International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(1): January-February 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 19-12-2011; Accepted: 25-12-2011

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF SILIBININ IN BULK DRUG AND IN ITS FORMULATIONS

Prakash S. Sarsambi*, Anupama K, Sravanthi Gudavarthi

H.K.E.S' Society College of Pharmacy, Sedam road, Gulbarga, Karnataka- 585105, India

Keywords:

Silibinin, RP-HPLC, Isocratic, Validation

For Correspondence:

Prakash S. Sarsambi

H.K.E.S' Society College of Pharmacy, Sedam road, Gulbarga, Karnataka- 585105, India

E-mail:

prakash.sarasambi@gmail.com

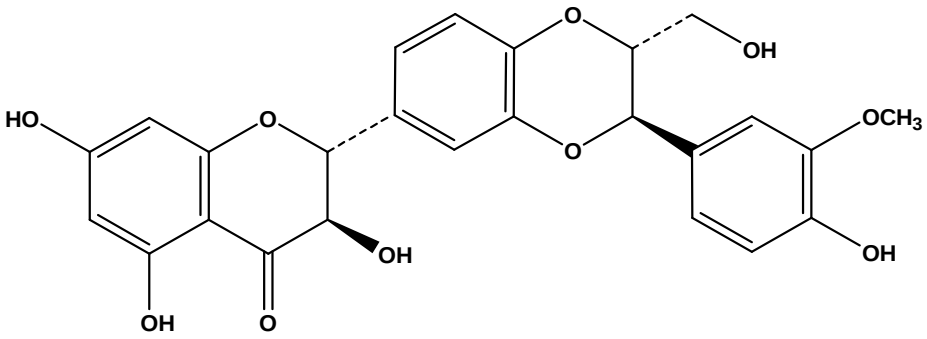
ABSTRACT

A simple, accurate and rapid RP-HPLC method has been developed for estimation of silibinin in bulk drug and pharmaceutical dosage form using C_{18} phenomenex 250 × 4.5mm i.d, 5µm particle size in isocratic mode with mobile phase comprising of HPLC grade methanol and water in the ratio of 90:10 (v/v). The flow rate was set at 1ml/min and detection was carried out by U.V detection at 288 nm.the retention time for silibinin was found to be 2.667 min. the proposed method was permitted the quantification of silibinin over linearity in the range of 20-100µg/ml and its percentage recovery was found to be 98.87 %.The percentage RSD of the intraday and interday precision was found to be less than 1.

INTRODUCTION

Silibinin[1-3] is chemically, 3,5,7-trihydroxy-2-[3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benodioxan-6-yl]-4-chromaone. It shows a hepatoprotective action[4]. Since, it is widely used in the hepatoprotective therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage forms. The present work reports the development and validation of a RP-HPLC method for the estimation of Silibinin in bulk drug and in formulations. so far no systematic RP-HPLC method has been reported, however only few- physicochemical methods were reported for silibinin (concerned with biological fluid sample) i.e., Two HPLC assay for the determination of free and total silibinin diasteromers switching with ECD and RP chromatography with UV detection[5]. Applications of LC-electrospray ionization-ion trap mass spectrometry to investigate the metabolism of silibinin in human liver microsomes[6]. Method development for analysis of silibinin from human plasma by HPLC[7]. The present work reports a rapid and sensitive RP-HPLC determination method using UV detection, useful for the routine quality control of Silibinin in bulk drug and its pharmaceutical formulations. The method was validated by parameters such as accuracy, precision.

Figure1: structure of Silibinin



EXPERIMENTAL

HPLC system is used for this study, the specifications are given below. A shimadzu's HPLC equipped with SPA- 20A detector, phenomenox C_{18}(250 × 4.6mm; 5µm) column and spinchrom software was used. Analytical balance and various volumetric flask of required capacity and other glassware (Borosil) were used.

REAGENTS AND CHEMICALS

Pure sample was obtained as a gift sample from (Sun pharmaceuticals Ltd, Himachalpradesh, India), methanol (HPLC grade, S.D. fine chemicals, Mumbai), triple distilled water (HPLC grade, thermo fisher scientific India pvt.), nylon 0.45µ membrane filter (German laboratory, Mumbai, India) were used for the study. Silibinin drug product (silibostin) was procured from the local market.

PREPARATION OF STANDARD STOCK SOLUTION OF SILIBININ:

Procedure: About 100 mg of Silibinin was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in 50 ml HPLC grade methanol and sonication for about 15 min and then made up to the volume with HPLC grade methanol. For this, a working standard solution of 500µg/ml of strength was prepared, from this dilution of 20, 40, 60, 80, 100 µg/ml were prepared. 20 µl of each dilution was injected each time into the column at a flow rate of 1ml/min. each dilution was injected into the column and the corresponding chromatograms were obtained.

Assay of Silibinin in capsules: Ten capsules were weighed. An accurately weighed portion of the powder equivalent to 100 mg of Silibinin was transferred to 100 ml volumetric flask containing 10 ml of methanol and the content of the flask were sonicated for 15min, to ensure the complete solubility of the drug. The mixture was then made up to 100ml with methanol.

The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. This solution (20µl) was injected three times into the column. The mean values of peak areas of five such determinations were calculated and the drug content in the tablet was quantified using the regression equation.

Chromatographic conditions: The content of the mobile phase were HPLC grade methanol and HPLC grade water in ratio of 90:10 percent (v/v). the content of mobile phase is filtered before use through 0.45µm membrane filter and sonicated for 15 min .the flow rate of the mobile phase was maintained at 1.0 ml/min the column temperature was set at 25±1^{o}c and the detection was carried out by UV- detector, wave length was set at 288 nm the run time was set at 10 min and the volume of the injection loop was 20 µl prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The data were acquired, stored and analysed with the software class spinchrome (SHIMADZU).

Calibration procedure: The calibration curve plotted with five concentrations of the standard drug solution 20- 100µg/ml solution and chromatography was repeated thrice for each dilution. The linearity was evaluated by linear regression analysis, before injecting solutions; the column was equilibrated for at least 30 min with the mobile phase flowing through the system five determinations were carried out for each solution, peak area ratios were recorded for all the solutions. The correlation graph was constructed by plotting the peak area ratios obtained at the optimum wave length of detection versus the injection amounts of the respective concentrations.

RESULTS AND DISCUSSION:

The applied chromatographic conditions permitted a good resolution of Silibinin (60 µg/ml) in the sample solution (fig: 2) and in standard solution (fig: 3) .No drug decomposition was observed during the analysis. .The LC method was validated for the parameters reported below.

Figure 2: Model chromatogram for Silibinin(Bulk drug) –R.T: 2.667

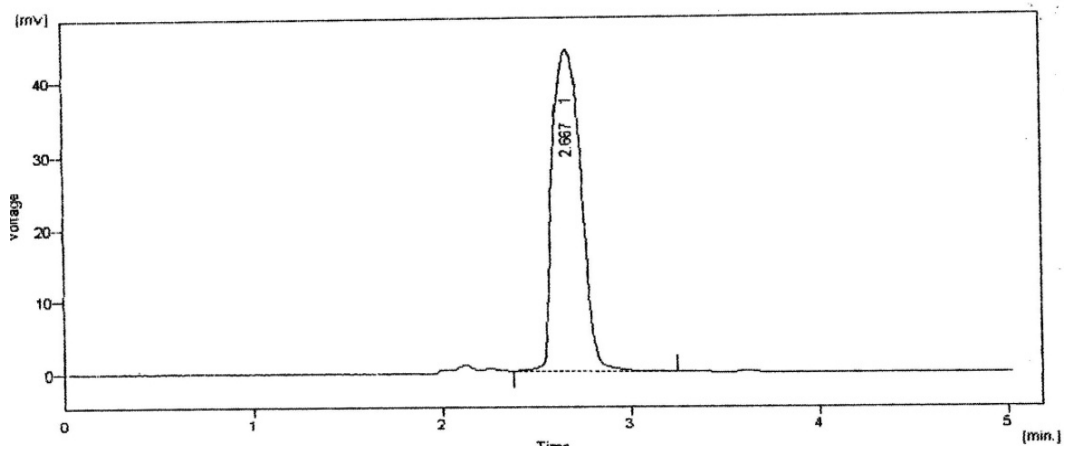
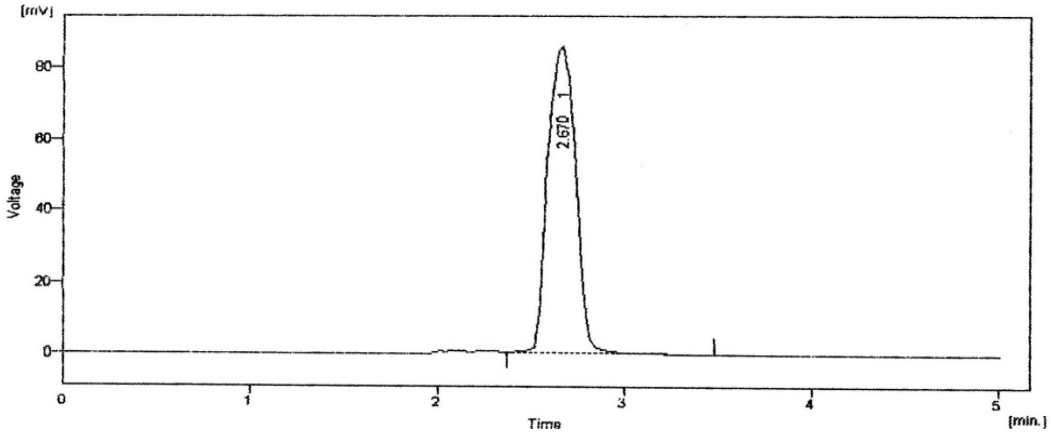


Figure 3:Model chromatogram for Silibinin(Pharmaceutical formulation) – R.T : 2.670



Linearity: The Silibinin were chromatographed using the mobile phase, the linearity of peak area responses versus concentration was studied from 20- 100µl for Silibinin .a linear response was observed over the examined concentration range(Fig:4). The results are tabulated in the (Table: 1)

Fig: 4 Calibration graph of silibinin

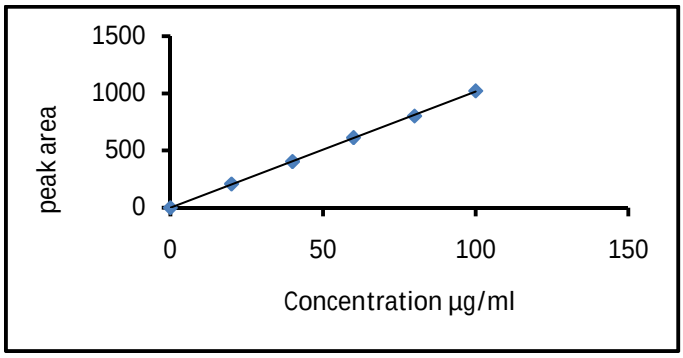


Table1: performance calculation,detection charecteristics precison and accuracy of the proposed method.

PARAMETER	SILIBININ
Retention time (t) min	2.667
Theoretical plates (n)	1265
Plates per meter(N)	5439
Height equivalent to theoretical plate (HETP)	1.976×10^{-4}
Linearity range (µg/mL)	20-100
LOD (µg mL^{-1})	0.00102
LOQ (µg mL^{-1})	0.00309
Regression equation (Y=bC+a)	
Slope (b)	10.122
Intercept (a)	2.67
Correlation coefficient (r)	0.9997
Relative standard deviation	0.000512
Range of errors** Confidence limits with 0.05 level (±) Confidence limits with 0.01 level (±)	 0.0033 0.0048

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount of the drug solution of known concentration and subjecting the samples to the proposed HPLC method the known amount of the drug solution was also added to the volumetric flask containing the powder sample of the capsule formulation with known amount of the drug. The drug was estimated as the procedure described above for the estimation of Silibinin in the capsule formulation. In both cases the recovery studies were replicated three times. The accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of the measured drug concentration with 100, so as to given the percentage recovery (table: 2 and 3)

Table 2:- Assay result of silibinin in pharmaceutical formulations

Sample	**Labeled amount (mg)**	**Amount found by the proposed method *±SD**	**Reference method (UV)**	**%Recovery by the proposed method± SD**
C_1	120	118.15 ± 0.144 t = 0.452 F = 0.673	119.52 ± 0.181	99.48 ± 0.48
C_2	120	119.14 ± 0.63 t =0.823 F = 0.683	119.47 ± 0.025	99.51 ± 0.67

C_1 (silibostin) and C_2 (levalon) are capsules from different manufactures, *Average ± SD of 5 determinations, the t and F-values refer to comparison of the proposed method with reference method. Theoretical values at 95% confidence limits t=2.365 and F=4.88.

Table 3:- Determination of accuracy of silibinin

Level of %recovery	Amount of formulation added(mg)	Amount of standard drug added (mg)	Total amount found(mg)	%Recovery
80 %	119.74	96	214.92	99.74
	118.90	96	214.9	99.04
	118.53	96	214.53	98.73
100 %	117.87	120	237.87	98.18
	118.15	120	237.89	98.41
	117.95	120	237.45	98.40
120 %	119.89	144	263.39	99.86
	118.47	144	262.27	98.68
	118.65	144	262.65	98.83

Precision: the precision of the assay was determined in terms of intra and inter day variation in the peak area for a set of drug solution (60 and 100 µg/ml) assayed five times on the same day and on three different days. The intra (Table:4) and interday (Table:5)variations in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100 (CV=SD/MEAN × 100) .

Table 4: inter day precision

Conc. of SIL(µg/mL)	Peak Area					Mean*	SD*	%CV*
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
60	612.58	611.24	612.24	612.67	612.24	612.19	0.567	0.092
100	1021.9	1021.6	1021.1	1020.9	1020.7	1021.4	0.84	0.082

Table 5: Intra day precision

Conc. of SIL (µg/mL)	Peak Area					Mean*	SD*	%CV*
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
60	607.78	608.23	607.25	607.35	607.24	607.57	0.429	0.070
100	1011.8	1011.3	1010.9	1010.8	1011.2	1011.2	0.393	0.038

TABLE: 4 and 5 Inter and intraday precision for Silibinin assay in the pharmaceutical dosage forms by the proposed RP- HPLC method.

CONCLUSIONS:

The proposed reverse phase high performance liquid chromatographic method has been evaluated over the linearity ,precision, accuracy and proved to be convenient and effective for the quality control of Silibinin in given application. The measured signals were shown to be precise, accurate, and linearity over the concentration range tested (20-100µg/ml) with correlation coefficient of 0.9997. Thus proposed method is rapid, selective, requires a simple sample preparation procedure, Moreover, the lower solvent composition leads to a cost effective and represents a good procedure of silbinin determination in bulk drug and in pharmaceutical dosage forms.

ACKNOWLEDGEMENT:

The authors are thankful to Sun pharmaceutical Pvt Ltd. Himachal Pradesh For providing the drug sample. And to the Principal, H.K.E.S s' college of pharmacy, Gulbarga for providing the facilities to carry out the research work.

REFERENCES:

1. Neil OMJ. The Merck Index: An Encyclopedia of Chemicals, Drug and Biologicals. 14th ed. Merck & Co. Inc., 2006; 4363.

2. Sweetman SC. Martindale: The Complete Drug Reference. 35th ed. Pharmaceutical Press, London. 2007; 788-90.

3. British pharmacopeia, Medicines and Health care products regulatory agency (MHRA), London, 2008, A IV 106.

4. Bertram G, Katzang. Basic and clinical pharmacology. 10th ed: 1993; 1056

5. Rickling B, Hans B, Kramarczyk R, Krumbiegel G, Weyhenmeyer R. Two high performance liquid chromatographic assay for the determination of free and total silibinin diasteromers in plasma using column switching with electrochemical detection and reversed- phase chromatography with ultraviolet detection. Journal of chromatography B: Biomedical applications. 1995; 670: 267-77.

6. Gunaratna C, Zhang T.Application of liquid chromatography- electrospray ionization-ion trap mass spectrometry to investigate the metabolism of silibinin in human liver microsomes. Journal of chromatography B.2003; 794: 303-10.

7. Radjabian T, Rezazadeh SH, and Fullah Huseinin H. Analysis of silymarin components in seed extracts of some milk thistle ecotypes from Iran by HPLC. Iranian journal of science and technology .2008; 32: 141-46.