ACCELERATED STABILITY STUDIES OF A POLYHERBAL PREPARATION (EAZMOV) CAPSULE
S.K. Chauhan, A. Tyagi, B. Singh and S. Agarwal
R&D centre, Indian herbs, Post Box No 5
Saharanpur (UP) India

Received: 6th August, 1997
Accepted: 4th January, 1999

ABSTRACT: The stability of Eazmov capsule in accelerated condition i.e. by exposing it to the temperature at 45°C and 40°C with 75% relative humidity was studied. The samples were periodically analysed up to six months for their organoleptic characteristics, assay of active plant ingredients and the DPTLC fingerprinting 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 a stability of the product for more than three years at room temperature.

Key words: Stability study, Eazmov, s-sitosterol, Kutkin, Glycyrrhizin, HPTLC

INTRODUCTION:
Shelf life is an important parameter while formulating any drug product. Lots of effects, technical expertise and experience is required for formulating a stable product. Self life of any drug product can be defined as the time period or duration up to which it is expected to retain its active ingredients i.e. 90% of label claim when stored in recommended conditions. 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 labeled amount at room temperature. On account of this reason, accelerated stability testing is normally carried out for assigning shelf life of the drugs.

We have attempted to study accelerated stability of Eazmov, a herbal product of M/S Envin Biocuticals Pvt. Ltd., Saharanpur It has been prepared using selected, herbal ingredients in optimum combination which includes Picrorhiza kurroa, Tinospora cordifolia, Glycyrrhiza glabra, Zingiber officinale, Cyperus rotundus and Sasselurea lappa.

EXPERIMENTAL
The samples of Eazmov capsule Batch No.03 Mfg date-June 97 packed in PVDC coated PVC/Aluminium blister of ten capsule were randomly taken for study. Enough blisters in duplex board were kept in oven at 45°C and 75% Relative Humidity. Required blisters were withdrawn after one month, three months and six months in triplicate for analysis.

EVALUATION PARAMETERS:
The parameters studies are all those readily quantifiable and are not necessarily only the active moieties, which includes organoleptic, physical characters, HPTLC.
finger printing and their peak area analysis and assay of selective ingredients viz. *Picrorrhiza kurroa, Glycyrrhiza glabra and Cyperus rotundus* with reference to their biologically active compounds.

**PROCEDURE:**

The physical parameters of Eazmov capsule were evaluated at different time interval in different storage conditions and the data is shown in table.

**ESTIMATION OF GLYCYRRHIZN**

Glycyrrhizin was estimated using HPTLC technique as per the procedure of chauhan, et. al\(^3\). The results are provided in Table -2.

**ESTIMATION OF β SITOSTEROL**

The samples (one gram each) were dispersed in 20 ml of water, transferred to seperating funnel and extracted wit chloroform (10ml x6) or till colour persist. The chloroform extract was passed through anhydrous sodium sulphate and the solvent was evaporated completely over water bath. The residue thus obtained were acetylated using the mixture of acetic anhydride and pyridine as per usual procedure. He solvent was evaporated and the residue was dissolved in 1 ml of chloroform. 0.5ul of these test samples along with four different concentration of standard β –Sitosterol were injected into a Varian 3800 Gas Chromatograph. The column used was VA 17, 30 mx 0.25 mm id. The flame ionization detector was set at 330oC. The oven was programmed as follows.

| Temp °C | Rate (°C/min) | Hold (min) | Total (min) |
|---------|---------------|------------|-------------|
| 200     | 0.0           | 1.0        | 1.0         |
| 220     | 5.0           | 5.0        | 10.0        |
| 270     | 2.0           | 15.0       | 35.0        |

The injector temperature was set at 250°C the carrier gas was nitrogen at a flow rate of 5ml/min. The contents of β –Sitosterol were quantified using the linear regression equation obtained from calibration curve plotted between concentration and area of standard β –Sitosterol. The results have been provided in Table 2.
### TABLE -1 PHYSICAL PARAMETERS OF DIFFERENT SAMPLES OF EAZMOV CAPSULE

| DETAILS OF SAMPLE                                      | Parameters | Initial | Kept at 45 C for 1 month | Kept at 40C /75% RH for 1 month | Kept at 45 C for 3 month | Kept at 40C /75% RH for 3 month | Kept at 45 C for 6 month | Kept at 40C /75% RH for 6 month |
|-------------------------------------------------------|------------|---------|--------------------------|---------------------------------|-------------------------|---------------------------------|-------------------------|---------------------------------|
| 1 Appearance                                          | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ | Hard gelatin capsule of size ‘0’ |
| 2 Appearance of capsule powder                        | Brown coloured fine powder | Brown coloured fine powder | Brown coloured fine powder | Brown coloured fine powder | Brown coloured fine powder | Brown coloured fine powder | Brown coloured fine powder |
| 3 Average weight (mg)                                  | 690        | 687     | 675                      | 680                             | 687                      | 685                             | 682                      |
| 4 Moisture content of capsule powder                   | 4.40       | 4.45    | 4.45                     | 4.40                            | 4.40                     | 4.40                            | 4.50                     |
| 5 Disintegration time (minutes)                        | 8          | 10      | 10                       | 8                               | 10                       | 10                              | 10                       |

### TABLE -2 ESTIMATION OF KUTKIN, GLYCYRRHIZIN AND B – SITOSTEROL IN EAZMOV CAPSULE

| DETAILS OF SAMPLE                                      | KUTKIN CONTENT (% W/W) | GLYCYRRHIZIN CONTENT (% W/W) | B – SITOSTEROL CONTENT (% W/W) |
|-------------------------------------------------------|-------------------------|-------------------------------|--------------------------------|
| 1. Initial sample                                      | 0.890                   | 0.979                         | 0.1793                         |
| 2. Kept at 45°C for one month                          | 0.857                   | 1.042                         | 0.1789                         |
| S. No | Details of Sample                                  | Total Area |
|-------|---------------------------------------------------|------------|
| 1.    | Initial sample                                    | 22460.7    |
| 2.    | Kept at 45°C for one month                        | 21976.7    |
| 3.    | Kept at 45°C & 75% RH for one month               | 21829.1    |
| 4.    | Kept at 45°C for three month                      | 21839.0    |
| 5.    | Kept at 45°C & 75% RH for three month             | 20966.9    |
| 6.    | Kept at 45°C & 75% RH for six month               | 20983.3    |
| 7.    | Kept at 45°C & 75% RH for six month               | 20866.7    |

**TABLE -3 TOTAL AREA OF HPTLC CHROMATOGRAMS OF DIFFERENT SAMPLES OF EAZMOV CAPSULE**
ESTIMATION OF KUTKIN:

The samples (one gram each) were dissolved in 40 ml of water by shaking the contents over steam water bath. The samples were filtered and the volume was made upto 40ml. 5 and 10ml of each samples were applied on precoated silica gel 60 F$_{254}$ alluminium plate (E.Merck. Cat No. 5554) withalong 1,2,5 and 10ml of standard kutkin (1 mg/ml) using camag’s Linomat IV. The plat was developed in Ethyl acetate; Methanol; water – 77: 15:8 upto 80mm using twin through development chamber under chamber saturation condition. The plate was air dried and scanned at 260 nm using Camag’s TLC Scanner III. The contents of Kutkin were quantified using linear regression equation obtained from calibration graph plotted between concentration and area of standard kutkin. The results are provided in table -2.

HPTLC FINGER PRINTING

For HPTLC analysis, test samples (one gram each) were dispersed in 20 ml of water and 5ml of 5N HCl was added to each sample which were then refluxed for 2 hrs on heating mantle. The samples were cooled to
room temperature and transferred to separating funnel which were then extracted with chloroform (6ml x 8) or till colour persisted. The samples were filtered, passed through anhydrous sodium sulphate and concentrated to 10ml over steam water bath. 20 ml of each, were applied on precoated silica gel 60 F254 aluminium plate (E.Meck, cat no.5554) which was developed in chloroform: Methanol – 95:5 upto 80 mm under chamber saturation condition. The plate was air dried and scanned at 260 nm using camag’s TLC scanner III. The fingerprinting of different sample have been provided in figure 1 while the total area are provided in Table -3

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\(^2\). By this method the self life of an drug product can be predicted in a sort period of time, Unlike allopathic drugs the selection of testing parameters is critical for herbal drugs because in most of 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\(^4\), which includes organoleptic, physical chemical parameters, HPTLC finger printing with their peak area analysis and assay of selective ingredients wherever Possible. All the individual ingredients of Eazmov contains the complex chemical compounds of different nature. We have selected β – Sitosterol from Cyperus rotundus, kutkin from Picrorrhiza jurroa and Glycyrrhizin from Glycyrrhiza glabra as the active principle and quantified them in different sample of Eazmov. 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 w physical characteristics of Eazmov does not produce any significant changes (Table -1). The similar results are indicated by table 2 where the assay of β – Sitosterol, Glycyrrhihizin and Kutkin in different samples of Eazmov are Kutkin in different samples of Eazmov are within the limits. On t 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 eh chromatograms which is within the limit of 90% of t initial area indicating the overall stability of Eazmov. The above study indicates that Eazmov is stable at room temperature for more than three years. However, real time studies are underway to confirm these findings.

ACKNOWLEDGEMENT:

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

REFERENCES:

1. Pediyar, A., Ali, J and Khar, R.K Pharmatimes, 1998, 30(8), 35-38

2. Cannors K.A., Amidon G.L. and Kennon L. Chemical stability of pharmaceuticals A hand book for pharmacists 1979, Jon wiley & sons, New York.
3. Chauhan, S.K., Singh B.P., Kimothi, G.P. and Agrwal, *S.Ind.J. J.Pharma Sci* 1998, 60(4), 251-252.

4. Chauhan, S.K., and Agrwal, *S. the eastern Pharmacist* 1999, XLII (498), 35.

5. Chatterjee, S. and Das S.N. *Ind. J. Pharmacol* 1996, 28(2), 116-119

6. Chatterjee, S. and Das S.N. *Ind. J. Ind Med.* 1994, 11(1), 39-42.

7. Chatterjee, S. and Das S.N. Agrwal, *S. Ind. J. Med.* 1996, 18(1), 83-85.