IJPSR (2017), Volume 8, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 November, 2016; received in revised form, 21 August, 2017; accepted, 29 August, 2017; published 01 September, 2017

DEVELOPMENT OF A POLYHERBAL CAPSULE FOR THE TREATMENT OF ASTHMA

Anand Gupta[*1], Santosh Kumar Singh [1], Ravindra Pal Singh [1] and Suman Jain [2]

Department of Pharmaceutical Sciences [1], Suresh Gyan Vihar University, Jaipur - 302017, Rajasthan, India.
School of Studies in Pharmaceutical Sciences [2], Jiwaji University, Gwalior - 474022, Madhya Pradesh, India.

Keywords:

Ipomoea aquatica, *Piper longum*, *Cinnamomum zeylanicum*, Polyherbal capsule, Asthma, Preformulation studies, Stability studies

Correspondence to Author:
Anand Gupta

Department of Pharmaceutical Sciences, Suresh Gyan Vihar University, Jaipur – 302017, Rajasthan, India.

E-mail:anjuanandgupta0007@gmail.com

ABSTRACT: 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, flexibility of formulation *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. Present study was envisaged to develop a polyherbal formulation as capsule. The results obtained by the thermo analytical techniques showed no chemical incompatibility between capsule shell and herbal extract. The results were further confirmed by IR spectroscopy. The herbal capsule was stored at different temperatures *viz*., 25 ^{0}C, 40 ^{0}C and 50 ^{0}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 herbal capsule showed maximum pharmaceutical elegance and remained stable throughout the observation period. The shelf life of the herbal capsule was found to be 3.2 years which is much more than sufficient to satisfy the regulatory norms of many countries.

INTRODUCTION: Major Pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(9).4006-13

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(9).4006-13

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.

During the past decade, a dramatic increase in exports of medicinal plants attests to worldwide interest in these products as well as in traditional health systems. Instead of that the remedies provided either by allopathic, by homeopathic or herbal, the sole objective of all types of medicine is to restore the patient to good health.

Therefore, now herbal drugs are formulated in primary dosage form for the ease and acceptability of patients [1, 2]. 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]. Thermo analysis 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 favourable directions to pursue for a successful formulation [6, 7, 8]. The most widely used thermo analytical techniques are Differential Scanning Calorimetry (DSC) and Thermo-gravimetry/Derivative Thermo-gravimetry (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. 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[11]. 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. In the present study an attempt was made to study accelerated stability of herbal capsules, which are containing the extract of *Ipomoea aquatic* (leaf), *Cinnamomum zeylanicum* (bark) and *Piper longum* Linn. (fruit).

MATERIALS AND METHOD:

Plant Material: The *Cinnamomum zeylanicum* (dried bark), *Piper longum* (dried stem) were procured from the AHS Enterprises, Gwalior, Madhya Pradesh and certificate of authentication was provided by them. The plant of *Ipomoea aquatica* was collected in the month of the September 2008 from the village Gormi and it was authenticated from CDRI Lucknow (U.P.). (Voucher specimen no.-J-31)

Herbal Capsules: Empty hard gelatin capsules were received as gift from Sarazen Research & Development Organisation, 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.

Preparation of Extracts: Fifty grams of each of the air-dried and coarsely powdered plant material was extracted with 200 ml each of chloroform and water using a soxhlet apparatus for 48 h. Extracts of plant materials either in water or chloroform are concentrated. The concentrated herbal extract, now in the form of a viscous liquid, is piped into a flow coater.

The granulator sprays the concentrate onto minute particles of a base material as the moisture slowly dissipates. With the moisture evaporated, the granules are dry and ready to be packaged. These granules were stored at 4oC in airtight bottles for further investigations. The granules can then be pressed into easy-to-swallow tablets or encapsulated [12].

Preparation of Formulation: Five hundred milligram granules of each plant extract was encapsulated into hard gelatin capsule of 000 size.

Thermo-gravimetric 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 Calorimetric 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 aluminum 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 semilog 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 [17, 18, 19].

Quantitative Alkaloid Determination Method: 5g (10 capsules) of the sample was weighed into a 250ml beaker and 200ml 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 [20, 21].

RESULT: It was evident from the results of pharmacological evaluation that extract namely IA+CZ+PL-W is the most potent and safe (result not shown). Hence herbal capsule was prepared using Hard Gelatin Capsule (Size 000).

Thermo-gravimetric Analysis: The TGA thermo grams of unused and used hard gelatin capsules were recorded and given in **Fig. 1**. 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 unused and used hard gelatin capsules shoeds no difference indicating there is no interaction between extract and the capsule shell.

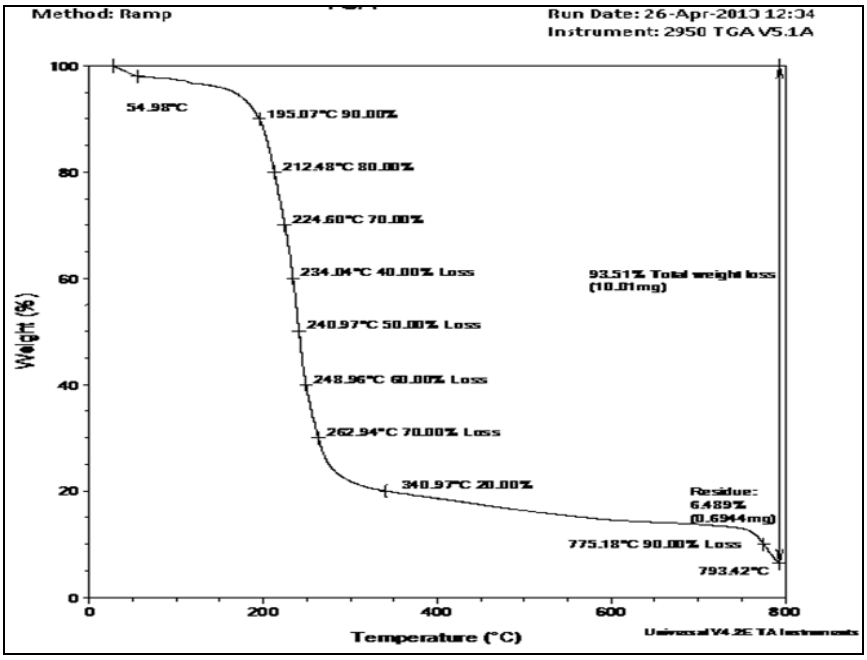

FIG. 1: TGA THERMOGRAM OF HARD GELATIN CAPSULE

Differential Scanning Calorimetry Analysis: DSC curve of unused and used hard gelatin capsules were recorded and given in **Fig. 2**. The DSC curve shows the endothermic peak due to fusion of the compound at 93.15 ^{0}C, and it is followed by a sharp exothermic peak at 309.45 ^{0}C ascribed to the thermal decomposition in consecutive stages. No remarkable interaction was found between unused and used hard gelatin capsules indicating no interaction between extract and the capsule shell.

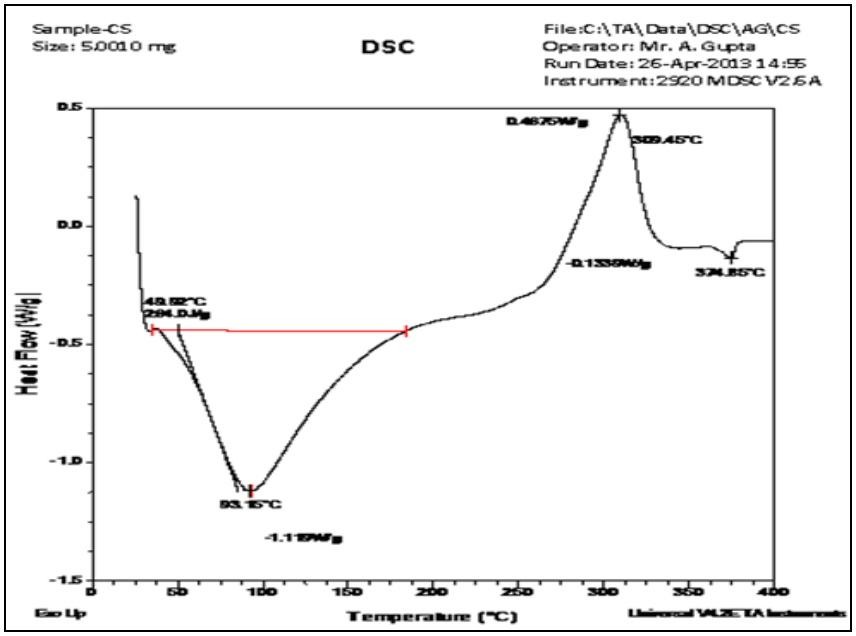

FIG. 2: DSC THERMOGRAM OF HARD GELATIN CAPSULE

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 hard gelatin capsule was given in **Fig. 3**. 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.

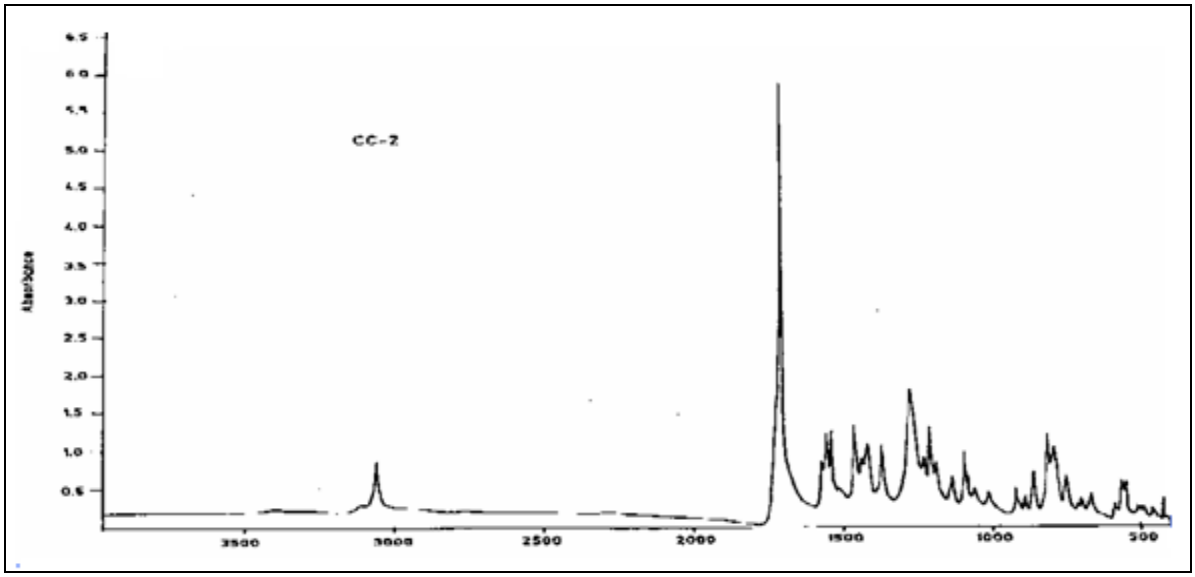

FIG. 3: FT-IR SPECTRA OF HARD GELATIN CAPSULE

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**.

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%	554.23	553.57	553.31	552.97	553.45
3	Locking length	20 - 21 mm	20.72	20.75	20.73	20.74	20.74
4	Disintegration	NMT - 30min.	11.21	11.29	11.28	11.30	11.23
5	pH (1% solution)	5.00-7.00	5.21	5.21	5.23	5.22	5.21

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 ^{0}C	40 ^{0}C	50 ^{0}C
0 month	100±0.9	100±8.45	100±1.63
1 months	100±1.7	94.73±8.34	73.59±1.36
2 months	99.93±1.6	88.21±20.66	50.57±1.29
3 months	99.21±3.2	86.46±3.42	14.50±0.01
6 months	98.43±2	85.83±2.01	5.94±0.30
9 months	97.65±1.1	70.51±8.29	-/-
12 months	96.87±1.5	55.62±1.09	-/-
18 months	95.31±0.3	-/-	-/-
24 months	93.75±5.2	-/-	-/-
36 months	90.62±0.6	-/-	-/-

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 AND 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	-0.26	100	1.0
2	40	-3.29	98.57	0.94
3	50	-15.71	86.63	0.83

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**). 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 ^{0}C was calculated. The shelf life for the suspension at 25 ^{0}C can be calculated using the formula:

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

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	-0.26	0.58
2	313	0.003195	-3.29	0.517
3	323	0.003096	-15.71	1.19

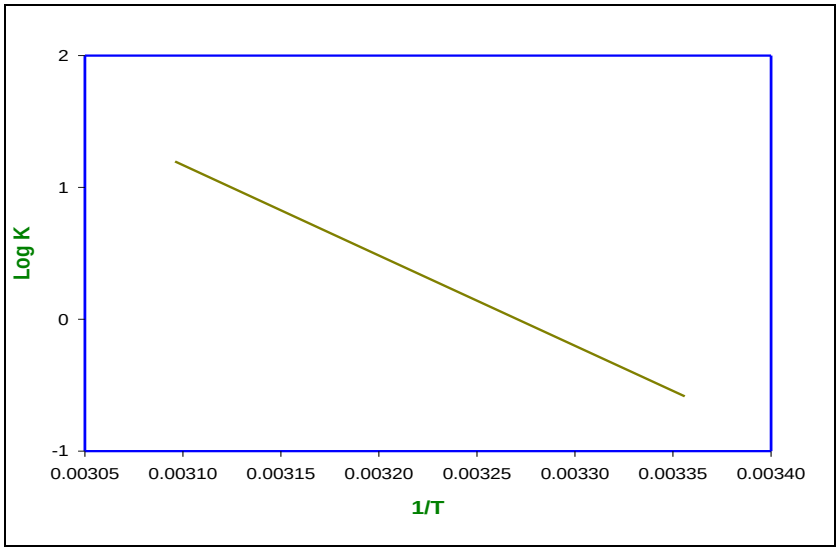

FIG. 4: ARRHENIUS PLOT

The shelf life of herbal capsule was calculated (**Table 5**) and found to be 3.2 years.

TABLE 5: DATA FOR THE SHELF LIFE DETERMINATION

Formulations	Slope K_0 (month^{-1})	Intercept C_0 (%)	r^2 Values (Unit less)	$k_{0,\ 25}$ (month^{-1})	Shelf life at 25 °C (Years)
Herbal capsule	-6857.36	22.42	1.0	0.259	3.2

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.

Thermo analysis 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 thermo analytical 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 (IA+CZ+PL-W) was selected for filling in the herbal capsule. The results obtained by the thermo analytical techniques showed no chemical incompatibility. The results were further confirmed by IR spectroscopy.

The herbal capsule was stored at different temperatures *viz*., 25 ^{0}C, 40 ^{0}C and 50 ^{0}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 remains 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 1 month and less than 1 month 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 3.2 years which is much more than sufficient to satisfy the regulatory norms of many countries.

CONCLUSION: Present study was envisaged to develop a polyherbal formulation as capsule. The results obtained by the thermo analytical techniques showed no chemical incompatibility between capsule shell and herbal 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. Likewise, pH values were fairly constant and appear not to be influenced by temperature. 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 3.2 years which is much more than sufficient to satisfy the regulatory norms of many countries.

ACKNOWLEDGEMENTS: The authors are thankful and grateful to SRCP Banmore, Gwalior, for their continuous support and inspiration.

CONFLICT OF INTEREST: Authors do not have any conflict of interest.

REFERENCES:

1. Qasim M, Khalid M, Sayyed A, Din I Hayat K, Ahmad S and R Ahmad: Phytochemical potentials and medicinal uses of twenty-four selected medicinal plants from Swabi. Journal of Rural Development and Agriculture 2016; 1(1): 49-58.
2. Farag M, Mohammed MS, Foud I, Ahmed WJ and Mohamed MS: The role of natural products in drug discovery and development. World Journal of pharmaceutical Research 2015; 4(3): 22-33.
3. Peter Borocz: Thermo-analytical technique to analize the quality of paper for packaging. Journal of Applied packaging research 2016; 8(1): 6.
4. Cardoso TM, Rodrigues PO, Stulzer HK, Silva MAS and Matos JR. Drug Dev. Ind. Pharm.2005; 31: 631-637.
5. Rodrigues PO, Cardoso TM, Silva MAS and Matos JR: Acta Farm. Bonaerense 2005; 24: 383-387
6. Bruni, G, Amici L, Berbenni V, Marini A and Orlandi A: J. Therm. Anal. Calorim. 2002; 68:561-573.
7. Rodante F Vecchio S Catalani G and Toassetti, M: Il Farmaco. 2002; 57: 833-843.
8. Vueba ML, Veiga F, Sousa JJ and Pina ME: Drug Dev. Ind. Pharm. 2005; 31: 943-949.

9. Araujo AAS, Storpirtis S, Mercuri LP, Carvalho FMS, Filho M Santos and Matos JR: Int. J. Pharm. 2003; 260: 303-314.
10. Kiss D, Zelko R, Novak CS and Éhen ZS: J. Therm. Anal. Calorim. 2006; 84: 447-451.
11. Sambasivarao A, Chandra SRB and Reddy MH: Accelerated stability testing of dosage forms as per international conference of hormonization (ICH) guidelines. World Journal of Pharmaceutical and Medical Research 2016; 2(3): 99-103.
12. Hardik KS: International Journal of Applied Biology and Pharmaceutical Technology 2010; 3(8): 1899-1902.
13. Ram VR, Ram PN, Khatri TT, Vyas SJ and Dave PN: Thermal analytical characteristics by TGA-DTA-DSC analysis of *Carica papaya* leaves from Kachchh, International Letters of Natural Sciences IRCS medical Science 2014; 21: 12-20.
14. Gunasekaran S, Venkatesan P, Anand G and Kumaresan S: Thermal properties of pure and doped BTMC and BTZS Single Crystals 2012; 4: 1072-1076.
15. Malar GSPL and David SB: Development of novel citric acid based biodegradable polymers from seasame oil. International Journal of Chemistry and Applications. 2014; 6:133-138.
16. Wunderlich B: Thermal analysis. New York Academic Press 1990; 137-140.
17. Punam T, Shubhangi W, Rahul S, Sargar A, Bhujbal A and Shinde A: Stability study of dosage form: World Journal of Pharmacy and Pharmaceutical Science.2014; 1031-1050.
18. Khalid H, Zhari I, Amirin S and Pazilah I: Accelaerated stability and chmical kinetics of ethanol extracts of fruit of *Piper sarmentosum* using high performance liquid chromatography. Iranian Journal of Pharmaceutical Research. 2011; 10(3): 403-413.
19. Carstensen JT: Stability and dating of solid dosage forms. Pharmaceutics of solids and solid dosage forms Wiley-Interscience, 182-185.
20. Shao J and Chow SC: Statistical inference in stability analysis. Biometrics1994; 50:753-763.
21. Ganapaty S, Ramaiah M, Yasaswini K, Nuthakki VJ and Reddy DH: Quantitative phytochemical estimation and evaluation of hepatoprotective activity of methanolic extract of *Dendrobium ovatum* (L.) Kraenzl. Journal of Pharmacognocy and Phytochemistry 2013; 2 (3): 113-118.

How to cite this article:
Gupta A, Singh SK, Singh RP and Jain S: Development of a polyherbal capsule for the treatment of asthma. Int J Pharm Sci Res 2017; 8(9): 4006-13.doi: 10.13040/IJPSR.0975-8232.8(9).4006-13.