RESEARCH PAPER

Accepted 12 February 2000
Revised 14 December 1999
Received 9 August 1999
Indian J. Pharm. Sci., 2000, 62 (3) 181-184

Accelerated Stability Studies of a Polyherbal Preparation (Eumil[R]) Capsule

S.K. CHAUHAN*, B.P. SINGH, A.TYAGI AND S. AGRAWAL
R&D Centre, Indian Herbs, Post Box No.5,
Saharanpur-247 001, UP, India.

The stability of Eumil capsule by exposing it to elevated conditions of temperature at 45° and at 40° with 75% relative humidity was studied. The samples were periodically analysed upto six months for their organoleptic characteristics, assay of active plant ingredients and the HPTLC finger printing and their peak area analysis, which were found to be stable/consistent during the period of study. The change in quantifiable components was within 90% of the initial amount, Indicating the stability of product for more than three years at room temperature.

Shelf life of any medicine can be defined as the time period or duration upto which it is expected to retain its active ingredients i.e. 90% of label claim when stored in recommended conditions[1]. Every product has a definite shelf life which depends on various physical, chemical, environmental and biological factors. Real time stability study is a long procedure. The manufacturers find it difficult to wait till the drug degrades naturally to 90% of its labelled amount at room temperature. On account of this reason, accelerated stability testing is normally carried out for assigning shelf life of the drugs[2].

We have attempted to study accelerated stability of Eumil, a herbal product which is used as an antioxidant and free radical scavenger[3]. It has been prepared using selected, time tested herbal ingredients in optimum combinations which include *Ocimum sanctum, Withania somnifera, Emblica officinalis* and *Asparagus racemosus*.

MATERIAL AND METHODS

The Samples of Eumil capsule (M/s Envin Bioceuticals Pvt. Ltd. Saharanpur) Batch No. 05, Mfg. Date – June'97 packed in PVDC coated PVC/ Aluminium blister of ten capsules were randomly taken for study.

Enough blisters in duplex board were kept in oven at 45° and in relative humidity chamber adjusted at 40° and 75% Relative Humidity. Required blisters were withdrawn after one month, three months and six months in triplicate for analysis.

Evaluation Parameters:

The parameters studied are all those readily quantifiable and are not necessarily only the active moieties, which include organoleptic, physical characters, HPTLC finger printing and their peak area analysis and assay of selective ingredients viz *Ocimum sanctum, Emblica officinalis* and *Withania somnifera* with reference to their biologically active compounds. Eugenol for *Ocimum sanctum, gallic acid for Emblica officinalis and* withanolide glycoside for *Withania somnifera* were selected as the active compounds. The physical parameters of Eumil capsule were evaluated at different time interval in different storage conditions and the data is presented in Table-1.

Estimation of Eugenol:

The samples (1 g each) were dispersed in 20 ml of water and transferred into a seperating funnel. It was extracted with chloroform (6 ml x 8), or till colour persisted. The chloroform extracts were pooled, passed through anhydrous sodium sulphate and concentrated over steam water bath to make the volume to 10 ml. Twenty microlitres of each test sample was applied on precoated silica gel

*For correspondence

TABLE 1 : PHYSICAL PARAMETERS OF DIFFERENT SAMPLES OF EUMIL CAPSULE

PARAMETERS	INITIAL	DETAILS OF SAMPLES (STORAGE CONDITIONS)					
		45° for 1 month	40° 75% RH for 1 month	45° for 3 months	40° 75% RH for 3 Months	45° for 6 months	40° 75% RH for 6 months
1. Appearance	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'	Hard gelatin capsule Size '0'
2. Appearance of capsule powder	Brown, fine powder	Brown, fine powder	Brown, fine powder	Brown, fine powder	Brown, fine powder	Brown, fine powder	Brown, fine powder
3. Average weight (mg)	672	677	678	675	677	678	682
4. Moisture content of capsule powder	4.60	4.65	4.65	4.60	4.64	4.65	4.63
5. pH of 2% aqeous suspension	4.95	4.95	4.98	4.93	4.97	4.98	4.98
6. Disintegration time (min)	8	10	10	10	10	10	10

60 F254 aluminimum plate (E. Merck. Cat. No. 5554) alongwith 1, 2, 5 and 10 µl of standard eugenol (1mg/ 10ml). The plate was developed in tolune:ethyl acetate - 93:07 upto 80 mm under chamber saturation condition. The plate was air dried and scanned at 260 nm using a Camag TLC Scanner III. The contents of eugenol were calculated using the linear regression equation obtained from calibration graph plotted between concentration and area of standard eugenol. The equation for eugenol was found to be Y = 1951. 8x + 706 with a correlation coefficient of 0.995 where Y is the response in peak area and x is the concentration in mg/ml. The results have been documented in Table-2.

Estimation of Gallic Acid:

The samples (1 g each) were extracted with methanol (15 ml x 6) over steam water bath. The extracts were filtered and volume of respective sample was made up to 100 ml with methanol, 10µl of each sample was applied on precoated silica gel 60 F254 aluminium plate (E. Merck Cat. No. 5554) alongwith 2, 5, 10 and 20 µl of standard gallic acid (1 mg/10 ml) using a Camag Linomat IV. The plate was developed in ethyl acetate: formic acid: acetic acid:water - 100:11:11:27 (upper layer) upto 80 mm under chamber saturation condition. The plate was air dried and scanned at 260 nm using Camag TLC Scanner III. The contents of gallic acid were quantified using the linear regression equation obtained from calibration graph plotted between concentration and area of standard gallic acid. The equation for gallic acid was found to be Y=5828x + 1277 with a correlation coefficient of 0.9997 where Y is the response in peak area and x is the concentration in mg/ml. The results have been provided in Table-2.

TABLE 2 : ESTIMATION OF EUGENOL, GALLIC ACID AND WITHANOLIDE GLYCOSIDE IN EUMIL CAPSULE

S. No.	Details of Samples	Eugenol (% w/w)	Gallic Acid (% w/w)	Withanolide Glycoside (% w/w)
1.	Initial sample	0.0375	1.260	1.620
2.	45° for one month	0.0385	1.198	1.668
3.	40° and 75% RH for one month	0.0380	1.290	1.577
4.	45° for three months	0.0370	1.198	1.670
5.	40° and 75% RH for three months	0.0349	1.189	1.590
6.	45° for six months	0.0348	1.210	1.640
7.	40° and 75% RH for six months	0.0345	1.222	1.660

Estimation of withanolide glycoside:

The samples (1 g each) where dissolved in 100 ml of

TABLE 3 : TOTAL AREA OF HPTLC CHROMATOGRAMS OF DIFFERENT SAMPLES OF EUMIL CAPSULE

S. NO.	DETAILS OF SAMPLES STORAGE CONDITIONS	TOTAL AREA
1.	Initial sample	31053.7
2.	45° for one month	30413.8
3.	40° and 75% RH for one month	30559.9
4.	45° for three months	29499.6
5.	40° and 75% RH for three months	30327.3
6.	45° for six months	30158.2
7.	40° and 75% RH for six months	30025.1

water by shaking the contents over steam water bath and filtered. Ten microlitre of each sample was applied on precoated silica gel 60 F254 aluminium plate (E.Merck. Cat. No. 5554) alongwith 1,2,5 and 10µl of withanolide glycoside (1 mg/ml) using a Camag Linomat IV. The plate was developed in n-butanol:acetic acid:water - 4:1:2 upto 80mm using twin trough development chamber under chamber saturation condition. The plate was dried under the current of hot air and sprayed with Liebermann Burchard spray reagent[4] which was then dried at 100° for 10 min and scanned at 520 nm using Camag's TLC Scanner III. The contents of withanolide glycoside were quantified using linear regression equation obtained from calibration graph plotted between concentration and area of standard withanolide glycoside. The equation for Withanolide glycoside was found to be Y=861x +811 with a correlation coefficient of 0.998 where y is the response in peak area and x is the concentration in mg/ml. The results are presented in Table-2.

HPTLC Finger Printing:

For HPTLC analysis, test samples (1 g each) were dispersed in 20 ml of water and 5 ml of 5 N Hcl was added to each sample which were then refluxed for 2h on a heating mentle. The samples were cooled to room temperature and transferred to seperating funnel which were then extracted with chloroform (6 ml x 8) or till colour persisted. The samples were filtered, passed through anhydrous sodium sulphate and concentrated to 10ml over steam water bath. Twenty microlitres of each where applied on precoated silica gel 60 F254 aluminium plate (E. Merck, Cat. No.5554) which was developed in chloroform:methanol - 95:5 upto 80mm under chamber saturation condition. The plate was air dried and scanned at 260nm using a Camag TLC Scanner III. The finger printing of different samples have been presented in Figure-1 while the total areas are provided in Table-3.

TABLE 4 : TIME AT WHICH POTENCY MUST BE ATLEAST 90% OF LABEL CLAIM WHEN STORED AT 45°

Shelf life (years)	Time in months (minimum)
2	2.9
3	5.2
4	6.1
5	9.0

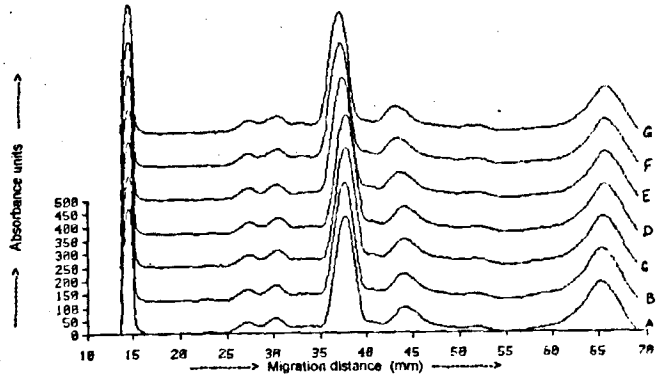

Fig. 1: HPTLC patterns of Eumil capsules under different storage conditions

Eumil capsules were kept for different periods under different conditions such as A. Initial sample; B. 45° for 1 month; C 40°; 75% RH for 1 month; D 45° for 3 months; E. 40°, 75% RH for 3 months; F. 45° for 6 m and G. 40°, 75% RH for 6 months

RESULTS AND DISCUSSION

It is a normal practice to study the stability of pharmaceutical preparations at accelerated conditions of temperature and humidity, the experimental findings of which can be transformed into a reliable shelf life or expiry date at room temperature by adopting certain assumptions and criterion[2]. By this method the shelf life of any drug product can be predicted in a short period of time. Table-4 provides the minimum time which is required

for assessing the stability of drug products. Unlike allopathic drugs the selection of testing parameters is critical for herbal drugs because in most of the cases, biologically active compounds and their testing procedure are not well defined and hence the parameters should be such that can be quantified and provides the overall stability of the formulation[5], which includes organoleptic, physico-chemical parameters, HPTLC finger printing with their peak area analysis and assay of selective ingredients wherever possible. All the individual ingredients of Eumil contains the complex chemical compounds of different nature. We have selected eugenol from *Ocimum sanctum,* gallic acid from *Emblica officinalis* and withanolide glycoside from *Withania somnifera* as the active principle and quantified them in different samples of Eumil. The physical parameters of initial sample and sample analysed after 1, 3 and 6 months of storage at accelerated conditions of temperature and humidity are found similar, indicating that gross physical characteristics of Eumil does not produce any significant changes (Table-1). The similar results are indicated by Table-2, where the assay of eugenol, gallic acid and withanolide glycoside in different samples of Eumil are within the limits. On comparing the HPTLC finger printing of initial as well as samples stored at accelerated temperature and relative humidity for 1, 3 and 6 months, we see from Figure -1 that all the chromatograms are essentially similar which get further confirmed from the total area of the chromatograms which is within the limit of 90% of the initial area indicating the overall stability of Eumil. The above study indicates that Eumil is stable at room temperature for more than three years. However, real time studies are underway to confirm these findings.

ACKNOWLEDGEMENTS

Technical assistance of Mr. B.P. Bhatt is thankfully acknowledged.

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