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NOTE

Spectrophotometric Determination of Silymarin

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Two simple spectrophotometric methods (A and B) have been developed for the determination of silymarin in pure form and in its pharmaceutical formulations. Method A is based on the formation of blood red coloured complex with ferric chloride and 1,10-phenanthroline having absorption maximum at 510 nm. In method B, silymarin forms blue coloured complex with Folin-cioCalteu (FC) reagent in the presence of sodium hydroxide exhibiting maximum absorption at 740 nm. The chromogens obey Beer's law in the concentration ranges of 2.5 to 25 μg/mL and 2.5 to 20 μg/mL for methods A and B respectively.

Silymarin (SLN) is chemically 2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one, 2-[(2R,3R)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxy methyl)-1,4-benzodioxin-6-yl] having hepatoprotective effects *via* several mechanisms including antioxidation[1] and inhibition of lipid peroxidation[2]. Very few analytical methods have been reported for the determination of silymarin which include HPLC,[3] TLC-Photodensitometry[4], colorimetry[5] and TLC-spectrophotometry[6] methods. The authors have developed two simple, sensitive and reproducible spectrophotometric methods (A and B) for the determination of silymarin. In method A, silymarin reacts with $FeCl_3$ and 1,10-phenanthroline and forms a blood red coloured complex having absorption maximum at 510 nm, whereas in method B it reacts with Folin-ciocalteu (FC) reagent in presence of sodium hydroxide to form a blue coloured chromogen exhibiting maximum absorption at 740 nm.

All the chemicals used were of analytical grade. Ferric chloride (Loba Chemie) (0.0033 M), 1,10-phenanthroline (Loba Chemie) (0.1 M), FC reagent (Loba Chemie) (1 N), sodium hydroxide (Qualigens) (1 N) were prepared in distilled water. The commercially available tablets and capsules were procured from the local market. Spectral and absorbance measurements were made on Systronics UV/Vis spectrophotometer model 117 with 10 mm matched quartz cells.

Standard and Sample Solutions: About 100 mg of silymarin (pure or formulation) was accurately weighed and dissolved in 100 mL of methanol for

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method A. For method B, it was first dissolved in 2.5 mL of 1 N NaOH and then made up to 100 mL with distilled water. The above stock solutions were further diluted with distilled water to get a working standard solution of 100 μg/mL for both methods.

Method A: Aliquots of working standard solution of silymarin ranging from 0.25 to 2.5 mL (1 mL = 100 μg) were transferred in to a series of 10 mL volumetric flasks. To that 0.5 mL of $FeCl_3$ (0.0033 M) and 2.5 mL of 1,10-phenanthroline (0.1 M) were successively added and the final volume was brought to 10 mL with distilled water. The absorbance of the blood red coloured species formed was measured at 510 nm against reagent blank and the amount of silymarin present in the sample solution was computed from its calibration curve.

Method B: To a series of 10 mL graduated test tubes aliquot sample of working standard solutions of silymarin ranging from 0.25 to 2.0 mL (1 mL = 100 μg) were transferred and to that 2.0 mL of NaOH (1 N) and 1 mL of FC reagent (1 N) were added and mixed well. The total volume of each tube was made up to 10 mL with distilled water. The absorbance of blue coloured chromogen formed was measured at 740 nm against reagent blank. The amount of silymarin present in the sample solution was computed from its calibration curve.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, per cent relative standard deviation (calculated from the eight measurements containing 3/4th of the amount of the upper Beer's law limits of silymarin), % range of error (0.05 to 0.01 confidence limits) were calculated for both the methods and the results are summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

Parameters	Method A	Method B
Beer's law limit (μg/mL)	2.5–25	2.5–20
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.02941	0.02491
Molar extinction coefficient (1 mole^{-1} cm^{-1})	1.6403×10^4	1.9362×10^4
% Relative standard deviation	0.4729	0.6542
% Range of error		
0.05 Confidence limits	±0.3960	±0.5478
0.01 Confidence limits	±0.5859	±0.8105
Correlation coefficient	0.9997	0.9998
Regression equation (Y*)		
Slope (a)	0.0032	0.0038
Intercept (b)	0.0245	0.0137

$Y^* = b + aC$, where 'C' is concentration in μg/mL and Y is absorbance unit.

The values obtained for the determination of silymarin in several pharmaceutical formulations (Tablets and capsules) by the proposed and reported methods are compared in Table-2. To evaluate the validity and reproducibility of the

methods, known amount of pure drug was added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by proposed methods and the per cent recoveries are given in Table-2. Interference studies revealed that the common excipients and other additives usually present in the dosage form such as parabens, lactose, sucrose, starch, sodium benzoate, sodium phosphate, calcium gluconate, gelatin, talc, magnesium stearate did not interfere in the proposed methods.

TABLE-2
ESTIMATION OF SILYMARIN IN PHARMACEUTICAL FORMULATIONS

Sample	Labelled amount (mg)	Amount obtained (mg)			Per cent recovery of the proposed method	
		Reported method[5]	Proposed method			
			A	B	A	B
1.	140	139.4	139.7	139.5	99.78	99.64
2.	140	139.2	139.8	139.7	99.85	99.78
3.	70	69.5	69.6	69.8	99.42	99.71

The blood red coloured complex formed in method A may be due to the fact that each of the two nitrogen atoms in 1,10-phenanthroline has an unshared pair of electrons that can be shared with Fe(II) ion [formed by reaction of SLN with Fe(III)]. Three such molecules of 1,10-phenanthroline attaches themselves to the metallic ion to form ferrion complex. In method B, the blue coloured complex formed may be due to the reduction of 1, 2 or 3 oxygen atom of FC reagent and the formation of molybdenum blue or tungsten blue. In conclusion the proposed methods are simple, sensitive and accurate and can be used for the routine determination of SLN in bulk as well as in its pharmaceutical preparations.

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REFERENCES

1. H. Wagner, in: J.L. Beal and E. Reinhard (Eds.), Natural Products as Medicinal Agents, Hippokrates-Verlag, Stuttgart (1981).
2. E. Bosisio, C. Benelli and O. Pirola, *Pharmacol. Res.*, **25**, 147 (1992).
3. M. Steinigen, *Pharm. Ztg.*, **128**, 783 (1983).
4. M.C. Guinea-Lopez and A. Pizzaro-Sanz, *An. R. Acad. Farm.*, **53**, 413 (1987).
5. A. Folkman and K. Kowalewska, *Herb. Pol.*, **33**, 17 (1987).
6. M. Simova and T. Pangarova, *Farmatsiya*, **30**, 39 (1980).

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