RESEARCH PAPER

Received 3rd December, 1996.
Accepted 17th May, 1997.
Indian J. Pharm. Sci., 1997, 59(5) pp. 230-231

Spectrophotometric determination of Silymarin

A. RAJASEKARAN,* M. KUMAR, G. KRISHNAMOORTHY AND B. JAYAKAR
Periyar College of Pharmaceutical Sciences for girls, Trichy - 620 021.

A simple and sensitive method has been developed for the estimation of silymarin in pharmaceutical formulations. The method is based on the reaction of silymarin with diazotised sulphanilic acid in alkaline medium to form orange red colored chromogen which exhibits absorption maximum at 460 nm. The chromogen formed is stable and Beer's law is obeyed in the concentration range of 2-10 mcg/ml. The probable mechanism of reaction is the coupling of diazo group in diazotised sulphanilic acid at 8th position of flavone ring which imparts intense orange red colour in alkaline medium. The proposed method is precise, accurate and reproducible and it is extended to the analysis of silymarin in pharmaceutical solid formulations.

SILYMARIN[1-4] has been used for the treatment of hepatic disorders and disodium silibinin dihemisuccinate has been used in *Amanita phalloides* poisoning. Silymarin[5] is 2-[2,3-dihydro- 3 (4-hydroxy-3 methoxy phenyl) - 2 - (hydroxy methyl) -1, 4- benzodioxin -6-yl]- 2,3-dihydro-3,5,7 - trihydroxy-4H-1- benzopyran-4-one. Reported analytical methods include a TLC photodensitometric method[6], a colorimetric method[7], a potentiometric titration method[8], and HPLC method[9]. In the present communication, a new, simple, selective and sensitive spectrophotometric method is reported for the determination of silymarin in solid formulations.

EXPERIMENTAL

Materials

Pure silymarin was obtained from Micro Labs Ltd, Hosur, T.N, India. Sulphanilic acid of analytical grade was obtained from S.D. fine chemicals Ltd. Diazotised sulphanilic acid was prepared by dissolving 0.3 g of sulphanilic acid with a small quantity of 8% v/v HCl in a 25ml standard flask to which 1.5 ml of freshly prepared 5% sodium nitrite solution was added and the volume made upto 25 ml with 8% v/v HCl. Twenty percent sodium carbonate solution was prepared by dissolving 20 g of anhydrous sodium carbonate with distilled water to 100 ml in a standard flask. Tablets and capsules were obtained from Micro labs, Hosur and Ranbaxy labs, Dewas respectively. A systronics single beam UV-VIS Spectrophotometer was used for analysis.

Stock standard solution : Stock solution of pharmaceutical grade silymarin was prepared by dissolving 50 mg of the sample in 50 ml with ethanol in a standard flask.

Calibration curve : Aliquots of standard solution representing 2-10 mcg of silymarin were transfered into five separate 100 ml standard flasks which are numbered previously. One ml of freshly prepared diazotised sulphanilic acid followed by 0.5 ml of 20% sodium carbonate solution was added. A reaction time of 15 min was given for the completion of reaction and it was made upto 100 ml with distilled water. A blank also prepared in the same manner as described above.

*For correspondence

Table 1 : Analysis of Silymarin Tablets

Formulation	Labelled amount (mg)	Amount found		% Recovery[c]
		Proposed a method (mg)	HPLC[b]method (mg)	
Tablet-sample1	70	69.88	70.04	100.17
Tablet-sample 2	70	70.26	70.18	100.19
Capsule-sample 3	70	70.12	70.43	100.11

a Average of 3 determination

b Industrial method in which the mobile phase is the mixture of 5% v/v acetic acid and methanol (60:40) and the liquid chromatograph is equipped with 288 nm detector and 4.6 mm x 25 cm column that contains L-1 packing (Macherg-Nagel)

c Recovery of 0.5 mg, 1 mg and 1.5 mg added to pharmaceutical preparations

Procedure for Sample Solution : For analysis of tablets, 50 mg equivalent of the tablet/capsule content was transfered into a 50 ml standard flask and it is dissolved and made upto to 50 ml with ethanol. The solution was heated in a waterbath for 10 min and filtered to obtain a clear solution. Required quantity of the filterate was treated with 1 ml of freshly prepared diazotised sulphanilic acid solution and 0.5 ml of 20% sodium carbonate solution and allowed it to stand at room temperature for 15 min and the volume was made upto to 100 ml with distilled water and absorbance was measured at 460 nm against a reagent blank.

RESULT AND DISCUSSION

Silymarin reacts with diazotised sulphanilic acid to form an orange red colored chromogen which exhibits absorption maximum at 460 nm. This orange colored chromogen has been found to be stable for more than 6 h and Beer's law obeyed in the concentration range of 2-10 mcg/ml. (Slope = 0.04925, Intercept = 0.0175, r value = 0.9970 and molar absorptivity = $2.5411 \times 10^4 mol^{-1} cm^{-1}$). The optimum concentration of the reagent was found to be 1.2%. The percentage recovery ranged from 100.11 to 100.19 and is indicative of non-interference of excipients in the determination of the drug. The low value of % Relative standard deviation indicated that the proposed method is very accurate and precise. Hence the proposed method is quite simple, fast and economical so that it can be used in routine analysis of silymarin in its solid formulations.

ACKNOWLEDGEMENT

The authors are thankful to Micro Labs Ltd for providing gift sample of silymarin for our study and to the President and Correspondent of Periyar College of Pharmaceutical Sciences for girls, Trichy for providing facilities.

REFERENCES

1. Cavalieri, S., **Gazz Med Ital.**, 1974, 133, 628.
2. Salim, H. A. and Sarna, S., **Scand J Gasteroenterol.**, 1982, 17, 517.
3. Ferenci, P., **J Hepatol.**, 1989, 9, 105.
4. Hruby, K., **Hum Toxicol.**, 1983, 2, 183.
5. Budavari, S. The Merck Index, 11th Ed, Merck and Co Inc. Rahway, USA, 1989, 1350.
6. Guinea, M.C. and Pizarro, A., **An R. Acad Farm.**, 1987, 53, 413.
7. Folkman, A. and Kowalewska K., **Herba Pol.**, 1987, 33, 17.
8. Koerbl, H., Jiri, F., Jancik, D., Fedir, K., Jkaczk, F., Martin, J., Havel, U. and Karel, L., Czech CS 234387 (IPC GO1N - 027 126) 1986, Appl., 8314077, 1983, 3.
9. Mascheri, H., Kikuta, C. and Wehenmeyer, R., **J. of Chromatography.**, 1993, 16, 2777.