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DEVELOPMENT OF A POLYHERBAL CAPSULE FOR THE TREATMENT OF HYPERLIPIDAEMIA

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Keywords:

Murraya koenigii, *Achyranthus aspera*, Polyherbal Capsule, Preformulation studies, Stability studies

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ABSTRACT: Hyperlipidaemia is mentioned as one of the life-style-related diseases as much as so for diabetes. In the present study an attempt was made to develop herbal capsules, which are containing the extract of *Murraya koenigii* and *Achyranthus aspera.* One of the major concerns in designing new formulation is that the active ingredient should be compatible with all the excipients and packaging material components. Incompatibilities will affect the efficiency of the drug. Thermoanalytical techniques were effectively employed for the preformulations studies. The results obtained by the thermoanalytical techniques showed no chemical incompatibility between hard gelatin capsule shell and extract. The results were further confirmed by IR spectroscopy. The herbal capsule was stored at different temperatures *viz*., 25 °C, 40 °C and 50 °C for shelf life evaluation. The organoleptic properties of the herbal capsule remains unchanged at tested temperatures throughout the stability study. The shelf life of the herbal capsule was found to be 6.4 years. The Harborne's Quantitative Alkaloid determination method proved to be selective, precise, linear, sensitive, and adequate for the determination of alkaloid content in the extract. The percentage alkaloid content in the herbal capsule remained above 90% throughout the 3 year study for room temperature samples. The percentage alkaloid content remained above 90% for a period of 6 months and 3 months for the samples kept at 40 °C and 50 °C. The herbal capsule showed maximum pharmaceutical elegance and remained stable throughout the observation period.

INTRODUCTION: Hyperlipidaemia is mentioned as one of the life-style-related diseases as much as so for diabetes. Hyperlipidemia means status in which levels of lipids in plasma are increased over normal ranges.

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Coronary heart disease (CHD) is the leading cause of death and disability worldwide. It is a complex condition resulting from numerous gene-gene and gene-environment interaction. According to WHO, 16.6 million people around the globe die of CHD each year. WHO also estimates that low and lower-middle income countries contributed more than 70 percent to the global distribution of chronic disease death and the projected death rates from CVD in countries like India and Pakistan would be much higher than the death rates from HIV / AIDS, tuberculosis and malaria.

In India, CHD prevalence in urban population rose from 3.5% in 1965 to 9% in 1990. Rates appear to be higher in southern India with highest in Kerala. Major Pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value [1, 2]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants. The valuable medicinal properties of different plants are due to presence of several constituents *i.e.* saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters. Among them some are act as synergistic and enhance the bioactivity of other compounds.

One of the major concerns in designing new formulation is that the active ingredient should be compatible with all the excipients and packaging material components. Incompatibilities will affect the efficiency of the drug. The identification of possible incompatibilities between drug and excipients is one of the basic tasks to be dealt with in a pre-formulation study. Thermo analytical techniques measure changes in physical or chemical properties of the sample as a function of temperature [3]. Thermoanalysis has been used for the rapid evaluation of purity, kinetics decomposition and physical property of drugs [4, 5]. Moreover, this technique provided an alert for compatibility problems and it was indicated the most favorable directions to pursue for a successful formulation [6, 7, 8]. The most widely used thermoanalytical techniques are Differential Scanning Calorimetry (DSC) and Thermogravimetry / Derivative Thermogravimetry (TG / DTG) in which a physical property of a substance and/or its reaction product is measured as a function of a controlled temperature program [9, 10].

Stability study provides evidence on how quality of a drug substance or product varies with time under influence of variety of environmental factors such as, temperature, humidity and light and also to establish a retest period for the drug substance or product and recommended storage conditions. So we can say stability study is necessary as an assessment of product quality. Pharmaceutical products are generally studied for stability profile at accelerated temperature and humidity, the experimental findings of which can be very helpful to predict reliable self life or expiry date at room temperature by adopting certain assumptions and criterions [11].

Every product has definite self-life which depends on various physical, chemical, environmental and biological factors. Real time study is a long procedure. The manufacturer finds it difficult to wait till the drug degrades naturally to 90% of its labelled amount at room temperature. On this account stability study is normally carried out for assigning self-life of the drugs. Quality guidelines known as ICH guidelines have established a series of guidelines acceptable to multiple countries for the drug approval process. It is a normal practice to study the stability of pharmaceutical preparations at accelerated conditions of temperature and humidity, the experimental findings which can be transformed into reliable shelf life or expiry date by adopting certain assumptions or criterions. By this method the shelf life of any drug product can be predicted in a very short period of time. In comparison to conventional preparations herbal product represents number of unique problems when quality and stability are considered. To ensure proper reproducibility, proper control is essential; an important part of quality control is to ensure the chemical stability of final product during storage product [12].

In the present study an attempt was made to develop herbal capsules, which are containing the extract of *Murraya koenigii* and *Achyranthus aspera*.

MATERIAL AND METHODS:

Herbal Capsules: Empty hard gelatin capsules were received as gift from Sarazen Research & Development Organization, Gwalior. The capsules were filled with pharmacologically most effective extract or combination of extracts. The capsules were then dedusted, transferred into polybags, labelled and the samples were evaluated as per the testing requirements.

Preformulation Studies:

Thermogravimetric Analysis: The TG / DTG measurement was performed on Thermo balance, TGA-2950 (TA Instruments, USA), under dynamic nitrogen atmosphere with the flow rate of 50

ml/min. Approximately 5 mg of sample was placed in platinum pan and heated from 25 °C to 900 °C at a heating rate of 10 °C/min [13, 14].

Differential Scanning Calorimetry Analysis: The DSC measurement was performed in DSC-2920 cell (TA Instruments, USA), under dynamic nitrogen atmosphere with the flow rate of 50 ml/min. Approximately 2 mg of sample was weight out and placed in a sealed aluminium pan and scanned from 25 °C up to 500 °C with heating rate of 10 °C/min [15, 16].

Fourier Transform Infrared Spectroscopy (FT-IR): Fourier transform infrared (FTIR) spectra was recorded on a Nicolet IMPACT 410 FT-IR apparatus using KBr discs in the range of 400 - 4000 cm^{-1}.

Effect of Storage Temperature and Shelf Life Determination: To study the effect of temperature on the stability of herbal capsules, 100 filled capsules were stored at 25 °C, 40 °C, and 50 °C. For evaluation different parameters were taken that were organoleptic evaluation, Average weight content, locking length, disintegration test, pH and assay of alkaloids. The amount of alkaloid on day zero was taken as 100%; and accordingly, the percentages were calculated at regular intervals. The shelf life of the herbal capsule was evaluated at elevated temperatures in accordance with the Arrhenius equation:

$$K_{app} = Ae^{-Ea/RT}$$

Where;
K_{app} = The apparent rate of constant for the reaction
A = The frequency factor
E_a = The activation energy for the reaction
R = The gas constant (1.987 cal./deg.mole)
T = Absolute temperature (degree Kelvin)

The Arrhenius equation can be rewritten as

$$\text{In } K_{app} = \text{InA} - E_a/R \cdot 1/T$$

Again an equation of the form y = mx + c is generated, indicating that a semi log plot of K_{app} *v/s* the reciprocal of temperature (1/T) should yield a straight line with a negative slope of $-E_a/R$. this line can be extrapolated to the value of 1/T that corresponds to room temperature and predict rate constant for the reaction at room temperature can be taken from the y-axis.

Quantitative Alkaloid Determination Method: 5 g (10 capsules) of the sample was weighed into a 250 ml beaker and 200 ml of acetic acid in ethanol was added and covered. It was allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

RESULTS: The extracts of leaves of *Achyranthus aspera* and *Murraya koenigii* were prepared. The most potent extract was formulated into Herbal capsule. It was evident from the results of pharmacological evaluation that extract namely MK+AA-W is the most potent and safe. Hence herbal capsule was prepared using Hard Gelatin Capsule (Size 000).

Preformulation Studies: Preformulation studies were carried out to determine incompatibility between hard gelatin capsule shell and polyherbal extract. The results are as follows:

Thermogravimetric Analysis: The TGA thermo grams of unused hard gelatin capsule and used hard gelatin capsule are given in **Fig. 1** and **2**. Charring of the sample and decrease in mass that occurred between 107.4 °C and 794.75 °C indicated chemical decomposition occurred in all samples above 794.75 °C. The overlay **Fig. 2** of the unused hard gelatin capsule and used hard gelatin capsule shows no difference indicating there is no interaction between extract and the capsule shell.

Differential Scanning Calorimetry Analysis: DSC curve of both unused hard gelatin capsule and used hard gelatin capsule were recorded and given in **Fig. 3**. The DSC curve shows the endothermic peak due to fusion of the compound at 170.48 °C (ΔH= 24.26 J/g), and it is followed by a sharp exothermic peak at 199.96 °C ascribed to the thermal decomposition in consecutive stages. No remarkable interaction was found between unused hard gelatin capsule and used hard gelatin capsule indicating no interaction between extract and the capsule shell.

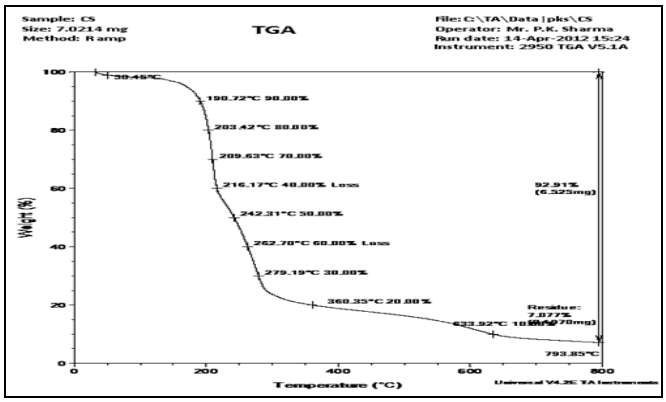

FIG. 1: TGA THERMOGRAM OF HARD GELATIN CAPSULE (UNUSED)

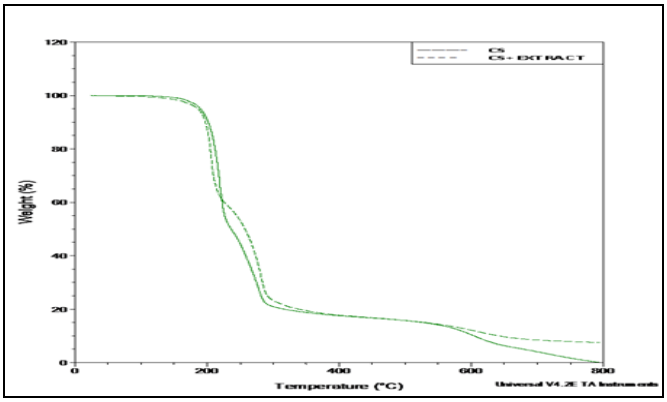

FIG. 2: TGA THERMOGRAM OF HARD GELATIN CAPSULE (UNUSED AND USED)

Fourier Transform Infrared Spectroscopy (FT-IR): The subsequent step of the present study was to analyse the FT-IR spectra of unused hard gelatin capsule and used hard gelatin capsule in order to identify a possible chemical interaction between extract and hard gelatin capsule. The FTIR spectra of unused hard gelatin capsule and used hard gelatin capsule did not show evidence on chemical interaction in the solid state. Moreover, the spectra of binaries did not show the absence or shift of vibration bands of hard gelatin capsule.

Shelf Life Determination of Various Suspensions: The herbal capsules were evaluated for different parameters *i.e*., organoleptic evaluation, and Average weight content, locking length, disintegration test, pH and assay of alkaloids. The Results of different parameters used for accelerated stability study are given in **Table 1**.

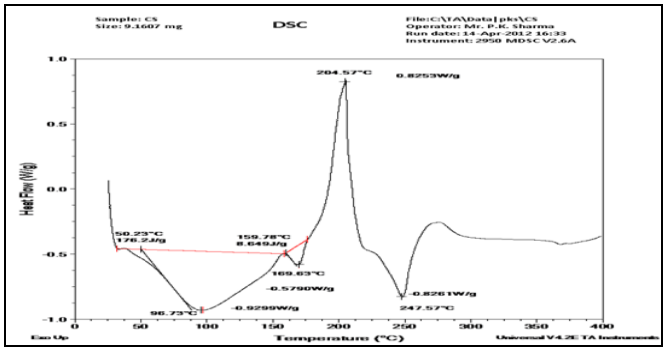

FIG. 3: DSC THERMOGRAM OF HARDGELATIN CAPSULE

TABLE 1: RESULTS OF DIFFERENT PARAMETERS USED FOR ACCELERATED STABILITY STUDY TEST FOR HERBAL CAPSULE

S. no.	Parameters	Limits	0 Month	6 Months	12 Months	24 Months	36 Months
1.	Description	Green smooth capsule with characteristic odour and taste	Complies	Complies	Complies	Complies	Complies
2.	Average weight(mg)	550 ± 5%	557.73	559.14	558.21	557.93	558.25
3.	Locking length	20 - 21 mm	20.83	20.86	20.84	20.85	20.85
4.	Disintegration	NMT - 30min	12.32	12.40	12.39	12.41	12.34
5.	pH (1% solution)	5.00-7.00	5.18	5.18	5.20	5.20	5.20

The amount of alkaloid on day zero was taken as 100%; and accordingly, the percentages were calculated at regular intervals. The result is shown in **Table 2**.

TABLE 2: ALKALOID CONTENT (%) IN HERBAL CAPSULE KEPT AT DIFFERENT TEMPERATURES ASSAYED AT DIFFERENT TIME INTERVALS (MONTHS)

Time	Percentage of alkaloid content		
	25 °C	40 °C	50 °C
0 month	100±1.2	100±0.92	100±0.99
1 months	100±1.3	95.92±1.72	94.87±2.80
2 months	99.73±1.9	95.68±2.53	89.06±1.01
3 months	99.60±3.8	95.38±1.13	76.12±1.09
6 months	99.21±0.8	95.68±2.45	51.91±1.12
9 months	98.81±1.1	94.81±0.2	-/-
12 months	98.42±0.9	90.00±0.69	-/-
18 months	97.63±1.4		-/-
24 months	96.85±1.0	-/-	-/-
36 months	95.27±1.0	-/-	-/-

Values are Mean ± SEM (n = 10)

The percentage of alkaloid content was plotted against time. The values obtained from the regression analysis are given in **Table 3** for different formulations.

TABLE 3: SLOPE, INTERCEPT r^2 VALUES FOR THE REGRESSION LINES FOR THE DEGRADATION OF ALKALOID CONTENT

S. no.	Temperature (°C)	Slope K_0 (month$^{-1)}$	Intercept C_0 (%)	r^2 Values (Unit less)
1	25	-0.13	99.99	1.0
2	40	-2.21	96.19	0.87
3	50	-12.59	96.23	0.88

The logarithm of the zero-order rate constants was plotted against the reciprocal of the absolute temperature to obtain the Arrhenius plot **Table 4** and **Fig. 4**.

TABLE 4: DATA FOR THE ARRHENIUS PLOT OF *ln* k *vs* (1/T) FOR ALKALOID CONTENT

S. no.	T (k)	1/T (k^{-1})	K_0 (day^{-1})	Log K
1	298	0.003356	-0.13	0.88
2	313	0.003195	-2.21	0.34
3	323	0.003096	-12.59	1.10

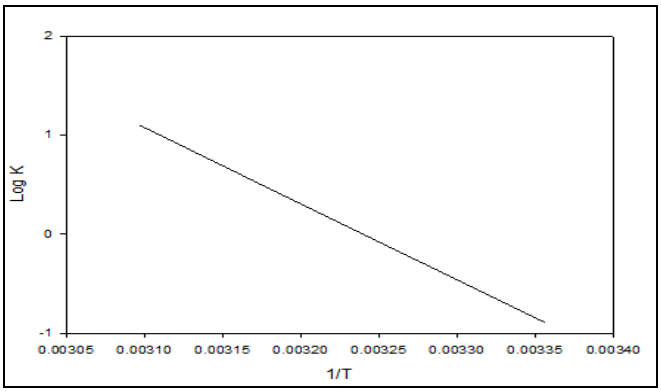

FIG. 4: ARRHENIUS PLOT

The values for the slope and intercept were inserted into Arrhenius equation were

$$Y = mx + c$$

From the Arrhenius equation, the zero-order degradation rate constant ($k_{0,\ 25}$) at 25 °C was calculated.

The shelf life for the suspension at 25 °C can be calculated using the formula.

Shelf life = 0.10 (initial concentration as a percentage)/ $k_{0,\ 25}$

The shelf life of herbal capsule was calculated **Table 5** and found to be 6.4 years.

TABLE 5: DATA FOR THE SHELF LIFE DETERMINATION

Formulations	Slope K_0 (month$^{-1)}$	Intercept C_0 (%)	r^2 Values (Unitless)	$k_{0,\ 25}$ (month^{-1})	Shelf life at 25°C (Years)
Herbal capsule	-7632.67	24.73	1	0.130	6.4

DISCUSSION: A drug dosage form is very important in delivering the drug in proper dose in most appropriate form. The capsule shell is an excellent barrier to air, and it also has some other advantages such as being easy to swallow and tasteless, may allow rapid release, flexibility of formulation, short manufacturing steps, *etc*. The use of the hard gelatin capsule dosage form may perhaps be an alternative to overcome the problem, which the hygroscopicity of the extracts may pose.

The preparation of a capsule containing most potent extract, thus lead to products of acceptable pharmaceutical quality. To test this hypothesis (*i.e.* to test for the presence of the quality of the capsules) appropriate marker constituent (Alkaloid) in the plant material was monitored. During the formulation of new products or reformulation of existing products, it is advantageous to have knowledge on any physical and/or chemical interactions between drug and excipients.

Thermoanalysis has been used for the rapid evaluation of purity, kinetics decomposition and physical property of drugs. Moreover, this technique was provided an alert for compatibility problems and it was indicated the most favourable directions to pursue for a successful formulation. The most widely used thermoanalytical techniques are DSC and TG / DTG in which a physical property of a substance and / or its reaction product is measured as a function of a controlled temperature program. If the drug is compatible with excipient or with hard gelatin capsule shell as in our case, at high temperature it is necessarily compatible at room temperature. But if there is incompatibility at high temperature it may or may not be incompatible at room temperature.

The extract should be investigated for the compatibility at room temperature by other independent measurements. The additional prominent DSC peaks in the extract is a positive indication of chemical interaction of the drugs with capsule shell. During our study the most potent and safe extract (MK+AA-W) was selected for filling in the herbal capsule. The results obtained by the thermoanalytical techniques showed no chemical incompatibility. The results were further confirmed by IR spectroscopy. The herbal capsule was stored at different temperatures *viz*., 25 °C, 40 °C and 50°C for shelf life evaluation. The percentage alkaloid content for day zero was taken as 100%; and, accordingly, the percentages were calculated for all the other days. The organoleptic properties of the herbal capsule remain unchanged at tested temperatures throughout the stability study. Likewise, pH values were fairly constant and appear not to be influenced by temperature.

The Harborne's Quantitative Alkaloid determination method proved to be selective, precise, linear, sensitive, and adequate for the determination of alkaloid content in the extract. The percentage alkaloid content in the herbal capsule remained above 90% throughout the 3 year study for room temperature samples. The percentage alkaloid content remained above 90% for a period of 6 months and 3 months for the samples kept at 40 °C and 50 °C. The herbal capsule showed maximum pharmaceutical elegance and remained stable throughout the observation period.

The shelf life of the herbal capsule was found to be 6.4 years which is much more than sufficient to satisfy the regulatory norms of many countries. We can conclude that the present study has been results into a herbal capsule which can be used for its hypolipidemic property.

CONCLUSION: Thermoanalytical techniques were effectively employed for the preformulations studies. The results obtained by the thermoanalytical techniques showed no chemical incompatibility between hard gelatin capsule shell and extract. The results were further confirmed by IR spectroscopy. The herbal capsule was stored at different temperatures *viz*., 25 °C, 40 °C and 50 °C for shelf life evaluation. The organoleptic properties of the herbal capsule remain unchanged at tested temperatures throughout the stability study. The shelf life of the herbal capsule was found to be 6.4 years.

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CONFLICT OF INTEREST: The authors have no conflict of interests to declare regarding the publication of this paper.

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