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NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF LECITHIN AND SILYMARIN

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Keywords:

Lecithin and Silymarin, Method development, Validation, Simultaneous estimation, RP- HPLC

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ABSTRACT: Objective: A simple, rapid, accurate, precise and reproducible validated RP- HPLC method was developed for the determination of Lecithin and Silymarin in bulk and tablet dosage forms. **Methods:** A simple double beam UV spectrophotometric method has been developed and validated with different parameters such as linearity, precision, repeatability, the limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. **Results:** The quantification was carried out using Symmetry PremsilC_{18} (250mm x 4.6mm, 5µm) column run in an isocratic way using mobile phase comprising of acetonitrile: water (0.1% OPA with PH 3) 70:30 v/v and a detection wavelength of 236 nm and injection volume of 20µL, with a flow rate of 1ml/min. The retention times of Lecithin and Silymarin were found to be 6.58 min and 3.616min. The method was validated in terms of linearity, precision, accuracy, LOD, LOQ, and robustness in accordance with ICH guidelines. The assay of the proposed method was found to be 99-101%. The recovery studies were also carried out, and the mean % recovery was found to be 99-101%. The % RSD from reproducibility was found to be <2%. **Conclusion:** The proposed method was statistically evaluated and can be applied for routine quality control analysis of Lecithin and Silymarin in bulk and in the tablet dosage form.

INTRODUCTION: Lecithin (LCN) is chemically (2-nonanoyloxy – 3 – octadeca - 9, 12 - dienoyloxy propoxy) - [2-(trimethyl - azaniumyl) ethyl] phosphinate **Fig. 1** and Lecithin is used for treating memory disorders such as dementia and Alzheimer's disease. It is also used for treating gallbladder disease, liver disease, certain types of depression, high cholesterol, anxiety, and a skin disease called eczema [1]. Silymarin (SYL) is chemically 3, 5, 7- trihydroxy-2- [3-(4-hydroxy-3-methoxy phenyl) -2-(hydroxyl methyl) -2, 3-dihydro-1, 4-benzodioxin-6-yl]-2, 3-dihydro-chromen-4-one **Fig. 1** and Silymarin is a natural polyphenolic flavonoid derived from milk thistle seeds. It has long been used as a traditional herbal hepatoprotective agent. It is one of the most widely used flavonoids compounds because of its extensive therapeutic properties [2].

Recently, there has been a growing interest in using herbal medicines to treat skin disease [3-5]. The literature review reveals that no HPLC methods for the estimation of Lecithin and Silymarin in tablet dosage. Hence the present study develops an accurate and reliable RP- HPLC method for simultaneous estimation of Lecithin and Silymarin in tablet dosage form as per ICH norm.

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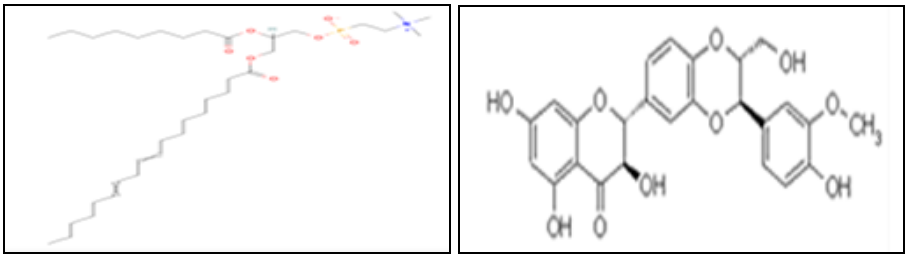

FIG. 1: STRUCTURE OF LECITHIN AND SILYMARIN

MATERIALS AND METHODS:

Materials and Reagents: The drug analysis was carried out on Agilent (S. K.) Gradient System UV Detector. Equipped with Reverse Phase (Grace) C18 column (4.6mm x 250mm; 5µm), an SP930D pump, a 20µl injection loop, and UV730D Absorbance detector and running autochro-3000 software.

The API of both drugs Lecithin and Silymarin procured from R. S. I. T. C Jalgaon, India. Orthophosphoric acid (OPA) (HPLC grade Avantor Performance material India Ltd. Thane, (Maharashtr). Methanol, acetonitrile, water (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), 0.45µm filter (Millipore, Bangalore). A combination of Lecithin 525mg and Silymarin 140mg in tablet formulation was procured from a local pharmacy (Shamliv Tab, Shalman pharmaceuticals Pvt. Ltd. Gujarat.

Chromatographic Conditions: The analysis of the drug was carried out on Agilent (S.K.) Gradient System UV Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 100mm; 2.5µm), a SP930Dpump, a 20µl injection loop, and UV730D (DAD) Absorbance detector and running Chemstation software. Temperature ambient 26 ^{0}C; sample size 20 µl; mobile phase MeOH: Water (0.1% with OPA) 70: 30; detection wavelength 236 nm; flow rate 1ml/min.

Preparation of Standard Stock Solution:

Preparation of Std. Silymarin Solution: (Stock - I): An accurately weighed quantity, 10 mg of Silymarin (SYL) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1000 µg/ml. From the freshly prepared standard stock solution (1000 µg/ml), 0.1ml stock solution was pipette out in 10 ml of a volumetric flask, and volume was made up to 10 ml with mobile phase to get a final concentration of 10 µg/ml.

Preparation of Std. Lecithin Solution: (Stock-II): An accurately weighed quantity, 3.75 mg of Lecithin (LCN) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 40 µg/ml. From the freshly prepared standard stock solution (3750 µg/ml), 0.1 ml stock solution was pipette out in 10 ml of the volumetric flask, and volume was made up to 10 ml with mobile phase to get a final concentration of 0.4µg/ml.

Preparation of std. Silymarin and Lecithin solution: (Stock III): From the freshly prepared standard stock solution (1000 and 3750 µg/ml), 0.1 ml stock solution was pipette out in 10 ml of the volumetric flask, and volume was made up to 10 ml with mobile phase to get final concentration 10– 50 and 37.5 – 187.5 µg/ml **Fig. 2**.

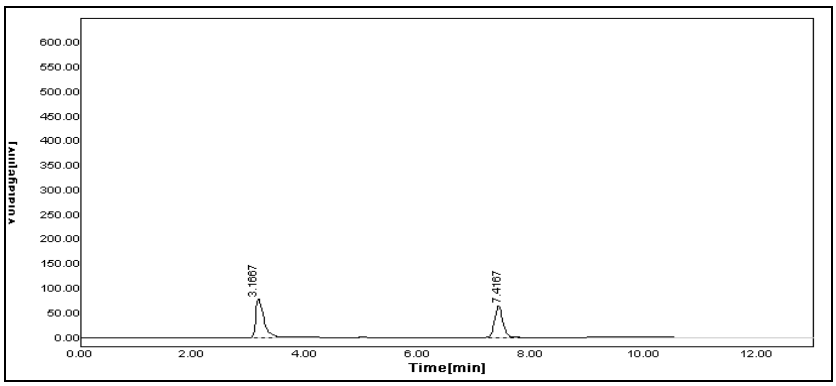

FIG. 2: CHROMATOGRAM OF STANDARD COMBINATION OF LCN AND SYL

Method Development and Validation: The working standard of various concentrations was prepared by taking aliquots of standard solution and diluting to get the required concentration for calibration plot and injecting [6-10].

Assay Preparation for Commercial Formulation: Weigh 20 Lecithin and Silymarin combination tablets, calculate the average weight, and accurately weigh and transfer the sample equivalent to 10 mg Lecithin and 37.5mg Silymarin into 10 ml volumetric flask.

Add about 10ml MEOH of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through a 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10 µg/ml). The simple chromatogram of test Lecithin and Silymarin is shown in **Fig. 3.**

The amounts of Lecithin and Silymarin per tablet were calculated by extrapolating the area value from the calibration curve.

The analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for % RSD Calculated, result was shown in **Table 1.**

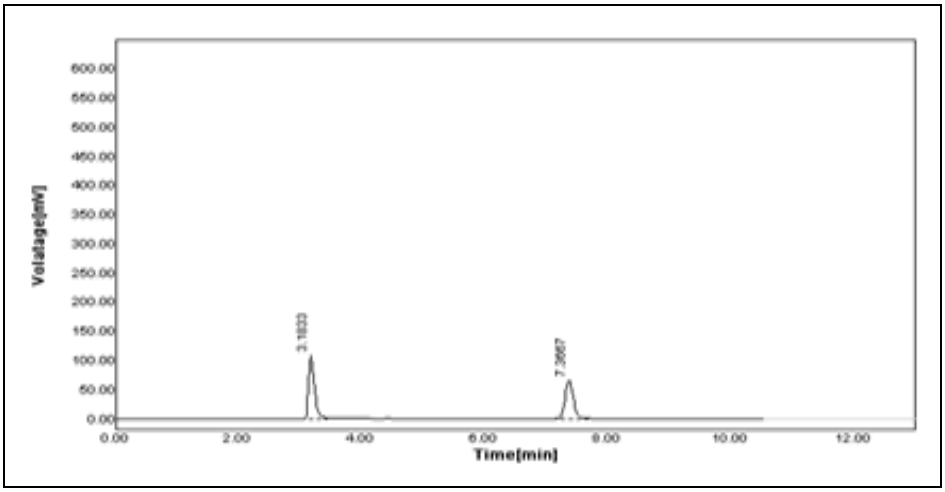

FIG. 3: CHROMATOGRAM FOR MARKETED FORMULATION

TABLE 1: ANALYSIS OF MARKETED FORMULATION

Drug	RT [min]	Area [mV*s]	Area%	TP	TF	Resolution
Lecithin	3.1833	784.4373	54.47	4128.5	1.4000	0.0000
Silymarin	7.3667	615.5257	45.53	10833.3	1.1111	14.7647

RESULTS:

Linearity and Range: The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 37.5-187.2 µg/ml for Lecithin and 10-50µg/ml for Silymarin **Table 2 and 3** depict the calibration data of Lecithin and Silymarin. The respective linear equation for Lecithin was y = 71.605x + 55.119 and Silymarin equation y = 16.549 x + 0.5469 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Lecithin and Silymarin is depicted in **Fig. 4 and 5.**

TABLE 2: LINEARITY DATA FOR LECITHIN

Method	Conc. µg/ml	Peak area (µV.sec) 1	Peak area (µV.sec) 2	Average peak area (µV. sec)	S.D. of peak area	% RSD of peak area
RP-HPLC Method	37.5	623.5154	600.2597	611.8876	16.44	2.69
	75	1220.399	1298.359	1259.379	55.13	4.38
	112.5	1819.49	1902.357	1860.924	58.60	3.15
	150	2441.527	2498.368	2469.947	40.19	1.63
	187.2	3050.95	3168.265	3109.607	82.95	2.67
	Equation			y = 16.549 x + 0.5469		
	R^2			0.999		

TABLE 3: LINEARITY DATA FOR SILYMARIN

Method	Conc. µg/ml	Peak area(µV.sec) 1	Peak area(µV.sec) 2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
RP-HPLC Method	10	775.2776	788.365	781.8213	9.25	1.18
	20	1470.337	1459.327	1464.832	7.79	0.53
	30	2202.554	2165.296	2183.925	26.34	1.21
	40	3008.865	2956.354	2982.609	37.13	1.24
	50	3638.126	3568.265	3603.195	49.40	1.37
	Equation			Y= 71.605 x + 55.119		
	R^2			0.999		

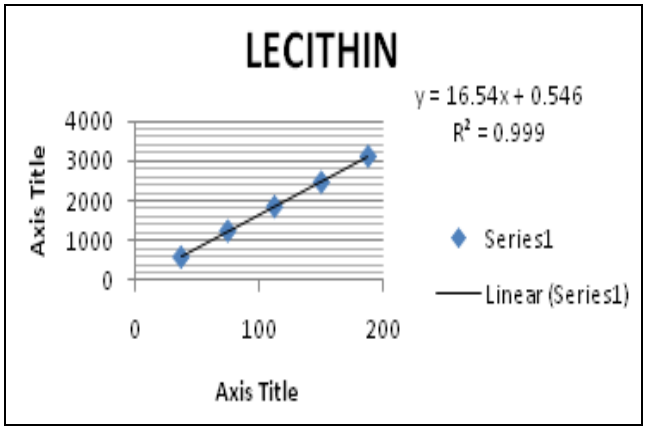

FIG. 4: CALIBRATION CURVE OF LECITHIN

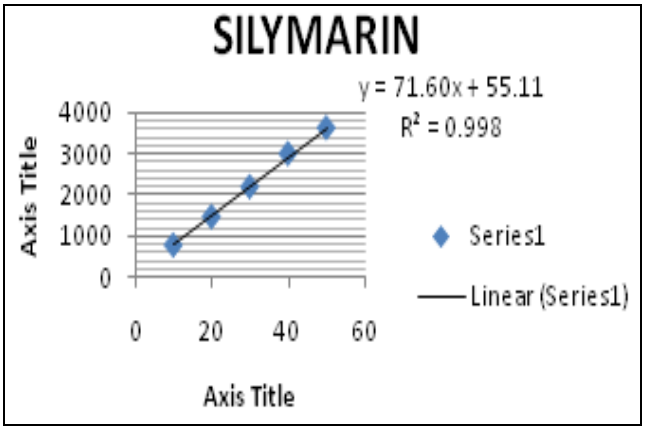

FIG. 5: CALIBRATION CURVE OF SILYMARIN

Accuracy: It is defined as the closeness of agreement between the actual (true) value and analytical value and obtained by applying the test method a number of times.

The accuracy of the methods was determined at three different concentration levels, *i.e.*, 80%, 100%, and 120% **Fig. 6 - 8** in triplicate for each drug as per ICH guidelines.

From the total amount of drug found, the percentage recovery was found in a range of 99-101% **Table 4 and 5.**

TABLE 4: RESULT OF RECOVERY DATA FOR LECITHIN AND SILYMARIN

Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
SLY	80%	10	8	18.02±0.021	8.02±0.021	100.333±0.26
	100%	10	10	19.98±0.007	20.58±0.007	99.80±0.007
	120%	10	12	21.96±0.022	20.58±0.022	99.67±0.18
LCN	80%	75	60	135.29±0.69	60.29±0.69	100.48±1.14
	100%	75	75	150.33±0.15	20.58±0.15	100.44±0.20
	120%	75	90	164.42±0.59	20.58±0.59	99.36±0.66

*Mean of each 3 reading for RP-HPLC method

TABLE 5: STATISTICAL VALIDATION OF RECOVERY STUDIES LECITHIN AND SILYMARIN

Method	Level of Recovery (%)	Drug	% RSD	S.D.*	Mean % Recovery
RP-HPLC Method	80%	SYL	0.26	0.26	100.33
		LCN	1.14	1.14	100.48
	100%	SYL	0.07	0.07	99.80
		LCN	0.20	0.20	100.44
	120%	SYL	0.18	0.18	99.67
		LCN	0.66	0.66	99.36

*Denotes average of three determinations for RP-HPLC

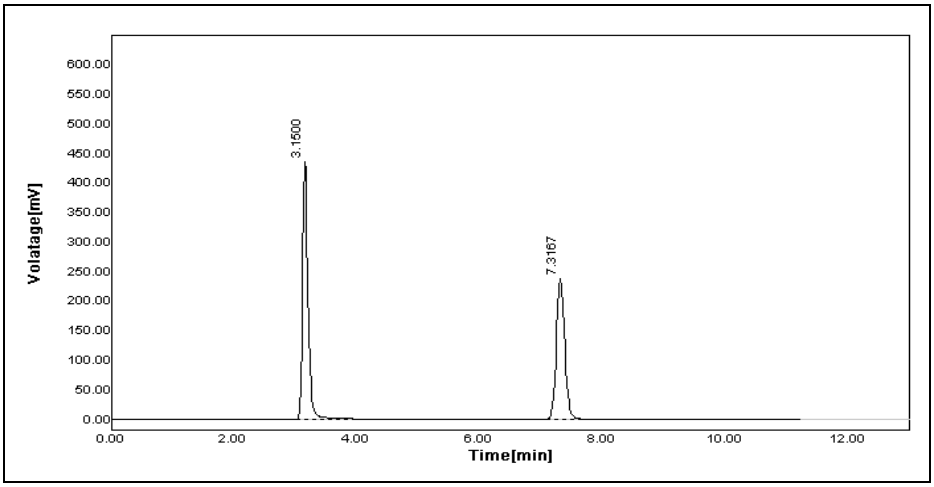

FIG. 6: CHROMATOGRAM OF ACCURACY 80%

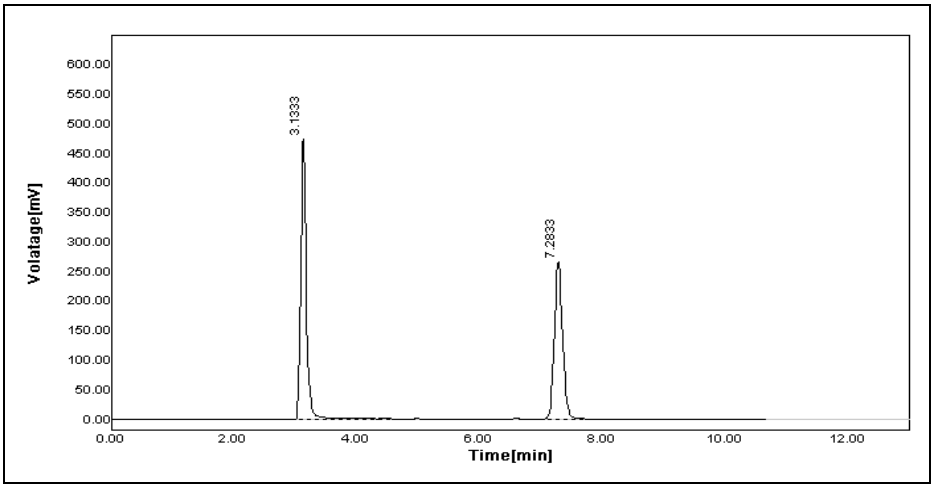

FIG. 7: CHROMATOGRAM OF ACCURACY 100%

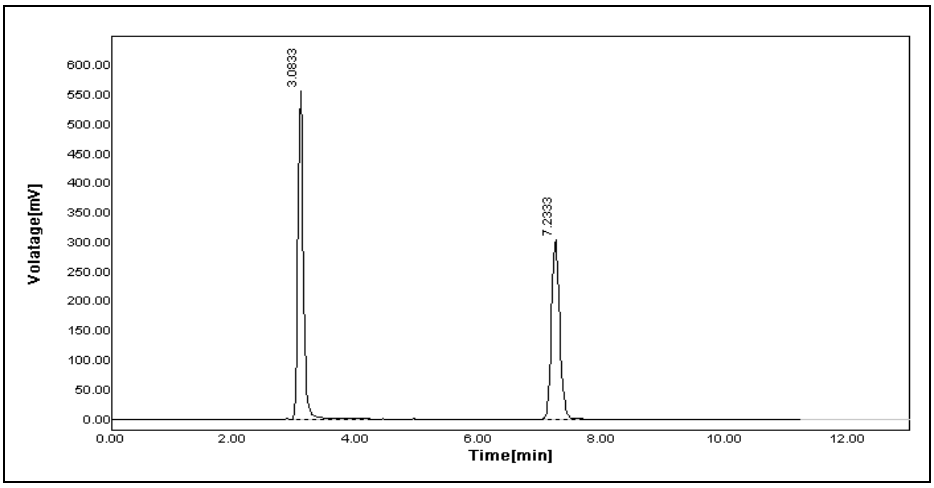

FIG. 8: CHROMATOGRAM OF ACCURACY 120%

Precision: Precision was studied to find out intra and inter-day variations in the test method of Lecithin and Silymarin.

Intra-day precision was determined by analyzing three concentrations in three replicate measurements of within the linearity range of drugs three different times in the same day.

Inter-day precision was conducted during routine operation of the system over three consecutive days. Intraday and Inter day Precision studies on RP- HPLC method for Lecithin and Silymarin, which shows the high precision % amount in between 97% to 101%, indicates the analytical method that concluded **Table 6.**

TABLE 6: INTRADAY AND INTER DAY PRECISION STUDIES ON HPLC METHOD FOR LCN AND SYL

Drug	Conc. (µg/ml)	Interday Precision		Intraday Precision	
		Mean± SD	%Amt Found	Mean± SD	%Amt Found
SYL	10	9.89±0.96	98.93	10.00±0.65	100.00
	15	15.37±0.11	102.51	15.45±0.28	102.05
	20	19.76±0.15	98.80	19.87±0.14	99.38
	75	105.55±0.9	99.82	105.41±0.65	99.69
LCN	112.5	161.51±0.8	102.39	161.02±0.73	101.44
	150	209.37±0.17	98.84	209.37±0.17	98.84

Mean of each 3 reading for HPLC method

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD is the lowest analyte in a sample that can be detected but not necessarily quantified under the stated experimental conditions.

LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions.

The LOD and LOQ of Lecithin were found to be 0.033 µg/ml and 0.01µg/ml, the analytical method.

The LOD and LOQ of Silymarin were found to be 0.2375µg/ml and 0.7199 µg/ml, the analytical method that concluded.

Robustness: The robustness is a measure of its capacity to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability during normal usage; hence the following are performed by slight variations in parameters. The assay content of the sample was measured by the change in the flow rate 0.6ml/min to 0.8ml/min **Table 7.**

TABLE 7: RESULT OF ROBUSTNESS STUDY

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD	Amount of detected (mean ±SD)	%RSD
		For Lecithin		For Silymarin	
Chromatogram of flow change 0.9 ml	50+187.2	1296.26± 1.61	0.12	1689.44± 1.16	0.07
Chromatogram of flow change 1.1 ml	50+187.2	969.58±1.39	0.14	1260.09±2.11	0.17
Chrom. of comp change wavelength change 235nm	50+187.2	1247.9±1.50	0.12	1674.5±1.24	0.07
Chrom. of comp change wavelength change 237nm	50+187.2	975.56±1.45	0.15	1233.80±2.49	0.20
Chrom. of mobile phase change 69+31 ml	50+187.2	1673.9±0.64	0.47	1447.2±0.41	0.03
Chrom. of mobile phase change 71+29 ml	50+187.2	1108.46±2.51	0.23	1448.21±2.84	00.20

Repeatability: Repeatability studies on RP-HPLC method for Lecithin and Silymarin were found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded **Table 8.**

TABLE 8: REPEATABILITY STUDIES ON RP-HPLC FOR LECITHIN AND SILYMARIN

Method	Conc. of LCN and SYL (mg/ml)	Peak area	Amount found (mg)	% amount found
HPLC SYL Method	20	42.47	19.78	98.90
	20	43.11		
	Mean	42.80		
	SD	0.45		
	%RSD	0.16		
	150	209.06	148.07	98.71
HPLC LCN Method	150	208.88		
	Mean	208.98		
	SD	0.12		
	%RSD	0.16		

DISCUSSION: The proposed methods for simultaneous estimation of LCN and SYL in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for LCN and SYL were linear

with correlation coefficients (r^2) values in the range of the selected wavelengths, and the values were average of three readings. The values of % RSD are within the prescribed limit of 2%, showing high precision of methods, and recovery was close to 101% for both the drugs. The analysis of pharmaceutical formulations reveals that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully to estimate LCN and SYL in formulations simultaneously.

CONCLUSION: Simple, rapid, accurate and precise RP-HPLC methods have been developed and validated for the routine analysis of LCN and SYL in API and tablet dosage forms. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations.

The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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CONFLICTS OF INTEREST: Authors have no conflicts of interest to declare.

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