Ancient Science of Life Vol. No. 18 (3&4) January & April 1999 pages 210 - 217

ACCLERATD STABILITY STUDIES OF A POLYHERBAL PREPARA – TION (EAZMOV^{R}) CAPSULE

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

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

Key words: Stbility study, Eazmov, s-sitosterol, Kutkin, Glycryrrhizin, 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 stale product. Self life of any drug product can be defined as the time period or duration upto which it is expected o retain its active ingredients ie. 90% of label claim when stored in recommended conditions. Every product as a definite shelf life which depends on various physical chemical environmental and biological factors'. Real time stability study is long procedure. The manufacturers, find I 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[2].

We have attempted to stud accelerated stability of Eazmov, a herbal product of M/S Envin Bioceuticals Pvt. Ltd., Sharanpur It is used as analgesic, anti-inflammatory, antiarthritic and Immune modulater [5,6,7]. It has been prepared using selected, herbal ingredients in optimum combination which includes *Picrorrhiza kurroa, Tinospora cordifolia, Glycyrrhiza glabra, Zingiber officinale, Cyperus rotundus and sassurea 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 45oC 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 Required blisters were withdrawn after one month, three months and six months in triplicate for analysis.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 ^{o}C	**Rate (^{o}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^{o}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

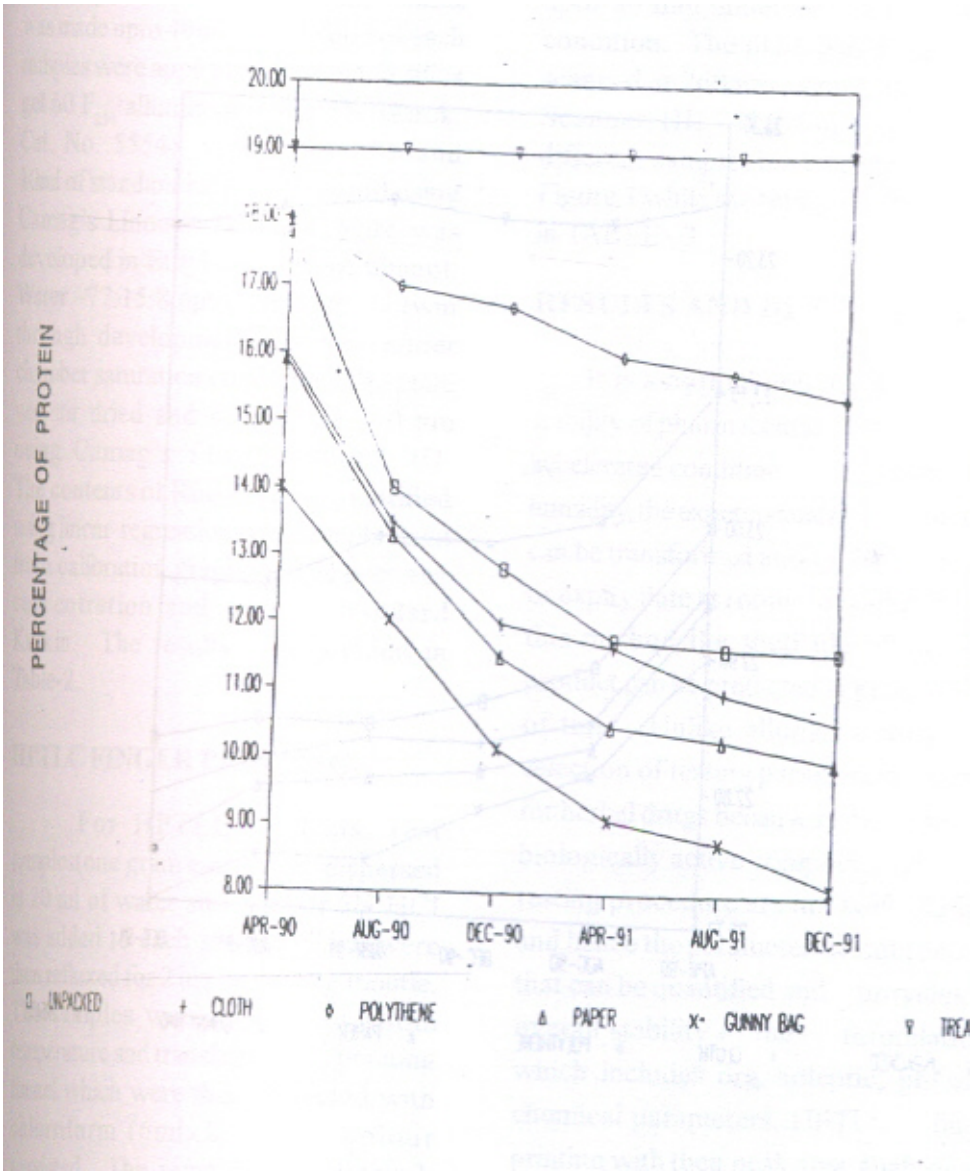
TABLE -2 ESTIMATION OF KUTKIN, GLYCYRRHIZIN AND B – SITOSTEROL IN EAZMOV CAPSULE

S. NO	DETAILS OF SAMPLE	KUTKIN CONTENT (% W/W)	GLYCYRRHIZIN CONTENT (%W/W)	- SITOSTEROL CONTENT (% W/W)
1.	Initial sample	0.890	0.979	0.1793
2.	Kept at 45^{o}C for one month	0.857	1.042	0.1789

3.	Kept at 45^{o}C & 75% RH for one month	0.904	0.969	0.1782
4.	Kept at 45^{o}C for three month	0.817	0.926	0.1705
5.	Kept at 45^{o}C & 75% RH for three month	0.839	0.937	0.1698
6	Kept at 45^{o}C for six month	0.824	0.916	0.1702
7.	Kept at 45^{o}C & 75% RH for six month	0.822	0.922	0.1652

TABLE -3 TOTAL AREA OF HPTLC CHROMATOGRAMS OF DIFFERENT SAMPLES OF EAZMOV CAPSULE

S. NO	DETAILS OF SAMPLE	TOTAL AREA
1.	Initial sample	22460.7
2.	Kept at 45^{o}C for one month	21976.7
3.	Kept at 45^{o}C & 75% RH for one month	21829.1
4.	Kept at 45^{o}C for three month	21839.0
5.	Kept at 45^{o}C & 75% RH for three month	20966.9
6	Kept at 45^{o}C for six month	20983.3
7.	Kept at 45^{o}C & 75% RH for six month	20866.7


PERCENTAGE OF PROTEIN
20.00
19.00
17.00
16.00
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
APR-90
AUG-90
DEC-90
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AUG-91
DEC-91
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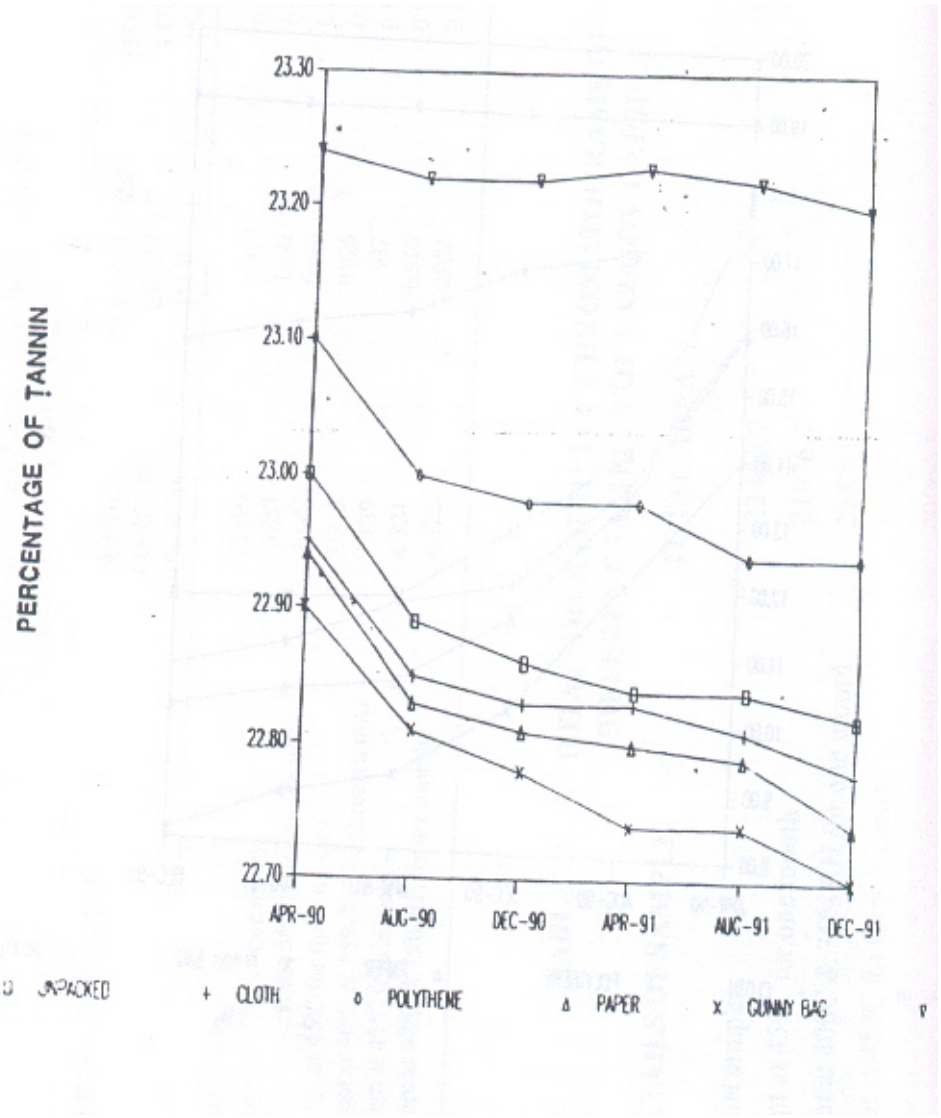
CLOTH
POLYTHENE
PAPER
GUNNY BAG



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 HCI 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 x8) or till colour persisted. The samples were filtered, passed through anhydrous sodium sulphate and concentrated to 10ml over steam water bat. 20 ml of each, were applied on precoated silica gel 60 F_{254} 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 finger printing of different sample shave 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 trans formed 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 Glycyrrhin 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 tat gross wphysical 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, Glycyrrihizin 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.

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