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Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF LAMIVUDINE AND SILYMARIN IN MIXTURE

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ABSTRACT

A novel, simple, rapid and sensitive spectrophotometric method has been developed for simultaneous estimation of Lamivudine and Silymarin. The method employs formation and solving of simultaneous equation using 270.9 nm and 326.4 nm as two analytical wavelengths. Both the drugs obey Beer's Law in the concentration ranges 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 drugs.

Keywords: Lamivudine, Silymarin, Spectrophotometric estimation, UV-Visible spectrophotometer.

INTRODUCTION

Lamivudine (3TC) is a cytosine analog with potent activity against human immunodeficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity. Lamivudine is used in treatment of HBV infections and it has strongly been recommended for the treatment of HIV infections in combination with other antiviral drugs[1]. Silymarin is a group of flavonoid compounds obtained from *Silybum marianum,* the chemically defined mixture of three isomers, silibinin (major isomer), silycristin and silydianin. The drug is a unique hepatoprotective agent that has a positive effect on metabolism and physiology of liver cells, influencing their regenerative capacity due to two main actions: antioxidant and protein restoring activities. The drug also prevents toxic and foreign substances from penetrating liver cells by stabilizing the outer membranes of liver cells[2]. Clinical efficacy and reduced dosage regimen can be obtained with a dual therapy of antiviral and hepatoprotectant agents.

Literature survey reveals several methods that have been used for the quantitative determination of the two drugs individually, such as for Lamivudine(LAMI) UV-spectrophotometry, HPLC, estimation in pharmaceutical dosage forms by UV-spectrophotometry and RP- HPLC and in plasma determination of LAMI by HPLC[3-5]. For Silymarin (SILY) direct spectrometric assay, colorimetric estimation, in body fluids by TLC with fluorometric detection and HPLC with UV detection[6-9].

MATERIALS

Reference standard of LAMI and SILY were procured from Shreeyam Labs, Ahmadabad and Aurobindo Pharma, Hyderabad respectively. Methanol (Qualigens, Mumbai), disodium hydrogen orthophosphate, potassium dihydrogen phosphate, sodium chloride were of AR grade and distilled water were used in the present study. A GBC Cintra 10 uv/vis spectrophotometer 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 of 1200.00 and fixed slit width of 2.0 nm.

EXPERIMENTAL

Preparation of standard stock solution

Stock solutions (100µg/ml) of SILY and LAMI were prepared by dissolving separately 10 mg of drug in 3 ml of methanol and making up the volume with phosphate buffer saline (pH 7.4). The stock solution were suitably diluted to produce solution of concentration 10 µg/ml, these working solutions were scanned in the entire UV range (200-400 nm) to determine the λ_{max}. Absorption maxima of SILY and LAMI were detected at 270.9 nm (λ1) and 326.4 nm (λ2), respectively and overlain spectra was recorded. A series of standard dilutions of each drug were prepared having concentration range of 2-20 µg/ml. Both LAMI and SILY showed linearity with absorbance in the range 2-20 µg/ml at their respective maxima. The absorbances were measured at 270.9 nm and 326.4 nm and calibration curves were plotted at these wavelengths.

Recovery studies

Recovery studies were done so as to check the accuracy of the method. The accuracy of the method was assessed by taking known amounts of LAMI and SILY in standard mixture solution and absorbance were determined at 270.9 nm and 326.4 nm. Concentration of the drugs in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates.

Sensitivity

Sensitivity of the method for both drugs was individually determined by calculating Sandell's sensitivity (µg/cm^2/0.001 Abs unit), which can be defined as the smallest weight of the substance that can be detected in column of unit cross section.

RESULTS

Absorption maxima

Absorption maxima of SILY and LAMI were detected at 270.9 nm (λ1) and 326.4 nm (λ2), respectively and overlain spectra was recorded (Fig. 1).

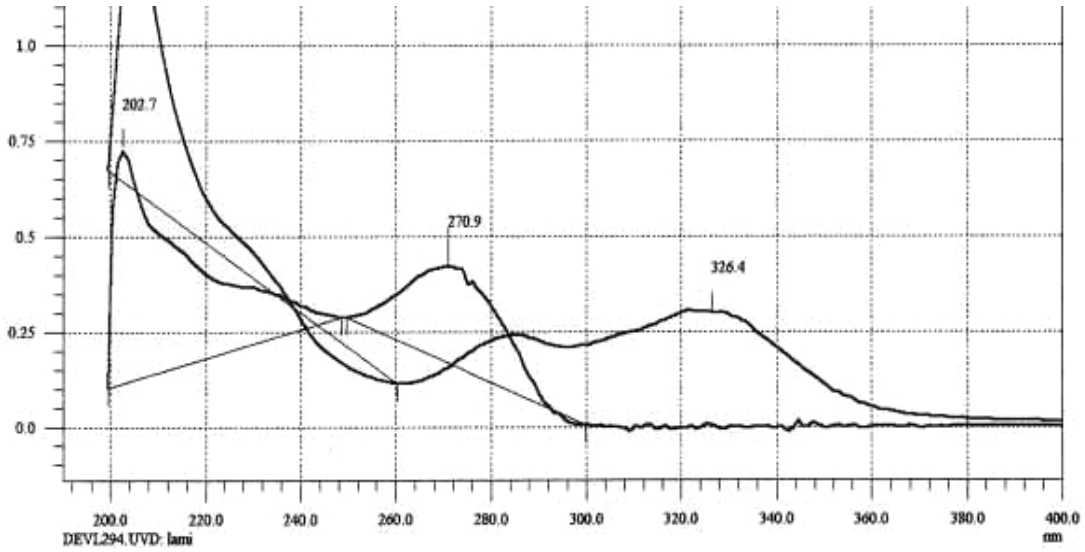

Fig. 1 : Overlain spectra of LAMI and SILY

Absortivity coefficients

The absorptivity coefficients of the two drugs were determined by using Beer's law:

A = E (1%, 1cm) CL

The absorptivity coefficients of LAMI at 270.9 nm and 326.4 nm were 0.0380± $2.5539X10^{-3}$ and 0 and for SILY were 0.0164± $1.900X10^{-3}$ and 0.0313± $1.8196X10^{-3}$. The observations are presented in Table 1.The optical characteristics and regression values for the calibration curve are presented in Table 2.

Table 1 : Absorptivity values for LAMI and SILY

Concentration(µg/mL)		Absorptivity		
		270.9 nm		326.4 nm
LAMI	**SILY**	**LAMI**	**SILY**	**SILY**
2	2	0.0315	0.0115	0.0360
4	4	0.0361	0.0149	0.0327
6	6	0.0377	0.0161	0.0316
8	8	0.0384	0.0167	0.0311
10	10	0.0389	0.0170	0.0308
12	12	0.0392	0.0172	0.0305
14	14	0.0394	0.0174	0.0304
16	16	0.0396	0.0175	0.0303
18	18	0.0397	0.0176	0.0301
20	20	0.0397	0.0176	0.0301
MEAN		0.0380	0.0164	0.0313
SD		$2.5539X10^{-3}$	$1.900X10^{-3}$	$1.8196X10^{-3}$

(n=3)

Table 2: Optical characteristics

S.No.	Characteristics	LAMI	SILY
1.	λ_{max} (nm)	270.9	326.4
2.	Beer's Law Limit (µg/mL)	2-20	2-20
3.	Molar Absorptivity (L/mol/cm)	$8.7321x10^3$	$1.5150x10^4$
4.	Sandell's Sensitivity	0.0263	0.0318
5.	Regression Equation	y = 0.0408x + 0.0185	y = 0.0295x + 0.013
i	Slope	0.0408	0.0295
ii	Intercept	0.0185	0.0130
iii	r^2	0.9992	0.9998

n=3

Partial simultaneous equation method

A set of two simultaneous equations were framed using the mean absorptivity coefficients values, as given below:-

At λ1 (270.9 nm) $A1 = ax_1bC_x + ay_1bC_y$

$A1 = 0.038C_x + 0.0164C_y$

At λ2 (326.4 nm) $A2 = ax_2bC_x + ay_2bC_y$

$A2 = 0.0313C_y$

Where A1 and A2 are absorbances at 270.9 nm and 326.4 nm, C_x and C_y are the concentration of LAMI and SILY respectively (µg/ml).

Recovery studies and sensitivity

Recovery studies were done so as to check the accuracy of the method. The analysis was done in a set of 3 replicates and results are summarized in Table 3.Recovery was close to 100% stating the accuracy and reproducibility of the method. Sandell's sensitivity for LAMI was 0.0263 and for SILY was 0.0318.

Table 3: Recovery Studies

Drug in standard mixture solution(µg/ml)		% Recovery ± SD		Coefficient of variance %	
LAMI	**SILY**	**LAMI**	**SILY**	**LAMI**	**SILY**
4	4	99.07±0.78	100.83±0.35	0.7873	0.3471
6	6	102.24±0.80	101.58±1.07	0.7824	1.0533
8	8	99.63±0.66	99.03±0.78	0.6624	0.7876

(n=3)

DISCUSSION

The validation parameters were studied at both the wavelengths for the method. Accuracy and reproducibility was determined by calculating the recovery that was close to 100%. Precision was calculated as repeatability (SD and %CV). The proposed 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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