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CHARACTERIZATION AND PHARMACODYNAMIC EVALUATION OF DEVELOPED MICROEMULSION FOR NASAL DRUG DELIVERY

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Keywords:

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ABSTRACT: In the present study, we had developed Asenapine maleate (ASNM) loaded microemulsion (ME) to increase the solubility of Asenapine maleate by components of microemulsion including formulation concern, characterization, mucosal diffusion, stability and nasal ciliotoxicity of Asenapine maleate microemulsion (AME). For nasal delivery of Asenapine maleate, a challenge existing in formulation development is the solubilization of poorly water-soluble Asenapine maleate. The intrinsic solubility of Asenapine maleate is about 0.0312 mg/ml. The purpose of this study was to improve the solubility and to enhance the brain uptake of Asenapine maleate through an o/w microemulsion, with suitable intranasal delivery. The optimal microemulsion formulation consisted of Oleic acid, Tween 80: Propylene glycol (PG) (3:1) and water, with a maximum solubility of Asenapine maleate and no ciliotoxicity, was developed and characterized. AME was characterized for % Transmittance, pH, viscosity, globule size, zeta potential, drug content with *in-vitro* release studies and *Ex-vivo* diffusion studies using Standard Franz Diffusion cell. Further, the behavioural studies were assessed in rats by inducing hyperactivity. The optimized microemulsion was found to be stable and transparent with average globule size 94.40 nm, PDI 0.293 and without showing any ciliotoxicity during its histopathological evaluation on goat nasal mucosa.

INTRODUCTION: Asenapine maleate (ASNM), (3aRS, 12bRS)- 5- Chloro- 2- methyl-2, 3, 3a, 12b-tetrahydro-1H-dibenzo [2, 3, 6, 7] oxepino [4,5-c] pyrrole (2Z)-2-butenedioate (1:1) is an approved atypical antipsychotic drug belonging to chemical class of dibenzo-oxepino pyrroles with combination antagonist activity at dopamine D2 and serotonin 5-HT2A receptors in the limbic system alleviates symptoms of schizophrenia and bipolar I disorder [1, 2].

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ASNM is classified as BCS class - II drug with less than 2% oral bioavailability because of extensive first-pass metabolism. Bioavailability of marketed sublingual tablet was found to be increased up to 35% with the drawbacks of the restrictions of eating and drinking before and after dose administration and extrapyramidal side effects.

It is tough for psychic patients to take the dosage form via a suggested route as the patients are not in a normal state of mind. The drawback related to extensive metabolism is required to defeat which leads to increase the bioavailability of ASNM and for that few approaches and advancement were taken up by some of the research workers, including mouth dissolving film, sublingual film, intranasal formulations and injectable formulations of ASNM [3-6].

ASNM loaded nanostructured lipid carriers reported for a nose to brain targeted delivery to enhance the therapeutic efficacy of ASNM in the treatment of Schizophrenia. Pharmacodynamic and pharmacokinetic studies showed high brain bioavailability, better therapeutic and safety profile of ASNM loaded nanostructured lipid carriers *via* intranasal route [17]. Also, microemulsion and mucoadhesive microemulsion of ASNM was reported with the increased retention time of formulation due to polycarbophil, which leads to a significant effect on ASNM diffusion through nasal mucosa [21].

The systemic drug delivery *via* nasal route has received great attention, as it offers some advantages, including rapid absorption, avoidance of hepatic first-pass metabolism, and preferential drug delivery to the brain via the olfactory region [25]. Previous studies have demonstrated that intranasal administration offers a simple, practical, non-invasive, convenient, cost-effective, and an alternative route for rapid drug delivery to the brain/CNS [7].

Some of the pharmaceutical challenges like low bioavailability, local irritation, and toxicity upon long term usage; can be resolved by the synthesis of more lipophilic analogs, permeation enhancers, enzyme inhibitors, colloidal, bioadhesive and novel approaches for drug delivery systems like microemulsion, liposomes and nanoparticles [8].

The microemulsion is a thermodynamically stable, isotropically clear product represents a promising prospect for the development of formulations suitable for the incorporation of poorly water-soluble drugs due to the high solubilization capacity, as well as the potential for enhanced absorption and good thermodynamic stability. Microemulsion under their lipophilic nature and low globule size are widely explored as a delivery system to enhance uptake across nasal mucosa [9].

MATERIALS AND METHODS:

Materials: Asenapine maleate was provided as a gift sample by Sun Pharmaceuticals, Silvassa. Oleic acid and Tween 80 were purchased from S.D. fine chemicals, Mumbai. All other chemicals and reagents were of the highest grade commercially available.

Methods:

Solubility and Screening of Components: The solubility of the drug in the microemulsion (ME) components was determined by adding an excess of the drug to 1 ml each of selected oils, surfactants and co-surfactants in Eppendorf tubes. The Eppendorf tubes were maintained at 37 ± 1 °C in a shaker water bath for 48 h to reach equilibrium. The equilibrated samples were centrifuged at 10,000 rpm for 15 min, and the supernatant was then analyzed, and the drug was quantified by using UV spectrophotometer at λ_{max} 269 nm. The amount of drug present was calculated with the help of fitting the calibration curve constructed with the reference of drug [10].

The Solubility of Drug in o/w microemulsion: The solubilization capacity of selected micro-emulsion for ASNM was investigated. An excess amount of ASNM was introduced to 5 ml micro-emulsion, and the mixture was stirred for 24 h at 25°C under light shielding. After 24 h, the undissolved drug was removed by centrifugation at 10,000 rpm for 10 min, at room temperature then 0.5 ml supernatant was taken, and the content of ASNM was quantified by spectrophotometer at 269 nm after suitable dilution with methanol.

Construction of Phase Diagram: Pseudo ternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: co-surfactant ratio, which can result in large existence of ME area. The water titration method was used to construct the phases. Surfactant (Tween 80) and co-surfactant (PG) were blended (Smix) in fixed weight ratios (1:1, 1:2, 3:1, and 4:1). Oil (oleic acid) and Smix (Tween 80 & PG) were mixed thoroughly in different weight ratios from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w) in different glass vials and diluted with distilled water in a dropwise manner.

After equilibrium, the samples were visually checked and determined as clear microemulsions, or emulsions, or gels. By joining the change points, the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogeneous state were assigned to a ME region in the phase diagram. The pseudo ternary phase diagrams were constructed by using Chemix software [11].

Optimization of Microemulsion: Considering the amount and solubility of the drug in Oil, Surfactant and Co surfactant, certain oil- Smix-water mixtures within the microemulsion region were prepared and the final composition of the formulation was optimized based on % Transmittance, dilution characteristics, and globule size. For process optimization, the microemulsions were prepared by varying the stirring speed and stirring time for different batch sizes, and the % Transmittance and globule size were taken as a response.

Preparation of Drug-Loaded Microemulsion: The preparation of ASNM loaded microemulsion was performed by the water titration method. Smix of 3:1 was selected based on a maximum region of ME found from phase diagrams. Few formulations from the phase diagram were selected based on the particular selection criteria. ME was prepared by dissolving ASNM powder into the oil, stirring magnetically on the stirrer, and then adding Smix. The defined portion of water was added to the mixture dropwise and stirred to form a clear and transparent liquid. The resulting microemulsions were tightly sealed and stored at room temperature, and their physical stability was measured by observing periodically (after 24 h & 48 h) the occurrence of phase separation [12].

Characterization of Microemulsion: [13, 14] The analysis of globule size and Poly Dispersity Index (PDI) was done by using a dynamic light scattering method through Malvern Zeta sizer (Nano ZS-90 Malvern Instrument, UK). The influences of the ratio of surfactant to cosurfactant (Smix) and ASNM contents on the droplet size of microemulsion were evaluated. Zeta Potential measurement was carried out using Malvern Zeta sizer. Drug content of the formulations was measured by taking about 1 ml of the ME formulations in 10 ml volumetric flask and was diluted with methanol. It was diluted appropriately and analyzed by UV spectroscopic method at 269 nm.

The Dilution test was done by diluting 1 ml of microemulsion formulation to 10 ml and 100 ml with water to examine whether these systems could be diluted with an external phase of the system without phase separation or not. % transmittance of plain ME and drug-loaded MEs was measured at 650 nm using U.V. spectrophotometer by using double distilled water as a blank solvent. The pH of the formulations was measured with a calibrated digital pH meter (Elico LI 120, India), standardized with pH 4 and 7 buffers before use. The rheological behaviour of optimized microemulsion was evaluated using Brookfield Viscometer (Brookfield, USA). The conductivity of ME was measured using a conductometer (Elico, Hyderabad, India) by inserting the probe in 10 ml of the prepared microemulsion. An Abbe refractometer (INCO, Ambala) was used to determine the Refractive index of ME.

***In-vitro* Release Study:** [15, 16] For *in-vitro* release study, a Franz diffusion cell was used with the activated dialysis membrane (Dialysis membrane 110, Himedia, cut off 12000 Da). Dialysis membrane was pretreated with the diffusion media (Phosphate Buffer Saline pH 6.4) and then was mounted on a Franz diffusion cell with PBS (pH 6.4) filled in receptor compartment of the capacity 25 ml. The Franz diffusion cell had a diameter of 10 mm and maintained at a temperature of 37 ± 0.5 °C throughout the study. The cells were placed on a magnetic stirrer, and AME (1 ml) and AS (1 ml) were placed in the donor compartments. Aliquots were withdrawn from the receptor compartment at periodic time intervals for 8 h, replaced with the same volume of fresh diffusion medium to maintain the sink condition. The aliquots were analyzed using UV spectrophotometer.

***Ex-vivo* Diffusion Study:** [17] The use of a natural membrane is vital for envisage the potential drug release characteristic. Freshly excised goat nasal mucosa was used, procured from the slaughterhouse. It was dipped immediately in phosphate buffer (pH 6.4). The nasal membrane was removed carefully, made free from adhered tissues, and was washed with phosphate buffer (pH 6.4). The receptor compartment was filled with 25 ml diffusion media (PBS pH 6.4) to maintain perfect sink condition while 1 ml AME and AS was placed in the donor compartment. Continuous stirring was maintained in the receptor compartment with Teflon coated bead. Similarly, *ex-vivo* diffusion of the pure drug was conducted by taking 1 ml of drug solution. At periodic time intervals, the withdrawn samples from the receptor compartment were analyzed using UV

spectrophotometer at 269 nm. An equal volume of diffusion medium was added to replace each withdrawn sample. The cumulative amount of ASNM diffused through the membrane was determined.

Nasal Ciliotoxicity: [15] Nasal ciliotoxicity studies were carried out using the freshly excised goat nasal mucosa collected from a slaughterhouse in phosphate buffer saline pH 6.4. The excised superior nasal membrane was sectioned in pieces. One-piece was treated with PBS pH 6.4 (as negative control), the other with Isopropyl alcohol (1% w/v solution), a nasal mucociliary toxicity agent (as positive control) and other with 1 ml of optimized microemulsion formulation containing ASNM for 1 h, then rinsed with saline. The membranes were sent to the pathological laboratory in 10% formalin for the preparation of pathological slides and then subjected to histological studies with the microscope (Nikon ELWD) to evaluate the toxicities of formulations.

Drug Excipient Compatibility Studies: [22] Compatibility of ASNM and excipient added in the formulation was assessed by FT IR spectra of ASNM and AME. The IR spectra were taken using the Fourier Transform Infrared Spectrophotometer (Bruker, Germany). The liquid formulations were spread as a thin layer on potassium bromide cell and then scanned between 4000 and 400 cm^{-1}.

Pharmacodynamic Study: [18, 19] All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of the Parul Institute of Pharmacy, Parul University, following the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Protocol no. PIPH19/18 CPCSEA. Wistar Albino rats (180-220 g) were used for behavioral studies of AS and AME. The rats were divided into five separate groups (Six animals per group) and housed in a polypropylene cage at normal room temperature in 12 h light/dark cycle. They had free access to food and water. AS and AME (40 µl each) equivalent to 1.0 mg/kg of Asenapine was given in behavioral studies via nasal route using a micropipette. The group administered with intranasal blank microemulsion was considered as vehicle control for induced locomotor test and paw test.

Another group administered with intra-peritoneal L-dopa (10 mg/kg) and carbidopa (2.5 mg/kg) was considered as a positive control in induced locomotor activity test. The experiments were performed for 21 days and observations were recorded on 1st, 7th, 14th and 21st days of treatment to account for any discrepancy. The data were reported in mean ± SEM for each group.

Induced Locomotor Activity Test: [20] This behavioral model is based on a proposition that an increase in locomotor activity is due to increased dopaminergic activity in the mesolimbic system. All antipsychotics have antagonistic effects on dopamine agonist-induced hyperactivity. The locomotor counts were determined by Digital Actophotometer. On the day of the examination, the AS and AME groups received the respective formulation intranasally followed by administration of intra-peritoneal L-dopa (10 mg/kg) and carbidopa (2.5 mg/kg). The other group received AME by intravenous route. The locomotor activity was measured for 10 min by placing the animals in Actophotometer 1 h after drug administration. For AME by i.v. route, the activity was measured after 2 min of dosing for a period of 10 min.

Paw Test: [21] The paw test is a good model for a prediction of both therapeutic potential and extrapyramidal side effects (EPS) associated with any antipsychotic drug. Also found to have unique characteristics for differentiating classical antipsychotics. The increase in hind limb retraction time (HRT) is related to the antipsychotic potential, whereas the increase in forelimb retraction time (FRT) is linked with the potential to induce EPS. Classical antipsychotics are equipotent in prolonging both the FRT and HRT and atypical antipsychotics, which are much more effective in prolonging HRT than FRT. The paw test was performed on a Perspex platform measuring 30 cm × 30 cm, with a height of 20 cm. The top of the platform had two holes of 3.5 cm diameter for the forelimbs and two larger holes of 4.5 cm diameter for hind limbs and a slit for the tail.

The test was performed 1 h after the intranasal administration of the saline (control group) or the formulations by slowly lowering the hind limbs of the rats in the holes, followed by forelimbs. The time took the rat to withdraw either of the

forelimbs was called FRT and the time took the rat to withdraw either of the hind limbs was called HRT. For both FRT and HRT, the minimum time was set to 1 s and maximum time to 60 s. Experiments were performed in triplicate in the five-minute interval. The average FRT and HRT were then calculated for each rat.

Stability Studies: Stability studies for ASNM loaded microemulsion were carried out as per ICH guidelines for 6 months. Optimized formulations were kept at 30 ± 2 °C, 65 ± 5% RH, and physicochemical parameters were evaluated.

Statistical Analysis: All data are reported as the mean ± SD, and each group was compared using the ANOVA with $p<0.05$ considered statistically significant.

RESULTS AND DISCUSSION: The solubility of ASNM was determined in different oils to find out the oil with a maximum solubility of ASNM. It was found to be highest in oleic acid (35 ± 0.241 mg/ml) **Fig. 1**, so it was selected as an oily phase for the preparation of ME. As per the selection criteria of surfactant, hydrophilic-lipophilic balance (HLB) value greater than 10 is an important criterion for the selection of surfactant to form stable oil in water (O/W) ME along with the non-irritancy of surfactant [23].

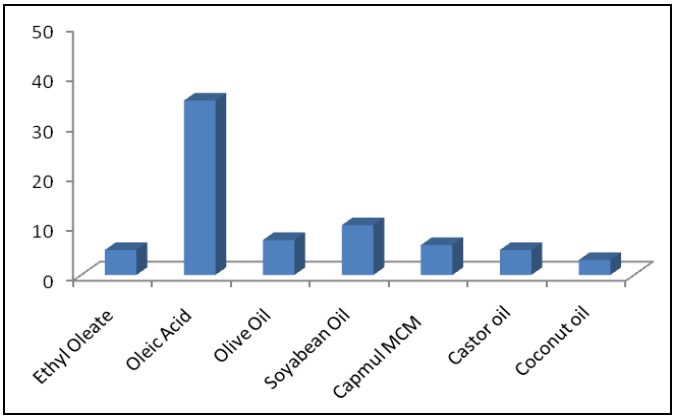

FIG. 1: GRAPHICAL PRESENTATION OF SOLUBILITY OF DRUG IN VARIOUS OILS

From the results of the solubility study, it was found that ASNM had the highest solubility (69.14 ± 0.114 mg/ml) in Tween 80 (HLB 15) as compared to Cremophore RH 40, Labrafil M 1944 CS, Pluronic F 68 and Pluronic F 127. Tween 80 is a non-ionic surfactant and found to be less affected by pH and ionic strength changes while acting as a solubilizing agent. Tween 80 was selected as a surfactant for the preparation of ASNM loaded microemulsions. The presence of co-surfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form micro-emulsions over a wide range of compositions. To form ME short to medium chain length alcohols are required as co-surfactant as most of the single-chain surfactants do not lower the oil-water interfacial tension sufficiently. Co surfactants interpose between surfactant molecules and decrease polar head group contacts as well as increase the flexibility of interfacial film around nanodroplets. So, from the observed literature, propylene glycol with ASNM solubility 1.5 ± 0.11 mg/ml and good miscibility with Tween 80 was selected as a co-surfactant.

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations. The components that showed maximum solubility was further optimized using pseudo ternary phase diagram, as shown in **Fig. 2**. Ternary phase diagrams were constructed by varying Tween 80 and PG ratios as 1:1, 2:1, 3:1, and 4:1 **Fig. 2**. The shaded areas in the phase diagrams show the microemulsion regions, and the other unshaded is the emulsion region. The ternary phase system of Tween 80: PG (3:1) that exhibits maximum microemulsion formation area was selected for the optimization of microemulsion batches. It could be seen that as the concentration of surfactant increases, the emulsion area increases. Hence, Tween 80: PG (3:1) ratio was selected for optimization studies. From the zone of ME obtained, six formulations were taken from each corner arbitrarily, and the best formulation was characterized systematically. The optimization of microemulsion was carried out based on % transmittance and particle size. The results are recorded in **Table 1**.

Depending on the solubility of the drug in Oil, Surfactant and Co surfactant, 1%, 2%, and 3% of oil as a fixed oil phase for microemulsion formation was chosen. From the ternary phase diagram, microemulsions were made with different surfactant and the co-surfactant ratio [(1:1), (2:1), (3:1) and (4:1)]. Different batches for different Oil to Surfactant ratios and Surfactant to Co surfactant

ratios were prepared. From the different batches made for optimization, 1% by weight of the oily phase was required to fulfill the dose requirement, was selected, and surfactant to co-surfactant ratio was maintained at 3:1 and water at 60 to 70 % by weight, six batches were prepared.

All the batches have droplet size in the nano range, which is very well evident from the low polydispersity values. Polydispersity is the ratio of the standard deviation to mean droplet size indicates the uniformity of droplet size within the formulation. The higher the polydispersity, the lower the uniformity of the droplet size in the formulation.

The polydispersity value of batch AF2 is the least. The batch AF2 was selected as an optimized batch as it was shown the transparency of 99.8%, and low globule size (94.40 nm).

TABLE 1: SELECTED COMPOSITION AND CHARACTERIZATION PARAMETERS OF BLANK MICROEMULSIONS

Batch Code	Oleic acid (% w/w)	Tween 80 (% w/w)	PG (% w/w)	Water (% w/w)	% T (DF=10)	Globule size (nm) ± SD	PDI ± SD
AF1	1	20.25	6.75	72	98.1 ± 0.165	90.35 ± 0.47	0.457 ± 0.002
AF2	1	23.25	7.75	68	99.8 ± 0.247	92.19 ± 0.85	0.212 ± 0.001
AF3	1	26.25	8.75	65	99.4 ± 0.215	101.57 ± 0.87	0.359 ± 0.004
AF4	2	20.25	6.75	71	98.7 ± 0.746	97.17 ± 0.24	0.417 ± 0.003
AF5	2	23.25	7.75	67	97.2 ± 0.358	117.57 ± 0.34	0.635 ± 0.002
AF6	2	26.25	8.75	63	94.7 ± 0.751	124.11 ± 0.46	0.629 ± 0.001

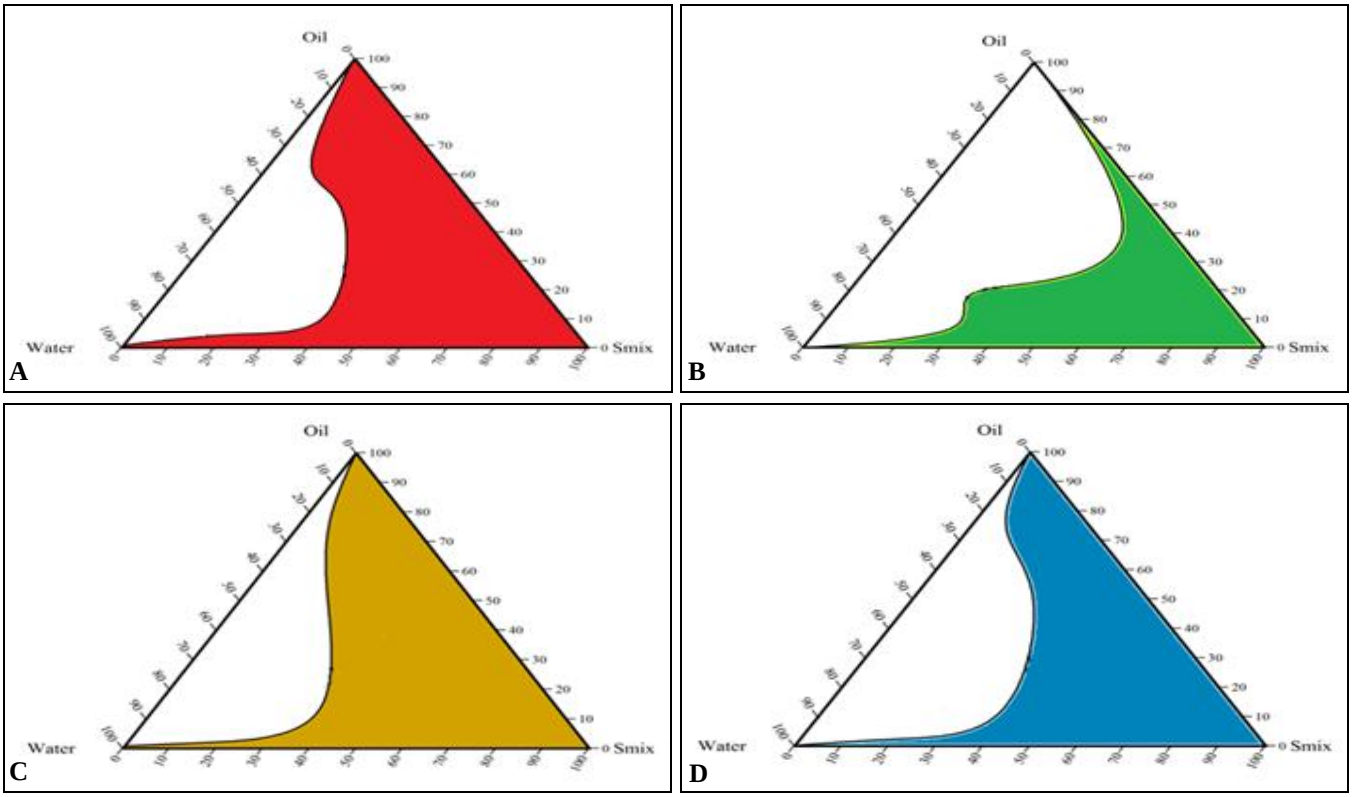

FIG. 2: PSEUDOTERNARY PHASE DIAGRAMS OF DIFFERENT SURFACTANT-CO SURFACTANT (S_{MIX}) RATIO. A. S_{MIX}(1:1), B. S_{MIX}(2:1), C. S_{MIX}(3:1), D. S_{MIX}(4:1)

Process Optimization: The process for the microemulsion preparation was optimized by making three formulations of three different batch sizes (10 ml, 20 ml and 50 ml) evaluated by % Transmittance and then by varying the stirring time (10 min, 15 min and 20 min) and stirring speed (100, 200 and 300 rpm) followed by the % Transmittance and globule size determination.

There was no significant effect on % Transmittance on increasing batch size of the same composition. At 100 rpm, there was not proper mixing of components and/or take more time for microemulsion preparation. At 300 rpm foam was forming, which was taking hours to become the formulation clear. So, the optimum speed of stirring was kept 200 rpm for the preparation of

microemulsion. Microemulsion preparation for 10 min shows % Transmittance of less than 98%, while for 15, 20 and 30 min there was no significant effect on as no remarkable changes in % Transmittance and particle size of ME found. So the optimum time of microemulsion preparation was kept 15 min. Based on lower globule size and % Transmittance (98.9%), a stirring speed of 200 rpm and stirring time of 15 min were selected as the optimized process parameters for drug-loaded microemulsion. There was no significant effect on % Transmittance on increasing batch size of the same composition.

Characterization of Microemulsion: The micro-emulsion formulations for nasal administration are expected to show a higher permeation rate with minimum droplet size. Developed microemulsions are expected to have good physical stability concerning phase separation and/or flocculation. It can be attained if zeta potential values are negative. The normal pH range of nasal secretions is 4.5-6.5. An acidic pH of formulation causes nasal irritation, followed by an increase in the nasal mucociliary clearance rate, whereas alkaline pH leads to the microbial susceptibility and infection.

Therefore, it is advised to maintain the nasal formulation pH in the physiological range of 4.5 to 6.5 [25]. Viscosity and pH are important factors affecting mucociliary action which may be the bases to another set of complications. Higher viscosity is preferred as it increases residence time but permeation rate also decreases if viscosity increases and hence formulation should have moderate viscosity.

The globule size of the ME is a critical issue for its performance as it determines the rate and extent of drug release and drug absorption. From the literature, it has been proven that the smaller droplet size of the MEs may lead to good and rapid absorption and so better bioavailability. Globule size was found to decrease with increase in surfactant as a reduction in interfacial tension and solubilization of oil. Mixtures of surfactants form a film around dispersed droplets and maintain droplet stability by strengthening the interfacial film. The globule size of selected MEs was found to be in the range of 90.35-124.11 nm, and globule size of ASNM loaded ME was found to be in the range of 100.57-143.51 nm **Fig. 3**. The increased globule size was due to the more proportion of oil. The PDI of ASNM MEs was found to be in the range of 0.289-0.671, which indicates the uniformity of globule size in the ME formulations **Table 2**. The ASNM content in the ME formulations was found in the range of 92.19-98.34%. From all six formulations, AF2 was found with less globule size and PDI values with drug content 98.34%. So, it was considered an optimized batch. The inverted microscopic image of AF2 is shown in **Fig. 9**.

The dilution tests are based on the fact that the emulsion is only miscible with the liquid that forms the continuous phase. On dilution, the formulations retained the clarity indicating the MEs are oil in water type. The refractive index of AF2 was 1.33 and % transmittance was found to be greater than 99% which confirmed that prepared ASNM ME was transparent. The viscosity of the optimized formulation (115.25 ± 0.31 cPs) is suitable for nasal administration. The conductivity value 159.2 µS of the optimized formulation indicates the microemulsion to be of oil in water type. Zeta potential was negative -5.68 which indicated the stability of formulation as there were fewer chances of globules aggregation. After centrifugation cycle, it was found that AF2 was stable and no phase separation was observed which indicates centrifugation stability. The optimized ME AF2 was found to be clear and transparent even after 3 & then 6 months of storage. ME AF2 was optimized from the developed ME formulations as per the formulation aspects decided.

TABLE 2: CHARACTERIZATION PARAMETERS OF SELECTED MICROEMULSION FORMULATIONS

Batch code	% T (DF=10)	Globule size (nm)	PDI	pH	Viscosity (cPs)	Drug content (%)
AF2	99.8 ± 0.342	100.57 ± 0.15	0.289 ± 0.003	5.75 ± 0.01	115.25 ± 0.31	98.34 ± 0.056
AF3	98.9 ± 0.378	109.31 ± 0.41	0.384 ± 0.001	5.67 ± 0.05	124.58 ± 0.28	98.32 ± 0.245
AF4	98.3 ± 0.845	115.14 ± 0.18	0.448 ± 0.002	5.69 ± 0.08	125.73 ± 0.14	92.19 ± 0.078
AF5	96.5 ± 0.989	129.24 ± 0.11	0.671 ± 0.003	5.73 ± 0.10	137.18 ± 0.32	96.57 ± 0.230
AF6	94.6 ± 0.989	143.51 ± 0.32	0.547 ± 0.002	4.78 ± 0.12	129.37 ± 0.27	97.72 ± 0.478

Mean S.D. (n=3) [% T = % Transmittance; DF = Dilution factor; PDI = Polydispersity index, S.D. (n=3)]

TABLE 2: CHARACTERIZATION PARAMETERS OF SELECTED MICROEMULSION FORMULATIONS

Batch code	% T (DF=10)	Globule size (nm)	PDI	pH	Viscosity (cPs)	Drug content (%)
AF1	98.4 ± 0.265	110.24 ± 0.17	0.412 ± 0.001	5.23 ± 0.04	101.13 ± 0.21	97.12 ± 0.135
AF2	99.8 ± 0.342	100.57 ± 0.15	0.289 ± 0.003	5.75 ± 0.01	115.25 ± 0.31	98.34 ± 0.056
AF3	98.9 ± 0.378	109.31 ± 0.41	0.384 ± 0.001	5.67 ± 0.05	124.58 ± 0.28	98.32 ± 0.245
AF4	98.3 ± 0.845	115.14 ± 0.18	0.448 ± 0.002	5.69 ± 0.08	125.73 ± 0.14	92.19 ± 0.078
AF5	96.5 ± 0.989	129.24 ± 0.11	0.671 ± 0.003	5.73 ± 0.10	137.18 ± 0.32	96.57 ± 0.230
AF6	94.6 ± 0.989	143.51 ± 0.32	0.547 ± 0.002	4.78 ± 0.12	129.37 ± 0.27	97.72 ± 0.478

Mean S.D. (n=3)

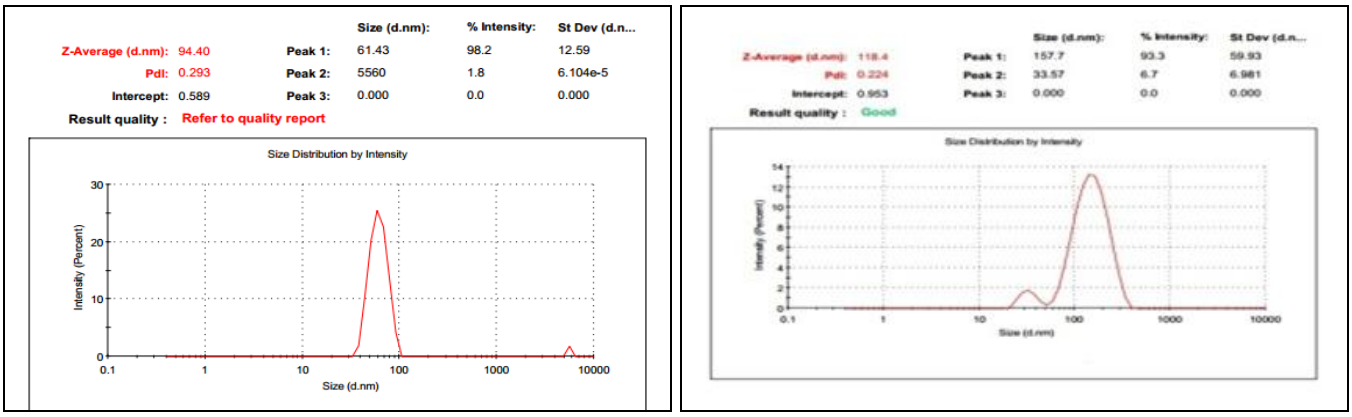

FIG. 3: (A) GLOBULE SIZE AND PDI OF BLANK OPTIMIZED BATCH. (B) GLOBULE SIZE AND PDI OF OPTIMIZED BATCH

In-vitro release study of optimized microemulsion batch was successfully done for 8 h. The results of AS and AME diffused through the dialysis membrane are shown in **Fig. 4**. In the release study, it was found that more than 80% of drug released from AME due to increase solubility of the drug in ME components, oil, and Smix. AS shows about 60 % drug releases in 8 h. Further, AF2 was subjected to the *ex-vivo* drug diffusion study. The results of AS and AME diffused through the goat nasal mucosal membrane are shown in **Fig. 5**. It can be seen from the release profiles that ASNM permeation is better from AME than AS. The higher permeation in the ME may be attributed to the high solubilizing power of ME system, which increases the absorption potential of ME [24].

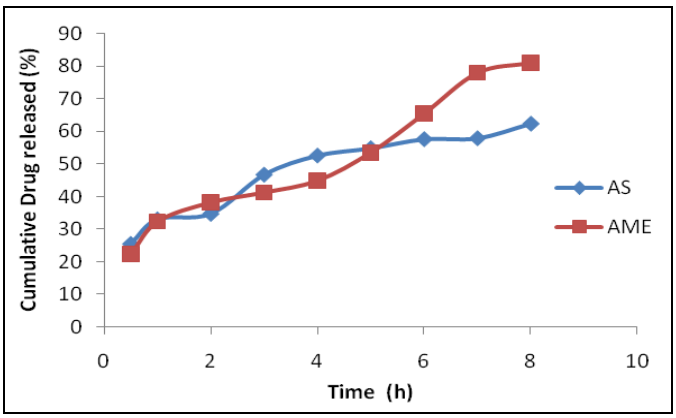

FIG. 4: *IN-VITRO* DRUG DIFFUSION PROFILE OF ASENAPINE LOADED MICROEMULSION (AME) AND SOLUTION (AS)

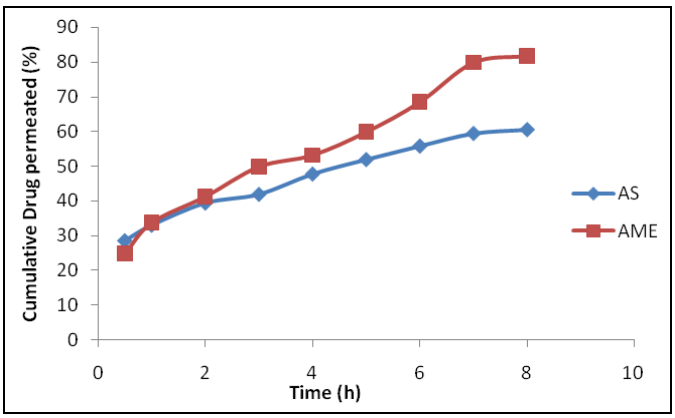

FIG. 5: *EX-VIVO* PERMEATION STUDY OF THE ASENAPINE LOADED MICROEMULSION (AME) AND SOLUTION (AS)

Nasal Ciliotoxicity Study: An important requirement in formulation development is no nasal mucosal irritation from the microemulsion. Optical microscopic results shown in figure revealed that there was no nasociliary damage, and nasal membrane remained intact with PBS pH 6.4 and optimized microemulsion, indicating that micro-emulsion had no obvious effect on the cilia movement, whereas extensive damage to nasal mucosa observed with the positive control, Isopropyl alcohol **Fig. 6**.

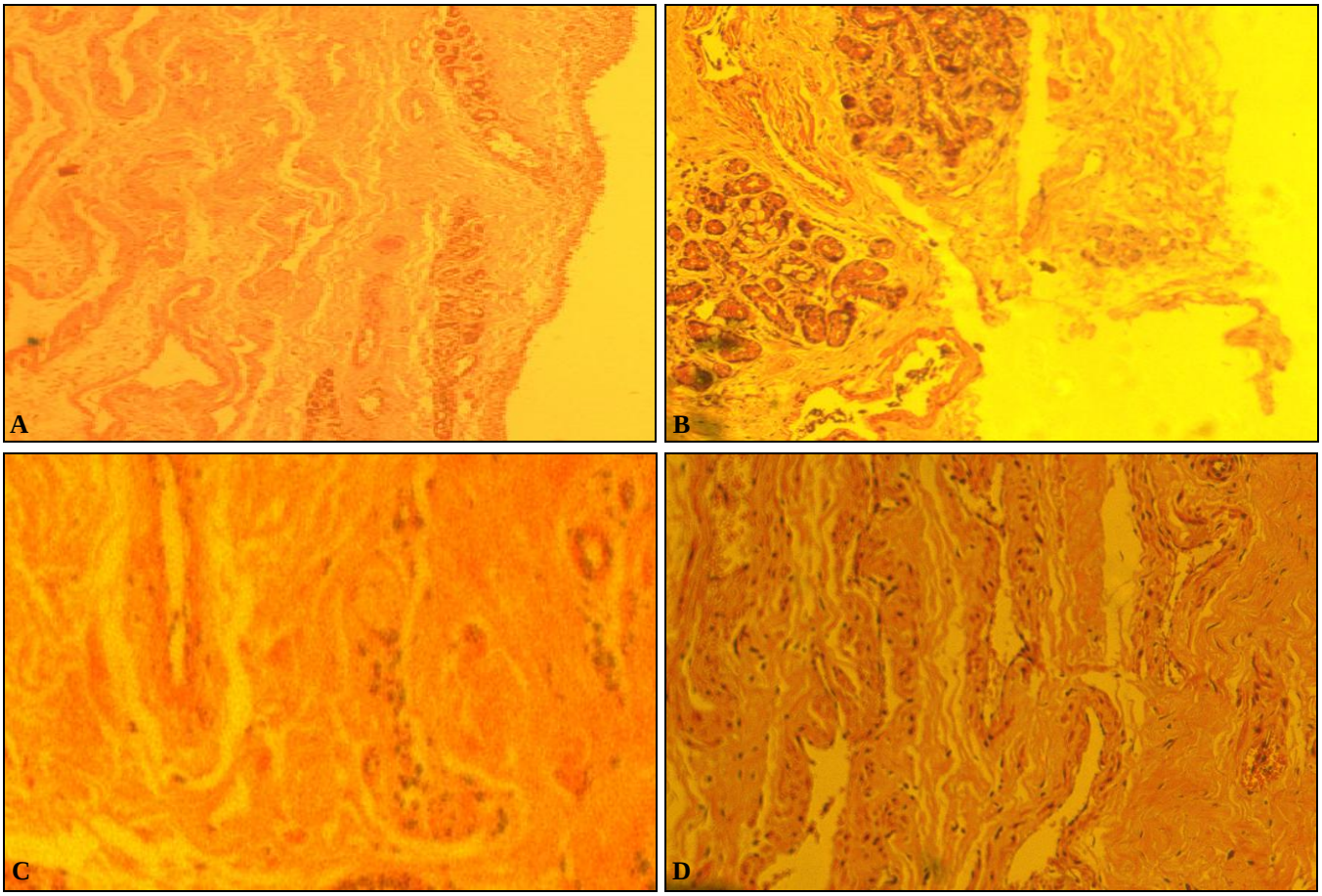

FIG. 6: MICROSCOPIC IMAGES OF GOAT NASAL MUCOSA DEMONSTRATING HISTOLOGICAL CHARACTERISTICS. (A) POSITIVE CONTROL (B) NEGATIVE CONTROL (C) BLANK MICROEMULSION (D) ASNM MICROEMULSION

Drug Excipients Compatibility Study: The major peaks of the pure drug were retained as such in the spectra of the drug-containing microemulsion, shown in **Fig. 7**. Any other degradation peaks were not found indicated that nonexistence of any possible interaction between drug and excipients used in the formulation. It was concluded that the ASNM was compatible with the formulation components to formulate AME.

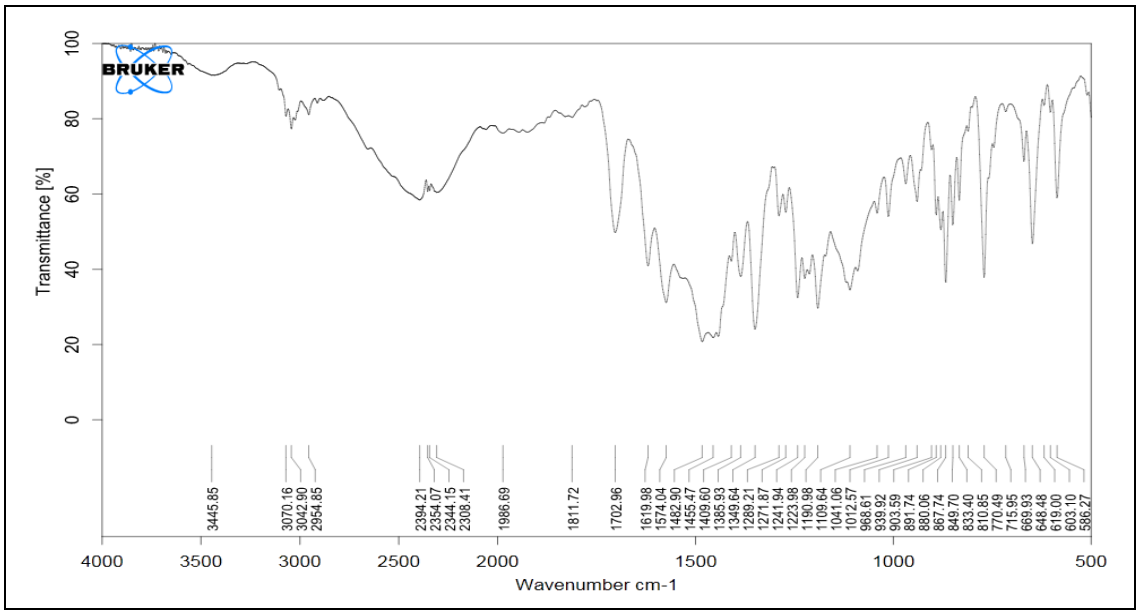

FIG. 7: FTIR OF ASENAPINE MALEATE

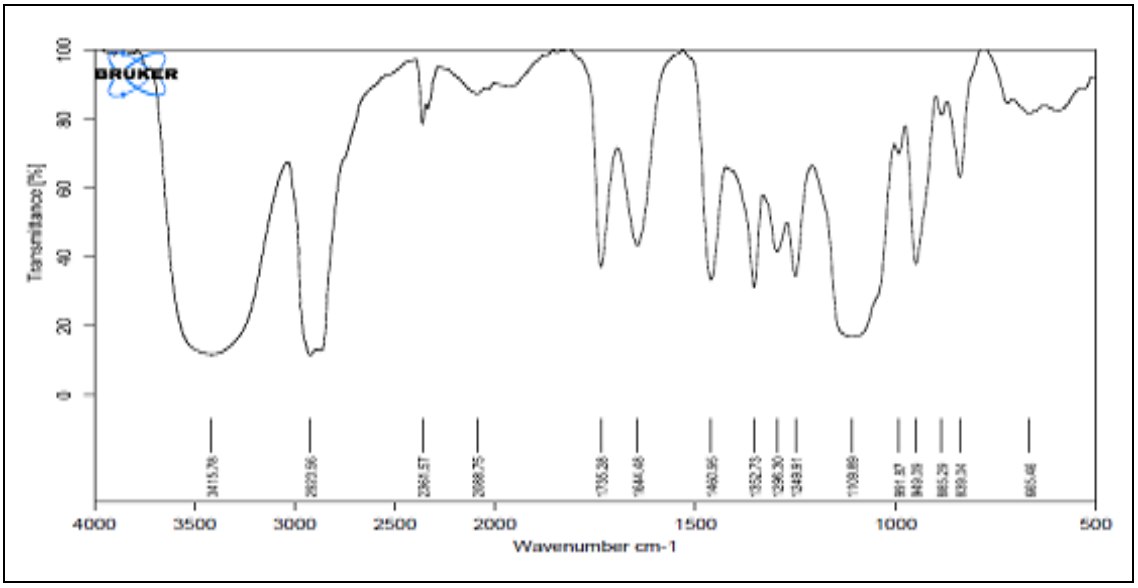

FIG. 8: FTIR OF ASENAPINE MALEATE LOADED MICROEMULSION

Pharmacodynamic Evaluation: The results of the locomotors activity test are shown in **Table 3**. The one way ANOVA demonstrated a significant difference among the group ($p<0.05$) and no significant interaction between groups. The Bonferroni's test revealed that maximum locomotor counts in the control group due to dopamine hyperactivity. The groups administered with vehicle control (blank microemulsion), AS, and AME intranasal revealed significant reduction ($P<0.05$) in the locomotor counts. Reduction in locomotor activity upon intranasal administration of AME was more as compared to blank ME and AS **Table 3**. The results are suggesting the superiority of the intranasal route for brain delivery of ASNM and that the antagonistic activity was achieved by both AS and AME, revealed the sensitivity of Asenapine against dopamine D2 receptor.

From a variety of research reports, it is evidenced that atypical antipsychotics drugs like ASNM do not change the FRT but specifically influence the HRT. As a result, insignificant ($p<0.05$) difference was observed in FRT on AME formulation. Typical antipsychotics like haloperidol are known to influence both HRT and FRT [21]. Interpretation of HRT indicated AME to have higher HRT value than AS, which may be due to increased passage of ASNM from AME either by a transcellular or paracellular mechanism to the CNS via the olfactory region of the nasal cavity. The animal group that received intranasal AME showed the highest HRT values in comparison to other groups, indicating the superiority of intranasal ME. It shows that developed microemulsion has the potential to target the brain resulting in higher HRT in the AME group and better uptake of ASNM to the brain by the intranasal route.

TABLE 3: ANIMAL BEHAVIOURAL STUDIES OF ASENAPINE MALEATE FORMULATIONS IN RATS

Parameters	Control group (Saline - i.n.)	Vehicle control (Blank ME – i.n.)	AS (i.n.)	AME (i.n.)	AME (i.v.)
Locomotor counts	235 ± 4	139 ± 3	123 ± 2	71 ± 2	113 ± 3
FRT (s)	7 ± 0.49	5 ± 0.42	6 ± 0.33	3 ± 0.25	5 ± 0.44
HRT (s)	2 ± 0.30	2 ± 0.31	14 ± 0.66	17 ± 0.71	14 ± 0.71

Mean ± SD, n = 3

Stability Study: The stability study was carried out as per ICH guidelines. During 6 months period of stability studies, on visual observation, it was found that AF2 showed no signs of any precipitation of drug, no creaming, no phase separation, and no flocculation and it was also found stable after centrifugation (at 3000 rpm for 15 min) at 30 ± 2 °C, 65 ± 5% RH. The results of stability studies are shown in **Table 4**, indicates that there was statistically no significant difference observed for various parameters evaluated. So it was concluded that the developed microemulsion was stable for a period of 6 months at 30 ± 2 °C, 65 ± 5% RH.

TABLE 4: STABILITY STUDIES RESULTS OF ASENAPINE MALEATE LOADED MICROEMULSION

Time (months)	Appearance	Phase separation	% Transmittance	Particle size (nm)	PDI	Drug content (% w/v)
0	White milky Dispersion	No Phase separation	99.1 ± 1.14	119.5 ± 1.2	0.232 ± 0.002	98.34 ± 0.056
1	No significant Change	No Phase separation	98.6 ± 0.84	124.9 ± 2.1	0.244 ± 0.004	98.12 ± 1.024
3	No significant Change	No Phase separation	98.1 ± 1.74	123.5 ± 2.6	0.261 ± 0.008	98.27 ± 1.047
6	No significant Change	No Phase separation	99.6 ± 1.17	123.5 ± 1.4	0.267 ± 0.002	98.21 ± 1.210

Mean ± SD, n = 3

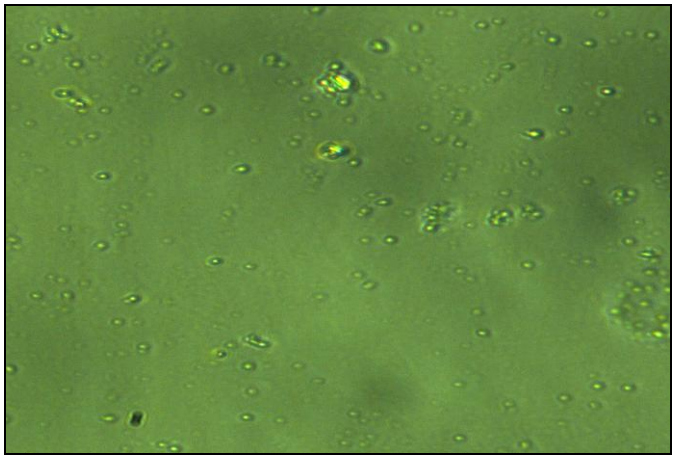

FIG. 9: INVERTED MICROSCOPIC IMAGE OF ASENAPINE MALEATE LOADED MICROEMULSION

CONCLUSION: The formulation of microemulsion containing oleic acid, Tween 80, and PG with high solubilization for the Asenapine maleate was successfully developed. The predicted response from optimized formulation was found to be good concerning % Transmittance (99.8%), globule size (94.40 nm), PDI (0.293), zeta potential (-5.68), and drug content (98.34%). Other physicochemical parameters showed the suitability of the formulation for intranasal administration. The formulation was found to be free from nasal ciliotoxicity. The Pharmacodynamic evaluation also showed lowest locomotor counts with optimized microemulsion designated with antagonistic effect of ASNM towards dopamine-induced hyperactivity and highest HRT in rats treated with optimized formulation in comparison to rats treated with intranasal Asenapine maleate solution (AS) and intravenous AME which suggested AME intranasal delivery system as an effective alternative therapy for treatment of Schizophrenia. The long term animal behavioral studies confirmed remarkable brain bioavailability with the better safety profile of antipsychotic activity of Asenapine maleate microemulsion compared to microemulsion *via* i.v. route and pure drug *via* the intranasal route. So, that the developed formulation finds application in the treatment of schizophrenia and may also exhibit an advantage over conventional formulation (tablet) as better brain uptake of the drug by nasal route, one of the major limitations of commercially available preparation.

More brain selective drug delivery possibly reduces the dose and/or dosing frequency as well as systemic side effects of the drug and, consequently, the cost of therapy for treating the disease. Indeed, as the ASNM microemulsion is prepared by an inexpensive process through auto emulsification or supply of energy, is stable and have a high capacity of dissolving the drug. However, the therapeutic benefits of Asenapine intranasally over conventional treatment in these types of disorders need further preclinical and clinical studies.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

ANIMAL STUDIES: All institutional and national guidelines for the care and use of laboratory animals were followed.

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