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Validated method for Silymarin by Spectrophotometry in Bulk drug and Pharmaceutical formulations

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Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of silymarin-flavonolignans (a compound from *Silybum marianum*) in pure form and in pharmaceutical formulations is described. The method is based on the simple solubility of silymarin in methanol. The absorbance maximum of silymarin measured at wavelength 287nm. The drug obeys Beer's Law in the concentration range 6-16 µg/ml employed for this method. Accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The method is found to be rapid, precise and accurate and can easily be employed in the laboratory for the routine estimation of drug and it's extended to the analysis of silymarin in pharmaceutical formulations.

Key words: Silymarin, Validation parameters, Spectrophotometric Estimation, UV-Visible spectrophotometer

Introduction

Silymarin i.e. 3, 5, 7-trihydroxy-2-[3-(4-hydroxy-3-methoxy phenyl)-2-(hydroxy-methyl)-1, 4-benzodioxan-6-yl]-4-chromanone (CAS: 22888-70-6) is a free radical scavenger and an important antihepatotoxic drug. It has been used for the treatment of liver diseases of different etiology due to its hepatoprotective activity [1-4] that is considered to involve antioxidation, inhibition of lipid peroxidation and the membrane stabilizing effects. Thus, its broad pharmacological effects have generated a keen interest in the drug and therefore, the analysis of its dosage form is very important. The drug is officially listed in Martindale the Extra Pharmacopoeia [5]. The assay of the drug in pure and dosage forms is only listed in the

monograph of The Italian Pharmacopoeia which describes UV-vis spectrophotometric method [6] and therefore requires much more investigation. The different analytical methods that have been reported for its determination include high performance liquid chromatography [7-11], thin layer chromatography [12-13], and UV spectrophotometry [14]. Few spectrophotometric methods have been reported for the assay of silymarin based on the formation of colored complex of the drug with [15-19]. The literature is still poor in analytical assay methods based on flavonoid content for the determination of silymarin in dosage forms. Advantage of developed method over existing method is Simple and fast methods because some experimental steps such as filtration, extraction, color development etc. are avoided prior to absorbance measurements.

Materials and Methods

Reference standard of silymarin were purchase from Sigma, USA. Methanol and chloroform were of AR grade. SILYBON-70 tablet (micro labs limited), LIMARIN-70 capsule (serum international) were purchased from the market. Simandzu uv-1700 & 1800 uv/vis spectrophotometers with 10 mm matched quartz cells was used for experiment. Absorption and overlain spectra were recorded over the wavelength range of 200-400 nm, using 1cm quartz cells at a scan speed medium and fixed slit width of 1.0 nm.

Experimental work

Preparation of standard stock solution:

Standard silymarin 100mg was dissolved in 100ml methanol to make 1000µg/ml stock solution.

Procedure for the Determination of Silymarin standard:

From the above solution aliquots of 0.6 ml, 0.8 ml, 1 ml, 1.2 ml, 1.4 ml, 1.6 ml were taken in a separate 10 ml volumetric flasks then make up the volume with methanol.

Procedure for the Determination of Silymarin in Drug Formulations:

An amount of the powdered tablet and capsule equivalent to 100 mg of silymarin was weighed accurately, and extracted into 3 × 20 ml portions of chloroform with shaking. The residue was filtered using Whatmann No. 42 filter paper. The filtrate was evaporated to dryness under vacuum and the remaining drug was dissolved in methanol and diluted to 100 ml.

Results and Discussion

Absorption maxima of silymarin were detected at 287 nm and overlay spectra of concentration range 6-16 (µg/ml) was recorded (Figure 1). Absorbance at different concentration showed in (Table 1). Linearity graph was showed in (Figure 2). Optical characteristics data showed in (Table 2), Repeatability data for analysis of silymarin in (Table 3). Reproducibility data for analysis of silymarin were in (Table 4). The absorptivity coefficient of drug was determined by using equation A=abc. Recovery studies were done so as to check the accuracy of the method which was mentioned in (Table 5). Results of analysis of silymarin in marketed formulation were showed in (Table 6).

Figure 1: Overlay spectrum of standard silymarin

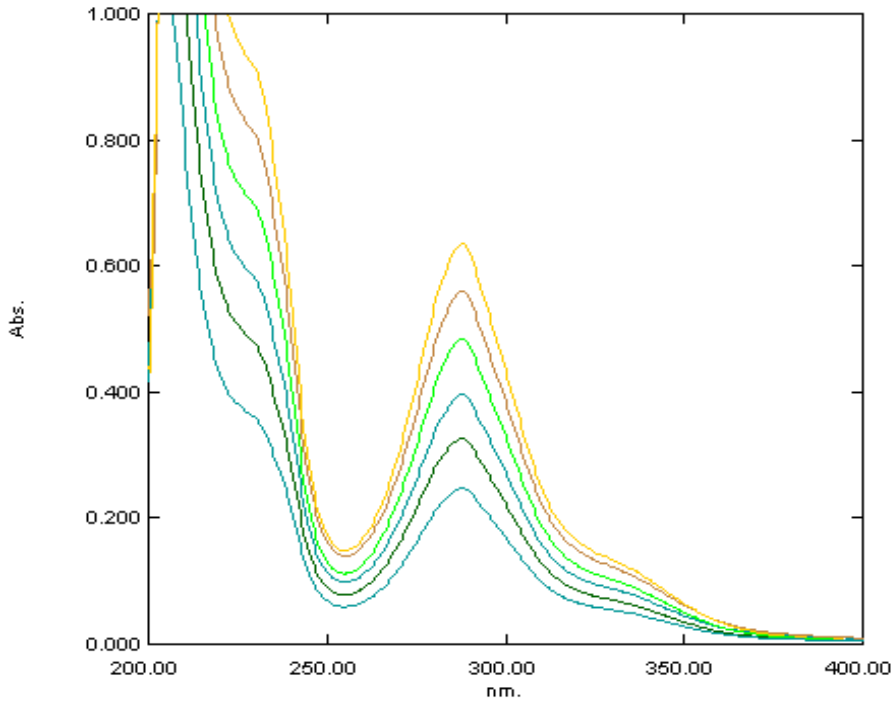

Table 1: Calibration data for analysis of Silymarin at 287nm

Concentration (µg/ml)	Absorbance Mean ± S.D.
6	0.247±0.003
8	0.326±0.002
10	0.398±0.002
12	0.483±0.001
14	0.559±0.001
16	0.634±0.001

(n=3)

Figure 2: Results of linearity graph

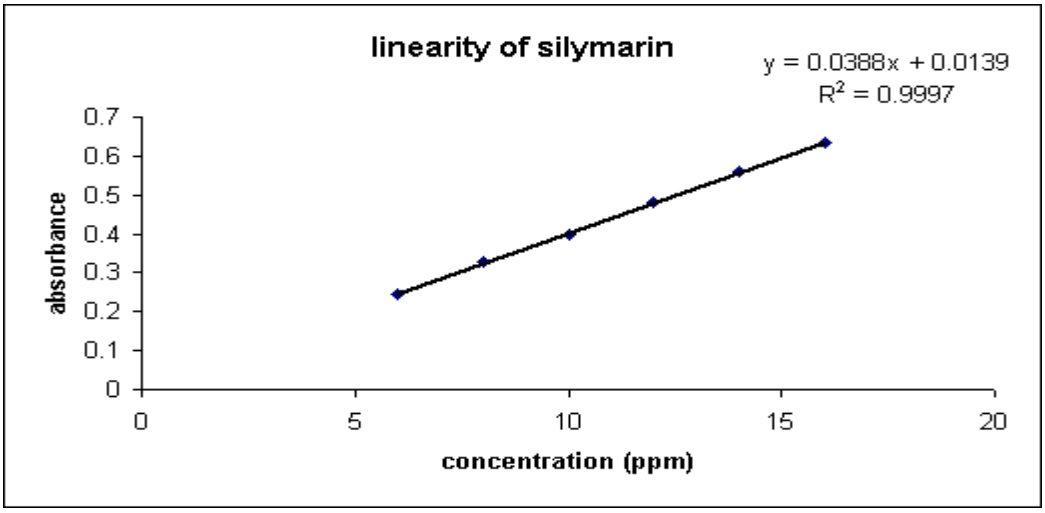

Table 2: Optical characteristics

Parameters	Value
λmax (nm)	287
Beer's law limit (µg/ml)	6-16
$A^{1\%}_{1cm}$	402.53
Correlation coefficient (r^2)	0.9997
Regression Equation (y=a+bc)	Y=0.0388x-0.0139
Intercept (a)	0.0139
Slope (c)	0.0338
Limit of quantification (µg/ml)	0.9
Limit of detection (µg/ml)	0.29

Table 3: Repeatability data for analysis of silymarin

Concentration (µg/ml)	Intraday	Interday
	Absorbance (Mean ± S.D.)	Absorbance (Mean ± SD)
8	0.325 ± 0.0037	0.327 ± 0.0031
12	0.485 ± 0.0053	0.483 ± 0.0049
16	0.636 ± 0.0048	0.639 ± 0.0053

Table 4: Reproducibility data for analysis of silymarin

Concentration (µg/ml)	Absorbance	
	UV 1700 (Mean ± S.D.)	UV 1800 (Mean ± S.D.)
8	0.324±0.002	0.325±0.003
12	0.486±0.002	0.490±0.002
16	0.634±0.003	0.638±0.003

Table 5: Accuracy data for analysis of silymarin (Recovery studies)

Amount of sample taken (µg/ml) (A)	Amount of standard added (µg/ml) (B)	Total Amount (A + B) (µg/ml)	Total amount found (µg/ml)	% Recovery
10	8	18	17.825	99.03
10	10	20	20.337	101.69
10	12	22	22.129	100.59

Table 6: Analysis of marketed formulations

Formulation	%Amount Found ± SD
SILYBON-70	99.84±0.0039
LIMARIN-70	101.97±0.0031

Conclusion

The developed method is useful due to high tolerance limit for common Excipients found in drug formulations. The developed method does not require any elaborate treatment of the drug and tedious extraction procedure for the formation of colored chromophore of the drug with the interacting reagents also. The method which we developed for the validation was studied at 287 nm wavelength. Accuracy and reproducibility was determined by calculating the recovery study that was close to 100%. The developed method is simple, precise, accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis.

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