

Method development and validation of silymarin in bulk and pharmaceutical formulation by UV- spectrophotometric area under curve method (AUC)

Sandip D.Firke*¹, Nikhil Patel², Sanjay J.Surana¹, Sunjaykumar B.Bari²

¹R.C.Patel Institute of Pharmaceutical Education and Research, Shirpur, (INDIA)

²H.R.Patel Institute of Pharmaceutical Education and Research, Shirpur, (INDIA)

E-mail : sandipfirke@rediffmail.com

ABSTRACT

A new, simple, rapid and novel UV- spectrophotometric method has been developed for estimation of Silymarin (SLY) by using Area under Curve method (AUC). The method involved measurement of absorbance at wavelengths 288 nm for measurement of area under curve in the wavelength range 265.60 – 311.80 nm for SLY. Linearity was observed in the concentration range of 5-25 µg/ml, with correlation coefficient value 0.9985. The proposed method was recommended for routine analysis since it is a rapid, simple, accurate, sensitive and specific. The results obtained are reproducible with a coefficient of variation less than 2%. This method was validated for precision, reproducibility, linearity and accuracy as per ICH guidelines.
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KEYWORDS

Silymarin;
Area under curve method;
UV- spectrophotometry;
Method validation;
ICH guidelines.

INTRODUCTION

Silymarin (Figure 1), is 3, 5, 7-trihydroxy-2-[3-(4-hydroxy-3-methoxy phenyl)-2-(hydroxy-methyl)-1,4-benzodioxan-6-yl]-4-chromanone. Silymarin is natural flavonolignans, isolated from the milk thistle plant, silybum marianum^[1]. Clinically, silymarin is often used to treat acute hepatitis, chronic hepatitis and persistent hepatitis. Recently silymarin/silybin received attention due to its alternative beneficial activities like anticancer and chemopreventive actions, as well as hypocholesterolemic, cardioprotective, neuroactive and neuroprotective activities. But due to its hepatoprotective activity that is considered to involve antioxidation, inhibition of lipid peroxidation and the membrane stabilizing effects^[2,3]. The different analytical

methods that have been reported for its determination include high performance liquid chromatography, thin layer chromatography, and UV spectrophotometry^[4-7]. Advantage of developed method over existing method is simple and rapid, accurate method for determination of Silymarin by UV-Spectrophotometric area curve (AUC) technique.

MATERIAL AND METHODS

Instrumentation

A Shimadzu UV-VIS Spectrophotometer 1700 (UV-Probe 2.21) with 1.0 cm matched quartz cell was used for all spectral measurements.

Chemicals and reagents

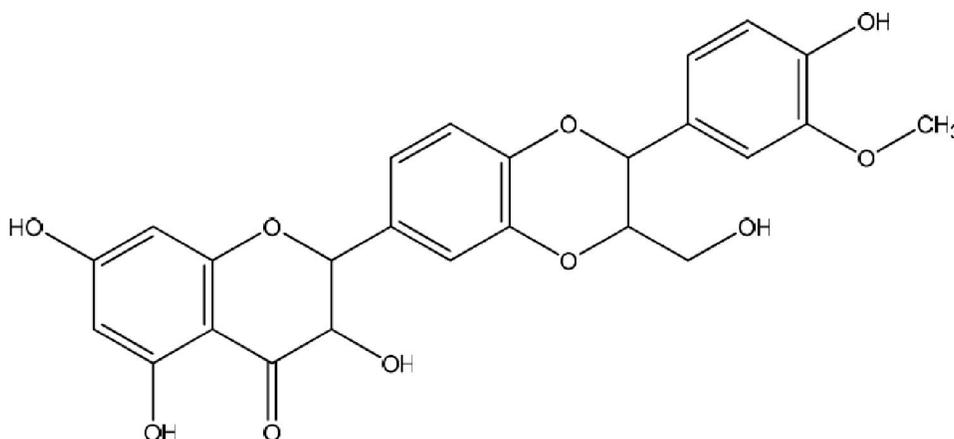


Figure 1 : Structure of silymarin

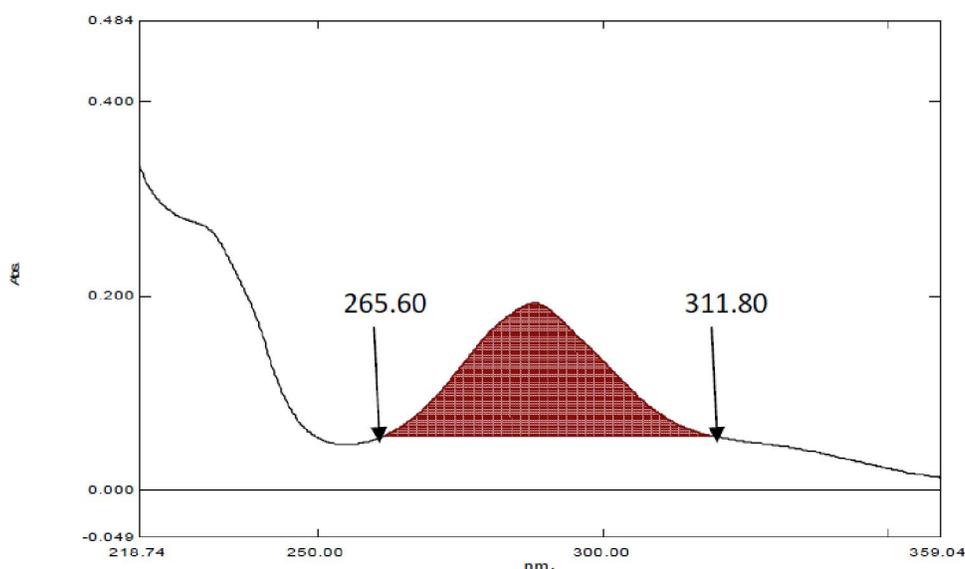


Figure 2 : UV-spectrum of silymarin

TABLE 1 : Results of analysis of SLY in bulk material

Drug	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Amount found
SLY	25	24.96 \pm 0.28	99.84 \pm 0.85
	25	24.68 \pm 0.34	98.72 \pm 0.91
	25	25.1 \pm 0.32	100.4 \pm 0.93
	25	25.05 \pm 0.37	100.2 \pm 0.86
	25	24.98 \pm 0.29	99.42 \pm 0.87
	25	25.03 \pm 0.35	100.12 \pm 0.96
	Mean \pm SD	24.91 \pm 0.32	99.78 \pm 1.06
% RSD	0.19	0.19	

Standard gift samples of SLY were procured from Microlab Ltd. Bangalore. Marketed formulation containing SLY were purchase from local market having brand name Silybon 70, Microlab Ltd. Bangalore.

TABLE 2 : Results of analysis of SLY in tablet formulation
Brand Name: Silybon 70 Mfg. By: Microlab Ltd

Drug	Label claim (mg)	Amount found (mg)	% Amount found
SLY	70	70.03 \pm 0.56	100.04 \pm 0.95
	70	69.68 \pm 0.63	99.54 \pm 0.81
	70	70.08 \pm 0.62	100.1 \pm 0.83
	70	69.9 \pm 0.63	99.85 \pm 0.92
	70	69.83 \pm 0.64	99.75 \pm 0.96
	70	69.94 \pm 0.69	99.91 \pm 0.88
	Mean \pm SD	69.91 \pm 0.62	99.86 \pm 0.89
% RSD	0.681	0.681	

Preparation of standard stock solution (100 $\mu\text{g/ml}$)

Accurately weighed 10 mg of SLY transferred to 100ml volumetric flasks. It was dissolved in 100ml of

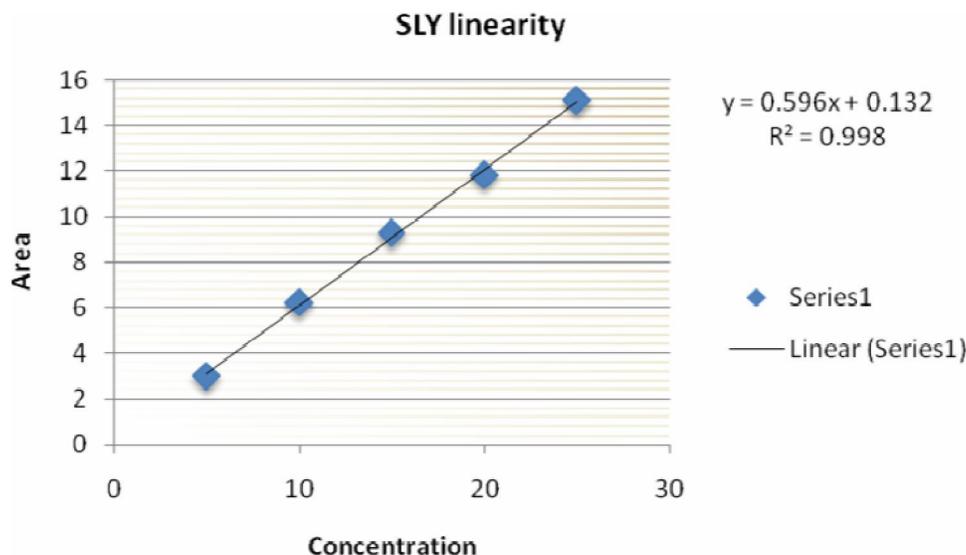


TABLE 3 : Results of recovery studies

Drug	Initial amount ($\mu\text{g/ml}$)	Amount added ($\mu\text{g/ml}$)	Amount recovered ($\mu\text{g/ml}$, n = 3)	% Recovered	% RSD
SLY	5	4	8.92	99.92	0.0145
	5	5	8.91	99.94	0.0152
	5	6	8.98	99.95	0.0159

TABLE 4 : Results of repeatability studies

Drug	Amount taken ($\mu\text{g/ml}$)	Amount found	% Amount found
SLY	15	15.32 \pm 0.081	102.12 \pm 0.61
	15	15.34 \pm 0.076	100.72 \pm 0.63
	15	15.36 \pm 0.079	100.29 \pm 0.67
	15	15.34 \pm 0.080	100.42 \pm 0.64
	15	15.33 \pm 0.084	101.03 \pm 0.68
	15	15.39 \pm 0.072	102.12 \pm 0.61
	Mean \pm SD	15.34 \pm 0.081	101.11 \pm 0.65
% RSD	0.64	0.64	

TABLE 5 : Results of intra-day and inter-day precision study

Drug	Concentration ($\mu\text{g/ml}$)	Intra-day (n = 3)	%RSD	Inter-day (n = 3)	%RSD
SLY	10	6.22	0.056	6.24	0.065771
	15	9.283	0.037	9.281	0.028278
	20	11.827	0.030	11.741	0.181378

TABLE 6 : Results of ruggedness study

Drug	Analyst -I		Analyst -II	
	% Amount Found \pm SD (n = 3)	% RSD	% Amount Found \pm SD (n = 3)	% RSD
SLY	101.2 \pm 0.12	1.30	100.5 \pm 0.01	1.35

methanol and shaken manually for 10 min. The volume was making up to the mark with distilled water to obtain final concentration 100 $\mu\text{g/ml}$.

Determination of λ_{max} and selection of wavelengths

From the stock standard solution, 1.5ml of SLY was transferred in to 10ml volumetric flask and the volume was adjusted to the mark to obtain strength 30 $\mu\text{g/ml}$. The solution was scanned in the UV range 200-400 nm. SLY showed maximum absorbance at 288 nm. Two wavelengths 265.60 – 311.80 nm were selected to record the AUC (Figure 2).

Analysis of bulk material

Accurately weighed quantity of 10mg SLY were taken in 100ml volumetric flask and dissolved in 100ml methanol by vigorous shaking. The aliquot portions of this stock solution were further diluted with methanol to get final concentration of about 100 $\mu\text{g/ml}$ of SLY and the AUC was measured at selected wavelengths and results are shown in TABLE 1.

Analysis of tablet formulation

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent

TABLE 7 : Summary of validation parameters

Parameter	Result
λ max	288
Linearity range	5-30 $\mu\text{g/ml}$
Regression Equation ($y= mx+c$)	$y=0.5963x+0.1323$
Correlation Coefficient (R^2)	0.9985
Precision (% R.S.D)	
Repeatability (n= 6)	0.64
Intraday (n=3)	0.041
Interday (n=3)	0.091
Accuracy (Mean % Recovery)	
LOD	0.018
LOQ	0.056

to 10 mg of SLY was accurately weighed, transferred to a 100 ml of volumetric flask and diluted up to mark with methanol. The solution was filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10 $\mu\text{g/ml}$ solution with same solvent and subjected for UV analysis. The results of tablet analysis are shown in TABLE 2.

Validation

The proposed method was validated as per the ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantification and ruggedness.

Linearity

From the stock standard solution, aliquots portion in the range 0.5-2.5ml were transferred to five separate 10ml volumetric flasks. The volume was adjusted to the mark with same solvent to obtain concentrations in the range of 5-25 $\mu\text{g/ml}$. The AUC for each concentration in between selected wavelengths was recorded. (Figure 3)

Accuracy and precision

To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, area under curve was recorded at selected wavelengths. (TABLE 4)

Precision of the method is studied as repeatability, intra-day and inter-day precision. Repeatability was determined by analyzing SLY (15 $\mu\text{g/ml}$) for six times.

(TABLE 4)

Intra-day precision was determined by analyzing the 5-25 $\mu\text{g/ml}$ of SLY for three times in the same day. Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days. (TABLE 5)

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by the use of the following equation,

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Where, SD = Standard deviation of peak areas of the drug (n=3) and S = Slope of the corresponding calibration plot. LOD and LOQ were found to be 0.018 μg and 0.056 μg , respectively.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions. (TABLE 6)

RESULTS AND DISCUSSION

An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of SLY in bulk and tablet dosage form. The generated regression equation was,

$$\int_{311.80}^{265.60} Ad \lambda = 0.5963C + 0.1323 (R^2 = 0.9985)$$

Where, $\int_{311.80}^{265.60} Ad \lambda$ is Area under curve between 265.30 to 311.80n, C is concentration and R is correlation coefficient. The R^2 value as 0.9985 indicates that developed method was linear. The method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed good recoveries (100.72 % to 101.11 %). Hence, it can be said that this method was accurate. The LOD and LOQ

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were calculated as 0.0181g/ml and 0.0561g/ml, respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the SLY in tablet dosage form. (TABLE 7)

CONCLUSION

The proposed method was accurate, precise and consistent for the determination of SLY in tablet dosage form. This method was validated as per ICH guidelines. Results suggest that this method can be used for routine estimation of SLY in bulk and pharmaceutical dosage forms.

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