility to seizures and affect quality of life [1]. Lamotrigine, tiagabine, felbamate, pregabalin, stiripentol and topiramate are commonly used antiepileptic drugs (AEDs) which are effective toward only 60-80% of Quinazolinone derivatives having varied pharmacological profile such as antimalarial, antimicrobial, anti-inflammatory, anti-hypertensive, anti-diabetic, cholinesterase inhibition and anticancer activities [2].

Materials and Methods:
All chemicals and solvents were supplied by Sigma-Aldrich Chemical Company. The reactions were monitored with the help of thin layer chromatography (TLC, silica gel-G) with the help of solvent system ethyl acetate: hexane (6:2) and iodine vapours as a detecting agent to observe the spot. Melting points of the synthesized compounds were recorded on the Veego melting point apparatus. UV spectrum of synthesized compounds was determined on UV Shimadzu 1700s Spectrophotometer. Obtained spectra show $\lambda_{max}$ of corresponding compounds. The IR and $^1$H-NMR spectra obtained from sophisticated analytical instrumental facility (SAIF), Panjab University, Chandigarh. IR spectra of synthesized compounds showed characteristic peaks for the functional group present in the compounds. The anticonvulsant profile of biological activity was established after i.p. injection in the MES pattern test and the subcutaneous injection in pentylenetetrazole (scPTZ) seizure model. In The
MES test there is electrical induction of the seizure, whereas PTZ test having a chemical induction to generate the convulsion. Some selected compounds were further tested for their neurotoxicity. Study was conducted in the Department of Pharmacology, VNS Faculty of Pharmacy, Bhopal.

**Results and Discussion:**

**Chemistry** Anthranilic acid (2.74g, 0.02 mol) (1) dissolved in to the dry pyridine at room temperature and then the solution was cooled to 0°C and a solution of Benzoylchloride (2.4 mL, 0.02 mol) and dry pyridine was added slowly with constant stirring. After the completion of reaction the solution was poured into ice cold water to obtained 2-Phenyl-beno[d] [1, 3] oxazin-4-one (2). The equimolar amount of 2-phenyl-4H-3, 1-benzoxazin-4-One (2.23g, 0.01mol) and p-amino acetophenone (1.35 g, 0.01mol) was heated together upon fusion at 150°C on sand bath for 2 h. After cooling, the crude mass crystallized twice from ethanol to obtain dark brown crystal of 2-phenyl-3-(4- acetylphenyl0-4(3H) quinazolinone (3). To a mixture of 2-phenyl-3-(4-acetylphenyl0-4(3H) quinazolinone (0.68 g, 0.002mol) and appropriate aromatic aldehyde namely methoxybenzaldehyde, dimethoxybenzaldehyde, nitrobenzaldehyde, chlorobenzaldehyde, dimethylaminobenzaldehyde and hydroxybenzaldehyde (0.002mol) in ethanol (10mL), 5% NaOH in ethanol (10mL) was added dropwise within 15 min. The reaction mixture was refluxed for 3 h. Then the mixture was cooled and the precipitated product was filtered, air dried and re-crystallized to get the targeted compounds 3-(4-(3-(substituted phenyl) acryloyl) phenyl)-2-phenylquinazoline-4(3H)-one (4a-4f) [3-4]. The structures of synthesized compounds with their %yield, melting point and molecular formula are summarized in Table 1. All the synthesized compounds were characterized by their physical and spectral data.

**Neurotoxicity screening** The rotorod test was used to evaluate neurotoxicity. The rats were trained to stay on an accelerating rotorod that rotates at six revolutions per minute. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

**Anticonvulsant screening** The test compounds were administered intraperitoneally (i.p.) into the rats at the doses of 100 mg/kg in the MES screen and in the scPTZ screen. Phenytoin was selected as standard drugs for the comparison. All the synthesized compounds had shown significant activity against the tonic seizure with decreased mean duration of tonic hindleg extension. In case of scPTZ screening synthesized compounds had shown significant protection with increased onset time clonic convulsion While evaluating the anticonvulsant activity, it was observed that compounds having electron withdrawing group is beneficial for anticonvulsant activity. Among these compound 4f was found to be potent. Some selected compounds were further tested for their neurotoxicity. Not one of them showed any sign of neurotoxicity as shown in Table 2.
Table 1: List of synthesized compounds:

<table>
<thead>
<tr>
<th>Compounds Code</th>
<th>R₁</th>
<th>R₂</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>-OCH₃</td>
<td>-H</td>
<td>78.12</td>
<td>280-282</td>
<td>458.2</td>
</tr>
<tr>
<td>4b</td>
<td>-OCH₃</td>
<td>-OCH₃</td>
<td>74.36</td>
<td>276-278</td>
<td>488</td>
</tr>
<tr>
<td>4c</td>
<td>-NO₂</td>
<td>-H</td>
<td>69.83</td>
<td>292-294</td>
<td>473.2</td>
</tr>
<tr>
<td>4d</td>
<td>-Cl</td>
<td>-H</td>
<td>79.37</td>
<td>&gt;340</td>
<td>462.1</td>
</tr>
<tr>
<td>4e</td>
<td>-N(CH₃)₂</td>
<td>-H</td>
<td>68.54</td>
<td>329-330</td>
<td>471.2</td>
</tr>
<tr>
<td>4f</td>
<td>-OH</td>
<td>-H</td>
<td>83.44</td>
<td>312-313</td>
<td>444.1</td>
</tr>
</tbody>
</table>

Table 2: Anticonvulsant and neurotoxicity screening of synthesized compounds:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Electroshock Mean duration of tonic hindleg extension±SEM (sec)</th>
<th>PTZ-induced seizure Onset time clonic convulsion duration±SEM(sec)</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.90 ±0.53</td>
<td>76.80 ± 2.41</td>
<td>ND</td>
</tr>
<tr>
<td>4a</td>
<td>9.5 ±0.53*</td>
<td>90.41 ± 1.34*</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>8.60 ±0.65*</td>
<td>92.4 ± 1.51*</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>7.40 ±0.56*</td>
<td>94.45 ± 1.43*</td>
<td>-</td>
</tr>
<tr>
<td>4d</td>
<td>9.5 ±0.53*</td>
<td>85.60 ± 1.51*</td>
<td>ND</td>
</tr>
<tr>
<td>4e</td>
<td>10.9 ±0.80*</td>
<td>80.10 ± 1.028*</td>
<td>ND</td>
</tr>
<tr>
<td>4f</td>
<td>7.35 ±0.52*</td>
<td>96.65 ± 1.05*</td>
<td>-</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>NIL*</td>
<td>111.6 ± 2.015*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values with * are statistically significant (p < 0.05) from the control group by using one way ANOVA followed by Dunnett’s post tests. ND indicates not done; dash (-) indicates non neurotoxic.

Conclusion:
6 novel 3-(4-(3-(substituted phenyl) acryloyl)phenyl)-2-phenylquinazoline-4(3H)-one were synthesized for their anticonvulsant activity. The proposed work is to effort towards the development and identification of novel molecules as anticonvulsant agents by synthesis of some novel quinazolinone derivatives with improved biological activity.

Acknowledgement:
The author would like to thank panjab university, Chandigarh for providing analytical data of synthesized compounds and VNS faculty of Pharmacy for providing facilities to carry out the research work.

References:
Structure aided drug design, synthesis and evaluation of potential neuraminidase inhibitors as antiviral agents and probing their molecular mechanism using NMR techniques

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Abstract: The upsurge of resistance to currently available NA inhibitors against swine flu caused by H1N1 necessitates search for new NA inhibitors. We have developed compounds by molecular docking computational approach to explore active site of H1N1-NA in both standard and pandemic virus. Promising ones were synthesized and further evaluated using in-vitro enzyme based study on standard influenza virus. Based on their antiviral activities we have studied their interaction with model membranes prepared from DPPC by NMR techniques. Results obtained explained the effect of synthesized molecules on membrane stabilization, fluidity and mobility which further strengthen molecular basis of their antiviral action.

Introduction: Swine flu virus type A- H1N1 is the world’s largest pandemic threat. The causative Influenza virus contains two major membrane glycoproteins namely, Hemagglutinin (HA) and Neuraminidase (NA). HA is a lectin that mediates binding of the virus to target cells and entry of the viral genome into the target cell by binding to the terminal sialic acid of the receptor, while NA is involved in the release of progeny virus from infected cells, by cleaving sugars that bind the mature viral particles by digesting sialic acid [1]. NA inhibitors inhibit NA activity and receptor binding activity of HA thus affecting attachment and fusion of cell membrane. The increasing emergence of resistance against currently used drugs: Oseltamivir and Zanamivir calls for development of newer NA inhibitors. Reports indicate that natural compounds, which are abundant with chalcones, possess antiviral activity [2] and therefore can be novel scaffolds to be developed as newer NA inhibitors.

Materials and Method:
Different substituted acetophenone and benzaldehyde were purchased from S D fine-chem. Ltd. India. L-α-Diapalmitoyl phosphatidyl choline (DPPC) was purchased from Sigma Chemicals Co., U.S.A. All other solvents used for synthesis were of LR grade. Oseltamivir was a gift sample from Cipla Ltd., India. Standard H1N1 virus obtained from National Institute of Virology, Pune, India.
**Computational studies:** In order to understand binding interaction of our candidate molecules with H1N1 virus (standard and pandemic), we have carried out computational studies with the modeling package Discovery Studio (DS) 3.1 (Accelrys Inc., USA) running on a Red Hat Enterprise platform. Docking studies were carried out with GOLD v 3.1 (CCDC, UK) running on a separate Red Hat Enterprise platform.

**Synthesis:** We have synthesized sixteen derivatives of chalcone based on Claisen-Schmidt condensation reaction.

**Drug-lipid interaction studies:** Multilamellar vesicles (MLV) were prepared by the standard procedure wherein the desired quantity of DPPC was dissolved in chloroform. The solvent was then evaporated with a stream of nitrogen gas, so as to deposit a thin film on the walls of the container. The last traces of the solvent were removed by vacuum drying for a period of 1 h. The lipid film was hydrated with the required amount of D$_2$O (pH 7.2); this was then incubated in a water bath at 50°C with intermittent vortexing. The lipid concentrations were maintained at 100 mM. Unilamellar vesicles were prepared by sonicating the above dispersions with a Branson Sonicator (Model 450) at 50% duty cycles, till solution was optically clear [3]. NMR experiments were recorded on 500 and 600 MHz BRUKER AVANCE NMR spectrometer and data was processed using Bruker Topspin2.1 software. Experiments performed were $^1$H, $^{31}$P, $^{13}$C NMR.

**Antiviral activity testing:** Neuraminidase inhibitory activity was determined by the NA-Star Influenza Neuraminidase Inhibitor Resistance Detection Kit (Applied Biosystems, Foster City, CA) on influenza A virus H1N1 (sH1N1) neuraminidase. Oseltamivir carboxylate (OMVC) [4] was used as a standard alongwith Quercetin (QR) as a standard for noncompetitive inhibition. Two wells containing only assay buffer (instead of neuraminidase inhibitor) and culture medium (instead of virus) were used as negative controls. The substrate was diluted at 1:1000 in assay buffer immediately before use. Then 10 μL of the diluted substrate were added to each well. The chemiluminescent signal was quantified immediately by microplate reader. The 50% inhibitory concentration (IC$_{50}$) was determined by regression analysis (Prism; version 6.00; GraphPad Software).

**Results and Discussion:**

We have used molecular docking to investigate the binding interactions of various chalcone derivatives with NA enzyme considering both standard as well as pandemic influenza virus. The H1N1-NA active site of standard is an open conformation while pandemic is a closed conformation of the enzyme [5]. Based on docking results, modifying the molecular volume and decreasing the lipophilicity may strengthen the binding interactions and overcome the crisis of drug resistance, which is seen in case of available NA inhibitors. For this study, we have selected four derivatives based on their docking results and antiviral activity. They are 2′, 4′-dihydroxy-3-chloro chalcone (A), 2′, 4′-
dihydroxy-3-methoxy chalcone (B), 2', 4'-dihydroxy-4-methoxy chalcone (C) and 2', 4'-dihydroxy-4-nitro chalcone (D). We have evaluated antiviral activity of selected candidates by enzyme based study on standard influenza virus (H1N1). It was observed that the antiviral activity is in the order: C>A>D>B.

Docking studies of compound C with standard (open) enzyme reveals that it shows interactions quite different from that of Oseltamivir and it fits well into the cavity. $^{31}$P NMR studies indicate that it shows membrane stabilizing effect. Thus, the antiviral activity of compound C as observed from the enzyme inhibition assay among all. The other derivatives A, B and D do not fit as well into the cavity of the standard enzyme as the 4-methoxy derivative. The $^{31}$P NMR of these derivatives show that though the CSA is affected, the parallel peak is not completely faded. The enzyme inhibition studies reveal that the activity of these compounds is comparable with that of compound C.

**Conclusion:**
On the basis of these results, we conclude that chalcone derivatives synthesized by us have promising activity against standard H1N1 virus. All the candidates under study showed different mode of binding in the active cavity and showed good enzyme inhibition. These compounds have a better scope for further development in the pandemic and oseltamivir carboxylate mutant viruses.

**Acknowledgement:**
MA. Kanyalkar thanks Indian Council of Medical Research (ICMR), New Delhi for funding. The authors gratefully acknowledge the National facility for High Field NMR located at TIFR for providing NMR Facility and Haffkine Institute for providing antiviral testing facility.

**References:**
Estimation of Ranolazine in Pharmaceutical Dosage Form by High Performance Liquid Chromatography

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Abstract: A new simple, precise and accurate reverse phase high-performance liquid chromatographic method was developed for the estimation of Ranolazine in tablet dosage form. Chromatogram was run through sunfire C-18 (250x4.6 mm, 5 μm) column with a mobile phase consisting of methanol and water (70:30 v/v) at a flow rate of 1 ml/min. Optimized wavelength for Ranolazine was used 271nm by using PDA detector. The retention time was 3.36 min. The detector response was linear in the range of 30 to 150 μg/ml with coefficient correlation 0.999. The method was validated by using ICH guideline.

Introduction:
Ranolazine is chemically N-(2,6-dimethylphenyl)-2-[4-{2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazin-1-yl}acetamide. Ranolazine used as antianginal agent. Literature survey reveals that several methods have been reported for Ranolazine as HPTLC [1], in human plasma LLE HPLC [2], spectrophotometric [3]. No liquid chromatographic method for estimation of ranolazine in mobile phase (methanol: water at ratio 70:30 v/v) is reported. Hence a simple, sensitive and accurate reverse phase high performance liquid chromatography method was developed for estimation of ranolazine in its tablets.

Materials and method:
Material: Analytical pure Ranolazinewas obtained as gift sample from Unichem Laboratories Ltd, Mumbai, India. The formulation Rancad tablet (ranolazine500, Lupin Laboratories Ltd, India) was procured from the local market. Methanol and water were used of HPLC grade.
Instrument: Waters HPLC, 2996 PDA detector module equipped with 515 Binary pump and sunfire C-18 (250x4.6 mm, 5 μm) column with Empower software was used.
Chromatographic conditions: The mobile phase consisting of methanol and water (70:30 v/v) with flow rate of 1 ml/min was used.
Standard stock solution: A standard stock solution 1000 μg/ml of ranolazinewas prepared methanol.
Analysis of Tablet formulation: Twenty tablets were weighed accurately, powdered and powder equivalent to 100 mg of ranolazine was transferred to a 100 ml volumetric flask. It was dissolved in mobile phase and filtered through a 0.45 μm membrane filter. The filtered solution was suitably diluted and used for the analysis. The sample solution was injected five times and chromatograms were recorded. The content of ranolazine was found to be 99.91 percent with % relative standard deviation 0.9321.

Results and Discussion:

Figure 1 Chromatogram of Ranolazine

Linearity
Linearity was observed in the range of 30-150 μg/ml solutions of ranolazine at retention time 3.36

Preparation of calibration curve
From aliquots of standard stock solution of ranolazine 0.3, 0.6, 0.9, 0.12, and 1.5 ml solution was peppered out in triplicate and transferred to series of 10 ml volumetric flask and volume was made up to the mark with using mobile phase to get the concentration range from 30-150 µg/ml. Chromatograms were recorded at wavelength 271 nm with retention time 3.36. Calibration graph was constructed by taking concentration verses area of the peak. LOD and LOQ was found to be 0.652 and 1.976 for ranolazine, with regression equation Y=9370x + 51760, x=concentration, y=intercept. (figure1).

Accuracy
Recovery studies used to find the accuracy of the method. This study was carried out in 80, 100 and 120 % level. Percentage RSD was very low below 2 shows method is accurate [4]

Precision
The Intra and Inter day variation show % RSD less than 2 means method is precise.
Conclusion:
The proposed method is selective and sensitive. It can be used in industry.

Acknowledgement:
The authors are thankful to the Management and Principal, M. G. V.’s Pharmacy College, Nasik for providing necessary facilities for the research work.

References:
Method development and validation for estimation of Butorphanol tartrate in marketed formulation by UV spectrophotometry and RP-HPLC

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Abstract-The present work is concerned with application of a simple, precise, reliable, rapid, sensitive and validated RP-HPLC method has been developed to determine butorphanol tartrate in marketed formulation. Separation of butorphanol tartrate was successfully achieved on a Chromatographic separation was achieved isocratically at 25°C ± 0.5°C on Luna C18 column (250 x 4.6 mm i.d.) with a mobile phase composed of 50 mM KH₂PO₄ : Acetonitrile in the ratio of 80: 20% v/w at flow rate of 1.0 ml/min and eluate was monitored at 280 nm. The retention time of butorphanol tartrate was found to be 5.25 ± 0.3 min. The method was found to be linear in the range of 5-25μg/ml with mean recovery of 99.35%. The correlation coefficients for all components are close to 1. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method was successfully applied for determination of butorphanol tartrate in routine analysis.

Introduction:
Butorphanol is a morphinan-type synthetic opioid analgesic. Butorphanol is most closely structurally related to levorphanol. Butorphanol is available as the tartrate salt in injectable, tablet, and intranasal spray formulations. Butorphanol is listed under the Single Convention on Narcotic Drugs 1961 and in the United States is a Schedule IV Narcotic controlled substance with a DEA ACSCN of 9720; being in Schedule IV it is not subject to annual aggregate manufacturing quotas. The free base conversion ratio of the hydrochloride is 0.69. It is used in the management of severe pain.[1] The opiate antagonistic effect may result from competitive inhibition at the opiate receptor, but may also be a result of other mechanisms. Literature survey reveals that a few HPLC methods, Potentiometric method, UV spectrophotometric, LC-MS, method have been used.[2,3,4] The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP HPLC method with UV detection for the determination of Butorphanol tartrate in marketed formulation.

Materials and method:
Chemicals:- Butorphanol tartrate was obtained from marketed formulate (Denocrin enamelal drop) and was used as such without further purification.
Reagents:- Methanol (HPLC) (Merck), Acetonitrile (HPLC) (Merck), Water(HPLC) (Loba Chemi).
**Instruments and Equipments:** WATERS HPLC, Model: Alliance 2695UV- Visible Dual absorbance Detector 2487, with an Automated Sample injector. The output signal was monitored and integrated using Empower 2 software. ASymmetry Luna C18 (4.6 x 150mm, 5 Waters), UV-3000+ LABINDIA Doublebeam with UV win 5 software UV-Visible spectrophotometer with 1cm matched quartz cells, Weighing Balance, Sonicator, pH Meter, Heating Mantle, Filter Paper 0.45 microns.

**Preparation of standard solution:** -10 mg of Butorphanol Tartrate was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluent (water), to give a stock solution of 1000 ppm.

**Sample preparation for Nasal drop Formulation**

Weight equivalent to 10 mg of Butorphanol tartrate and dissolved with 5 ml solvent methanol in 10 ml Volumetric Flask and sonicate it for 10 min by ultrasonicator, after that volume was made up to 10 ml with solvent to obtain concentration of 1000 µg/ml from this take 0.1 ml and dilute up to 10 ml with methanol and take the absorbance of the sample solutions at 280.0 nm and the concentration of drug in the sample solution was determined by using Regression equation, After obtaining the value of concentration, Calculated the Percentage estimation of drug.

**Result and Discussion:**

*(Estimation of butorphanol tartrate by UV spectroscopy and RP-HPLC)*

The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

**Validation:**

The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

**Conclusion:**

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of butorphanol Tartrate from pure and its dosage forms. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of butorphanol Tartrate.
Table-1. Selection of Separation Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td></td>
</tr>
<tr>
<td>Dimension.</td>
<td>250mm x 4.60mm</td>
</tr>
<tr>
<td>Particle Size</td>
<td>5µm</td>
</tr>
<tr>
<td>Bonded Phase</td>
<td>Octadecylsilane (C\textsubscript{18})</td>
</tr>
<tr>
<td><strong>Mobile Phase</strong></td>
<td></td>
</tr>
<tr>
<td>50mM KH\textsubscript{2}PO\textsubscript{4}</td>
<td>80</td>
</tr>
<tr>
<td>ACN</td>
<td>20</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1ml/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temp.</td>
</tr>
<tr>
<td>Sample Size</td>
<td>20 µl</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>280.0 nm</td>
</tr>
<tr>
<td>Retention time</td>
<td></td>
</tr>
<tr>
<td>Butorphanol tartrate</td>
<td>5.254 ± 0.3 min</td>
</tr>
</tbody>
</table>

Table-2. Summary of analytical method validation of rifampicin by HPLC

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Acceptance criteria</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td>The correlation coefficient should be NLT 0.999</td>
<td>0.989</td>
</tr>
<tr>
<td><strong>Repeatability</strong></td>
<td>% RSD of 6 injections</td>
<td>0.551</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>% Recovery at each level should be between 98-102%</td>
<td>80% 98.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% 100.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120% 100</td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td>Change in mobile phase ratio</td>
<td>-2% 0.78</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>+2% 0.26</td>
</tr>
<tr>
<td></td>
<td>% RSD of 6 injections</td>
<td>-5% 0.95</td>
</tr>
<tr>
<td></td>
<td>Change in flow rate</td>
<td>+5% 0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ml/min 0.1230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7 ml/min 0.2421</td>
</tr>
</tbody>
</table>

Acknowledgement:
I greatly acknowledge the Dr. S. K. Jain of Director SIRT-Pharmacy, Bhopal for their kind help, support and providing all necessary facilities.

References:

Development and validation of new stability indicating HPLC method for determination of Irbesartan

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Abstract: A simple, specific, accurate and precise new stability indicating reverse phase high performance liquid chromatographic method was developed and validated for the determination of irbesartan. The mobile phase containing Acetonitrile: Phosphate buffer pH 3.5 (50:50 v/v) was used. The flow rate was 1.0 ml/min and effluents were monitored at 240 nm. The retention time for irbesartan was 1.50 min. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification and robustness. The proposed method was successfully applied for the quantitative determination of irbesartan. A linear response was observed in the range of 5-40µg/ml. Linear regression of absorbance on concentration gave the equation y = 101.9x + 195.3 with a regression coefficient r²=0.993. The method was then validated for different parameters as per the ICH guidelines. The degradation studies were carried out by using the developed method. Thus the method is useful for the determination of irbesartan in bulk and pharmaceutical formulations.

Introduction:
It is mandatory requirement from regulatory authorities to show the proper qualification of its degradation pathways and characterization of known degraded product that are present. Degradation can arise during the storage of the drug substances and their acceptance up to certain limits are based on pharmaceutical studies or known safety data. In the present study we describe an ideal stability indicating spectroscopic method should estimate the drug and also be able to resolve the drug from its degradation products. Hence an attempt has been made to develop an accurate, rapid and reproducible method for the determination of irbesartan in presence of its degradation products for its content analysis in pharmaceutical dosage form as per ICH guidelines.

Materials and Method:
Preparation of stock solution: Irbesartan (50 mg) weighed and transferred to a 25 ml volumetric flask. Volume was made upto 25 ml with HPLC grade methanol, further dilutions were made with mobile phase to get the solutions of 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml. These solutions were injected into HPLC column.
The chromatographic conditions followed were as follows:

Detector: Cyberlab UV/VIS detector.
Column: C-18 (250 mm length x 4.6 mm internal diameter and 5 μm particle size, Make-SMT SAM)
Mobile Phase: Acetonitrile: Phosphate buffer pH 3.5 (50:50 v/v)
Flow rate: 1 ml/min.
Detection: 240 nm
Loop size: 25 μl
Pressure: 8 psi
Temperature: 22°C

Method Validation: The developed method was validated for linearity, precision, robustness, limit of detection and limit of quantitation. Forced degradation study of irbesartan in bulk was carried out under the conditions of acid hydrolysis, alkaline hydrolysis, oxidation degradation.

Results and Discussion:
Standard curve for irbesartan was obtained by plotting AUC verses concentration in μg/ml. The retention time for irbesartan was found to be 1.50 min. The linear relationship between AUC and concentration was found in the range of 5 – 40 μg/ml (y = 101.9x + 195.3, r = 0.993).

![HPLC chromatogram of irbesartan in Mobile Phase](image)

**Figure 1** HPLC chromatogram of irbesartan in Mobile Phase

Method Validation: The developed method was found be valid for the linearity, precision, robustness, LOD and LOQ parameters.

**Forced Degradation of Irbesartan:** Significant degradation were observed in acidic, basic, oxidative condition for irbesartan. The additional peaks of degradation products were observed. No additional
peak was observed in case of oxidation studies but significant change in retention time has been observed.

**Table:** Following table highlights the degradation of irbesartan under stress conditions:

<table>
<thead>
<tr>
<th>Stress Degradation</th>
<th>Amount of irbesartan Degraded</th>
<th>Amount of irbesartan Recovered</th>
<th>Retention time of degraded product</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M HCL</td>
<td>67.26</td>
<td>32.73</td>
<td>1.75, 2.17, 6.10</td>
</tr>
<tr>
<td>0.1 M NaOH</td>
<td>67.13</td>
<td>32.86</td>
<td>1.75, 6.60</td>
</tr>
<tr>
<td>3 % H₂O₂</td>
<td>77.94</td>
<td>22.05</td>
<td>2.09</td>
</tr>
</tbody>
</table>

**Conclusion:**

Proposed study describes new HPLC method for the estimation of irbesartan. The method was validated and found to be simple, sensitive, accurate and precise.

**References:**


Development of RP- HPLC method for estimation of Rosuvastatin in tablet dosage form

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Abstract: The method is simple, accurate and precise for estimation of Rosuvastatin in tablet dosage form by HPLC. The Rosuvastatin is an anti-hypertensive drug soluble in water, methanol, chloroform, ethanol, acetonitrile. New method has been developed for estimation of Rosuvastatin in tablet dosage form by using Potassium (dihydrogen) orthophosphate and Acetonitrile in the ratio of 50:50. The Beers-Lamberts law was obeyed in the range of 5-30µ/ml with a regression coefficient 0.992. Accuracy of proposed method was obtained by recovery studies by standard comparison method and the results were obtained on the basis of statistical parameters as per ICH guidelines.

Introduction:
Rosuvastatin (statin) HMG-CoA reductase inhibitor [1] used, in the primary and secondary prevention of coronary heart disease, carotid artery disease and other atherosclerotic vascular diseases. In US guidelines, the lowering of low-density lipoprotein cholesterol (LDL-C) is the primary goal of lipid-modifying therapy in patients with atherosclerotic disease and those at risk for atherosclerotic disease due to dyslipidaemia. However, in patients with primary hyperlipidemia [2] Present study involves development of UV- spectrophotometric method which is simple, economical, sensitive and rapid for quantification of Rosuvastatin and Niacin in individual as well as combined tablet dosage forms as well as subsequent validation of developed method according to ICH guidelines [3-6].

Materials and Method:
Instrument: - RP-HPLC, Make – Cyberlab LC-100, Injection Volume – 20 µl using Intertsil ODS C-18 column with 15cm x 4.6mm internal diameter and 5µm particle
Preparation of Standard Stock Solution:-
A stock solution of Rosuvastatin was prepared by accurately weighed 50mg of drug, transferred to 50ml of volumetric flask, containing 50ml of mobile phase dissolving it to obtain final standard solution of 1mg/ml of Rosuvastatin.
Preparation of Sample Solution:- The formulation tablets of Rosuvastatin were crushed to give finely powdered material. Powder equivalent to 50mg of Rosuvastatin was taken in 50 ml of volumetric
flask containing 50ml of solvent and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent and was further diluted to obtain concentration of 100µg/ml.

Chromatographic Conditions: The mobile phase consisting of Potassium (dihydrogen) orthophosphate buffer: acetonitrile were filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 50:50 v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 254nm with a run time of 6min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Preparation of buffer solution: Weigh accurately and dissolve 2.74gm of potassium (dihydrogen) phosphate buffer (monobasic) (KH2PO4) In 1000ml volumetric flask and make up the volume with double distilled water. filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter.

Method Validation: The proposed method was validated by studying several parameters such as Specificity, linearity, precision, accuracy and limit of detection (LOD), limit of quantitation (LOQ),and system suitability [7,8,9].

Results and Discussion: The proposed method was found to be linear in the concentration range of 5-40µg/ml for Rosuvastatin Calcium. The method was specific since excipients in the formulation did not interfere in the estimation of Rosuvastatin Calcium. Regression coefficient was found 0.98 for drug. Accuracy of the method was indicated by the recovery values 98-105%. Precision is reflected by %RSD as 1.45 for ROS. The LOD and LOQ values were obtained in the given range.

Conclusion: The proposed method is found to be simple, sensitive and reproducible and hence it can be used in routine analysis for determination of Rosuvastatin in pharmaceutical preparation. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The results of linearity, precision, accuracy and specificity, proved to be within the limits.

Acknowledgement: Authors are thankful to project guide and Pharmaceutical chemistry department and quality assurance department of Marathwada Mitra Mandal’s College of Pharmacy Pune-33, Pune University for providing equipment and facility during entire duration of research /project work. Words of gratitude...
also expressed for Ranbaxy lab. Ltd. Gurgaon, India for providing gift sample of Rosuvastatin calcium and Analab fine chemicals, Mumbai- 53 for providing niacin as bulk drugs.

References:
[4] ICH, Q2(R1) Validation of Analytical Procedures : Text and Methodology, ICH Harmonized tripartite guideline; november.2005
Development of classical least square method for the determination of Candesartan and Hydrochlorothiazide in tablet dosage form

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Abstract: A new simple spectrophotometric method was developed for the determination of binary mixtures of Candesartan cilexetil (CAND) and Hydrochlorothiazide (HCTZ), without prior separation. The method is based on the multi-wavelength technique i.e. classical least square (CLS) method. The tablet is determined by the multi-wavelength technique (CLS), at the wavelengths range of 215-225 nm over the concentration ranges of 2.5–50 and 1–30 µg/mL with mean recovery more than 98% for both drugs CAND and HCTZ, respectively. The proposed spectrophotometric method was validated and successfully applied for the assay of drug combination in several laboratory-prepared mixtures and commercial tablets.

Introduction:

Candesartan cilexetil (CAND) is an angiotensin II receptor antagonist. It is used in the management of hypertension and may also be used in heart failure in patients with impaired left ventricular systolic function [1]. Hydrochlorothiazide (HCTZ) is a moderately potent diuretic. It exerts its effect by reducing the re-absorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water.[2]

In this study, a new classical least square method was described for the determination of binary mixtures of CAND and HCTZ without prior separation. The method eliminates the derivative step, and does not require searching for zero-crossing points. The developed method was validated and successfully applied for the assay of drug combinations in their commercial tablets.

Materials and method:

A Shimadzu (UV-1800) spectrophotometer (Japan) was used as instrument. All chemicals were of analytical reagent grade, 0.1M Hydrochloric acid (Merck India, Mumbai. Candesartan cilexetil (CAND) and Hydrochlorothiazide (HCTZ) were obtained as gift samples from Zim Laboratories Limited, Nagpur (India).

Results and discussion:

Spectrophotometric Characteristics
Fig. 1. Shown the overlain zero order spectra of CAND (λmax=251 nm), HCTZ (λmax=272 nm) and their mixture against a blank, spectra were detected in the spectral region 200-340 nm. Since the spectra of two drugs overlap in the working wavelength range, it is not possible to determine CAND and HCTZ simultaneously in their mixture by conventional spectrophotometric methods.

**Classical Least Square methods (CLS)**

The zero-order absorption spectra for CAND and HCTZ and their binary mixture in 0.1M HCl were shown in Fig.1 as could be seen, a considerable degree of spectral overlapping occurs in the region from 210-230 nm for CAND and HCTZ. The absorption data matrix and concentration matrix were obtained by measurement of absorbance between the ranges of 215-225 nm in the interval with Δλ=1 nm at 11 selected wavelengths in their zero-order spectra. As indicated in Table 1. The highest values for the regression coefficients (r) were obtained for all regression equations.

**Conclusion:**

The contents of several laboratory prepared mixtures and commercial tablets were simultaneously determined using UV-spectrophotometric measurements together with CLS method. The good recoveries obtained in all cases as well as the reliable agreement with the reported procedures proved that, the proposed procedures could be applied efficiently for determination of studied drugs simultaneously in their binary mixtures as well as in the commercial dosage forms with satisfactory precision. Hence, the proposed procedures are rapid and sufficiently precise and suitable for quality control laboratories, where the economy and time are important factors.

**Fig. 1.** zero order overlain absorption spectra of CAN 20 μg/mL (––), HCT 20 μg/mL (—) and a mixture of CAN 20 μg/mL and HCT 20 μg/mL (–––)

**Table 1.** Data for calibration graph for binary mixtures of CAND and HCTZ by using CLS method
### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAND</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>215-225</td>
<td>215-225</td>
</tr>
<tr>
<td>Concentration range (μg/mL)</td>
<td>2.5-50</td>
<td>1-30</td>
</tr>
<tr>
<td>Intercept&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.011</td>
<td>-0.005</td>
</tr>
<tr>
<td>Slope&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.020</td>
<td>0.034</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.121</td>
<td>0.354</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>LOD&lt;sup&gt;c&lt;/sup&gt; (μg/mL)</td>
<td>0.55</td>
<td>0.32</td>
</tr>
<tr>
<td>LOQ&lt;sup&gt;d&lt;/sup&gt; (μg/mL)</td>
<td>1.66</td>
<td>0.97</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard deviation of the intercept.  
<sup>b</sup>Standard deviation of the slope  
<sup>c</sup>Limit of detection.  
<sup>d</sup>Limit of quantification  
S.D.-standard deviation

### References:


Virtual screening and synthesis of 1, 3, 5-trisubstituted-1H-pyrazolines as antifungal agents

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Abstract: Molecular shape and electrostatic profile based virtual screening strategy was adopted to identify new antifungal agents. The shape and electrostatic query was built with most active antifungal agents and then screened the database to identify novel templates. The tri-substituted pyrazoline was identified and synthesized for antifungal evaluations. In SAR, it was found that introduction of electron withdrawing (EW) substituents (Cl group) in ortho and para position in one of the aromatic rings yielded active compounds. In contrary, introduction of electron donating (ED) substituents (OCH3 group) in meta and para position in the same ring produced less active compounds.

Introduction:
Fungal infections are continuously setting severe threats to human health, which are of great consequence.1 In the developed world, the incidence of life-threatening fungal infections has markedly increased in recent years due to extensive use of immunosuppressive drugs, prolonged use of broad spectrum antibiotics, widespread use of indwelling catheters. Fungal infections have emerged as a major cause of death among cancer patients and transplant recipients.2 Clinically among all type of fungal infection, candidiasis and aspergillosis account for between 80% and 90% of infections in immune compromised patients.3 There are three main families of antifungal drugs currently in clinics use: the polyenes, (such as amphotericin B and nystatin), the azoles (such as fluconazole, ketoconazole and itraconazole) and allyl amines (such as nifitine and terbinafine).3 These antifungal drugs are not able to meet the increasing requirements of managing infection in the complex patient populations. The development of new antifungal drugs has been constantly required in the clinical therapy. Therefore, we aimed to design and synthesize the effective anti-fungal agents against candidosis, in the immune-compromised individuals.

Materials and Method:
Shape and electrostatic profile based virtual screening strategy was employed to identify novel antifungal agents. The molecular shape and electrostatic query was built with most active antifungal agents and then screened the database to identify new biologically active templates. Further, the template was modified and synthesized for antifungal evaluation. Condensation of substituted
chalones with phenyl hydrazine yielded the title compounds as in Scheme 1. The trisubstituted compounds were evaluated in vitro for antifungal activity against *C. albicans* MTCC227 by disk diffusion method.

**Results and Discussion**

We choose shape and electrostatic profile based virtual screening strategy as the molecular shape, surface and electrostatic plays an important role in antifungal agents. The molecular shape and electrostatic query was built with most active antifungal agents and then screened the database to identify novel active templates. The trisubstituted pyrazoline template was found most promising and further modified with varying substituents and synthesized for antifungal evaluations.

**Table. 1. Molecular Shape & Surface Area of Query Molecule and the Hit.**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Shape &amp; Surface Area (Connolly molecular surface)</th>
<th>Electrostatic Topography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1.</td>
<td>Connolly Accessible Area: 504.409 Angstroms Squared</td>
<td>Query-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1</td>
<td>Connolly Accessible Area: 619.136 Angstroms Squared</td>
<td>87% similarity with Query-1 &amp; 91% similarity with Query-2</td>
</tr>
</tbody>
</table>

In the structure-activity relationship, it was found that introduction of electron withdrawing (EW) substituents (Cl group) in *ortho* and *para* position in ring A yielded active compounds. In contrary, introduction of electron donating (ED) substituents (OCH$_3$ group) in *meta* and *para* position in ring A produced less active compounds. The presence of a single methoxy group was effective at *para*
position in ring. However, introduction of dimethoxy group at meta and para position in ring B led to very low active compounds. Chain elongation of the amine system by introduction of one additional methyl group to the dimethyl amine moiety (diethyl amine moiety) produce more active antifungal compounds.

**Conclusion:**
A shape and electrostatic based virtual screening strategy was employed to screen the database and identified trisubstituted pyrazoline template as hit for antifungal agent development. Then, a series of six novel trisubstituted pyrazoline derivatives were synthesized and evaluated for their antifungal activity. Compound 6 showed good antifungal activity against C. albicans (9μg/ml). In the structure-activity relationship, it was found that introduction of electron withdrawing (EW) substituents (Cl group) in ortho and para position in ring A yielded active compounds.

**Acknowledgements:**
Author thankful to SAIF, Punjab University, Chandigarh for NMR and MASS spectral analysis.

**References:**
Simultaneous determination and method development for assay of Losartan Potassium and Hydrochlorothiazide drugs in solid dosage form by RP-HPLC

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Abstract: A simple, specific, accurate and precise RP HPLC method has been developed for the simultaneous determination of Losartan Potassium (LOS) and Hydrochlorothiazide (HCTZ) from combined dosage form by reverse phase C18 column (Zorbax CN (250mm x 4.6mm) 5μ). The sample was analysed using Triethylamine: Acetonitrile: Methanol in the ratio of 33:27:40 (pH adjusted to 7.0 with orthophosphoric acid) as a mobile phase at a flow rate of 1.0ml/min and detection at 270nm. The retention time for Losartan potassium (LOS) and Hydrochlorothiazide (HCTZ) was found to be 11.869 min and 7.893 min respectively. The stability assay was performed for this combination and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible.

Introduction:
Losartan Potassium\(^1\) is a Angiotensin II receptor Antagonist used as an anti-hypertensive. Hydrochlorothiazide\(^2\) is a Loop Diuretics used as an anti hypertensive by reducing symtomatic oedema. Literature survey reveals that there is method developed for the combination Losartan Potassium and Hydrochlorothiazide\(^1\). Present work emphasizes on the stability testing of Losartan Potassium and Hydrochlorothiazide in their combined dosage form by RP-HPLC.

Materials and Method:
A High Performance Liquid Chromatography system, the purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5μm diameter reverse phase C18 column (Luna CN (250mm x 4.6mm) 5μ). Optimized chromatographic conditions are listed in Table 1.

Validation of the Method\(^2\)
The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Losartan Potassium, Hydrochlorothiazide and measured the absorbance at 270nm. Calibration curves where constructed by plotting the area against the concentration. Losartan Potassium shows the
linearity in the concentration range from 35-65ppm with correlation coefficient of 0.9999 and Hydrochlorothiazide shows the linearity in the concentration range from 8.75-16.75ppm with correlation coefficient of 0.9998. Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to reanalyzed tablet powder, at three level and the percentage recoveries were calculated.

Table 1: Optimized Chromatographic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Shimadzu- LC-10Atvp/Empower software/PDA detector</td>
</tr>
<tr>
<td>Column</td>
<td>Luna C18 (250mm x 4.6mm) 5μ</td>
</tr>
<tr>
<td>Mobile phase*</td>
<td>Methanol : Acetate Buffer I.P. in the ratio of 70:30 (pH adjusted to 3.7 with acetic acid)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>248nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20μl</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

*Filtered through a 0.45μ membrane filter (Millipore), degassed and sonicated

Results and Discussion:

1. Estimation

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard deviations in formulation were calculated.

2. Validation of the method

The recovery studies were carried out 6 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in Table 1. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.
Table 1: Analysis of Formulation and Recovery studies

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Mean Peak Area for Losartan 50ppm</th>
<th>Losartan Potassium Assay (%)</th>
<th>Mean Peak Area for HCTZ 12.5ppm</th>
<th>HCTZ Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>847410</td>
<td>102.2</td>
<td>966274</td>
<td>99.8</td>
</tr>
<tr>
<td>2</td>
<td>852145</td>
<td>102.8</td>
<td>973023</td>
<td>100.5</td>
</tr>
<tr>
<td>3</td>
<td>840808</td>
<td>101.4</td>
<td>960635</td>
<td>99.2</td>
</tr>
<tr>
<td>4</td>
<td>860665</td>
<td>103.8</td>
<td>984912</td>
<td>101.7</td>
</tr>
<tr>
<td>5</td>
<td>847918</td>
<td>102.3</td>
<td>969477</td>
<td>100.1</td>
</tr>
<tr>
<td>6</td>
<td>847101</td>
<td>102.2</td>
<td>968552</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean</td>
<td>849341.02</td>
<td>102.5</td>
<td>970478.8</td>
<td>100.2</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.78</td>
<td>0.78</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Table 2: System Suitability Parameters

<table>
<thead>
<tr>
<th>System Suitability test</th>
<th>Analytes</th>
<th>RT(N=5)</th>
<th>Tailing Factor(N=5) Limit(NMT 2.0)</th>
<th>Theoretical Plates (N=5) Limit (NLT 2000)</th>
<th>%RSD (N=5) Limit (NMT 2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Losartan potassium</td>
<td>11.85</td>
<td>1.03</td>
<td>19440</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>HCTZ</td>
<td>7.88</td>
<td>1.02</td>
<td>57704</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Conclusion:
From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages. The standard and sample preparation requires less time. No tedious extraction procedure was involved in the analytical process and suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 15 times. Hence, the chromatographic method developed for Losartan Potassium and Hydrochlorothiazide were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

References:
Development and validation of stability-indicating method for assay of Linezolid immediate release tablet by reversed-phase HPLC

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Abstract: A stability indicating reverse-phase high performance liquid chromatographic method was developed for the assay of linezolid as a bulk drug and in pharmaceuticals. LC was carried out by an isocratic LC method using reversed phase technique on a C18 column. Eluents were monitored by UV detection at 251 nm using the mobile phase methanol–ammonium dihydrogen phosphate (0.002M) (50:50, v/v). The method was statistically validated and yielded good results and included linearity, accuracy, precision, robustness, ruggedness and specificity. The linearity of linezolid peak area responses was demonstrated within the concentration range of 25-75µg/mL and ($r^2 = 0.9999$). The results indicated that the proposed method is a simple, rapid and useful for linezolid determination in routine quality control and stability assay.

Introduction:
Linezolid is a synthetic antibiotic of oxazolidinone class used as antibacterial and anti-infective. It is used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several antibiotics. It is chemically known as N-[(5S)-3-[3-fluoro-4-(4morpholinyl)phenyl] – 2 – oxo – 5-oxazolidinyl] methyl] acetamide. It is official in the Indian Pharmacopoeia[1]. Literature Survey revealed that some methods are reported for determination of Linezolid in human serum by HPLC and LCMS/MS, HPTLC, HPLC and UV spectrophotometric method are cited in literature for the determination of linezolid.However, very less work has been reported of a stability indicating method for linezolid by HPLC. The paper describes the development and validation of a stability indicating RP-LC method for the assay of Linezolid as a bulk drug and in its pharmaceutical dosage form [2].

Materials and Method:
Chromatography was performed at 50°C temperature under isocratic conditions at a flow rate of 1.0 mL/min. Detection was done at 251 nm. A stock solution of linezolid (500 µg/mL) was prepared in diluents (Mobile Phase) and diluted further with diluent to obtain a standard solution of 50 µg/mL.

Forced degradation of linezolid
Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted using heat, oxidation, acid, base and humidity. For thermal degradation, the drug was exposed for 3 days at 100°C in oven. Oxidative degradation was induced by refluxing on a boiling water bath for 15 minutes, after treating with 2.5 mL 30 % H₂O₂. Acid degradation was attempted by refluxing linezolidon a boiling water bath for 30 minutes, after treating with 2.5 mL 1N HCl. Base degradation was performed by placing on bench top for 45 minutes, after treating with 5 mL 1N NaOH. Humidity degradation were performed by exposing the drug for 3 days at 40°C and 75% RH.

**Results and Discussion:**

Linezolid were quantitatively oxidized in 30 % H₂O₂ and was found more susceptible under the conditions employed for oxidation conditions than basic and acidic conditions. As evidenced from the percentage intact drug and difference in hydrolysis and oxidation time. The drug was found to be stable to heat and humidity under the conditions of the study. No degradation was observed upon refluxing the drug with methanol, suggesting that the degradation under oxidation, acidic and basic conditions was the result of oxidation and hydrolysis with no influence of heat and humidity on the degradation.

**Table 1: Optimization of chromatographic condition**

<table>
<thead>
<tr>
<th>Mobile phase composition</th>
<th>Flow rate ml/min</th>
<th>Temp. °C</th>
<th>Retention time(min) Linezolid</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (0.04M KH₂PO₄ pH 3.5): ACN: MeOH(20:60:20)</td>
<td>1.5</td>
<td>30</td>
<td>--</td>
<td>Peak was not proper; retention time comes under void time.</td>
</tr>
<tr>
<td>Buffer (0.02M KH₂PO₄ pH 3.5): ACN(35:65)</td>
<td>1.3</td>
<td>45</td>
<td>2.500</td>
<td>Peak Shape was not good and shouldering observed; retention time needs to be adjusted.</td>
</tr>
<tr>
<td>Buffer (0.02M NH₄H₂PO₄): MeOH(40:60)</td>
<td>0.6</td>
<td>45</td>
<td>5.587</td>
<td>Peak Shape was not good and broad peak obtained.</td>
</tr>
<tr>
<td>Buffer (0.002M NH₄H₂PO₄): MeOH(65:35)</td>
<td>1</td>
<td>45</td>
<td>8.057</td>
<td>Peak Shape was good and sharp but retention time needs to be reduced.</td>
</tr>
<tr>
<td>Buffer (0.002M NH₄H₂PO₄): MeOH (50:50)</td>
<td>1</td>
<td>50</td>
<td>3.827</td>
<td>Peak Shape was good and sharp with suitable reduced run time 5 min.</td>
</tr>
</tbody>
</table>
Fig.1. Chromatogram of linezolid

**Conclusion:**
The RP-HPLC assay method developed for linezolid is rapid, precise, accurate, specific and stability indicating. The method may be used for assessing the stability of linezolid as a bulk drug and in its pharmaceutical formulations. Hence, based on the statistical data these methods can be easily and conveniently adopted for routine estimation of assay of Linezolid drug in tablet dosage form.

**Acknowledgements:**
One of the authors is grateful to the management of Zydus Cadila Healthcare Ltd. for supporting this work.

**References:**
Validated HPLC method for estimation of Misoprostol in bulk drug and formulation

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Abstract: In this research work an attempt has been made for estimation of Misoprostol in tablet formulation through HPLC. This method is using ODS C8 column (250 mm × 4.6 mm, 5.0 μ) and mobile phase containing acetonitrile, methanol and water (60:30:10 % v/v/v) at flow rate of 1.6 ml/min with UV detection at 224 nm. This method has been applied to formulation without interference of excipients of formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 1-20 µg/ml and retention time 7.60 min. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.60 µg/ml and 0.95 µg/ml. Statistical analysis showed that the method is repeatable and selective for the estimation Misoprostol.

Introduction:
Misoprostol, methyl-7-([1R,2R,3R]-3-hydroxy-2-((S,E)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl) heptanoate is used for the prevention of non-steroidal anti-inflammatory drug (NSAID) induced gastric ulcers, for early abortion, to treat missed miscarriage, and to induce labor. Misoprostol may be dependent on guanosine-5-triphosphate (GTP). [1]

Literature review reveals that methods have been reported for analysis of Misoprostol, stability indicating HPLC method in raw materials and solid dosage form, RP-HPLC method for determination in combination with other drugs [2,3] and few bioanalytical methods are also reported [4,5,6]. Stability Indicating HPLC Assay method for Misoprostol, RP-HPLC method for determination of Misoprostol in combination with other drugs, HPTLC method for quantitation of Misoprostol in combination with other drugs is also reported. To date, there have been no published reports about the quantitation of Misoprostol by HPLC in bulk drug and in tablet dosage form. This present study reports for the first time quantitation of Misoprostol by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines [5,6]

Materials and Method:
All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India. The HPLC system consisted of a Pump (LC-10 ADVP Winchrome ltd.), with sampler programmed at 20 µL capacity per injection was used. Data was integrated using Jasco Borwin
version 1.5, LC-Net II/ADC system. For estimation of misoprostol reversed phase chromatography was preferred. As almost any separation can be achieved on an ODS C₈ column, it was the first choice for this method. An ODS C₈ column of length 250 mm and internal diameter 4.6 mm was selected for the analysis.

After series of trials with different composition of mobile phase, it was found that a mixture of acetonitrile, methanol and water in a ratio of 60:30:10 % v/v/v is most suitable mobile phase for resolution of misoprostol. The mobile phase was filtered through a membrane filter of 0.2 µ and then sonicated for 20 min in an ultrasonicator. Filtration and ultrasonication was carried out to remove particulate matter and to degas the mobile phase. Flow rate of 1.6 ml/min was employed.

Wavelength selected for estimation of drugs was 224 nm. The column was saturated with the mobile phase for about an hour at a flow rate of 1.6 ml/min, monitoring the eluent at 224 nm so as to obtain a steady base line. After the chromatographic conditions were set and the instrument was stabilized to obtain a steady baseline, 20 µL of standard drug solution of misoprostol (10µg/ml) made in mobile phase was loaded into the injection port of the instrument and injected after filtration through a 0.2 µ membrane filter. The injection was repeated three times. This was done to check retention times of drug. The mean retention time for misoprostol was found to be 7.60 min.

The linearity was observed in the concentration range of 1- 20 µg/ml for misoprostol.

**Method validation**

Linearity, accuracy, precision (interday, intraday), Reproducibility, specificity, LOD, LOQ, Sample solution stability, robustness, System suitability tests were performed. The data from peak area versus drug concentration plots were treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility. The experiment was performed in triplicate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>1 - 20 µg/ml</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope</td>
<td>6542.5</td>
</tr>
<tr>
<td>Intercept</td>
<td>210781</td>
</tr>
<tr>
<td>Retention time</td>
<td>7.60 min</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.95 µg/ml</td>
</tr>
<tr>
<td>LOD</td>
<td>0.60 µg/ml</td>
</tr>
</tbody>
</table>

**Results and Discussion:**

In the present work HPLC method has been developed for estimation of misoprostol from its tablet dosage form. The developed HPLC method for estimation of misoprostol make use of C₈ column. Mobile phase used for this method was acetonitrile, methanol and water (60:30:10 % v/v/v) and
detection of eluent was carried out at 224.0 nm. The total run time of this method was less than 10 min and retention time for misoprostol was found to be at 7.60 min. at a flow rate of 1.6 ml/min. Percentage label claim of tablet formulation using this method was found to be 99.66%. Standard deviation was found to be 0.255. The results of analysis of two drugs from their tablet formulation using developed method were found close to 100%. The low values of standard deviation indicate accuracy and reproducibility of methods. The recovery studies were satisfactory which shows no interference from the excipients. Thus this developed method can be used for the routine analysis of two drugs from combined dosage form.

References:
Abstract: Isatins (21-30) were prepared by using Sandmeyer method and the substituted 3-(2-morpholinoethylimino)indolin-2-one derivatives (32-41) were obtained by reacting substituted isatins (21-30) with 2-morpholinoethanamine (31). The structures of all the synthesized compounds were deduced on the basis of spectral data and elemental analysis. All the synthesized compounds were screened for in vitro antimicrobial activity.

Introduction:
Morpholine derivatives are known to exhibit a wide range of biological activities such as antimicrobial, antitubercular and anticancer. Some isatin derivatives were reported to possess antimicrobial and antitubercular activity. In view of all these observations and in continuation to our work on isatin and piperazine we decided to synthesize Schiff bases of these two scaffolds and to evaluate them for antimicrobial activity.

Materials and Method:
Synthesis of substituted isatins (21-30)
Substituted isatins (21-30) were synthesized by following Sandmeyer method.

Synthesis of 2-morpholinoethanamine derivatives (32-41)
Equimolar quantity of substituted/unsubstituted isatin (21-30, 0.1mol) and 2-morpholinoethanamine (31, 0.1mol) in acetonitrile (20mL) was stirred for 5h at room temperature or refluxed for 10h. The reaction mixture was cooled in ice bath. The precipitate thus obtained was filtered, washed with acetonitrile, dried and was purified by recrystallization.

Antimicrobial activity
All the synthesized compounds were screened for antibacterial and antifungal activities by determining MIC’s in µg mL⁻¹ against selected strains. The MIC’s was determined by broth dilution method. The Minimum Inhibitory Concentration (MIC) was determined for each compound along with ciprofloxacin and fluconazole as reference standards and the results are presented in Tables 2.

Results & Discussion:
In present work, novel ten Schiff bases of 2-morpholinoethanamine were synthesized as outlined in the scheme I. The synthesized compounds (32-41) were characterized by their physical properties and spectral data. The formation of the Schiff bases was evident by appearance of bands/peaks as mentioned in Table 1.

**Scheme I: Synthesis of 3-(2-morpholinoethylimino)indolin-2-one derivatives (32-41)**

![Scheme I: Synthesis of 3-(2-morpholinoethylimino)indolin-2-one derivatives (32-41)](image)

**Table 1: Spectral data of compounds (32-41)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (cm⁻¹)</th>
<th>¹H NMR (δ ppm)</th>
<th>¹³C NMR (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 - 41</td>
<td>1039-1114 (C-O-C), 1616-1660 (C=N), 1660-1742 (C=O), 2800-2958.80 (Al C-H) 3000 (Ar C-H) 3165-3246 (N-H)</td>
<td>2.67-2.76 (t, CH₂-N-CH₂), 2.817-4.40 (Al C-H), 4.41-4.49 (t, CH₂-O-CH₂ ), 6.70-7.55 (Ar C-H), 10.05-10.83 (s, N-H)</td>
<td>48.74-66.19 (Al C), 116.6-133.53 (Ar C), 155-159 (C=N), 162.9-165.4 (C=O)</td>
</tr>
</tbody>
</table>

The antibacterial activity results showed that the compound 33, 34, 35, 36 and 41 exhibited MIC value ranging between 12.5-25 μg/mL. The antifungal activity results revealed that all the compound exhibited MIC value ranging between 0.2-6.25 μg/mL.

**Conclusions:**

In the present work the Schiff bases of 2-morpholinoethanamine 32-41 were satisfactorily synthesized and characterized by various spectral analysis. By comparing the *in vitro* antimicrobial of these compounds, we conclude that electron withdrawing substituents at 5th and 7th position of isatin ring enhance the activity. In contrast the compounds having electron withdrawing substituents at 6th position show moderate activity. Hence it is predicted that the position of the substituents either
electron releasing/electron withdrawing groups do affect the biological activity of a particular derivative.

**Table 2:** Antimicrobial activity of morpholine derivatives (32-41)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Minimum Inhibitory Concentration (MIC) in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>32</td>
<td>H</td>
<td>50</td>
</tr>
<tr>
<td>33</td>
<td>5-CH₃</td>
<td>12.5</td>
</tr>
<tr>
<td>34</td>
<td>5-F</td>
<td>12.5</td>
</tr>
<tr>
<td>35</td>
<td>7-Cl</td>
<td>50</td>
</tr>
<tr>
<td>36</td>
<td>5,7-diCl</td>
<td>12.5</td>
</tr>
<tr>
<td>37</td>
<td>7-CH₃</td>
<td>50</td>
</tr>
<tr>
<td>38</td>
<td>5-Cl</td>
<td>50</td>
</tr>
<tr>
<td>39</td>
<td>6-Cl</td>
<td>50</td>
</tr>
<tr>
<td>40</td>
<td>6-CH₃</td>
<td>50</td>
</tr>
<tr>
<td>41</td>
<td>5-Br</td>
<td>25</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

References:


Synthesis, characterization and biological evaluation of some new 3, 5 Diaryl substituted Pyrazoline derivatives

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Abstract: Pyrazoline derivatives have been explored extensively by various researchers in past years and investigations supported their therapeutics importance as antimicrobial agents also; considering this fact present study aimed to synthesize some new 3, 5 diaryl substituted pyrazoline derivatives and their screening for anti-bacterial activity. Various substituted pyrazoline derivatives were synthesized by cyclization of substituted chalcone derivatives in presence of hydrazine hydrate.

Introduction:
Pyrazolines are nitrogen containing heterocyclic compounds play important role in medicinal chemistry [1]. Literature review suggested that pyrazoline derivatives possess considerable biological activities like; analgesic and anti-inflammatory, antiamoebic, antimicrobial, antidepressant, anti-tubercular and antimalarial activity [2-5]. The present study involves synthesis and screening of new pyrazoline derivatives as potent anti-bacterial agents against Escherichia coli and Bacillus subtilus.

Materials and Method:
Melting points were determined by thieis tube and were uncorrected. Synthesized derivative were characterized by FT-IR, ¹HNMR and MS spectrometry.

Synthesis of Substituted Pyrazoline Derivatives:

Synthesis of Chalcone [1a-1b]:
Substituted acetophenones were dissolved in ethanol. Substituted benzaldehydes were added and the solution was heated to boiling. To this solution 40% NaOH was added with constant stirring. A yellow-orange coloured solid mass was obtained which was kept overnight and acidified by 10% HCL, washed with 10% NaHCO₃ followed by water and crystallized from ethanol to give substituted chalcone.

Synthesis of pyrazoline [2a-2b]: Substituted Chalcones were dissolved in ethanol and that after hydrazine hydrate was added to it. The reaction mixture was refluxed for 2 hr, cooled concentrated and allowed to stand overnight. The solid mass obtained which then separated out and crystallized from ethanol to get substituted pyrazoline.
Synthesis of N- substituted Pyrazoline [3a-3b]: Substituted pyrazoline and acetic acid were refluxed for 2 hr. The reaction mixture was then concentrated, allowed to cool, the solid product filtered, washed with water and recrystallized from ethanol to get N- substituted pyrazoline.

Synthesis of N- substituted Pyrazoline [4a-4b]: Substituted pyrazoline and benzyl chloride was dissolved in pyridine and stirred at room temperature for 1 hr. then reaction mixture was treated with cold dilute HCL. The resulting solid was filtered, washed successively with water, NaOH (2%) and finally crystallized from glacial acetic acid to get N- substituted pyrazoline

Biological evaluation:

In-vitro Anti-bacterial activity of synthesized derivatives: The define volume of nutrient broth (3 gm) were dissolved in 200 ml of distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving for 15- 20 min. Bacterial culture were prepared in nutrient broth one day prior to test and incubated at 37°C for 18 -24 hr. All derivatives were dissolved in dimethyl sulphoxide (DMSO) (100mg/ml) and different dilutions of derivatives in each well were filled, such as 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, 300µg/ml using DMSO as solvent. DMSO was used as a control.

Results and Discussion:

Substituted Pyrazoline derivatives were synthesized via cyclization of substituted chalcones. Structure of all the synthesized derivatives have been established on the basis of their consistent IR, $^1$H-NMR and Mass spectral data. Compound 3a [1-(3-(4-hydroxybenzyl)-5-phenyl-4, 5-dihydropyrazole-1-yl) ethanone] possess most potent antibacterial activity while other compounds showed mild to good activity against the tested microbes.

Table 1. Result of in-vitro Anti-bacterial activity of synthesized products.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Product code</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>1</td>
<td>3a</td>
<td>0.78</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>3.125</td>
</tr>
<tr>
<td>3</td>
<td>4a</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>4b</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Conclusion:

These results suggested that the 3, 5 diaryl substituted pyrazoline derivatives have an excellent scope for further development as commercial anti-bacterial agent
Figure 1. Scheme for the synthesis of proposed pyrazoline derivatives.

References:

Synthesis, characterization and biological evaluation of new substituted Isoxazole derivatives as antiobesity agents

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Abstract: Various substituted Isoxazole (A-E) derivatives were synthesized. The structures of these compounds were established by spectral (IR, $^1$H-NMR, Mass) analysis. The synthesized compounds were screened for their antiobesity activities. Compound J [4-(3-(3, 4-dimethoxyphenyl) isoxal-5-yl) phenol] was found to possess significant antiobesity activity since it has shown the minimum gain in body weight when compared with the control group.

Introduction:
Isoxazole derivatives are well known five membered heterocyclic compounds and possess various biological activities such as PTP IB inhibitors, antifungal, anti-inflammatory, anticonvulsant, antitubercular, immunomodulatory activity [1-3]. The present investigation involves synthesis of various isoxazole derivatives via cyclization of substituted chalcone and their screening for antiobesity activities.

Materials and Method:
The progress of the reaction was monitored by TLC and products were purified through recrystallization. The spectral analysis was performed by IR, $^1$H-NMR and Mass spectrometry.

1. General procedure for synthesis of Chalcones: (A-E)
A solution of sodium hydroxide (30%) in water and rectified spirit was placed in a flask provided with a mechanical stirrer. The flask was immersed in a bath of crushed ice. Substituted acetophenone was poured with constant stirring that after substituted benzaldehydes was also added. The mixture stirred vigorously until it became thick enough (Approx. 6 hr). The reaction mixture was kept at 8°C overnight. The product was filtered with suction on a buchner funnel, washed with cold water until the washings were neutral to litmus and then with ice cold ethanol. The crude product was recrystallized from ethanol.

1. 1. General procedure for synthesis of Isoxazole derivatives: (F-J)
A mixture of substituted Chalcones (A-E) and hydroxylamine hydrochloride in ethanol was taken in a round bottom flask. The reaction mixture was refluxed for 6 hrs on a water bath followed with addition of ice cold water at room temperature. The mixture was kept overnight at 8°C. The
precipitates were filtered, washed with distilled water and dried. The product was recrystallized with ethanol to obtain the final product (F-J).

**Biological activity (Antiobesity activity):**

*Effect on diet induced obesity model:* Compounds were tested for the decrease in body weight in rats of average body weight 150-180 gm. The body weight of all animals were checked and randomly divided into groups of three animals each. Animals of experimental group were administered the suspension of synthesized compounds with 0.9% saline and high fat diet. Animals of standard group were given Sibutramine suspended in 0.9% saline and high fat diet. Animals of control group were given only 0.9% saline; whereas animals of diet control group were given only high fat diet. The mentioned schedule was followed for the respective groups of animals for 20 days. The body weight was recorded on day 1 and then on alternate days for 20 days.

**Results and Discussion:**
All the compounds were evaluated for their antiobesity activity by diet induced obesity model. The present pharmacological investigation revealed that high fat diet significantly increased body weight. The treatment with synthesized compounds showed significant reduction in body weight of rats fed high fat diet, indicating that the synthesized compounds possess weight reducing property. The compound J [4-(3-(3, 4-dimethoxyphenyl) isoxal-5-yl) phenol] possessed most potent response as compared to other derivates.

**Conclusion:**
Study concluded that synthesized compounds possessed potent antiobesity activity. The results of present study have suggested that the Isoxazole derivatives have excellent scope for further development as commercial antiobesity agents.

**Table 1. Result of In-vivo Antiobesity activity of synthesized derivatives**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Weight on day 1 (gm)</th>
<th>Weight on day 20 (gm)</th>
<th>Weight gain (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F</td>
<td>161.24±3.185</td>
<td>170.24±2.027</td>
<td>9.0±1.158</td>
</tr>
<tr>
<td>2.</td>
<td>G</td>
<td>157.58±3.296</td>
<td>167.58±2.138</td>
<td>10.0±1.267</td>
</tr>
<tr>
<td>3.</td>
<td>H</td>
<td>159.11±3.317</td>
<td>168.61±2.249</td>
<td>9.50±1.068</td>
</tr>
<tr>
<td>4.</td>
<td>I</td>
<td>162.18±3.428</td>
<td>169.68±2.350</td>
<td>7.5±1.078</td>
</tr>
<tr>
<td>5.</td>
<td>J</td>
<td>161.93±3.519</td>
<td>169.28±2.461</td>
<td>7.35±1.168*</td>
</tr>
<tr>
<td>6.</td>
<td>Sibutramine</td>
<td>163.24±3.489</td>
<td>171.56±4.360</td>
<td>8.32±1.129</td>
</tr>
<tr>
<td>7.</td>
<td>Diet Control</td>
<td>160.33±3.094</td>
<td>185.83±4.524</td>
<td>25.50±1.429</td>
</tr>
<tr>
<td>8.</td>
<td>Control</td>
<td>156.12±3.562</td>
<td>158.23±4.154</td>
<td>2.11±1.407</td>
</tr>
</tbody>
</table>

Data presented as Mean ± SEM, n=3, p<0.05    * indicate most potent compound
Figure 1. Scheme for the synthesis of proposed Isoxazole Derivatives.

References:
Synthesis, antimycobacterial screening and ligand-based molecular docking studies on novel pyrrole derivatives bearing Pyrazoline, Isoxazole and Phenyl Thiourea moieties

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Abstract: We report here the synthesis, antibacterial and antitubercular evaluation of 61 novel pyrrolyl derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties. Docking analysis of the crystal structure of ENR performed using Surflex-Dock in Sybyl-X 2.0 software indicates the occupation of substituted pyrrolyl derivatives into hydrophobic pocket of InhA enzyme. Compounds 9b and 9d exhibited the highest antitubercular activity almost close to isoniazid (0.4 µg/mL) with a MIC value of 0.8 µg/mL. The compounds were further tested for mammalian cell toxicity using human lung cancer cell-line (A549) and were nontoxic.

Introduction:
Tuberculosis is one of the chronic disorder caused by Mycobacterium tuberculosis, it is know a day becoming an alarm for medical ferternity, as it is developing resistance viz., multi-drug resistance (MDR-TB), extensive-drug resistance (XRD-TB) and HIV infection. There are so many enzymes which are involved in the resistance but our main concentration is on the synthesis of mycolic acid, which is the key enzyme of the fatty acid synthase system of M. tuberculosis the synthesis of mycobacterial cell wall. InhA, the enoyl acyl carrier protein reductase (ENR) from M. tuberculosis is the key enzyme for type II fatty acid synthesis (FAS II), which catalyses NADH-dependent reduction of 2-trans-enoyl-ACP (acyl carrier protein) to yield NAD+ and reduced enoyl thioester-ACP substrate, which in turn, helps the synthesis of mycolic acid [1]. In our previous studies [2, 3], we have synthesized the potential inhibitors of InhA bearing pyrrole as a central core with different pharmacophores in a single molecular framework along with 2D and 3D-QSAR studies. In this work, we have undertaken to develop new chemical entities containing pyrrole as the core that inhibits enoyl ACP reductase enzyme along with their in vitro antibacterial and anti-TB activities bearing pyrazoline, isoxazole and phenylthiourea.

Materials and Methods:
All the compounds were synthesized as per steps outlined in Schemes 1, 2 and 3. The Paal-Knorr reaction was performed to synthesize (4-pyrryl-1-yl)acetophenone (2) by condensing 4-amino acetophenone (1) with 2,5-dimethoxytetrahydrofuran. The required key intermediates viz., chalcones
were obtained by Claisen-Schmidt condensation of (4-pyrrol-1-yl)acetophenone (2) with the substituted aldehydes in the presence of sodium hydroxide catalyst in ethanol. Chalcones (3a-r) were treated with hydrazine hydrate and glacial acetic acid in a solvent free condition to obtain the corresponding N-acetyl pyrazolines (4a-s). The reaction of chalcones with hydroxylamine hydrochloride and sodium acetate in the presence of glacial acetic acid led to the synthesis of 5-(4-(1H-pyrrol-1-yl)phenyl)-3-substituted phenylisoxazoles (5a-r) as per schemes 1 and 2.

o-Phenylenediamine (6) reacted with acetonyl acetone (7) to afford 2-(2,5-dimethyl-1H-pyrrol-1-yl)aniline (8). Next, different phenylisothiocyanates were reacted with intermediate 8 in dry chloroform to get the final desired 1-(2-(2,5dimethyl-1H-pyrrol-1-yl)phenyl)-3-(substituted phenyl)thioureas (9a-k) with good yields (Scheme 3).

Results and discussion:

**Figure 1** Synthetic route of a novel series of pyrrole chalcone derivatives.

**Figure 2** Synthetic route of a novel series of pyrrole isoxazole and pyrazoline derivatives.

Conclusion:

These pyrrole derivatives were further explored in search of novel antitubercular and antibacterial agents, identifying several derivatives with reasonable inhibitory activities against *M. tuberculosis*. Of all the compounds tested, 9a-k displayed better activities against both Gram positive and Gram negative bacteria with the MIC value of 0.2 - 1.6 µg/mL. Compounds 3d, 3e, 3g, 3m, 3o, 4d, 4e, 4i, 4m, 4p, 4q and 5m displayed asignificant activities (6.25 µg/mL) against *M. tuberculosis* H37Rv strain. The two compounds viz., 9b and 9d exhibited interesting anti-TB activities with the MIC of
0.8 µg/mL and no apparent cytotoxicities towards human lung cancer cell-line (A549). Furthermore, compounds 9b and 9d displayed good inhibition activities InhA.

**Figure 3**: Synthetic route of a novel series of pyrrolyl phenyl thiourea.

Molecular docking of the compounds was carried out for better understanding of the drug-receptor interaction. Docking simulation studies have shown that these compounds are bound mainly with the substrate binding site of InhA and the scoring function for most of the compounds is similar to that of the reference inhibitor. The anti-TB activity of these compounds was fully supported by in silico molecular docking calculations. The synthesized compounds will be quite useful as the lead compounds for developing InhA inhibitors.

**Acknowledgements:**
The authors acknowledge the financial support from the Council of Scientific and Industrial Research, New Delhi, India (Letter No. 02(0139)/13/EMR-II dated- 12/04/2013). We thank Mr. H. V. Dambal, president, S. E. T’s college of Pharmacy for his support and Dr. Christian Lherbet, Universite de Toulouse, UPS, Laboratoire de Synthese et Physico-chimie de Molecules d’Interet Biologique, France, for providing enzyme inhibition studies and Dr. K.G.Bhat of Maratha Mandal’s Dental College, Hospital and Research Centre, Belgaum, for providing anti-tubercular and cytotoxic activities. Prof. Kap Seung Yang, Department of Polymer and Fiber System Engineering, Chonnam National University, South-Korea and the Director, SAIF, Panjab University, Chandigarh, Panjab, India have provided some of the NMR and mass spectral data. The authors also appreciate the technical assistance from Mr. Ravi Nadagir.

**References:**
Structural requisites for Aurones as antimalarial agents

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Abstract: In the present paper, an effort has been made to correlate the antimalarial activity of the aurones to the physicochemical descriptors through QSAR studies. kNN-MFA and other advanced methods of analysis were opted for a set of thirty five aurone derivatives to figure out the correlation-ship between steric and electrostatic descriptors. In 2D-QSAR, amongst the several models obtained, statistically significant model 2 was selected with $r^2=0.8140$, $q^2=0.7590$ and $\text{pred}_r^2=0.7933$ with three descriptors $\text{chiV5chain}$, $\text{RotatableBondCount}$ and $\text{T}_2_2_2$ which showed significant contributions in the model. The model was found to be significant within the corresponding limits of internal and external validation parameters. In 3D-QSAR, statistically significant model 4 was obtained using kNN-MFA algorithm, with the corresponding $q^2$ and $\text{pred}_r^2$ values 0.6535 and 0.6674 respectively, showing internal and external predictivities 65% and 66% respectively.

Introduction:
Malaria kills over 1.5 million people a year and it is responsible for human misery in tropical countries. Malaria is caused by a protozoal parasite of the genus Plasmodium and remains world-wide problem and there is an urgent need to identifying new class of anti-malarial agents [1].

Materials and Method:
A set of 35 compounds used in 2D QSAR were now subjected to 3D QSAR analysis by advance kNN molecular field analysis. The 3D QSAR analysis resulted in two models for different combinations of test and training sets. Sphere exclusion algorithm method was opted for division of test and training set. Three models (Model 1, 2, 3) were obtained using MLR, PLS and PCR. The training set included 22, 26, 30, 17, 29, 18, 37, 32, 33, 34, 36, 11, 4, 14, 7, 3, 8, 10, 9, 20, 16, 15, 12, 35 and 21. The compounds that comprised of test set are 23, 24, 25, 28, 31, 27, 19, 6, 5 and 13. Two advanced kNN molecular field analysis methods (SWFB and SA) were employed in 3D QSAR analysis. The test set included 37, 15, 23, 26, 28, 31, 12, 18, 3, 8 rest molecules were placed in the training set for 3D QSAR study with dissimilarity value set to 7[2].

Result and Discussion:

D QSAR approach: The model 1 determined by multiple linear expression showed in Table 1 has an internal predictive power $q^2=0.7678$ of 77 % and predictivity for external test set $\text{pred}_r^2=0.7925$ about 79%. Model 2 was generated by partial least square analysis with three significant parameters
with $r^2 = 0.8140$ as coefficient of determination. It is capable of explaining 81% of variance in the observed activity values. The model showed an internal predictive power $q^2 = 0.7590$ of 75% and predictivity for external test set $\text{pred}_r^2 = 0.7933$ about 79%. Model 3 a tri-parametric model generated with principle component analysis with same distribution of test and training set.

Table 1: Statistical Analysis of Multiple Regression, Partial Least Square and Principle Component analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1 (MLR)</th>
<th>Model 2 (PLS)</th>
<th>Model 3 (PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptor_1</td>
<td>chiV5chain 116.4690</td>
<td>chiV5chain 118.3850</td>
<td>chiV5chain 121.6420</td>
</tr>
<tr>
<td></td>
<td>($\pm$23.2877)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Descriptor_2</td>
<td>RotatableBondCount 0.1401</td>
<td>RotatableBondCount 0.1397</td>
<td>RotatableBondCount 0.1354</td>
</tr>
<tr>
<td>Descriptor_3</td>
<td>T_2_2_1 -0.1174($\pm$0.0002)</td>
<td>T_2_2_2 -0.0837</td>
<td>T_2_2_1 -0.1135</td>
</tr>
<tr>
<td>Constant</td>
<td>2.5846</td>
<td>2.3733</td>
<td>2.3990</td>
</tr>
<tr>
<td>Deg. of freedom</td>
<td>21</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Opt. Comp.</td>
<td>--</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>n (training/test)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.7925</td>
<td>0.7933</td>
<td>0.7901</td>
</tr>
<tr>
<td>$q^2$</td>
<td>0.8140</td>
<td>0.7933</td>
<td>0.7840</td>
</tr>
</tbody>
</table>

It confirmed that one alignment independent descriptors $T_2_2_2$ play pivotal role in determining activity. In the same way $T_2_2_2$ is the count of number of double bounded atoms (i.e. any double bonded atom, $T_2$) separated from any other double bonded atom by 2 bonds in a molecule. The physico-chemical chiV5chain descriptor signifies atomic valence connectivity index for five membered ring as depicted in Figure 1. The descriptor namely RotatableBondCount is the number of rotatable bonds indicating physicochemical properties of the molecule. The alignment Independent descriptor $T_2_2_1$ is defined as the count of number of double bounded atoms (i.e. any double bonded atom, $T_2$) separated from any other double bonded atom by 1 bonds in a molecule.

Figure 1: Contribution Chart for Model 1

3D kNN Molecular field approach

In 3D QSAR analysis the descriptors that get selected in a given model were the field points either of steric or electrostatic nature at particular locations in a common grid around reported set of molecules. The extrema of field values of compounds in the cluster of most active compounds decide range of field values which is preferred and recommended for new compound design. In the analysis, Model 4 as illustrated in Table 2 the two parametric model obtained by SWFB. The electrostatic descriptor (E_525 -2.7441 -2.6196) suggests that the electronegative atoms will always be conducive to activity. The descriptor (S_940 -0.7498 -0.3508) has shown negative range inferring that analogues with large aromatic and aliphatic substituents will decrease
biological activity at and hence less bulky aromatic groups are required for enhancing the biological activity, bulky groups can be detrimental to activity.

**Table 2:** Statistical Analysis of kNN-MFA and SA

<table>
<thead>
<tr>
<th>Descriptor Range</th>
<th>kNN-MFA (Model 4)</th>
<th>Simulated Annealing (Model 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptor Range:</td>
<td>E_525 -2.7441 -2.6196</td>
<td>S_1069 -0.1126 -0.1122</td>
</tr>
<tr>
<td>Descriptor Range:</td>
<td>S_940 -0.7498 -0.3508</td>
<td>S_960 30.0000 30.0000</td>
</tr>
<tr>
<td>k Nearest Neighbour</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Degree_of_freedom</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>q^2</td>
<td>0.6535</td>
<td>0.5890</td>
</tr>
<tr>
<td>pred_r^2</td>
<td>0.6674</td>
<td>0.4875</td>
</tr>
</tbody>
</table>

**Conclusion:**
A QSAR study on 35 of 1-Azaaurones derived from the naturally occurring aurones as potential antimalarial drug analogues acting is described in this research. The model was investigated for reliability and stability by using statistical analysis criteria. The resultant Models 2 (r^2=0.8140) suggest importance of chiV5chain, RotatableBondCount and T_2_2_2 of the molecules in determining the activities in 2D QSAR analysis. Since PLS approach utilizes simultaneously information of available dependent response (i.e., activity) and independent variables (molecular descriptors) while building the QSAR model, it offers a significant advantage to compare behaviour of common subset descriptors toward individual activity. Similarly in 3D QSAR analysis, advanced kNN-MFA Model 4 Step Wise Forward Back Ward kNN Method (q^2 = 0.65) yielded a two descriptor model with contributing descriptors E_525 -2.7441 -2.6196, S_940 -0.7498 -0.3508 provided confidence in robustness of descriptors toward data distribution. QSAR model validation becomes an essential part in the development of a statistically valid and predictive model, because the real utility of a QSAR model was to design and predict accurately the modelled properties of the newly synthesized compounds as antimalarial agents.

**Acknowledgement:**
The author wishes to acknowledge V-life Science Technologies Pvt. Ltd for providing the software for the study, and Head, School of Pharmacy, Devi Ahilya Vishwavidyalaya for providing facilities to carry out the work.

**References:**


“Solid as solvent”- novel spectrophotometric analysis of Piroxicam tablets using solids (eutectic mixture of phenol and Metformin Hydrochloride) as solubilizing agents (mixed solvency concept)

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Abstract: The present attempt is a research to show that solid can also be used as solvents in various spectrophotometric methods. In present study we are using eutectic mixture of Phenol and Metformin hydrochloride (PMHCl 41) which is obtained by trituration of crystals of phenol and metformin hydrochloride employed to extract (dissolve) Piroxicam from fine tablet triturate powder. Distilled water is used for dilution to carry out spectrophotometric analysis at 358nm without using any toxic and carcinogenic organic solvents. The solubility of Piroxicam in distilled water at room temperature was found to be 0.4 mg/ml while the solubility of Piroxicam in PMHCl 41 was more than 125mg/ml (of PMHCl 41). This new innovative approach can be used in day to day spectrophotometric analysis of compounds. The corresponding approach is accurate and reproducible. The method provides ecofriendly way for the estimation of the compound. The accuracy, reproducibility and precision of the method were confirmed by recovery studies and statistical data. The presence of excipients, eutectic mixture (PMHCl 41) did not interfere in spectrophotometric analysis estimation at 358nm. PMHCl 41 does not interfere above 300nm.

Introduction:

Poor water solubility is the major issue for most of the existing and upcoming pharmaceutical products. The drug shows poor solubility in their analytical estimation and in the liquid dosage form in solutions. All substances present on the earth possess solubilizing properties. Each substance shall show solubilizing power for some solutes and non-solubilizing power for other. For water insoluble drugs, commonly used organic solvents for spectrophotometric analysis includes Methanol, Ethanol, Chloroform, Benzene, Dichloromethane, Dimethyl formamide, Acetonitrile, Ethyl acetate, Toluene, Carbon tetrachloride, Acetone, Hexane, etc. The main problems of organic solvents include high cost, toxicity and pollution. They should be replaced by eco-friendly and alternative sources. The use of “solids as solvents” shall prove a boon in pharmaceutical field. The solvents action of solids can be demonstrated nicely by mixed solvency concept. By application of this concept innumerable solvent systems can be developed. The advantage of mixed solvency concept is employing combination of pharmaceutical excipients in small concentration as a result of which toxicity is reduced. The present research is an attempt to prove that solid can also be wisely used to act as solvents, precluding the use of organic solvents. The main objective of present study is to demonstrate solvent action of solids.
Materials and method:
Piroxicam bulk drug sample was generous gift by M/S Shree Pharmaceuticals, Indore (India). Metformin hydrochloride was generous gift from M/S IPCA Laboratories Ltd., Ratlam (India). Commercial tablets of Piroxicam (Piroxits DT of Intas Pharmaceuticals Limited, Ahmedabad and Nesprex-DT of Nestor Pharmaceuticals Limited, Goa) were procured from local market. Other chemicals used for research were of analytical grade. A Shimadzu-1700 UV Visible spectrophotometer with 1 cm matched silica cell was used for spectrophotometric analysis.

Preparation of eutectic liquid: Phenol and Metformin hydrochloride were triturated in (4:1) ratio of their respective weight quantity and eutectic liquid (PMHCL 41) was prepared.

Calibration curve: Accurately weighed 50mg of Piroxicam standard drug was transferred to a 500ml volumetric flask and 10ml of PMHCL 41 was added to it. The flask was shaken to solubilize the drug. Then, about 400ml distilled water was added and the flask was shaken for 5min to solubilize the contents. The volume was made up to the mark with distilled water. This stock solution (100µg/ml) was suitably diluted with distilled water to obtain standard solutions of 5, 10, 15, 20 and 25µg/ml. The absorbances of these standard solutions were noted at 358nm against respective reagent blanks to obtain the calibration curve.

Proposed method of analysis: To carry out spectrophotometric analysis, twenty tablets of tablet formulation I were weighed and crushed to get a fine powder. Tablet powder equivalent to 50 mg Piroxicam was transferred to a 500 ml volumetric flask. Then, 10 ml of PMHCL 41 was transferred to it and the flask was briskly shaken for 10 minutes to extract the drug from tablet powder. Then, 400 ml distilled water was added and the flask was shaken for 5 minutes to homogenize the contents. The volume was made up to the mark with sufficient distilled water. Filtration was carried out through Whatmann filter paper #41 to remove the tablet excipients. Ten ml of the filtrate was diluted to 50 ml with distilled water. Then, the absorbance of the filtrate was noted at 358 nm against reagent blank. Using the calibration curve, the drug content was calculated. Same procedure was repeated for tablet formulation II. The results of analysis were reported as in table 1.

Recovery studies: The recovery studies were performed in which standard Piroxicam drug was added (15 mg and 30 mg, respectively) to the pre-analyzed tablet powder equivalent to 50 mg Piroxicam and drug content was determined by the proposed method. Results of analysis were reported as in table 2 with statistical evaluation.

Table 1: Analysis data of Piroxicam tablet formulations with statistical evaluation (n=3)

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label Claim (mg/tablet)</th>
<th>Percent drug estimated (mean ± SD)</th>
<th>Percent coefficient of variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>101.39 ± 1.419</td>
<td>1.399</td>
<td>0.819</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>99.57 ± 1.914</td>
<td>1.922</td>
<td>1.105</td>
</tr>
</tbody>
</table>
Results and discussion:

The Solubility of Piroxicam in distilled water at the room temperature was found to be 0.4 mg/ml. The solubility of Piroxicam in PMHCl 41 was more than 125 mg/ml. It is evident from table 1 that the percent drug estimation in tablet formulation I and II were 101.39 ± 1.419 and 99.57 ± 1.914, respectively. The values are very close to 100, indicating the accuracy and precision of the proposed analytical method. In addition to this, the table 2 emphasizes on the percent recoveries studies which varies from 99.81 ± 1.082 to 101.74 ± 1.290 which are again very close to 100.00, indicating the accuracy and precision of the proposed method. Proposed analytical technique is further supported remarkably by small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error as indicated in table 2.

Conclusion:

The evidence that supports the solvency of Piroxicam in Eutectic liquid of phenol and Metformin Hydrochloride in 4:1 ratio on weight basis was suitably demonstrated by the above research. This supports that the extraction (dissolution) of Piroxicam from fine powder of tablets can be carried out by use of “solid as solvent concept”. The presence of PMHCl 41 does not interfere in spectrophotometric estimation at 358 nm. Phenol and metformin hydrochloride do not interfere above 300 nm. The proposed research opens the new dimensions of eco-friendly and safe methods of estimations in pharmaceutical field. The research evokes and builds potential for use of such novel methods for use of “solid as solvents”.

References:

Synthesis and characterization of block polymer of poly (polyethylene glycol-glycerol-co-citrate) for drug delivery system

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Abstract: From the synthetic biodegradable polymers, aliphatic polyesters have attracted the attention to the various researchers, as they have combined features of biocompatibility, biodegradability and bioreabsorbability. Citric acid based polymers are comparatively new and having better profile in hydrolytic resistant and controlled degradation. Thereby, a better controlled release pattern can be achieved from them. In the present work, block polymer of citric acid, polyethylene glycol and glycerol was synthesized using condensation polymerization method in three steps. Although the synthesis of block polymer in the melt is more challenging without catalyst, it is beneficial from both a green chemistry standpoint and a biocompatibility perspective, since unnecessary additives were eliminated. From the standpoint of biodegradation, a catalyst free reaction eliminates concerns related to leaching unwanted products. Characterization of synthesized polymers for structure and molecular weight was confirmed by different analytical techniques like FTIR, NMR, MALDI and thermal properties were analyzed by DSC and TGA analysis. The performance of the synthesized polymer for drug delivery system was studied by the formulation development of enteric coated tablets of the polymer synthesized.

Introduction:
The development of the biodegradable materials has stimulated interest in the range of biotechnological and biomedical applications. Recently, there has been an increasing interest for the development of synthetic biodegradable polymers which can be used as different drug delivery systems for the controlled and sustained release of drugs. From the synthetic biodegradable polymers, aliphatic polyesters have attracted the attention to the various researchers, as they have combined features of biocompatibility, biodegradability and bioreabsorbability. Polyesters are most studied group of biocompatible, biodegradable and bioreabsorbable polymers[2]. The use of crosslinking in polymers, effectively generating thermoset materials, has received widespread attention as a means to tailor device properties for use in vascular and osseous tissue. The improved mechanical performance of crosslinked biomaterials aids as a scaffold for cell growth, as well as varying degrees of controlled
drug release, or biodegradability\[1,4\]. Poly (glycerol-co-citrate) (PGC), a glassy polymer, has been proposed for applications in drug delivery systems due to its desired mechanical and physicochemical properties, biocompatibility and controlled degradation\[3\]. Despite interesting physical and chemical properties, PGC shows limited water uptake capacity, thus confining its utility. Therefore, a modification of PGC that would mimic the water uptake and water retention characteristics is beneficial for enhancing its utility for biomedical applications. Hence, the block polymer poly(polyethylene glycol-co-glycerol-co-citrate) was synthesised by condensation polymerisation and characterized by various spectroscopic methods, and it was found to be used for drug delivery systems.

Materials and Method:

Materials : Table 1: Materials for synthesis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Name</th>
<th>Batch No.</th>
<th>Manufacturer</th>
<th>CAS No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glycerol (99% Pure)</td>
<td>356350</td>
<td>Merck</td>
<td>56-81-5</td>
</tr>
<tr>
<td>2.</td>
<td>Citric Acid (99.5% Pure)</td>
<td>201-069-1</td>
<td>Merck</td>
<td>77-92-9</td>
</tr>
<tr>
<td>3.</td>
<td>Polyethylene glycol 600</td>
<td>807486</td>
<td>Merck</td>
<td>25322-68-3</td>
</tr>
<tr>
<td>4.</td>
<td>Polyethylene glycol 1450</td>
<td>807488</td>
<td>Merck</td>
<td>25322-68-3</td>
</tr>
<tr>
<td>5.</td>
<td>Polyethylene glycol 4000</td>
<td>807490</td>
<td>Merck</td>
<td>25322-68-3</td>
</tr>
</tbody>
</table>

Methods: Table 2: Reaction Parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Monomers</th>
<th>Molar ratio</th>
<th>Temp(°C)</th>
<th>Vacuum (mm Hg)</th>
<th>Stirring Rate(rpm)</th>
<th>Time(Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CA+PEG1450+Glycerol</td>
<td>PF1(10:1:4)</td>
<td>145°C</td>
<td>400-500</td>
<td>300-400</td>
<td>36</td>
</tr>
<tr>
<td>2.</td>
<td>CA+PEG600+Glycerol</td>
<td>PF2(5:1:4)</td>
<td>140°C</td>
<td>400-500</td>
<td>300-400</td>
<td>36</td>
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<tr>
<td>3.</td>
<td>CA+PEG600+Glycerol</td>
<td>PF3(8:1:7)</td>
<td>155°C</td>
<td>300-600</td>
<td>300-400</td>
<td>48</td>
</tr>
<tr>
<td>4.</td>
<td>CA+PEG600+Glycerol</td>
<td>PF4(2:1:1)</td>
<td>145°C</td>
<td>350-500</td>
<td>300-600</td>
<td>24</td>
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<tr>
<td>5.</td>
<td>CA+PEG4000+Glycerol</td>
<td>PF5(20:1:10)</td>
<td>150°C</td>
<td>400-600</td>
<td>300-400</td>
<td>12</td>
</tr>
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</table>

Results and Discussion:
Conclusion:

Different ratios of PPEGGC polymers were synthesized successfully to observe the effect of molar ratios on physical properties of the polymer. Characterization of synthesized polymers for structure and molecular weight was confirmed by different analytical techniques like FTIR, NMR, MALDI and thermal properties were analyzed by DSC and TGA analysis. The synthesized block polymer was used for enteric coating of tablets for drug delivery system. The evaluation of coating was studied and the invitro release profile data confirmed that the polymer remain intact in gastric fluid and can prolong the drug release.

References:

Designing of new derivatives through CoMFA analysis on Trifluorophenyl β-amino amide as dipeptidyl peptidase IV inhibitors for treatment of diabetes

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Khandwa Road, Indore-452001, M.P., India
Email address: swarajpatil2006@gmail.com

Abstract: Diabetes mellitus affected million people worldwide and India is going to world capital of diabetics in few years. The above findings provoked us to undertake the proposed study of CoMFA analysis, performed on fifty nine trifluorophenyl β-amino amide derivatives for the optimization and design of novel compounds as potential DPP IV inhibitors by using Sybyl X2.1.1. Models with good statistically significant parameters values were selected, validated and optimized by a test set of ten compounds. The q², r² and pred. r² values are 0.874, 0.721 and 0.765 for CoMFA respectively. The suggestion from statistical best models, contour maps and binding affinity analysis possibly will be efficiently utilized for designing of new DPP IV inhibitors.

Introduction:
Diabetes mellitus is a non-communicable and non-infectious, metabolic disease chosen as an epidemic by the world health organization. In 2004, an estimated 3.4 million people died from consequences of fasting high blood sugar. A related number of deaths have been estimated more than 80% for 2010 of diabetes in low and middle income countries. It has been expected that 347 million people would be affected with diabetes worldwide by the year 2030.

Materials and Method:
A dataset of fifty nine derivatives with DPP IV inhibitory activity value (IC₅₀ nM) was utilized for the CoMFA analysis. The structures were sketched using the sketch module of Sybyl X2.1.1 software. The alignment was done through template based method and for analysis, energy minimization (tripos force field) of structure and calculation of charges for performed for better prediction.

The CoMFA model depends on the steric (S) and electrostatic (E) potential field interaction, calculated using the tripos force field with 2 Å grid space, sp³ carbon atom energy cut-off 70 kcal/mol, column filtering 2.0 kcal/mol and charge of +1.0.

Results and discussion:
The best CoMFA model included steric and electrostatic fields and gave a $q^2$ of 0.754 with an optimized component of 6. An elevated non-cross-validated $r^2$ of 0.924 with a low SEE of 0.092, $r^2$ bootstrapping of 0.854, F value of 104.71, $r^2$ pred of 0.732 and standard deviation prediction of 0.303 was obtained. The contributions of steric and electrostatic fields were 0.557 and 0.443, respectively. The ComFA steric interactions are represented by green and yellow colored contours, while electrostatic interactions are represented by blue and red colored contours. In the green regions of the steric contour plot, bulky substituents enhance biological activity, while bulky substituent’s in the yellow regions are likely to decrease activity. Blue colored contours represent regions where electropositive groups increase activity, whereas red colored regions represent areas where electronegative groups enhance activity Figures 1. The CoMFA, models, contour generated exhibited reliable correlative and predictive abilities, provided enough information to understand the structure-activity relationship and to identify structural features influencing inhibitory activity.

Figure 1 Compound most active mapped on CoMFA: (A) steric contour; (B) electrostatic contour

Conclusion:
In present study successfully applied ComFA analysis to predict and characterize a dataset of recently synthesized trifluorophenyl-β-amino-amide derivatives as DPP IV inhibitors. Based on the structure-activity relationship revealed by this analysis, we successfully designed a set of new inhibitors with excellent predicted activities in molecular modeling models. These results will serve as a useful guideline for designing novel DPP IV inhibitors with desired activities.
Acknowledgement:
The authors are thankful to the Head, School of Pharmacy for providing facilities and University Grants Commission, New Delhi for fellowship and financial assistance.

References:

“Solid as solvent”- novel spectrophotometric analysis of Satranidazole tablets using solids (eutectic mixture of phenol and Metformin hydrochloride) as solubilizing agents (mixed solvency concept)

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Abstract: The pollution and toxicity caused by most of the organic solvents is a big challenge. Using mixed solvency concept, innumerable solvent systems can be developed based on an assumption that each substance possesses solubilizing power which can be further explored to develop eco-friendly methods in the area of drug estimation and formulation precluding the use of any toxic organic solvents. In present study we used eutectic mixture of phenol and metformin hydrochloride (PMHCl 41) which is obtained by vigorous trituration of crystals of phenol and metformin hydrochloride (in 4:1 ratio) employed to extract (dissolve) Satranidazole from fine tablet triturate powder. Distilled water is used for dilution to carry out spectrophotometric analysis at 320nm without using any types of organic solvents. The solubility of Satranidazole in distilled water at room temperature was found to be 6.41mg/ml while the solubility of Satranidazole in PMHCl 41 was more than 150mg/ml (of PMHCl 41). The accuracy, reproducibility and precision of the method were confirmed by continuous recovery studies and statistical data. The presence of excipients, eutectic mixture PMHCL 41 did not interfere in spectrophotometric analysis at 320nm. Phenol and metformin hydrochloride PMHCL 41 does not interfere above 300nm.

Introduction:
The present study is an attempt to show that solids can also be wisely used to act as solvent precluding the use of organic solvents. The main objective of the present study is to demonstrate the solvent action of solids. The poor water solubility is the major issue for most of the existing and upcoming pharmaceutical product. All substance present on the earth possess solubilizing power, each substance shall show solubilizing power for some solutes and non-solubilizing power for other. Present study describes the application of solvent character of eutectic liquid consisting of phenol and Metformin hydrochloride in 4:1 ratio (PMHCL 41) on the weight basis for spectrophotometric estimation of Satranidazole tablets. Solubility of Satranidazole in distilled water is 6.41mg/ml at room temperature. In the present investigation, PMHCL was utilized to extract out (dissolve) the drug from powder of tablets. Distilled water was used for dilution purpose. Absorbance was noted at 320 nm against reagent blank for determination of drug content. Proposed method is novel, economic, eco-friendly,
rapid, free from toxicity of organic solvent, accurate and reproducible. Recovery studies and statistical data proved the accuracy, reproducibility and precision of the proposed method. The presence of tablet excipients, phenol and metformin did not interfere in the spectrophotometric estimation of Satranidazole at 320 nm. Phenol and metformin hydrochloride do not interfere above 300 nm in spectrophotometric analysis.

Materials and method:
Satranidazole bulk drug sample was a generous gift by Alkem Laboratories Limited Mumbai. All other chemicals used were of analytical grade. Commercial tablets of Satranidazole were produced from local market. A Shimadzu-1700 UV visible spectrophotometer with 1cm matched silica cells was used for spectrophotometric analysis.

Preparation of eutectic liquid: Phenol and metformin hydrochloride were triturated in (4:1) ratio of their respective weight quantity and prepared the eutectic liquid (PMHCL 41).

Preparation of calibration curve: Accurately weighed 50 mg of Satranidazole standard drug was transferred to a 500ml volumetric flask and 10ml of PMHCL 41 was added to it. The flask was shaken to solubilize the drug. Then, about 400ml distilled water was added and the flask was shaken for 5min to solubilize the contents. The volume was made up to the mark with distilled water. This stock solution (100µg/ml) was suitably diluted with distilled water to obtain standard solutions of 10, 20, 30, 40 and 50µg/ml. The absorbances of these standard solutions were noted at 320 nm against respective reagent blanks to obtain the calibration curve.

Recovery studies: The recovery studies were performed in which standard Satranidazole drug was added to the pre-analyzed tablet powder equivalent to 50 mg Satranidazole and drug content was determined by the proposed method. Results of analysis were reported as in table 2 with statistical evaluation.

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label Claim (mg/tablet)</th>
<th>Percent drug estimated (mean ± SD)</th>
<th>Percent coefficient of variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>300</td>
<td>98.72 ± 1.229</td>
<td>1.245</td>
<td>0.710</td>
</tr>
<tr>
<td>II</td>
<td>300</td>
<td>99.64 ± 0.871</td>
<td>0.874</td>
<td>0.503</td>
</tr>
</tbody>
</table>

Table 2: Results of recovery studies with statistical evaluation (n=3)
### Results and discussion:

The solubility of metronidazole in distilled water at room temperature was found to be 6.41 mg/ml. The solubility of metronidazole in PMHCl 41 was more than 150 mg/gm of PMHCl 41. It is evident from Table 1 that the percent drug estimated in tablet formulation I and II were 98.72 ± 1.229 and 99.64 ± 0.871, respectively. The values are very close to 100.0 indicating the accuracy of the proposed analytical method. Small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error further validated the method. Further, Table 2 shows that the range of percent recoveries varied from 99.04 ± 1.555 to 100.79 ± 1.739 which are again very close to 100.0, indicating the accuracy of the proposed method which is further supported by significantly small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error.

### Conclusion:

The proposed method is new, simple, environment friendly, accurate and reproducible. The proposed method can be successfully employed in the routine analysis of Satranidazole tablets. Phenol does not interfere above 300 nm. Obtained accuracy of the proposed analytical method is also indicative of the proof that the solids possess solvent character.

### References:


<table>
<thead>
<tr>
<th>Formulation</th>
<th>powder (mg)</th>
<th>drug added (mg)</th>
<th>± SD)</th>
<th>variation</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50</td>
<td>15</td>
<td>100.79 ± 1.739</td>
<td>1.725</td>
<td>1.004</td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>30</td>
<td>100.14 ± 1.207</td>
<td>1.205</td>
<td>0.697</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>15</td>
<td>99.04 ± 1.555</td>
<td>1.570</td>
<td>0.898</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>30</td>
<td>99.17 ± 0.882</td>
<td>0.889</td>
<td>0.509</td>
</tr>
</tbody>
</table>
Enzyme (novozyme-435) catalysed synthesis and characterization of poly lactide-co-glycolides (PLGA) polymer

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Abstract: The copolymer poly lactide-co-glycolides is one of the most interesting polymers for medical applications. In this study the copolymer poly lactide-co-glycolides were synthesized at 55°C -75°C via ring opening polymerization of the D,L-lactide and glycolide using Lipase (Novozyme 435) enzyme as a catalyst. The structures, chemical composition and the ratio of the monomers of these copolymers were characterized by NMR and FTIR analysis. The molecular weight and polydispersity index were determined by gel permeation chromatography (GPC). The results of NMR and FTIR analysis showed that the copolymer PLGA was successfully synthesized and GPC analysis results the number average molecular weight (Mn) 2.40×10³ and 2.24×10³ with polydispersity index of 1.491 and 1.452 for 50:50 and 75:25 ratio PLGA polymer respectively.

Introduction: [1-2]
Poly lactide-co-glycolides (PLGA) is one of the polymer used extensively in the marketed formulations. It is the best defined or most attractive biomaterial available for the drug delivery or tissue engineering with respect to design and performance.

PLGA can be synthesized in a wide range of molecular weights by following two processes:
1) Direct polycondensation reaction of lactic acid and glycolic acid which leads to low molecular weight PLGA.
2) Ring-opening polymerization of cyclic oligomers, i.e. d, l-lactide and glycolide in the presence of catalysts to synthesize high molecular weight polymers.

The synthesis is usually based on metallic initiator (tin-salts widely used) at high temperatures which have improved physicochemical properties of these polymers for drug delivery. Strategies to replace metallic initiator are interesting due to the toxicity of metals for potential problematic effects in higher organisms as U.S. F.D.A.CFR 21 mentions that stannous 2-ethylhexanoate can only be used as catalyst at a level not exceeding 1 percent by weight of the polymer used in coatings that are intended for contact with food under conditions of use not more than 150°C. This study extends to investigate nontoxic metal free catalysts (LIPASE) and their potential to facilitate ring-opening polymerizations (ROP).
Materials and Method:
Lactic acid was purchased from Loba chemicals, glycolic acid was purchased from Himedia lab., Novozyme-435 was procured as sample from Novozymes Biopharma, India.

Synthesis of PLGA: Firstly, the cyclic dimer of lactic acid i.e. D.L- lactide was synthesized by heating the lactic acid at an appropriate temperature for 10-12 hours in the presence of vacuum and nitrogen. The synthesized crude D,L-lactide was then purified to get pure lactide. Similarly, cyclic dimer of glycolic acid i.e. glycolide was synthesized by heating the glycolic acid at optimum temperature for about 10 hours. The crude glycolide was purified to get pure glycolide. After this, the D,L-lactide and glycolide were copolymerized at specified temperatures in the presence of enzyme (Novozym 435) as a catalyst for about 60-72 hours. The crude copolymer i.e. PLGA was further purified to get a pure PLGA.

Results and Discussion:
The poly lactide-co-glycolides (PLGA) was confirmed by FTIR and NMR analysis. In the first part of the study, copolymer of lactic acid & glycolic acid i.e. PLGA in two different ratio were synthesized i.e. 50:50 and 75:25 lactide, glycolide respectively. It has been confirmed that the NOV-435 immobilized system can replace conventional synthesis based on metallic catalyst i.e. stannous octoate. Characterization of synthesized polymers for structure and molecular weight was confirmed by different analytical techniques like FTIR, NMR, GPC.

FTIR spectrum (Fig.1) of PLGA 50:50 and 75:25 showed all characteristics peaks related to poly lactide-co-glycolide.
\(^1\)H NMR spectrum (Fig.3) of PLGA copolymers showed characteristic peak of methyllic proton at 1.498-1.601 for PLGA 50:50 and 1.499-1.59 for PLGA 75:25 and methine proton at 5.17-5.237 for PLGA 50:50 and 5.179 for PLGA 75:25. The PLGA was formed with the ratio PLGA 82.5/17.5 and PLGA 86.77/13.22.

GPC analysis (Fig.2) results number average molecular weight (Mn) 2.40\times10^3 and 2.24\times10^3 with PDI of 1.491 and 1.425 respectively for 50:50 and 75:25 ratio PLGA polymer.

**Conclusion:**

From the above studies it can be concluded that poly lactide-co-glycolides can be efficiently synthesized using Lipase (Novozyme-435) as a catalyst and can be further used for drug delivery system.

**Acknowledgement:**

Authors wish to thank Indian Institute of Science Education and Research (IISER) Bhopal for carrying out NMR analysis and Sophisticated Instrumentation Centre for Scientific Research and Testing (SICART) Gujarat for carrying out GPC analysis.

**References:**


FT-IR Spectroscopy: A tool for quantitative analysis of Famciclovir tablets

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| STES’s Sinhgad college of Pharmacy, Vadgaon (Bk), Pune-41 Maharahstra, India 425405. |
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Abstract: In this study a latest IR spectroscopic procedure was narrated for quantitative determination of Famciclovir from solid dosage form. For IR Spectroscopic method (KBr disc technique, base line method) has been used. The specific absorption bands at 1731 cm\(^{-1}\) and 1746 cm\(^{-1}\) were chosen as C=O stretching mainly for Ester respectively. In this method Beer’s law was followed in the concentration range 0.8 to 2.0% w/w in KBr disc. The regression equation was found to be y = 0.195x + 0.037 with correlation coefficient 0.998, and the assay was found to be 98.5% with RSD of 1.023%.

Introduction:
Famciclovir is an orally administered prodrug of the antiviral agent penciclovir. Chemically, famciclovir is known as 2-[2-(2-amino-9H-purin-9-yl) ethyl] - 1, 3-propanediol diacetate (Fig. 1). Famciclovir is marketed as a white, film-coated tablet. The 125-mg and 250-mg tablets are round; the 500-mg tablets are oval. Few analytical methods have been reported for the estimation of Famciclovir in biological fluids or pharmaceutical formulations include liquid chromatography [1,2] and UV-visible spectrophotometry [3,4]. The earlier methods are expensive, time consuming and high technical skills are required. The objectives of this study were firstly to develop and then validate the FTIR analytical methods for the quantification of Famciclovir containing solid dosage form. [5]

Materials and Methods:
Apparatus and chemicals: FT-IR spectrophotometer: Perkin Elmer, Spectrum Bx-II (Operated on HP computer with Windows 2003). Famciclovir supplied by Dhanuka Laboratories Ltd., Gurgaon. Potassium bromide (IR spectroscopy grade), reagents and chemicals were of analytical grade. Penvir tablets containing 250 mg of Famciclovir were purchased from local pharmacies in Pune-India.

IR spectroscopic method: The stock solution of Famciclovir (2 mg ml\(^{-1}\)) was prepared in chloroform. This solution was stable at 2-8 °C for 2 weeks.

Base line technique: The baseline technique was used for the quantitative analysis of drug substance (Figure 1). The values of P\(_0\) and P\(_T\) were calculated using the infra-red spectrum of the samples and, using the equation of regression, the quantitative analysis was carried out.

Method validation
Linearity: 1.0, 1.5, 2.0, 2.5, 3 ml of solution of Famciclovir. The dishes were kept in a hot air oven for 20 min at 60 °C. Each disk of 125 mg contained 2 mg, 3 mg, 4 mg, 5 mg and 6 mg Famciclovir.
respectively so a linear curve could be plotted.

Accuracy and precision: Varying concentrations of Famciclovir were prepared in KBr disk range from 80%, 100% and 120%. P₀ and Pₜ points of the absorption peaks were determined and LogP₀-LogPₜ values of Famciclovir calculated by base line technique.

Recovery study: 80%, 100% and 120%. 4 mg of Famciclovir and concentration 3.2 mg, 4 mg, and 4.8 mg added into the disk containing 4 mg Famciclovir.

Ruggedness: Analysis of aliquots from homogeneous slot in by different analysts.

Assay of Famciclovir tablet: Ten tablets were weighed powdered and a portion of powder equivalent to 100 mg Famciclovir was accurately weighed and extracted with chloroform. Then 2ml of solution of Famciclovir poured into 250 mg of KBr powder in porcelain dish.

![Figure 1](image1.png)

**Figure 1** Measurement of P₀ and Pₜ points of the absorption peak by base line technique.

**Results and Discussion:**

IR spectroscopy method; Linearity: The calibration curve of Famciclovir was prepared using the LogP₀-LogPₜ values Famciclovir standard powder. The correlation of coefficient for Famciclovir was found to be 0.9980 with equation line of \(y = 0.1950x+0.0370\) (Figure 2 and Table 1).

![Figure 2](image2.png)

**Figure 2** Linearity of Famciclovir

Accuracy and precision: The developed method of Famciclovir show high level of accuracy and precision at 80%, 100%, and 120% levels as given in Table 2 and 3.

The intra-day and Inter-day percentage RSD for three levels is found below 1.75.

Recovery study: At the level of three concentrations of 3.2mg, 4mg, 4.8mg the recovery was more than 80% and the % RSD was found to be less than 0.325.

Ruggedness: Ruggedness of the proposed method shows that the different analysts and accuracy of the method is more than 98% and %RSD was found below 3.728.

Assay of Famciclovir tablet by IR Spectroscopic method: The proposed method was used for the tablet formulation of Famciclovir and found the % assay is 98.57% and % RSD of 1.023.
Table 1 Log P₀–Log Pₜ values found for Famciclovir in standard

<table>
<thead>
<tr>
<th>Std</th>
<th>Disk Wt./mg</th>
<th>Conc./mg</th>
<th>P₀ – PT</th>
<th>Log P₀ – Log PT</th>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>5</td>
<td>124.7</td>
<td>6</td>
<td>47</td>
<td>1.2100</td>
</tr>
</tbody>
</table>

In this method, the linear concentration range was obtained as 0.8%–2.5% w/w. The regression equation was calculated by using concentration/absorbance of Famciclovir Table 1. At 1731 cm⁻¹ and 1746 cm⁻¹ the regression equation was found y = 0.195x + 0.037, R² = 0.998 and the assay was found to be 98.57% with RSD 1.023%.

Table 2 Assay of result of commercial sample (Penvir tablets 250 mg by IR spectroscopy)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IR Spectroscopy Famciclovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label Claim</td>
<td>250mg</td>
</tr>
<tr>
<td>%Drug content</td>
<td>98.576</td>
</tr>
<tr>
<td>SD</td>
<td>0.00824</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.023</td>
</tr>
</tbody>
</table>

The methods which are available like HPLC-UV, LC-MS/MS, HPTLC are available, these are expensive due to use of expensive instruments, columns, solvents, materials and more time needed to develop and validate the method. The present IR spectroscopy method is a simple, precise, accurate and rapid.

Conclusion:
This paper depicts the application of IR spectroscopic method to determine Famciclovir in formulation. The proposed method is simple, precise, accurate and rapid for quantitative determination of Famciclovir in tablet dosage form. Analysis of authentic sample containing Famciclovir showed no interference from common additives and excipients, expressing that the recommended procedure is well suited for assay and evaluation of drugs in pharmaceutical preparations. This developed method can be easily and competently adopted for routine quality control analysis.

Acknowledgements:
We are thankful to Dhanuka Laboratories Ltd. Gurgaon and Dr. K. N. Gujar, Principal, SCOP, Vadgaon (Bk.), Pune for providing the necessary samples & facilities.

References:
Development of stability indicating assay method for antidiabetic drugs in pharmaceutical dosage form by RP-HPLC

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Government Polytechnic, Department of Pharmacy, Gadge nagar, Amravati, MS, India.
Government College of Pharmacy, Kathora Naka, Amravati, MS, India.
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Abstract: A force degradation profile was developed of Metformin (MET) & Pioglitazone (PIOG) in pharmaceutical combine tablet dosage form on RP-HPLC using Comosil RP-C18 (4.6 x 150mm, 5µm) in an isocratic mode with mobile phase comprising of Acetonitrile: Pott. Dihydrogen Phosphate (pH 2.5 using OPA). The flow rate was 0.7 mL/min and effluent was monitored at 254 nm. The stress conditions selected on the basis of literature review and the drug profile. For both estimations 99.81% for MET and 99.33% for PIOG mean % recovery was found with % RSD was NMT 2 which fully agrees by system suitability in good agreement with labeled claimed of formulation. All the parameters of system suitability were fully obeyed during force degradation profile generation.

Introduction:
Forced degradation studies are also called as stress decomposition studies, stress studies, stress testing, forced decomposition studies, etc. To determine the stability of the molecule forced degradation is carry out that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied. As indicated by ICH guideline to identify the likely degradation products that stress testing is intended which further helps in determination of the intrinsic stability of the molecule and to validate the stability indicating procedures used and establishing degradation pathways.

Materials and Method:
Standard samples of MET & PIOG were obtained as gift samples from Madras Pharmaceuticals/Maral lab (Chennai) India. The marketed formulation Pioz-MF30 (USV LIMITED, B.S.D. Marg Govandi, Mumbai-400088) was purchased from the local market containing MET 500 mg and PIOG 30 mg and all the chemicals were used are of analytical grade.

Optimization of Mobile Phase and Chromatographic Conditions: The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (A)was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by
permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents.

**Analysis of Marketed Formulation:** Equal volume (20.0 μL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured.

**Force Degradation Studies:** To determine whether the analytical method for assay was stability indicating and in order to establish the force degradation profile, the Tablet formulation of MET and PIOG were subjected to various stress conditions to perform forced degradation studies. Stress studies were carried out under the condition of acid hydrolysis, alkali hydrolysis, oxidation and neutral degradation in accordance with ICH Q1A (R2) guideline. On the basis of literature review and drug profile the selection of stress conditions was primarily depends.

**Results and Discussion:**

### Analysis of Marketed Formulation

- **Figure 1 Optimized Chromatogram of MET & PIOG**

### Force Degradation Studies

<table>
<thead>
<tr>
<th>Condition</th>
<th>%Assay MET</th>
<th>%Degradation MET</th>
<th>%Assay PIOG</th>
<th>%Degradation PIOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial sample</td>
<td>98.85</td>
<td>-</td>
<td>98.96</td>
<td>-</td>
</tr>
<tr>
<td>1N HCL</td>
<td>95.33</td>
<td>3.52</td>
<td>97.68</td>
<td>1.28</td>
</tr>
<tr>
<td>1N NaOH</td>
<td>96.63</td>
<td>2.22</td>
<td>98.15</td>
<td>0.81</td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td>71.90</td>
<td>26.95</td>
<td>96.69</td>
<td>2.27</td>
</tr>
<tr>
<td>Neutral</td>
<td>98.85</td>
<td>-</td>
<td>98.61</td>
<td>-</td>
</tr>
</tbody>
</table>
Conclusion:

As per the ICH Q1A (R2) guideline the force degradation studies were conducted. On the primary basis of literature review and drug profile selection of stress conditions was primarily based and results of the stress studies were undergo full agreement with drug profile and review of literature. Selectivity of the developed method indicated due to the degraded products was well resolved on under optimized an chromatographic condition. Also the specificity of the developed method indicated by the results of marketed formulation analysis, hence for the stability studies on pharmaceutical preparations within pharmaceutical industry the developed method could be employed.

Acknowledgement:

The authors are thankful to The Principal, Government College of Pharmacy, Amravati

References:

Synthesis and Evaluation of 1, 2, 3, 4-Tetrahydropyrimidine-5-Carbahydrazide Derivatives as Antimicrobial Agents

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Abstract: A series of new isoniazid cyclo condensed 1, 2, 3, 4-tetrahydropyrimidine-5-carbahydrazide derivatives were efficiently synthesized by N’-(3-oxobutanoyl) isonicotino hydrazide with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of p-tolunesulphonic acid. The structures of newly synthesized compounds were elucidated using IR, ¹H-NMR, and Mass spectra. The progress of reaction, homogeneity and purity of the compounds were confirmed by melting point and TLC. All the synthesized compounds were evaluated for in vitro antimicrobial activity against one Gram positive S. aureus (ATCC25923), Gram-negative E. Coli (ATCC25922) and fungal strain C. albicans (ATCC 24433) by dilution method. Norfloxacin & Fluconazole were used as reference standard for bacterial & fungal strain respectively. Compound SJ 5 & SJ 6 are most potent against Gram positive strain of S. aureus (ATCC 25923) and fungal strain of C. albicans (ATCC 24433).

Introduction:
Infectious diseases are leading cause of the death worldwide. The incidence of fungal and bacterial infection has increased in the recent years. Dihydropyrimidine and their derivatives have attracted increasing interest owing to their diverse therapeutic and pharmaceutical properties, such as antiviral, antibacterial and antitubercular etc.
The emergence of resistance to most of antibacterial and antifungal agents poses a significant threat to human health care, and novel therapeutic agents are urgently needed. The aim of this research work is to discover new agents with potent antimicrobial activity.

Materials and Methods:
Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G). IR spectrums were obtained on IR affinity-1 Shimadazu 8400 s spectrometer (KBr Pellets). ¹H -NMR spectra were recorded or a Bruker DRX 400 MHz spectrometer using TMS as internal standard in DMSO. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique.
Synthesis of N’-(3-oxobutanoyl) isonicotinohydrazide
Isoniazid (0.01M) and ethylacetoacetate (0.01M) were mixed in a RBF and then refluxed for 6 hours. A white semisolid liquid was formed then it was placed in hot water bath to remove ethanol which was formed during the reaction. Then reaction mixture was allowed to cool and the solid obtained was filtered to get the desired product. Purification of the product was done by the cold diethyl ether. The completion of reaction was monitored using thin layer chromatography (TLC).

Preparation of 1, 2, 3, 4-tetrahydropyrimidine-5-carbahydrazide derivative by microwave irradiation
(SJ1-SJ10)

The mixture of the N’- (3-oxobutanoyl) isonicotinohydrazide (0.005M) and urea/thiourea (0.0075M) with various substituted aldehydes (0.005M) in the presence of the catalytic amount of p-toluenesulphonic acid, with ethanol as a solvent was exposed to the microwave radiation for 10 min at 80-110°C. The completion of reaction was checked by thin layer chromatography, (ethyl acetate and hexane was used as solvent system). Then cooled reaction mixture was filtered and washed with water to remove the unreacted urea/thiourea. The final product was recrystallized using ethanol.

![Chemical Structure](image)

**Figure 1 Reagents & Conditions:** i Reflux 6 hr ii Ethanol, p-toluenesulphonic acid Microwave irradiation (80-110°C) for 10min.

**Evaluation of Antimicrobial activity:** The antimicrobial activity was evaluated against three strains of microbes one Gram positive *S. aureus* (ATCC25923), Gram-negative *E. Coli* (ATCC25922) and fungal strain *C. albicans* (ATCC 24433) by two dilution methods. The result was obtained by MICs (minimum inhibitory concentration). MICs of the synthesized compounds were determined by serial dilution method. A stock solution of synthesized compounds was made with water as solvent.

**Results and Discussion:**

The structure of compounds was confirmed by IR, $^1$HNMR and Mass spectrometry. IR spectra of all the synthesized compounds shows strong N-H stretching band around 3490-3410 cm$^{-1}$ and C=O stretching absorption band at 1690-1630 cm$^{-1}$ indicates presence of carbonyl group. $^1$H NMR spectra confirm the structures of all the synthesized compounds by number of proton present in compounds. The $^1$H NMR spectra of all the synthesized compounds shows presence of 8.00-8.90 δ ppm (s, 2H,
NH), these peaks clearly indicates the formation of hydrazide. All the compounds show [M]+ peak in mass spectra. After confirmation of the compounds to anticipated structures, the antimicrobial activity of synthesized compounds was done.

All the synthesized compounds show good to moderate activity against the tested organism. Among all the compounds, two compounds SJ5 and SJ6 were most potent against S. aureus (ATCC25923) and C. albicans (ATCC 24433). Compound SJ5 & SJ6 with MIC of 25 µg/ml and 40 µg/ml against S. aureus (ATCC25923) and MIC of 40 µg/ml and 55µg/ml against C. albicans (ATCC 24433) respectively, this indicates unsubstituted phenyl is most favourable for antimicrobial activity.

N′-isonicotinoyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbahydrazide (SJ5):
Yield: 75%, MP: 187-192°C, IR (KBr pellets): cm-1 3432 (N-H stretch.), 3035 (C-H stretch., Aromatic ring), 1650 (C=O stretch.), 1620 (N-H bend.), 1590 (C=N stretch.), 1564 (C=C stretch.), 1348 (C-N stretch.), 1H NMR (400 MHz, DMSO, δ, ppm): 1.71 (s, 3H, CH3), 5.56 (s, 1H, CH), 6.0 (s, 2H, NH), 7.06-7.14 (m, 5H, ArH), 7.96-9.06 (d, 4H, ArH), 8.0 (s, 1H, NH), 8.76 (s, 1H, NH) MS (ESI): 351.36 (C18H17N5O3).

N′-isonicotinoyl-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbahydrazide (SJ6):
Yield: 61%, MP: 190-195°C, IR (KBr pellets): cm-1 3436 (N-H stretch), 3045 (C-H stretch. Aromatic ring), 1860 (C=S stretch), 1650(C=O stretch., amide), 1630 (N-H bend), 1580 (C=N stretch.), 1560 (C=C stretch.), 1350 (C-N stretch.) 1H NMR (400 MHz, DMSO, δ, ppm): 1.81 (s, 3H, CH3), 5.50 (s, 1H, CH), 6.11 (s, 2H, NH), 7.08-7.16 (m, 5H, ArH), 7.99-9.03 (d, 4H, ArH), 8.02(s, 1H, NH), 8.75 (s, 1H, NH) MS (ESI): 367.42 (C18H17N5O2S).

Conclusion:
Ten new 1, 2, 3, 4-Tetrahydropyrimidin-5-Carbahydrazide derivatives were synthesized and antimicrobial screening was done against S. aureus (ATCC 25293), B. subtilis (ATCC 13597) & C. albicans (ATCC 24433). Compounds (SJ5) N′-isonicotinoyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimid -ne-5- carbahydrazide and (SJ6) N′-isonicotinoyl-6-methyl-4-phenyl -2-thioxo-1,2,3,4-tetrahydropyrimdi -ne-5- carbahydrazide were found to be the most potent against the tested bacterial strains and fungal strain. Further exploration and optimization of 1, 2, 3, 4-Tetrahydropyrimidine-5-Carbahydrazide derivatives could provide novel antimicrobial agents which can eliminate the issue of resistance to existing drugs.

References:
Development of force degradation profile of Metformin and Glimepiride in combine tablet dosage form by high performance liquid chromatography

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Government College of Pharmacy, Kathora Naka, Amravati – 444604, MS, India
E-Mail address: pndhabale@rediffmail.com

Abstract: A force degradation profile of Metformin HCl & Glimepiride in combine tablet dosage form on RP-HPLC was developed using Grace RP-C18 (4.6 x 150mm, 5µm) in an gradient mode with mobile phase comprising of Acetonitrile: Dihydrogen Pott.Phosphate (pH 2.5 using 0.1% OPA)
The flow rate was 0.7 mL/ min and effluent was monitored at 242 nm. The stress conditions selected on the basis of literature survey and drug profile. The analysis of the marketed formulation shows the % RSD of 0.37 and 0.99 for MET&GLIM which fully agrees with system suitability. All the system suitability parameters were fully obeyed during generation of force degradation profile

Introduction:
The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing. Although forced degradation studies area regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program.

Materials and Method:

Optimization of Mobile Phase and Chromatographic Conditions

Procedure The chromatographic conditions were set as per the trial of various optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by combinations and also by varying column, different flow rate, column temperature and type of buffers with varying pH and solvents.

Force Degradation Studies
In order to establish the force degradation profile and to determine whether the analytical method for assay was stability indicating, the Tablet formulation of MET and GLIM were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the
condition of acid/alkali hydrolysis, oxidation, neutral and thermal degradation in accordance with ICH Q1A (R2) guideline. Selection of stress conditions was primarily depends on the literature review and drug profile

**Results and Discussion:**

**Optimization of Mobile Phase and Chromatographic Conditions**

![Chromatogram of MET and GLIM using ACN: Pot.Phosphate (70:30 %, v/v pH 2.5)](image)

**Figure 1:** Chromatogram of MET and GLIM using ACN: Pot.Phosphate (70:30 %, v/v pH 2.5)

**Analysis of Marketed Formulation**

**Table 1:** Results of Marketed Formulation Analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration in µg/ml</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>GLIM</td>
</tr>
<tr>
<td>1</td>
<td>250.0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>500.0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>750.0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1000.0</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>1250.0</td>
<td>5</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlative Coefficient ( R² )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion:
A force degradation studies were conducted as per the ICH Q1A (R2) guideline. Selection of stress conditions was primarily based on literature review and drug profile and results of the stress studies were undergo full agreement with literature review and drug profile. The developed RP-HPLC method was found to be linear over wider concentration range. Therefore the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of MET and GLIM in bulk and pharmaceutical formulations like tablets. The developed RP-HPLC method was validated as per the ICH guidelines. The developed RP-HPLC method has a stability indicating nature hence the proposed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

Acknowledgement:
The authors are thankful to The Principal, Government College of Pharmacy, Amravati.

References:
Development of validated analytical method for Metformin and Pioglitazone in pharmaceutical dosage form

Anup K. Chakraborty, Sk. JafarAmjad Sk, M.S. Charde
School of Pharmacy, People’s University, Bhopal, MP, India
Government College of Pharmacy, Kathora Naka, Amravati – 444604, MS, India.
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Abstract: A RP-HPLC method was developed using Cosmosil RP-C18 (4.6 x 150mm, 5µm) as stationary phase with younling (S.K.) isocratic system UV detector in a gradient mode with mobile phase comprising of Acetonitrile : Pott. Dihydrogen Phosphate (adjusted pH-2.5 using OPA). 0.7ml/min flow rate and monitoring of effluent were done at 254.0 nm for MET (Metformin) and PIOG (Pioglitazone) estimation in combined dosage form.2.1 min for MET and 7.53 min for PIO G retention times were found. The dynamic range of linearity 50- 250 µg/ml for MET and 3- 15 µg/ml for PIOG were exhibited for the assay. The linear calibration curves were found over the entire range linearity (r² = 0.996 for MET and r² = 0.995 for PIOG) and 99.81% for MET and 99.33% for PIOG mean % recovery was found with % RSD was NMT 2 for both estimations which fully agrees by system suitability in good agreement with labeled claimed of formulation. The % RSD for Intra & Inter-Day Precision was NMT than 2 for both drugs. The developed method was accurate, precise, rugged and linear as per ICH guidelines

Introduction:
HPLC is a physical separation technique carried out in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds.

Materials and Method:
HPLC System of Younglin Quaternary pump with UV- VIS detector (190-990 nm) Software – Autochro-3000. Ultrasonicator servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose. Analytical balance of citizen model CY 104 (micro analytical balance) was used for weighing purpose.
The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (A) was injected in the Rheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents.

### Analysis of Marketed Formulation

Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured.

### Results and Discussion:

#### Optimization of Mobile Phase and Chromatographic Conditions

![Figure 1: Optimized Chromatogram of MET & PIOG](image)

#### Analysis of Marketed Formulation

![Table 1: Results of Marketed Formulation Analysis](image)
Conclusion:
The developed RP-HPLC method was found to be simple, accurate, sensitive, precise, rugged, robust, economical and rapid. The developed RP-HPLC method shows the good resolution between MET and PIOG within the run time of 10 min. The developed RP-HPLC method was found to be linear over wider concentration range. Therefore the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of MET and PIOG in bulk and pharmaceutical formulations like tablets. The developed RP-HPLC method was validated as per the ICH guidelines. The developed RP-HPLC method has a stability indicating nature hence the proposed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

Acknowledgement:
The authors are thankful to The Principal, Government College of Pharmacy, Amravati.

References:
Development of force degradation profile of Ibuprofen and Famotidine by HPLC in combine dosage form

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E-Mail address: avinashnugale@gmail.com

Abstract: A force degradation profile by RP-HPLC method was developed for Ibuprofen (IBU) & Famotidine (FMT) in combine tablet dosage form using Cosmosil RP-C18 (4.6 x 250mm, 5µm) in an gradient mode with mobile phase comprising of Methanol: Water (pH 2.5 using OPA) The flow rate was 0.7 mL/ min and effluent was monitored at 240 nm. The stress conditions were selected on the basis of literature review and drug profile. The analysis of the marketed formulation shows the % RSD of 0.061 and 0.35 for IBU & FMT which fully agrees with system suitability. All the system suitability parameters were fully obeyed during generation of force degradation profile.

Introduction: The present work was undertaken with an objective to develop the force degradation profile for the above combination on RP-HPLC so as to support the development of stability testing program.

Ibuprofen. Chemically is (RS)-2-(4-(2- methylpropyl) phenyl) propanoic acid. It is white crystalline powder used as analgesic having solubility in methanol, ethanol and in water 21 mg/Lt.

While famotidine chemically is 3-[(2-[(diaminomethylidene) amino]-1,3-thiazol-4-yl]methyl)sulfanyl]-N'-sulfamoylpropanimid amide. It is white to pale yellow crystalline. Used as anti-ulcer having solubility in methanol and freely soluble in glacial acetic acid, slightly soluble in water.[1,6]

Materials and Method:
Optimization of Mobile Phase and Chromatographic Conditions

Procedure The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in theRheodyne injector (20.0 µl) and the respective chromatograms were recorded. Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents.

Force Degradation Studies
In order to establish the force degradation profile and to determine whether the analytical method for assay was stability indicating, the Tablet formulation of IBU and FMT were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the condition of acid/alkali hydrolysis, oxidation, neutral degradation in accordance with ICH Q1A (R2) guideline. Selection of stress conditions was primarily depends on the literature review and drug profile.

Results and Discussion:
Optimization of Mobile Phase and Chromatographic Conditions

![Figure 1 Optimized chromatogram of Ibu &Fmt](image)

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Assay IBU</th>
<th>% Degradation IBU</th>
<th>% Assay FMT</th>
<th>% Degradation FMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial sample</td>
<td>99.82</td>
<td>-</td>
<td>99.91</td>
<td>-</td>
</tr>
<tr>
<td>1N HCL</td>
<td>95.53</td>
<td>4.29</td>
<td>97.68</td>
<td>2.23</td>
</tr>
<tr>
<td>1N NaOH</td>
<td>99.82</td>
<td>100</td>
<td>98.15</td>
<td>1.76</td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td>70.42</td>
<td>29.4</td>
<td>91.69</td>
<td>8.3</td>
</tr>
<tr>
<td>Neutral</td>
<td>99.68</td>
<td>0.14</td>
<td>98.61</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusion:
A force degradation studies were conducted as per the ICH Q1A (R2) guideline. Selection of stress conditions was primarily based on literature review and drug profile and results of the stress studies were undergo full agreement with literature review and drug profile. The degraded products were well resolved on under optimized an chromatographic condition which indicates selectivity of the developed method. Also the results of marketed formulation analysis indicate the specificity of the
developed method hence the developed method could be employed for the stability studies on pharmaceutical preparations within pharmaceutical industry.

Acknowledgement:
The authors are thankful to The Principal, Government College of Pharmacy, Amravati

References:
Validated RP-HPLC method development for the simultaneous estimation of Metformin and Glimepiride in combine tablet dosage form

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E-Mail address: diwakse95@gmail.com

Abstract: A force degradation profile of Metformin HCl & Glimepiride in combine tablet dosage form on RP-HPLC was developed using Grace RP-C18 (4.6 x 150 mm, 5 µm) in an gradient mode with mobile phase comprising of Acetonitrile: Dihydrogen Pott. Phosphate (pH 2.5 using 0.1% OPA). The flow rate was 0.7 mL/min and effluent was monitored at 242 nm. The retention times were found to be 2.06 min for MET and 5.80 min for GLIM. The assay shows a linear dynamic range of 250-1250 µg/mL for MET and 1.0-5.0 µg/mL for GLIM. The calibration curves were linear (r² = 0.999 for MET and r² = 0.998 for GLIM) over the entire linear range. Mean % recovery was found to be 99.80% for MET and 98.93% for GLIM with % RSD was NMT 2 for both estimations which fully agrees with system suitability which is in good agreement with labeled amount of formulation. The % RSD for Intra-Day & Inter-Day Precision was NMT than 2 for both the drugs. The developed method was validated as per ICH guidelines.

Introduction:
HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. The parameters for method validation as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantitation, Linearity, Range, Robustness and Ruggedness.

Materials and Method:
MET (5 gms) supplied as gift sample by CiplaPharma Ltd. (Mumbai, India) and its claimed purity was 99.4% and GLIM (5 gms) supplied as gift sample by Ranbaxy Laboratories Limited (Haryana), India and have 99.6 % purity. The marketed formulation Metpride (Alkem Laboratories) was purchased from the local market containing MET 500 mg and GLIM 2.0 mg and all the chemicals used were are of analytical grade.

Optimization of Mobile Phase and Chromatographic Conditions
Analysis of Marketed Formulation: Take the powder weight of tablet equivalent to 250 mg of MET in 100.0 mL of volumetric flask and add sufficient mobile phase and sonicate it for 15 min. Make up the volume up to the mark with mobile phase and filtered it with 0.24μ to get 5000.0 μg/mL and 200 μg/mL of MET and GLIM respectively. Take 0.05 mL of GLIM and 1.0 mL of MET from above solution of GLIM and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 1.0 μg/mL GLIM & 250.0 μg/mL MET.

Results and Discussion:
Optimization of Mobile Phase and Chromatographic Conditions

![Optimized Chromatogram of MET & GLIM](image)

Analysis of Marketed Formulation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration in μg/ml</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>GLIM</td>
</tr>
<tr>
<td>1</td>
<td>250.0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>500.0</td>
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<tr>
<td>3</td>
<td>750.0</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>1250.0</td>
<td>5</td>
</tr>
<tr>
<td>Slope</td>
<td>12.12</td>
<td>76.15</td>
</tr>
<tr>
<td>Intercept</td>
<td>141.3</td>
<td>11.46</td>
</tr>
<tr>
<td>Correlative Coefficient (R²)</td>
<td>R² = 0.999</td>
<td>R² = 0.998</td>
</tr>
</tbody>
</table>

Conclusion:
The developed RP-HPLC method was found to be highly specific. The developed RP-HPLC method was found to be linear over wider concentration range. The developed RP-HPLC method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. The developed RP-HPLC method shows the good resolution between MET and GLIM within the run time of 10 min. The developed RP-HPLC method is very simple involving no complicated sample preparations. Therefore
the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of MET and GLIM in bulk and pharmaceutical formulations like tablets. The developed RP-HPLC method was validated as per the ICH guidelines.

Acknowledgement:
The authors are thankful to The Principal, Government College of Pharmacy, Amravati.

References:
Development of a validated stability indicating HPTLC method for Tadalafil Hydrochloride

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Prin. K M Kundnani College of Pharmacy, Mumbai, Maharashtra.
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Abstract: An accurate, precise, and stability-indicating high performance thin layer chromatographic method has been developed and validated for analysis of Tadalafil hydrochloride. The separation was performed on pre-coated silica gel 60 F254 plates with hexane: 2-propanol: acetonitrile (5:4:1). Densitometric analysis was performed at 285 nm and the linearity range was between 200ng/ml to 600ng/ml with regression value (r)² = 0.995. Stress studies were performed and drug was subjected to acid, basic hydrolysis and oxidation. Statistical analysis proved the method is repeatable and selective for estimation of tadalafil hydrochloride. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability-indicating method.

Introduction:
The chemical designation of tadalafil hydrochloride is pyrazino-[1’,2’:1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5yl)-2,3,6,7,12,12a-hexahydro-2-methyl- (6R,12aR). It is used for the treatment of erectile dysfunction, and is a potent, reversible, competitive inhibitor of phosphodiesterase 5. Inhibition of PDE5 in the corpus cavernosum of the penis increases intracellular cGMP levels, thereby facilitating relaxation of smooth muscle leading to penile erection.

The parent drug stability test guidelines (Q1A) issued by International Conference on Harmonisation (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability indicating. The main intention of the work is to build up a stability indicating high-performance thin-layer chromatography for determination of tadalafil hydrochloride. This paper mainly deals with the forced degradation of tadalafil hydrochloride under stress conditions like acid hydrolysis, base hydrolysis, peroxide hydrolysis, heat and light.

Materials and Method:
Tadalafil hydrochloride was supplied by Ipca Laboratories, India as a gift sample.
Table 1 List of Chemicals

<table>
<thead>
<tr>
<th>Name of Chemicals</th>
<th>Grade</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>HPLC Grade</td>
<td>MERCK</td>
</tr>
<tr>
<td>Hexane</td>
<td>AR Grade</td>
<td>MERCK</td>
</tr>
<tr>
<td>2-propanol</td>
<td>HPLC Grade</td>
<td>MERCK</td>
</tr>
</tbody>
</table>

Table 2 List of instruments:

<table>
<thead>
<tr>
<th>Name of Instrument</th>
<th>Make</th>
<th>Model</th>
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</thead>
<tbody>
<tr>
<td>HPTLC applicator</td>
<td>LINOMAT 5</td>
<td>CAMAG</td>
</tr>
<tr>
<td>Scanner</td>
<td>DESAGA TLC SCANNER</td>
<td>DESAGA 60</td>
</tr>
<tr>
<td>Balance</td>
<td>ANALYTICAL BALANCE</td>
<td>AB 104-S</td>
</tr>
</tbody>
</table>

Results and discussion:
The HPTLC method was developed and optimized in view to developing a stability indicating assay method. Several easily available solvents in different combinations and concentrations were tried to obtain good resolution, compact spot and better separation of degradation products. Lastly, the mobile phase consisting of Hexane: 2-propanol: Acetonitrile (5:4:1) was selected which gives a sharp and well defined peak of Tadalafil at Rf of 0.65. It was found that the solvent system has very good resolution for separation of drug and degradation products.

Analytical Method Validation:

LOD and LOQ: The LOD of proposed method, with a signal-to-noise ratio of 3:1, was found to be 30 ng per spot where as LOQ, with a signal to noise ratio of 10:1, was found to be 90 ng per spot.

Linearity: The linear regression data for calibration curves (n=6) showed good linear relationship over the concentration range of 200-600 ng/ml. Correlation coefficient, r²= 0.995±0.0001.

Precision and Accuracy: The precision (intraday and interday) and accuracy of the developed method were expressed in terms of % RSD. The results depicted revealed excellent accuracy and high precision of method.

Repeatability of sample application: 400 ng/ml samples were applied over the plate for six times. Area for six injections was recorded and % RSD is not more than 1%.

Specificity: The method was quite selective for Tadalafil Hydrochloride. There was no other interfering peak around the retention factor of Tadalafil Hydrochloride. The baseline did not show any significant peak.
Optimized chromatographic conditions were validated for standard solution. The linearity range was found to be 200ng/ml to 600ng/ml with regression value \( r^2 = 0.995 \). The Limit of Detection (LOD) was 30ng per spot and Limit of Quantification (LOQ) was 90 ng per spot. The precision of the method for the standard solution shows Relative Standard Deviation (RSD) for intraday and for interday were within limits.

**Conclusion:**

The developed HPTLC method is simple, precise, sensitive, fast and stability indicating. Stress conditions indicate that the drug is susceptible to acid, base hydrolysis and oxidation. All the degradation products were fully resolved, this indicate specificity of the method. Thus the method can be employed for monitoring the stability of Tadalafil Hydrochloride in bulk drug.

**Acknowledgement:**

The author is thankful to Ipca Laboratories and other those who helped in successful completion of work.

**References:**


In silico docking and in vitro hepatoprotactive activity of Embelia Ribes

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Abstract: Present investigations aimed to carry out In silico docking and in vitro hepatoprotactive activity of hydroalcoholic extract of Embelia ribes (HAER) against ethanol induced hepatic damage. In silico docking analysis of active constituents form Embelia ribes was carried out on potent target receptors NFkB and Pregnane X using Autodock 4.2 software. Hepatotoxicity was induced on Goat (Capra hircus) liver slices with ethanol to study hepatoprotective activity. Goat liver slices were incubated at 37°C with different concentrations (400, 1000 and 2000μg/ml) of the HAER in the presence of ethanol for 2 hours. The hepatoprotective effects were quantified by the leakage of alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) to the medium. In-silico studies revealed that the compound embeliaribyl ester from Embelia ribes fruits is the best target compound (drug) for NFkB receptor and similarly Vilangin is best target for Pregnane X receptor. Hydroalcoholic extract of Embelia ribes decrease the elevated percentage release of three enzymes AST (59.13%), ALT (54.08%), LDH (43.68%) at concentration of 2000μg/ml when compared to hepatotoxic control group AST (71.3), ALT (72.81%), LDH (54.25%).

Introduction:

Embelia ribes is a large woody tropical forest scendent shrub with slender branches and gland dotted leaves. It is commonly known as False Black Pepper. In Ayurveda, it is considered widely beneficial in variety of diseases and is also used in homeopathy. It is distributed throughout hilly parts of India from Himalayas to Western Ghats. A scrutiny of exploration of literature revealed some notable pharmacological activites of the plant such as antibacterial activity, anticancer activity, wound healing activity, antihyperglyceamic activity. The present work was therefore undertaken to correlate the claims of traditional medicinal practitioners of using Embelia ribes as hepatoprotective agent.

Materials and Method:

Collection and Extraction

Fruits of Plant Embelia ribes were collected from Spice market Khari Bawli, Old Delhi and fruits were authenticated by Prof. B.D Vashisth, Dept. of Botany Kurukshetra University, Kurukshetra and Haryana. The coarsely powdered plant fruits were extracted with hydroalcoholic solvent in ratio 70:30
(Ethanol:Water) till the colorless solvent comes out. The extract obtained was filtered and then concentrated with the help of rotary evaporator under reduced pressure and then dried in open air. The concentrated extract was stored in airtight container at cool place at temperature 4-8°C.

**In silico docking studies:** Active constituents such as Embelin, Embelinol, Embeliaribyl ester, Potassium embelate, Vilangin were selected. Chem Sketch of ACDLABS 10.00 software was used to design the ligands followed by 3D optimization. The potent target receptors which is involved in the hepatoprotection are NFkB and Pregnane X receptor which were elicited from literature survey and their respective pdb files ID: 1VKX and ID: 1ILG were retrieved from Protein data bank.. Docking analysis was done using Autodock 4.2[^2]

**In vitro studies:** Hepatotoxicity was induced on Goat (Capra hircus) liver slices with ethanol to study hepatoprotective activity. Goat liver slices were incubated at 37°C with different concentrations (400, 1000 and 2000μg/ml) of the HAER in the presence of ethanol for 2 hours. The hepatoprotective effects were quantified by the leakage of alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) to the medium[^3].

**Statistical Analysis:** A minimum of three independent experiments were carried in triplicates unless otherwise specified. Students T test was performed for statistical analysis and results are presented as mean ± standard error mean (Mean ± SEM). Value of p <0.05 was considered as significant.

**Results and Discussion:**

**In-silico docking studies**

The docking analysis of the lead molecules in this present study showed important interactions which is operating at the molecular level like hydrogen bond interactions, hydrogen bond distance, amino acid residues. There is considerably low binding energy for all the active constituents present in the plant *Embelia ribes*. Among the leads, Embeliaribyl ester was found to be the best target drug for NFkB receptor as it exhibited lowest binding energy of -8.8kcal/mol Similarly Vilangin for Pregnane X receptor having binding energy of -9.1kcal/mol. Embeliaribyl ester with lowest binding energy -8.8kcal/mol & having 3 hydrogen bond interactions at residues ARG187, ARG248, PHE607. Vilangin with lowest binding energy -9.1 kcal/mol having 2 hydrogen bond interactions at residue GLN285, HIS407. Thus in silico studies revealed that the compound embeliaribyl ester is the best target compound for NFkb receptor and similarly Vilangin is best target for Pregnane X receptor.

**In-vitro Hepatoprotective activity**

The results revealed that the activities of the three enzymes ALT, AST and LDH were increased in the goat liver slices treated with ethanol (5M) which indicates cellular leakage, and loss of functional integrity of the cell membrane indicating liver damage. The percentage release of AST, ALT and LDH in hepatotoxic control group (liver slices treated with ethanol for 2 hours) was 71.3%, 72.81%
and 54.25% and in untreated liver slices (negative control group) values were 26.59%, 23.69% and 19.81% respectively. There was a significant (p <0.05) reduction in percentage release of ALT (59.13%), AST (54.08%) and LDH (43.68%) after treatment with HAER at a concentration of 2000μg/ml as compared to hepatotoxic control group. This reduction in percentage release of three enzymes indicates the hepatoprotective effect of *Embelia ribes* fruits against ethanol induced hepatotoxicity.

**Table.1 Effect of various doses of HAER on liver enzymes leakage (%) due to ethanol induced hepatotoxicity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
<th>LDH(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>26.59±0.571**</td>
<td>23.69±0.692**</td>
<td>19.81±0.003**</td>
</tr>
<tr>
<td>Hepatotoxic control Ethanol (5M)</td>
<td>71.3±1.732</td>
<td>72.81±1.270</td>
<td>54.25±1.299</td>
</tr>
<tr>
<td>HAEER 400μg/ml + Ethanol 5M</td>
<td>69.4±0.461ns</td>
<td>65.47±0.635**</td>
<td>51.69±0.058ns</td>
</tr>
<tr>
<td>HAEER 1000μg/ml + Ethanol 5M</td>
<td>64.39±1.166*</td>
<td>59.39±2.309*</td>
<td>48.71±1.132*</td>
</tr>
<tr>
<td>HAEER 2000μg/ml + Ethanol 5M</td>
<td>59.13±2.875**</td>
<td>54.08±0.006**</td>
<td>43.68±1.160**</td>
</tr>
</tbody>
</table>

Values are expressed as (Mean ± SEM), **P<0.01, *P<0.05 when compared with control group (statistically analysed by Dunnet’s t-test)

**Conclusion:**

Reduction in increased percentage release of three liver marker enzymes namely AST, ALT, LDH by the treatment of HAER confers the heptoprotective activity by the plant. Results obtained from the present study obviously demonstrates that fruits of *Embelia ribes* are suitable for developing hepatoprotective agents.

**Acknowledgement:**

The authors are grateful to the Director, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, India for providing necessary facilities to carry out the work.

**References:**


Synthesis and screening of antimicrobial activity of novel 2,5-Dimethyl Pyrrolyl-Pyrazole derivatives

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Abstract: New series of 2,5-dimethylpyrrolyl-pyrazole derivatives were synthesized by refluxing a mixture of substituted chalcones in ethanol with 4-(2,5-dimethylpyrrol-1-yl)benzoic acid hydrazide. The reaction mixture was heated under reflux for 16 hrs on a water bath followed by addition of ice cold water at room temperature and mixture was kept overnight resulting in formations of substituted 2,5-dimethylpyrrolyl-pyrazole derivatives (5a-g). Purity of newly synthesized compounds was confirmed by TLC and melting point. The structure of the all newly synthesized compounds was confirmed by spectral study such as FTIR, 1H NMR and Mass spectroscopy. The newly synthesized compounds were screened for their antimicrobial and antitubercular activities using broth microdilution and MABA methods respectively.

Introduction:
Chalcones are useful synthons in the synthesis of a large number of bioactive molecules such as pyrazolines that are well known nitrogen containing heterocyclic compounds. Considerable interest has been focused on the pyrazoline structure which is known to possess a broad spectrum of biological activities such as antiamoebic, antimicrobial, monoamine oxidase inhibitors, antimycobacterial, antidepressant, anticonvulsant, and anti-inflammatory activities.

The pyrrole ring is a part of many biological compounds such as the enzyme catalase, the bile pigment bilirubin and the mould pigment prodigiosin; it is also a significant part of macrocyclic porphyrin ring system of chlorophyll and hemin. Apart from these properties pyrrole and its derivative possess a number of biological activities such as antiallergic, antitumor, antibacterial, antifungal, antiinflammatory, analgesic, anticonvulsant, antitubercular, anticancer and anti HIV [1,2].

MATERIALS AND METHODS:
General procedure for the synthesis of substituted chalcones (3a-g) [3]: A solution of sodium hydroxide (40%) in water and rectified spirit was placed in a flask provided with mechanical stirrer. The flask was immersed in a bath of crushed ice. Acetophenone (0.6 g, 0.005 mol) was added with constant stirring then appropriate benzaldehydes (0.005 mol) were added to the solution. The
temperature of mixture was kept at about 25 °C and stirred vigorously until the mixture was thick enough to retard stirring (4h). Stirrer was removed and the mixture was kept at 8 °C overnight. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and further washed with ice cold ethanol. The crude product was recrystallized from ethanol.

**SCHEME-1**

**Procedure for the synthesis of 4-(2,5-dimethyl pyrrol-1-yl)benzoic acid hydrazide (4)** [4]: Synthesis of compound 4 was carried out by procedure described by Joshi et al. A mixture of ethyl 4-(2,5-dimethyl pyrrol-1-yl benzoate (3.64 g, 0.015 mol) with hydrazine hydrate (10 ml) in absolute ethanol (10 ml) was refluxed for 3 h. The reaction mixture was cooled and crystalline mass obtained was recrystallized from ethanol and obtained as yellow crystals in 80% yield. M.p. 170-172°C.

**General procedure for the synthesis of (4-(2,5-dimethylpyrrol-1-yl)phenyl)(5-substitutedphenyl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methanones (5a-g):** In a mixture of appropriate chalcones (3a-g) (0.01 mol) in ethanol, 4-(2,5-dimethyl pyrrol-1-yl)benzoic acid hydrazide (4) (0.01 mol) was added in a round bottom flask. The reaction mixture was heated under reflux for 16 h on a water bath followed by addition of ice cold water at room temperature. The mixture was kept overnight at 8 °C. The precipitate was filtered washed with distilled water and dried. The crude crystals were recrystallized from ethanol.

**Antibacterial activity:** The MIC determination of the tested compounds was carried out simultaneously in comparison with ciprofloxacin, norfloxacin against Gram-positive (*Staphylococcus aureus, Streptococcus faecalis and Bacillus subtilis*). Gram-negative bacteria (*Klebsiella pneumonia,*)
Escherichia coli and Pseudomonas aeruginosa) by broth microdilution method. Compounds showed antibacterial activity between MIC of 100-3.125 μg/ml.

**Antitubercular activity:** MIC values were determined for the newly synthesised compounds against *M. tuberculosis* strain H$_{37}$Rv using the Microplate Alamar Blue assay (MABA) using isoniazid as the standard drug.

**Result and Discussion:**

Previously we have synthesised substituted pyrrolyl-pyazole derivatives which were found to be possess potent antibacterial and antitubercular activities. In present work, we thought to combine 2,5-dimethylpyrrole with pyrazole, for that precursor substituted chalcone derivatives (3a-g) were prepared by reaction between various substituted benzaldehydes (1a-g) with acetophenone (2) in 40% NaOH and ethanol. Further, 3a-g was reacted with 4-(2,5-dimethyl pyrrol-1-yl)benzoic acid hydrazide (4) yielded the corresponding 2,5-dimetyl pyrrolyl-pyrazole derivatives (5a-g).

Compound 5c showed MIC value of 6.25 μg/ml against Streptococcus faecalis and Bacillus subtilis, of 12.5 μg/ml against Escherichia coli. Compound 5d showed MIC value of 3.125 μg/ml against Streptococcus faecalis and Bacillus subtilis, of 6.25μg/ml against Escherichia coli. Compound 5g showed MIC value of 6.25 μg/ml against Streptococcus faecalis and Escherichia coli, of 3.125 μg/ml against Bacillus subtilis. Compound 5d showed MIC value of 3.25 μg/ml and compound 5g showed MIC value of 6.25 μg/ml against *M. tuberculosis*.

**Conclusion:**

Based on screening results, it can be concluded that incorporation of methoxy group and dichloro group on phenyl ring and presence of 2,5-dimethyl pyrrole, pyrazole fragments helped to improve antibacterial and antitubercular activities of synthesized compounds.

**Acknowledgement:**

Authors immensely thank research support from the Board of Research in Nuclear Sciences (BRNS), Bhabha Atomic Research Centre (BARC), Mumbai (File No. 2013/37B/17/BRNS/0417 dated-14/05/2013).

**References:**

Computer assisted design, synthesis and biological evaluation of some 1,3,5, triazine substituted benzene sulfonamides as carbonic anhydride inhibitors

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Abstract: A series of benzene sulfonamides incorporating 1,3,5-triazinyl moiety have been synthesized and investigated as inhibitors of the metalloenzymes carbonic anhydrase (CA. E.C 4.2.1.1) isoenzymes I, II (cytosolic) and XII (transmembrane). Against the human CA I, Synthesized compounds showed Kᵢ values from 184.7 to 291.9 nM, toward hCA II at range of 4.65 to 11.89 nM whereas against hCA XII in the range of 0.84 to 11.1 nM. Most of the synthesized compounds exhibited excellent CA inhibitory activity against the CA isoenzymes as compared to the reference drug acetazolamide.

Introduction:
Carbonic anhydrase (carbonate dehydratase, carbonate hydro-lyase; EC 4.2.1.1) is a zinc containing metalloenzyme that catalyzes a simple physiological reaction, the conversion of CO₂ to the bicarbonate ion and protons. Most Carbonic anhydrases contain a zinc ion at the active site, which is essential for catalysis. Carbonic anhydrase inhibitors are well established drugs as diuretics and anti-glaucoma agents, but recent research has shown that several CA isozymes are drug targets for cancer, epilepsy and infective diseases. CA II is responsible for increasing sodium bicarbonate secretion in anterior uvea of eye leading to visual dysfunctioning and glaucoma. Sulfonamide and sulfamate carbonic anhydrase inhibitors were reported to show substantial anti-glaucoma as well as anti-tumor activity in-vitro and in-vivo, thus constituting interesting leads or new therapeutic approaches, when targeting either CAII or CA XII. Earlier studies reported that incorporation of 1,3,5-triazine moiety in benzene sulfonamide scaffold leads to compounds with enhanced specificity and efficacy against the membrane-bound isoforms hCA IX and XII over cytosolic hCA I and II.

Materials and Method:
2D QSAR analysis was performed on a dataset of 1,3,5 Triazine derivatives (51 compounds) and models were generated for carbonic anhydrase inhibition activity. The best model was selected for designing of new series of compounds. Virtual screening of ZINC database was performed to obtain some potential hits. Out of seven hits, one hit was selected as a lead compound.
In order to know the binding affinity and binding orientation of designed compounds within the active site of enzyme, docking of all the designed compounds was performed using the AUTODOCK VINA software. The crystal structure of CAI, CAII, and CAXII was retrieved from RCSB protein data bank (1AZM, 3V2J and 1JDO). Protein and ligand files were prepared using DOCKPREP tool in CHIMERA software. Grid size was 120 Å for the three coordinates (X, Y, Z) with grid spacing set to 0.365 Å. Docking was carried out using Lamarckian Genetic Algorithm (LGA). The free energy of binding for all ligands were calculated.

The compounds were synthesized using nucleophilic aromatic substitution; the reaction was monitored by Thin Layer Chromatography. The compounds were purified by column chromatography and characterized on the basis of various physical properties such as melting temperature range, UV spectroscopy, Rf value and LogP value. The structure conformation of synthesized compounds was done by Infrared spectroscopy, 1H NMR and Mass spectroscopy.

The In-silico Molecular properties and ADME prediction of synthesized compounds was performed by Qikprop software. Drug score was calculated using Molsoft software.

For in-vitro evaluation, stopped-flow instrument was used and CA catalyzed CO2 hydration activity assay was carried out at 25°C, pH 7.4. Triplicate experiments were done for each inhibitor concentration, and the values reported were in the form of inhibition constant.

Results and Discussion:

The compounds were designed based on virtual screening as well as QSAR studies. Docking analysis showed that nitrogen atom of the sulfonamide moiety coordinated to the Zinc ion and an extended network of hydrogen bonds involving Thr, His and Leu residue. The structure confirmation of synthesized compounds was done by IR, 1H NMR and Mass spectroscopy. Characteristic bands at anticipated position in IR spectrum confirmed the formation of synthesized compounds. NMR spectrum of the synthesized compounds showed anticipated chemical shift value. Structure of synthesized compounds was also confirmed by mass spectroscopy using LC/MS technique. The molecular ion peak or M+/M-1/M+1/M+2 peaks observed in the mass spectrum for compounds confirmed their molecular weight. The Binding energy and Inhibition Constant K_i (nM) of synthesized compounds are shown in table 1.

Table 1: Binding energy in kcal/mol and biological activity of synthesized compounds
<table>
<thead>
<tr>
<th>S.NO</th>
<th>CMP</th>
<th>R₁</th>
<th>R₂</th>
<th>Binding Energy (kcal/mol) of human CAs</th>
<th>Kᵢ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CAI</td>
<td>CA II</td>
</tr>
<tr>
<td>1</td>
<td>A1</td>
<td>Cl</td>
<td>NHCH₃</td>
<td>-1.44</td>
<td>-6.88</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>Cl</td>
<td>NHCH₂CH₃</td>
<td>-0.73</td>
<td>-6.84</td>
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<td>3</td>
<td>A3</td>
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<td>-7.35</td>
</tr>
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<td>A4</td>
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<td>-7.49</td>
</tr>
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<td>5</td>
<td>A5</td>
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<td>H</td>
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<td>H</td>
<td>-2.10</td>
<td>-6.32</td>
</tr>
<tr>
<td>7</td>
<td>A7</td>
<td>Cl</td>
<td>OCH(CH₃)₂</td>
<td>1.34</td>
<td>-5.03</td>
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<td>9</td>
<td>A9</td>
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<td>-6.98</td>
</tr>
<tr>
<td>10</td>
<td>A10</td>
<td>Cl</td>
<td>NH₂H₂</td>
<td>-1.70</td>
<td>-7.02</td>
</tr>
<tr>
<td>11</td>
<td>AAZ</td>
<td>Cl</td>
<td>NH⁻NH⁻</td>
<td>-5.12</td>
<td>-7.13</td>
</tr>
</tbody>
</table>

**Conclusion:**

The new derivatives inhibit the carbonic anhydrase enzyme in low nanomolar range and some compounds showed better activity in low concentration as compared to Acetazolamide.

**Acknowledgement:**

We thankfully acknowledge AICTE New Delhi and Dr. C.T. Supuran, Italy for carrying out in-vitro biological evaluation of synthesized compounds.

**References:**

Synthesis and biological evaluation of some novel glycogen synthase kinase-3β for the management of Type II diabetes

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RKDF Institute of Pharmaceutical Sciences
Email address: sumeet6882@gmail.com

Abstract: Diabetes mellitus (DM) is a progressive disease characterized by hyperglycemia due to insulin deficiency and insulin resistance or both. In the present article, Rhodanine analogs were screened for their anti diabetic activity. Rosiglitazone is used as a reference standard. The compounds R2, R5 and R7 shows better activity than the reference compound and was found to be effective as compared to the other synthesized compounds. The determined fasting blood glucose levels were monitored and the decrease in the blood glucose level was calculated. Some of the synthesized Rhodanine derivatives have shown considerable improved efficacy and improved biological response which is enough reason to believe that Glycogen synthase kinase-3β is a promising target for the management of type II diabetes and that Rhodanine analogs have it in them to illicit a biological response out of the receptor.

Introduction:
Diabetes mellitus (DM) is a progressive disease characterized by hyperglycemia due to insulin deficiency and insulin resistance or both. The insulin-insensitive form of diabetes, type 2 diabetes mellitus (T2DM), characterized by hyperglycemia (elevated blood glucose concentrations), most frequently arises as a consequence of obesity, represents approximately 95% of the overall incidence of diabetes. Additionally, diabetes-related complications exert a heavy toll on patients with poor metabolic control [11], [14], [16]. Therefore it is worthwhile to develop new Glycogen synthase kinase-3β activators. Thus for this purpose identification of compounds is essential that are aimed at resolving this issue. The objective is to synthesize the compounds which are efficacious than those present in the market and possess minimum side effects.

Materials and Method:
Synthesis:
Step I – Synthesis of acid chloride
Substituted Benzoic Acids

Acid Chloride

Step II: Synthesis of aldehyde ester:

1. Triethylamine
2. DCM

Aldehyde Ester

Step III: Synthesis of Phenyl Benzoate Derivatives:

Rhodanine

\((Z)-4-((4\text{-}\text{oxo}\text{-}2\text{-}\text{thioxothiazolidin}\text{-}5\text{-}\text{ylidene})\text{ methyl})\text{ phenyl substituted benzoate}\)

The biological activity data chart of the compounds is as follows
Results and discussion:
On the basis of literature study Rhodanine analogs were designed. Compounds (Der01-Der07) were synthesized by Knoevenagel condensation reaction. The progress of the reaction was monitored through thin layer chromatography. The synthesized compounds were characterized by TLC, melting point, IR, NMR & Mass spectroscopy. Structure of synthesized compounds was elucidated by $^1$H NMR, Mass spectral data and IR findings.

In-vivo Biological Activity:
Rhodanine analogs were screened for their antidiabetic activity by alloxan induced diabetic model and blood glucose level was measured by taking out blood from the retro orbital plexus and blood glucose was measured using a glucometer. There were ten groups each consisting of six rats. Male rats were used only.

References:
Development and validation of RP-HPLC method for the simultaneous estimation of Naloxone Hydrochloride and Oxycodone Hydrochloride in bulk and tablet dosage form

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Email address: surekha_dhokane@rediffmail.com

Abstract: A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated as per ICH guidelines for the simultaneous estimation of Naloxone HCl and Oxycodone HCl in bulk and tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry C18 column. The mobile phase consisted of 65:35 (v/v) methanol: buffer (10mM, (KH$_2$PO$_4$), pH 3 (adjusted with ortho phosphoric acid). The UV detection wavelength was at 260 nm. The R.T for Naloxone HCl was 2.7 min and Oxycodone HCl was 5.7 min.

Introduction:
Oxycodone is a semi-synthetic opioid synthesized from thebaine, an opioid alkaloid. It is an analgesic indicated for relief of moderate to severe pain. Combination products with non-narcotic ingredients such as NSAID and paracetamol; a combination with naloxone is available in managed-release tablets and used for managing moderately severe acute or chronic pain$^{[1,2,3]}$. Naloxone hydrochloride is an opioid antagonist, synthetic congener of oxymorphone and indicated for complete or partial reversal of opioid and respiratory depression, induced by natural and synthetic opioids including propoxyphene, methadone, and certain mixed agonist-antagonist analgesics: nalbuphine, pentazocine, butorphanol, and cyclazocine$^{[4]}$.

Materials and method:
Chromatographic Conditions: Chromatographic separation of Nalo HCl and Oxy HCl were performed by use of an isocratic M.P prepared from 65:35 (v/v) methanol: buffer (10mM, KH$_2$PO$_4$), pH 3 (adjusted with ortho phosphoric acid) giving well resolved, sharp peak for Nalo HCl and Oxy HCl with a retention time (tR) 2.7 and 5.7 min. The flow rate was 1.0 ml/min, UV detection was performed at 260 nm.

Preparation of Stock, working standard solutions and Sample solutions
Nalo HCl and Oxy HCl was weighed separately and std solution with conc. Of 2.5 to 15 ppm prepared, injected and calibration curve was plotted.
The formulation tablets of Nalo HCl and Oxy HCl were powdered, weighed and sample solution was prepared containing concentration of Nalo HCl 5 ppm and Oxy HCl 10ppm.
Fig. 1 Standard chromatogram of Naloxone hydrochloride and Oxycodone hydrochloride

**Method Validation procedure:** The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and robustness and system suitability.

**System suitability parameters:** System suitability parameters i.e. theoretical plates and tailing factor were determined before any analysis was carried out and shown in Table No.1.

**Linearity:** Calibration curve of Nalo HCl and Oxy HCl was obtained by plotting the peak area versus the conc. The linear correlation coefficient for Nalo HCl and Oxy HCl was shown in Table No. 2.

**Calibration curve of Naloxone hydrochloride and Oxycodone hydrochloride**

![Calibration curve of Naloxone hydrochloride](image1.png)  
![Calibration curve of Oxycodone hydrochloride](image2.png)

**Fig.2:** Calibration curve of Naloxone hydrochloride  
**Fig.3:** Calibration curve of Oxycodone hydrochloride

**Precision:** Repeatability was checked by injecting replicate injections of 5 ppm and 10ppm of Nalo HCl and Oxy HCl solution for six times on the same day. Results shown in Table No.2.

**Assay of tablet formulation:** Assay was checked by injecting replicate injections of sample solution for six times and results are shown in Table No.3

**Accuracy (Recovery study):** The accuracy/recovery studies checked by addition of std drug to pre-analyzed sample solution at three different conc. levels (80 %, 100 % and 120 %) results shown in Table No.2

**Robustness:** It should show the reliability of an analysis with deliberate variations in method parameters. The parameters included flow rate, pH of M.P. The results are shown in Table No.2.

**Results and Discussion:**

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Nalo HCl and Oxy HCl in bulk and tablet formulation.

**Table 1:** System suitability parameter

<table>
<thead>
<tr>
<th>Name</th>
<th>R.T</th>
<th>Area</th>
<th>Tailing factor</th>
<th>Theoretical plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalo HCl</td>
<td>2.72</td>
<td>208265</td>
<td>1.61</td>
<td>7477</td>
</tr>
<tr>
<td>Oxy HCl</td>
<td>5.73</td>
<td>350812</td>
<td>1.55</td>
<td>15831</td>
</tr>
</tbody>
</table>
Table 2: Validation Parameters

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Acceptance criteria</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Naloxone HCl</td>
</tr>
<tr>
<td>Linearity</td>
<td>The correlation coefficient should be NLT 0.999</td>
<td>0.9991</td>
</tr>
<tr>
<td>Repeatability</td>
<td>% RSD of 6 injections</td>
<td>0.027</td>
</tr>
<tr>
<td>Accuracy</td>
<td>% Recovery at each level should be between 98-102% and RSD NMT 2.0%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120%</td>
</tr>
</tbody>
</table>

Robustness

<table>
<thead>
<tr>
<th>Change in flow rate</th>
<th>% RSD of R.T NMT 2%</th>
<th>0.8 ml/min</th>
<th>1.2 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in mobile phase pH</td>
<td></td>
<td>2.5</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusion:
In the present study proposed method is validated as per ICH guidelines and is simple, precise, specific, accurate and less time consumption for analysis, so it can be employed for routine analysis for API and formulations and can be employed for bioequivalence studies in same formulation.

References:
Study of stability indicating assay method and quantitative estimation of Dabigatran Etxilate Mesylate by HPTLC

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Email address: deeptijain@rgtu.net

Abstract: A sensitive Stability indicating high-performance thin layer chromatographic method was developed and validated for quantitative determination of dabigatran etexilate mesylate. Chromatographic method separation was performed on a silica gel 60F254 HPTLC plate using MeOH: CHCl3: hexane (4:2:4, v/v) as a mobile phase. A TLC scanner set at 316 nm was used for direct evaluation of the chromatograms in absorbance mode. Dabigatran etexilate mesylate was satisfactorily resolved with Rf value of 0.87 ± 0.05. Calibration curve was polynomial in the concentration range 200-1000 ng/band. The high correlation coefficient (r2 = 0.998) values indicated clear correlation between the drug concentration and their peak area within the test ranges. The developed method was validated according to ICH [1-3] guidelines. The repeatability and intermediate precision, expressed by the % RSD were <1.20. The accuracy and validity of the method were further ascertained by performing recovery and was satisfactory (99.58 %). The proposed HPTLC method was utilized to investigate the acidic, alkaline and neutral degradation of dabigatran etexilate mesylate.

Introduction:
Dabigatran etexilate mesylate [5] [Methanesulfonic acid ethyl 3-(1-[(4-amino ([(hexyloxy) carbonyl] imino)]) methyl] phenyl] amino) methyl]-1-methyl-1H-1, 3benzodiazo-5-yl}-N-pyridin-2-yl) formamido) propanoate] is a salt of dabigatran etexilate.

Figure 1: Structure of dabigatran etexilate mesylate

Materials and Method:
Instrumentation and chromatographic conditions: The samples were spotted in the form of bands with a Cammag microliter syringe under nitrogen stream using a CAMAG Linomat V sample applicator (Switzerland). Precoated TLC silica gel aluminum plates 60 F254 (20 x10 cm, 200 μm thickness, Fluka, Switzerland) were used. Densitometric scanning was performed on CAMAG TLC scanner operated by WINCATS software (V 3.15 CAMAG). The spots were scanned at 316 nm.
**Chromatography**: Standard stock solution of 100 µg/ml of DEM was prepared and filtered through a 0.2 µm membrane filter. The working spots were made in the concentration range 200-1000 ng/spot. Triplicate applications were made for each concentration. The plate was then developed using chloroform: n-hexane: methanol (2:4:4, v/v/v) mobile phase and peak areas at 316 nm were spotted against the corresponding concentrations to obtain the calibration graph.

**Forced degradation studies** were performed in **Neutral, Acidic and Alkaline**

For **oxidative condition** studies were performed in 3% hydrogen peroxide at room temperature for 24 hours. The resultant solutions after all stress conditions were applied to TLC plate in a concentration of 800 ng/spot.

**Results and Discussion**: 

**Development and optimization of method**: Initially the drug was analyzed on HPTLC using methanol: chloroform: n-hexane in the ratio of 4:2:4 at 20 x10 TLC plate. Under this condition the shape of the peak of drug and its degradation products was good, and all the degradants and drug was resolved.

The developed method was validated with various validation parameters and results obtained are given below:

**Linearity**: The response for the drug was linear in the concentration range between 200 to 1000 ng/spot.

**Accuracy and Precision** for intra-day and inter day precision studies at three different concentration range were studied. The % RSD value for inter-day is less than 1%, for intra-day is less than 2%, indicating that the method is sufficiently precise.

**LOD and LOQ** of the proposed method were found to be 0.025ng/spot and 0.078 ng/spot respectively, which indicated that the proposed method can be used in wide range for detection and quantification of dabigatran etexilate mesylate effectively.

**Table 2**: Data for linearity of DEM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.998</td>
</tr>
<tr>
<td>Slope (M)</td>
<td>2765</td>
</tr>
<tr>
<td>Y-Intercept</td>
<td>7.976</td>
</tr>
<tr>
<td>Linearity range (ng/spot)</td>
<td>200-1000</td>
</tr>
</tbody>
</table>

**Degradation behavior of DEM**

The forced degradation study on DEM shows that the drug degraded under alkaline, neutral and acid condition. The specificity and selectivity of method under these conditions was demonstrated through the evaluation of Rₚ resolution and purity data for all peak area in these chromatograms. Dabigatran etexilate mesylate was stable in H₂O₂ and photolytic stress condition. In a mixture of stressed, samples total degradants were observed. Stability-indicating HPTLC studies of the DEM under

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different stress conditions, using methanol: chloroform: N-hexane (4:2:4) as the mobile phase suggested the following degradation behavior. The drug gradually decreased with time on heating at 60°C. DEM was found to be liable to acidic and alkaline hydrolysis. The rate of hydrolysis in acid was slower as compared to that of alkali. No degradation was observed even on exposure of the drug to 3% H₂O₂ for 24 hours, showing that it was stable against oxidative stress.

Acidic degradants

Alkaline degradants

Neutral degradants

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>Degradants</th>
<th>Concentration (ng/ml)</th>
<th>Rf</th>
<th>Area</th>
<th>Avg remaining % quantity of DEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic Hydrolysis</td>
<td>Deg-2</td>
<td>800</td>
<td>0.02</td>
<td>474.9</td>
<td>39.89</td>
</tr>
<tr>
<td></td>
<td>Deg-3</td>
<td>800</td>
<td>0.20</td>
<td>2811.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deg-4</td>
<td>800</td>
<td>0.09</td>
<td>634.7</td>
<td></td>
</tr>
<tr>
<td>Alkaline Hydrolysis</td>
<td>Deg-2</td>
<td>800</td>
<td>0.03</td>
<td>520.8</td>
<td>32.71</td>
</tr>
<tr>
<td></td>
<td>Deg-3</td>
<td>800</td>
<td>0.23</td>
<td>3737.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deg-4</td>
<td>800</td>
<td>0.10</td>
<td>2430.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deg-5</td>
<td>800</td>
<td>0.29</td>
<td>313</td>
<td>45.14</td>
</tr>
<tr>
<td>Neutral Hydrolysis</td>
<td>Deg-7</td>
<td>800</td>
<td>0.42</td>
<td>367.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deg-8</td>
<td>800</td>
<td>0.40</td>
<td>178.2</td>
<td></td>
</tr>
<tr>
<td>Oxidative</td>
<td>No degradants were found in that hydrolysis condition</td>
<td></td>
<td></td>
<td>99.95</td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td>No degradants were found in that hydrolysis condition</td>
<td></td>
<td></td>
<td>98.15</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:
The proposed method provides sensitive, selective, accurate and reproducible means for determination of DEM in the presence of its degradation products using HPTLC technique. HPTLC method has the advantage that several samples can be run simultaneously using a small quantity of developing system and that it can provide high sensitivity and selectivity. After the degradation study we found that DEM is stable in thermal and oxidative condition, and is unstable in acidic, basic and neutral condition.

References:
**De novo design for identification of leads as dipeptidyl peptidase-IV inhibitors**

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Email address: piyushghode@gmail.com

**Abstract:** A de novo design approach has been applied for identification of compounds which can serve as lead for development of novel and selective dipeptidyl peptidase-IV (DPP-IV) inhibitors. The active site of enzyme was utilized for construction of molecules through fragment connection approach using e-LEA3D de novo drug design pipeline. The molecules were “grown” inside the active site and the output contained compounds that exhibited interactions with active site residues. Several such runs resulted in a library of compounds which were further filtered on the basis of scores and interactions with essential residues. The study exhibited that (S)-2-(4-(((S)-2-((5-(quinazolin-7-yl)furan-2-carbonyl oxy) propyl) amino) phenyl) propanoate among H-bonding interactions with the important active site amino acid residues apart from others. Therefore this compound can serve as lead for development of a new class of DPP-IV inhibitors.

**Introduction:**
The enzyme dipeptidyl peptidase-IV (DPP-IV) is a potential target for effective treatment of type-2 diabetes mellitus (T2DM). DPP-IV is a 766 amino acid homodimer in its active state in which it curtails the proteins and peptides with penultimate proline or alanine [1]. Glucagon like peptide (GLP) and glucose dependent insulinotropic polypeptide (GIP) are among such peptides. GLP and GIP are secreted postprandially and regulate insulin secretion. Consequently the blood glucose levels remain under control in healthy individuals. Their truncation by DPP-IV abbreviates their half life (GLP t1/2 =1-2 minutes, GIP t1/2 = ~7minutes). Thus inhibition of DPP-IV results in increased t1/2 of these peptides and eventually higher blood insulin levels. The objective of current study is to apply de novo design strategy for identification of new compound(s) that may serve as lead(s) for development of novel DPP-IV inhibitors.

**Materials and Method:**
The DPP-IV enzyme in complex with 1-biphenyl-2-yl methanamine (PDBentry: 3CCB) [2] was retrieved from Protein Data Bank (PDB). The protein structure was processed using standard molecular mechanics protocol which comprised of removal of crystallographic water molecules, addition of polar hydrogens, assignment of bond orders and Kollman charges. All N-acetyl-D-
glucosamine molecules were removed. e-LEA3D [3] *de novo* drug design pipeline was employed for development of DPP-IV inhibitors. The protein structure in PDB file format was converted to Mol2 format after assignment of amino acid charges via automated PDB2PQR protonation scheme. The 1-biphenyl-2-yl methanamine bound site was defined as the binding site with a radius of 10 Å for designing molecules. e-LEA3D combines drug-like or lead-like molecular properties and Tversky similarity fingerprint measure leading to the fragment selection from the database containing 5283 building blocks. It utilizes the docking algorithm PLANTS (Protein-Ligand ANT System) [4] to find the interactions between the receptor and ligands. PLANTS is based on a class of stochastic optimization algorithm known as ant colony optimization (ACO), which is conceived on the behavior of real ants to find the shortest path between their nest and food source. Analogously, an ant colony is virtually formed in the binding site of a protein to find a minimum energy conformation of the ligand and subsequent iterations are performed to generate low-energy conformations with a high probability.

**Results and Discussion:**

In search of molecules having different scaffolds other than the commercially available DPP-IV inhibitors a fragment based approach was applied for lead identification. It comprised of construction of diverse set of molecules by fragment connection approach in the active site of the receptor followed by scrutinizing the molecules on the basis of their interactions with important active site residues. The active site of DPP-IV comprises of S1 and S2 pockets. The interactions with S2’ pocket extending from S2 pocket is also considered for stability of the protein ligand complex. The most important amino acids responsible for DPP-IV activity are SER630 (S1), GLU205 (S2) and GLU206 (S2). Consequently any ligand binding to these amino acids can cause inhibition of DPP-IV activity. Interaction with above mentioned residues was preferred over binding affinity as the selection criterion. Thus compounds with lower values of binding affinity but spurious interactions were assumed to be trivial.

The docking scores and interactions of top 5 compounds are given in Table 1. Among these compounds (S)-2-(4-(((S)-2-((5-(quinazolin-7-yl) furan-2-carbonyl oxy) propyl) amino) phenyl) propanoate was found to have hydrogen bonding interactions with GLU205 in S2 and ARG125 and ARG358 in S2’. The interactions with S2’ subsite residues contribute towards the stability of the complex. The other common interactions among the compounds are with GLU668 and ARG669 but these interactions do not contribute towards the enzymatic inhibitory activity. In view of the interactions, compound 3 appears to be the most significant. Figure 1 shows the structure of compound 3 along with its interactions with active site residues.
Conclusion:
In the present investigation an attempt was made to discover new leads for efficient DPP-IV inhibition using de novo drug design approach. Different fragments were linked to form molecules inside the active site of enzyme that interacted with residues in the active site cavity. Among these compounds (S)-2-(4-(((S)-2-((5-(quinazolin-7-yl) furan-2-carbonyl) oxy) propyl) amino) phenyl) propanoate is estimated to have the most favorable interactions. Therefore this compound may provide the impetus for development of a new class of DPP-IV inhibitors. Further efforts in this direction are in progress.

Table 1: The binding affinity (expressed as PLANTS score) and interactions with active site residues of top five compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PLANTS score</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-115.580</td>
<td>GLU206, ARG669</td>
</tr>
<tr>
<td>2</td>
<td>-101.350</td>
<td>GLU205, GLU206, ARG358, ARG669</td>
</tr>
<tr>
<td>3</td>
<td>-104.230</td>
<td>ARG125, GLU205, ARG358, GLU668, ARG669</td>
</tr>
<tr>
<td>4</td>
<td>-106.770</td>
<td>ARG125, Y553, SER630, Y662, HIS672</td>
</tr>
<tr>
<td>5</td>
<td>-108.360</td>
<td>GLU206, ARG669</td>
</tr>
</tbody>
</table>

Figure 1: (a) Structure and (b) Binding mode of (S)-2-(4-(((S)-2-((5-(quinazolin-7-yl) furan-2-carbonyl) oxy) propyl) amino) phenyl) propanoate. The ligand carbon atoms are green while the interacting amino acids are magenta among the otherwise blue active site.

References:
Development and validation of RP-HPLC method for simultaneous estimation of Atorvastatin calcium and Ezetimibe tablet dosage form

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College of Pharmacy, IPS Academy, Indore (M.P.) India
Email address: anamika_02@rediffmail.com

Abstract: Simultaneous determination of Ezetimibe and Atorvastatin calcium in tablet dosage form by RP-HPLC method was developed and validated using C18 column(250mm x 4.6mm) 5μ Shimadzu- LC-10 which was simple, specific, accurate and precise. The sample was analysed using Methanol: Acetate Buffer (7:3) pH 3.7 as a mobile phase at detection wavelength 248nm. The retention time was observed to be 5.82 min and 6.96 min for EZ and AC respectively. Calibration curve was plotted and plots were linear for both the drugs of EZ and AC, and recoveries obtained between 99.8% and 101.8%. The method can be used for estimation of EZ and AC in bulk and combined dosage form.

Introduction:
Ezetimibe is a therapeutically beneficial drug that works by a unique mechanism and differs from traditional lipid lowering agents. Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase [1-5].

Materials and Method:
HPLC parameter optimized and shown in Table No. 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Shimadzu- LC-10Atyp/Empower software/PDA detector</td>
</tr>
<tr>
<td>Column</td>
<td>Luna C18 (250mm x 4.6mm) 5μ</td>
</tr>
<tr>
<td>Mobile phase*</td>
<td>Methanol : Acetate Buffer(70:30) adjusted to pH 3.7 with acetic acid</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>248nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20μl</td>
</tr>
</tbody>
</table>

Preparation of Standard Stock Solution:
10 mg of EZ and AC WS (working standards) was accurately weighed and transferred to a 50 ml volumetric flask and dissolved in M.P to get a solution of concentration 200 mg/ml (stock A). 12.5 ml of stock A was taken and diluted with diluent to 25ml (10 0mg/ml stock B). Further dilutions were made from stock B to get a series of dilutions ranging in concentration from 5 – 20 mg/ml.
Preparation of test sample:
Tablets were crushed and weighed powder equivalent to 10 mg of AC and EZ was taken in 10ml volumetric flask and dissolved in 7ml in M.P and sonicated and diluted upto 10 ml with M.P. Then filtered through Whatmann filter paper (No.41). This solution was further diluted to get final concentrations of both the drugs in the working range.

Results and Discussion:
1. Estimation
A RP-HPLC method was developed and validated for estimation of Ezetimibe and Atorvastatin Calcium in combined dosage forms. The method is simple, precise, accurate and which can be conveniently employed for routine analysis in pharmaceutical dosage forms.

![Figure 1: Typical chromatogram of Ezetimibe and Atorvastatin Calcium](image)

2. System Suitability Parameters
The system suitability studies were performed for the standard solutions and were shown in Table no.2. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

<table>
<thead>
<tr>
<th>System suitability parameters</th>
<th>Mean</th>
<th>R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC X 10^6</td>
<td>EZ</td>
<td>AC</td>
</tr>
<tr>
<td></td>
<td>0.766577</td>
<td>0.66205</td>
</tr>
<tr>
<td>Retention Time</td>
<td>6.975</td>
<td>5.826</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.23</td>
<td>1.266</td>
</tr>
<tr>
<td>No. of Theoretical plates</td>
<td>4476</td>
<td>4680</td>
</tr>
</tbody>
</table>

3. Accuracy or Recovery studies
The accuracy/recovery of the method was determined by adding a known quantity of the pure drug to the pre-analyzed sample at 80%-120% levels. The results are shown Table No.3.

Table 3: Results of the Recovery Tests

<table>
<thead>
<tr>
<th>Level</th>
<th>% Recovery</th>
<th>Mean S.D</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. Assay:

Sample solution was injected in replicate 5 times and results are mentioned in table no. 4.

**Table 4:** Analysis of Formulation and Recovery studies

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Standard Deviation of AC</th>
<th>Atorvastatin calcium Assay (%)</th>
<th>Standard Deviation of EZ</th>
<th>Ezetimibe Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02301</td>
<td>100.691</td>
<td>0.1154</td>
<td>100.36</td>
</tr>
<tr>
<td>2</td>
<td>0.08299</td>
<td>99.84</td>
<td>0.1222</td>
<td>100.25</td>
</tr>
<tr>
<td>3</td>
<td>0.06394</td>
<td>100.128</td>
<td>0.1081</td>
<td>99.95</td>
</tr>
<tr>
<td>4</td>
<td>0.11368</td>
<td>100.115</td>
<td>0.1463</td>
<td>99.98</td>
</tr>
<tr>
<td>5</td>
<td>0.13043</td>
<td>99.902</td>
<td>0.0991</td>
<td>100.15</td>
</tr>
<tr>
<td>Mean</td>
<td>0.08281</td>
<td>100.135</td>
<td>0.1182</td>
<td>100.138</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.78</td>
<td>0.78</td>
<td>0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of Ezetimibe and Atorvastatin Calcium were found to be 0.998 and 0.999 respectively.

**Conclusion:**

The chromatographic method developed for Ezetimibe and Atorvastatin Calcium were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, biopharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

**References:**


Novel square wave polarographic method for estimation of rabeprazole sodium in bulk, formulation and human plasma

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E mail address: richa_dayaramani@yahoo.co.in

Abstract: The presented work is development of voltammetric method of analysis for estimation of Rabeprazole Sodium in bulk, formulation as well as in human plasma. The voltammetric method used is square wave polarographic technique. The developed method is accurate, precise, reproducible, quick, inexpensive, simple yet highly sensitive, up to 1ng/ml levels. The method uses a hanging dropping mercury electrode (HMDE) system and Ag/AgCl electrode as reference electrode. Square wave polarography is a highly sensitive voltammetric technique and concentration levels of part per billion are easily detectable. This method was explored for analysis of the drug in order to estimate it with better sensitivity levels.

Introduction:
Voltammetry is concerned with current potential relationship in an electrochemical cell and in particular, with the current time response of an electrode at a controlled potential. Square wave polarography (SqWP) is an advanced voltammetric technique, which is highly sensitive where concentration levels of parts per billion are easily detectable. The drug chosen is Rabeprazole Sodium (RS) which is a proton pump inhibitor. Chemically it is a substituted benzimidazole essentially a prodrug that in acidic biophase of he parietal cell form an active metabolite that irreversibly interact with an essential thiol (-SH) function on ATPase of the proton pump. Chemical name of RS is 2-[[3-(3-methoxy – propoxy) – 3 – methyl 2 – pyridinyl] methyl] sulfinyl] – 1H – benzimidazole sodium salt. As compared to the modern chromatographic methods like HPLC, HPTLC etc the voltammetric method is sensitive, quick and economical as no costly reagents and chemicals are required. The method allows the determination of limit of quantification (LOQ) with RSD less than the value reported for the chromatographic technique.

Materials and Methods:
The instrument used was Metrohm 757 Computrace VA voltammeter incorporating a Teflon stirrer and a 3 electrode working system consisting of a Hg electrode system HMDE as working electrode, a Platinum wire as auxillary electrode and a Ag/AgCl electrode as reference electrode. The reagents used were double distilled water (ddw); 0.1M LiCl; solution, IP’66; aqueous solution of concentration 10 µg/ml of RS, adjusted to pH 10.5 with 0.2M NaOH and 0.2M NaOH, IP’66. A standard stock
solution of 100 µg/ml of standard RS was prepared in ddw adjusting pH 10.5 with 0.2M NaOH. For sample solution for formulation, average weight of 10 tablets was taken and accurately weighed tablet powder equivalent to about 2.4 mg of RS was taken in 100ml volumetric flask and dissolved in ddw and volume made upto mark with ddw after adjusting pH 10.45 with 0.2N NaOH. A final sample solution of concentration 2 ng/ml was prepared.

<table>
<thead>
<tr>
<th>Table 1: Experimental setup parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode</td>
</tr>
<tr>
<td>End potential</td>
</tr>
<tr>
<td>Voltage step</td>
</tr>
<tr>
<td>Pulse amplitude</td>
</tr>
<tr>
<td>Pulse time</td>
</tr>
<tr>
<td>Voltage step time</td>
</tr>
<tr>
<td>Stirrer speed</td>
</tr>
<tr>
<td>Initial purge time</td>
</tr>
<tr>
<td>Additional purge time</td>
</tr>
<tr>
<td>Equilibration time</td>
</tr>
<tr>
<td>Deposition potential</td>
</tr>
<tr>
<td>Deposition time</td>
</tr>
<tr>
<td>Blank purging time</td>
</tr>
<tr>
<td>Purging gas</td>
</tr>
</tbody>
</table>

The voltammetric responses were obtained by OVAT technique employing the parameters given in table 2. The optimum values of the parameters are selected on the basis of linearity, peak shape, repeatability and accuracy of results when a known concentration of drug solution was analyzed as sample in standard addition mode.

<table>
<thead>
<tr>
<th>Table 2: Optimum values of voltammetric parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirring rate</td>
</tr>
<tr>
<td>Start potential</td>
</tr>
<tr>
<td>End potential</td>
</tr>
<tr>
<td>Voltage step</td>
</tr>
<tr>
<td>Pulse amplitude</td>
</tr>
<tr>
<td>Pulse time</td>
</tr>
<tr>
<td>Voltage step time</td>
</tr>
<tr>
<td>Deposition potential</td>
</tr>
<tr>
<td>Deposition time</td>
</tr>
<tr>
<td>Equilibration time</td>
</tr>
</tbody>
</table>

For stripping procedure, 10 ml of 0.1M LiCl3 was used as supporting electrolyte and with the optimized parameters a cathodic differential pulse potential scan was applied. The adsorptive stripping voltammetric cycle was repeated with a new drop for each solution analyzed and the mean of these voltammograms was used for subsequent data handling. Peak heights were evaluated as the difference between each voltammogram and background electrolyte voltammogram.
Results and Discussion:

The material was validated with respect to accuracy, precision, LOD and LOQ and the RSD for all these parameters was found to be excellent that is in the order of 0.001%. By method of standard addition a linear relationship between peak height and analyte concentration was found. Solutions up to 2ng/ml concentration could be successfully analyzed.

Conclusion:

The developed novel method of estimation of RS is suitable for estimation in bulk, formulation as well as in human plasma. It is economical, simple and quick and doesn’t require any sample preparation. It can be used in routine analysis and gives consistent results.

References:

Analgesic and anti-inflammatory activities of 5, 6-di(pyridine-3-yl-anhydride)-bi cyclo[2:2:1]hept-2-ene in experimental rodent model

Vishwakarma Singh*, Sarika Shrivastava, Anjeeta Singh
Radharaman College of Pharmacy, Fatehpur Dobra, Ratibad, Bhopal- 462 002, M.P., India.

Abstract: The natural product epibatidine is one of the most potent nicotinic acetylcholine receptors ligands to date. Its exceptionally potent analgesic activity has prompted an intense study of this unique alkaloid and renewed interest in the search of non-narcotic nAChR mediated analgesic agents. The discovery that epibatidine, isolated from the skin of the frog *Epipedobates tricolor* is an extremely potent efficacious antinociceptive agent and the subsequent observation by our group that its activity is mediated via nicotinic receptors stimulated research into the discovery of epibatidine analogs without toxic side effects. We synthesized 5,6-di(pyridine-3-yl-Anhydride)-bi cyclo [2:2:1] hept-2-ene. The present study assessed analgesic and anti-inflammatory activities of the synthesized compound at the doses of 0.2 µg/kg and 0.5 µg/kg body weight in experimental animal model. Analgesic activity was evaluated by hot plate method in Swiss albino mice and acute anti-inflammatory activity was evaluated by carrageenan, induced rat paw edema in Wister albino rats. Diclofenac and Aspirin were employed as reference drugs for analgesic and anti-inflammatory studies respectively.

Introduction:
Implications of nAChRs in a number of other relevant physiological and pathological processes, like appetite, schizophrenia, epilepsy, depression and analgesia. The structural similarity to nicotine suggested that epibatidine would have activity at nicotinic receptors. Epibatidine was found to exhibit potent analgesic effects (200-500) times more potent than morphine) that were not mediated through opioid receptors. This is clearly established by the fact that epibatidine was not blocked by administration of the potent opiate antagonist naloxone. The discovery of epibatidine and its exceptionally potent analgesic activity had prompted an intensive study of the pharmacological activity of this unique alkaloid and has renewed the search for non-narcotic nAChR mediated analgesic agents. The Compounds with similar structures often tend to have similar pharmacological activity. In our study the compound was synthesized by the complete SAR study of the Epibatidine(exo-2-(6-chloro-3-pyridyl)-7-azabicyclo[ 2.2.1] heptane) ring system.

Materials and Methods:
Dicyclopentadine, maleic anhydride, ethyl acetate, ligroin, n-hexane, calcium chloride, nicotinic acid, thionyl chloride(SnCl₂), aluminium trichloride were petroleum ether used

**Analytical method:** The synthesized chemical compound was detected by

- NMR spectroscopy,
- Thin layer chromatography.
- Elementary Analysis.
- The melting point determination.
- UV spectroscopy was performed and the λ max of the product was recorded at 290 nm.

**Experimental Animals:** Albino mice of either sex with a mean weight of 30 g ± 5 were used. The mice were allowed free access to standard food (Pranab Agroindustries limited) and water *ad libitum.*

**Acute oral toxicity study and selection of doses:** The acute toxicity of the chemical compound was determined OECD guideline no. 423 (Acute toxic class method). It was observed that the ethanolic extract and the fractions were not mortal even at 1 µg/kg dose. Hence, (0.1-0.7 µg /kg) of this dose was selected for this study.

**Hot Plate Test Method:** Hot plate method was employed to evaluate the analgesic activity of the analouge.

**Carrageenan induced Paw edema method:** The lyophilized extract on carrageenan induced inflammation in mice paw) with some modifications according to our laboratory conditions.

**Results and Discussion:**

![Graphical representation of analgesic effect by hot plate method and volume of displacement (ml) by carrageen induced rat paw edema method](image)

Figure 1: Graphical representation of analgesic effect by hot plate method and volume of displacement (ml) by carrageen induced rat paw edema method

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in the world today. The present study evaluated the analgesic and anti-inflammatory activities of 5,6-di(pyridine-3-
yl-Anhydride)-bi cyclo[2:2:1]hept-2-ene in experimental rodent models. It has been mentioned in the specialized literature that prostaglandins, particularly prostaglandin PGE2 plays a great role in the advent of pain and inflammation. Carrageenan-induced edema involves the synthesis or release of mediators at the injured site. Among these mediators we can distinguish prostaglandins, histamine, bradykinins, leucotriene and serotonin which also cause pain and fever. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorates the inflammation. The compound 5,6-di(pyridine-3 yol-Anhydride)-bi cyclo[2:2:1]hept-2-ene is structurally similar to the Epibatidine which works by possessing potent agonistic effect on nAchR (nicotinic acetyl colinergic receptors) which finally results Analgesic effect (200) times more potent then morphine (a non opioidal mechanism). The Analgesic effect of the chemical compound is greater than the standard drug (diclofinac). So it is suggested that the mechanism of action of the synthesized compound may be by the nACh receptor mediated.

References:
Estimation of Lurasidone in bulk and formulation by first order derivative area under curve uv- spectrophotometric method

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¹Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education & Research, Karwand Naka, Shirpur, Dist: Dhule (MS) India 425 405
²Department of Pharmaceutical Chemistry, R.C. Patel Institute of Pharmaceutical Education & Research, Karwand Naka, Shirpur, Dist: Dhule (MS) India 425 405
E mail address: khanzamir.5588@gmail.com

Abstract: Simple, fast, precise and reliable spectrophotometric method was developed for determination of lurasidone in bulk and pharmaceutical dosage form. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the first order derivative Area Under Curve method, values measured at 320 - 343 nm. Linearity found in the concentration range of 2 – 12 μg/ml (r²=0.9980). The proposed method has been extensively validated as per ICH guidelines.

Introduction:
Lurasidone (LSD) is chemically (3aR, 4S, 7R, 7aS) - 2-hexahydro-4, 7-methano-2H-isooindole- 1, 3-dione hydrochloride. Lurasidone hydrochloride is a benzisothiazole derivative and an atypical antipsychotic drug.[1] Lurasidone is practically insoluble in water, has poor bioavailability and slow onset of action and as a result could not be given in emergency clinical situations like schizophrenia. Hence there is need to enhance the solubility of LSD. Techniques like UV – Visible Spectrophotometry[2], high - performance liquid – chromatography (HPLC)[3], high - performance thin - layer chromatography (HPTLC) [4], and Liquid chromatography – mass spectroscopy (LC-MS) with ESI have been used for analysis of lurasidone. To our notice, no UV- Visible spectrophotometric method using first order derivative area under curve has been reported for the determination of lurasidone in bulk and tablets. Hence an attempt has been made to develop new first order derivative area under curve spectrophotometric method for estimation of lurasidone in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy as per ICH guidelines[5].

Materials and Methods:
Derivative Spectrophotometric Method
Zero order spectrum of lurasidone is derivatised to the first derivative spectrum used in the wavelength ranges from 320 and 343 nm. [(dA/dλ=f(λ))]: first order. The first derivative spectrum of an
absorption band is regarded as by a maximum, a minimum, and a cross-over face at the $\lambda$ absorption band.

A shimadzu 1700 Pharma Spec UV/VIS double beam spectrophotometer (Shimadzu).

Reference standard of lurasidone (Active Pharmaceutical Ingredient) was supplied as gift sample by Glenmark Pharmaceuticals Limited, Andheri (E), Mumbai. Methanol was obtained from Merck-Lab (B.No.-SB5SF65051). Tablet with label claim 40 mg were prepared in pilot plant (in house).

**Preparation of Standard stock solution:** Stock solution of 10$\mu$g/ml of lurasidone was prepared in Methanol, sonicated for 12 min. The standard solutions were prepared by dilution of the stock solution with double distilled water in concentration ranges of 02, 04, 06, 08, 10 and 12$\mu$g/ml.

**Calibration curve for lurasidone:** The dilutions were scanned from 800 to 200 nm and first order derivative area under curve values was integrated in the range of 320 – 343 nm. The calibration curve was plotted between areas under curve values against concentration.

**Assay of tablet formulation:** Twenty tablets each containing 40 mg of lurasidone were weighed crushed to powder and averaged weight was calculated. Powder equivalent to 10 mg of lurasidone was transferred in 10 ml of volumetric flask. A 7 ml of methanol was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with methanol. The solution was filtered using Whatmann filter paper no. 41; first 3 ml of filtrate was discarded. This solution was further diluted to obtain 06$\mu$g/mL solution with double distilled water, subjected for UV analysis using methanol as blank. This procedure was repeated three times.

**Method Validations**
The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

<table>
<thead>
<tr>
<th>Summary of validation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>$\lambda$ range (nm)</td>
</tr>
<tr>
<td>Regression Equation ($y= mx+c$)</td>
</tr>
<tr>
<td>Linearity range ($\mu$g/ml)</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Correlation coefficient ($R^2$)</td>
</tr>
<tr>
<td>Limit of Detection (LOD) $\mu$g/ml</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) $\mu$g/ml</td>
</tr>
<tr>
<td>Accuracy (Mean % Recovery)</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
</tr>
</tbody>
</table>

**Results and Discussion:**
The UV visible spectroscopic method for the lurasidone by first order derivative area under curve was found to be simple, economical, accurate reproducible. The drug concentrations were found to be linear in the range of 02 - 20 $\mu$g/ml with correlation coefficient value of 0.9980 indicating that
developed method was linear. For precision, percent relative standard deviation (% RSD) was found to be 0.0547 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.854 and 1.035 respectively, thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels. The values of standard deviation were acceptable and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.215 µg/ml & 0.648 µg/ml respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly reliable and reproducible. The method can be used for routine quality control analysis of lurasidone in bulk as well as pharmaceutical formulations.

Conclusion
The UV spectroscopic AUC method for the analysis of lurasidone by first order derivative area under curve was found to be simple, accurate and precise; can be used for analyse of bulk drug and pharmaceutical dosage formulations.

Acknowledgement
The authors are highly thankful to Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education & Research, Karwand Naka, Shirpur, Maharashtra, India for proving all the facilities to carry out the research work.

Figure: 1. Chemical structure of lurasidone  Figure: 2. First order derivative spectrum showin AUC in the range 320 - 343 nm

References:
Mixed hydro tropy solubilization approach for simultaneous quantitative estimation of Aceclofenac and Paracetamol in tablet dosage form by UV- spectrophotometer

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E-mail address: gmrdmishra@rediffmail.com

Abstract: Two accurate, precise, sensitive and economical spectroscopic methods for simultaneous estimation of Aceclofenac and Paracetamol in tablet dosage form have been developed by mixed hydro tropic solubilization technique. Method-I is Simultaneous Equation method and Method-II is Area under Curve method. In the present study, saturation aqueous solubility method in mixture of 20 ml (2M) Urea, 30 ml of (5M) Sodium Acetate and 50ml Distilled water was used to solubilize both poorly water soluble drugs Aceclofenac and Paracetamol to carry out their spectrophotometric analysis. The proposed methods were validated as per ICH guideline in terms of accuracy, linearity, precision. The Accuracy study result of both methods shows that the % of recovery for aceclofenac and paracetamol was within the range of 99-101%. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less than 2.0%.

Introduction:
Various organic solvents have been employed for the solubilization of poorly water soluble drugs for spectrophotometric estimations. The primary objective of this study was to employ hydro tropic solubilizing agents for the selected drugs to preclude the use of organic Solvents. The term “hydro tropy” has been used to designate the increase in aqueous solubility of various poorly water-soluble compounds due to the presence of a large amount of additives [1-3]. Chemically Aceclofenac (ACE) is 2-[2-[2,6-dichlorophenyl]amino]phenyl]- acetyl]oxyacetic acid[4] is a potent inhibitor of the enzyme cyclo-oxygenase, which is involved in the production of prostaglandins. Chemically Paracetamol (PCM) is N-(4-hydroxyphenyl) acetamide [5] a good and promptly acting antipyretic and anti-inflammatory action.. Literature survey revealed that no spectrophotometric methods has been reported hence an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate spectroscopic methods to analyze both drugs simultaneously by using hydro tropic technique.

Materials and Methods:
Working standards of pharmaceutical grade ACE and PCM were obtained as gift sample from Ranbaxy Pvt. Ltd. Dewas (M.P), India and were used without further purification. All the chemicals used were
of analytical reagent grade. The tablet dosage form Aclospar (containing ACE 100 mg and PCM 500 mg) was procured from the local market, Ujjain, India. Urea and Sodium Acetate were taken as hydrotropic agents and distilled water was used for whole experiment. Mixture of 20 mL (2 M) urea and 30 mL (5 M) sodium acetate was selected as solvent system.

**Analysis of Tablet:** Ten tablets of Aclospar were taken and their average weight was determined, they were crushed to fine powder. Then required quantity of powder was taken and dissolved in mixture of 20 ml (2M) Urea solution and 30 ml (5M) Sodium acetate solution then adjust the volume upto 100 ml with distilled water to prepare a stock solution. From the above prepared stock solution further sub stock solution of 100µg/ml of ACE and 500 µg/ml of PCM was prepared. From the sub stock solution 1 ml was taken and dissolved up to 10 ml with distilled water to prepare a solution contain 10µg/ml of ACE and 50µg/ml of PCM. Absorbances of the prepared dilutions were taken at 269.0 and 238.5nm wavelengths. AUC for above prepared solution was taken within the wave length range of 283-290 for ACE and 250-258 for PCM in triplicates and their concentrations were determined by using (Eq. 01, Eq.02) for Method-I and (Eq.03 and Eq.04) for Method-II respectively. The result was given in the Table 1.

\[
C_x = \frac{A_x a_y - A_y a_x}{a_x a_y - a_y a_x} \quad \ldots \text{Eqn.1} \\
C_y = \frac{A_y a_x - A_x a_y}{a_x a_y - a_y a_x} \quad \ldots \text{Eqn.2} \\
\int_{283}^{290} Ad\lambda = K_s C_1 + K_z C_2 \quad \ldots \text{Eqn.3} \\
\int_{250}^{258} Ad\lambda = K_s C_1 + K_z C_2 \quad \text{Eqn.4}
\]

**Results and Discussion:**

Two spectroscopy methods Simultaneous equation and Area under curve methods were developed for simultaneous estimation of ACE and PCM in tablet dosage form using mixed hydrotropic technique and validated as per ICH guideline in terms of accuracy, linearity, precision. The % of drug content was within the range of 99-101% for both ACE and PCM. The % of recovery for ACE and PCM was within the range of 98-102% . Precision study shows that the drug content for both drugs in market formulation were within the range of 98 -101% with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds.

<table>
<thead>
<tr>
<th>Replicate no.</th>
<th>Method</th>
<th>Label claim (mg/Tab)</th>
<th>Conc. Found (mg/Tab)</th>
<th>Percentage found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACE</td>
<td>PCM</td>
<td>ACE</td>
</tr>
<tr>
<td>Replicate-1</td>
<td>Simultaneous Equation</td>
<td>100</td>
<td>500</td>
<td>99.805</td>
</tr>
<tr>
<td>Replicate-2</td>
<td>Simultaneous Equation</td>
<td>100</td>
<td>500</td>
<td>100.058</td>
</tr>
<tr>
<td>Replicate-3</td>
<td>Simultaneous Equation</td>
<td>100</td>
<td>500</td>
<td>100.123</td>
</tr>
<tr>
<td>Replicate-1</td>
<td>Area Under Curve Method</td>
<td>100</td>
<td>500</td>
<td>99.915</td>
</tr>
<tr>
<td>Replicate-2</td>
<td>Area Under Curve Method</td>
<td>100</td>
<td>500</td>
<td>100.352</td>
</tr>
</tbody>
</table>

Table 1 Assay of tablet dosage form
Replicate-3 | 100 | 500 | 100.235 | 500.514 | 100.23 | 100.10

**Conclusion:**
The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less than 2.0% and give reliable and accurate results hence, utilized as quality control tool for the simultaneous estimation of both drug from their combined dosage form in quality control laboratory.

**Reference:**
Synthesis and biological evaluation of Nitrosothiol Ester prodrugs of Aspirin and Ibuprofen

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E-mail address: vijay2day@gmail.com

Abstract: From various studies it has been found that ulcerogenic potential of commonly used non-steroidal anti-inflammatory drugs (NSAIDs) can be reduced by concomitant administration of gastrointestinal (GI) cytoprotectants such as nitric oxide (NO). The present study reported the synthesis and analgesic, anti-inflammatory and anti-ulcerogenic activity of some novel nitrosothiol (-S-NO) ester prodrugs of aspirin and ibuprofen having NO releasing capability. At equimolar oral doses, all synthesized S-NO-Aspirin/ibuprofen derivatives have shown comparable analgesic and anti-inflammatory activity in carrageenan-induced paw edema test and acetic acid-induced writhing test, respectively and less ulcerogenic potential as compare to parent drugs, in rat models.

Introduction:
Aspirin and Ibuprofen are most widely used NSAIDs for the treatment of pain, fever, and inflammation. In general, NSAIDs suppress the biosynthesis of prostaglandin H₂ (PGH₂) .COX enzyme exists in two isoforms, COX-1 and COX-2. COX-1 exerts cytoprotection in GI tract whereas COX-2 mediates inflammation [1]. Two important causes of GI damage from NSAIDs are local GI irritation by the carboxylic acid moiety of most NSAIDs (“topical effect”) and decreased production of cytoprotective prostaglandins. [2]. One such approach for developing a gastric-sparing NSAID involves chemical coupling of a NO-releasing moiety to the parent NSAID. [3]. NO released from the S-NO-NSAIDs also causes a rapid and dose dependent inhibition in morphological conversion of H-pylori from replicating spiral form to non-replicating coccoid form which is incapable of inducing interleukin-8 which is responsible for inflammation. This causes down regulation of inflammatory activity. Acylation of NO-NSAID’S exerts protective effect in stomach [4].

Material and Methods:
Two classes of nitroso-thiols, presented in schemes 1 and 2, were synthesized. Aliphatic aldehydes with an enolizable proton (Such as isobutyraldehyde, cyclohexanecarboxaldehyde) reacted with sulfur monochloride (S₂Cl₂) in CCl₄ at 55 °C to give disulfide-dialdehydes. Synthesized disulfide-dialdehydes reduced with LiAlH₄ to afford thio-aldehydes. Subsequently, each thio-alcohol was nitrosylated with tert-butyl nitrite (t-BuONO) in dichloromethane to give nitrosothiol in good yield. The nitrosylated thiol was then acylated with parent drugs by drop wise addition of a solution of DCC
in CH2Cl2 to a mixture of aspirin/ibuprofen, nitrosylated tether, and DMAP in CH2Cl2 so as to minimize the lactam formation. Coupling was completed within 30–40 minutes at 0°C (scheme 1). Another nitroso-thiol, containing a tertiary amine was synthesized via Scheme 2.

**Synthetic Scheme 1**

![Diagram of Synthetic Scheme 1](image)

Reagents and conditions: (a) S2Cl2/CCl4, 55 °C; (b) LAH, THF; (c) t-BuONO, CH2Cl2; (d) DCC/DMAP, CH2Cl2, 0 °C

**Synthetic Scheme 2**

![Diagram of Synthetic Scheme 2](image)

Reagents and conditions: (a) S2Cl2/CCl4; (b) i. NH2(CH2)nOH, CHCl3, reflux, 6 h, ii. NaBH4, MeOH, rt; (c) i. 38% CH2O, MeOH, ii. NaBH4; (d) LAH, THF; (e) t-BuONO, MeOH, HCl; (f) DCC, DMAP, CH2Cl2, 0 °C, 30 min.

The synthesized compounds were tested for analgesic, anti-inflammatory and anti-ulcerogenic activity in rat models using carrageenan induced paw edema test, writhing test and gastric lesion test, respectively.

**Results and Discussion:**

The synthesized compounds were characterized by UV, IR, NMR, Mass spectroscopy and elemental analysis. The spectral data is as follows:

**AT1.** 2-Methyl 1-(2-methyl-2-(nitrosothio)propyl)phthalate (C13H15NO2S): white crystalline solid; RF, 0.82 (hexane : ethyl acetate; 7:3 ) λmax (277): IR (KBr): 1757, 1370, 1187, 1088, 653; 1H NMR (400 MHz, Chloroform) δ 8.65 – 5.88 (m, 4H), 7.32 (td, J = 7.5, 1.5 Hz, 1H), 7.40 – 5.88 (m, 2H), 7.17 (dd, J = 7.4, 1.3 Hz, 1H), 4.64 (s, 2H), 2.31 (s, 3H), 1.51 (s, 6H); EIMS m/z: 297.07, 298.07; Elemental analysis: calcd., C, 52.51; H, 5.09; N, 4.71; O, 26.91; S, 10.78; Found, C, 51.92; H, 4.89; N, 4.25; O, 25.99; S, 9.78.

**IT1.** 2-Methyl-2-(nitrosothio)propyl 2-(p-isobutylphenyl)propionate(C17H2O2NO5S): yellowish white crystalline powder; RF, 0.79 (hexane : ethyl acetate; 7:3 ) λmax (283): IR (KBr), 1735, 1379, 1178, 1081, 777, 591; 1H NMR (400 MHz, Chloroform) δ 7.38 (d, J = 7.4 Hz, 2H), 7.19 (d, J = 7.5 Hz, 2H), 4.47 (s, 2H), 3.42 (q, J = 6.6 Hz, 1H), 2.54 (d, J = 7.5 Hz, 2H), 2.29 – 1.95 (m, 1H), 1.54 (d, J = 6.6 Hz, 3H), 1.37 (s, 3H), 1.05 (d, J = 6.3 Hz, 6H); EIMS m/z: 323.16, 324.16, 325.15; Elemental Analysis: calcd., C, 63.13; H, 7.79; N, 3.83; O, 14.84; S, 9.91; Found, C, 62.92; H, 6.89; N, 3.89; O, 14.99; S, 10.01.

**AT2.** 1-(2-[N-Methyl[[1-(nitrosothio)cyclohexyl]methyl]amino]ethyl 2-methyl phthalates(C13H23NO3S): yellowish white crystalline powder; RF, 0.72 (hexane : ethyl acetate; 7:3 ) λmax (277): IR (KBr), 2851, 1737, 1373, 1083, 755, 651; 1H NMR (400 MHz, Chloroform) δ 8.36 – 7.50 (m, 1H), 7.45 (td, J = 7.5, 1.4 Hz, 1H), 7.32 (td, J = 7.5, 1.4 Hz, 1H), 7.18 (dd, J = 7.4, 1.5 Hz,
1H), 4.35 (t, J = 7.2 Hz, 2H), 3.03 (t, J = 7.2 Hz, 1H), 2.79 (t, J = 7.2 Hz, 1H), 2.54 (s, 2H), 2.38 (s, 3H), 2.31 (s, 3H), 1.99 – 0.93 (m, 10H); EIMS m/z: 394.16, 395.16; Elemental analysis: calcd., C, 57.85; H, 6.64; N, 7.10; O, 20.28; S, 8.13, Found, C, 56.70; H, 5.99; N, 7.72; O, 19.89; S, 7.77.

**IT2.2-(N-Methyl)[1-(nitrosothio)cyclohexyl][methyl]amino)ethyl2-(p-isobutylphenyl)propionate (C_{23}H_{36}N_{2}O_{3}S):** Brownish white crystalline powder; Rf, 0.75 (hexane : ethyl acetate; 7:3) \( \lambda_{max} \) (277); IR (KBr), 2854, 1748, 1378, 1091, 756, 651; \(^1\)H NMR (400 MHz, Chloroform) \( \delta \) 7.38 (d, J = 7.5 Hz, 2H), 7.19 (d, J = 7.4 Hz, 2H), 4.19 (t, J = 7.3 Hz, 2H), 3.71 (q, J = 6.6 Hz, 1H), 2.82 (t, J = 7.3 Hz, 1H), 2.55 (dd, J = 13.9, 6.8 Hz, 5H), 2.36 (s, 3H), 2.20 – 1.80 (m, 3H), 1.80 – 1.08 (m, 12H), 1.05 (d, J = 6.3 Hz, 6H); EIMS m/z: 420.24, 421.25, 422.24; Elemental analysis: calcd., C, 65.68; H, 8.63; N, 6.66; O, 11.41; S, 7.62, Found, C, 64.88; H, 7.99; N, 5.98; O, 11.22; S, 7.02.

The spectral data confirmed that the proposed prodrugs have been synthesized. The results of biological evaluation showed that synthesized prodrugs are effective in controlling ulcerogenic effect of parent drugs at higher doses without losing its therapeutic effect (Table 1).

**Table 3** Analgesic, anti-inflammatory, and anti-ulcerogenic activity of synthesized compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Analgesic activity (Writhing test)</th>
<th>Anti-inflammatory activity (Paw edema test)</th>
<th>Ulcerogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% inhibition Writhing in mice</td>
<td>% inhibition of edema volume</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>64</td>
<td>82</td>
<td>0.755±0.035</td>
</tr>
<tr>
<td>AT1</td>
<td>63</td>
<td>80</td>
<td>0.283±0.074</td>
</tr>
<tr>
<td>AT2</td>
<td>61</td>
<td>79</td>
<td>0.165±0.0035</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>69</td>
<td>76</td>
<td>2.590±0.063</td>
</tr>
<tr>
<td>IT1</td>
<td>68</td>
<td>75</td>
<td>1.090±0.572</td>
</tr>
<tr>
<td>IT2</td>
<td>65</td>
<td>72</td>
<td>0.595±0.017</td>
</tr>
</tbody>
</table>

**Conclusion:**

Some novel prodrugs of aspirin and ibuprofen bearing tert-nitroso-thiol moiety have been synthesized and evaluated. These NO-releasing prodrugs yielded the parent NSAID in vivo and showed comparable analgesic and anti-inflammatory activities as of parent drugs in rodent models. The study concluded that nitrosothiol esters of aspirin and ibuprofen may constitute a novel class of NO-donating compounds having therapeutic potential as NSAIDs with an enhanced gastric safety profile.

**Acknowledgement:**

Authors are thankful to Director, SGSITS Indore, for providing state-of-art facilities to successfully complete this research.

**References:**

**Synthesis, characterization and biological evaluation of some new Benzimidazole derivatives as anti-microbial agents**

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**Abstract:** Benzimidazole derivatives have been explored extensively by various researchers in past years and investigations supported their therapeutics importance as antimicrobial agents also; keeping in view the diverse therapeutic activities of Benzimidazole for the preparation of bioactive heterocycles, it was contemplated to synthesize a novel series of Benzimidazole. Attention has been focused on the modification of the Benzimidazole moiety to achieve a new antimicrobial profile.

**Introduction:**

Benzimidazole ring displays an important heterocyclic compound in drug discovery. These compounds carrying different substituents in the benzimidazole structure are associated with a wide range of biological activities including anticancer, antiviral, antibacterial, antifungal, antihelmintic, anti inflammatory, antihistaminic, proton pump inhibitor, antioxidant, antihypertensive and anticoagulant properties. As an outgrowth of our investigations of benzimidazole derivatives, which have displayed significant antifungal and antibacterial activity, we described new series of substituted benzimidazoles. The present study involves synthesis and screening of new benzimidazole derivatives as potent anti-microbial agents against *Escherichia coli* and *Candida albicans* [1-3].

**Materials and Methods:**

Melting points were determined by thiels tube and were uncorrected. Synthesized derivative were characterized by FT-IR, $^1$HNMR and MS spectrometry.

**Synthesis of 2-substituted benzimidazole derivatives:** O-Phenylene diamine (2.7 gm) was dissolved in a mixture of methanol/water (200 ml, v/v:1:1). To this, benzaldehyde (5.3 gm) in methanol (50 ml) was added sequentially while stirring the solution. The reaction mixture was then heated to reflux under vigorous stirring for three hours after this a radish pale precipitate was formed. The mixture was filtered hot and then washed with water to afford a yellow solid. The precipitate was re-dissolved in ethanol (150 ml) and to this; HCl (24 ml) was added. At this stage, the reaction mixture was heated at reflux for one hour, resulting in the formation of black slurry. Reaction mixture was allowed to cool to room temperature and filtered through a pad of celite. The filtrate was treated with ammonia solution to pH 8-9 and then concentrated to yield a reddish pale precipitate. After filtration and vacuum evaporation, compound was obtained as reddish solid.
Synthesis of 5-nitro-2-substituted benzimidazole derivatives: 10.75 ml of concentrated nitric acid was placed in three necked flask and equal quantity of concentrated sulphuric acid (1:1) was added slowly. The mixture was kept in the ice cold water then above compound (6.72 gm) was mixed in portions during ½ hour under room temperature. After stirred continuously for 12 hours and then the reaction mixture was poured slowly over crushed ice with stirring. The precipitated product was filtered out and washes with cold water. The final product was formed as yellowish pale.

Synthesis of ethyl 2-(2-substituted-5-nitro-1H-benzo (d) imidazol-1-yl) acetate: Ethylchloroacetate (0.01 mol, 1.06 mL) was added to a solution of above product (0.01 mol, 1.32 g) in dry acetone (20 mL). To that mixture, anhydrous K2CO3 (1 g) was added and the reaction mixture was refluxed for 10 hours. Acetone was removed after completion of reaction and the residue crystallized from ethanol to give compound.

Synthesis of ethyl 2-(2-substituted-5-nitro-1H-benzo (d) imidazol-1-yl) acetoxydrazide: To a solution of above compounds (0.01 mol) dissolved in dry methanol (50 ml), 99% hydrazine hydrate (1 ml) was added and the mixture was refluxed for 4–5 h. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol to give final hydrazide compound.

Biological evaluation: In-vitro Anti-microbial activity of synthesized derivatives:

In-vitro antifungal activity: In-vitro antifungal activity was performed by ITL Labs, New Dehli using Cup Plate Method. The newly synthesized compounds (DM1-DM5) were screened for their antifungal activity against candida albicans by using cup plate method.

In-vitro antibacterial activity: In-vitro antibacterial activity was performed by ITL Labs, New Dehli using same Cup Plate Method. The newly synthesized compounds (DM1-DM5) were screened for their antibacterial activity against gram negative bacteria viz., Escherichia coli by using cup plate method.

Results and Discussion:
Substituted Benzimidazole derivatives were synthesized via Condensation Reaction of substituted benzaldehydes. Five such compounds were synthesized. They were evaluated for their in-vitro antibacterial activity against E.coli. They were also evaluated for their in-vitro anti-fungal activity against C.albicans. They showed potent anti-microbial activity and greater zone of inhibition than control which is comparable with the standard drug at similar concentration.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Result of in-vitro Anti-bacterial activity of synthesized products.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of Inhibition (mm)</td>
</tr>
</tbody>
</table>

“Strategic Approaches to Strengthen Academic and Industrial Collaboration” www.ijpsr.com
<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 (µg/ml)</td>
</tr>
<tr>
<td>DM1</td>
<td>25.06</td>
</tr>
<tr>
<td>DM2</td>
<td>11.87</td>
</tr>
<tr>
<td>DM3</td>
<td>11.62</td>
</tr>
<tr>
<td>DM4</td>
<td>25.06</td>
</tr>
<tr>
<td>DM5</td>
<td>13.26</td>
</tr>
<tr>
<td>Ciprofloxacin (STD)</td>
<td>25.62</td>
</tr>
</tbody>
</table>

Table 2 Result of in-vitro Anti-fungal activity of synthesized products.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. albicans</td>
</tr>
<tr>
<td></td>
<td>50 (µg/ml)</td>
</tr>
<tr>
<td>DM1</td>
<td>10.43</td>
</tr>
<tr>
<td>DM2</td>
<td>10.02</td>
</tr>
<tr>
<td>DM3</td>
<td>10.50</td>
</tr>
<tr>
<td>DM4</td>
<td>10.43</td>
</tr>
<tr>
<td>DM5</td>
<td>10.19</td>
</tr>
<tr>
<td>Fluconazole (STD)</td>
<td>11.68</td>
</tr>
</tbody>
</table>

Conclusion:
These results suggested that the substituted benzimidazole derivatives have an excellent scope for further development as commercial anti-bacterial agent.

References:
Models for the prediction of anti-bacterial activity of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones

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Abstract: An in-silico approach comprising decision tree (DT), random forest (RF) and moving average analysis (MAA) was successfully employed for development of models for the prediction of anti-bacterial activity of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones. A data set consisting of 41 analogues of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones was selected for the present study. The values of molecular descriptors (MD) for each analog in the data set were calculated using E-Dragon software and an in-house computer program. Finally, a total of 46 2D and 3D molecular descriptors of diverse nature, from a large pool MDs (>400) were selected for further analysis with DT, RF and MAA. DT was employed to determine the importance of molecular descriptors. DT classified the analogues of the dataset with an accuracy of ~95% in training set and 78% in 10 fold cross validated set. Random forest correctly classified the analogues with an accuracy of 85.4%. Four independent models developed through MAA predicted the activity of analogues of the data set with an accuracy of 87.8% to >99.9%. The statistical significance of proposed models was assessed through intercorrelation analysis, sensitivity, specificity and Matthew’s correlation coefficient (MCC). The proposed models offer a vast potential for providing lead structures for development of potent anti-microbial agents for treatment of infectious diseases.

Introduction:
Resistance to pathogenic bacteria to currently known antibiotics is quickly becoming a major problem in the community and hospital based healthcare settings [1].
Quantitative structure-activity relationship (QSAR) studies of antibiotics represent an emerging and exceptionally important topic in the area of computer-aided drug design (CADD). Although the demand for ‘in silico’ drug discovery is clear in all areas of human therapeutics, the field of anti-infectives has a particular need for computational solutions enabling rapid identification of novel therapeutic leads. An urge for new antibiotic and antiviral candidates is driven by critical situations with the spread of multi-drug resistant bacteria.
The goal of a (Q)SAR model is to encode the relationship between molecular structure and biological activity or physical property. In drug discovery, (Q)SAR methodologies have proved to be a powerful tool for the prediction of simple chemical-physical properties as well as complex pharmacodynamic,
pharmacokinetic and toxicological profiles, and these provide the basis for directional synthesis of novel compounds possessing desired biological activity [2].

**Materials and Methods:**
In the first step of a typical (Q)SAR study, one needs to find a set of MDs representing the higher impact on the biological activity of interest [3]. Together with other classes of MDs (i.e., LFER substituent constants, geometrical descriptors, and quantum indices), topological indices (TIs) are extensively used in (Q)SAR/SPR studies as effective numerical descriptors of the chemical structure. In the present study, MDs of diverse nature have been successfully utilized for development of suitable models through decision tree (DT), random forest (RF) and moving average analysis (MAA) for the prediction of anti-bacterial activity of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones as inhibitors of UDP-N-acetylenolpyruvyl glucosamine reductase: the MurB enzyme.

**Dataset:** A dataset comprising 41 analogues of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones was selected for the present study [4].

**Computation of Molecular Descriptors (MD):** The Dragon software was used to calculate molecular descriptors for the compounds in the data set from minimized energy molecular models as described below. The structures of all the compounds in the dataset were drawn in SYBYL 7.1 using a standard procedure.

**Decision tree (DT):** Decision tree analysis also known as recursive partitioning, is a simple, yet powerful, statistical method that seeks to uncover relationships in large complex data sets. A decision tree gets its name because it is shaped like a tree and can be easily used to make decisions. Technically, a tree is a set of nodes and branches and each branch descends from a node to another node. A node that has no parents is termed as root node, and a node that has no children is termed as leaf node. The nodes represent a feature in an instance to be classified, and the branches represent a value that the node can assume. The feature that best divides the training data would be the root node of the tree. The resulting classifier is then used to assign class labels to the testing instances where the values of the predictor features are known, but the value of the class label is unknown. In the present study, R, open source statistical computing software from the R Project for Statistical Computing was utilized to create a classification tree using the default parameters.

**Random Forest (RF):** Taking the tree paradigm a step further, Breiman introduced “Random Forest”, which is an ensemble of single decision trees. It creates a preset number of unpruned trees, 100 in this application, with randomly selected features during learning. In the present study, RF was trained using the Random Forest library in the statistical computing environment, R, using default parameters.
Moving average analysis (MAA): Moving average analysis was employed, in order to develop single MD based models for classifying data set into active and inactive analogues. Index values of all the 46 chosen descriptors were analyzed and suitable models were developed after identification of the active ranges by maximization of moving average with respect to active compounds (<35% = inactive, 35-65% = transitional, >65% = active). Subsequently, each analogue of the data set was assigned a biological activity using these models, which was then compared with the reported antibacterial activity. Anti-bacterial activity was reported quantitatively in terms of IC$_{50}$ (µM), which is defined as the concentration of analogues required for 50% growth inhibition of UDP-N-acetylenolpyruvyl glucosamine reductase (MurB) enzyme.

Model validation: There are many ways to present the performance of a classifier. Sensitivity, specificity, overall accuracy of prediction and Matthews correlation coefficient were used in the present study to evaluate the performance of the models. The value of Matthews correlation coefficient (MCC) which takes into account both the sensitivity and specificity is always between -1 and +1. A value of -1 indicates total disagreement (all-false predictions), and +1 indicates total agreement (perfect predictions). The MCC is 0 (zero) for completely random predictions and, therefore, it yields an easy comparison with regard to random baseline. Statistical significance of TIs used in building predictive models was assessed by intercorrelation analysis by using index values of analogues of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones. The degree of correlation was appraised by the correlation coefficient r.

Conclusion:
Four topological models based upon MDs of diverse nature have been successfully developed for predicting anti-microbial activity of pyrazolidine-3, 5-diones and 5-hydroxy-1H-pyrazole-3(2H)-ones as inhibitors of MurB enzyme. The overall accuracy of prediction of proposed models derived through DT, RF and MAA varied from 87.8% to >99.9%. High positive rate in the cross-validation run of DT, low out of bag (OOB) error of RF and high MCC values suggest satisfactory performance, robustness and high predictability of proposed models. Thus, proposed models offer vast potential for providing lead structures for the development of potent antibacterial agents for treatment of infectious diseases which have emerged as a global public-health problem.

References:
Molecular docking study of sulfonamide derivatives as DPP IV inhibitors

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Abstract: Type 2 diabetes (T2D) is a disease which affecting millions of people worldwide. Different treatments of T2D are available and now the inhibition of enzyme dipeptidyl peptidase-IV (DPP-IV) has come out as a promising treatment of T2D. In this research work we designed fifteen sulfonamide derivatives fused with pyrrolidine ring and subjected for molecular docking. Amongst all the designed derivatives, compound 07 and 09 showed good binding affinities in the active site of the enzyme DPP-IV.

Introduction:
Dipeptidyl peptidase IV (DPP-IV) is an extensively expressed enzyme occur in many tissues of mammals and present either as a soluble enzyme or in a membrane bound form. It is mainly found on the vascular endothelium, epithelial cells of kidney, liver, intestine, pancreas, lymphoid and myeloid cells and contributes to the extracellular matrix binding [1,2]. The serine protease DPP-IV quickly renders glucagon-like peptide 1 (GLP-1) inactive through cleavage of its N-terminal two amino acids. Inhibition of DPP-IV extend the half life of GLP-1 and thus, increased the levels of active endogenous GLP-1[3,4]. Hence, inhibition of DPP-IV is a promising new treatment for T2D. In the present investigation we designed a new series of sulfonamide derivatives fused with pyrrolidine ring and carried out molecular docking study with enzyme DPP-IV.

Materials and methods:
Designing of Compounds
All the compounds were designed by substituting different halogen group at R₁ position while R₂ position was substituted with different alkyl and substituted aromatic ring. 2D Structure of all the designed compounds were draw with the help of Chem Draw ultra version 8.0.3 and exported to window of Chem 3D ultra version 8.0.3. Energy of all the 3D structures were minimized through MOPAC up to RMS gradient 0.0001 and saved in MDL Mol file (.Mol) format.

Molecular docking study
The molecular docking studies of the designed compounds were carried out through Molegro Virtual Docker (MVD version 5) software. For the docking purpose protein model of DPP-IV was downloaded from protein data bank (PDB code; 3W2T) and designed compounds were docked in the
active site of the enzyme to evaluate their binding affinities. Then through an automatic procedure possible binding cavities were detected. During this process the maximum number of cavities was fixed to 5, the grid resolution was 0.80 Å, and probe size was 1.20 Å, while other parameters were set default. Then ligands (designed thiolactone derivatives) were imported in work space area, necessary bonds, bond orders, hybridizations, hydrogen atoms, charges were assigned and flexible torsions were detected in the ligands.

From the docking wizard ligands were selected and the scoring functions used in the docking were moldock score and rerank score. The search algorithm is taken as moldock SE and numbers of runs were taken 10 and maximum iterations were 1500 with population size 50 and the energy threshold for pose generation was 100.00 and similar poses were ignored. Similar poses were clustered depending upon an RMSD threshold of 1.00Å°. After docking simulation the generated poses were sorted on the basis of their moldock scores and rerank scores. The reranking score function is computationally more expensive than the scoring function used during the docking simulation but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand. Docking output of MVD as moldock score and rerank score (Kcal/mol)

Results and Discussion:

3W2T was the only structure of human DPP-IV co-crystallized with Vildagliptin in protein data bank and hence this 3D structure was selected for the molecular docking study. The binding score of compound 07 having fluoro and methyl group at R1 and R2 group respectively was found to be -146.30 (Mol dock score) and -132.56 (Rerank score) while the compound 09 possessed chloro and methyl group respectively at R1 and R2 group showed Mol dock score -169.36 and rerank score – 155.20 which comparable to that of standard drug of Vildagliptin (Moldock score -176.31; Rerank score -165.32). Other designed molecules also showed comparable binding affinities for the enzyme as compared to standard (Vildagliptin). The docking score are given in following table 1.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>R2</th>
<th>Moldock score</th>
<th>Rerank score</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Br</td>
<td>C2H5</td>
<td>-115.27</td>
<td>-105.24</td>
</tr>
<tr>
<td>02</td>
<td>F</td>
<td>C2H9</td>
<td>-121.23</td>
<td>-106.28</td>
</tr>
</tbody>
</table>
Conclusion:
In exploration of novel DPP-IV inhibitors, sulfonamide derivatives fused with pyrrolidine were designed and their binding affinities for the DPPIV were compared with standard drug Vildagliptin through molecular docking study. In the docking studies compound 07 and 09 showed encouraging results. The molecular docking discussed above may provide valuable information for further design and synthesis of compounds with enhanced DPP IV inhibitory activity.

References:
Terminalia catappa – a promising Anti-Arthritic plant

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Abstract: In vivo anti-inflammatory activity of selected safe dose (500 mg/kg p.o) of TCE - Terminalia catappa ethanolic extract and TCW - Terminalia catappa water extract was performed by Carrageenan induced paw edema and Complete Freund’s adjuvant (CFA) induced arthritis in Wister rats and was compared with Diclofenac sodium at the dose of 10 mg/kg. Results indicates that ethanolic extract has shown promising anti arthritic activity and comparable with Diclofenac sodium

Introduction:
Terminalia catappa Linn (T. catappa) [1], commonly known as Bengal Almond, belonging to the family Combretaceae, is reported to possess anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic and hepato-protective activities. However, there are no reports of the scientific work being carried out on this plant

Materials and Methods:
Acute inflammation [2] was induced in male Wister rats weighing between 180-200 g in 5 groups of 6 rats in each. Drug treatment was done by administering as detailed below
Group I Negative control
Group II Positive control
Group III Diclofenac sodium – 10 mg/kg p.o
Group IV TCE – 500 mg/kg p.o
Group V TCW – 500 mg/kg p.o
All the animals belonging to group II to V were given subcutaneously 0.1 ml of 1% solution of carrageenan in paraffin oil in the sub plantar region of the right hind paw and paw volume was measured at 0, 1, 3, 5 h using a digital plethysmometer. Results of the study is shown in Graph I (a)
Chronic inflammation [3]
Randomised CFA-induced arthritic rats were divided in to 5 groups with 6 animals in each group. Drug treatment was done orally from day 14 to 21 and as detailed below
Group I Negative control – 0.25% CMC
Group II Positive control – 0.25% CMC
Group III Diclofenac sodium – 10 mg/kg p.o
Group IV TCE – 500 mg/kg p.o
Group V TCW – 500 mg/kg p.o

Paw volume of injected (ipsilateral) rats was measured on day 14 and day 21. Percent increase in volume was calculated using the formula

\[
\text{Increase in paw volume} = \frac{A - B}{B} \times 100
\]

Where

- A – Paw volume on day 21
- B – Paw volume on day 14

Evaluation of severity of arthritis was done by recording arthritis score in the range of 0 to 4. Zero is least but definite swelling and 4 maximum swelling. The results of the study is indicated in Graph 1(b)

**Results and Discussion:**

In *In vivo* paw oedema model, the TCE reduced paw edema significantly than TCW suggesting its inhibitory effect on prostaglandins – mediated inflammatory pathway. CFA arthritis is thought to occur through cell-mediated auto-immunity by structural mimicry between mycobacterium and cartilage proteoglycans. TCE, the appearance of secondary lesions on rats after 7 days is the manifestation of cellular immunity. Reduction in the secondary lesions by ethanolic extract suggests its immune-modulatory activity and reveals potent suppression of cell-mediated immunity. Ethanolic extract also reduced arthritic score significantly compared to aqueous extract.

**Conclusion:**

Based on the present results, it was demonstrated that bark of *T. catappa* possess anti-inflammatory activity and TCE showing better activity when compared with that of TCW. Our studies have shown
that the bark extract of *T. catappa* possesses moderate anti-inflammatory and anti-arthritic activity with minimal toxicity and thus having a promising role in the treatment.

**Acknowledgement:**

Authors thank the authorities of Manipal University for providing necessary facilities to carry out this work

**References:**


HPTLC determination of Piperine in Balacaturbhadrika Churna

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Abstract: The present work aimed at the high performance thin layer chromatography (HPTLC) method for the analysis of Piperine content of In-house Balacaturbhadrikachurna (BC). Balacaturbhadrikachurna alcoholic extract (BCAL) was prepared by extracting 30 g of BC with ethanol using soxhlet extraction process. HPTLC was performed for BCAL using Piperine as the reference standard. The solvent system used was Acetone-Petroleum ether (3.5:6.5) as the mobile phase. The chromatographic system afforded a satisfactory R_f value of 0.57±0.01 for Piperine of the test extract which was comparable with the standard. The Piperine content of BCAL was found to be 0.48% w/w.

Introduction:
Balacaturbhadrikachurna (BC) is an ayurvedic preparation used in the treatment of atisara (diarrhea), chardi (emesis), kasa (cough), savsa (asthma), jvara (fever), balasosa (emaciation in children). The ingredients of churna are Musta (Cyperus rotundus), Pippali (Piper longum), Ativisa (Aconitum heterophyllum), and Sringi (Pistacia integerrima) in equal parts. The present work aimed at the high performance thin layer chromatography (HPTLC) method for the analysis of Piperine content of self-prepared In-house churna.

Materials and Methods:
Plant materials and marker compound: The crude drugs used in the In-house BC formulation were procured from the local market of Udupi, Karnataka, India, and authenticated by the botanist. The marker compound Piperine (purity 99%) was purchased from Sigma Aldrich, USA. All other chemicals used in the experiment were of analytical grade.

Preparation of churna: The In-house churna was prepared in three different batches according to The Ayurvedic Formulary of India [1]. All the ingredients were powdered and passed through the sieve #80 and mixed together in specified proportions to get uniformly blended churna.

Preparation of alcoholic extract: Balacaturbhadrika churna alcoholic extract (BCAL) was prepared by extracting 30 g of BC with ethanol using soxhlet extraction process. The ethanolic extract was evaporated to dryness and the dried extract (28.93%) was stored in desiccator until its use.
High performance thin layer chromatography (HPTLC): The standard Piperine solution (0.1 mg/ml) was prepared by dissolving Piperine in methanol. Extract sample solution was prepared by dissolving 100 mg of BCAL in methanol in a 10 ml volumetric flask. HPTLC was performed for BCAL using Piperine as the reference standard. The standard and test extract were spotted in triplicate on silica gel 60F254 HPTLC plates and developed with the solvent system Acetone-Petroleum ether (3.5:6.5) as the mobile phase[2]. After development, the plate was dried and scanned at 366 nm with Camag TLC Scanner-3.

Results and Discussion:
One of the best methods of standardizing herbs and herbal formulations is chromatographic techniques. HPTLC is the most frequently used technique for fingerprinting as well as quantifying the compounds with the help of a densitometer. Piperine has been achieved as the marker compound for the plant Pippali (Piper longum) [3]. Therefore, Piperine has been determined qualitatively and quantitatively in our experiment. The chromatographic system afforded a satisfactory Rf value of 0.57±0.01 for Piperine of the test extract which was comparable with the standard. The Piperine content of BCAL was found to be 0.48% w/w. Chromatograms of standard Piperine and BCAL are represented in Figure 1a and 1b respectively.

![Figure 1a](image1.png) Chromatogram of standard Piperine.  
![Figure 1b](image2.png) Chromatogram of BCAL.

Conclusion:
HPTLC was performed for the identification and quantification of Piperine in BCAL. The method used was found to be simple and accurate for the routine analysis. The results obtained can be used as reference while setting the pharmacopoeial standards for Balacaturbhadrikachurna to ensure the quality of the medicine.
References:


Tabernaemontana Divaricata linn. (r.br.) leaves extract shows spermicidal activity in Rats

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Abstract: The objective of the present study was to prepare and evaluate a potent, safe, effective and easily acceptable contraceptive containing herbal drug. Efforts were made to evaluate the contraceptive potential of the ethanolic extract from the leaves of Tabernaemontana divaricata and the herbal vaginal gel containing the extract. Spermicidal effect was evaluated in vitro using healthy human spermatozoa and in vivo in rats. In vitro results demonstrated that herbal vaginal gel is an effective spermicide. At a 5 mg/mL dose, complete immobilization of human spermatozoa was observed within 10 s. None of the treated animals conceived, indicating 100% contraceptive effect as compared to Gynol II, a nonoxynol-9-containing marketed formulation, which showed only 62% contraceptive effect in vivo.

Introduction:
The status of herbal medicine has been fast growing all over the world during the last few decades. The World Health Organization (WHO) has set up a Task Force on Plant Research for fertility regulation with an objective to find new orally active non-steroidal contraceptive compounds. In India, phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties [1]. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. The quest for oral contraceptive agents that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents [2] are available, these cannot be used continuously due to their severe side effects [3]. Hence, people are looking back to the age-old tradition of using herbal medicines, which have minimal side effects.

Materials and Methods:
Tabernaemontana divaricata (leaves) was collected from roadside area of Mandsaur District India and authenticated using references and authoritative books. The leaves were shade-dried and then powdered. The powder was then used for extraction using solvents (ethanol). Ethanolic extract was thus prepared by using a laboratory scale Soxhlet extraction apparatus.
Bull semen was collected from the healthy volunteer. The semen samples showed sperm count ranging from 80 to 110×10⁶/mL, motility of 60%–70%, morphology of 55%–60% and viability of 70%–80%. The rate of dilution was such that, a milliliter (ml) of the diluted semen was tested for percentage of progressively motile sperms, drop diluted semen was taken on the slide motility was checked on the stage of microscope. The ethanolic leaves extracts of Tabernaemontana divaricata (Linn.) at different concentrations (5.0%, 2.5%, 2.0%, 1.5%, 1.0%, 0.5%, and 0.1%) have been examined for spermicidal activity [4]. To all the test samples showing 80.23% arrest of sperm motility at the 10 s time point, 500 μL of buffered glucose saline was added and incubated at 37°C for 30 min. Sperm motility was observed by placing a drop of the mixture on a slide and viewing under phase contrast microscope (400X objective).

Sexually mature, 2-month-old male and female Wistar rats (180–220 g) were used in the present study. Six healthy female rats were used in each group. Vaginal smears were obtained from these animals to determine the stage of estrous cycle of the animals. A total of 0.3 mL of the herbal vaginal gel was taken in 1 mL syringe and administered into the vagina during the proestrus estrus transition phase. Control animals received the placebo gel and were compared with the marketed spermicidal formulation Gynol II containing nonoxynol-9 as the active ingredient [5]. The animals were observed for any vaginal leakage post administration of gels. The animals were allowed to mate with young proven fertile males (1:1). During cohabitation, mounting was seen, and mating was confirmed by the presence of spermatozoa in the vaginal lavage.

**Results and Discussion:**

The ethanolic extract from the leaves of Tabernaemontana divaricata was obtained as a brown-colored, odorless, thick viscous mass. The yield of the extract was found to be 17%–22% of the total weight of the initial leaves. The effect of the extract from the leaves of Tabernaemontana divaricata on sperm motility was found to be time and dose dependent. The concentration required for complete immobilization of human spermatozoa within 10 s was observed to be 5 mg/ mL. The motility could not be restored in the treated spermatozoa even after incubation in buffered glucose solution for 30 min (Table 1).

Herbal vaginal gel applied intra vaginally before mating during proestrus estrus resulted in complete arrest of sperm motility and none of these animals became pregnant.

The in vitro results clearly demonstrated that the ethanolic extract from the leaves of Tabernaemontana divaricata, as well as the herbal vaginal gel containing the ethanolic extract from the leaves of
Tabernaemontana divaricata, is an effective spermicide with a dose and time dependent effect. At a 5 mg/mL dose, complete immobilization of human spermatozoa was observed within 10 s.

**Table 1** Effect of Tabernaemontana divaricata leaves of ethanolic extract on sperm mortality  
Control percentage mortality - 84.54±6.25

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentrations (Mg/ml)</th>
<th>Mortality ethanolic extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5</td>
<td>80.23±6.15*</td>
</tr>
<tr>
<td>2.</td>
<td>2.5</td>
<td>65.21±5.75**</td>
</tr>
<tr>
<td>3.</td>
<td>2</td>
<td>62.78±4.82**</td>
</tr>
<tr>
<td>4.</td>
<td>1.5</td>
<td>59.44±5.95***</td>
</tr>
<tr>
<td>5.</td>
<td>1</td>
<td>58.93±4.48***</td>
</tr>
<tr>
<td>6.</td>
<td>0.5</td>
<td>56.78±5.32***</td>
</tr>
<tr>
<td>7.</td>
<td>0.1</td>
<td>55.58±5.3***</td>
</tr>
</tbody>
</table>

Values are given in Mean±Sem, *P<0.05, **P<0.01, ***P<0.001 compare to control.

**References:**

Comparative account of Astangavaleha

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Abstract: Astangavaleha is an Ayurvedic preparation belonging to class of formulations commonly known as Avaleha. Astangavaleha is widely used in cases like asthma, bronchitis, cough, dyspnea, vatakaphajvara. The recent study was performed for the preparation and evaluation of Astangavaleha. Standardisation of Ashtangavaleha has not been carried out yet and hence an attempt is made to formulate Ashtangavaleha & to standardize the same with specific relevant physicochemical parameters & compare it with marketed formulation.

Introduction:
Ayurvedic medicine is a system of Hindu traditional medicine. Ayurvedic compound formulations are mainly divided into two groups’ viz. (1) Kasthausadhi (predominantly plant drugs) and (2) Rasausadhhi (predominantly metals and minerals). There are several categories of Kasthausadhi formulations such as Asavarishta, Avleha, Churna, Taila etc. and of Rasausadh is such as Bhasma, Pisti, Lauha, Rasayana etc. Avaleha is the semi-solid dosage form, having long shelf-life in comparison to primary dosage forms, and can be administered to all the age groups. The basic material required for this dosage form includes Drava Dravya (liquid substance), Madhura Dravya (sweet substance), Prakshepa Dravya (condiments) and Kalka Dravya (paste of drugs). Diseases like Gulma, Kasa, Shwasa, Pandu, Shotha, Arsha, and Raktapitta, have been treated with different Avalehas.

Materials and Methods:
Collection of Plant Materials:
Various raw materials required for preparation of Astangavaleha namely Katphala, Pauskara,Sringi,Yamini, Karavi,Sunthi, Marica, Pippali, Madhu, Adraka were purchased from local Market from Mankarnika Ayurvedic Aushadhalaya, Pune in the month of October 2014 and Authentication was confirmed by Department of Pharmacognosy, Marathwada Mitra Mandal’s College of Pharmacy, Pune.

Method of Preparation:
All above raw materials used for preparation of Astangavaleha were washed with distilled water. Then it was properly dried & made into fine powder. All the powder ingredients were passed through sieve
number 85. These powders were separately weighed and added into the 250ml of beaker. The fresh juice of ginger was added in it and this mixture was kept in hot air oven. The dried mixture passed through grinder and mixed with sufficient quantity of honey.

**Results and Discussion:**

**Organoleptic Evaluation:** The Organoleptic evaluation of in house formulation and marketed formulation concluded that both the formulations were dark brown in color, having aromatic and pleasant odor, with slightly bitter and sweet taste.

**Physicochemical Description:** Physicochemical constants of the in house formulation were determined to be within a limit; the values were 1.00±0.15% for total ash, 0.76±0.05% for acid insoluble ash, 2.03±0.98% for water soluble ash and 1.21±1.5% for sulphated ash; which were within the range. Water soluble extractive value was found to be 2.9±0.05, alcohol soluble extractive value was found to be 2.3±0.05.

Table-1- Physicochemical constants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Result%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In house formulation</td>
</tr>
<tr>
<td>1.</td>
<td>Ash Value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Ash</td>
<td>1.00±0.15</td>
</tr>
<tr>
<td></td>
<td>Acid Insoluble Ash</td>
<td>0.76±0.05</td>
</tr>
<tr>
<td></td>
<td>Water Soluble Ash</td>
<td>2.03±0.98</td>
</tr>
<tr>
<td></td>
<td>Sulphated Ash</td>
<td>1.21±1.5</td>
</tr>
<tr>
<td>2.</td>
<td>Extractive Value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water Soluble</td>
<td>2.9±0.05</td>
</tr>
<tr>
<td></td>
<td>Alcohol Soluble</td>
<td>2.3±0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Moisture Content</td>
<td>0.17±0.45</td>
</tr>
</tbody>
</table>

**Determination of Rf value (Thin Layer Chromatography):**

The Thin Layer Chromatography of both in house formulation & marketed formulation were performed. Results of TLC study indicate that Rf values of in house formulation were found to be similar with standard Rf values reported in Ayurvedic Pharmacopoeia.
Conclusion:
From the present investigation various standardization parameters such as organoleptic standards, analytical parameters and physicochemical evaluations were carried out, it can be concluded that the in house formulation of Astangavaleha contains all good characters of an ideal avaleha and is easy to formulate. The study shows that the contents of the formulation are of good quality and purity.

References:
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Email: sunita_pharma2008@rediffmail.com

Abstract: For chemoprofiling of the bark, TLC were performed for preliminary identification of constituent in solvent system Toluene:Chloroform:Acetone (40:25:35 v/v/v), gallic acid was used as standard for phenolic compound. Phenolic compound was estimated in hydro-alcoholic extract of Saraca asoca Roxb. (stem bark) by high performance thin layer chromatography (HPTLC). Precoated silica gel 60 F254 (E. Merk) TLC plates were used as stationary phase and Toluene: Chloroform: Acetone (40:25:35 v/v/v) was used as mobile phase. Detection and quantification were performed by densitometry at λ 254 nm. The linear range was 100 ng to 700 ng. This HPTLC method was found to reproducible, accurate and precise.

Introduction:
Chromatographic techniques have been used for a long time in identification of herbal drugs or extracts and in assessment of single chemical entity drug substances. High performance thin layer chromatography (HPTLC) has preferred as a modern analytical tool for qualitative and quantitative estimation of marker compounds in herbal drugs because of its high throughput screening, sensitivity and reliability at nanogram level [1-2].

Ashoka is wonderful herb that claims to cure several diseases. According to Ayurvedic medicine, it is the one herb that stands out as especially useful for treating excessive uterine bleeding. It is useful in stimulating the uterus, the endometrium and the ovarian tissues, uterine bleeding associated with fibroids, leucorrhoea, menstrual disturbances without producing any side effects. Apart from this it is also useful for other ailments such as internal piles, diabetes, dyspepsia, indigestion, burning sensation, blood disorders, fractures, tumors, bites, ulcerations, and skin discoloration [3].

It mainly contains glycosidic principles, non-phenolic, sapogenetic glycoside, sterols and aliphatic alcohols. The bark yields alkanes, esters and primary alcohols. It gave n-octacosanol, tannin, catechin, (+)-catechol, (−)-epicatechin, (−)-epicatechol, leucocyanidin, leucopelargonidin, procyanidin derivatives, methyl-and ethylcholesterol derivatives. It also contains flavonoids and sterols [4].

Material and Methods:
The stem barks of *Saraca asoca* Roxb. were collected from rural areas of Udaipur (Raj.) and were dried at room temperature under a well ventilated shade by distributing them homogeneously. Drug sample was identified by Dr. S.S. Katewa, College of Science, Mohanlal Sukhadia University, Udaipur (Raj). *Saraca asoca* Roxb. De Wilde was authentified from Botanical Survey of India (BSI), Dehradun (UK), letter no. BSI/NRC/Tech (Indent)/2011/166 and acc no. is 113548.

The dried powdered of stem bark of *Saraca asoca* Roxb. was weighed and extracted with an appropriate solvent (Ethanol:Water-60:40) in a soxhlet apparatus. The extract was then filtered and dried in vacuum evaporator at 38°C. Phytochemical screening of hydro-alcoholic extract of *Saraca asoca* Roxb. showed the presence of various chemical constituents mainly alkaloids, flavonoids, tannins, phenolic compounds and steroids.

TLC of the hydro-alcoholic extract of *Saraca asoca* Roxb. performed on Silica gel GF 254 plates using Toluene: Chloroform: Acetone (40:25:35 v/v/v) solvent system. The spots were observed in UV chamber.

HPTLC was performed using the reference standard solution of gallic acid in methanol (100 ng to 700 ng.) using Silica gel 60 F₃₅ (E. Merck) as stationary phase and Toluene: Chloroform: Acetone (40:25:35 v/v/v) as mobile phase. Sample volume was kept 2 µl at temp.60°C, Migration Distance, 70 mm and detection wavelength, 254nm.

Linearity was performed by applying standard solution at different concentrations ranging from 100 ng to 700 ng on 20 × 20 cm HPTLC plates, precoated with silica gel 60 F₃₅ (E. Merck) in the form of sharp 8 mm bands. The plates were developed in a solvent system of Toluene: Chloroform: Acetone (40:25:35 v/v/v), up to a distance of 70 mm, at 60°C. The detector response for gallic acid was measured for each band at wavelength of 254 nm, using Camag TLC Scanner and win CAT software.

**Results:**

Phytochemical investigations (qualitative chemical analysis, TLC and HPTLC) were carried out with hydro-alcoholic extract of *Saraca asoca* Roxb. prepared by soxhlet extraction method. Qualitative chemical analysis and TLC determination showed the presence of several phytoconstituents like phenolic compounds, flavonoids, steroids, tannins, carbohydrates and the presence of phenolic compound was confirmed by HPTLC. (Tables 1 and 2)

**Table 1 HPTLC profile of standard gallic acid**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Rₜ Value</th>
<th>Conc.</th>
<th>Peak Height</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.54</td>
<td>200 ng</td>
<td>150.11</td>
<td>1614.40</td>
</tr>
</tbody>
</table>
Table 2 HPTLC profile of hydro-alcoholic extract of *Saraca asoca* Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>R_f Value</th>
<th>Peak Height</th>
<th>Peak Area</th>
<th>Quantitative Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.55</td>
<td>259.21</td>
<td>3247.10</td>
<td>350.06 ng</td>
</tr>
<tr>
<td>2.</td>
<td>0.56</td>
<td>400 ng</td>
<td>273.07</td>
<td>3339.71</td>
</tr>
<tr>
<td>3.</td>
<td>0.56</td>
<td>600 ng</td>
<td>150.20</td>
<td>1454.76</td>
</tr>
</tbody>
</table>

Conclusion:

In conclusion, the proposed HPTLC method was found to be precise, specific, accurate and robust and can be used for identification and quantitative determination of extracts of asoca plant and its formulations. HPTLC method is especially suitable for the fingerprinting and high throughput analysis of botanical samples and herbal formulations.

References:

Development and evaluation of selected Indian medicinal plant based Polyherbal Anti-aging cream

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Abstract: Current study aimed to prepare and evaluate a poly herbal cream containing standardized extracts of Aloe vera (A), Ocimum sanctum (O) and Punica granatum peel (P) at different ratios. Santalum album, Citrus aurantium, Olea europaea, Prunus amygdalus oils, Cetyl alcohol and Stearic acid were used in a ratio of 0.1:0.1:1.0:0.5:0.5 and 3.0 respectively, to formulate creams F1-F6. pH, viscosity, spreadabilty, extrudability, stability, anti-oxidant, anti-microbial and advanced glycation end product inhibition properties were evaluated. From the studies F4 with A: O: P (each 1.6% w/w) has shown significant anti-aging potential as a poly herbal anti aging cream.

Introduction:
In human body all the tissues are subjected for ageing as the human age goes on. Clinically significant and visible changes take place in the aged skin when the individual ages over 60 years. Skin, more than any other organ, is also subject to environmental influences which can lead to extrinsic ageing. One such environmental factor is chronic exposure to sunlight which results in phenotypic changes termed photo ageing—inevitably a combination of intrinsic ageing and photodamage.

By comparison with intrinsic ageing, photoaged skin is rough, dyspigmented and exhibits both fine and deep wrinkles. Aging as a complex of natural circumstance is exhibited by an augmentation in the chance of illness and finally death, due to cellular oxidative stress. Despite the strong efforts, no effective drug has been discovered yet for anti aging, this revisited importance of folk medicine with antioxidants.

Present study is aimed to prepare and evaluate a poly herbal cream containing standardized extracts of Indian folk medicinal plants Aloe vera (A), Ocimum sanctum (O) and Punica granatum peel (P) at different ratios. Santalum album, Citrus aurantium, Olea europaea, Prunus amygdalus oils as an anti-aging cosmeceutical.

Materials and Methods:
Water in Oil (W/O) emulsion-based cream (semisolid formulation) was formulated by using emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, *Santalum album*, *Citrus aurantium*, *Olea europa*, *Prunus amygdalus* oils) which were dissolved in the oil phase and heated to 75 °C. The preservatives and other water soluble components (methyl paraben, propyl paraben, triethanolamine, *Aloe vera*, *Ocimum sanctum* and *Punica granatum* peel were dissolved in the aqueous phase and heated to 75 °C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring.

The poly herbal cream containing standardized extracts of *Aloe vera* (A), *Ocimum sanctum* (O) and *Punica granatum* peel (P) at different ratios. *Santalum album*, *Citrus aurantium*, *Olea europa*, *Prunus amygdalus* oils, Cetyl alcohol and Stearic acid were used in a ratio of 0.1:0.1:1.0:0.5:0.5 and 3.0 respectively, along with preservatives to formulate a cream base for formulations F1 to F6. Six formulations were prepared by using 10, 20, 30, 40, 50 and 60 mg of each extract in formulations.

These formulations were evaluated for formulation parameters like pH, viscosity, spreadability, extrudability and stability. Further, formulations were also evaluated for anti-oxidant, anti-microbial and advanced glycation end product inhibition (AGE) properties. In addition, the formulations were tested in human volunteers to evaluate its compatibility and efficacy.

**Results and Discussion:**

The prepared anti aging creams F1-F6 were exhibited easy removal with plain water to give better compliance. Formulations F3-F6 has shown no phase separation upon storage. Formualtions F3 and F6 have featured lower spreadability and extrudability.

The order of anti-oxidant ability of the all formulations found as F6>F5>F4>F3>F2>F1. Formulations F4 and F5 shown comparable AGE inhibitory and anti-microbial effects to standards Aminoguanidine and Neomycin sulphate cream, respectively, however F4 shown higher AGE inhibitory effect vis-a-vis with F5.

In human volunteer’s study, the formulations F4 and F5 shows no redness, edema, inflammation and irritation during irritancy studies. The synergistic effects of formulation F4 containing A: O: P (each 1.6% w/w) has addressed hyperpigmentation, erythema, wrinkles and firmness of skin more significantly than F5.

**Conclusion:**
Present research work suggests that poly herbal anti-aging composition was found to be consistent in safety, efficacy and quality. Further formulation and qualitative parameters were found within the acceptable ranges. These findings indicate the significant synergistic anti-aging potential of formulation F4.

References
Abstract: Azadirachta indica belong to family Meliaceae and is an evergreen of the tropics and sub-tropics. This plant with an extensive and deep root system can grow in marginal and leached soils. Its uses antiseptic, ophthalmic, malaria, astringent and intermittent fevers, wounds diabetics insecticidal, demulcent, refrigerant appetizer. Cassia fistula L. belongs family Leguminosae and contains sennosides A and B, rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid, their ethyl esters and oxalic acid, sap, acetyl acid, iod, thiocyanogen and unsapon matter, tannins, phlobaphenes, reducing sugars and oxyanthraquinones. Eucalyptus leaves consists of the dried, mature leaves from older trees of Eucalyptus globulus [Fam. Myrtaceae]. In the present study, the aqueous leaves extracts of A. indica, C. fistula and E. globulus was investigated for its phytochemical constituents.

Introduction:
A. indica A. Juss., popularly known as “neem”, is a multipotential tree that originally belongs to South India and Myanmar and is cultivated on the southern coast of Iran. C. fistula is a fast-growing tree which reaches 30 to 40 feet in height and 30 to 40 feet wide The well-spaced branches are clothed with avis will drop from the tree for a short period of time and are quickly replaced by new leaves. Eucalyptus is a deciduous tree up to 40 m tall with silver-gray bark, which has scattered warts. [1-2].

Materials and Methods:
To study phytochemical constituents and physical parameters selected plant parts were collected from various sources e.g. A indica leaves, C. fistula leaves and E. globulus leaves from, Bhopal, during the month of Sep-Oct and were authenticated by the department of Pharmacy, SIRT-P. Bhopal. Herbarium no. SIRTPH-no.1A (A. indica, SIRTPH no. 2B (C. fistula), SIRTPH no.3C (E. globulus).

Preparation of Extracts: Plant parts were dried under shade, coarsely powdered using mechanical grinder and stored in airtight containers. Preparation of extract of various plant parts mentioned above was done by using water as solvent shown in table no.1. by maceration method.
Determination of Physical Parameters: Physical standards are to be determined, wherever possible. These are rarely constant for crude drugs, but may help in evaluation, specifically with reference to moisture content, density, foreign organic matter, crude fiber content, ash value, extractive value etc. [3-5]. Part used: Leaves of A. indica, Leaves of C. fistula & Leaves of E. globulus. Sample - Dry powdered samples of above mentioned plant parts were used. Month of experiment – November & December 2011.

Moisture content [3-5] - equipment used: Infra-red Moisture Balance. Procedure: Approximately 5 gm exactly weighed powdered samples were kept in IR moisture balance. The loss in wt. was recorded as percentage moisture with respect to air-dried sample of crude drug.


Preliminary phytochemical studies: Chemical tests are available for use in the examination of plant extract for the presence of a range of chemical groups. [3-5]. All extracts were mixed with water, before subjected to various chemical reagents.

General tests [3-5]

I. Test for detection of Steroids. II. Legal’s test. III. Test for detection of Alkaloids. IV. Test for Flavonoid. V. Test for Tannins: Ferric-chloride test. Potassium dichromate test Potassium permanganate test. VI. Test for Protein: Xanthoprotic test. VII. Test for carbohydrates.

Results and Discussion:
The aqueous leaves extracts of A. indica, C. fistula and E. globulus on phytochemical screening showed the presence of different chemical constituents. The results are shown in Table 2. Other physical parameters showed in Table 1.

Table 1 Physical parameters

<table>
<thead>
<tr>
<th>S.N o.</th>
<th>Plant</th>
<th>Part</th>
<th>Solvent</th>
<th>% Yield (w/w)</th>
<th>Moisture content (% w/w)</th>
<th>Total ash content (% w/w)</th>
<th>Acid insoluble ash content (% w/w)</th>
<th>Water soluble extractive value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. indica</td>
<td>Leaves</td>
<td>Water</td>
<td>18.7</td>
<td>12.3</td>
<td>9.3</td>
<td>1.0</td>
<td>24.0</td>
</tr>
<tr>
<td>2.</td>
<td>C. fistula</td>
<td>Leaves</td>
<td>Water</td>
<td>26.1</td>
<td>8.8</td>
<td>9.0</td>
<td>1.0</td>
<td>10.2</td>
</tr>
<tr>
<td>3.</td>
<td>E. globulus</td>
<td>Leaves</td>
<td>water</td>
<td>20.3</td>
<td>10.0</td>
<td>6.0</td>
<td>1.0</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Table 2 Phytochemical tests

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for steroids</td>
<td>Salkowski test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Test for Glycoside</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Legal’s test</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Test for alkaloids

<table>
<thead>
<tr>
<th>Method</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dragendorff’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hager’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Test for Flavonoid

<table>
<thead>
<tr>
<th>Method</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Test for Tannins

<table>
<thead>
<tr>
<th>Method</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dil KMnO₄ sol test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pot. Dichromate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Test for Protein

<table>
<thead>
<tr>
<th>Method</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthoproteic test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

### Test for carbohydrates

<table>
<thead>
<tr>
<th>Method</th>
<th>A. indica</th>
<th>C. fistula</th>
<th>E. globulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molish test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fehling test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- indicates the presence of the constituent.  - indicates the absence of the constituent.

### Conclusion:

It may be concluded from this study that the aqueous leaves extracts of *A. indica*, *C. fistula* and *E. globulus* on phytochemical screening showed the presence of different chemical constituents. These plants extracts screening can be carry out in further study.

### References:

Anti-hepatotoxic activity of fractions of Erythroxylum monogynum against alcohol induced damage

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Department of Pharmacognosy, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune
E-mail address: agnamdeo@gmail.com

Abstract: Erythroxylum monogynum (E. monogynum) has been traditionally used for liver diseases. However, no previous study investigated for antihepatotoxic against ethanol induced toxicity. In the present study, ethanol (3 ml/100 g/day) was administered for 21 days and fractions of leaves of methanolic extract given orally from day 15 to day 21 along with ethanol and blood collected from retroorbital plexus after 24 hours of last dose. Levels of biochemical markers along with histopathological changes were monitored to evaluate the extent of hepatotoxicity. A significant decrease in the biochemical parameters was evident by the hydroalcoholic fraction as compared to toxic group which is also confirmed by histopathological changes observed. The results of the present study indicate the significant antihepatotoxicity activity by the hydroalcoholic fraction of E. monogynum against alcohol induced toxicity.

Introduction:
Liver is the largest gland in the human body, performs various significant physiological activities like synthesis, metabolism, secretion and excretion of xenobiotics. In this process various free radicals are generated which are detoxified by endogenous antioxidants, but once endogenous antioxidants are saturated it will lead to injuries. Herbal drugs have gained importance in the treatment of such liver injuries as synthetic drugs cause severe side effects [1].

Erythroxylum monogynum Roxb (Erythroxylaceae) leaves are traditionally reported for liver disease [2]. The present study was aimed to prove the traditional usage of leaves scientifically by inducing alcohol damage to liver.

Materials and Methods:
Chemical and drugs: Silymarin (Yucca Enterprises, Mumbai, India). Assay kits for AST, ALT, ALP and TB (AccurexBiomedical Pvt. Ltd., Maharashtra, India). All other chemicals and reagents used were of analytical grade.
**Extraction and fractionation:** The authenticated dried leaves of the plant were powdered and extracted with methanol by cold maceration technique for 7 days with intermittent shaking. The obtained methanol extract (50g) was suspended in 1:1 ratio of methanol and water (500 ml) and fractionated successively with pet ether (3x 500 ml), chloroform (3x 500 ml) and the left over extract acts as hydroalcoholic extract. Yield of pet ether fraction PE-MEEM (12.4g), chloroform fraction CF-MEEM (7.2g) and hydro alcoholic fraction HA-MEEM (26.4g).

**Experimental protocol**

The screening of the fractions of MEEM for antihepatotoxic activity was performed according to the literature [3]. The rats were divided into nine groups of six animals each. Hepatotoxicity was induced by administering ethanol (3 ml/100 g/day in 2 divided doses) for 21 days in toxic group and corn oil (1 ml/100 g/day, in a single dose) orally for normal group. The administration of extracts PE-MEEM (100mg/kg), PE-MEEM (200mg/kg), CF-MEEM (100mg/kg), CF-MEEM (200mg/kg), HA-MEEM (100 mg/kg), HA-MEEM (200mg/kg) orally from day 15 to day 21 along with ethanol and standard group (silymarin 50mg/kg). Blood samples were collected from the retroorbital plexus are subjected to centrifugation at 2500 rpm for 15 min to separate serum which is used for analysis of various biochemical parameters.

**Results:**

**Hepatoprotective Evaluation:**

**Table 1** Estimation of rat’s biochemical profile of fractions of MEEM on ethanol intoxicated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>TB (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184.45 ± 8.49</td>
<td>145.39 ± 9.69</td>
<td>59.95 ± 3.12</td>
<td>0.30 ± 0.09</td>
</tr>
<tr>
<td>Toxic group</td>
<td>330.57 ± 7.84</td>
<td>319.69 ± 9.48</td>
<td>145.34 ± 7.30</td>
<td>2.38 ± 0.05</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg)</td>
<td>249.07 ± 8.85(^a)</td>
<td>235.78 ± 8.57(^a)</td>
<td>73.13 ± 6.32(^a)</td>
<td>0.58 ± 0.08(^a)</td>
</tr>
<tr>
<td>PE-MEEM (100 mg/kg)</td>
<td>285.16 ± 10.48(^m)</td>
<td>282.19 ± 10.89(^m)</td>
<td>121.35 ± 5.23(^m)</td>
<td>1.85 ± 0.09(^m)</td>
</tr>
<tr>
<td>PE-MEEM (200 mg/kg)</td>
<td>272.10 ± 5.48(^c)</td>
<td>275.08 ± 7.89(^c)</td>
<td>111.56 ± 6.34(^c)</td>
<td>1.80 ± 0.07(^c)</td>
</tr>
<tr>
<td>CF-MEEM (100 mg/kg)</td>
<td>260.14 ± 6.12(^b)</td>
<td>262.40 ± 8.17(^b)</td>
<td>100.14 ± 5.30(^b)</td>
<td>1.60 ± 0.03(^b)</td>
</tr>
<tr>
<td>CF-MEEM (200 mg/kg)</td>
<td>255.45 ± 7.30(^b)</td>
<td>255.50 ± 11.87(^b)</td>
<td>95.15 ± 2.23(^b)</td>
<td>1.58 ± 0.06(^b)</td>
</tr>
<tr>
<td>HA-MEEM (100 mg/kg)</td>
<td>250.12 ± 4.16(^a)</td>
<td>245.17 ± 8.10(^a)</td>
<td>91.17 ± 1.13(^a)</td>
<td>1.18 ± 0.05(^a)</td>
</tr>
<tr>
<td>HA-MEEM (200 mg/kg)</td>
<td>245.08 ± 3.19(^a)</td>
<td>240.09 ± 5.98(^a)</td>
<td>89.12 ± 1.30(^a)</td>
<td>1.01 ± 0.06(^a)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6)

\(^a\)p<0.001 compared to paracetamol intoxicated group, \(^b\)p< 0.01 compared to paracetamol intoxicated group, \(^c\)p< 0.05 and ns > using one way ANOVA followed by Bonferroni’s multiple comparison test.
Figure 1: Histopathological changes occurred in rats during ethanol intoxication and prevention by the treatment with fractions of methanolic extract fractions of leaves of *E. monogynum*.

**Conclusion:**
The hydroalcoholic extract of leaves of methanolic extract of *E. monogynum* showed significant antihepatotoxic activity as evident from biochemical and histopathological finding. Therefore, the hydroalcoholic extract should be considered for possible clinical application in the treatment of liver diseases.

**Acknowledgement:**
The grants received to one of the author, Sabeena Hussain Syed from University Grants Commission (UGC) for this project under Maulana Azad National Fellowship (MANF) Scheme.F1-17.1/2010/MANF-MUS-AND-4007/ (SA-III/Website) has been duly acknowledged.

**References:**
Aristolochia indica leaves as an Anthelmintic

Abstract: Aristolochia indica L. (Family: Aristolochiaceae) has long been used in Indian subcontinent as traditional system of medicine. Most of the diseases like cholera, fever, bowel troubles, ulcers, leprosy, skin diseases, menstrual problems and snakebites can be treated using these leaves. Aristolochia indica (Linn) also called Iswarmul is a rare endangered medicinal plant of India. The present research work was aimed to investigate the anthelmintic activity of the aqueous, ether and ethanol extract of Aristolochia indica. The anthelmintic activity was evaluated on adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal parasites of human being.

Introduction:
Aristolochia indica linn. (Family: Aristolochiaceae). World Health Organization (WHO) has listed 21,000 plant species used around the world for medicinal purpose. In India about 2,500 plants species, belongs to more than 1000 genera are being used in the indigenous system of medicine. India is tenth plant rich countries among the world and fourth among the Asian countries. Different diseases can be cured using Aristolochia indica linn. plant. It is also used in case of snake bite as the leaf of the plant & roots have specific antidote against the cobra poisoning. It has also stimulant property, intermittent fever etc. effect of A. indica. Aristolochia Species refers to several members of genus Aristolochia indica (Indian Birthwort) is a perennial climber with greenish white woody stems found very rarely in India. The leaves are glabrous and very variable, usually obviate –oblong to sub-pandurate entire with somewhat undulate margins somewhat cordate acuminate. Leaves are simple, alternate, entire, with undulate margins, acute; flowers greenish white, in axillary cymes; fruits rounded oblong. The term anthelmintic is frequently restricted to drugs acting locally to expel parasites from the GI tract. However, there are several types of worms that penetrate other tissues; drugs that act on these parasitic infections are also known as anthelmintics. Further more, drugs that kill worms are refer to commonly as vermicides; those that effects the worms in such a manner that peristaltic activity or catharsis expels it from the intestinal tract are referred to as vermifuges. Therefore, the anthelmintics are defined more properly as drugs used to combat any type of helminthasis. The worm parasits of man belong to two phyla L: Nemathelminthes (round worms ) and platy helminthes (flat worms), The round worms include tha hook worms, whip worm, pin worm, strogylodes steroralis, Trichinella spiralis, and Wuchereria bancrofti [1,2,3].
Materials and Methods:
This work deals with the methods utilized to perform different experiments to investigate the anthelmintic activity.

Procurement of plant material: Aristolochia indica linn. is collected from local area of Daund dist., Pune (Maharashtra).

Identification and authentication: The plant was identified & authenticated at Botanical Survey of India (BSI) Office Pune by Dr. V.K. Rawat, (Scientist “C” & H.O.D) Department of Botany.

Phytochemical studies:

Extraction of drugs: The plant materials were dried and powdered. The obtained powder was weighed and then used for extraction. Then aqueous, Ether, Ethanol extracts of Aristolochia indica Linn was prepared. The extract were weighed and stored in amber colored airtight bottles.

Anthelmintic activity: The anthelmintic activity was performed on adult Indian earthworm Pheritima posthuma as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Pheritima posthuma worms are easily available and used as a suitable model for screening anthelmintic drugs. In the 50 ml of formulation containing different concentration of Aqueous, Ether and Ethanol extract (50,100mg/ml in saline water), standard using Albendazole (20mg/ml) and normal saline as control were prepared and approximately equal size five earthworms were released in each group. Observations were made for the time taken to paralysis or death of individual worms. Paralysis was said to occur when the worms do not revive or stimulate even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color [5].

Results and Discussion:
The Anthelmintic activity on earth worm by using Aristolochia indica linn. leaf was observed and identified.

Pharmacognostical studies:
Effect of Ether, Ethanol and Aqueous extracts of leaf of Aristolochia indica linn on earth worms
Experimental data shows that, the aqueous, ether and ethanol extracts of Aristolochia indica linn. was found the anthelmintic activity in dose dependent manner as shown in table no 2.
Adaptation of locally available herb or herbal product in the treatment of human aliment has great advantages as far as cost of treatment concern. Hence the present study planned to investigates utility of locally and abundantly available plant that is Aristolochia indica linn. for the treatment helminthes infection. Albendazol acts by increasing chloride ion conductance of worms muscle membrane that produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.
The aqueous, ether and ethanol extracts of *Aristolochia indica linn*. Not only showed paralysis but also caused the death of worms at different concentration. The result indicates that as the concentration of the extracts increases anthelmintic activity also increases, and decreases time required for paralysis and death. By observing the reports the Ether extract shows fast action against earth worm as compared with Aqueous and Ethanol extract of *Aristolochia indica linn*.

**Table 1** Preparation of extract of Leaf of *A. indica* Linn. by maceration has yielded.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>Colour &amp; Consistency</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ether</td>
<td>Brown and waxy</td>
<td>2.5%</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>Brown and waxy</td>
<td>4.75%</td>
</tr>
<tr>
<td>3</td>
<td>Water</td>
<td>Brown and Waxy</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

**Table 2** Anthelmintic activity of aqueous, ether, and ethanol leaf extract of *A. indica* linn

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Concentration (mg/ml)</th>
<th>Time taken for paralysis (min.)</th>
<th>Time taken for Death (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>20</td>
<td>15.44 ± 0.14</td>
<td>19.48 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>42.54 ± 0.17</td>
<td>50.43 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>48.11 ± 0.00</td>
<td>56.11 ± 0.13</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>50</td>
<td>38.30 ± 0.13</td>
<td>44.24 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>21.44 ± 0.05</td>
<td>29.34 ± 0.07</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>50</td>
<td>20.22 ± 0.04**</td>
<td>24.38 ± 0.06**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18.34 ± 0.05**</td>
<td>22.10 ± 0.09**</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett’s test n=5 in each group

**Conclusion:**

The present study has shown that, the Aqueous, Ether and Ethanol extract of *Aristolochia indica linn*. Have been confirmed to show anthelmintic activity. By observing the reports it may conclude that the Ether extract shows fast action against earth worm as compared with Aqueous and Ethanol extract of *Aristolochia indica linn*. Further detail information regarding identification of the possible chemical constituent responsible for anthelmintic potential need to be carried out in future.

**References:**

Studying the Testosterone lowering potential of *Vitex negundo* (nirgundi) seed extract in hyperandrogenic female rats induced with Polycystic ovaries

Funde Snehal S, Shetty Divya S, Patil Swati S
K.M.Kundnani College of Pharmacy, Cuffe Parade, Mumbai-5, India.
E-mail address: funde.snehal@gmail.com

Abstract: Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder characterised by high testosterone levels, chronic anovulation & metabolic disturbances. This study was conducted to assess testosterone lowering potential of *Vitex negundo* L (Nirgundi) in hyperandrogenised female Sprague Dawley rats induced with PCOS. Histopathology reports showed least follicular cysts & lesions being found in nirgundi group. Nirgundi has promising potential as an anti-androgen from this research study.

Introduction:
Polycystic ovarian syndrome (PCOS) is a condition characterized by accumulation of numerous cysts on the ovaries associated with high male hormone levels, chronic an ovulation and numerous metabolic disturbances. The goal when using nirgundi is to reduce circulating androgens, optimize ovarian functions and support optimal endocrine function. This research study aims at exploring the testosterone lowering potential of nirgundi in comparison to conventional medicines in order to provide an insight into how herbal medicines could contribute to the future of treating disorder such as PCOS.

Materials and methods:

Materials: *Vitex negundo* Linn. seeds, Test drug: Hydro-alcoholic extract of *Vitex negundo* seeds, Standard drug: Finast 5mg, Hormone drug: Aquaviron Testosterone 25mg/ml suspension.

Animal Model:
Group I: Served to be Normal group receiving only animal feed and water orally, Group II : Received 0.1 ml Aquaviron testosterone suspension intramuscularly, Group III : Received testosterone suspension intramuscularly & hydroalcoholic nirgundi extract 400 mg/kg orally, Group IV : Received testosterone suspension intramuscularly & Standard drug, Finasteride 20 mg/kg suspended in water orally.

Methods: *Vitex negundo* seeds were collected, processed and subjected for extraction using ethanol and water (70:30) as solvent. The study employed nirgundi seed extract for evaluating its testosterone lowering potential which was conducted on 24 female Sprague Dawley rats for one month. Finasteride, a Type II 5α-reductase inhibitor used to lower testosterone levels in PCOS women was used as standard
The experiment was carried out as per CPCSEA guidelines. Nirgundi seed extract was administered in a suspension form. Testosterone suspension was given intra-muscularly to animals in Groups II, III, IV prior to administering the plant drugs as discussed hereafter. After a gap of 6 days i.e on Day 7, animals belonging to Groups II, III, IV were again administered the same dose of Aquaviron and were kept under observation. During the 6 day gap, animals were observed for any signs of hypertension, since exogenously given androgens can cause hypertension. After another gap of 7 days, blood was withdrawn and collected from one animal in each group via the retro-orbital plexus and used for serum testosterone testing using Radio Immuno Assay technique (RIA). Vaginal smears were obtained. Once the excessive Testosterone levels in blood were confirmed it was essential to confirm PCOS like condition at ovarian level. This can be verified by considering the nature of estrous cycle in female rats. Characteristics of each phase were studied from the vaginal smears obtained.

**Results and discussion:**

**Serum testosterone analysis**

![Figure 1](image-url) Serum testosterone levels before and after one month.

The section of ovary of animals from Normal group showed normal Follicles and corpus luteas. In group 2 there were increased primordial follicles, hemorrhage and follicular cysts indicative of induction of PCO. In group 4 the severity was less as compared to group 2 and well comparable to group 3.

**Table 1** Histopathological studies

<table>
<thead>
<tr>
<th>Groups</th>
<th>Microscopic Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>N1: Normal follicles, Corpus Lutea Normal</td>
</tr>
<tr>
<td></td>
<td>N2: Normal follicles, Corpus Lutea Normal</td>
</tr>
<tr>
<td>Group 2</td>
<td>Testosterone</td>
</tr>
<tr>
<td></td>
<td>T1: Increased number of primordial follicles (2+), follicular cysts (3+), Hemorrhage (2+)</td>
</tr>
<tr>
<td></td>
<td>T2: follicular cysts (3+), Hemorrhage (2+)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Standard (Finastat)</td>
</tr>
<tr>
<td></td>
<td>S1: Follicular Cysts (+), Primordial follicles (+)</td>
</tr>
<tr>
<td></td>
<td>S2: Follicular Cysts (+)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Nirgundi</td>
</tr>
<tr>
<td></td>
<td>V1: Follicular Cysts (+), Primordial follicles (+)</td>
</tr>
<tr>
<td></td>
<td>V2: Follicular Cysts (+), Hemorrhage (+)</td>
</tr>
</tbody>
</table>
Hydroalcoholic extract of seeds of *Vitex negundo* has potential of lowering excessive androgen levels when ingested on daily basis. Also it may possess activity at ovarian levels, as was derived from the histopathological studies. The probable mechanism of action for antiandrogenic action of the extract may be due to the inhibition of 5α-reductase.

**Table 2** Determination of Serum Glucose, Total Cholesterol, HDL Cholesterol and Triglycerides.

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Glucose (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>106.92 ± 10.24</td>
<td>36.33 ± 6.83</td>
<td>24.70 ± 1.32</td>
<td>147.62 ± 17.87</td>
</tr>
<tr>
<td>Nirgundi</td>
<td>63.70 ± 5.26a</td>
<td>38.52 ± 3.56b</td>
<td>36.69 ± 1.39b</td>
<td>91.27 ± 17.32a</td>
</tr>
<tr>
<td>Testosterone</td>
<td>159.18 ± 8.88a</td>
<td>55.79 ± 1.39a</td>
<td>23.16 ± 1.54a</td>
<td>231.88 ± 39.31c</td>
</tr>
<tr>
<td>Standard</td>
<td>67.46 ± 3.61a</td>
<td>31.47 ± 0.18a</td>
<td>32.86 ± 1.98a</td>
<td>78.74 ± 37.65a</td>
</tr>
</tbody>
</table>

Significant at *p* <0.001 , Significant at *p* <0.01 , Significant at *p* <0.05 , ns: not significant.

Along with reducing androgen levels in hyperandrogenised female rats, *Vitex negundo* was also found to balance blood glucose, total Cholesterol and triglycerides levels and also increase HDL Cholesterol level at the same time.

**Conclusion:**

*Vitex negundo* has anti-androgenic potential which may be due to the presence of phytosterols and flavonoids. It also showed considerable potential in balancing metabolic parameters. Also it may possess activity at ovarian levels.

**REFERENCES**

HPLC chemical fingerprinting of ethanol fraction of *Plumbago zeylanica*

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**Abstract:** India has an ancient heritage of traditional medicine. Ayurveda is accepted to be the oldest treatise on medical system. In Ayurvedic formulations different parts of the plants are used. Although it is admitted that the herbal medicines are having lesser side effects than other system of medicines but the herbal formulations and ayurvedic formulations required standardization in order to optimize their stability and efficacy so that they can have safe use. For standardization of plants and their formulation the most effective, rapid and economic method is the chromatographic techniques. The present were carried out on standardization and HPLC fingerprinting analysis of the roots of *Plumbago zeylanica* which is of immense medicinal values. In this context we observed various parameters of their standardization and developed their TLC and HPLC profile.

**Introduction:**

*Plumbago zeylanica* (Chitraka) consists of dried mature root of *Plumbago zeylanica* Linn. (Fam. Plumbaginaceae), a large perennial sub-scandent shrub, found throughout India in wild state and occasionally cultivated in gardens. It has a number of properties like antimicrobial, hyperglycemia, hypolipidemic, and antiatherosclerotic [1]. Herbal drugs are used in all over the world since ancient times especially in China, Japan and India. Because of their high pharmacological action, low toxicities and less side effects herbal and ayurvedic drugs have attracted great interests. But in context of the standardization most of the herbal drugs and ayurvedic formulation are untouched and in order to specify their quality and efficacy they need standardization. For this purpose there are various analytical methods but the most commonly used methods for the standardization of herbal drugs are chromatography and chromatographic fingerprinting techniques.

Chromatographic techniques are dynamic processes wherein a mobile phase transports the sample mixtures across or through the stationary phase medium. Chitrakadi Churna is an important formulation mentioned in ayurvedic formulary of India used for G.I. disorders *P. zeylanica* is a essential constituent of the formulation [2].

**Materials and Methods:**

**Herbs, Chemicals and reagents:** Crude drugs were procured from local market and identification was confirmed by macroscopic and microscopic characters in the department of pharmacognosy. All the herbs
procured from the local market all the chemicals and solvents were used of AR grade; Standard Plumbagin (98% pure) was procured. Methanol (MeOH) and ethyl acetate was procured from Merck and used as a mobile phase.

**Methods:** Experiments were performed on a HPLC system Shimadzu-10AT, binary gradient equipped with detector Shimadzu UV -VIS SPD-10 A Vp, software Spinchrom, Chennai. The separations were performed by using methanol: ethyl acetate (85:15%v/v) mobile phase with flow rate 1.0 ml min⁻¹. Detector was set at 280 nm, attenuation adjusted.

**Preparation of the formulations:** Plumbago zeylanica, three laboratory batches (named CVL-I, CVL-II and CVL III) were prepared in the institutional laboratory. The available commercially brand CVM-A, CVM-B and CVM-C of Plumbago zeylanica was procured from local Pharmacy.

**Preparation of reference solution of Plumbagin:** Accurately weighed Plumbagin (10 mg) was transferred to 100 ml volumetric flask and dissolved in and diluted to 100 ml with ethanol. The final solution contained 100 µg of the Plumbagin per ml of the solution.

**Standard plot of Plumbagin:** Serial dilutions containing 2-10µg/ml Plumbagin in ethanol were prepared from a stock solution of Plumbagin (10 mg/100ml). Each dilution was chromatographed on HPLC and area under the peak of Plumbagin recorded (Table I). Retention time of Plumbagin was observed to be 2.810 min. A standard curve of Plumbagin was prepared by plotting the actual amount of Plumbagin present in 10 ml of the dilutions against the area under the peaks of Plumbagin observed by injecting above serial dilutions. The intercept and the slope of the standard plot were observed to be 44.04 and 37.804, respectively, with coefficient of correlation as 0.998 (R²). The amount of Plumbagin was calculated in the test material using the regression equation.

**Results and Discussion:**

**Optimization of HPLC Condition:** For better separation in the chromatograms, mobile phase composition on chromatographic separation was investigated and found there was a sharp peak obtained by methanol: acetic acid as compared to methanol alone so the mobile phase selected as methanol: ethyl acetate (85:15%v/v) wavelength for constituents in the formulation was selected by the UV.

There are some different peaks obtained and different wavelength but peaks were neither well in shape nor well separated. Therefore, 280 nm was selected as detection wavelength for Plumbagin.

**Method validation of quantitative analysis:** The method was validated in terms of linearity (2-10µg/ml), limits of detection and quantification (0.002846 mg/ml and 0.009823 mg/ml), precision (0.784%), repeatability (0.6907%) and recovery (99.5 %) test.
Figure 1 RP-HPLC Chromatogram of standard Plumbagin at 280 nm.

Table 1 Plumbagin content determined by HPLC

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plumbagin content [content ± SD; n=3]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contents (mg/g)</td>
</tr>
<tr>
<td>CVL-I</td>
<td>44.2</td>
</tr>
<tr>
<td>CVL-II</td>
<td>44.2</td>
</tr>
<tr>
<td>CVL III</td>
<td>44.27</td>
</tr>
<tr>
<td>CV-A</td>
<td>39.8</td>
</tr>
<tr>
<td>CVM-B</td>
<td>39.66</td>
</tr>
<tr>
<td>CVM-C</td>
<td>38.6</td>
</tr>
<tr>
<td>P. zeylanica</td>
<td>52.7</td>
</tr>
</tbody>
</table>

Conclusion:
The developed HPLC method, allows rapid, simple, precise, and quantitative estimation. The method is successful in providing quantitative differentiation of Plumbago zeylanica raw material and its formulations, and in rapidly detecting inconsistencies in the product. This method can potentially differentiate herbs or complex samples based on their chromatographic profiles. It can also determine samples. Therefore, it is a potentially useful tool in ensuring the quality and safety of herbal products containing Plumbagin.

Acknowledgement:
The authors acknowledge the Chhattisgarh science and technology Raipur [F. no. Ednt – 1922/CCOST/MRP/2012], Raipur, INDIA, for financial support.

References:
Chemo-genetic fingerprinting analysis of *Bridelia retusa*

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Abstract: The aim of the present work was to study the molecular and phytochemical methods for correct identification *Bridelia retusa* (*B. retusa*). In our current study, High performance thin layer chromatography (HPTLC) and Restricted fragment length polymorphism (RFLP) technique was employed to develop fingerprinting of *B. retusa*. The HPTLC method was found to give compact spots for Rf = 0.62, ellagic acid; Rf = 0.84, quercetin. RFLP analysis of the rbcL gene was developed to discriminate *B. retusa* from others. Only the 590-bp PCR product from *B. retusa* could be digested with restricted enzyme (Ssspl) into two fragments of 452 and 138-bp.

Introduction:
Molecular techniques play an increasingly important role in the management and utilization of plant genetic resources. Gene based coding of medicinal plants has become an important international intellectual property rights issue along with tracking biodiversity. Development and enforcement of quality control standards have become crucial for regulatory authorities and industries due to global interest in botanicals as drug and dietary supplements. In assuring quality of botanical drugs correct identification and characterization of plant species and plant part is important. Both, molecular phylogenetic studies and studies of the secondary metabolite profile of higher plants have received considerable attention in recent years. Molecular biological methods have been used for DNA analysis of medicinal plants, including randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and sequence characterized amplified region (SCAR), multiplex amplification refractory mutation system (ARMS), and polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) [1]. *Bridelia retusa* Spreng. Syn: Bridelia airyshawii (Family-Euphorbiaceae) found growing throughout hotter parts of India. Phyto-chemical study of different parts of Bridelia retusa have revealed the presence of sesqui-terpenes, tri-terpenoids, flavonoids and phenolic compounds. The fruit pulp and seeds contains gallic acid, quercetin, ellagic acid and β-sitosterol [2].

Based on our literature survey the present investigation was aimed to develop an efficient protocol to develop the genetic and chromatography fingerprint using PCR-RFLP and HPTLC of *B. retusa*.

Materials and Methods:
Collection and Preparation of extract: The plant *B. retusa* was collected from forest, managed by Government of Chhattisgarh State Forest Division in November, 2013. A voucher specimen (CNH/Tech.II/2014/70/139) was submitted to the Central National Herbarium, Howrah, India. Shade dried *B. retusa* fruits was powdered (10 gm) and extracted using buchi speed extractor with 90% methanol. Yield of the extract was 4.2%.

Chromatographic analysis

Preparation of standard and sample solution: Stock solution of 1000µg/mL-200 µg/mL of ellagic acid and quercetin standard was prepared and was serially diluted. Ellagic acids and quercetin were extracted by hydrolysis of 10 g of the sample with 30 mL of methanol, refluxed for 1 hour. Extract obtained was filtered using Whatman filter paper No 42. 10 mL of distilled water was added to the filtrate and evaporated to a volume of 10 mL [3].

Chromatography method: The standard and four different sample solutions were applied to TLC plates as 8.0 mm band with 9.0 mm space between two band using a Camag Linnomat IV sample applicator. The plate were developed with a mobile phase of Hexane: ethyl acetate: formic acid: methanol (5:4:1:0.5) in a TLC twin trough chamber previously saturated with the solvent for 30 minutes and quantification of the standards and samples were performed by mean of Camag TLC scanner III controlled by WinCATS 1.4.3 version software at 225 nm.

Validation of the method: Linearity, Precision and Accuracy [4] are determined over the concentration range of 200-800 ng/spot.

DNA extraction: Modified CTAB method [5] was used for DNA isolation using mixture of (chloroform: Isoamyl alcohol) followed several sequential steps. RNase A (10 – 15 ml; 50 mg/mL) was added into the DNA samples and incubated at 37°C for 1 h to degrade RNA.

Restriction digestion of the *rbcL* gene: Aliquots of 10 µL of PCR purified products were digested with SsspI ( Genie, Bangalore, India ) at 60° C for 2 h in a total volume of 20 µL. PCR products were subjected to agarose gel (1.5% [w/v]) electrophoresis in 0.5X TAE buffer, along with DNA markers (Genie, Bangalore, India) as size markers. DNA was stained with Ethidium Bromide and photographed under UV light. The sequences were verified by BLAST search

Results and Discussion:
The development of solvent system for chromatography (HPTLC) analysis was carried out by using different solvents in hit and trial method. A wavelength of 225 nm was chosen for quantification. The Rf value of Ellagic acid and Quercetin after development with the mobile phase Hexane: ethyl acetate: formic acid: methanol (5:4.5:1.5:0.5, v/v/v / v) was 0.62 and 0.84 respectively. When the concentrations of Ellagic acid and Quercetin and their respective peak areas were subjected to regression analysis by
least squares method. A good linear relationship ($r^2 = 0.998; 0.992$) was observed between the concentrations of Ellagic acid and Quercetin and the respective peak areas in the range 200 - 800 ng / spot. The regression of Ellagic acid and Quercetin was found to be $Y= 6.537X+ 1624$ and $Y= 4.823X+ 1611$ respectively, where ‘Y’ is the peak area and ‘X’ is the concentration of Ellagic acid and quercetin respectively. PCR-RFLP of *B. retusa* with the Ssp1 restriction enzyme, specifically digest species specific polymorphic site found in rbcL region. The partial rbcL PCR product (590 bp partially amplified) of *B. retusa* was digested with Ssp1 and resulted into two fragments of 452 and 138 bp (figure 1)

**Conclusion:**

Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary. A method based on PCR-RFLP and HPTLC is describing here to establish genetic and chemical quality control of selected plant. Here we could suggest that chemogenetic fingerprinting technique is convenient for authentication of *B. retusa* and its adulterant in the local herbal markets.

**Acknowledgement:**

Author would like to grateful acknowledge to the Director, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh for providing research facilities.

**References**


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In vitro antioxidant activity of Solanum xanthocarpum fruit

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Abstract: To evaluate the in-vitro free radical scavenging activity of fruits of Solanum xanthocarpum. Petroleum ether, ethanol, aqueous extracts of Solanum xanthocarpum were prepared, with successive extraction in soxhlet apparatus. Each extracts were selected to study the free radical scavenging activity by superoxide scavenging assay method. Ethanolic extract of Solanum xanthocarpum has showed 69.2±0.41% inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (51.3±0.18% compared to ethanolic extract), while petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. This investigation revealed that the Solanum xanthocarpum contains pharmacologically active substances such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the superoxide scavenging activity.

Introduction
Solanum xanthocarpum (Solanaceae) has been reported in the ayurvedic system of medicine for the treatment of fever, cough, asthma and costiveness [1]. It has also been reported that its powder has showed marked effect in the treatment of bronchial asthma [2]. In the present investigation ethanolic extract of fruits of Solanum xanthocarpum has been evaluated for its effect on free radical scavenging activity for there antioxidant property. Literature reveals that, the carbonyl groups are responsible for free radical scavenging activity. Free radicals are atoms or groups of atoms with an odd number of electrons and can formed when oxygen interacts with certain molecules. To prevent free radical damage, the body has a defense system of antioxidants. Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene alteration leading to cancer. Medicinal plants have attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines. In recent years, these have been a great interest in herbal remedies for the treatment of number of ailments. Plants are promising source of drugs. In continuation of search in potential free radical scavenging agents, the present investigation was aimed to determine free radical scavenging activity of Solanum xanthocarpum fruits. Free radical scavenging properties help in strengthening the immune system of the body, which helps to overcome cancer.
Materials and Methods
Petroleum ether, ethanolic and aqueous extracts were screened for anti-oxidant activity using superoxide free radical scavenging activity in dose and time dependent manner [3]. The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains 50 mM phosphate buffer, pH 7.6, 20µg riboflavin, 12 mM EDTA, 0.1 mg/3 ml NBT, added in that sequence. The reaction was started by illumination the reaction mixture with different concentrations (5-100 µg/ml) of samples for 15, 30 and 45 min. immediately after illumination the absorbance was measured at 590 nm. Ascorbic acid was used as standard drug.

Results and Discussion:
Ethanol extract of Solanum xanthocarpum had showed 69.2±0.41% inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (51.3±0.18%), while Petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed time dependent inhibition of superoxide scavenging activity. The results are reported in figure 1.

![Figure 1](image_url)  
**Figure 1** Anti-oxidant activity of petroleum ether, ethanolic and aqueous extracts using superoxide free radical scavenging activity.

Conclusion:
The results explore the potential of antioxidant activity of Solanum xanthocarpum plant against free radical scavenging and DPPH model. Ethanolic extract shows the maximum antioxidant activity compare to standard drug in both the model. The present studies clearly indicated that plant Solanum xanthocarpun plays an important role in maintenance of free radicals generate into the body.
References:


Effect of Pre-sowing pulsed magnetic exposure in secondary metabolite content of *Allium sativum*

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Abstract: Present study has been drawn to understand the relationship between plant secondary metabolite content and pre-sowing pulsed magnetic exposure. In the present study, garlic seed material exposed at different pulsed magnetic frequencies like T1(0.1Hz), T2(1Hz), T3(10Hz), T4(100Hz) and T5(untreated sample) for 15 days at 1500nT magnetic field intensity. After the treatment all the samples has been cultivated and after its cultivation period samples collected from the field for analysis. Analysis of alliin content in all the samples performed through HPTLC. Results of the analysis shown that there is no significant impact of pulsed magnetic exposure on *Allium sativum* with reference to their alliin content.

Introduction:
Garlic has been utilized as, both food and medicine, for more than 5000 years. Thousands of studies have been published showing the beneficial effects of garlic and garlic powders. Most of the marketed garlic powders are standardized on the basis of its alliin content [1].

There are so many studies revealed that pulsed magnetic exposure to the plant seed material, produces high impact on growth and yield of plant material but not so much work has been carried out on its secondary metabolite content. The purpose of this study was, therefore, to determine if pulsed magnetic field affect the growth and yield of the plant, could also be used for increasing the plant secondary metabolite content like alliin in garlic. HPTLC method used for this study because this method is suitable for the analysis of plant secondary metabolite content [2].

Materials and Methods:
The present investigation was carried out with pulsed magnetic field exposure enclosure unit on garlic (*Allium sativum* L.) at different frequency T1(0.1Hz), T2(1Hz), T3(10Hz), T4(100Hz) and T5 (untreated samples) 5hr per day for 15days@1500nT field intensity in Nanjanad farm, Institute of Commercial Horticulture, TamilNadu Agricultural University, Udthagamandalam, The Nilgiris. After the treatment garlic seed materials has been cultivated in the field under the control of T.A.U. Udthagamandalam, The Nilgiris.
After its cultivation period samples has been collected from the field and analysis done by HPTLC (Camag) through the alliin marker ≥90% which was purchased from (Fluka).

**Quantification of alliin in fresh raw samples of garlic by HPTLC [3,4,5].**

**Sample preparation** - Accurately weighed 500 mg fresh garlic clove sample (T1,T2,T3,T4,T5) crushed in methanol at room temperature and kept for 15 minutes for sonication. The pooled extracts were transferred separately to 50 ml volumetric flask to obtain sample solution of 10 mg/ml.

**Standard preparation** - Accurately weighed 5 mg of standard alliin of purity ≥90%w/w was dissolved in methanol-distilled water (7+3, v/v) in a 10 ml volumetric flask. From this stock solution 2 ml was transferred into a 10 ml volumetric flask to obtain standard solution of 100 µg/ml alliin. Stationary phase-Pre-coated silica gel F\textsubscript{254} TLC aluminum plates. (250 µm thickness, E, Merk), Mobile phase-n-Butanol-glacial acetic acid water-water (6:2:2 v/v), Sparying reagent-Ninhydrin reagent were used.

**Procedure:** Applied Specific (20µl) quantity of the samples and standard solution were applied with the help of Camag Linomat IV applicator (Camag, Switzerland) as bands on the TLC plate. The standard and samples, in the form band of 6 mm, were spotted 15 mm from the bottom, 12 mm from the left margin of the plate and 6 mm (for alliin) at a constant application rate using nitrogen aspirator. Then the plate was developed with mobile phase up to about 7.5 cm. and the developing time was 1h. The developed chromo-plate was dried by hair dryer and then the bands were detected by spraying the plate through ninhydrin reagent, followed by heating at 110°C for 5-10 minutes in a hot air oven. Densitometry scanning was performed on Camag TLC scanner III at 540nm and operated by win CATS Planar Chromatography version 1.4.3. The source of radiation utilized was deuterium lamp. Evaluation was done via peak area of standard and sample. Alliin was calculated in fresh samples by comparing the peak areas of sample (T1,T2,T3,T4,T5) and standard spots.

**Results and Discussion:**

Alliin content in different samples of fresh raw garlic clove were quantified. The results are given Table number 1. The results indicated that the untreated control sample has highest amount of alliin as compared to pulsed magnetic treated samples. The amount of alliin with respect to treated samples are in the sequence T5>T3>T4>T2>T1.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples and standard ID (20 µl)</th>
<th>Rf value</th>
<th>Peak area</th>
<th>Amount detected in sample %w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alliin standard</td>
<td>0.30</td>
<td>6001.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>T1(0.1Hz)</td>
<td>0.30</td>
<td>1408.2</td>
<td>0.2346 %</td>
</tr>
<tr>
<td>3</td>
<td>T2(1Hz)</td>
<td>0.30</td>
<td>1750.2</td>
<td>0.29165 %</td>
</tr>
<tr>
<td>4</td>
<td>T3(10Hz)</td>
<td>0.30</td>
<td>2272.1</td>
<td>0.37862 %</td>
</tr>
<tr>
<td>5</td>
<td>T4(100Hz)</td>
<td>0.30</td>
<td>2059.3</td>
<td>0.34315 %</td>
</tr>
<tr>
<td>6</td>
<td>T5(untreated sample)</td>
<td>0.30</td>
<td>2852.2</td>
<td>0.47528 %</td>
</tr>
</tbody>
</table>

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Figure 1 Overlay of all alliin track and Standard alliin track at 540nm through Camag HPTLC

Conclusion:
The objective of present study was to develop a possible method for increasing the content of secondary metabolite of garlic by applying pulsed magnetic exposure on plant seed material. However, pulsed magnetic field exposure on garlic seed material did not produce any significant change in its alliin content, could be observed. Relationship between pulsed magnetic field treatment on plant seed materials and secondary metabolite content seems to depend, on the nature of the plant.

References:
Pharmacognostic and Pharmacological evaluation of the alcoholic extract of pericarp of 
*Albezia lebbeck*

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**Abstract:** *Albizia lebbeck* benth, known as *Shirisha in Ayurveda* is an important medicinal plant belonging to the family Fabaceae (Formerly Leguminosae) and a member of subfamily Mimosaceae. The plant is useful in many disease conditions and is known for its anti-inflammatory, anti-histaminic, anti-anaphylactic, anti-asthmatic and anti-microbial activities. The present study deals with morphology, microscopic studies, preliminary physico-chemical and phytochemical analysis of pericarp of Shirisha along with its pharmacological activity. Preliminary analysis for the presence of various functional groups revealed the presence of carbohydrates, phenols, tannins, flavonoid and proteins. The ethanolic extract was further evaluated for its analgesic and antipyretic activity.

**Introduction:**

*Albezia lebbeck* commonly known as shiris is widely used in Ayurvedic medicines for the cure of various diseases. *Albezia lebbeck* is member of Fabaceae family. The family of around 730 genera and 19,500 species. Member of the Fabaceae can be found in most all parts of globe. The genus Albezia belongs to subfamily Mimosoideae, whose distribution is concentrated within tropical and temperature regions. The plant is found throughout India, Bangladesh, ascending to 900m in the Himalaya and Andmans [1] A. *lebbeck* is used in Indian folk medicine to treat several inflammatory pathologies such as asthma, arthritis and burns [2,3]. Mature pods remain on the tree for long period and are available till May-July. There is a need for documentation of research work carried out on traditional medicines; hence it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. Hence this work attempts to bring out the pharmacognostic and pharmacological study of the pericarp of *Albezia lebbeck*.

**Materials and Methods:**

The dried pods of *Albezia lebbeck* were collected from Purander Taluka near Pune and authenticated from Agarkar Research Institute Pune. The pericarp of the pods was studied for macroscopic and microscopic characters. The seeds were separated from the pods and the pericarp was powdered. It was then subjected to extraction process in Soxhlet extractor using various solvents like Petroleum ether,
Chloroform and methanol. It was then subjected to chemical evaluation along with analgesic and antipyretic activity.

Results and Discussion:

A macroscopic character of pod was done by naked eye observations like shape, colour, taste and odour. Microscopic study showed the presence of epicarp, mesocarp and endocarp. The powder microscopy showed warty trichomes, lignified fibers, tannin containing cells and prismatic crystals. Powder was then subjected to extraction process in Soxhlet extracter using various solvents. It was then subjected to chemical evaluation which showed the presence of carbohydrates, proteins, amino acids, saponins and flavonoids.

Table 1 Chemical test of the extract

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chemical Tests</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids ( Dragendorff’s test )</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates ( Molisch test )</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Proteins ( Biuret’s test )</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Amino acids ( Ninhydrin test )</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins ( Foam test )</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids ( Salkowski test )</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Tanins ( Ferric chloride test )</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2 Physicochemical evaluation of extract

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Observation (% w/w )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash value</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash value</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol soluble extractive value</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble extractive value</td>
<td>36.0</td>
</tr>
<tr>
<td>5</td>
<td>Crude fibre contains</td>
<td>1.63</td>
</tr>
<tr>
<td>6</td>
<td>Loss on drying</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Table 3 Analgesic activity of the ethanolic extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment (gm/kg)</th>
<th>Reaction time on hot plate zero time</th>
<th>Reaction time on hot plate 90 min after administration</th>
<th>% Increase in pain threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloroform, 1</td>
<td>7 ± 0.7</td>
<td>8 ± 0.9</td>
<td>14.2 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol, 1</td>
<td>7 ± 0.2</td>
<td>8.8 ± 0.7</td>
<td>25.7 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous, 1</td>
<td>6.8 ± 0.2</td>
<td>6.6 ± 0.7</td>
<td>Not active</td>
</tr>
<tr>
<td>4</td>
<td>Aspirin 0.2</td>
<td>5.7 ± 0.2</td>
<td>9.6 ± 0.9</td>
<td>68.4 ± 8</td>
</tr>
</tbody>
</table>

Antipyretic activity of various extracts showed significant decrease in temperature. Maximum decrease of 8\(^\circ\) was observed for ethanolic extract.
Table 4 Antipyretic activity of various extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Basal body temperature</th>
<th>Temperature after 90 min</th>
<th>Decrease in temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloroform (1 gm/kg)</td>
<td>36.5</td>
<td>34.2</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>Ehanol (1 gm/kg)</td>
<td>36.4</td>
<td>28.2</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous (1 gm/kg)</td>
<td>36.6</td>
<td>33.3</td>
<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>Aspirin (200 mg/kg)</td>
<td>37</td>
<td>33.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Conclusion:
The pericarp of *Albezia lebbeck* was studied for its morphological microscopical and physicochemical evaluation. After screening the various extracts for analgesic and antipyretic effects the ethanolic extract showed maximum activity.

Acknowledgement:
We are very much thankful to Sinhgad Technical education Society’s Smt Kashibai Navale College of pharmacy Kondhwa and APTI for giving us opportunity for this presentation.

References:
Preparation and evaluation of Vasadi kasaya

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Abstract: Kasaya is a method of extraction by boiling of dissolved chemicals from herbal or plant material. Medicinal plant parts obtained from Azadirachta indica, Adhatoda vasica, Picrorhiza kurroa, Swertia chirata and Tinospora cordifolia, in a given proportion were boiled for three hours and about 300 ml of the decoction were prepared and evaluated. Pharmacognostic studies on each drug and powder characteristics were performed. Various physicochemical parameters such as water-soluble extractive, alcohol-soluble extractive, loss on drying and total ash values were determined. The present formulation was investigated for pH, total solid content; limits of microbial count of species Escherichia coli and detection of specific species.

Introduction:
Ayurvedic medicine is a prehistoric system of health care which is indigenous to the Indian subcontinent. These preparations are widely acceptable due to their efficacy and desirable features. They are generally prepared by soaking the drug, either in powdered form or in the form of decoction (Kasaya), for a specified period of time. In modern systems of medicine these herbal formulations should undergo for quality assessment in order to justify their acceptability. The major drawbacks of herbal drug industry are the unavailability of inflexible quality control profiles for herbal drug and their preparations. It is obligatory to perform microbiological limit test to make sure whether the herbal drug and their formulations is free from risk. The existing herbal formulations in the market are usually not accurately standardized and are not assessed for their quality. The demand for herbal formulations is increasing tremendously; therefore it becomes an urgent need for pharmacists and physicians to have knowledge about the safety and efficacy of these preparations. [1, 2].

Materials and Methods:
1. Materials: Medicinal plant parts of Azadirachta indica, Adhatoda vasica, Picrorhiza kurroa, Swertia chirata and Tinospora cordifolia were locally collected. All samples were stored, washed, dried and fine powdered in a mixer.
2. Methods [1, 2, 3, 4]:

zhou
Pharmacognostic study: The each drug was studied for pharmacognostic study and powder characteristics were performed.

Preparation of decoction: Take 4 gram of each powdered drug & add sufficient amount of water (320ml). Concentrate the solution to 40-50ml. Filter and concentrate solution. Collect the filtrate & evaluate.

Physical and Physiochemical evaluations: Physical evaluations like pH, alcohol content, total solid content, water and alcohol soluble extractive values were determined as per the method prescribed in the Indian Pharmacopoeia.

Microbial evaluations by pour plate method [5]:

1 ml of sample as described in described in pre-treatment of sample

15 ml of casein soyabeans digest agar (medium 2) 15 ml of Sabouraud’s dextrose agar (medium 3)

Temperature: NMT 45°C Temperature: NMT 45°C

Empty petridish (Sterile) solidify agar Empty petridish (Sterile) solidify agar

Incubate 30°C- 35°C for 3 days Incubate 30°C- 35°C for 3 days

Calculate total aerobic count (TAC) Calculate total fungal count (TFC)

Test for Escherichia coli: Ten grams of the sample was dissolved in 100 mL of buffered lactose broth by shaking in a jar. 0.1% w/v of polysorbate 80 was added to prepare the suspension of poorly wettable substances. 1.0 milliliter of the preparation was transferred into container and to it 50 mL of nutrient broth added. The mixture was then shaken and allowed to stand for 1 h and then shake again. The cap was loosened and the jar was incubated at 37°C for 24 h. The dishes were tested for the presence of acid and gas.

Results and Discussion:
The pharmacognostic study of each drug and powder characteristics was studied. Physical and physiochemical parameters such as water soluble extractive, alcohol soluble extractive, loss on drying and ash values were determined using standard pharmacopoeial methods. Vasadi kasaya formulation was
evaluated for pH, solid content and microbial count. The pH of formulation was found to be 3-4 [Acidic]. The solid content formulation was found to be 25 %w/w. The total number colonies were found to be 164 IU. Standard limit = 100-250 IU. Decoctions from medicinal plants had been used for the treatment of hepatotoxicity.

Conclusion:
The present investigation found microbial count by pour plate method that was within the acceptable limits in Vasadi kasaya formulations. Stringent regulation has crop up to determine the consistency of these new formulations to determine their physicochemical, pharmacological, clinical activities besides with their safety and efficacy studies.

Acknowledgement:
Authors are thankful to Founder President Prof. M. N. Navale, S.T.E.S for encouragement and constant support throughout study & Dr. S. D. Sawant, Principal, Smt. Kashibai Navale College of Pharmacy, Kondhwa (Bk), Pune for permitting for the poster presentation.

Reference(s):
Pharmacological assessment of *Acacia nilotica* L. effect on sexual behavior of Male Rats

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Abstract: The aim of present study was to investigate the potential of ethanolic extracts of *Acacia nilotica* L. on male rat sexual behavior and its effects on androgenic hormones. Ethanolic extracts of *A. nilotica* stem part at the dose of 250 and 500mg/kg body weight were administered in male rats. Mount frequency (MF), intromission frequency (IF), ejaculatory frequency (EF), mount latency (ML), intromission latency (IL), ejaculatory latency (EL) and post-ejaculatory interval (PEI) were the parameters observed during the study. Results observed from the study revealed that alcoholic extract at 500mg/kg bw, significantly enhanced the sexual behavior as evident from increased MF, IF, EF and reduced ML, IL, EL and PEI. The results indicated that ethanolic extract of *A. nilotica* have a potential to enhance sexual performance of male rats.

Introduction:

*Acacia nilotica* is one of the most widely used medicinal plants in Indian system of medicine and traditionally used as antiplasmodial, anti-inflammatory, analgesic and antipyretic. It has been reported to have antidiabetic, antioxidant and considerable inhibitory effects against HIV-1 protease.

In traditional system of medicines, *Acacia nilotica* is often recommended as male sexual performance enhancing agent. However, convincing scientific data to support the aforesaid claim is lacking. Thus in the absence of any scientific evidence, an attempt was made to investigate the effect of *Acacia nilotica* extract on sexual behavior of male rats.

Materials and Methods:

**Preparation of extracts:** The dried bark of *Acacia nilotica* was reduced to moderately coarse powder using mechanical grinder and subjected for extraction with 95% v/v alcohol in a soxhlet extractor. The extract was filtered while hot and the filtrate was distilled under reduced pressure in order to remove solvent completely. The residue was dried and used for subsequent experiments.

**Experimental animals:** Adults, healthy albino rats (male and female) of Wister strain weighing 150-200g were used during the study. The animals were housed individually, maintained under standard conditions (12 hr light and 12 hr dark cycle, 25 ± 3°C, 35-60% relative humidity), the animals were fed with
standard rat pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental design.

**Preparation of the dose:** The suspension of alcoholic extracts was prepared by using tween 80 (0.5%) in normal saline, for oral use.

**Mating behaviour analysis:** Mating behaviour test was carried out according to method given by Chouhan *et al.*, 2008. In mating behaviour test effect of alcoholic extracts was evaluated in male rats, by analyzing and comparing Mount frequency (MF), intromission frequency (IF), ejaculatory frequency (EF), mount latency (ML), intromission latency (IL), ejaculatory latency (EL) and post-ejaculatory interval (PEI). For mating analysis female rats were brought to oestrus by sequential administration of suspension of ethinyl oestradiol orally at the dose of 100 μg/animal and progesterone 1 mg/animal, through subcutaneous injections, 48 hr before the experiment.

**Experimental animals were divided in four groups and each group consisted of six rats.**

Group 1 Control, received vehicle, group 2 Standard, received Testosterone Propionate (2 mg/day), group 3, 4 received ethanolic extract suspension (250 and 500mg/kg bw). Dosing frequency was once in a day for 28 days. At 0th, 14th and 28th day, after 30 min of dosing, oestrus female was introduced into respective cages of the male animal with 1 female to 1 male ratio and observed for mating performance and results were recorded and statistically analyzed.

**Statistical Analysis:** All the observed values were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad InStat. All values are expressed as mean ± SEM, n = 6; ²p<0.001 and ³p<0.01 as compared to control.

**Results and Discussion:**

Results of mating behavior at 0th, 14th and 28th day are statistically analysed, compared and summarized in Figure 1A-1G. Results observed from the study revealed that that alcoholic extract at 500mg/kg bw, significantly enhanced the sexual behavior as evident from increased MF, IF, EF and reduced ML, IL, EL and PEI. MF, IF and EF are useful indices of vigour, libido and potency. Increase in MF reflects sexual motivation and increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated. Therefore the increase in MF and IF suggests enhanced mating behavior of male rats. Mount latency, intromission latency and ejaculatory latency are indicators of sexual motivation. ML, IL and EL are inversely proportional to sexual motivation, and PEI is considered an index of potency, libido and the rate of recovery from exhaustion after first series of mating. Therefore the reduction in ML, IL, EL and PEI inferred that alcoholic extract at 500mg/kg bw of possess maximum potential to stimulate sexual behavior in male rats.
Conclusion:
The present study leads us to conclude that alcoholic extract of *A. nilotica* stem exhibited significant potential to enhance sexual performance in male rats. However, the field is further open to identify the active phytoconstituent and possible mechanism of action.

References:
Evaluation and comparative screening of antihyperglycemic activity of some Indian medicinal plants

Sapna Malviya, R V Sheorey, Javed Khan Pathan, Narendra Vyas, Anil Kharia
Modern Institute of Pharmaceutical Sciences, Indore
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Abstract: The present evaluation was undertaken to evaluate hydroalcoholic extracts of Ocimum canum (Lamiaceae) (HAOC), Acacia nilotica (Mimosaceae) (HAAN) and Catharanthus roseus (Apocynaceae) (HACR) leaves through oral glucose tolerance and hypoglycemic activities. Oral glucose tolerance test (OGTT) was tested in glucose loaded rats and hypoglycemic activity in fasted normal rats at same dose level of 200 mg kg\(^{-1}\) respectively. Glimepride 0.1 mg kg\(^{-1}\) was used as the reference drug for both the activities. Preliminary phytochemical screening of leaf extracts of three plants revealed the presence of carbohydrates, tannins, flavonoids, steriods, alkaloids and terpenoids. Results clearly demonstrated that the hydroalcoholic extracts of every plant tested, exhibited significant blood-glucose lowering potential in OGTT and hypoglycaemic activity with minimum toxicity. The study explains the traditional use of plant extracts in lowering the level of glucose in blood.

Introduction:
India is the diabetes capital of the world with 41 million diabetic patients; every fifth diabetic in the world is an Indian. The prevalence of diabetes for all age groups world wide was estimated to be 2.8% in 2000 and 4.4% in 2030. Diabetes mellitus is a chronic metabolic disorder as ancient as human race and its incidence is considered to be high all over the world characterized by hyperglycemia, glycosuria, negative nitrogen balance sometime ketonemia [1].

Materials and Methods:
Plant material: The plant OC, AN, CR were collected from the Medicinal Garden of Modern Institute of Pharmaceutical Sciences, Indore and authentified by Head of department of Botany, Holkar Science College, Indore, M.P. The Voucher Specimen was deposited for future reference.
Preparation of hydroalcoholic extracts: The collected plants were dried and powdered. The powdered material (500 gm) was defatted with petroleum ether and extracted with hydroalcohol (50:50) for 72 hrs in soxhlet apparatus. The extract was evaporated under reduced pressure to obtain solid mass, Percentage yield of concentrated hydroalcoholic extract of leaves was calculated as HAOC (13.47%), HOCR...
Preliminary phytochemical screening of leaf extracts of three plants revealed the presence of carbohydrates, tannins, flavonoids, steriods, alkaloids and terpenoids.

**Animals:** Healthy adult wistar albino rats (150-200 g) and swiss albino mice (25-30 g) of either sex were used. The animals were stabilized for 1 week, housed in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 30°C, 35–60 % relative humidity). The normoglycemic animals were selected for this experiment having the fasting blood glucose level around 80 mg/dl.

**Drugs and Chemicals Used:** Glibenclamide was procured from Aventis Pharma, Mumbai, India. All other chemicals were obtained local sources and were of analytical grade.

**Methods:**

**Acute oral toxicity studies:** Acute toxicity was performed according to the fixed dose procedure of OECD Guideline No. 420. The suspension of alcoholic and aqueous extract of various parts in tween 80 was administrated orally to overnight fasted swiss albino mice at two different doses 1000 and 2000 mg/kg b.w. respectively. The animals were observed continuously for the initial 4hrs for behavioral changes and mortality and intermittently for the next 6 h and then again at 24 h and 48 h after dosing for a total of 14 days[2].

**Oral glucose tolerance test:** Overnight fasting normal rats were divided into ten groups of five animals per group (n=6). Group I served as control received Tween 80. Group II served as standard group and received Glimepride (0.1 mg kg-1p.o.) suspended in vehicle, Group 3-5 received oral 200 mg kg-1 dose of hydroalcoholic extract of OC, AN and CR All animals were loaded with glucose (1.5 g kg-1, p.o.), 30 min after the extract and drug administration. Blood glucose was determined just prior to glucose administration (0h) and 1, 2, 3 and 6h after glucose administration. Blood glucose concentration was estimated by the glucose oxidase enzymatic method, using Accu-check Active TM Test strips [3].

**Hypoglycemic activity:** Experimental animals were divided into five groups of six animals per group (n = 6). Group I served as a control received vehicle Tween 80. Group II served as standard group received Glimepride (0.1 mg kg-1p.o.) suspended in vehicle and Group 3-6 received oral 200 mg kg-1 dose of hydroalcoholic extract of OC, AN and CR The blood glucose level of all experimental rats was determined at 0 hr (before oral administration), by snipping tail with surgically sterilized needle and then after 1, 2, 3, 4 and upto 6 h respectively [4].

**Statistical analysis:** The values are expressed as Mean±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet’s test. A value of p<0.001 was used as a criterion of significance

**Results and Discussions:**The changes in blood glucose level of different plant OC, AN and CR Linn extract, glibenclamide and vehicle treated albino rats after oral administration of glucose (1.5 mg kg-1)
are summarized in Figure 1 and Figure 2. The blood glucose levels of normoglycemic rats acquired significant hyperglycemia after oral administration of glucose and gradually decrease to the pre-glucose load level. Glibenclamide decreased blood glucose level of animals even below the normal values in 3rd and 6th hours. In glucose-hyperglycemic rats, hydroalcoholic extract of three extracts of leaves at dose of 200 mg kg-1 induced a potent reduction in glycaemia with maximum fall of (62.41%), (60.21%) and (59.21%). Hydroalcoholic extract of OC, AN and CR leaves extracts transiently decreases basal glucose 49.18%, 47.20%, 46.32% after 6 hr of oral administration.

**Figure 1** Effect of hydroalcoholic extract of OC, AN and CR on oral glucose tolerance test in glucose loaded hyperglycemic rats

![Figure 1](image1)

**Figure 2** Effect of hydroalcoholic extract of OC, AN and CR on hypoglycemic activity in normoglycemic rats

![Figure 2](image2)

References:
Effect of plant growth regulators on suspension culture of Adhatoda Vasica Nees

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2Hi-Tech College of Pharmacy, Hi-Tech Medical College and Hospital campus, Bhubneswar, Odisha, 751025
3PG Department of Pharmacology, M.K.C.G. Medical College, Brahmapur, GM, Odisha.
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Abstract: Suspension culture is one of common technique use in plant tissue culture for production of secondary metabolites. In the present study the callus was established using Adhatoda vasica cells and then used to prepare suspension culture. two auxin and one cytokine used to manipulate the media and the effect was studies in terms of cell growth. Secondary metabolite production was analyzed by HPLC.

Introduction:
Plant-produced secondary compounds have been incorporated into a wide range of commercial and industrial applications. Manipulation of the auxin to cytokinin ratio in the medium can lead to the development of shoots, roots, or somatic embryos from which whole plants can subsequently be produced. [1,2]. Adhatoda vasica is a well-known plant drug in Ayurvedic and Unani medicine. Adhatoda leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders. [3,4,5]. Vasicine and vasicinone is major alkaloid responsible for pharmacological activities.

Materials and Methods:
For the preparation of callus culture studies Murashige and Skoog medium (MS medium) and Gamborg B5 were selected. In the present study three plant growth regulators were selected in which one belongs to auxin class of plant growth regulator i.e. 2,4-Dichlorophenoxyacetic acid (2,4-D) and Indole 3 acetic acid (IAA) and kinetin (kin.), a kind of cytokinin, was selected. For raising callus, Auxiliary leaves explants from Adhatoda vasica were collected and washed under running tap water to remove dirt traces, surface sterilized with 0.1% mercuric chloride for 4-5 minutes, and then cut in to 1-2 mm² small pieces. The cut segments were then cultured individually on MS and B5 medium containing different concentration of 2, 4-D, Kin. and IAA. The developed callus was weighed, and homogenized with tissue homogenizer, under aseptic conditions. After weighing the concentrated cell
mass, selected plant tissue culture media was added in the cell suspension in this manner to get 1gm fresh weight of cells /ml.

The production media was collected from cell culture flasks at every 7-day interval for the estimation of alkaloid, and several other studies were perform i.e. cell growth, cell viability etc. Cell growth was measured in terms of fresh weight.

**Table 1:** Fresh weights Study in Suspension culture

<table>
<thead>
<tr>
<th>Time in Days</th>
<th>Fresh weight (gm) / 10 ml of suspension culture in MS Media Supplemented with</th>
<th>Fresh weight (gm) / 10 ml of suspension culture in B5 Media Supplemented with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 ppm 2,4-D 1 ppm Kin. 0.5 ppm IAA</td>
<td>2 ppm 2,4-D 1 ppm Kin. 0.5 ppm IAA</td>
</tr>
<tr>
<td>0</td>
<td>0.45</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>0.81</td>
<td>0.65</td>
</tr>
<tr>
<td>14</td>
<td>1.68</td>
<td>1.23</td>
</tr>
<tr>
<td>21</td>
<td>1.80</td>
<td>1.31</td>
</tr>
</tbody>
</table>

**Quantitative estimation of Vasicine (By HPLC)**

The Quantitative estimation of Vasicine, the major bioactive constituent in culture medium was done by High Performance Liquid Chromatography (HPLC) method given in Indian Herbal Pharmacopoeia, 1998.

**Table 2:** Vasicine content in suspension cell culture.

<table>
<thead>
<tr>
<th>Time in Days</th>
<th>Vasicine content (µg /ml / gm fresh weight) of <em>A. vasica</em> cells in suspension culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS medium supplemented</td>
</tr>
<tr>
<td></td>
<td>2,4-D (1 ppm) + Kinetin (1.0 ppm) + IAA(0.5 ppm)</td>
</tr>
<tr>
<td>Initial day</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>0.48 ± 0.11</td>
</tr>
<tr>
<td>14</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>21</td>
<td>0.67 ± 0.08</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE (n=3)

**Results and Discussion:**
The above studies with various combinations of plant growth regulators are tabulated, and it was observed that the combination of 1.0 ppm 2,4-D, 0.5 ppm Kinetin and 0.5 ppm IAA has increased cell number significantly. MS media was more capable in vasicine synthesis as compared to B5 media, this might be due to more organic and inorganic sources in MS medium, which provide necessary ions and energy for vasicine synthesis.

References:
Isolation of Bioactive Antidiabetic Components from Endophytic Fungi of Musa Paradisiaca

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2Tolani Institute of Pharmacy, Adipur, Kachchh-370205, Gujarat, India.
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Email address: drharaj@yahoo.com

Abstract: The aim of the research work is to screen fractions endophytes of Musa Pardisiaca in rats with induced diabetes. Antidiabetic activity of fraction of endophytes of Musa pardisiaca was evaluated in induced colon diabetes in rats. After antidiabatic treatment blood sample was collected and hematological parameters like RBC, WBC, Hemoglobin were evaluated. Colons were dissected for histopathological study and ACF assay was performed. % inhibition in rats was calculated and the data was analysed statistically. The treatment showed significant % inhibition and normalized hematological profiles as compared to those of pathological control rats. After completion of treatment, the rats showed significant improve in diabetic condition. The chloroform extracts of endophytic fungi from Musa Pardisiaca showed potent antidiabetic activity. These data support the claim that the secondary metabolites from the endophytic fungi Musa Pardisiaca can be explored for development of novel secondary metabolites.

Introduction:
Biologically active natural substances have played an important role in discovery of new beneficial chemical entities. A traditional plant which is used since decades, having multiple therapeutic use known as "Musa Paradisiaca L" belonging to family Musaceae. It provides an important source of carbohydrate all over the world. Commonly it is used to treat diarrhoea, diabetes, uraemia, hypertension, nephritis, gout, cardiac disease. Around the world there are more than 100 common name used for Musa Paradisiaca but in most of the regions it is commonly known as "Banana". More than 70 species of banana have been identified. Studies show that all the parts of the plant bear almost the same therapeutical activity.

The details regarding the chemical constitute along with their functional group in table

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Functional Group</th>
<th>Chemical Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ripe Pulp</td>
<td>Amino acids &amp; Alkaloids</td>
<td>Tryptophan, pectin, Indol compound</td>
</tr>
<tr>
<td>Unripe pulp</td>
<td>Flavanoids</td>
<td>Leucocyanidin, quercetin and its 3-Ogalactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside</td>
</tr>
</tbody>
</table>
Materials and Methods:
The flowers (Fig.1) were collected from *Musa Pardisiaca* available in the local regions of Surat, Gujarat, in the month of September of year 2014. Albino rats of Wistar strain of either sex weighing between 80-120 gm procured from Shree Dhanvantary Pharmacy College, Kim were used for the present investigation. Based on the morphology characters like surface, texture, pigmentation and spores present at the hyphal tips which were used to identify the endophytic fungi at species level. The endophytic fungi was fermented in 3000 ml reagent bottle containing 2000 ml of PDA (potato infusion from 200g potatoes + 20g of dextrose, pH 5.1±0.2, 24g/L Himedia) for 21 days at 23°C under static condition, in two replicates. The yield of the extract ranged from 0.1-0.2 mg/L fermented medium. The chloroform extract was tested for antidiabetic activity. The acute toxicity tests for the endophytic fraction from *Musa Pardisiaca* was performed on albino rats of male rats weighing between 20-30 gm as per OECD Guidelines. The animals were fasted overnight prior to the experiment. The animals did not show any mortality up to 5000mg/kg. The oral LD$_{50}$ was assumed to be more than 5000mg/kg.

Laboratory scale fermentor (Bio Age) with anaerobic condition will be used for the improve yield of fungal growth after received identification of fungi.

Results and Discussion:
The flower of *Musa Pardisiaca* was divided into pieces of 1 cm and transferred to Petri dishes containing 2.5% water agar medium under aseptic conditions. Plates were observed at regular intervals. After 1 week the Red colored colonies (hyphal structures) emerged out from the flower of *Musa Pardisiaca*. (Fig.5.1)
Colony of fungi appears exactly like *Rhizopus*, having loose, cottony and fluffy growth. Colonies are white or brown in color but as it ages numerous pin headed structure develop on aerial mycelium (Fig.2). PDA broth was used as mass production medium. Mass of fungal growth was observed, after 12 -14 days in *Musa Pardisiaca*.

Fungal mycelia were separated from the culture broth by passing through four layer of cheesecloth. The fermented broth was extracted with equal volumes of chloroform by cold maceration and dried by evaporation.

**Figure 2:** Fungi Growth from Flowers of *Musa Pardisiaca*

The yield of the extract ranged from 0.1-0.2 g/L fermented medium. Primery identification with chemical test and TLC profiling was done.

Chloroform extract of fungi shows the significant antidiabetic activity. The fungal strains were sent for identification to NCCR Pune.

**Conclusion:**

The fungal strains isolated from the flowers of *Musa Pardisiaca* can be further screened for bioactive metabolites and new chemical constituents having potent antidiabetic activity can be developed.

**Acknowledgment:**

Author is thankful to Gujarat Science and Technology Department (GUJCOST), DST for providing financial assistance to carry out the research work.

**References:**


Frequency analysis of ATP1B1 gene polymorphism in hypertensive subjects

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Abstract: The pathogenesis and pathophysiology of hypertension is a complex heterogeneous disorder that can coexist with other cardiovascular and metabolic abnormalities. ATPase Na+/K+ transporting gene (ATP1B1) has been found as a candidate gene in BP-related loci. Objective of this study was to investigate the association of ATP1B1 polymorphisms with hypertension in Rajkot population. Genotype frequencies of the SNP gene (rs3766031) were analysed in the 46 Non Hypertensive objects and 57 Hypertensive Patients by using Gel Electrophoresis and PCR technique. Results: In present study, no significant association was found between Hypertension and the SNP gene (rs3766031) in selected candidates.

Introduction:
Essential hypertension (EH) is a complex polygenetic disease in which the onset and development involve both genetic and environmental factors [1]. Genetic elements contribute blood pressure variability in human. ATP1B1 have been associated with hypertension [2]; ATP1B1 gene has been found as a candidate gene in BP-related loci by a recent genome-wide linkage study in an American population [3]. This interesting finding needs to be repeat in other ethnic populations.

Materials and Methods:
Materials: TE: Tris-Cl, EDTA, RBC lysis buffer, DNA extraction buffer, Proteinase-k (20mg/ml) , 5M NaCl solution, Ethanol, Cetus buffer, dNTPs , Taq Polymerase, primer Forward (5’CCATGCTTGT TG GT TAGCGCC3’), Primer Forward (mutant) (5’CTATGCTT GTTG GGTAGC GCC3’) ,Primer Reverse (5’GGGCCAGGGCTTGAGGCCT3’), Bromophenol Blue, Glycerol, Acetic Acid, Agarose , Ethidium bromide.
Methods:
DNA isolation from blood: The number of subjects were 103 having both hyper tension and non-hypertension, from Rajkot, Gujarat. DNA isolation method was adopted from Molecular Cloning, A Laboratory Manual Sambrook [4] with some changes.
**PCR and Gel Documentation:** Mutation study in the ATP1B1 gene was carried out by the ARMS-PCR method with program of initial Denaturation 94°C for 7 minute 1 cycle, 28 cycles of denaturation at 94°C for 45 seconds, primer annealing at 63°C for 45 seconds extension at 72°C for 45 sec and final Extension 72°C for 7 minute 1 cycle. Amplification products were electrophoreses in 2.5% agarose in TAE buffer (a buffer solution containing a mixture of Tris base, acetic acid and EDTA). The gels were stained with ethidium bromide and Load the digested DNA (digested with Hae III - a Restriction Enzyme from Haemophilus aegyptius) and loading dye 1 μL (3X bromophenol blue and glycerol). Gel was documented using gel documentation system. Internal control was primer sequence which gives known band at 861 bp.

**Results and Discussion:**
The clinical characteristics of hypertensive and Non Hypertensive subjects summarized in Table 1. There was significant higher incidence of diabetes (52.70% vs 36.84%) and family history of hypertension (40.35% vs 34.78%) in case group than control. Genetic analysis of rs3766031 mutation in INTRON 2 of ATP1B1 gene using ARMS-PCR technique is shown in figure1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hypertensive Patients (Mean ± SD)</th>
<th>Non-Hypertensive Patients (Mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.56 ± 10.95</td>
<td>45.94 ± 9.33</td>
<td>0.00023</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.34 ± 2.83</td>
<td>23.73 ± 4.13</td>
<td>0.84220</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>142.10 ± 19.15</td>
<td>125.08 ± 5.18</td>
<td>0.01474</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.89 ± 10.05</td>
<td>82.27 ± 3.23</td>
<td>0.18680</td>
</tr>
</tbody>
</table>

**Figure 1** Genetic analysis of rs3766031 mutation in INTRON 2 of ATP1B1 gene
Lane 1: standard Ladder, Lane 2, 4: standard Ladder the wild type genotype (CC) is represented as 261bp amplification with normal primer Lane 4, 5: heterozygous genotype (CT) is represented as 261bp
amplification with normal and mutant primer and Lane 5, 7: homozygous genotype (TT) represented as 261bp amplification with mutant primer. Lane 3, 6 No amplification at 261 bp.

**Study on the frequencies analysis of rs3766031of the ATP1B1 gene**

Genotype frequencies of the rs3766031 were analyzed in the 46 Non Hypertensive Patients and 57 Hypertensive Patients. Frequencies of CC, CT and TT genotype of rs 3766031 were 69.56 %, 28.26%, 2.18% in Non Hypertensive subjects and 66.66%, 29.84% and 3.50% in Hypertensive patients respectively.

The T allelic frequency of rs3766031 in Non Hypertensive subjects and Hypertensive patients were 16.50% and 18.50% respectively. Distribution of rs3766031 allelic frequencies was found to be 0.175 in Rajkot (Gujarat) population and p value was found 0.596.P value assessed by chi-square test.

Present study was conducted to check the Minor allele frequency distribution of intronic variant rs 3766031SNP of ATP1B1. The minor allele frequency of rs 3766031was 0.170 in Chinese population [5]. While in present study, minor allele frequency was found 0.175 in Rajkot population.

**Conclusion:**

The frequency of mutated T allele showed no significant difference between the Non Hypertensive group (0.164) and Hypertensive patient group (0.185) rs 3766031. SNP of ATP1B1 not directly associated with an increased risk for Hypertension in Rajkot (Gujarat, India) population. Further study is required to check the actual frequency of the rs 3766031.

**Acknowledgement:**

This work was supported by Department of Pharmaceutical Sciences and Milestone Hospital, Rajkot.

**References:**

Investigation of anti-diabetic and hypolipidemic activity of Dykure in streptozotocin induced diabetic wistar rats

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Abstract: In the present investigation, Dykure, a polyherbal formulation of 13 different herbs significantly decreased the Fasting serum glucose (FBG), glycosylated hemoglobin, total cholesterol, triglyceride and LDL cholesterol in Streptozotocin (STZ) (60mg/kg i.p) treated animals. Oral administration of Dykure (500mg/kg and 1gm/kg) for 30 days significantly improved the decreased liver glycogen content, HDL cholesterol level, liver prooxidant and antioxidants like Superoxide dismutase (SOD), Catalase and Reduced glutathione (GSH) in STZ intoxicated rats. Our results suggest that Dykure may be useful as hypoglycemic, hypolipidemic and anti-oxidant; however, further extensive study is required to validate its efficacy.

Introduction:
Diabetes mellitus is a disorder of carbohydrate metabolism with multiple etiological factors. Hyperglycemia is the landmark of this syndrome involving insulin deficiency or insulin resistance or both. The current therapy of insulin and oral hypoglycemic agents may have adverse effects. Polyherbal formulation is the combination of various types of herbs having different pharmacological actions, which can work synergistically or potentially to produce maximum therapeutic efficacy with minimum side effects.

Materials and Methods:
Animals: Male albino Wistar rats weighing 200-260 g were used in this study. The animals were fed on a normal pellet diet and water ad libitum. The protocol (Ref: MSU/ IAEC/2008) of the experiment was approved by the IAEC, Pharmacy dept., M.S. University of Baroda, Vadodara as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Dykure solution preparation: Aqueous solution of fine powder of Dykure was prepared in required doses.

Experimental design: (n=30 animals divided equally into following groups)
1) Normal control:- Animals orally administered with drinking water daily for 30 days. 2) Normal rats + Dykure:- Animals orally administered with Dykure 1gm/kg/day in divided doses at 0.5ml p.o for 30 days.
3) Diabetic control (STZ):- Animals injected with STZ 60mg/kg intraperitoneally. 4) Diabetic rats+ Dykure 500mg:- Animals orally administered with Dykure 500mg/kg/day in divided doses at 0.5ml p.o for 30 days. 5) Diabetic rats+ Dykure 1gm:- Diabetic animals orally administered with Dykure 1gm/kg/day in divided doses at 0.5ml p.o for 30 days.

Results and Discussion:

Table 1 Effect of Dykure on serum fasting glucose, glycosylated hemoglobin, total cholesterol, triglyceride LDL cholesterol and HDL cholesterol content in control and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>FBG mg/dl</th>
<th>Gly. Hemooglobin (HbA1c)(mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>LDL Cholesterol (mg/dl)</th>
<th>HDL Cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>72.04±2.59</td>
<td>4.66±0.33</td>
<td>81.68±1.81</td>
<td>81.12±1.81</td>
<td>15.28±1.43</td>
<td>50.18±1.64</td>
</tr>
<tr>
<td>II</td>
<td>C + Dy (1g/kg)</td>
<td>65.58±2.71</td>
<td>4.30±0.22</td>
<td>72.28±1.12</td>
<td>71.35±1.01</td>
<td>11.18±1.75</td>
<td>46.84±1.26</td>
</tr>
<tr>
<td>III</td>
<td>DB (STZ)</td>
<td>302.0±6.3*</td>
<td>8.77±0.23*</td>
<td>114.4±2.2*</td>
<td>114.6±2.9*</td>
<td>55.10±1.4*</td>
<td>36.34±1.3*</td>
</tr>
<tr>
<td>IV</td>
<td>DB+Dy 500mg/kg</td>
<td>194.9±2.6*</td>
<td>6.56±0.25*</td>
<td>100.3±1.5</td>
<td>90.37±1.6*</td>
<td>44.55±3.2*</td>
<td>37.82±1.82</td>
</tr>
<tr>
<td>V</td>
<td>DB+Dy 1gm/kg</td>
<td>96.6±2.2*</td>
<td>5.13±0.2*</td>
<td>80.23±2.5*</td>
<td>73.71±2.8*</td>
<td>18.57±2.6*</td>
<td>46.91±2.1*</td>
</tr>
</tbody>
</table>

*- significant as compared to normal control  + significant as compared to diabetic control  $- significant as compared to diabetic rats + Dykure 500mg/kg

Table 2 Effect of Dykure on liver glycogen, lipid peroxidation and tissue antioxidants in control and experimented rats.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Treatment</th>
<th>Liver Glycogen (mg/gm of tissue)</th>
<th>Lipid peroxidation (MDA/gm of tissue)</th>
<th>SOD Unit/gm of hepatic tissue</th>
<th>Catalase µM of H2O2 consumed/ min/gm of hepatic tissue</th>
<th>GSH µg /gm of hepatic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>10.45±2.32</td>
<td>17.43±1.42</td>
<td>9.64±2.08</td>
<td>13.86±1.81</td>
<td>138.29±2.80</td>
</tr>
<tr>
<td>II</td>
<td>C + Dy (1g/kg)</td>
<td>12.93±2.14</td>
<td>16.44±1.72</td>
<td>9.92±1.28</td>
<td>14.77±1.01</td>
<td>143.75±2.21</td>
</tr>
<tr>
<td>III</td>
<td>DC (STZ)</td>
<td>4.21±1.42</td>
<td>68.24±2.87</td>
<td>5.39±2.21</td>
<td>7.51±1.9</td>
<td>102.38±4.31</td>
</tr>
<tr>
<td>IV</td>
<td>DB rats 500mg/kg +Dy</td>
<td>6.99±1.39*</td>
<td>50.39±2.41*</td>
<td>7.27±1.50 *</td>
<td>11.19±1.65*</td>
<td>118.11±2.43*</td>
</tr>
<tr>
<td>V</td>
<td>DB rats 1gm/kg +Dy</td>
<td>8.59±2.11*</td>
<td>22.26±2.13*</td>
<td>8.93±2.5*</td>
<td>13.46±2.84*</td>
<td>135.54±3.02*</td>
</tr>
</tbody>
</table>

*- significant as compared to normal control  + significant as compared to diabetic control  $- significant as compared to diabetic rats + Dykure 500mg/kg
The results showed that Dykure produced significant (p<0.001) decrease in elevated serum glucose and glycosylated hemoglobin level in STZ treated animals, which might be attributed to its composition of 13 different plants. Azadirachta indica was reported to have increased insulin secretion from existing beta cell and inhibition of intestinal glucosidase inhibitory activity. Terminalia chebula might be regenerating β-cells and stimulating insulin release [1]. Tinospora cordifolia and Coccinia indica were found to inhibit the activity of gluconeogenic enzymes [2]. Hypoglycemic effects produced via different mechanisms by various plants present in Dykure might be responsible for improvement in liver glycogen level in diabetic animals. Deficient action of insulin in diabetes stimulates lipolysis, which results in hypertriglyceridemia and subsequent increase in blood cholesterol level. In present investigations, STZ intoxicated rats showed significant increase in serum cholesterol, triglyceride and LDL cholesterol level, which was reduced to a significant level (p<0.001) by Dykure. HDL cholesterol, a reverse cholesterol transporter was significantly (p<0.05) increased in diabetic rats treated with Dykure. Our results clearly indicate the hypolipidemic activity of Dykure, which might be due to various active constituents of its different plants such as alkaloid and pectin from C. indica, alkaloids from T. cordifolia, saponins from Trigonella foenum graecum and various glycosides present in A. indica [2] [3] [4]. Excessive Reactive oxygen species (ROS) can cause oxidative damage to cell membrane and important biomolecules like DNA, phospholipid, protein etc. In the present study, generation of oxidative stress indicated by increased lipid peroxidation in STZ treated rats was reversed by Dykure treatment. The decreased level of liver antioxidant enzymes SOD, GSH and Catalase were improved significantly (p<0.01) after Dykure administration. The results clearly indicate the anti-oxidant potential of Dykure owing to its different plants composition. Polyphenols of T. chebula and T. bellerica and picroside of Picrorrhiza kurroa were reported to have potential antioxidant activity [5]. T. cordifolia was also previously reported to possess potent free radical scavenging activity [3].

Conclusion:

On the basis of the results of the present study, it can be concluded that Dykure, a combination of 13 different herbs exerts a significant antidiabetic, hypolipidemic and antioxidant effect, which may play beneficial role in management of diabetes mellitus and associated metabolic abnormalities.

References:

Antifertility activity of traditional herbal formulation

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Abstract: Plumbago zeylanica root and their traditional formulation was selected for evaluation of antifertility activity. Plant collection, identification, successive solvent extraction, phytochemical examination, acute toxicity study, antifertility activity, isolation and characterization of biocompounds were carried out. The results of reproductive outcome study indicated that formulation exhibited 70% antifertility activity. Two bioactive compounds namely; 2-{17-(5-(4-Ethyl-3-hydroxy-phenyl)-tetrahydrofuran-2yl)-14-hydroxy-5-hydroxymethyl-hexadecahydro-cyclo-penta(a)phenanthren-3-floxy}-4,6-bis-hydroxymethyl-5-(6-hydroxymethyl-5-(6-hydroxymethyl-4-methyl-tetrahydro-pyran-2-yloxy)-tetrahydropyran-2-ol and 3-{2-Hydroxy-4,6-bis-hydroxymethyl-5-(6-hydroxymethyl-methyl-4-(5-carboxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy)-5-hydroxymethyl-17-(5-propyl-tetrahydro-furan-3-yl)-hexadecahydro-cyclopenta(a)phenanthrene-14,16-diol isolated from alcoholic extract which contain steroidal ring may be responsible for antifertility activity of formulation.

Introduction:
On the basis of traditional knowledge of herbal healers existing in the Bastar region and literature survey, one plant and their traditional formulation was selected for present proposed research. The purpose of present work is to identify the chemical constituents of the selected plant, evaluate further their claimed antifertility effect [1, 2].

Materials and Methods:
Plant material and extraction: Plant material was collected, authenticated and pulverized to coarde powder. Coarse powders of drugs were subjected to successive solvent extraction and various chemical tests to detect the presence of different phytoconstituents.

Animals: Wistar albino rats of either sex were used for this study. The animal experiment was performed as per CPCSEA (Registration No.: 1275/ac/09/CPCSEA; Date: 19.08.2010). Acute toxicity study was performed in two steps, namely, pilot study and main study.

Screening of antifertility activity of formulation: Formulation was prepared as per the method described by the local vaidys and traditional herbal healers prevalent in Bastar. One gm root powder of
Plumbago zeylanica was mixed with equal amount of rice water and kept overnight. Formulation was screened for reversible antifertility activity according to the method described by Ganguly, 2007 [3, 4].

**Isolation and characterization of phytoconstituents:** Extracts were subjected to column (50 x 2.5 cm) chromatography using different solvent systems. The fractions collected were further chromatographed using silica gel as a stationary phase to isolate constituents and characterized by spectroscopic methods [5].

**Results and discussion:**

All the results are presented in Tables and figures. Structure elucidation was carried out with the help of IR H & C-NMR and MASS spectroscopy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Litters No.</th>
<th>% Antifertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>10.33 ± 0.72</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PZ + RW</td>
<td>1000</td>
<td>6.00 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Post Extract</td>
<td>-</td>
<td>5.16 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

N=6, Values are mean ± S.E.M. Data are analysed one-way ANOVA followed by Dunnet multiple comparison test. b = p<0.01, significantly different from control.


**References:**


An in vitro study on anticancer potential of fungal chondroitinase

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Abstract: Recently there is a growing interest in exploring Chondroitinases which can find potential application in treatment of cancer and spinal cord injuries. The work on fungal organisms which produce these enzymes is sparse. The present work was carried out using the enzyme extracted from a fungal strain MSSS-1 isolated from marine soil, Udupi. The isolate was cultured on chondroitin sulfate—dextrose- yeast extract-peptone medium (pH 8.0) for 5-7 days and the enzyme was precipitated using ammonium sulfate. The precipitated enzyme was dissolved in tris-buffer (pH 7.5), dialysed and passed through Amicon® 100, 50 and 30 ultra-filters. The retentates had enzyme activities of 7.8, 15.75 and 2.13 U/ml respectively. Retentate from Amicon® 50 gave a prominent protein band on SDS PAGE. In vitro Anticancer studies were carried out using MTT assay, DNA fragmentation and nuclear staining techniques.

Introduction:
Chondroitinase catalyses depolymerisation of chondroitin sulphate into n-acetyl galactosamine and glucuronic acid. This enzyme can find therapeutic use in controlling tumor progression, glial scar removal and in treatment of vitreous detachment. Commercially available enzyme is from bacterial sources viz., Arthrobacter, Flavobacterium and Proteus species. Very little work is focused on isolation of fungal chondroitinase. Fungal proteins (being eukaryotic) pose less post translational mechanisms compared to bacterial enzymes and can have better acceptability (1).

Materials and Methods:
Culture and Cultivation conditions-
A fungal isolate MSSS-1 (from marine beach soil, Udupi) was maintained on SD Agar and preserved at 4°C. The organism was cultivated on Chondroitin sulfate- dextrose-yeast extract- peptone medium given by de Assis et.al. For 5-7 days at 150 rpm.

Enzyme activity: Chondroitinase activity was assessed by measuring the amount of N-acetyl galactosamine released using Muir et al method (2). The activity of chondroitinase is defined as the...
quantity of enzyme that catalyses the formation of 1µmol of unsaturated disaccharide from the substrate per minute at 37°C at pH 8.0.

Supernatant was obtained by centrifuging the harvest at 5000 g for 15 min. The enzyme was precipitated using 80% ammonium sulfate, collected after centrifugation at 9000 g for 20 min, suspended in tris buffer (pH 7.5) and dialysed for 12 h. The enzyme obtained was passed through Amicon® 100, 50 and 30 kDa ultra filters. Retentate from kDa 50 having higher enzyme activity was subjected to SDS-PAGE and also studied for invitro anticancer activity.

**In-vitro anti-cancer studies:** Cytotoxicity of the enzyme against HepG2, HCT 116, and MCF 7 and Vero cells was studied using MTT assay. Each of the 96 well plate wells was seeded with cells at 1 x 10^4 concentration and allowed to proliferate for 24 h at 37°C in 5 % CO₂ in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% Fetal Bovine Serum (FBS). The cells were then exposed to various concentrations of the test enzyme and incubated for 48 h. After incubation the medium was removed and incubated with 50 µl of MTT solution for 4 h. The supernatant was removed and the insoluble dye was solubilized in 50 µl of DMSO. The absorbance was measured at 540 nm using a microplate reader and the percentage cell-growth inhibition was calculated.

DNA fragmentation assay was performed using MCF 7 cells. Each of the six well tissue culture plate was seeded with MCF 7 cells (1 x 10^6) and incubated with DMEM having 10% FBS for 24 h. At the end of incubation period, cells were treated for 48 h with the enzyme and incubated at 37°C. After the incubation the cells were fixed and DNA fragmentation assay was carried out (3). Next, the nuclear morphology and membrane integrity test was performed using MCF 7 cells. Each well of the 24 well tissue culture plate was seeded with MCF 7 cells (5 x 10^4) and incubated for 24 h with DMEM having 10% FBS. At the end of incubation the cells were fixed with 90% ice cold methanol and air dried. The wells were washed with phosphate buffer saline (PBS) and ethidium bromide (10 µg/ml) was added to each well. The plate was incubated at 37°C for 20 mins. The unbound dye was removed by washing with PBS. The stained cells were observed under fluorescent microscope.

**Results and Discussion:**

The ammonium sulfate precipitate of the MSSS-1 culture supernatant had an enzyme activity of 15.75 U/ml. After dialysis, retentates on Amicon® 100, 50 and 30 ultra-filters, had enzyme activities of 7.8, 15.75 and 2.13 U/ml respectively. Retentate from Amicon® 50 gave a prominent protein band on SDS PAGE. In vitro cytotoxicity assay by MTT method showed that the test enzyme inhibited the proliferation of tested cancerous cell lines. Cells when treated with test enzyme at low concentration showed DNA fragmentation, while the untreated cells showed intact DNA. In the present study nuclear
staining with ethidium bromide showed that the test enzyme induces apoptosis in MCF-7 cells at the tested concentration.

**Figure 1** Cytotoxicity of 50 kD aretentate against various cell lines

**Figure 2** Determination of nuclear morphology and membrane integrity, Ethidium bromide staining of MCF-7 cells.

**Conclusion:**
Studies on in vitro anticancer activity showed that partially purified fungal chondroitinase was able to inhibit/control proliferation of cancer cells.

**Acknowledgement:**
The authors are thankful to Manipal University for providing the facilities needed to carry out the above study.

**References:**

“Strategic Approaches to Strengthen Academic and Industrial Collaboration” www.ijpsr.com
Antioxidant activity of the ethyl acetate extract of *Oroxylum indicum* leaves and its isolated compounds

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Abstract: Overproduction of free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes and other degenerative diseases in humans. Epidemiological studies showed that many of the antioxidant compounds possess anti-inflammatory, anticarcinogenic or antiviral activities. In the present study, antioxidant activities of ethyl acetate extract of *Oroxylum indicum* leaves and its isolated compounds were carried out by using DPPH assay method. The extract showed promising results. IC$\text{50}$ value of *Oroxylum indicum* ethyl acetate extract was found to be 213 µg and IC$\text{50}$ of isolated compound 2 was found to be 126.9 µg.

Introduction:
Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes and other degenerative diseases in humans [1].
Antioxidants are the agents which inhibits oxidation of the molecules. Oxidation is a chemical reaction which results in the formation of free radicals or in a broader sense reactive oxygen species (ROS), which further carry out chain reaction and can cause damage to cell, which can be a causative agent of many diseases like cancer, cardiovascular diseases. Antioxidants are capable of deactivating or neutralizing the effects of Reactive oxygen species (ROS). *Oroxylum indicum* (Bignoniaceae), also known as Sonapatha or Shyonaka is commonly used herbal medicine in Ayurvedic system. Alkaloids, flavonoids, glycosides, terpenoids are found to be the active constituents of *Oroxylum indicum* (L.). The leaves have been reported containing flavones and their glycosides baicalein and scutellarein. Leaves also contain anthraquinones, aloe emodin. In the present study, antioxidant activities of ethyl acetate extract of *Oroxylum indicum* leaves and its isolated compounds were carried out by using DPPH assay method.

Materials and Methods:
**Compounds tested:** Ethyl acetate extract of *Oroxyllum indicum* leaves, Isolated Compound 1, Compound and Compound 3 of *Oroxyllum indicum*.

**DPPH assay:** DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical having deep violet color in solution, showing maximum absorption at 517 nm. When it is mixed with a solution it is capable of donating hydrogen which leads to change in coloration to yellow. The change in color is in stichometry to the number of hydrogen captured [2].

**Procedure:** Six dilutions were prepared in the concentration of 40, 80, 120, 160, and 200 µg/ml. 6.0 mg of DPPH was mixed with 100 ml of methanol. 1ml of extract and compounds from each dilution were mixed with 2ml of DPPH solution. 1 ml of methanol was added with 2 ml of DPPH solution to get control. Ascorbic acid was used as standard. All the mixtures prepared were shaken thoroughly and wrapped with aluminium foil and kept in dark for 30 minutes. The absorbance of resulting solutions was taken at 517 nm.

**Results and Discussion:**
The IC_{50} value for standard ascorbic acid was found to be 0.81µg/ml. The antioxidant activity of the *Oroxyllum indicum* leaves ethyl extract and its isolated compounds were tabulated in the table

**Figure 1** Antioxidant activity of the *Oroxyllum indicum* ethyl extract and its isolated compounds

<table>
<thead>
<tr>
<th>Sample Conc. (µg/ml)</th>
<th><em>Oroxyllum indicum</em> Ethyl acetate extract</th>
<th>Isolated Compound 1</th>
<th>Isolated Compound 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>Percent inhibition</td>
<td>Absorbance</td>
<td>Percent inhibition</td>
</tr>
<tr>
<td>40</td>
<td>0.916</td>
<td>1.92</td>
<td>0.896</td>
</tr>
<tr>
<td>80</td>
<td>0.842</td>
<td>9.85</td>
<td>0.638</td>
</tr>
<tr>
<td>120</td>
<td>0.726</td>
<td>22.26</td>
<td>0.548</td>
</tr>
<tr>
<td>160</td>
<td>0.604</td>
<td>35.33</td>
<td>0.368</td>
</tr>
<tr>
<td>200</td>
<td>0.498</td>
<td>46.68</td>
<td>0.300</td>
</tr>
</tbody>
</table>

- IC_{50} value of *Oroxyllum indicum* leaves ethyl acetate extract was found to be 213 µg
- IC_{50} of compound 1 isolated from *Oroxyllum indicum* ethyl acetate extract was found to be 142 µg
- IC_{50} of compound 2 isolated from *Oroxyllum indicum* ethyl acetate extract was found to be 126.9 µg

**Conclusion:**
*Oroxyllum indicum* leaves ethyl extract and its isolated compounds showed good antioxidant activities.
The percentage inhibition of 200 µg of Compound 1 was 67.88% which is a good indication of antioxidant activities.

References:
Effect of Solanum torvum on doxorubicin induced testicular toxicity in rats

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Abstract: Oxidative stress is the main factor in Doxorubicin induced toxicity. The protective effect of S. torvum against doxorubicin induced testicular toxicity was investigated in male rats. S. torvum was administered orally to rats at a dose of (100 mg/kg and 300 mg/kg, p.o.) daily for 28 days along with doxorubicin. Doxorubicin was administered i.p. to the animals at a dose of 3mg/kg, for 4 weeks on day 1, 7,14,21,28. Testicular toxicity was assessed by recording changes in body weight, testis weight, epididymal sperm count. The results suggest that S. torvum has the potential in preventing the Doxorubicin induced testicular toxicity.

Introduction:
Doxorubicin is well known anthracycline glycoside antibiotic that possesses a potent broad spectrum antitumour activity against a variety of cancers including severe leukemias, lymphomas, human solid tumours and haematological malignancies However its use in chemotherapy has been limited largely due to its diverse toxicities, including kidney, renal, hematological and testicular toxicity [1]. Oxidative stress has been associated with DXR induced tissue damage. Although the exact mechanism of DXR induced toxicity remains unknown, it is believed to be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules and membrane lipid peroxidation [2]. The production of free radicals as a byproduct of DXR metabolism is considered to be the primary mechanism of DXR induced toxicity, which can be ameliorated by the use of natural antioxidants.

Materials and Methods:
Animals: Albino rats (Wistar strain) of either sex weighing between 200-250 g, were obtained from Serum Institute, Pune. Animals were housed into groups of five under standard laboratory conditions of temperature 25 ± 1°C with free access to food (Amrut rat and mice feed, Sangli, India.) and water. The experiments were performed during the light portion. The experiments were carried out according to the guidelines of (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.
Drugs and chemicals: Doxorubicin (ADRIM, 50 mg) was purchased from Dabur Pharma Ltd. Baddi, India. All the chemicals used were of analytical grade were obtained from Merck, India. Solanum torvum extract was dissolved in distilled water and administered orally.
**Experimental protocol:** The animals were divided into six groups each consisting of five rats and received the following treatment.

**Group I (Control):** Animals received distilled water for injection.

**Group II (DXR):** Animals received DXR injection (3 mg/kg, i.p.) on day 1, 7, 14, 21, 28.

**Group III (DXR + ST):** Animals received ST (100 mg/kg /day p.o. for 28 days) and DXR injection (3 mg/kg, i.p.) on day 1, 7, 14, 21, 28. Group IV (DXR + ST): Animals received ST (300 mg/kg /day, p.o. for 28 days) and DXR injection (3 mg/kg i.p.) on day 1, 7, 14, 21, 28. Group V (ST): Animals received ST (100 mg/kg /day, p.o. for 28 days)

**Group VI (ST):** Animals received ST (300 mg/kg /day, p.o. for 28 days)

**Estimation of body and organ weight:** In each group, body weight of rats was taken before and after DXR treatment. The testis was weighed after keeping them in ice-cold saline.

**Epididymal sperm count:** After 48 hours of the last injection of either DXR or vehicle, Epididymis was removed, after the adhering tissues were cleared the epididymal sperm count was done immediately. Epididymal sperm was collected by slicing the epididymis in 5 ml phosphate buffer saline (pH 7.2). An aliquot of the epididymal sperm suspension was used for spermatozoon count using Neubauer hemocytometer [3].

**Results and Discussion:**

**Table 1** The Effect of methanolic extract of *Solanum torvum* on body weight, testes weight and sperm count in DXR treated animals.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment groups (mg/kg)</th>
<th>Final Body Weight (gm)</th>
<th>Absolute testes weight (gm)</th>
<th>Sperm count (millions/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>239.4 ± 2.34</td>
<td>1.46 ± 0.02</td>
<td>44.76 ± 1.79</td>
</tr>
<tr>
<td>2</td>
<td>DXR (3)</td>
<td>197.9 ± 4.93*</td>
<td>0.89 ± 0.01*</td>
<td>10.80 ± 0.44*</td>
</tr>
<tr>
<td>3</td>
<td>ST (100) + DXR (3)</td>
<td>219.8 ± 4.54#</td>
<td>1.09 ± 0.02#</td>
<td>27.44 ± 0.66#</td>
</tr>
<tr>
<td>4</td>
<td>ST (300) + DXR (3)</td>
<td>225.2 ± 4.05#</td>
<td>1.15 ± 0.06#</td>
<td>30.80 ± 0.59#</td>
</tr>
<tr>
<td>5</td>
<td>ST (100)</td>
<td>211.4 ± 5.65*</td>
<td>1.34 ± 0.08</td>
<td>36.01 ± 1.25*</td>
</tr>
<tr>
<td>6</td>
<td>ST (300)</td>
<td>220.6 ± 5.56*</td>
<td>1.37 ± 0.03</td>
<td>39.92 ± 1.21*</td>
</tr>
<tr>
<td>7</td>
<td>F value</td>
<td>8.89</td>
<td>22.89</td>
<td>118.61</td>
</tr>
</tbody>
</table>

N = 5, All values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test. *P< 0.05 against control group, # P< 0.05 against DXR group. ST = methanolic extract of *Solanum torvum*, DXR = Doxorubicin.

**Testis weight and sperm count:** Co-treatment with methanolic extract of Solanum torvum (100 and 300 mg/kg/day, p.o.) in DXR treated (3 mg/kg, i.p. on day 1, 7, 14, 21, 28) animals significantly (p<0.05) restored the isolated testes weight and sperm count as compared to DXR treated group (Table 1)

“Strategic Approaches to Strengthen Academic and Industrial Collaboration” [www.ijpsr.com](http://www.ijpsr.com)
Doxorubicin a widely used anticancer drug is associated with multiple organ toxicity. Doxorubicin is known to disturb spermatogenesis in a dose dependent manner in animal studies. Ward and his co-workers have also reported doxorubicin induced reductions in testicular sperm count. Administration of DXR to rats significantly decreased epididimal sperm count [4]. Treatment with *S. torvum* extract (100 mg/kg and 300 mg/kg) has resulted in significant (p<0.05) change in all parameters as observed in DXR treated animals thus offering considerable protection against testicular toxicity [5].

**Conclusion:**

The antioxidant properties of flavonoids and their ability to chelate free iron could be effective in reducing toxicity of DXR. In view of this, though the antioxidant activity of *S. torvum* is well known, its protection against DXR- induced testicular damage is not reported. Therefore the present study was aimed at investigating the possible protective effect of *Solanum torvum*, against DXR-induced testicular toxicity in rats.

**Acknowledgement:**

Mrs. Mahalaxmi Mohan, HOD, Dept Pharmacology, M.G.V.M’s Pharmacy College, Nashik.

**References:**

Antioxidant activity of polyherbal formulation

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Abstract: Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention and treatment of diseases. Present study was such an attempt to develop polyherbal formulation for antioxidant activity with minimum ingredients in order to ensure standard parameters. Formulation was standardized by different analytical parameters. Assessment of the synergism and therapeutic efficacy of *Ocimum sanctum*, *Centella asiatica*, *Withania somnifera* was done by different in vitro antioxidant models. Study shown that formulation was superior than individual plants

Introduction:
Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. [1]. Drug discovery need not be always confined to the discovery of a single molecule. Many analysts believe that the current ‘one drug fits all’ approach may be unsustainable in the future. The growing interest in polypill concept is indicative of the need to collectively address multiple targets, risk factors or symptoms. [2]. Thus, by considering all these facts the present study was such an attempt to develop polyherbal formulation for antioxidant activity with minimum ingredients in order to ensure standard parameters

Materials and Methods:
Review of literature: Collection of updated review of plant and diseases.

Procurement and authentication of the plant materials: The plant *O. sanctum* was obtained from Pimpri, *C.asiatica* Roots of *W somnifera* from local market of Pune The plants were identified and authenticated from in Regional Research Institute (AY), Pune

Phytochemical screening: (Hydroalcoholic extracts of *C.asiatica, W .somnifera and O. sanctum*), Preliminary phytochemical screening and TLC profile

Results and discussion:
Extraction phytochemical tests and TLC of extracts: C. asiatica whole herb- Yield 15.12% color greenish black, W.somnifera yield 17.21%,color cream, O.Samctum leaves-yeild 12.10% color-brownish. In qualitative chemical tests and TLC were carried out. C. asiatica extract reveals the presence of reducing sugars, steroids, alkaloid, tannins, flavonoids and saponins. W. somnifera extract reveals the presence of steroids, alkaloids and saponins. O. sanctum extract reveals the presence of reducing sugars, tannins, flavonoids, saponins.

In vitro antioxidant study: 1. DPPH free radical scavenging activity:

Table 1 Effect of ascorbic acid and formulation in DPPH free radical scavenging assay.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Concentration μg/ml</th>
<th>% Scavenging of Ascorbic acid Mean ± S.E.M.</th>
<th>IC₅₀</th>
<th>% Scavenging of Formulation Mean ± S.E.M.</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 μg/ml</td>
<td>43.82 ± 2.000</td>
<td></td>
<td>42.28 ± 2.1200</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>100 μg/ml</td>
<td>48.55 ± 2.000</td>
<td></td>
<td>46.51 ± 1.90</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>150 μg/ml</td>
<td>55.22 ± 3.464</td>
<td></td>
<td>49.12 ± 2.47</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200 μg/ml</td>
<td>62.30 ± 3.464</td>
<td></td>
<td>53.21 ± 4.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>250 μg/ml</td>
<td>67.24 ± 2.000</td>
<td></td>
<td>59.67 ± 3.464</td>
<td></td>
</tr>
</tbody>
</table>

2. Determination of reducing power and hydrogen peroxide scavenging activity.

Table 2 Effect of ascorbic acid and formulation in hydrogen peroxide assay reducing power assay.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. μg/ml</th>
<th>H₂O₂ antioxidant assay</th>
<th>Reducing power assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ascorbic acid</td>
<td>Formulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% Scavenging</td>
<td>IC₅₀</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>41.04 ± 0.01</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>46.66 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>56.33 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>61.33 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>67.66 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>
The present study was an attempt to develop synergistic polyherbal formulation for stress management from plant used in the Indian System of Medicine (ISM) and which are scientifically proved as potential antioxidant. *C. asiatica*, *W. somnifera*, *O. sanctum* were selected for antioxidant activity. It is well known that intensive stress response results in creation of reactive oxygen species e.g., hydrogen peroxide, hydroxyl radical and superoxide anion radical that cause lipid peroxidation, especially in membranes and can play an important role in tissue injury. Formulation showed significant antioxidant effect in DPPH assay, reducing power assay and H$_2$O$_2$ assay. This may be due to presence of Phenolic compounds, trieterpenes *C. asiatica*, withanolides, glycowithanoloids of *W. somnifera*, triterpenes, flavonoids, phenolics of *O. sanctum*. Study shown that formulation was superior than individual plants i.e. *C. asiatica W. somnifera* and *O. sanctum* this clearly suggest that synergism take place and hence formulation is advised.

**Conclusion:**

Formulation showed superior activity than individual drugs. Further detail formulation related aspects need to be carrying out. Quantitative evaluation of phytoconstituents needs to carry out in order to ascertain quality of formulation. Detailed biological investigation and mechanism of actions of synergistic effect of formulation and clinical trials on human being need to be carry out in order to explain therapeutic use of formulation.

**References:**

Effect of dimethyl fumarate (DMF) on haloperidol induced parkinson’s disease like syndrome in mice

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Abstract: In the present study healthy Albino mice were divided in four groups each consist of six mice. Group I (Normal), Group II (Treated with 1mg/kg Haloperidol IP), Group III & Group IV (Treated with Haloperidol IP & 10 mg/kg, 20 mg/kg DMF Orally respectively). The effect of DMF on haloperidol induced Parkinson's like disease in mice was studied by using Actophotometer test (Locomotor activity), Rotarod test (Motor activity) & Bar test (muscle rigidity). DMF has shown good protective activity in Haloperidol induced Parkinson Disease like syndrome in mice dose dependently. Further research is required to establish the exact mechanism of action of DMF.

Introduction:
In 1817 James Parkinson, a medical doctor, for the first time described the disease as “involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured”. Since the existing anti Parkinson’s drugs encountered many side effects and need for prolonged treatment including questionable efficacy in the treatment, may cause Parkinson related movement problems, hallucinations and orthostatic hypotension. These reasons force the area of research to find improved treatments which will counteract the side effects and the draw backs of the existing treatment. As Dimethyl Fumarate (DMF) is a promising oral therapeutic option for neurodegeneration in multiple sclerosis which is linked to oxidative stress, our present study is aimed to find out the effect of DMF on haloperidol induced Parkinson's like disease in mice by using Bar test (muscle rigidity), Rotarod test (Motor activity) and Actophotometer test (Locomotor activity). The exact mechanism of action of Dimethyl Fumarate (DMF) in MS is unclear; it has anti-inflammatory and neuroprotective activity. As DMF has neuroprotective activity it may help to protect the cells against haloperidol induced damage as well as Parkinson Disease like syndrome.

Materials and Methods:
Animal: Adult male Swiss albino mice (25-30 g) were used. The animal housing and handling in accordance with CPCSCA guidelines, the prior permission for the study was obtained from institutional
animal ethics committee (IAEC).

**Drugs and chemicals:** Haloperidol (Sigma Aldrich, USA), DMF (Sigma Aldrich, USA). Solutions are prepared freshly on the days of experiment by dissolving in physiological saline and administered. All experiments are carried out under the guidelines of CPCSEA.

**Experimental Protocol:** Healthy mice were selected and divided in following groups, each group consist of six mice. The test drug DMF was given to group III & IV at the dose of 10mg/kg as well as 20mg/kg orally respectively for 21 days and all three tests were performed as per the protocol.

- Group I: animal treated with 1% w/v sod CMC (1gm/100 ml)
- Group II: animals treated with haloperidol (1mg/ kg) I.P
- Group III: animals treated with DMF (10mg/kg) P.O. + Haloperidol (1mg/ kg) I.P
- Group IV: animals treated with DMF (20mg/kg) P.O. + Haloperidol (1mg/ kg) I.P

**Assessment of Locomotor activity (Using Actophotometer)**
**Assessment of Motor coordination (Using Rotarod)**
**Assessment of Muscle Rigidity (Using Bar test).**

Measurement of activity was done on day 21, DMF was administered on just one hour prior to the challenge with haloperidol. Effect of test drug was measured at 30th min, 60th min, 90th min, 120th min and at 240th min.

**Results and Discussion:**
Haloperidol induced catalepsy in rodents has long been used as an animal model for screening of drugs for the treatment of Parkinsonism, same model was used in this study. DMF treated Group III & Group IV shown decrease in catalepsy in all three test mentioned above when compared with Group II. Results were almost similar to Group I which was normal group. This protective effect of DMF against Haloperidol induced damage might be due to its antioxidant or dopamine facilitatory action or dopamine agonist activity etc. This effect of DMF will be a great tool for the treatment of Parkinson’s disease.
Conclusion:

In the present study we found that Dimethyl Fumarate has shown good protective effect against Haloperidol induced catalepsy in mice. In all three tests mentioned in experimental protocol DMF has shown dose dependent effect when administered orally at the dose of 10mg/kg & 20mg/kg. The exact mechanism of action of DMF for this effect is not clear. Further research is required to establish its exact mechanism of action and utilization of DMF in the treatment of Parkinson’s disease.

References:


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Development and invivo evaluation of once a day chronotherapeutic drug delivery system of Gymnema sylvestre for treatment of diabetes

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Abstract: Gymnema sylvestre is a potent antidiabetic plant used in traditional systems of medicine. This study investigated the potential of applying technological advances in drug delivery to traditional medicine. The chronotherapeutic drug delivery is selected as diabetes requires a pulse of therapeutic concentration which coincides with high blood sugar levels. The system was designed to achieve three pulsatile drug deliveries at the time when the blood sugar level is high. The formulation was evaluated in vivoin rat model. The results showed significant improvement by treatment and curative action evidenced by control of blood sugar level even after stopping the therapy.

Introduction:
The potential of Ayurveda and herbal medicines is being realized in the recent years. These medicines have lot of advantages over chemical pharmaceuticals. However, the drug delivery system used for administering the herbal medicine to the patient is traditional and out-of-date, resulting in reduced efficacy of the drug. Gymnema sylvestre has long history as a treatment for diabetes and it not only prevents or delays the development of type 2 diabetes, but more important, reverses the effects of the disease. If the novel drug delivery technology is applied in herbal medicine, it may help in increasing the efficacy and reducing the side effects of various herbal compounds and herbs. A novel drug delivery approach can be employed and chronotherapeutic systems can be designed which are designed over the concept of chronopharmaceutics in which there is specificity in delivering higher amount of drug in a burst at circadian timings correlated with specific pathological disorder to achieve maximum drug effect. Diabetes is one of the disease where the constant drug levels are not preferred but needs a pulse of therapeutic concentration when the blood sugar levels are high i.e., after the meals which can be seen from the figure. The proposed therapy can combine the benefits of both herbal drugs and novel drug delivery technology to provide a more patient friendly, holistic treatment for diabetes and obesity with Gymnema sylvestre.

Materials and Methods:
There are various approaches to design chronotherapeutic systems to achieve pulsatile drug release. For the oral delivery of gymnema sylvestre time controlled chronotherapeutic systems are selected. The system can be visualized as having three sub-parts. Each sub part of the drug delivery system was designated to deliver a dose at the selected time interval from the dosage form in the small intestine. Gymnema sylvestre 75% extract at the dose of 300mg/day was used for developing the dosage form. The chronotherapeutic system was achieved by encapsulating all the three developed parts of the formulation in one capsule. All the three formulations were filled in a capsule and the capsule was sealed. The prepared capsules were evaluated for average weight of filled capsules, weight variation, in-vitro disintegration test and in-vitro release profile. The chronotherapeutic formulations were enteric coated so as to make the drug available at the site of action and probably the site of absorption, as well as to prevent the side effects in the stomach. The in vitro drug release profile of the developed system was done initially for two hours in 0.1N HCl and later in 6.8 pH Phosphate buffer. The stability study of the developed enteric coated Chrono system was performed as per ICH guidelines.

To evaluate the anti diabetic and anti obesity potential of developed chronotherapeutic drug delivery system of gymnema sylvestre an animal was generated and standardised. The model selected was a combination of HFD and low dose of STZ (35mg/kg, ip) treatment which mimic the metabolic characteristics of the common type 2 diabetes in humans. Weaned albino wistar rats were fed with high fat diet for 6 weeks and then challenged with low dose stz. After the confirmation of diabetic parameters, treatment was administered for a period of 6 weeks. During the treatment period, body weight was determined every week. The food consumption, water consumption, cage quality and the overall wellbeing of the animals was also monitored and recorded at weekly intervals. The blood glucose was determined at every 15 days interval during the treatment period. The biochemical parameters for diabetes and obesity viz. fasting blood glucose level, serum insulin, glycosylated hemoglobin levels, cholesterol, triglycerides, LDL and VLDL were measured after 4 weeks and 6 weeks of treatment. The glycosylated hemoglobin and serums insulin were determined after completion of the study period.

Results and Discussion:
The time controlled chronotherapeutic delivery system of Gymnema sylvestre was designed to contain three subparts which were individually developed and optimized. The chronotherapeutic system was achieved by encapsulating all the three developed parts of formulation in one capsule and the system was enteric coated. The invitro release study showed three pulse releases from the dosage form at the desired time interval coinciding with the high blood glucose level after breakfast, lunch and dinner.
The optimized drug loaded formulation was evaluated in the indigenously developed obese diabetic rat model. The results showed significant improvement by treatment of GS chrono formulation when compared with the extract at the same dose. This is attributed to the three pulsatile releases from the formulation. The therapeutic efficacy of the developed formulation was similar to the standard drug. Additionally the developed formulation when compared with the standard drug exhibited a curative action evidenced by the control of the blood sugar level even after stopping the drug administration. Further the results in table below showed that the developed formulation was therapeutically superior when compared to pure extract.

Table 1 The fasting blood glucose levels during the treatment period.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Group</th>
<th>Fasting blood glucose levels in the week mg/dl (Mean ± SD)</th>
<th>6 weeks therapy</th>
<th>8 weeks (Treatment discontinued after 6 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>78.50±2.08</td>
<td>76.56±4.76</td>
<td>78.67±4.20</td>
</tr>
<tr>
<td>2</td>
<td>DBT Control</td>
<td>248.50±18.15</td>
<td>321.54±21.34</td>
<td>338.31±24.5</td>
</tr>
<tr>
<td>3</td>
<td>TRT: EXT 50</td>
<td>243.12±15.30</td>
<td>153.34±7.45 c</td>
<td>169.45±5.98 c</td>
</tr>
<tr>
<td>4</td>
<td>TRT: CHR 50</td>
<td>239.54±9.67</td>
<td>104.54±6.54 c</td>
<td>138.6±7.76 c</td>
</tr>
<tr>
<td>5</td>
<td>TRT: GLB</td>
<td>242.45±13.60</td>
<td>94.21±6.70 c</td>
<td>206.5±9.51 c</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD; (n= 6). Statistical analysis – One-way ANOVA followed by Dunnett’s multiple comparisons test. GRP2Vs GRP 3-5 was shown as a (P< 0.05), b (P< 0.01) c (P< 0.001)

Conclusion:

The Gymnema sylvestre chronotherapeutic drug delivery system was developed to achieve three pulse releases from a single system. The invitro drug release indicated three pulse releases from the single system. The preclinical study proved the efficiency of the Gymnema sylvestre extract and formulations to treat diabetes, lipidic parameters and associated obesity.

Acknowledgement:

The authors are thankful to Al-Ameen College of Pharmacy for providing facilities for conducting this research work.

References:

Bioactive-loaded polymer dressings to improve healing in diabetic rat

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Abstract: In the present study, polymer based dressings were prepared to be applied as scaffold for the control release of a triterpenoid that acts as an inflammatory modulator in wound healing. The activity of bioactive loaded polymer matrices to treat wounds in streptozotocin diabetic induced rat was evaluated. There was a delay in wound healing in diabetic rats as compared to non-diabetic rats. The dressing exhibited potent wound healing activity as indication by the wound contraction in the excision wound model. The contents of hydroxyproline also correlated with the observed healing pattern. These results suggest that dressings can be an effective support for release into wound enhancing the healing process.

Introduction:
Normal wound healing is characterised by an organized progression of cell and tissue responses that can be grouped into four major phases i.e. Haemostasis, Inflammatory, Proliferation and Remodelling Phase [1]. In Diabetic wounds, a persistent inflammatory phase associated with an impediment in the formation of mature granulation tissue and reduction in wound tensile strength. In India, the diabetic foot represents a significant health problem, provoked by the high frequency of infection and the ever-rising incidence of diabetes. Inflammation and wound healing are complex processes and linked, and are disturbed in the situation of chronic, non-healing diabetic wounds [2, 3]. An ideal therapy for DWs should both suppress excessive inflammation while enhancing healing. It proposed that natural environment would clarify mechanisms and facilitate improve therapeutic approaches to DWs. We aimed to developed, a smart drug delivery system i.e. plant bioactive-loaded polymer dressings which reduce inflammation and improve healing in diabetic patient.

Materials and Methods:
Lupeol (Sigma aldrich), Arginine (Himedia) collagen (Sigma Aldrich) and Streptozotocin (Himedia). All other reagents and solvents were of analytical grade.
Arginine and collagen hydrogel containing lupeol were prepared by freeze thaw method.

Characterization
Morphology and Particle size analysis
Hydrogel were characterized by optical microscopy, SEM and water vapour transmission rate (WVTR).
In vitro release studies

*In-vitro* release studies were conducted in triplicate and mean values and standard deviations were calculated.

**Diabetes induction**

Diabetes was induced by intraperitoneal injections of STZ (50mg/kg body weight), in citrate buffer pH 4.5, during 5 consecutive days.

**Results and Discussion:**

**Characterization of Arginine and collagen hydrogel**

SEM revealed the uniform dispersion of Lupeol in a three-dimensional lamellar matrix of the arginine and collagen hydrogel formulation. The water vapour transmission rate of the hydrogel was found to be 1702 g/m²/day which is very close to the ideal value for wound dressing and indicating the suitability of film to be used as moisture retentive material as moist dressing.

**In vitro release studies**

In-vitro release data showed that hydrogel showed a typical biphasic release pattern with burst release followed by sustained release. The burst release is mainly due to plant actives entrapped on the surface.

**In vivo studies**

Significant positive differences (p < 0.01) between treated and control groups were observed at different aspects of diabetic wound healing process. Reduced inflammation and enhanced wound contraction, re-epithelialisation, regeneration of granulation tissue, angiogenesis and collagen deposition were detected in the treated wounds. This was also supported by hydroxyproline and protein content. A linear increase in total protein content was observed in all groups including control.

**Table 1** Effect of formulation on excision wound model

<table>
<thead>
<tr>
<th>Group</th>
<th>Epithelization Time (Days)</th>
<th>Hydroxyproline content (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-animals treated with streptozotocin only</td>
<td>21.00 ± 0.11</td>
<td>150.6 ± 2.69</td>
</tr>
<tr>
<td>II-with prepared arginine and collagen hydrogel (without any drug)</td>
<td>19.00 ± 0.77</td>
<td>162 ± 1.77</td>
</tr>
<tr>
<td>III-animals treated topically with lupeol formulation</td>
<td>18.80 ± 0.33</td>
<td>178 ± 1.55</td>
</tr>
<tr>
<td>IV-animals treated topically with ARG COL LU formulation</td>
<td>16.01 ± 0.35</td>
<td>198.16 ± 0.33</td>
</tr>
</tbody>
</table>

**Table 2** Effect of formulation on % wound contraction

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 9</th>
<th>Day 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-animals treated with streptozotocin only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-with prepared arginine and collagen hydrogel (without any drug)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-animals treated topically with lupeol formulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-animals treated topically with ARG COL LU formulation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th>Description</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-Animals treated with streptozotocin only</td>
<td>11.12 ± 2.17</td>
<td>34.34 ± 0.93</td>
<td>55.54 ± 0.37</td>
</tr>
<tr>
<td>II-with prepared arginine and collagen hydrogel (without any drug)</td>
<td>13.11 ± 3.22</td>
<td>61.96 ± 0.21</td>
<td>79.76 ± 0.44</td>
</tr>
<tr>
<td>III-Animals treated topically with lupeol formulation</td>
<td>12.13 ± 2.33</td>
<td>68.76 ± 0.36</td>
<td>88.39 ± 0.77</td>
</tr>
<tr>
<td>IV-Animals treated topically with ARG COL LU formulation</td>
<td>17.99 ± 3.12</td>
<td>80.01 ± 1.83</td>
<td>99.12 ± 0.88</td>
</tr>
</tbody>
</table>

### Conclusion:

We concluded that Lu–loaded polymer dressings are effective as wound healing accelerators in diabetic rat. Moreover, collagen and Arginine stimulated tissue granulation, collagen expression and deposition at the wound site, which lead to the production of a more organized collagen matrix and angiogenesis as compared with lupeol alone. The promising results obtained in this work need also to be complemented with human studies to further investigate the potential application of wound dressings.

### Acknowledgement:

The presenter is thankful to Director, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G), India for this study and UGC-BSR for financial assistance relating to this work.

### References:

Immunomodulatory activity of ethyl acetate fraction of *Calotropis procera* latex in mice

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Abstract: The influence of *Calotropis procera* (*C. procera*) active fraction for immunomodulatory activity in mice was studied. The dried latex was serially extracted with petroleum ether, chloroform, ethyl acetate and ethanol and screened by qualitative and quantitative phytochemical (Total flavonoids and total phenolics) analysis. The ethyl acetate extract showed maximum response was selected, screened by immunomodulatory activity using immunological tests in mice, viz. the humoral mediated antibody titre (AT), delayed-type hypersensitivity (DTH) showed dose dependently enhanced the serum primary and secondary antibody level and mean foot pad thickness at 0, 3, 24, and 48 h. The above findings suggest that latex of *C. procera* stimulate defence system by modulating primary immunological parameters and can be complementary medicine for the management of immunodeficiency disorders.

Introduction:
Immunomodulation using medicinal plants can provide an alternative especially when host defense mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders (Rheumatoid Arthritis) [1]. *Calotropis procera* (Ait.) known as Aak in Indian subcontinent, is a species from the Asclepiaceae family, used popularly to treat rheumatic diseases folklore, however, there is no scientific demonstration of its therapeutic efficacy [2].

Materials and Methods:
Plant material: The plant *C. procera* was identified and authenticated from Botanical Survey of India, Pune. Voucher specimen (BSI/WRC/Tech./2010/GAPCAP1) was kept at the centre.

Animals: The experiments were carried out on Swiss albino mice (*Mus musculus*, 20–30 g). All experimental procedures were carried out in accordance with the guidelines of IAEC following approval.

Preparation of extract: Latex was collected from the plant, protect from light to prevent any oxidative changes. Dried and ground to coarse powder, successively extracted with petroleum ether, chloroform and ethyl acetate and ethanol.

Qualitative and quantitative phytochemical screening[3].
Phytochemical tests were used to detect secondary metabolites by qualitative tests. Determination of the total phenolic content and total flavonoid content were used quantitatively.
Immunomodulatory activity[4]

**Haemagglutinating antibody (HA) titre:** Mice were divided into four groups each containing six mice. Group I control and given sodium carboxy methyl cellulose (0.5%, p.o.). Group II Standard drug levamisole (50 mg/kg, p.o.). Group III and IV were given ethyl acetate extract of (50, and 100 mg/kg p.o.) for eight days (day 0 to day 7) after sensitization with sheep red blood cells (0.1 ml of sheep RBC suspension containing 1x 10^8 cells i.p.). Antibody levels were determined by the haemagglutination technique on 7th and 14th day.

**Delayed type hypersensitivity (DTH):** After sensitization with sheep RBC on day +7, in the right hind foot pad, the thickness was measured. The foot thickness was measured at 0, 3, 24 and 48 h. The difference was taken as the measure of DTH response.

**Results and Discussion:**

After successive extraction of dried latex of *C. procera* g% yield of the extracts were obtained viz. Petroleum ether (0.32), Chloroform (2.2), Ethyl acetate (0.84), Ethanol (5.2) respectively

**Qualitative phytochemical screening:** Results from our phytochemical analysis suggest that the presence of biologically active compounds in the latex could be correlated to the immunomodulatory effects. Qualitative phytochemical study revealed us that, out of all extracts, ethyl acetate extract was rich in total flavonoid, phenolics contents.

**Quantitative phytochemical screening:** Ethyl acetate extract was selected for further study as confirmed by determination of the total phenolic content: Aluminum chloride and total flavonoid content: Folin–Ciocalteu’s method.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant Part</th>
<th>Extract</th>
<th>TPC Conc. Mcg/mg</th>
<th>TFC Conc. Mcg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Latex</td>
<td>PE</td>
<td>1.61</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>CH</td>
<td>5.14</td>
<td>3.12</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>EA</td>
<td>37.5</td>
<td>215.6</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>ET</td>
<td>20.4</td>
<td>103.7</td>
</tr>
</tbody>
</table>

**Immunomodulatory activity**

1. **Primary and secondary antibody titre:** The HA titre was used to assess humoral immune response. A dose-related augmentation in both primary and secondary antibody titre was observed in mice.
Tabulated values are mean ± S.D. *P < 0.01, highly significant; **P <0.05, significant Tukey-Kramer test (n=6) EA (ethyl acetate fraction of latex);

2. Delayed type hypersensitivity: The cell-mediated immune response was assessed by DTH reaction, i.e. foot pad reaction. T cell dependent antigen revealed the stimulatory effect of ethyl acetate extract.

Figure 1 Effect of Calotropis procera extracts on foot pad reaction of antigenically challenged mice.

Values are mean ± S.D. *P < 0.001, highly significant; **P <0.01, *P <0.05, significant as compare to control. Tukey-Kramer test (n=6)

Conclusion:

Present study shows that latex of C. procera markedly reduce inflammation and also have potent immunomodulatory property, has the potential to be used and has to be explored to its mechanism of action.

References:

Hepatoprotective efficacy of *Lannea coromandelica* against paracetamol treated experimental rats-an in vivo study

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**Abstract:** Liver disorders are one of the widespread current problems affects on the human being. In the current study, paracetamol was selected to induce hepato-toxicity in rats. *Lannea coromandelica* (*L. coromandelica*) leaf was extracted with methanol and ethyl acetate (100, 200 and 400 mg/kg body weight, p.o.), and is examined for the paracetamol (500 mg/kg bw) induced hepato-toxicity. Their activity was compared with standard hepatic drug silymarin (100 mg/kg b.w.) for 10 days. The hepatoprotective effect of different treatments was assessed by evaluating the changes in functional parameters like alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), serum bilirubin (SB), cholesterol and serum albumin (SA) in all groups of animals. From this study, it can be concluded that the both of extract of *L. coromandelica* possess anti-hepatotoxic action against paracetamol toxicity. Ethyl acetate extract showed the most significantly decrease in the liver enzymes.

**Introduction:**
Currently, peoples paying their attention towards the importance of dietary antioxidant components for the betterment of human health due to the uneasiness about the side effects of synthetic antioxidants. Liver, a vital organ of human body, playing a great role in metabolism and variedly exposed to xenobiotics be-cause of its strategic placement in the body. Hence there is growing interest in natural poly-phenolic antioxidants, present in medicinal and dietary plants that help assuage oxidative damage [1]. Paracetamol (PCM), an antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice at toxic doses. Hence this is commonly used to induce toxicity in liver. [2]. *Lannea coromandelica* (*L. coromandelica*) Houtt. Merrill. (Anacardiaceae), widely distributed in Bangladesh, India and some other tropical countries. It was commonly called as Woodier or Indian ash tree. *L. coromandelica* showed innumerable pharmacological activities such as hypotensive, antimicrobial, and wound healing, anti-cancerous and *in-vitro* antifilarial activities.

**Materials and Methods:**
*Collection of plant material:* The plant *L. coromandelica* were collected from forest, managed by Government of Chhattisgarh State Forest Division. The collected plant was botanically recognized by Dr.
V. P. Prasad. A voucher specimen (CNH/Tech.II/2014/70/139) was submitted to the Central National Herbarium, Howrah, India. The fresh leaves were carefully washed with de-ionized water and dried at room temperature. The dried leaves were manually ground into fine powder.

**Preparation of extract:** Air dried leaf powder (8 g) of *L. coromandelica* was extracted with 80% methanol by buchi speed extractor and extraction was repeated tree times. Same step is followed for ethyl acetate also. The methanol extract (ME) and ethyl acetate extract (EA) were filtered through filter paper Whatman no. 1. Then solvents were removed by using rotary evaporator (Ika RV 10). The yield of extract and its fractions was measured and maintained at 4 °C for further use.

**Animals:** Wistar rats (150–250 g) of both sexes were used for antioxidant activity. The animals were obtained from animal house of Rungta College of Pharmaceutical Sciences and Research, Bhilai, Chhattisgarh, India. The studies were performed as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India.

**Toxicity Studies:** Acute toxicity study was performed as per the protocols of OECD guidelines 423. Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg were administered to female rats as single dose. Animals were observed individually at during the first 30 minutes after dosing, periodically during the first 24 hr, and daily thereafter, for a total of 14 days. The dose of 100 mg/kg, 200 mg/kg and 400 mg/kg, were selected for the further activity.

**Paracetamol induced oxidative toxicity:** Rats were divided into nine groups (n= 5 in each group). Group I received vehicle only (0.3% CMC-Na; 1 mL/kg body weight, p.o.) for 5 days. Group II (Paracetamol) received 0.3% CMC-Na (1 mL/kg body weight, p.o.) for 5 days. Group III to VIII were treated with paracetamol and methanol and ethyl acetate extract of *L. coromandelica* (100, 200 and 400 mg/kg body weight, p.o.) respectively, for 5 days. Group IX received paracetamol and the standard drug silymarin (100 mg/kg body weight, p.o.). On day 6, animals were sacrificed by decapitation and blood and liver was carefully dissected, cleaned of extraneous tissue. 10.0% w/v homogenates were prepared by mincing and homogenizing 1 g of liver with cold 50 mM potassium phosphate buffer (pH 7.4) and centrifuged at 6000 rpm for 10 min at 4°C. The cell free supernatant was used for the biochemical testing [3, 4].

**Result and Discussion:**

PCM (500 mg/kg bw) given once orally showed hepato-toxicity after 24 h as evident from biochemical parameters. Presently it is hypothesized that herbal drugs inhibit the CYP2E1 enzyme activity in hepatic microsomes in vivo and in vitro and give protection against hepato-toxicity induced by PCM, CCl₄ and nitrosamines. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases. Pretreatment with both the herbal extracts restored the liver enzyme parameters showing a dose
dependent effect Table 1. The reduction of liver enzyme parameter, ALT was significant and showed as a specific marker of liver injury due to toxic drugs, alcohol and virus. This hepatoprotective activity of the plants can be attributed the presence of flavonoids like constituents.

**Table 1** Pretreatment with both the herbal extracts restored the liver enzyme parameters showing a dose dependent effect

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP(U/L)</th>
<th>SB (mg/dl)</th>
<th>CHL (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35.53± 1.12</td>
<td>91.82±5.04</td>
<td>149.54±12.36</td>
<td>0.27± 0.11</td>
<td>146.17± 21.31</td>
</tr>
<tr>
<td>II</td>
<td>323.23± 11.41</td>
<td>290.12 ±15.23</td>
<td>263.61 ± 39.61</td>
<td>1.54± 0.18</td>
<td>316.32± 13.54</td>
</tr>
<tr>
<td>III</td>
<td>169.18±14.45*</td>
<td>318.57± 11.25</td>
<td>364.33± 15.10</td>
<td>1.11± 0.13</td>
<td>308.17± 17.21</td>
</tr>
<tr>
<td>IV</td>
<td>119.38± 9.34*</td>
<td>250.93± 15.27*</td>
<td>201.78± 12.24</td>
<td>0.87± 0.05*</td>
<td>275.31± 18.95</td>
</tr>
<tr>
<td>V</td>
<td>54.76± 4.39***</td>
<td>139.19±18.28**</td>
<td>118.78± 8.32***</td>
<td>0.43± 0.09***</td>
<td>206.81±12.51**</td>
</tr>
<tr>
<td>VI</td>
<td>223.34± 15.05</td>
<td>378.48± 23.08</td>
<td>398.59± 15.21</td>
<td>1.27± 0.08</td>
<td>387.45± 27.12</td>
</tr>
<tr>
<td>VII</td>
<td>183.55± 13.71*</td>
<td>285.85± 19.45</td>
<td>303.31± 13.25*</td>
<td>0.96± 0.07</td>
<td>297.22± 22.76</td>
</tr>
<tr>
<td>VIII</td>
<td>110.51±12.07**</td>
<td>218.01± 16.55*</td>
<td>223.95± 17.36*</td>
<td>0.55± 0.08*</td>
<td>225.41± 12.51*</td>
</tr>
<tr>
<td>IX</td>
<td>44.52± 7.24***</td>
<td>116.16±11.36***</td>
<td>126± 13.12***</td>
<td>0.31± 0.08***</td>
<td>193.62±12.26**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n=5, analysed by one-way ANOVA followed by Student-Newman-Keuls test as post-hoc test. *P< 0.05, **P< 0.01 and ***P< 0.001 compared to solvent + PCM group I.

**Conclusion:**

Based on the results obtained from the study it can be concluded that both of the extract of *L. coromandelica* possess significant hepatoprotective activity. The present study indicates that the ethyl acetate extract showing more significant activity than the methanolic extract. This is possible due to presence of poly phenolic component present in ethyl acetate extract in higher amount than methanol extract. Further investigation is underway to determine the exact phyto-constituents in the extracts that are responsible for its hepatoprotective action.

**Acknowledgement:**

Author would like to grateful acknowledge to the Director, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh for providing research facilities.

**References:**


Effect of *aconitum heterophyllum* w. ethanol extract in experimentally induced hyperlipidemic rats

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Abstract: In the present study, ethanolic extract of *Aconitum heterophyllum* W. roots (EAR) was evaluated for exploring its antihyperlipidemic effect in rats. Hyperlipidemia was induced using Triton WR 1339 at a dose of 250mg/kg body weight by i.v. route in rats. The wistar rats were divided into control group, hyperlipidemic group, Gemfibrozil group, EAR treated groups (200, 400 mg/kg, p.o.). The groups treated with EAR showed significant reduction (p<0.001) in levels of triglyceride as compared to hyperlipidemic group after triton induction. Thus it was concluded that ethanolic extract of *Aconitum heterophyllum* W. roots was effective in treating hyperlipidemia.

Introduction:
Cardiovascular disorders are very common cause of mortality worldwide [1]. It is well known that hyperlipidemia represents major risk factor for atherosclerosis and cardiovascular complications [2]. The best approach for prevention or treatment of atherosclerosis and CVS complications is to target the lipid profile of hyperlipidemic patients with the help of lipid lowering drugs or improving the diet. Traditional systems medicine like Ayurveda, Unani, and Chinese prescribe many herbal remedies for these disorders. However a numbers of herbal drugs are still to be evaluated pharmacologically. *Aconitum heterophyllum* W. (family ranunculaceae) commonly known as *Atis* is a perennial herb. It is distributed over alpine pastures of Central Himalayas, ranging altitude from 3500m to 4000m. Various studies on traditional medicine system have shown that it is used in curing hysteria, dyspepsia, abdominal pain, throat infection, diabetes and is considered as a valuable febrifuge nerve tonic especially combating debility after malaria[3]. The plant contains alkaloids atisine, isoatisine, hetisine, heteratisine, hetsinone, heterophyllisine, heterophylline, heterophyllidine, benzoylheteratisine and atisenol a new entatisene diterpenoid lactone[4]. However, its antihyperlipidemic activity has not been investigated scientifically so far. Hence, the present study was designed to investigate the antihyperlipidemic activity of *Aconitum heterophyllum* W. on Triton WR- 1339 induced hyperlipidemia in rats.

Materials and Methods:
Collection and Extraction: The roots of *Aconitum heterophyllum* W. was collected from the local market of Bhopal, during the month of November. The specimens were identified and authenticated by Dr. Zia Ul Hassan, Assistant Professor, Department Of Botany, Safia College of Science and Education, Bhopal (voucher specimen no. 415/bot/safia/12). The roots were dried, coarsely powdered and then exhaustively extracted with 90% ethanol in soxhlet apparatus for 72 hr. Total extract was evaporated under vacuum.

Phytochemical screening: The ethanolic extract was then tested qualitatively for the identification of various plant constituents as according to the standard methods [5].

Drugs: All the chemicals used were of analytical grade. Triton WR 1339 (sigma chemicals), Gemfibrozil capsules USP 300mg, (Lopid Pfizer ltd), Erba diagnostic kit (Transasia bio medicals).

Experimental protocol: The wistar rats (130-200g) were housed with 12 h light and dark cycle at room temperature 22-23°C and fed with standard chow diet and water. The rats were divided into five different groups containing six animals in each group. Group I served as normal control received 0.5% CMC orally for 7 days. Group II was negative control treated with Triton WR 1339 (250mg/kg i.v.). Group III was positive control and received Gemfibrozil (50mg/kg/day p.o.) for 7 days. Group IV and V were treated with EAR (200 and 400mg/kg/day p.o.) for 7 days. After 7 days of treatment, Triton WR 1339 was administered 250mg/kg b.w. by i.v. route in group II, III, IV and V and blood was collected at time 7 h and 24 h from each group to compare the TG level between all the groups by using enzyme assay kit.

Results and Discussion:

The phytochemical analysis of the *Aconitum heterophyllum* root extracts showed the presence of alkaloids, carbohydrates, protein & amino acid, saponins, phenolic compounds and tannins, quinones, flavonoids, steroids, terpenoids. Glycosides were not present in the extract.

The level of triglycerides in serum for control, hyperlipidemic, Gemfibrozil and EAR treated groups (200, 400 mg/kg) after 7 and 24 hr from treatment are reported in table 1. The results showed that increased triglycerides levels were significantly (p<0.001) reduced by treatment of 200 and 400mg/kg of EAR.

Triton WR – 1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting into increased blood lipid concentration. *Aconitum heterophyllum* W. seems to have important role in preventing the development of triton WR 1339 induced hypertriglyceridemia. The lipid lowering effect of the root extract might be due to an early clearance of lipid from circulation in hyperlipidemic model. Its protective effect may be related to its anti-inflammatory property since administration of triton WR 1339 induces inflammation of hepatic portal triad and sinusoidal vein.
Conclusion:
It was concluded that ethanolic extract of *Aconitum heterophyllum* W. roots is helpful in decreasing increased triglycerides levels in blood and the study can be further explored for its chronic study and other lipid parameters.

**Table 1** Effect of EAR extract on serum TG level in Triton WR 1339 induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Groups</th>
<th>7 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control group</td>
<td>68.51±0.1</td>
<td>63.82±1.03</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic</td>
<td>98.14±1.40</td>
<td>120.39±1.34</td>
</tr>
<tr>
<td>III</td>
<td>Standard group</td>
<td>43.57±0.14**</td>
<td>78.66±0.13***</td>
</tr>
<tr>
<td>IV</td>
<td>EAR 200</td>
<td>58.88±0.2***</td>
<td>85.96±1.67***</td>
</tr>
<tr>
<td>V</td>
<td>EAR 400</td>
<td>53.81±0.15***</td>
<td>81.83±1.52***</td>
</tr>
</tbody>
</table>

Values are expressed as mg/dl, are mean±SEM of 6 animals in each group, ***p <0.001 when group III, IV, V were compared with group II.

Acknowledgement:
The authors are grateful to Faculty of Pharmacy, VNS Group of Institutions, Bhopal, India for providing all the necessary facilities, technical assistance during this work and Madhya Pradesh Council of Science and Technology, Bhopal for funding the project.

References:
Hydrogen sulfide synthesis enzyme inhibition in brain increases the severity of psychological stress induced ulcers in mice

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Abstract: The objective of the present work was to inhibit the synthesis of H₂S in brain and study its effect on psychological stress-induced ulcers in mice. Psychological stress was induced by a method, wherein mice in a group (responders) were allowed to visualize another group (senders) receiving electrical stimulus (1.6-2.0 mA/5 sec per min/ 3 hr for 3 days). The responder mice were grouped and given H₂S inhibitor (1 mg/kg; 5 mg/kg, i.p.). Diazepam was used as standard. The results revealed a dose-dependent increase in the incidence of ulcer as indicated by increased ulcer index, decreased mucin content and histopathological studies.

Introduction:
Stress is defined as an acute threat to the homeostasis of an organism. Stress evokes adaptive responses that serve to defend the stability of the internal environment [1]. Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. The peptic ulcers are erosion of lining stomach or the duodenum [2]. Psychological stress for prolonged period is reported to increase the incidence of gastric ulcers. Hydrogen sulfide along with carbon monoxide and nitric oxide is an important signalling molecule and it is involved in various physiological activities associated with vascular contractility, pro- and anti-inflammatory activities [3]. Endogenous hydrogen sulfide is produced from L-cysteine by cystathionine gamma lyase and cystathionine beta synthase (CSE and CBS), and 3-mercaptoppyruvate sulfurtransferase (3- MST).

Materials and Materials:
Materials: The drug O-carboxy methyl hydroxylamine hemichloride was purchased from Sigma Aldrich (MO, U.S.A.). All other chemicals were of AR grade.
Animals: Swiss albino mice of body weight 25g-30g were used in this experiment.
Psychological stress induced ulcer model: The experimental group consists of the following 2 groups: sender, and responder group. Sender animals received a foot shock of 10 sec duration at intervals of 50 sec for 3 hour. The electrical current for the shock is increased step-wise from 1.6 mA to 2.0 mA per hour for 3 day sender animals are changed daily. Both sender and responder animals were placed individually.
in each compartment of the communication box 15 min before beginning the shock period. On day-1, responder animals were returned to their home cages after the 3 hr foot shock period. On day-2, after the completing foot shock period, they were transferred to metal cages and were housed in the cages with 4 animals per cages under food deprivation condition. On day-3, just after the completing the foot-shock period, the responders were sacrificed by chloroform, and their stomach was removed. The stomach was visually inspected for lesions. Drug (O-carboxy methyl hydroxylamine hemihydrochloride) was administered intraperitoneally with low (2mg/kg), medium (5 mg/kg), high (10 mg/kg) dose daily for 3 days respectively, 30 min before the shock period.

**Determination of gastric pH:** After the collection of gastric juice from stomach in test tube 1-2 drops phenolphthalein indicator was added and titrated with 0.01 N NaOH until the colour changes from purple to colourless. The volume of NaOH consumed was noted and used to calculate the pH.

**Estimation of mucin:** After the collection of gastric juice the stomach were cut. The stomachs were drenched for 2 hr in 0.1% alcian blue 8 GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate to a pH with hydrochloric acid. Uncomplexed dye was detached by two consecutive washes of 15 min with 0.25 M sucrose solution. A dye complex with mucus was diluted by dipping it in 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The resulting blue solutions were shaken vigorously with an equal volume of diethyl ether and the absorbance of the aqueous phase was measured at 605 nm using UV Spectrophotometer [4]. The content was determined from the linearity.

**Histopathological analysis:** Histological studies were performed by taking a small piece of tissue, including ulcers; embedded in paraffin and sectioned at 5 µm in an automated microtone. Haematoxylin and eosin staining was done and tissue was observer under microscope.

**Results and Discussion:**

**Effect of O-carboxy methyl hydroxylamine hemichloride administration on the mean ulcer index of mice**

One way ANOVA revealed a significant influence of O-CHH treatment on the UI of mice [$F (3, 13) = 33.42; \ P < 0.001$]. Further, analysis revealed that CHH increased the gastric erosion significantly at 5 mg/kg doses ($P < 0.05$) with maximum increase observed at 10 mg/kg.

**Effect of O-carboxy methyl hydroxylamine hemichloride administration on the gastric pH of mice**

One way ANOVA revealed a significant influence of O-CHH treatment on the gastric pH of mice [$F (3, 13) = 38.05; \ P < 0.001$]. Further, analysis revealed that CHH increased the gastric pH significantly all doses ($P < 0.05$), with maximum increase observed at 10 mg/kg.

**Table 1** Estimation of mucin
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (saline)</td>
<td>0.2ml</td>
<td>5.491±1.14</td>
</tr>
<tr>
<td>II</td>
<td>Low dose (CBS Antagonist)</td>
<td>1 mg/kg</td>
<td>2.370±0.121*</td>
</tr>
<tr>
<td>III</td>
<td>Medium dose (CBS Antagonist)</td>
<td>5 mg/kg</td>
<td>2.754±0.125*</td>
</tr>
<tr>
<td>IV</td>
<td>Standard (Diazepam)</td>
<td>0.5 mg/kg</td>
<td>4.100±0.156#</td>
</tr>
</tbody>
</table>

*/# P < 0.05 w.r.t respective control group

Histopathological analysis:
In the histopathological examination, stomachs of control rat shows erosion in the upper part of epithelium and redness are seen in the eroded portion, stomachs of rats treated with standard drug (diazepam) showed less erosion with a minimal deviation from normal morphology. Stomach of rats treated with CBS antagonist showed small superficial erosion with minimal deviation from normal morphology.

Conclusion:
The present study revealed that inhibition of H$_2$S synthesis exacerbated the psychological stress induced ulcers implicating an important role of H$_2$S in regulation of stress induced effects on gastric physiology.

References:
Hepatoprotective activity of ethanolic extract of *Solanum surattense* root

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Abstract: The hepatoprotective effects of ethanolic extract of *Solanum surattense* root were studied in rats by inducing hepatotoxicity with paracetamol 0.5 gm/kg body weight in single oral dose. The ethanolic extract of *S. surattense* (600 and 800 mg/kg) was given orally in two divided dosage. The study parameters were conducted on day 9, which was evident by the decrease in serum aspartate amino transferase (AST/SGOT), alanine amino transferase (ALT/SGPT), Billirubin concentration and increase in hemoglobin when compared with the group of rats with paracetamol alone.

Introduction:

*Solanum surattense* Burm f. belongs to the family Solanaceae. It is a commonly growing perennial herbaceous weed [1, 2]. The plant showed antidiabetic [3, 4], wound healing activity [5], diuretic and antioxidant activities. In present study the hepatotoxicity was induced by administering a single oral dose of PCM (500 mg/kg bw) on 8th day. Serum alanine amino transferase (ALT), aspartate amino transferase (AST) activities and total bilirubin concentration was estimated.

Materials and Methods:

Plant materials: The entire plant of *Solanum surattense* (Kantkari) was collected from Bhander, distict Datia M.P. The plant material was authenticated by Dr. M. K. Panigrahi, Principal, Hi-Tech college of Pharmacy Bhubaneswar, Odisha.

Median effective dose (ED$_{50}$): The "median effective dose means the dose effective in producing certain expected response in 50% of the animal group. It helps in ascertaining the potency of a drug. It is also sometimes abbreviated as the ED$_{50}$, meaning “effective dose, for 50% of animals receiving the drug”.

Experimental protocol: 24 Wistar albino rats weighing between 200 to 250 g, of either sex, were used. The animals were allowed food pellets (Hindustan Lever, Mumbai) and water ad libitum. Animals were maintained in standard lab conditions (12 h: 12 h dark and light cycle & 25±2 0 C temperature).

The animals were randomized into four groups, each group comprising six animals.

Group I (G1) Normal control animals treated with 0.5% tween 8
Group II (G2) Paracetamol (0.5 gm/kg body weight, in 0.5% tween 80. Group III (G3) Animals treated with ethanolic extract of *S. surattense* (600mg/kg body Weight /day in 0.5% tween 80 orally in two divided doses for a period of
10 days), after administration of paracetamol (0.5gm/kg body weight) Group IV (G4) Animals was pre-treated with ethanolic extract of *S. surattense* (800mg/kg body Weight/day in 0.5% tween 80 orally in two divided doses for a period of 10 days), after administration of paracetamol (0.5gm/kg body weight) Blood was collected through tail vein of the rat.

**Ethanolic extract**: The powdered root was Soxhlet extracted successfully with 70% Ethanol following the complete removal of solvent and the final yield was 7.7% of the original.

**Haematological methods**: Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), haemoglobin concentration were measured.

**Statistical analysis**: The statistical significance of the data was calculated using ANOVA.

**Results & Discussion**: Results are tabulated as

**Table 1 Changes in Haemoglobin and Billirubin with Ethanolic extract of *S. surattense* root**

<table>
<thead>
<tr>
<th>Group</th>
<th>Hb (g/dl) (Mean ± S. E.)</th>
<th>Billirubin mg/dl (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
</tr>
<tr>
<td>G1</td>
<td>12.95 ± 0.77</td>
<td>12.50 ± 0.37</td>
</tr>
<tr>
<td>G2</td>
<td>12.98 ± 0.76</td>
<td>8.30 ± 0.41b</td>
</tr>
<tr>
<td>G3</td>
<td>13.80 ± 0.68</td>
<td>9.70 ± 1.60</td>
</tr>
<tr>
<td>G4</td>
<td>13.00 ± 0.83</td>
<td>12.80 ± 0.59c</td>
</tr>
</tbody>
</table>

Values are mean ± s.d., (n=6). Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.

P<0.05, bP<0.01, cP<0.001, with negative control dP<0.05, eP<0.01 with standard

**Table 2 Changes in SGOT and SGPT with Ethanolic extract of *S. surattense* root**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AST/SGOT IU/L (Mean ± S. E.)</th>
<th>ALT/SGPT IU/L (Mean ± S. E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 0</td>
<td>DAY 05</td>
</tr>
<tr>
<td>G1</td>
<td>23.00 ± 2.5</td>
<td>22.30±1.8</td>
</tr>
<tr>
<td>G2</td>
<td>20.00 ± 3.9</td>
<td>412 ± 25.3</td>
</tr>
<tr>
<td>G3</td>
<td>20.00 ± 3.9</td>
<td>107.7 ± 11.9</td>
</tr>
<tr>
<td>G4</td>
<td>17.50 ± 3.30</td>
<td>63 ± 9.9e</td>
</tr>
</tbody>
</table>

Values are mean ± s.d., (n=6). Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. aP<0.05, bP<0.01, cP<0.001, with negative control dP<0.05, eP<0.01 with standard

The results of these investigations showed that water ethanolic extract of *S. surattense* root possess hepatoprotective activity against paracetamol induced toxicity. Increases in haemoglobin, and decrease in SGOT, SGPT, and serum billirubin was observed in the paracetamol treated groups). These values were decreased especially in Group 4 (800 mg/kg) in a dose dependent manner indicating less damage to the liver.
References:


Wound healing activity of herbal cream formulation

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Abstract: An indigenous herbal cream formulation containing, curcumin, Aloe vera, Vitamin- A or Vitamin- E displayed antibacterial and antioxidant stimulatory activities thereby suggesting potential wound healing properties. The herbal cream formulation was evaluated for wound healing using in vivo excision, incision and dead space wound models in experimental rats. The wound healing contracting ability of animals treated with formulation 6 and formulation 7 topically was found to be significantly higher (p < 0.05) on day 12 and 16 as compared to the control. The present study provides scientific evidence that the topical application of herbal cream promoted wound healing. The activity was compared with that of the control and povidone iodine ointment as standard drug.

Introduction:
The aim of the present study was to identify potential ingredients for wound healing, formulate it in stable formulation and evaluate it’s for wound healing activity and evaluate activity of cream using in vitro cell culture like dermal fibroblasts, macrophage, and using in vivo excision, incision and dead space wound models in experimental rats. Herbal cream formulation of Curcumin, Aloe vera, Vitamin- A or Vitamin- E displayed antibacterial and antioxidant stimulatory activities thereby suggesting potential wound healing properties [1]

Materials and Methods:
Active ingredients: Curcumin (Family-Zingiberaceae) common name is haldi in north India, Aloe vera (Family-Xanthorroeaceae), Vitamin-A,Vitamin-E, and povidone iodine is used as a standard.
Preparation of cream: Herbal cream was prepared with cream base, oil in water type and active ingredients incorporate in appropriate quantity that is curcumin, Aloe vera, Vitamin-A, Vitamin-E.
Animals: The wistar rats of either sex weighing 150-200 g were used. Wistar rats were divided into four groups of six animals each. Group I: Negative control (cream base); Group II: Positive control (povidone iodine ointment); Group III: Formulation 6 Group IV: formulation 7 All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).
Excision wound: Excision wound of circular area (600 Mm²) was made on the back. The area of wound was measured on 0, 3, 6, 9, 12 and 15th post-wounding days. The period of epithelialization was calculated [2]

Incision wound: An incision wound of about 6 cm in length and about 2 mm in depth were made. The parted skin was stitched with sterilized needle at 0.5 cm intervals. The tensile strength of the skin was measured by tensiometer on the 11th day. Thereafter, euthanized animals and the tissues were processed for histopathological examination. [2]

Dead space wound model: Dead space wounds were created by subcutaneous implantation of sterilized cotton piths (10 mg) on the right side groin and axilla. The granulation tissue so harvested was subjected to hydroxyproline estimation, 10th day post wounding [3].

Statistical analysis: Data are expressed as a mean ± s.d. evaluation carried out using one-way ANOVA followed by Tukey’s test. The values of p < 0.05 were considered to be statistically significant.

Results and Discussion:

Excision wound study: The wound healing contracting ability of animals treated with herbal cream f1 & f2 topically was found to be significantly higher (p < 0.05) on day 12th and 16th as compared to the control.

Incision wound study: Herbal cream F2 topical, significantly increased (p<0.05) the tensile strength on 10th post wounding day (512 ± 3.4 g respectively when compared to control (232.5 ± 2.9 g).

Dead space wound study: The groups treated with F7 w/w topical, significantly increased weight of granuloma by 65.6 ± 0.4 mg/100g, respectively compared to control 32.5 ± 0.5 mg/100g.

### Table 1 Excision wound study of herbal cream formulation

<table>
<thead>
<tr>
<th>Treatment &amp; Doses</th>
<th>Percentage wound contraction (mean ± SEM)</th>
<th>Period of epitheliazation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 6</td>
</tr>
<tr>
<td>NC</td>
<td>25.2 ± 1.6</td>
<td>24.0 ± 1.5</td>
</tr>
<tr>
<td>PC</td>
<td>22.5 ± 0.8</td>
<td>18.8 ± 0.9</td>
</tr>
<tr>
<td>F1</td>
<td>24.2 ± 1.2</td>
<td>21.8 ± 1.0</td>
</tr>
<tr>
<td>F2</td>
<td>23.2 ± 1.0</td>
<td>19.8 ± 0.9</td>
</tr>
</tbody>
</table>

NC: Negative control; PC: Positive control, Values are mean ± s.d.,(n=6). Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. ap<0.05 with negative control

### Table 2 Incision and dead space wound study of herbal cream formulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incision wound</th>
<th>Dead space wound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tensile strength on 10th day (g)</td>
<td>Granuloma wt. (mg/100g)</td>
</tr>
<tr>
<td>NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NC: Negative control; PC: Positive control, Values are mean ± s.d.,(n=6) . Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. ap<0.05 with negative control

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>PC</th>
<th>F1(W/W)</th>
<th>F2(W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>232.5 ± 2.9</td>
<td>32.5 ± 0.5</td>
<td>1.5 ±0.7</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>614.4 ± 4.5a</td>
<td>74.6 ± 0.5a</td>
<td>5.8 ± 0.5a</td>
<td></td>
</tr>
<tr>
<td>F1(W/W)</td>
<td>453.7± 5.4a</td>
<td>57.5 ± 1.3a</td>
<td>2.1 ± 0.2a</td>
<td></td>
</tr>
<tr>
<td>F2(W/W)</td>
<td>512 ± 3.4a</td>
<td>65.6 ± 0.4a</td>
<td>4.3 ± 0.3a</td>
<td></td>
</tr>
</tbody>
</table>

\( NC \): Negative control; \( PC \): Positive control, Values are mean ± s.d.,\( n=6 \) . Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. \( ap<0.05 \) with negative control

**Figure 1:** Histological Examination (H & E Stains, 40 xs)

**A.** Negative control: Granulation tissue contains less collagen, fibroblasts, and blood capillaries and more inflammatory cells. **B.** Positive control: Granulation tissue contains lower number of lymphocytes and macrophages. Collagen fibers are organized and the tissue is aligned. **C.** Herbal cream (f2) Granulation tissue contains more collagen and fibroblasts with absence of inflammatory cells.

**Conclusion:**

The present study provides scientific evidence that herbal cream formulation has potential wound healing activity due to combination of fibroblast and macrophage activity as well the topical application of herbal cream formulation promoted wound healing. It can be used as a monotherapeutic wound healing agent or may be combined for synergistic effects.

**References:**

Inhibition potential of Ananas comosus and its constituent on CYP3A4 and CYP2D6 in human liver microsomes

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Abstract: Ananas comosus has been used since ancient time not only as dietary supplements but also treatment of the several ailments. In present study influence of extract of Ananas comosus on cytochrome isozymes (CYP3A4 and CYP2D6) were analyzed through fluorescence screening assay and their respective IC_{50} values were calculated. Standardized extract have higher IC_{50} value than the known positive inhibitors which indicated that extract has less interaction potential and may not cause significant herb-drug interactions relating to the inhibition of major CYP450 isozymes.

Introduction:
Ananas comosus (Family: Bromeliaceae), also named pineapple, has long been one of the most popular of tropical and subtropical fruits. A. comosus has been used as antitussive, antidiarrheal, alexipharmic and antidyspepsia in Indian Traditional Medicine [1]. Concomitantly administered dietary supplements or food substances may either induce or inhibit drug metabolizing enzymes, resulting in changes in the rate of drug metabolism [2]. Due to the central role of CYP catalyzed metabolism in drug clearance, the identification CYP substrate modulator (e.g., inhibitor and inducer) has been integrated and the results could be helpful in drug development [3]. The CYP enzymes inhibition might results alteration in drug metabolism and leading to various adverse effects [4]. Therefore an attempt has been made to investigate inhibition effects of A. comosus extract on major CYP450 isozymes (CYP2D6 and CYP3A4), which are responsible for most of drug metabolism.

Materials and Methods:
Chemicals and reagents: Ferulic acid was purchased from Sigma chemicals (Germany). Vivid® CYP450 Screening Kit was purchased from Invitrogen Drug Discovery Solutions, USA. Ketoconazole and quinidine were procured from Sigma chemicals and all other chemicals were of analytical grade.

Extraction of plant material: Air dried A. comosus (250 g) was extracted with 70% ethanol by cold maceration method. The obtained extract was lyophilized to get a dry powder and final yield was found to be 14.58% (w/w).

Standardization of Ananas comosus extract: A. comosus extract was standardized through reverse phase high performance liquid chromatography (RP-HPLC). The extract of A. comosus was prepared in
the concentration of 10 mg/mL. The optimum separation was achieved by solvent system of methanol: 0.5% acetic acid in water in ratio of 60:40 (v/v) with a flow rate of 1mL/min. Amount of the ferulic acid (biomarker) present in the extract was determined through the calibration curve.

**Cytochrome P450-CO complex assay:** Cytochrome P450-Carban monoxide complex (CYP450-CO) assay was performed with pooled rat liver microsome (RLM) in 96 well microplate [2]. Ketoconazole was used as positive control. **CYP450 enzymes inhibition assay:** This study was performed in black 96 microplates through Fluorogenic assays. The assay method was based on the previous reports by Ponnusankar et al., 2011 [5]. Product formation from the fluorogenic probes were determined from the fluorescence data at eight different concentrations of the inhibitors and the all measurements were performed in triplicate. The interaction potential of the *A. comosus* extract has been evaluated through two CYP isozymes like CYP3A4 and CYP2D6 to assay their inhibition potential. Percentage inhibition and IC₅₀ values were calculated on the basis of mean of curves for enzyme activity versus inhibitor concentration [2].

**Results and discussion:**

**Standardization of *A. comosus* extract:** Calibration curve of biomarker (ferulic acid) was plotted by plotting peak areas against five concentrations (100-500 μg/mL) of standard. Obtained chromatograms of marker and extract have been shown in Figure 1A & 1B respectively. Retention time (Rt) of ferulic acid was found to be 5.118 min and content of ferulic acid in extract was 1.64% (w/w).

![Figure 1](image_url) **Figure 1** RP-HPLC chromatograms of ferulic acid (A) and *Ananas comosus* extract (B)

**Herb-drug interaction study through CYP enzymes**

**CYP450-CO complex assay:** The results on cytochrome inhibition assay through cytochrome P450-CO complex method showed a concentration-dependent inhibition. Ketoconazole showed much more inhibition potential than *A. comosus* extract and its biomarker ferulic acid.

**CYP450 enzymes inhibition assay:** Drug interactions potential of CYP isozymes have been performed through specific high-throughput screening (HTS) assays in the concentration range of 50 to 200 μg/ml. *A. comosus* extract and its biomarker ferulic acid were evaluated for herb-drug interaction. Ketoconazole and quinidine were used as known inhibitors. Samples were assayed in triplicate and IC₅₀ values were calculated and have been represented in Table 1.

**Table 1:** IC₅₀ (μg/ml) value of *A. comosus*, ferulic acid and known inhibitors on CYP3A4 and CYP2D6 isozymes

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Graphs of percentage inhibitions of test solution on all the isozymes have been depicted in Figure 2. The results of present study revealed that the *A. comosus* extract and its biomarker have less inhibition potential on these tested isozymes compared to their respective known inhibitors. Enzyme inhibition studies showed that inhibition of *A. comosus* extract on CYP3A4 and CYP2D6 was concentration dependent with IC$_{50}$ 247.45±1.37 and 272.27±0.95 μg/ml respectively. The study showed that *A. comosus* extracts have much higher IC$_{50}$ value than known inhibitors. Further *in vivo* studies are necessary to evaluate the clinical significance of the interactions.

**Figure 2** Concentration dependant percentage inhibition of *A. comosus* extract, ferulic acid and positive inhibitors on drug modulating isozymes CYP3A4 (A) and CYP2D6 (B),(Values are in Mean± EM, n = 3)

**Conclusion:**
The present study demonstrates that *A. comosus* extract has no significant inhibition potential on the CYP3A4 and CYP2D6 isozymes activity compared to their respective positive controls. It concluded that drugs cleared by tested isozymes may not be influenced by the *A. comosus* extract.

**Acknowledgement:**
The authors would like to express their gratitude to the National Medicinal Plant Board (NMPB), Government of India, New Delhi, for providing financial support to School of Natural Product Studies, Jadavpur University, Kolkata.

**References:**
A comparative study of the different parts of *Pterocarpus marsupium roxb.* in diabetes mellitus and evaluation in wound healing

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Abstract: In the present study the *Pterocarpus marsupium Roxb.* was selected for studying different parts i.e. bark, heartwood and leaf for antidiabetic activity and diabetic complications. All the extracts were subjected to antidiabetic activity. Aqueous bark extract was found to have maximum antidiabetic activity and was selected for studying diabetic complications i.e. wound healing. Different extract of *Pterocarpus marsupium* administered at a doses of 200mg/kg, to Alloxan-treated diabetic rats caused significant ($p<0.01$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 7. Bark extract and heartwood extract exhibited maximum glucose lowering effect and promotes healing of wounds in alloxan induced diabetic rats

Introduction:
Diabetes mellitus is characterized by abnormal insulin secretion, derangements in carbohydrate or lipid metabolism [1]. Clinical studies indicate a direct relationship between hypoglycemia and long term complications such as neuropathy, retinopathy, atherosclerosis and coronary artery diseases [2]. The number of adults with diabetes mellitus in the world will increase to 300 million by the year 2025 [3,4]. The plant scientifically proved that it has antidiabetic activity [5], anticataract activity, antioxidant activity, cardiotonic activity, COX-2 inhibition activity.

Materials and Methods:
Experimental animals: The Wister albino rats of either sex (200-250g) were obtained. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad libitum*. The bark, leaf, and heart wood of *Pterocarpus marsupium* Roxb. were collected & The powdered materials were subjected to maceration. The water was used as solvent for extraction.

Dose: Therapeutic dose of “*Pterocarpus marsupium* Roxb” will be fixed on the basis of data obtaining from oral acute toxicity studies (OECD-423 guidelines).
Statistical Analysis: All the data were expressed in mean ± SEM. The significance of differences in mean between control and treated animals for different parameters determined by one way ANOVA followed by Dunnett’s multiple comparison tests.

Results and discussion:
Alloxan has been widely used to induce diabetes mellitus in experimental animal models allowing investigation of hypoglycemic agents in the treatment of diabetes. Alloxan Injection consistently produced symptoms characteristic of Diabetes mellitus including hyperglycemia, decreased insulin levels, polyuria and weight loss. The present study demonstrated that the aqueous extract of of *Pterocarpus marsupium* had an antihyperglycemic effect in the alloxan induced diabetic rats when administered orally. Extract of *Pterocarpus marsupium* showed significant reduction of blood glucose in alloxan induced diabetic rats. Groups treated with different extracts showed time dependent reduction in fasting serum glucose (FSG) level as shown in table. The standard group treated with glibenclamide also showed significant reduction in FBG level as compared to diabetic control group. Different extract of *Pterocarpus marsupium* administered at a doses of 200mg/kg, to Alloxan-treated diabetic rats caused significant (P<0.01) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 7. Bark extract and heartwood extract exhibited maximum glucose lowering effect in diabetic rats compared to the leaf extract. Glibenclamide exhibited significantly reduction in blood glucose levels at the end of the study when compared to diabetic control. The breaking strength of 10 day old resutured incision wounds in diabetic as well as non diabetic rats were estimated. The mean breaking strength of wounds in non- diabetic animals was 296.2 ± 7.715 where as in ALX-D was 192.0 ± 9.542, ALX-D treated animals was 350.8 ±12.71 and non diabetic treated animals with bark extract was 453.3 ± 13.78 indicating significant (p<0.01) increase in breaking strength of incision wounds in treated groups.

**Table 1** Effect of different extracts of *Pterocarpus marsupium* Roxb. on blood glucose levels of alloxan induced diabetic albino rats after prolonged treatment in chronic study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Blood glucose levels mg/dl (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>--</td>
<td>449.2+ 1.833</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10</td>
<td>450.7 + 1.745</td>
</tr>
<tr>
<td>Bark extract</td>
<td>200</td>
<td>448.7 +2.171</td>
</tr>
<tr>
<td>Heartwood extract</td>
<td>200</td>
<td>449.8+ 1.515</td>
</tr>
<tr>
<td>Leaf Extract</td>
<td>200</td>
<td>450.0 + 1.414</td>
</tr>
</tbody>
</table>
n=6, *p<0.01- more significant vs. control, SEM – Standard error mean, One way ANOVA followed by Dunetts‘t’ test.

Conclusion:
The active principles in the bark might have increased insulin secretion and thus decreased blood glucose level. From this study it is inferred that bark extract has beneficial effects on blood glucose level as compared to heartwood and leaf. Present study demonstrate that the bark extract of *Pterocarpus marsupium* Roxb. promotes healing of wounds in alloxan induced diabetic rats where healing is delayed. The healing is facilitated by significant increase in breaking strength of resutured incision wounds due to the presence of tannins.

References:
Systematic evaluation of anti compulsive activity of agomelatine for treatment of obsessive compulsive disorder

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Abstract: In the present work, a randomized, double-blind, placebo-controlled trial was performed to check the efficacy of agomelatine in treatment of anxiety disorder, prompting its therapeutic potential in treatment of obsessive-compulsive disorder (OCD). The effect of acute and chronic administration of agomelatine and other standard drugs diazepam, venlaflaxine, fluoxetine on the marble-burying behavior (MBB) of mice, which is reported to be an index of anticomulsive behavior, was performed. Results indicated a potent and dose dependent influence of agomelatine on MBB of mice, which was maintained after its chronic administration. However, the higher doses of agomelatine (40 and 50 mg/kg) were found to be locomotor depressant. In conclusion, agomelatine administration reduces the MBB in mice, which should be explored for its potential use in the treatment of OCD.

Introduction:
Agomelatine is a new melatonin analogue drug recently approved for treatment of major depression in adults including prevention of relapse. Agomelatine exhibits a new pharmacological mechanism of action, which combines its melatonin MT1 receptor and MT2 receptor agonist properties with a serotonin (5-HT) 2C receptor antagonist effect [1]. Owing to this novel mechanism of action there is a widespread excitement about its therapeutic potential in treatment of variety of central nervous system disorders. Obsessive compulsive disorder (OCD) is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions). In addition, it is also classified as a generalized anxiety disorder. Only potent serotonin reuptake inhibitors are consistently effective in patients with obsessive compulsive disorder. The pharmacodynamic profile of agomelatine and its efficacy in variety of CNS disorders prompted us to evaluate its effect on compulsive behavior.

Materials and Methods:
Materials, Drugs: Agomelatine was purchased from Sigma-Aldrich, MO, USA. Venlaflaxine was gifted
by Sun Pharmaceuticals, Baroda, India and diazepam injection were purchased from Svizera Health Care Ltd., Mumbai.

**Animals:** The animals were bred from an original stock purchased from Veterinary College, Mhow, India. The Adult male albino Swiss mice (22-25 g) were group housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Animals received standard rodent chow (Trimurti Feeds, Nagpur, India) and water ad libitum.

**Methods:**

All animals were acclimatized to laboratory conditions for at least seven days before carrying out the experiments, which were carried at 08.00 to 15.00 h daily. Separate group of mice (n=6/12) was used for each set of experiments. The studies were carried out in strict accordance with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi. Mice were individually placed in marble-burying behavior apparatus with 20 glass marbles for 30 min. The behavior of the mice during the test session was recorded by a video camera. At the end mice were removed, and unburied marbles were counted. A marble was considered ‘buried’ if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive-compulsive behavior.

**Results and discussion:**

**Influence of acute drug treatment on MBB**

**Agomelatine:** One-way ANOVA revealed that acute administration of agomelatine in different doses had a significant effect on the MBB of male mice \[F(5,53)=6.835, P<0.0001\]. Further the dunnett multiple comparison test revealed that agomelatine had a significant effect at 20mg/Kg (P<0.01), 30mg/Kg (P<0.05) and at 50mg/kg (P<0.001) however, the lower dose found to be non-significant at 10mg/Kg(P>0.05).

**Venlaflaxine:** One-way ANOVA revealed that acute administration of venlaflaxine in different doses had a significant effect on the MBB of male mice \[F(3,23)=5.505, P<0.0064\]. Further the dunnett multiple comparison test revealed that venlaflaxine had a significant effect at 5mg/Kg (P<0.01). However, the lower dose found to be non-significant at 1 and 3 mg/Kg (P>0.05).

**Fluoxetine:** One-way ANOVA revealed that acute administration of fluoxetine in different doses had a significant effect on the MBB of male mice \[F(2,17)=5.729, P=0.0412\]. Further the dunnett multiple comparison test revealed that fluoxetine had a significant effect at 10 mg/Kg (P<0.01). However, the lower dose found to be non-significant at 5 mg/Kg (P>0.05).
Diazepam: One-way ANOVA revealed that acute administration of diazepam in different doses had a significant effect on the MBB of male mice \( F (3,23)=13.26, P=0.0001 \). Further the dunnett multiple comparison test revealed that diazepam had a significant effect at 1 mg/Kg and .5 mg/Kg \( (P<0.001) \). However, the lower dose found to be non-significant at 0.25mg/Kg \( (P>0.05) \).

![Figure 1](image)

**Figure 1** Influence of agomelatine on anticomulsive activity

**Conclusion:**
In conclusion, the results of the present investigation support a potential strength of melatonergic drug, agomelatine on compulsive behavior in mice. Further this effect appears to be due to modulation of melatonergic receptors and not due to serotonergic system.

**Acknowledgement:**
The authors are very grateful to Faculty of Pharmacy, Pacific Academy of Higher Education and Research (PAHER), Pacific University, for providing all the necessary facilities for carrying out this study.

**References:**
Cytotoxic activity of methanolic and aqueous extract of *Eulophia nuda*

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**Abstract:** *Eulophia nuda* belonging to family orchidaceae investigated to evaluates the cytotoxic property of the methanolic and aqueous extracts of *Eulophia nuda* tuber using as three in-vitro models *Allium cepa* root, Brine shrimp lethality bioassay (BSLP) and MTT assay (A546 cell line). In the present bioactivity study, all of the extracts of *Eulophia nuda* (methanol, water) showed positive results indicating that the test samples are biologically active. Brine shrimp lethality bioassay (LC\textsubscript{50} = 450\mu g/ml and 600\mu g/ml), *Allium cepa* root meristem model and MTT assay (IC\textsubscript{50} =2.2 \mu g/ml and 9.8\mu g/ml) showed potent cytotoxic and antitumor activity of methanolic and aqueous extract of *Eulophia nuda* tubers.. Therefore, this plant has potential for the development of novel anticancer drug leads.

**Introduction:**
Over the past decade herbal medicine have been accepted universally, hence medicinal plants continue to play an important role in healthcare system of a large number of world’s population. Infact there are several medicinal plants all over the world which are being used traditionally in the prevention and treatment of cancer. Plant derived compounds have played an important role in the development of several clinically useful anti-cancer agents. The study demonstrated the cytotoxic activity of methanolic and aqueous extract of *Eulophia nuda* tubers reported by models Brine shrimp lethality bioassay (BSLB), *Allium cepa* root tip meristem model and MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) assay.

**Materials and Methods:**
The tubers of *Eulophia nuda* were collected from a supplier and authenticated by Dept. of Botany, R.T.M. Nagpur University, Nagpur, Maharashtra, India. The collected plant tubers were dried and pulverized into coarse material. The coarse plant material was used for preparation of extracts.

**Invitro cytotoxic activity:** Brine shrimp lethality bioassay: Brine Lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plant to determine toxicity through the estimation of medium lethal concentration (LC\textsubscript{50} values).
Alium cepa root meristem model: Locally available Onion bulb (Allium cepa 50 ± 10 g) were obtained and grown in the dark over 100 ml tap water at ambient temperature until the roots have grown to approximately 2-3 cm. The percentage root growth inhibition in relation to the negative control and the root growth after treating with different at 48 and 96 hr. extracts was determined.

III) MTT Assay: Cell proliferation activity which estimated the effect of various extracts the growth of cell in vitro. Measured of cell viability and proliferation forms are used as basis for this in vitro assay.

Results & Discussion:
Brine shrimp lethality bioassay: In the present bioactivity study, extracts of Eulophia nuda (methanol and aqueous) showed positive results indicating that; the test samples are biologically active. Plotting concentration versus percent mortality (% Mortality) for test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC50, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined. LC50 value of methanol and aqueous extract was found to be 450 (µg/ml) and 650 (µg/ml) respectively. All the values were compared with standard cytotoxic agent cyclophosphamide, who’s LC50 was found to be 300 (µg/ml).

![Graphical representation of % mortality of cyclophosphamide, methanolic & aqueous extract](image)

Figure 1 % mortality and LC50 value cyclophosphamide, methanolic & aqueous extract

Allium cepa root meristem model: Incubation of Bulbs in different concentration of cytotoxic agents produced a growth retardation effect that was associated with a decrease in root number. Both extracts specially the Methanolic extract and cyclophosphamide arrested the root growth. Methanolic extract has shown the maximum growth retarding effect at 10 mg/ml when compared to standard drugs. The root length after 0, 48, 96 hr. with significance at 10 mg/ml was found to be 3.78 ± 0.71 (n = 21), 2.16 ± 0.50 (n = 12) and 2.21 ± 0.71 (n = 8).

MTT Assay: Cell Proliferation activity of various extracts of Eulophia nuda carried out by MTT Assay, which estimated the effect of various extracts on the growth of cell in vitro. Measurement of cell viability and proliferation forms is used as basis for this in vitro assay. Analogous to the results obtained in
previous models, methanol extract was found to be active with IC$_{50}$ value of 2.2 µg/ml and also aqueous extract was found to be active with IC$_{50}$ value of 9.8 µg/ml

![Figure 3 A Dose response curves for Methanol Extract against A549. B Dose response curves for Water Extract against A549.](image)

**Conclusion:**
From the observation and results of cytotoxic assay of the extracts it was found that the methanol and aqueous extract of tubers had shown significant cytotoxic activity in three reported models (Brine shrimp, *Allium cepa* and MTT assay) data shows consistent results and potential for cytotoxic activity of methanol and aqueous extract. Based on the possible relationship between all these models used and plant bioactivity, this work could serve for further pharmacological research viz, isolation of constituents from the extracts and finding out the constituents responsible for activity.

**Acknowledgement:**
We would like to thank Deshpande Laboratories, Bhopal (India), for experimental analysis (MTT Assay).

**References:**
Antioxidant activity of methanolic and aqueous extract of *Eulophia nuda*

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Abstract: The ability of antioxidant activity of methanolic and aqueous extract of *Eulophia Nuda* was determined by 1,1-diphenyl-2-picryl-hydrazyl radical and nitric oxide scavenging assay. In the DPPH and nitric oxide scavenging assay the IC$_{50}$ value of methanol extract and aqueous extract in DPPH were obtained to be 24.62 µg/ml, 33.25 µg/ml and in Nitric oxide scavenging assay were obtained to be 25.54 µg/ml, 31.53 µg/ml. However the standard ascorbic acid were obtained to be 21.27 µg/ml and 22.43 µg/ml was determined in both DPPH and nitric oxide scavenging assay. The result obtained in this study it indicate that *Eulophia nuda* has a potential as natural antioxidant.

Introduction:
The traditional medicine all over the world is revalued by an extensive activity of research on different plant species and their therapeutic principals. Experimental evidence suggest that free radicals and reactive oxygen species can be involved in a number of diseases. The ability of antioxidant activity of methanolic and aqueous extract of *Eulophia Nuda* was determined by 1,1-diphenyl-2-picryl-hydrazyl radical and nitric oxide scavenging assay.

Materials and Methods:
The tubers of *Eulophia nuda* were collected from a supplier and authenticated by Dept. of Botany, R.T.M. Nagpur University, Nagpur, Maharashtra, India. The collected plant tubers were dried and pulverized into coarse material. The coarse plant material was used for preparation of extract.

**DPPH radical scavenging activity:** The free radicals scavenging activity of the *Eulophia nuda* tubers extracts and Ascorbic acid was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (10-100µl/ml). Thirty min later the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

**Nitric oxide scavenging activity:** Nitric oxide was generated from sodium nitroprusside and measured by the Griess Reagent. Sodium nitroprusside in aq. solution at physiological pH spontaneously generate nitric oxide, which interact with oxygen to produce to nitric oxide compete with oxygen leading to
reduce production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffer saline mixed with different conc. of each extract dissolved in respective solvent incubated at 25°C for 150 min. The same reaction mixture without extract but equivalent amount of ethanol served as control. At interval, sample (1.5 ml) of the incubated solution were removed and diluted with (1.5 ml) Griess Reagent (1% Sulphanamide, 2% H₃PO₄ and 0.1% Naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with Sulphanilamide subsequent coupling with Naphthyl ethylene diamine was read at 564 nm. Ascorbic acid was use as control.

Result & Discussion:

**DPPH radical scavenging activity:** These methods are most popular for determination of antioxidant activity. In DPPH method the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radicals scavenging activity. The capacity to scavenge the DPPH radicals was calculated. The antioxidant activity of the extracts was expressed as IC₅₀.

**Nitric oxide scavenging activity:** In nitric oxide method the nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphtylenediamine was read at 546 nm and % inhibition was calculated by using formula.
The effect of extracts on accumulation of Nitric Oxide radicals.

**Table 1** Antioxidant Activity of *Eulophia nuda* extracts

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Extracts</th>
<th>DPPH</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Inhibition</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>1</td>
<td>ASC</td>
<td>94.38</td>
<td>21.27</td>
</tr>
<tr>
<td>2</td>
<td>MEE</td>
<td>86.23</td>
<td>24.62</td>
</tr>
<tr>
<td>3</td>
<td>AQE</td>
<td>80.23</td>
<td>33.25</td>
</tr>
</tbody>
</table>

**Conclusion:**

The methanolic and aqueous extract of *Eulophianuda* tubers showed strong antioxidant activity by inhibiting DPPH and Nitric Oxide scavenging activity when compared with standard ascorbic acid. Thus it can be concluded that both extract of *Eulophianuda* tubers can be used as a source of natural antioxidant with consequent health benefits.

**References:**

Chronotherapeutic drug delivery system of metoprolol succinate for early morning rise in blood pressure

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Abstract: The objective of the present study was to formulate chronotherapeutic drug delivery system of metoprolol succinate. It has been known for nearly 50 years that blood pressure exhibits circadian variability with a rapid increase, in early morning hours. This early morning surge in blood pressure leads to serious cardiovascular complications like myocardial infraction and stroke. In the present work various core-in-cup compression coated formulations of metoprolol succinate were prepared using Polyox WSR 301, Kollicoat MAE 100P, Eudragit L100, Eudragit S100, guar gum and Methocel K100M at different ratios as release modulating layers. Formulation prepared with Kollicoat MAE 100P along with Eudragit L100 and S100 was considered as the best one with a lag time of 5 hrs followed by rapid and complete drug release within 1 h.

Introduction:
Since last 30 years, various technical advancements in biodegradable polymers, formulations and comprehensive understanding of pharmacokinetics have resulted new techniques of drug delivery. These techniques are capable of controlling the rate of drug release, sustaining the duration of therapy and targeting delivery of a medicinal agent to a specific organ or tissue. It is for this reason controlled or targeted drug delivery systems have been receiving more and more attention. Recently, however one type of drug delivery system, where delivery device is capable of releasing drug after predetermined time-delay, known as chronotherapeutic drug delivery system has drawn the attention of scientists. The present invention was aimed to bedtime dosing of metoprolol succinate for the treatment of early morning surge in blood pressure.

Materials and Methods:
The top release modulating layer of compression coated tablets were formulated using various erodiable and swellable polymers viz. Polyox WSR301 and Kollicoat MAE 100P and bottom layer was formulated using 1:1 ratio of guar gum and Methocel K100M i.e. hydrophobic and hydrophilic polymers respectively. Core tablets A, B and C were prepared with various formulae and core tablet C was selected
for further study based on dissolution and disintegration study. Compression coated tablets were evaluated for physical properties such as hardness, thickness, friability, weight variation and drug content. In-vitro drug release study of compressed coated tablets were done in three consecutive dissolution media (i.e. in 0.1 N HCl for 2 hrs, pH 6.8 phosphate buffer for next 4hrs and finally in pH 7.4 phosphate buffer solution) in order to mimic mouth to colon transit time and change in pH conditions.

Result and Discussion:
Among the various hydrophilic swellable and erodible polymers studied in this research work Kollicoat MAE 100P was selected for further study as this could produce a desired lag time in initial 6h of dissolution study. So attempts were made to further increase their efficacy with different ratios of pH dependent polymers i.e. Eudragit L 100 and Eudragit S 100. FTIR, DSC and XRD studies indicated that no solid-state interactions between drug, excipients and polymer or incompatibility problems occurred during formulation preparation. From the results of dissolution studies, formulation containing hydrophilic erodible polymer Kollicoat MAE100P(100mg) along with pH sensitive polymer Eudragit L100(40mg) and Eudragit S100(20mg) was considered as best formulation with a lag time of 5hrs followed by complete release of drug within 1hr. This formulation was then further evaluated for in-vivo studies including X-ray Studies in rabbits. The stability study of best formulation was carried out at 40 °C/75% RH for 6 months which indicated no change either in physical appearance, drug content and dissolution studies.

Table 1 Formulation of press coated tablets containing hydrophilic swellable and erodible polymers in combination with different ratios of pH sensitive polymers in top release modulating layer.

<table>
<thead>
<tr>
<th>Bottom Layer Wt (mg)</th>
<th>Core Tablet Wt(mg)</th>
<th>Top Layer Composition</th>
<th>Final Wt(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Polyox WSR301 Wt(mg)</td>
<td>Kollicoat MAE 100P Wt(mg)</td>
</tr>
<tr>
<td>250</td>
<td>150</td>
<td>100</td>
<td>-----</td>
</tr>
<tr>
<td>250</td>
<td>150</td>
<td>100</td>
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<td>100</td>
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<tr>
<td>250</td>
<td>150</td>
<td>-----</td>
<td>100</td>
</tr>
</tbody>
</table>
Conclusion:
Results indicated that compression coating technique using Kollicoat MAE 100P and Polyox WSR 301 polymers can be effectively used to have desired chronotherapeutic release of metoprolol succinate with lag time of 5-6 h.

References:
In-vitro screening of Curcuma longa linn for its antacid activity

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Abstract: The study was aimed to evaluate the antacid activity of Curcuma Longa Linn rhizomes hydroalcoholic extract under simulated conditions. Fresh, homogenized and dried rhizome of Curcuma Longa Linn was subjected for the screening of antacid activity. Antacid activity was evaluated using carbon dioxide induced acidity & Rossette Rice test. The hydroalcoholic extract showed significant activity agents’ gastric acid neutralizations (P < 0.05) at different concentration and results were compare to standard NaHCO3. Present study suggest that hydroalcoholic extract of curcuma longa linn significantly neutralized acid as well as resist change in pH and illustrate good antacid property.

Introduction:
Now a day’s use of the medicinal plants is increasing in many countries where as 35% of drugs contain natural products. Turmeric is one of most essential spices amongst. Stomach secretes hydrochloric acid which is necessary for the digestion of food. Excessive amount of hydrochloric acid condition is called as hyperacidity or acid dyspepsia. Symptoms are typical feeling of restlessness, nausea, vomiting, sour belching with an after taste of the already-eaten food, stiffness in the stomach, which is called as dyspepsia, lack of desire for any other type of food indigestion constipation. Turmeric recommended in food for its potential medicinal value, Its use as a coloring agent, drug in herbal medicine, preservative for food along with used as anti inflammatory antibacterial agents wield verity of uses are found with use of turmeric Alzheimer's, Arthritis, Cancer and Diabetes turmeric has been used traditionally for thousands of years as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its supposed antimicrobial property. In the Siddha system (since c. 1900 BCE) turmeric use as medicine for a range of diseases & conditions. It is used for treatment of various infections and as an antiseptic. Positive effect turmeric has against cancer or any disease [1, 2, 3, 4].

Materials and Methods:
Plant material: Fresh rhizomes of Curcuma longa collected and dried for7 days in small pieces. Grind dried rhizome to obtain a fine powder. Dried fine powder was ready for use.
Hydroalcoholic extraction: The grinded powder was extracted with 500 ml of hydroalcoholic solution by Soxhlation for 72h. The extract was concentrated at temperature <45°C. The residue was dried and refrigerated.

Aqueous extraction: The grinded powder was then extracted with 1000ml double distilled water containing 3-4 drops of chloroform for 48h. Concentrate extract at temperature less than 45°C.

Pharmacological study: Pharmacological study of the crude extract was carried out for determination the active pharmacological constituents which responsible for the antacid activity.

Determination of antacid potential: Three different quantities i.e. 2.5, 5 and 7.5 gm of hydroalcoholic extract of *curcuma longa* linn were taken for antacid evaluation using Rossett-Rice method and the results obtained were compared with standard sodium bicarbonate. The method adopted here in simulated the acidic environment of stomach and records the change in pH with the time followed by administration of the different doses of crude hydroalcoholic extract of *curcuma longa* linn and standard sodium bicarbonate were recorded. A jacketed reaction vessel made up of borosilicate glass containing 70ml HCl and 30ml of water approximating the acidity of the gastric contents, was heated till the temperature of this simulated fluid reached to 37°C. Immediately 2.5 gm of *curcuma longa* linn extract was added. Simultaneously pH meter and recorder were turned on and a pump calibrated to add 0.1N HCl at a rate of 4ml/min was activated. The flow rate simulates the normal acid secretion rate. The pH was noted & the Rosette-Rice time was determined. The procedure was repeated for 5and 7.5 gm of *curcuma longa* linn & 0.8 gm of sodium bicarbonate. The time during which the pH maintained 3-5 is the duration of effective pH control and termed as Rosette-Rice time. Rossett-Rice curve was prepared for drug extract and standard [5]

Result and discussion:

Antacid Profile: The antacid profile was evaluated in vitro using Rosette-rise test. The Rosette-rise time for 2.5gm of *Curcuma longa* linn was found to be (6.32±.220) while for 5gm dose it was 8.95±.242 min and for 7.5gm it was (11.32±.330) compared to standard 0.8gm NaHCO3 which maintained the pH for 1.508±.015 min. Rossett-Rice curve was prepared for drug extract and standard (fig.1). Assays of all samples were conducted in triplicate and averaged. All groups were compared employing one-way analysis of variance (ANOVA) followed by Bonferroni’s test. The results were termed significant statistically when probability was less than 0.05 (P<0.06).

Problem of acidity is very common and the main causes behind this are over stress life style, smoking and dependence on junk food. Antacids are agent that neutralizes the stomach acid responsible for maintain of acidity of stomach occasionally. They should not be taken continuously for more than two weeks unless
under a physician's directions as they produce serious side effects such as Milkalkali syndrome, loss of appetite, mood changes, muscular pain, nervousness, weakness, constipation, stones in kidney etc. Antacids are classified on the onset of action and influence. An ideal antacid should have adequate duration of action. This is related to gastric residence time i.e. how long a drug can maintain the pH of stomach. The drug extract of curcuma longa linn showed potent antacid property in terms of Rosette-Rice time. The results of present study indicate that the Rosette-Rice time is dose dependent.

![Graph](image_url)

**Figure 1** Rossett-Rice curve for drug extract and standard.

However, the Rossett-Rice dynamic test conditions can be fulfilled by drug extract of curcuma longa linn only at doses higher than the standard dose for antacid activity. But the higher dose of *curcuma longa linn* can be safely ingested in view of its safety profile. It is suggested that herbal remedy for acid reflux can be used as the treatment of choice firstly because they cure the symptoms by strengthening the digestive system and secondly they result little or no side effect.

**References:**

Hematopoietic effect of Beta vulgaris extract on dapsone induced experimental anemia in rats

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Abstract: Traditional oral report indicates than Beta vulgaris is used in the treatment of anemia. For this purpose, the extract of Beta vulgaris is evaluated on anaemia model of rat induces by intraperitoneal injection of dapsone at 20mg/kg for 2 days. Oral administration of Beta vulgaris extract at 200mg/kg/day and 400mg/kg/day, to the rat previously treated with dapsone, increased the concentration of hemoglobin, red blood cells number, haematocrit and reticulocytes rate. These results support partially the traditional use of Beta vulgaris.

Introduction:
Anemia is a Greek word that means lack of blood. It is defined as a decrease in total number of red blood cells. Iron deficiency anemia is a form of anemia due to the insufficient iron to from normal red blood cells. It is a very common cause of anemia which is generally affected 5% of women and 2 % of men. In the advancing age; anemia is increase according to prevalence. Anemia is increased with age like 26.1% in men and 20.1%in women

Causes of anemia is divided into three groups-Iron deficiency anemia (nutrient-deficiency anemia), anemia of chronic disease, unexplained anemia. Pro-inflammatory phenotype is present in aged mammals by increased immune responses in the tissues of old humans and gene linked to inflammation. Higher level of cytokines present in the serum like IL-6 and TNF-alfa, and regulation of inflammation responses by the activation of NF-B

Materials and Methods:
Determination of acute toxicity: The acute toxicity study of extract was performed using OECD guidelines .The animals were fasted overnight prior to the experiment and fixed dose was adopted for toxicity studies (OECD Guideline No. 423) The extracts were administered in doses of 5, 50, 200, 400and 500 mg/kg orally and mortality were observed after 24 hrs. The extract of Beta vulgaris was devoid of mortality of animal at dose of 2000 mg/kg orally and hence> 2000 mg/kg taken as LD50 cut off value and 1/10th and 1/5th of the same i.e. 200 mg/kg and 400 mg/kg were selected for screening dose for further studies.
**Induction of experimental Anaemia:** The control group administered 0.9% normal saline orally once daily for 15 days, whereas dapsone treated i.e. negative control group rats received 40mg/kg of dapsone once on day zero intraperitoneal. Third group which served as standard, received 150mg/kg folic acid once daily for 15 days and fourth and fifth groups received 200, 400mg/kg, respectively of ethanol extract of *Beta vulgaris* once daily for a 15 days, orally, accompanied by dapsone (40mg/kg) administered intraperitoneal on day zero. This induction by dapsone in rats is a well-accepted model which procedures anaemic reaction in human which leads to Anemia. The severity of anemia in rats was assessed by the volume of blood collected (0.27 to 0.45 ml). Moreover, the animals were weighed every day.

**Result and Discussion:**
When the dapsone intraperitoneal administration decreased RBCs, WBCs, Hb, and PCV, but Increased time of ESR. When the rats were treated with ethanolic extract of *Beta vulgaris* at 200 mg/kg and 400 mg/kg increased RBCs, WBCs, Hb, and PCV count but ESR time decreased. Results deficits that anemia induced in rat by dapsone and when treated with ethanolic extract of *Beta vulgaris* shows the improvement in hematological parameters of anemia in rat.

The result of these studies generally indicates the anti-anemic properties of the extract of *Beta vulgaris*, which provides for the various applications in traditional medicine especially in the treatment of anemia. Further studies to identify and isolate the active compounds are necessary. Therefore, our present in-vivo studies on *Beta vulgaris* extracts demonstrated the significant anti-anemic activity. Due to the presence of active principles such as flavonoids, glycosides, essential oils and carbohydrates and related resins may be responsible for this activity. Hence, *Beta vulgaris* can be used as a potent anti-anemic agent

**References:**
**In-vitro and in-vivo preliminary antilithiatic screening of Cynodon dactylon phenolic fraction**

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**Abstract:** Nephrolithiasis are recorded as painful health problem. It is a complex process that result from a succession of several physicochemical events in the kidneys including supersaturation, aggregation, growth, nucleation, and retention, or that characterized by the deposition of calcium oxalate crystals in kidney. Main causes of Nephrolithiasis are hypercalciuria, hyperoxaluria, hypocitraturia, hyperuricosuria, hypomagnesuria, gouty diathesis etc.

**Method:** In the present study *In vitro* inhibition of CaO₄ crystals growth Induced by Na₂C₂O₄ (2 mmol/L) was observed. *In vivo* ethylen glycol and ammonium chloride induced nephrolithiasis in rat was performed. After the treatment with the extract *in vitro* and *in vivo* both have anti-lithiatic potential was observed. The result was found to be very satisfactory and further pharmacological study was carried out on the samples so that it can be used as a drug for the treatment of urolithiasis. It was reveals from data that phytopharmaceuticals could be useful as either an complementary or alternative in the control of urolithiasis.

**Introduction**

Nephrolithiasis disorder is common worldwide, often debilitating disorder that has different etiology and pathophysiology. Nephrolithiasis is initiated by supersaturation of urinary salts and crystal retention in the urinary tract. Copiousness of promoter and inadequacy of inhibitors mainly produced, retention of crystals in renal tubules or stone formation in kidney. Urolithiasis affected approximately 10-12% population. with a recurrence rate of 70-80% on males and 48-61% in females. The kidney stones mainly made of, up to 80%, are of calcium oxalate.

**Material and Methods**

The plants were selected and reviewed for its antilithiatic activity and collected. The plants were then authenticated from Saifia College of Science, Bhopal, M.P.

**Preparation of extract and phytochemical screening:** The preparations of the extracts were by done maceration procedures. Phytochemical analysis was performed to determine the active ingredients in the extracts, and were used for the detection of the presence of saponins, flavonoids, carbohydrates, glycosides, resins, sterols, alkaloids and tannins.
Separation of ethyl acetate fraction: 50% hydro methanolic extract of *Cynodon dactylon* was taken and mix with 1 normal HCl boil 60°C at 30 min then cool 2 hrs in room temperature. Then supernatant phase were separated.

Quantitative estimation on ethyl acetate fraction of *Cynodon dactylon*: Total phenolic compound and total flavonoids fraction was separated and estimated.

Toxicological studies: LD₅₀ was determined according to the guidelines of organization for economic Co-operation & Development (OECD). The limit test was performed at 2000 mg/ kg, p.o, and found non-toxic. A dose range of 100, 300 and 500 mg/kg was selected for the pharmacological activity.

In-vitro anti-lithiatic potential (Nucleation assay): This activity crystallization without inhibitor and with was studied, to assess the inhibiting capacity. Solution of sodium oxalate and calcium chloride were used for the crystal formation.

In-vivo anti-nephrolithiasis in rodent: The 36 rats were divided into six groups comprising four animals each group. The treatment protocol was performed for 10 days. *Group 1*: Normal, Rats were administered 6 μl distilled water/g body weight by gavage (positive control). *Group 2*: Rats were administered 6 μl distilled water/g body weight by gavage (Negative control). *Group 3*: Standard drug (Cystone tab.750 mg). *Group 4*: Phenolic fraction of *Cynodon dactylon* (PFCD) Hydro-methanolic Extract; 100 mg/kg. *Group 5*: PFCD Hydro-methanolic Extract; 300 mg/kg. *Group 6*: PFCD Hydro-methanolic Extract; 500 mg/kg. Groups 2, 3, 4, 5 and 6 were access to drinking water containing 2% [w/v] ammonium chloride (AC) and 0.75% [v/v] ethylene glycol (EG) in order to formed CaOx and hyperoxaluria deposition in the kidneys.

In-vivo study parameters: Serum analysis of Sodium, Chloride, Potassium, Calcium, Phosphates, blood urea nitrogen (BUN), Uric acid, Creatinine, SGOT, SGPT, Body weight and relative organ wt of liver, kidney, heart & spleen. Anti-oxidant enzyme study on kidney homogenate for Malodialdehyde, Nitric Oxide, Reduced glutathione, Superoxide dismutase and histology of kidney.

Result and Discussion
The turbidity of solution of the treatment group was found to be lower as compared with control, showing the potential aspect on oxalate crystallization. As the concentration of the plant extract increased calcium oxalate crystal formation in % inhibition also increased proportionally. Urea and creatinine levels were found to be higher in Groups 3, 4, 5 and 6 known by serum analysis as compared to Group 2. There are marked renal damage in the EG/AC-treated rats as shown by data. The level of urea, creatinine, calcium and phosphorus were significantly constant near normal level in the treatment groups rats (Groups 4, 5 and 6) compare as in EG/AC (Group 1, positive control). At the completion of the experiment weight of
EG/AC-treated rats (Groups 3, 4, 5 and 6) was found to be less than the normal rats (Group 1). EG/AC (Group 5) was found to increased kidney calcium levels compared with the normal rats, the administration of PFCD extracts reduced this accumulation of calcium (Group 2) Histopathological studies clearly revealed single epithelial lining along the margin and were of normal size compared with the tissue samples from the control group (Group 1) shows tubules with. In Group 2, 3, 4 (test group) the specimen showed characters of the normal animals.

**Conclusion**
The extract showed potent antilithic ability and the percentage inhibition and amount of crystal formation. The Phenolic fraction of *Cynodon dactylon* has significantly reduced the elevated level of calcium oxalate ions which is consider as one of the inhibitor of crystallization. The histopathological findings also show sign of improvement after treatment with extract.

**References**
**Abstract:** The extract of the blume of *Arisema lechenaultii* obtained by extraction of mixture of equal proportions ethanol and water was chosen for pharmacological screening. In radiant heat tail-flick method and hot plate method the crude extract produced 14.3 sec ($p<0.001$) and 15.6 sec ($p<0.001$) elongation of tail flicking time 30 minutes after oral administration at the 200 mg/kg dose level, respectively.

**Introduction:**
*Ariseama lechenaultii* Blume (Araceae) puthuashak is distributed over the greater part of India on the hills of Assam, Karnataka, Kerala & Tamil Nadu. It is also distributed in Nilgiri’s, travancore, ceylone, Shimoga (Hulical); Palghat (Upper Ghat; Silent Vally). It is locally called as Dehi and its roots are used as a medicinally for urinary diseases, colitis, eczema, purging, gonorrhoea, piles, haemorrhoids, syphilis, round worms, fistula, sinus. The tubers of plant rich in fructosans [1]. It is Monoecious or dioecious tuberous herb.

**Materials and Methods:**

**Randall-Selitto assay:** Mechanical nociceptive thresholds, an index of mechanohyperalgesia, were estimated using an analgesiometer (Ugo Basile, Milan, Italy). A constantly increasing pressure was applied to the right hind paw until the rats vocalized or withdrew their hind paws. [2]. Suspension of extracts of *A. lechenaultii* (100 and 200 mg/kg) were administered orally 30 min prior to the induction of pain. The cut off pressure was 450 g.

**Tail flick method:** In this method immersing extreme 3 cm of rats tail in water bath containing water at a temperature of 55±0.5°C within a few minute, the rat reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. The reaction time following the administration of the extracts (100 and 200 mg/kg p.o.), pentazocine (20 mg/kg i.p.) and vehicle (10 mL/kg) was measured at an interval from 0.5-2 h after a latency period of 30 min [3].

**Hot plate method:** The temperature of the cylinder was set at 55±0.5°C [4]. Each rat (six per group) acted as its control before the treatment; the reaction time of each rat (licking of the fore paw or jumping response) was done at 0 and 10 min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of the alcoholic and aqueous extracts (100
and 200 mg/kg p.o.), pentazocine (20 mg/kg i.p.) and vehicle (10 mL/kg p.o.) was measured at an interval from 0.5-2 h after a latency period of 30 min.

Results and Discussion:

Analgesic activity: The analgesic activity of MEN was studied for central (narcotic) and peripheral (non-narcotic) effect. The analgesic activity of MEN against acute inflammatory pain was good as compared to potent inhibitory activity of ibuprofen. Ibuprofen offers relief from inflammatory pain by suppressing the formation of pain substances (prostaglandins and bradykinin) in the peripheral tissues. Therefore, it was expected that MEN might suppress the formation of these substances or antagonize the action of these substances and thus, exerts its peripheral and central analgesic activity. The central and peripheral analgesic activity may be due to free radical scavenging activity [6].

Randall–Selitto assay: The Randall–Selitto was used to measure inflammatory hyperalgesia. Table 1 shows analgesic activity of A. leschenaultii in Randall-Selitto assay. At the dose of 100-200 mg/kg body weight, MEN showed an early onset of analgesic effect to rat paw from 0 h to 5 h after drug treatment in Randall-Selitto assay. The EEAL showed maximum increase in pain threshold (281.2 g) followed by AEAL.

Table 1 Analgesic activity of A. Leschenaultii blume by Randall-Selitto assay.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Pain threshold (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Con</td>
<td>-</td>
<td>77.0±0.05</td>
</tr>
<tr>
<td>EEAL</td>
<td>100</td>
<td>153.3±0.01*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>171.3±0.05*</td>
</tr>
<tr>
<td>AEAL</td>
<td>100</td>
<td>130.3±0.02*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>147.2±0.04*</td>
</tr>
<tr>
<td>Ibn</td>
<td>100</td>
<td>170.0±0.01**</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SEM (n=6); One-way ANOVA followed by Dunnett's test; *P<0.05 and **P<0.01 considered significant as compared to control.

Hot plate and Tail flick test: The hot plate and tail flick method involve spinal reflexes and is regarded as one of the most suitable methods for studying the involvement of centrally acting analgesics. The neurogenic phase is probably a direct result of stimulation in the paw and reflects centrally mediated pain with release of substance P while the late phase is due to the release of histamine, serotonin, bradykinin and prostaglandins. The hot plate and tail flick test indicated that the pharmacological actions were mediated by (μ), opioid receptors rather than kappa (κ) and delta (δ) receptors.

In both test EEAL at the dose of 200 mg/kg exhibited maximum activity with reaction time (14.3 sec and 15.6 sec) respectively. Pentazocine at the dose of 20 mg/kg showed its maximum protective effect in hot plate (15.0 sec) and tail flick method (16.0 sec). The result of the hot plate and tail flick test revealed that
the reaction time was significant as compared to control (P<0.05). The results are presented in Table 2 and Table 3. On the basis of results of hot plate and tail flick test we can consider ethanolic extracts of *A. leschenaultii* as central analgesic.

**Table 2** Analgesic activity of *A. leschenaultii blume* extracts in hot plate method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Con</td>
<td>-</td>
<td>1.8±0.03</td>
</tr>
<tr>
<td>EEAL</td>
<td>100</td>
<td>9.8±0.01**</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.8±0.03*</td>
</tr>
<tr>
<td>AEAL</td>
<td>100</td>
<td>7.6±0.02*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9.6±0.01**</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>20</td>
<td>14.0±0.01**</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SEM (n=6); One-way ANOVA followed by Dunnett's test; *P<0.05 and **P<0.01 considered significant as compared to control.

**Table 3** Analgesic activity of *A. leschenaultii* blume extracts in tail flick method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Con</td>
<td>-</td>
<td>3.0±0.04</td>
</tr>
<tr>
<td>EEAL</td>
<td>100</td>
<td>9.7±0.001***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>12.7±0.07*</td>
</tr>
<tr>
<td>AEAL</td>
<td>100</td>
<td>8.5±0.006*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.5±0.1</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>20</td>
<td>11.0±0.010***</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SEM (n=6); One-way ANOVA followed by Dunnett's test; *P<0.05 and **P<0.01 considered significant as compared to control.

**Conclusion:**

Ethanolic extract of *A. leschenaultii* were subjected to analgesic activity using various *in vivo* models. The extracts were active against all peripheral and central pain mechanism. Among both extracts the ethanolic extract of blume showed better analgesic activity.

**References:**

Nootropic potential of *E. thymifolia* phytosterol in the treatment of various cognitive disorders in mice

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**Abstract:** *Euphorbia thymifolia* Linn (Family-Euphorbiaceae) is a commonly occurring annual herb found in waste lands and along roadsides under humid condition. The present study attempts to evaluate nootropic effect of ETTS by testing its effect on exteroceptive, interoceptive and cognitive memory related behavior of mice. ETTS was isolated following standard procedure and LD$_{50}$ was found to be 283.00 mg/kg (i.p) and the dose range of 25, 50 and 75 mg/kg was selected for evaluation of nootropic activity. ETTS 75 mg/kg showed significant reduction of response time against scopolamine induced amnesic response. Passive avoidance measured as step down latency was increased extreme significantly by ETTS. ETTS at all three doses showed significant reduction of TL against amnesic response of diazepam and scopolamine on elevated plus maze. ETTS significantly reduced lithium induced head twitches. The present study indicated nootropic activity of ETTS as it showed facilitatory effect on retention of acquired learning and enhanced spatial long term memory.

**Introduction:**

*Euphorbia thymifolia* Linn (Family-Euphorbiaceae) commonly known as Dudhi is an annual herb found in waste lands, along roadsides and wall sides under humid condition. The preliminary antianxiety and moderate depressant activity with modulating antidepressant property of *E. thymifolia* was the driving force to select the plant for assessing neurorejuvenating activity which probably may protect against the development of dementia or even slow the progression of dementia. The present study evaluates the neurorejuvenating effect of *E. thymifolia* phytosterol by testing its effect on exteroceptive, interoceptive and cognitive memory related behaviour.

**Materials and Methods:**

**Plant material:** The plant was collected from Bhopal (M.P.) India and authenticated by NBRI, Lucknow. The dried whole plant material was extracted with petroleum ether, refluxed with 5% alcoholic KOH and filtrate was extracted with diethyl ether to obtain *E. thymifolia* total sterol (ETTS).
**Experimental animals:** Male and female breed Swiss Albino mice weighing between 18-22 gm were used. ETTS was weighed and dissolved in 5% Tween 80 in water for injection and administered by intraperitoneal (i.p) route. The acute toxicity study was performed based on the OECD guideline 425. LD₅₀ was calculated as 283.00 mg/kg from graphical representation.

**Group division:** Group I: vehicle control (0.1 ml/20 gm WFI, i.p.), Group II-IV ETTS 25, 50, 75 mg/kg, i.p., Group V: negative control (amnesia inducing agent), Group VI: Piracetam 200 mg/kg, i.p., Group VII-IX ETTS 25, 50, 75 mg/kg, i.p. along with amnesia inducing agent as per the particular protocol (n = 6 per group).

**Exteroceptive behaviour model**

**Active avoidance paradigm (Shuttle box):** All the animals of group I to IV and VI to IX were treated with respective drugs for 15 days. On 15<sup>th</sup> day scopolamine hydrobromide (1 mg/kg, i.p.) was administered to animals group V to IX 30 min after dosing of standard/test drug to induce impairment of memory and repeat test was performed to observe the conditioned stimulus and unconditioned stimulus time [2].

**Passive avoidance paradigm (Step down type passive avoidance test):** Each mouse was gently placed on the wooden platform of the apparatus, shocks was delivered for 15 sec and the step-down latency (SDL) was recorded. Animals were administered with drugs and after successful first trial animals were treated with scopolamine hydrobromide (1 mg/kg, i.p.) after 45 min. SDL was measured after further 45 min and also after 24 hrs [3].

**Interoceptive behaviour model**

**Retention of learned-task:** Animals of group I to IV was administered with vehicle and test drug for 14 days and TL was noted after 45 min of last dose administration on 14<sup>th</sup> day and again after 24 hr that is on the 15<sup>th</sup> day. Retention of this learned-task was evaluated by calculating the inflexion ratio (IR) using the formula as follows.

\[ IR = \frac{(L_0 - L_1)}{L_0}, \text{where } L_0 \text{ is the initial TL on 14}^{th} \text{ day and } L_1 \text{ is the TL on the 15}^{th} \text{ day.} \]

**Diazepam-induced amnesia:** All the animals were treated with different doses of ETTS respectively for a period of 14 days. Diazepam (1 mg/kg, i.p) was administered 90 minutes after last dose to induce impairment of memory through GABAergic system activation and TL measured after 45 min and 24 hrs to calculate the IR [4].

**Scopolamine induced amnesia:** Done with similar protocol as diazepam induced amnesia using scopolamine as amnesic agent.

**Cognitive behavioural Model**
Lithium-induced (5-HT mediated) head twitches: Mice were treated with vehicle, test drug and Piracetam 30 min before i.p. administration of lithium carbonate and the number of head twitches was counted up to 60 min after lithium treatment [5].

Haloperidol-induced (DA mediated) catalepsy: Animals were treated with different doses 30 min before haloperidol treatment and the duration of catalepsy was noted at 15, 30, 60, 90, 120 and 150 min by means of Bar test [6].

Results and Discussion:
ETTS 75 mg/kg (P < 0.05) treated group showed significant reduction of response time against scopolamine induced amnesic response. Passive avoidance measured as step down latency (SDL) after 24 hrs of training was increased extreme significantly (P < 0.001) of all three doses of ETTS. ETTS at all three doses (25, 50, 75 mg/kg) showed significant reduction (P < 0.001) of TL against amnesic response of diazepam and scopolamine on elevated plus maze. ETTS significantly reduced (P < 0.001) head twitches in all doses but had non significant effect on haloperidol induced catalepsy.

ETTS treatment in all three doses showed higher SDL than the control group in passive avoidance test session. ETTS reversed scopolamine, induced deficit in learning and memory indicating its potential anti-cholinesterase role. The results showed that ETTS decreased the number of lithium induced serotonin mediated head twitches indicating diminished serotonergic function in brain reinforcing the anxiolytic effect of ETTS. ETTS neither reduced nor potentiated the haloperidol induced catalepsy indicating no interference with brain dopaminergic system.

Conclusion:
E. thymifolia steroid possesses nootropic activity in view of its facilitatory effect on retention of acquired learning and enhancing spatial long term memory and may be useful as a nootropic agent in the treatment of various cognitive disorders.

Acknowledgement:
The authors express their sincere thanks to the MPCST, Bhopal, MP for providing financial support (project grant no. A/R&D (BS)-02/31).

References:
A pharmacological evaluation of anti-diarrhoeal activity of bark extract of *Thespesia populnea* in diarrheal induced rats

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Abstract: The present study was performed to substantiate the traditional claim of antidiarrhoeal activity of stem bark extract of *Thespesia populnea* in rats. The effect of ethanolic extract of stem bark of *T. populnea* on castor oil induced diarrhoea, gastrointestinal motility test using charcoal meal method were examined. The extract was initially assayed for its affect in castor oil induced diarrhoea at different doses (100, 200 mg/kg, p.o.) in which significant activity (p<0.05) was observed at a dose level of 200 mg/kg. The extract was found to inhibit peristaltic movement in charcoal meal test and intestinal fluid secretion in castor oil induced enteropooling, confirming its antidiarrhoeal activity, which might be due to its high glycoside content. The results provide evidence that the ethanolic extract of *T. populnea* stem bark posses’ potent anti-diarrheal activity.

Introduction:

Diarrhoea is recognized as one of the important health problems in most of countries. In diarrhoea an increase in the frequency, fluidity, or volume of bowel movements and characterized by increased frequency, sound and movement of bowel, wet stools, and abdominal pain. Clinically it describes increased stool liquidity, associated with increased stool weight and frequency [1].

Materials and Methods:

Experimental animals: Albino Wistar rats weighing between 150-250 g of either sex were used. Experimental protocol was approved by IAEC, housed under standard conditions of temperature (24 ± 2°C), relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum*.

Plant Material: Fresh bark of *T. populnea* was collected from the Malva region of Madhya Pradesh. The bark were shade dried and converted in to fine cores powdered, packed into soxhlet column and successive extraction was perform with different solvent (petroleum ether (60-80°C) for 24 h chloroform (50-60°C) and ethanol for 24 h). The obtained extract were dried and stored in airtight container.
Phytochemical Investigation: The extracts of *T. populnea* were investigated for the various phytoconstituents like phytosterols, saponins, tannins, proteins alkaloids, carbohydrates, glycosides, amino acids and flavonoids [2].

Castor oil induced diarrhea: Rats (150-250gm) of either sex fasted over 16 h. Divides into four groups (n=6). First group which served as control was administered aqueous 1% tragacanth suspension. The second groups served as standard drug receive Loperamide (2 mg/kg) orally. The extract was administered orally at 100 mg/kg dose to third group serve as a lower dose group and 200 mg/kg dose to fourth group serve as higher dose group. After one hours of drug treatment, the animals of each group administered 1ml of castor oil orally and the watery fecal material and number of defecation was noted up to 4 h in the metabolic cages. Weight of stool before and after defecation was noted [3].

Charcoal meal test: Rats (150-250gm) of either sex fasted over 16 h. Divides into four groups (n=6). First group which served as control was administered aqueous 1% tragacanth suspension. The second groups served as standard drug receive atropine (0.1 mg/kg) sc. The extract was administered orally at 100 mg/kg dose to third group serve as a lower dose group and 200 mg/kg dose to fourth group serve as higher dose group. The animals were administered 1ml of 10% activated charcoal. Animals were anesthesed 30 min after charcoal meal administration. Cut abdomen and remove small intestine carefully. Measure distance travelled by charcoal plug from pylorus to caecum, expressed as percentage of the distance traveled by charcoal plug for each of animal [4].

Results and Discussion: Ethanolic extract of *T. populnea* (lower dose and higher dose) and the standard drug, atropine (0.1 mg/kg) decreased the intestinal movement in the charcoal meal test. The underlying mechanism appears to be anti-spasm and an anti-enteropooling property by which the extract shows relief in diarrhea. Tannins are present in many plants and they denature proteins forms protein tannate complex responsible for anti diarrheal activity. The complex formed over the intestinal mucosa and makes the intestinal mucosa more resistant and reduces secretion.

The anti-diarrhoeal activity of the extracts may be due to an inhibition of muscle contractility and motility, as observed by the decrease in intestinal motility by charcoal meal, in a reduction in intestinal tone. Extract inhibits the onset of time and severity of diarrhoea induced by castor oil [5]. It is well known that nitric oxide and prostaglandins are crucial mediators contributing to generation of inflammatory
response to castor oil. Alternatively, the effect of castor oil may be attributed to disordered motility and hence to an increase in intestinal transit of intra-luminal material. In this connection, castor oil could alter coordination of intestinal motility and could promote greater loss of fluid from intestine. The reduction of gastrointestinal motility is one of the mechanisms by which many anti-diarrhoeal agents.

Table 1 Evaluation of anti-diarrhoeal activity of alcoholic extract of *T. populnea* by castor oil induced diarrhea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean wet defecation</th>
<th>Mean increase in weight of paper (g)</th>
<th>Delay in defecation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.16±0.87</td>
<td>3.02±0.49</td>
<td>32.16±7.30</td>
</tr>
<tr>
<td>Loperamide (2 mg/kg)</td>
<td>1.83±1.05</td>
<td>0.53±0.29</td>
<td>188.00±22.50</td>
</tr>
<tr>
<td>Alcoholic extract (100 mg/kg)</td>
<td>4.66±0.99</td>
<td>2.14±0.25</td>
<td>70.83±2.84</td>
</tr>
<tr>
<td>Alcoholic extract (200 mg/kg)</td>
<td>3.66±0.80</td>
<td>1.40±051</td>
<td>132.33±29.26</td>
</tr>
</tbody>
</table>

Number of animals (N) = 6
Values are expressed as mean ± SEM

Table 2 Evaluation of anti-diarrhoeal activity of alcoholic extract of *T. populnea* by charcoal meal test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Movement of charcoal (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.25 ± 1.56</td>
</tr>
<tr>
<td>Atropine sulphate (0.1 mg/kg)</td>
<td>34.42 ± 3.10**</td>
</tr>
<tr>
<td>Alcoholic extract (100 mg/kg)</td>
<td>53.02 ± 4.35**</td>
</tr>
<tr>
<td>Alcoholic extract (200 mg/kg)</td>
<td>43.13 ± 4.10**</td>
</tr>
</tbody>
</table>

Number of animals (N) = 6, Values are expressed as mean ± SEM

**Conclusion:**

The antidiarrhoeal effect of ethanolic extract is due to reduction of gastrointestinal motility, inhibition of the synthesis of prostaglandin and NO. The extract has potential effect on the reduction of gastrointestinal motility than the other effects. The above effects of it may also be due to the presence of tannins and flavanoids in the extract.

**References:**

What difference can a pharmacist make in the future pharmaceuticals profession?

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Abstract: A pharmacist, being a part of the regulatory sector plays a vital role in the entire health care process. The government is taking major steps to address requirements of drug regulatory bodies by extending financial support. Although with rapidly growing market the Indian government regulatory agency stands in need of a number of modifications for its advancement in the global market. In this review we have attempted to find out vital roles of pharmacist at various steps right from approval to the dispensing of drugs. We have reviewed the various setbacks in the practice of pharmacy profession and thereby have attempted to find out how involvement of pharmacists can bring a change and deliver quality health products to the community.

Introduction:
Even though a pharmacist is generally pictured as a person clad in a white coat standing behind the desk, filling prescriptions, the role of a pharmacist has evolved just as much as the prescriptions he/she dispenses. My basic avenue is to review the various affairs of the regulatory agencies and what alterations can be made to enhance the pharmaceutical industry. The current trend in the pharmaceutical market is the use of generic drugs. These are not well recognized in the market and hence it is important that their use and advantage is pointed out and promoted among the common man. Another major area that needs to be looked into is the use of off label medicines in the market. This entails major health risks and legal liabilities thus certain norms and regulations should be implemented for cautious use of such medicines. OTC use of schedule H drugs and certain drugs that are banned in other countries is an area of concern.

Method:
Data has been sourced and collected from the following;
1) Interviewed patients
2) Interviewed community pharmacists
3) Discussed input with people involved in public health regulation
4) Talked with undergraduate pharmacy students
5) Referred online websites
Results:

Current situation in pharmaceutical industry

A lot of amendments and changes have been brought about in last few years, one of the recent being approval of gross budgetary allocation of Rs.1750 crores to the Central Drugs Standard Control Organisation (CDSCO) of India by the Union Finance Ministry which shall be utilised for strengthening the departments as well as to address the requirements of the state drug regulatory body by extending financial support as central government assistance. The drug regulatory boards are constantly facing a large number of issues relating to drug pricing, government subsidies, patent related issues, counter fees and many other things [1].

Ignorance in use of generic medicines

The latest trend in the pharmaceutical market is the use of generic drugs. These are drugs which are copies of the original branded drugs which are protected and patented by MNC and large manufacturers. These generic drugs are introduced into the market when the patent expires. In India a very large number of people are living below poverty line. They are not able to afford branded drugs because many a times these drugs are too expensive. Therefore, generic drugs become preferred options in such cases. Although there is a general misconception that generic drugs are low quality and hence people as well as physicians avoid opting for these. This image however does not hold true. Generic drugs are equivalent to the branded drugs and differ solely in their excipients. Hence there is no alteration in terms of their quality or strength and are perfectly safe as well as economic for use [2].

Off label prescribing of medicines and malpractices of unauthorized physicians

Off-label use means use of medicine which is outside the terms of product license. Off-label medicines are those with a different indication or a different route of administration, different age group or different dosage to that which is approved by the regulatory authorities. For example, some cancer drugs were found to work against many different kinds of tumours. Chemotherapy treatments often combine drugs. These combinations might include one or more drugs not approved for that disease. Thus off-label drugs can be used to good effect by physicians because they understand how to use them correctly. However it can entail health risks and legal liabilities [3].

OTC dispensing of Schedule H drugs

With the Union Heath and Family Welfare Ministry notifying amendments to drugs and cosmetics act 1940, a new provision, Schedule H1 has been introduced and brought into effect in 2014 to check the
indiscriminate use of drugs. As many as 46 drugs have been placed under this restriction category and yet many commonly used drugs are still being dispensed as OTCs by Indian pharmacies. These malpractices should be curbed and it should be ensured that the pharmacies abide by the rules by having thorough and frequent drug inspections carried out [4].

Use of banned drugs
Some drugs that are banned in other countries for proven adverse effects are still available in the Indian market. Some of these drugs are available as OTC drugs and people may take it without realizing the risk. For example, Nimesulide, an NSAID is a drug used for osteoarthritis and dysmenorrhea. It has been found to cause liver failure due to which it has been withdrawn from foreign markets but the ban has not been implemented in India. Few other drugs which are still in use and need to be banned in India are Oxyphenbutazone, Cisapride, Phenylpropanolamine, Cerivastatin, Analgin [5].

Conclusion:
The objective of pharmacists should be to bring about a major change in the above situations by introduction and implementation of regulations and laws and by taking severe actions against various malpractices being carried out in a time bound manner and in this way impact the country by providing the consumers with a better and more secure future in terms of heath care thus not only benefitting the consumers but also to rise India’s stand in the global pharmaceutical market.

Reference(s):
Designing of curriculum aspects of pharmacy undergraduate course in respect of graduate employability

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Introduction:
The graduate employability in India is a global concern as increasing number of graduates produced by higher education in pharmaceutical education each year. Graduate should acquire both theoretical and practical skills in order to become work deliverable and to perform well at work. The pharmacy degree curriculum varies from university to university. The governing body at university level also varies from university to university. The 1st B. Pharm. students were surveyed to know their inclination towards career orientation program and opinion about essential knowledge towards employability [1].

In the context of current scenario of lack of exposure to the latest development in pharmaceutical industry, intellectual property right and industry based regulatory needs, it is urged to inculcate awareness to update existing degree pharmacy curriculum, keeping in view of requirements and expectations of industry evaluating existing status of degree pharmacy curriculum at national level using questionnaire survey design from four category of professionals industrial, academic, industrial other than pharmacy and clinical professionals connected to the pharmacy profession.

Materials and Methods
Questionnaire survey design of fifteen multiple choice questions based on the undergraduate pharmacy curriculum, aspects of its regulation, existing state of art and improvements anticipated and inviting concerned human subjects for sampling for preferential opinions. Four types of human subject's samples were collected directly by face to face, visiting industrial organizations, from college academic staffs and prospective post graduates in pharmacy and indirectly by electronic mail service as fastest innovative service to generate the sampling data.

Sampling methods: Sampling by
1. Face to face direct interview, and distribution of questionnaire sheets to three industrial, two academic educational institutions, and industrial professionals other than pharmacy and delegates who attended meeting of Maharashtra Community Pharmacist Association held at Pune.
2. Indirectly by electronic mail. The clinical, academic and industrial pharmacy professionals were communicated indirectly by electronic mail.
Results:
The total seventy eight samples from human subjects were summarized category wise and alpha-beta (a), (b), (c) and (d) preference from multiple choice questions. The codified data of 15 questions generated from 78 human subject samples was transformed from alpha bet coding to the number of times i.e. frequency at which the respondents preferred the code. The data from three categories of human subjects was analyzed statistically for S.D., (S.D.)² i.e. variance (V) and evaluated for F statistics using ratio of variances as F calculated and F tabulated value to test null hypothesis and 0.05 level of significance.

Discussion:
The degree pharmacy education is generally an option to the students aspiring for challenging professional field in which both the faculties, Engineering and Medical equally contribute to develop the profession of qualified pharmacist. It was suggested to develop a degree pharmacy curriculum in two streams right from 1st year B. Pharmacy with inclination of student's attitude to choose their career profession in pharmacy, industry stream and health care stream [2]. Accordingly to mold syllabus as per the present need as well as students own liking in the profession. It is also important to inculcate knowledge to acquire various essential skills by tying up with industry and hospitals for seminars, lectures and training. There is an apparent need in the current global scenario that the pharmacy educational institutes must not only academic oriented but also professional activity oriented to mold their students so that they are accustomed to the present competitive and demanding industrial environment. In India there is a decline in the selection by pharmacy graduates to opt for hospital oriented postgraduate program because of less availability of job opportunities as compared to the industry and product oriented program.

As pharmacy is not independently developed or evolved as a separate state university of pharmaceutical education, opinions were generated about the affiliation to Technological, Health Sciences, Allied Health Science or faculty of technology affiliated to the university. Usually student opts for pharmacy undergraduate program as an alternative to medical or engineering. The student take up the course via state entrance examination specifically designed for pharmacy. As these students have medical and engineering orientation, the pool of student to take up pharmacy profession will be compromised. As a qualified pharmacist, job opportunities in industry are extensive in comparison with the job opportunities in health care program. It may be because we in India have given less attention to the American system of pharmacy education or in cutting age technology, Indian concern is more towards product development for the improvement of health and prevention of disease. However as certain fraction of students prefer to medical oriented subject areas during graduation program. These graduates after post-graduation degree
or post graduate diploma have opportunities to join the medical /health team in the area of clinical research. Recently there is an acute shortage of these candidates as students get easily accommodated in the industrial specialization, against vacancies in great many private colleges. It is suggestive to develop pharmacist during undergraduate course work based on their innate abilities, attitude and performance towards the professional education, to freely select the subjects which can offer them qualification B.Pharm (Pharmaceutical Industry) or B. Pharm (Clinical Industry). B. Pharm (Pharmaceutical Industry) would choose pharmaceutical industry oriented job and post graduate program whereas B.Pharm (clinical industry), would choose biological product development and manufacturing , jobs at clinical research centers and academic programs PG in biopharmaceutics, pharmacy practice, pharmacology and Pharm D.

**Conclusion:**
In conclusion the maximum percent respondents from different categories of profession have preferred undergraduate pharmacy curriculum affiliation to the Health Science University. For better employability and marketability with additional specialized electives, degree can be conferred as B.Pharm (Pharmaceutical Industry) and B. Pharm. (Clinical Industry). It is emphasized that regular lecture course, seminar or workshop besides one semester subject course work, is needed to constrict the gap of search for job and employability after graduation. The maximum percent respondents from three categories were of the view that prospectus of employability will be high with additional specialized training in clinical research, product development, quality management, regulatory affairs, analytical methods for scale up technique, research &development and health care profession. It is emphasized that the teachers engaged in training of pharmacy students, needs to be given adequate industrial exposure to train student and participation in the teacher training course concern with educating student with current industrial and technological development to bridge the gap college laboratory to industrial practice.

**Acknowledgements:**
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**References:**
Outcome based learning for overall development of pharmacy students

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Abstract: The study was carried out with an intention to evaluate student’s ability to effectively use their qualities, strengths, knowledge, and for their novel ideas. Student of Final year B. Pharm were grouped into different batches (6 students each batch) and were assigned the work of preparing a poster of the experiment specified for the day’s practical class. A validated questionnaire based evaluation was done to find out the students ability, perception and competency about outcome based learning process. 92% of students found that the activity made the subject interesting and 70% responded that it helped to plan their activities well in advance. Internet was the major source of information (93%) as compared to text books and journals (4%) and medical shops (3%). 90% students agreed that this learning process helped in their skill utilization, skill development and motivated them to come out with innovative ideas. 93% students expressed that it improved their subject knowledge and presentation skills and thereby reduced their stage fear (90%) and developed their self-confidence (85%). Subsequently each group was able to work as team (78%). 62% students revealed that activity did not affect their regular lab work and 72% expressed that activity made them to come to lab early. Based on validated supported data, it is concluded that OBL is very effective and useful tool to develop students and supports to outperform with respect to multiskilling activities and hence we recommend that this tool can be incorporated in pharmacy curriculum to make teaching-learning process effective.

Introduction:
Pharmacy graduates should have acquired an appreciation of the values of a broad range of intellectual disciplines as well as general knowledge, and within that wide spectrum, have gained a depth of knowledge within a specialty, not only as an end in itself but also as a vehicle for experience in serious study and enquiry.

To improve the innovativeness, competencies and abilities of the pharmacy students. The study comprises is a survey by students to assess their program’s educational outcomes that were used by many colleges either alone or in combination with other methods.

Experimental Methods:
Documentation and Strategies that contains findings from three surveys administered to the first year
(n=54 items), second year (n=58 items), and third year (n=42 items) and final year (n=58 items) professional students. A listing of instructional objectives for each year of the pharmacy curriculum was compiled and rated by the students.

Upon completion of each year of the program, the survey asked the respondent whether they were "taught" and "can perform" each listed objective. The following objectives were set to prepare the questionnaire for the survey of pharmacy students as well as the difficulty level of

All questions is also year wise decided.
1) Communication ability
2) Pharmaceutical care competency
3) Technical Competency
4) Multitasking abilities
5) Case Study

Results and Discussion:

Table 1 Communication ability (e.g., mapping subjects)

<table>
<thead>
<tr>
<th>Communication ability</th>
<th>N</th>
<th>Total</th>
<th>Mean P-1 n=67</th>
<th>P-2 n=58</th>
<th>P-3 n=42</th>
<th>P-1 to P-3 Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Write, speak and use data</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>15</td>
<td>3.36</td>
<td>3.95</td>
</tr>
<tr>
<td>A2 Interpret ideas, thoughts, and feelings.</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>19</td>
<td>3.42</td>
<td>3.87</td>
</tr>
<tr>
<td>A3 Identify personal strengths, weaknesses, barriers, and preferences</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>14</td>
<td>3.52</td>
<td>3.98</td>
</tr>
<tr>
<td>A4 Use writing, speaking, data and media</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>19</td>
<td>3.30</td>
<td>3.71</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>36</td>
<td>20</td>
<td>67</td>
<td>7.7</td>
<td>6(3.3)</td>
</tr>
</tbody>
</table>

Conclusion:OBL is very effective and useful tool to develop students and supports to outperform with respect to multiskilling activities and hence we recommend that this tool can be incorporated in pharmacy curriculum to make teaching-learning process effective.
References:


[2] Best Practice in outcome based teaching and learning by David Kember, University of Hong kong
A study on the health screening services for the rural population of adopted village of south India

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Introduction:
Health screening services (HSSs) are the services provided by the health care professionals (Doctors, Dentists, Pharmacists, Nurses, Physiotherapists, Health educators) to screen the health status of the individuals with or without positive sign and symptoms. Health screenings are quick and easy, does not hurts and it takes no special preparations. Screening services help to detect mild to severe diseases. All the detections of disease make a difference for referral to doctor for further management .Early diagnosis always better than late diagnosis. Screening services can be provided at a setup where adequate facility and space for performing services. Rural population in India lack in accessibility in health sector. This may be due to negligence of doctors to provide health care services and economic status of the population.

Pharmacist, a health care professional performs health screening services at hospital, community pharmacies. Health screen comprises of Blood pressure (BP) measurement, blood glucose measurement, health education materials, provision of lifestyle advice and dietary advice and advice on risk factors and smoking cessation support. The measurement is done by using different devices which are compact, digital, quick and easy to use. The study objective was to evaluate the status of the health in a rural population.

Materials and Methods:
A nearby village is adopted to screen the health status of individual a trained clinical pharmacist measured the blood pressure, random blood glucose the abnormal values of the individuals were asked for consulting doctor for the follow up. The data was analyzed by using Microsoft excel 10 version. A suitable identified easy accessible location used for the measurement of B.P and RBS .the study was a prospective population screening population. The apparatus used are automatic blood pressure monitor. Optima™ Blood Pressure Monitor, fully automatic upper arm style, Model No. U80B used. This device statement on the blood pressure measurements determined are equivalent to those obtained by a trained observer using the cuff/stethoscope auscultator method, within the limits prescribed by the American National Standard, manual sphygmomanometers.
Results and Discussion:

A total of 187 screened female 104(55.61%) and males 83(44.31%).age and occupation details shown in table 1. The details of blood pressure grades of the screened population are shown in table 2. The details of diabetic patients is shown in table no.3. The variations in demographics of screening of blood pressure and diabetic may be due to life style modifications and genetic makeup and not access to the primary health care system.

<table>
<thead>
<tr>
<th>Table 1 Demographic details of enrolled and/or screened population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descriptions</strong></td>
</tr>
<tr>
<td><strong>Gender (n=187)</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Age (Years) n=187</strong></td>
</tr>
<tr>
<td>&gt;20</td>
</tr>
<tr>
<td>21-30</td>
</tr>
<tr>
<td>31-40</td>
</tr>
<tr>
<td>41-50</td>
</tr>
<tr>
<td>51-60</td>
</tr>
<tr>
<td>61-70</td>
</tr>
<tr>
<td>71-80</td>
</tr>
<tr>
<td>Average</td>
</tr>
<tr>
<td><strong>Occupation (n=153)</strong></td>
</tr>
<tr>
<td>Male (n=83)</td>
</tr>
<tr>
<td>Agriculture</td>
</tr>
<tr>
<td>Housewife</td>
</tr>
<tr>
<td>Business/Driver</td>
</tr>
<tr>
<td>Employed</td>
</tr>
<tr>
<td>Student</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Comparison of blood pressure measurement among screened population with Hypertension (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descriptions</strong></td>
</tr>
<tr>
<td><strong>Systolic blood pressure</strong></td>
</tr>
<tr>
<td>Grade I (140-159)</td>
</tr>
<tr>
<td>Grade II (160-179)</td>
</tr>
<tr>
<td>Grade III (&gt;180)</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure</strong></td>
</tr>
<tr>
<td>Grade I (90-99)</td>
</tr>
<tr>
<td>Grade II (100-109)</td>
</tr>
<tr>
<td>Grade III (&gt;110)</td>
</tr>
</tbody>
</table>
Table 3 Comparison of random blood glucose measurement among screened population with Diabetic 
(n=60)

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Male (n=18) Number (Percentage)</th>
<th>Female (n=39) Number (Percentage)</th>
<th>Total Number(Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;140 mg/dL)</td>
<td>14(66.66)</td>
<td>12(30.76)</td>
<td>26(43.33)</td>
</tr>
<tr>
<td>Early diabetes (140-200 mg/dL)</td>
<td>08(38.09)</td>
<td>15(38.46)</td>
<td>23(38.33)</td>
</tr>
<tr>
<td>Establish (&gt;200 mg/dL)</td>
<td>04(19.04)</td>
<td>07(17.94)</td>
<td>11(18.33)</td>
</tr>
</tbody>
</table>

Conclusion:
This study revealed that the health status deteriorates with aging progression in rural population needs periodic monitoring of health status by the health care professional.

Acknowledgements:
Authors thanks to participants and B.V.B Balaji G. Ausha B. Mary Prasanna K. Arun Chand Roby, SK. Hussain, faculty, non teaching staff, students of VIPS for their active participation.

References:
A survey on alcohol consumption among college students

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Abstract: We conducted this study to find the prevalence, pattern and predictors of alcohol consumption among college students in Gwalior district of Madhya Pradesh. Students were (from professional and non professional colleges) were randomly selected for the present study. Information on socio-demographic details, family history of alcohol consumption, knowledge of health effects of alcohol consumption, frequency and quantity of alcohol consumption, age at initiation, reason for initiation and pattern of drinking was collected using an anonymous structured, and self administered questionnaire. The questionnaire was developed by the authors and pre-tested on a few college students in Gwalior. Data were entered and analyzed statistically.

Introduction:
Alcohol consumption has been steadily increasing in developing countries like India and decreasing in developed countries since the 1980s. With more than half of all alcohol drinkers in India falling into the criteria for hazardous drinking, alcohol abuse is emerging as a major public-health problem in the country. The country, which has seen a rapid proliferation of city bars and nightclubs in recent years, is fast shedding its inhibitions about alcohol as a lifestyle choice. This situation has led to fears of an undocumented rise in alcohol abuse not only among poorer classes but also in sections of society that were previously considered dry. The increasing production, distribution, and promotion of alcohol have already seen drink-related problems emerging as a major public-health concern in India. Sales of alcohol have seen a growth rate of 8% in the past 3 years. [1, 2]
The shifting composition of Indian drinkers has seen a rise in the number of Indian women drinking regularly and heavily. One recent study in the southern state of Karnataka found young women consumed similar amounts of alcohol to young men on any typical drinking occasion. Today 32 percent of our population consume alcohol and between 4 and 13 percent have it daily. [3] There is no distinction between rural and urban population and consumption is going up, particularly amongst the young. Urban youth in India is taking to alcohol in a big way and that is one of the reasons why Health Minister Study has revealed that the average age of alcohol consumption in India has fallen by nearly nine years over the past decade, from 28 to 19, and this is predicted to fall to 15 in another 5-7 years. [4, 5]
Research Methodology

The present investigation is comparative study of alcohol consumption among college students with survey being used as method for collecting data to complete the study.

Sampling Design:
1. Sample Population: population included all college students.
2. Sampling Frame: since the data was collected through personal contact the sample frame was the students of different colleges.
3. Sampling Elements: Individual respondents were the sampling element.

Self designed questionnaire was administered for evaluating the consumption of alcohol among college students. Various analytical and statistical methods were used for data analysis.

Result and Conclusion:

From the collected data, it is observed that nearly 72% boys reported drinking and 46.8% girls were indulged in drinking, so the ratio of boys to girls was nearly 3:2. This indicates that boys are more involved in consumption of alcohol than girls because boys are more in touch with their surroundings, the communities outside their residencies. 46% Hostellers and 43% day scholars accepted alcohol drinking. 72.54% college students aged between 18-25 year, 13.75% below 18 years, and 13.9% of above 25 years were reported as drinkers. It indicates that students of 18-25 age groups are more involved in alcohol drinking than other age groups, which signifies that when the students entered in college they start consuming alcohol either influenced by their seniors or by classmates who is already indulged in drinking. Students start drinking mostly because of influence of an adult and curiosity. 24.31% accepted of having history of alcohol or drug problems in their family. Most of the students drink alcohol on special occasions which include mostly parties. Beer is the most favorite beverage among students, is considered “cool” or “soft” drinks. Beer is easily available at most of the shops, in attractive packages which pull the students towards it. From the data, it is clear that most of the students can stop drinking if they want but then also they do not stop to drink because they don’t see any harm in drinking alcoholic beverages. Most of the students refused to become violent with fight after drinking but some accepted of becoming violent but no fight. It indicates that they have been able to control themselves after drinking.

72.54% of students have not been suggested to cut down consuming alcohol by relatives, friends or doctors. It indicates that either their relatives, friends or doctors are not serious towards their drinking or they are unaware of involvement of student in drinking. 83.15% of students have neither visited nor have knowledge about rehabilitation center. This remarks the indifferent behavior of the government towards
the awareness programmes and lack of advertisement about rehabilitation centers and treatment policies run under the Health Ministry. Most of the rehabilitation centers are located in urban areas, leaving large areas of rural India unserved by any organized activity in this field. There is a need of serious attention of government in this regards. 78.54% accepted of driving after drinking. Although such activities are not legitimate and are prohibited under the law, driving after drinking is rampant and so common that it is usually ignored. Some students who are regular drinker or addicted have reported that they use some alternatives (smack, codeine cough syrup, liquid whitener’s solvent, tobacco, and cigarette), if they do not get alcohol.

We hope as India strives to maintain its much hyped growth rate it also stops its infantile approach in dealing with alcoholism and many other health issues that are part of reason why people's quality of life is not going up at the same rate as economy. What is needed of college students is to be informed of the beneficial and harmful effects of alcohol, and understand that addiction is a disease that can be cured in most instances and the sooner one intervenes the better one can expect the outcomes to be. What is needed of the government is to have a smart regulation of alcohol that will add to revenue, ensure quality of alcohol, and spread information on the ills of alcoholism without making it the forbidden fruit.

References:
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