

A Validated specific Stability-Indicating Reversed-Phase High-Performance Liquid Chromatography Assay Method for L-Ornithine L-Aspartate and Silymarin, and their related Substances and its Application to Dissolution Studies

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ABSTRACT

The present study was aimed at the development and successive validation of a novel, simple, sensitive, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for quantitative calculation of L-ornithine L-aspartate (LOLA) and Silymarin (SL), and also their relevant substances in bulk and pharmaceutical dosage forms. The chromatographic technique was optimized using the impurity-spiked solution. The separation of all the two active components and their impurities was achieved by a chromatographic method with an Agilent Eclipse XDB-C18, 150 × 4.6 mm, 3.5 μ column, using gradient elution with mobile phase A consisting of a mixture of 0.1% orthophosphoric acid and water and acetonitrile as mobile phase B. The instrumental settings included a flow rate of 1 mL/min for both related substances and assay, a detector wavelength of 225 nm, by using a PDA detector. The established method was validated according to the current ICH requirements. The detection limit and the limit of quantification for the two active components and their related impurities were established with respect to test concentration. The calibration graphs plotted were linear with a regression coefficient $R^2 > 0.999$, indicates the linearity of the method was within the limits. Recovery studies were satisfactory and the parameters, such as, specificity, linearity, accuracy, precision, and robustness were determined as part of the method validation. Moreover, using the same method dissolution study was performed on active pharma ingredients to estimate the recovery. The obtained results were within the range of acceptance criteria. These results suggest that the developed method was found to be applicable for routine analysis for testing chromatographic purity of LOLA and SL and it can be utilized for the calculation of both active ingredients and their impurities in tablet dosage forms.

Keywords: Dissolution profile, Related substances, RP-HPLC, Validation.

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INTRODUCTION

It has been observed that the presence of any unwanted chemicals in drug substances can affect the quality, safety, and efficacy of the pharmaceutical product, thereby causing serious health hazards. Hence, it is very important to determine such unwanted chemicals. Therefore, as part of the method validation, we have identified four impurities in samples of LOLA and SL drug formulation products, characterized by HPLC analytical data. The structures are shown in Figure 1.

The LOLA is a stable salt of naturally occurring amino acids ornithine and aspartate. It has the capacity to increase ammonia removal by residual hepatocytes and skeletal muscle

of patients with cirrhosis.¹ It can stimulate the urea cycle and glutamine synthesis, which are key metabolic pathways in ammonia detoxification.^{2,3} LOLA has anabolic effects, wound-healing effects, and improves athletic performance and is believed to be beneficial in hepatic encephalopathy. It has confirmed its efficacy (reducing elevated ammonia levels) in some animal studies^{4,5} and both in uncontrolled clinical trials^{6,7} and in well designed controlled clinical trials with hyperammonemia and cirrhotic patients.^{8,9} LOLA has a direct hepatoprotective effect, exerts via an antioxidative mechanism, by stabilization of antioxidant balance of liver cells.^{10,11}

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SL, Figure 2, a flavonoid, which is classified as benzopyranone,¹² and is extracted from the plant *Silybum marianum* and is a mixture of three structural components, such as, silibinin, silydianine, and silychristine.¹³ It is isolated from the fruits and seeds of the milk thistle (*Silybum marianum*) and an extract of milk thistle plant has been used to treat chronic liver disease.¹⁴ The most prevalent component of the SL complex

is silybin (50–60% of SL), which is the major bioactive component of SL.^{15,16} SL supports healthy DNA function and is able to modulate the immune system. As a result of pharmacological activities of SL, including hepatoprotectant and anti-inflammatory agent, antibacterial, antifibrotic, antiallergic, antimutagenic, antiviral, anti-lipid-peroxidative, antineoplastic, antithrombotic agents, and vasodilatory actions,^{17,18} it has been claimed that SL has clinical applications in the treatment of toxic hepatitis, fatty liver, ischaemic injury, radiation toxicity, and viral hepatitis. Since no health hazards or side effects are known in conjugation with the proper administration of designed therapeutic dosages,¹⁹ SL may be accepted as a safe herbal product.

However, both LOLA and SL are hepatoprotective drugs and are highly effective against chemical-induced toxicity.^{20,21} The review of the literature has shown that no significant work has been carried out on the simultaneous determination of the related substances and an assay of LOLA and SL. To the best of our knowledge, dissolution studies of these active drugs have not yet been reported previously. It was, therefore, felt essential to introduce a new RP-HPLC method for the estimation of related substances, assay of LOLA, SL, and its application to dissolution studies. Therefore, we have attempted to develop an accurate, simple, specific, precise, and reproducible technique to determine the related substances and assay of LOLA and SL and its application to dissolution studies. This study is the first reported RP-HPLC method for the determination of related substances, assay, and dissolution study of LOLA and SL. The newly developed method was validated as per International Conference on Harmonization (ICH) guidelines.²²⁻²⁵

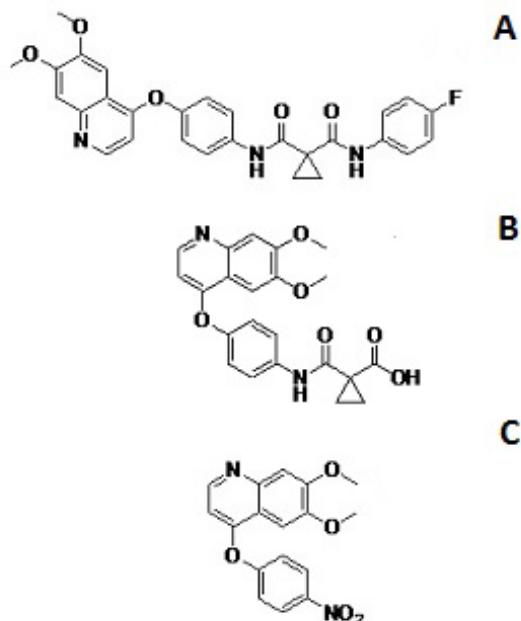


Figure 1: Chemical structures of LOLA (A); LOLA imp-1 (B); and LOLA imp-2 (C)

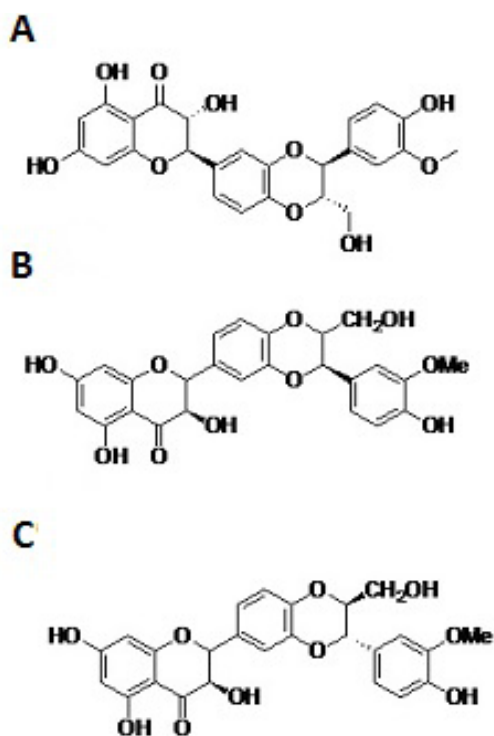


Figure 2: Chemical structures of SL (A); silybin-A (B); silybin-B (C)

EXPERIMENTAL

Chemicals and Reagents

Acetonitrile, orthophosphoric acid, and HPLC grade water were purchased from Merck (India) Ltd., Worli, Mumbai, India. LOLA and SL active pharmaceutical ingredients (APIs) as reference standards, were procured from Zydus Cadila, Ahmedabad, India (99.7–99.9% purity).

Instrumentation

HPLC was performed using a Water Alliance e-2696 chromatographic system with a quaternary pump and photodiode-array detection (PDA) detector 2996. The Empower-2.0 software was used to collect the data.

Chromatographic Conditions

Fine resolution between active pharma ingredients and their related impurities was obtained by employing Agilent, Eclipse XDB-C18 column (150 × 4.6 mm, 3.5 μm) with a flow rate of 1 mL/min and 10 μL injection volume. The UV detection wavelength was set at 225 nm.

Selection of Wavelength

Detection was carried out at various wavelengths from 200 to 400 nm in order to raise the sensitivity of the method, where scanning at 225 nm gave considerable sensitivity for the studied components (Figure 3).

Preparation of the Mobile Phase

Mobile phase A: 1 mL orthophosphoric acid was taken and introduced into 1-liter water. It is degassed and filtered by using a 0.45 μ membrane filter. Mobile phase B: Acetonitrile

Different mobile phases were evaluated in order to obtain adequate separation and short-time analysis. The above mentioned mobile phase with gradient elution provided longer retention time, for that reason it was selected for further method development. The gradient program is shown in Table 1.

Diluent

Mobile phase A and B in the ratio of 50:50 v/v was used as diluent.

Preparation of Standard Solution for Related Substances (RS)

Accurately weighed about 26.5 mg of LOLA and 7.4 mg of SL (working standards) and transferred in 100 mL volumetric flask and to this about 70 mL of diluent has been added and it was sonicated for 15 minutes to dissolve and diluted up to the mark with the diluent and it was mixed well.

Preparation of Sample Solution for RS

Ten tablets were weighed and triturated in a mortar. Then the weight equivalent to one tablet (350 mg of the sample) was transferred to a 100 mL clean and dry volumetric flask. It was sonicated after the addition of 70 mL of diluent for about 30 minutes with occasional shaking to dissolve and was made up to the mark with the diluent and it was filtered through 0.45 μ nylon syringe filter.

Impurity Standard Stock Solution for RS

Accurately weighed each 5 mg of impurity-1 of LOLA, 2.5 mg of its impurity-2, 5.5 mg of silybin-A, and 5.5 mg of silybin-B were taken into a 100 mL volumetric flask. To this, 50 mL of diluent was added and it was sonicated to for complete dissolution and diluted the solution up to the mark and the solution was filtered through 0.45 μ nylon syringe filter.

Preparation of Standard Solution for Assay

Accurately taken 25 mg of LOLA and 7 mg of SL (working standards) and it was transferred to a 100 mL volumetric

flask. To this, 70 mL of diluent solution was added and it was sonicated for about 15 minutes for complete dissolution and it was made up to the mark with a diluent solution and shaken thoroughly. 5 mL of this solution was pipette out into a 50 mL volumetric flask and made up to the mark with a diluent solution and it was filtered through a 0.45 μ nylon syringe filter.

Preparation of Sample Solution for Assay

Ten tablets were accurately weighed and triturated in a mortar. From this, the weight equivalent to one tablet (350 mg of the sample) was transferred to a 100 mL clean and dry volumetric flask. To this 70 mL of mobile phase was added and sonicated for 30 minutes with occasional shaking to dissolve and made the solution up to the mark. Further, 5 mL of the above solution was pipette out into a 50 mL volumetric flask and made up to the volume with diluent and it was filtered through a 0.45 μ nylon syringe filter.

RESULTS AND OBSERVATIONS

The main analytical challenge during the development of a new method was to separate active pharma ingredients from their impurities. A dissolution study was also performed to estimate the recovery using the same method. In order to provide good performance, the chromatographic conditions were optimized.

Method Optimization

Development trails were performed to obtain good resolution between LOLA and SL, and their impurities. To optimize the chromatographic conditions, several proportions of phosphate buffer and acetonitrile in the mobile phase with isocratic and gradient mode was tested. However, the mobile phase composition was modified at each trail to enhance the resolution and also to achieve acceptable retention times. Finally, 0.1% OPA buffer (mobile phase A) and acetonitrile (mobile phase B) with gradient elution were preferred because it resulted in a greater response to active pharma ingredients and their impurities, and the above mobile phase system was used as diluent. During the optimization of the method, various stationary phases, such as, C8, C18, phenyl, and amino columns were used. On the basis of these trails, it has been found that peak shapes of active pharma ingredients and all impurities were relatively good on Agilent, Eclipse XDB-C18, 150 \times 4.6 mm, 3.5 μ column connected to PDA detector. The flow rate of the mobile phase was 1 mL/min. UV detection has been done at 225 nm in order to obtain enough sensitivity among the analyte and all impurities. Under these conditions, the best response between impurities and analyte was achieved when an impurity-spiked sample solution was

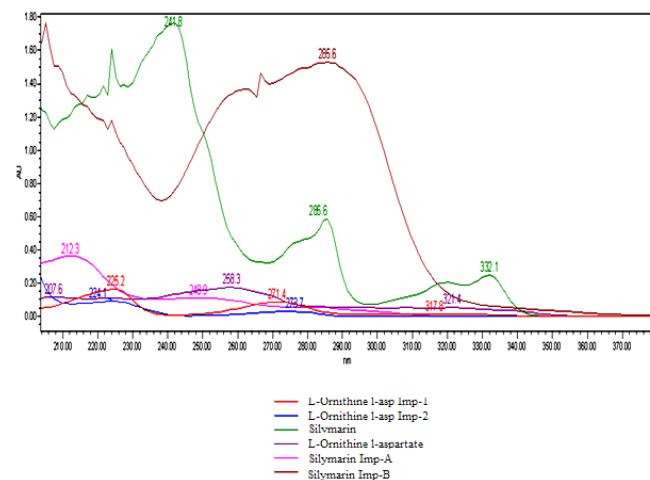


Figure 3: PDA spectra for LOLA and SL

Table 1: Gradient program for LOLA and SL

Time (min)	Mobile phase-A	Mobile phase-B
0	80	20
5	50	50
10	30	70
12	80	20
17	80	20

injected. The retention times of LOLA and SL were about 4.16 and 7 with a tailing factor of 1.04 and 1.07. The retention times of impurity-1 and impurity-2 of LOLA were 3.71 and 4.76 and impurity-A and impurity-B of SL were about 5.79 and 10.93, respectively. The number of theoretical plates for LOLA and SL peak was about 3,098 and 47,312, which indicates the efficient performance of the column. % RSD for six replicate injections was around 0.94%, which indicates that the proposed method is highly accurate. The method developed was validated as per ICH guidelines with all of the results within the limits, so that the method was selected for the purity of LOLA and SL. Moreover, under the optimized chromatographic conditions and using the solubility data of the drugs, various dissolutions were performed to optimize the parameters, like dissolution media and its volume, apparatus, and rpm to get the maximum % release of the drug.

Method Validation

The optimized RP-HPLC method was validated as per the ICH guidelines with respect to system suitability, linearity, and range, precision, accuracy, and robustness.

System Suitability

The chromatographic system must satisfy system suitability requirements before analysis. So the system has been stabilized for 60 minutes to get a stable baseline. Standard solutions of both RS and assay were injected into the system to check

the system suitability conditions and chromatograms were recorded (Figure 4). System suitability parameters were evaluated to measure whether the obtained result complies with the recommended limit. Results were within the acceptance criteria, which is shown in Table 2.

Specificity

It is the ability of the technique to determine accurately and specifically the analyte response in the presence of its impurities. The specificity of this kind was evaluated in the presence of impurities. According to the test method, placebo, sample, and standard solutions were analyzed individually to examine the interference. The solutions of standard, placebo, and blank were injected into the system, and chromatograms were shown in Figures 5. Figures 5 show that active ingredients were well separated from blank and their excipients, and there was no interference of placebo with the principal peak, hence, the method is specific (Figure 5).

Linearity

Linearity is its ability to get the test results, which were directly proportional to the analyte concentration of the sample and was determined by computing correlation coefficient from calibration curves. The Plot of the linearity of peak vs. different concentrations has been evaluated for LOLA and SL and their relevant substances. The test solutions were prepared for related substance methods from impurity stock solutions at various

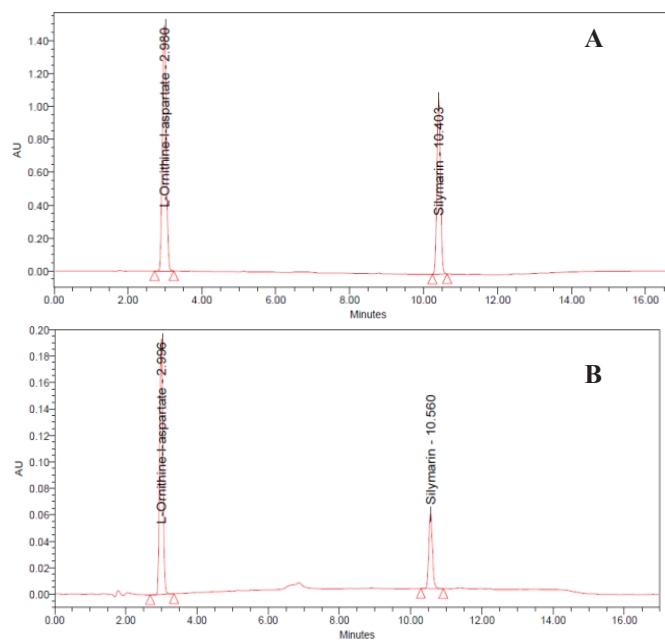


Figure 4: Chromatograms for SST (A); RS (B)

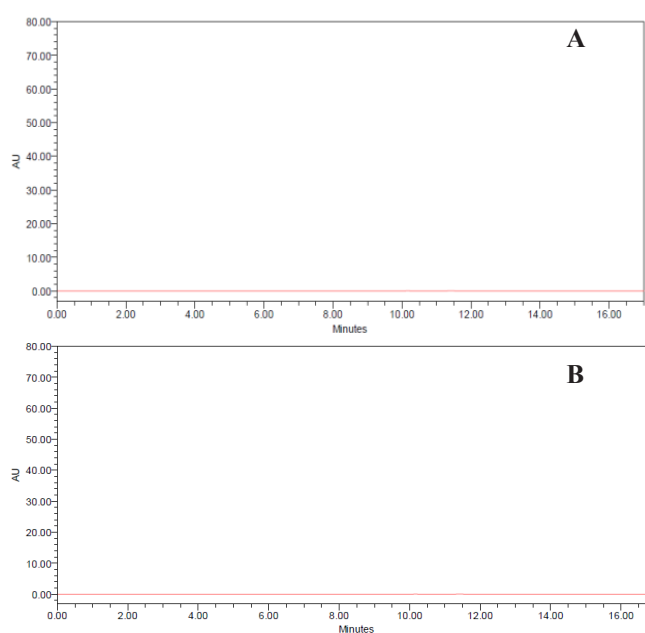


Figure 5: Chromatograms for Blank (A); Placebo (B)

Table 2: System suitability data

System Suitability parameter	Acceptance criteria	Drug name			
		LOLA		SL	
		RS	Assay	RS	Assay
% RSD	NMT 2.0	0.84	0.52	0.76	0.34
USP tailing	NMT 2.0	1.05	1.04	1.09	1.09
USP plate count	NLT 3000	3,474	4,280	47,609	49,059

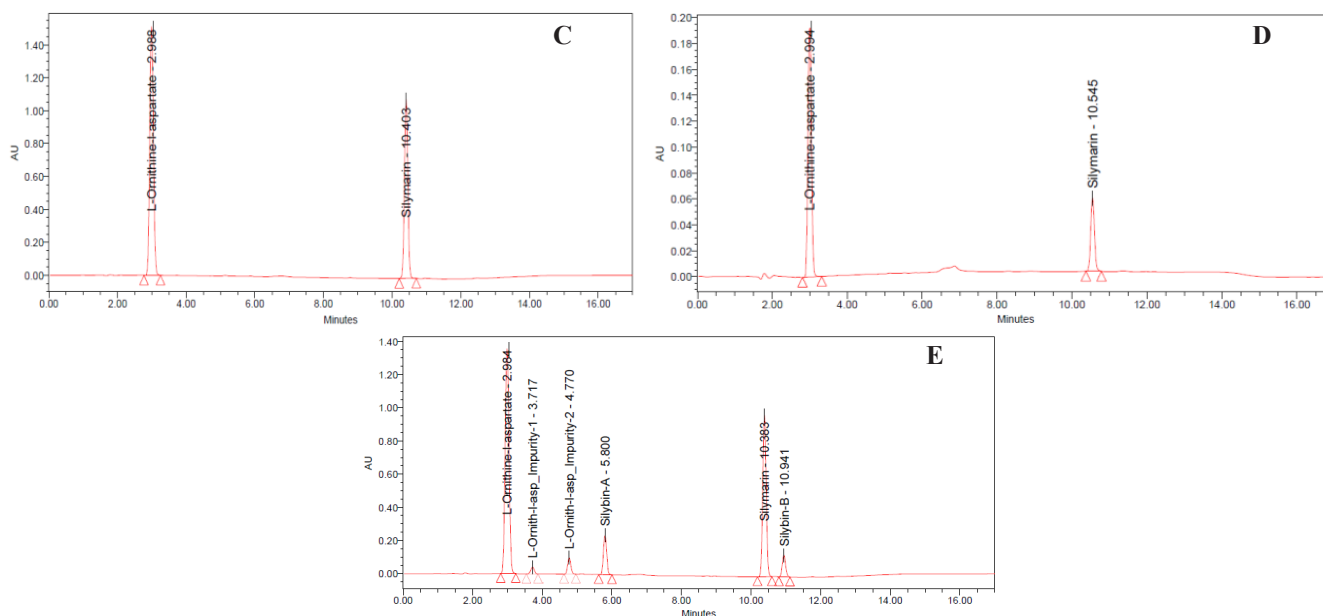


Figure 5: Standard solution (C); Sample solution (D); Spiked sample solution (E)

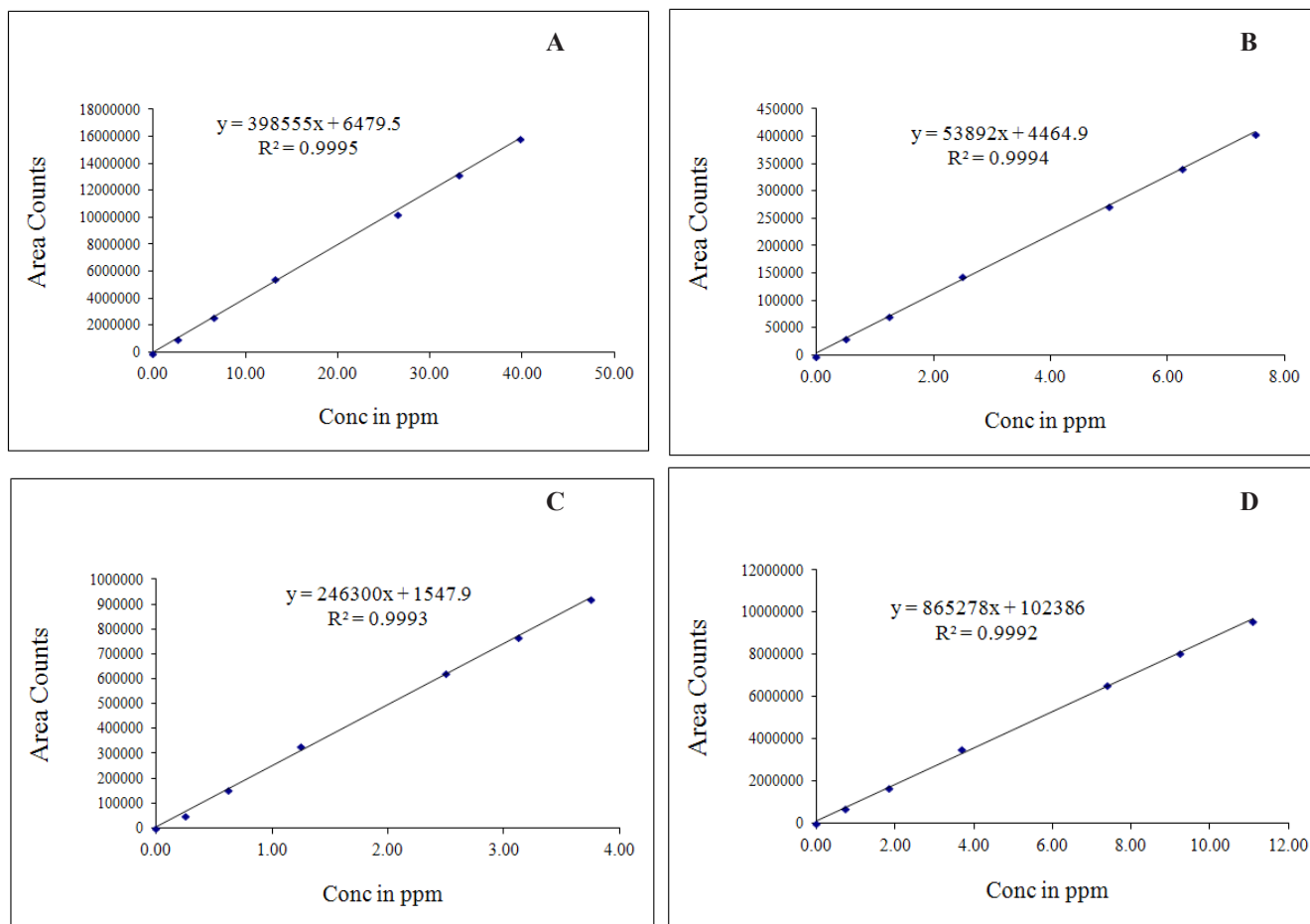


Figure 6: RS linearity plot for LOLA (A); LOLA imp-1 (B); LOLA imp-2 (C); SL (D)

concentration levels. The linearity range was found to be 2.65 to 39.75 $\mu\text{g/mL}$ for LOLA, 0.5 to 7.5 $\mu\text{g/mL}$ for LOLA impurity-1, 0.25 to 3.75 $\mu\text{g/mL}$ for LOLA impurity-2, and 0.74 to 11.1 $\mu\text{g/mL}$ for SL, 0.55 to 8.25 $\mu\text{g/mL}$ for SL impurity-A,

and 0.55 to 8.25 $\mu\text{g/mL}$ SL impurity-B. Under optimum chromatographic conditions, we got linear relationships between the peak areas and the corresponding concentrations (Figure 6). Slopes, correlation coefficients, and y-intercepts were

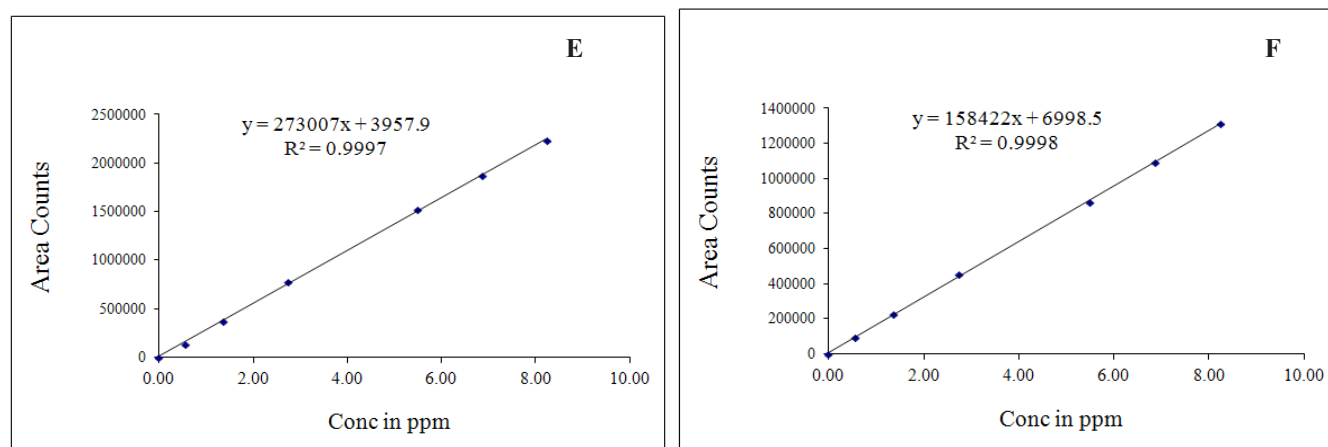


Figure 6: SL imp-A (E); SL imp-B (F)

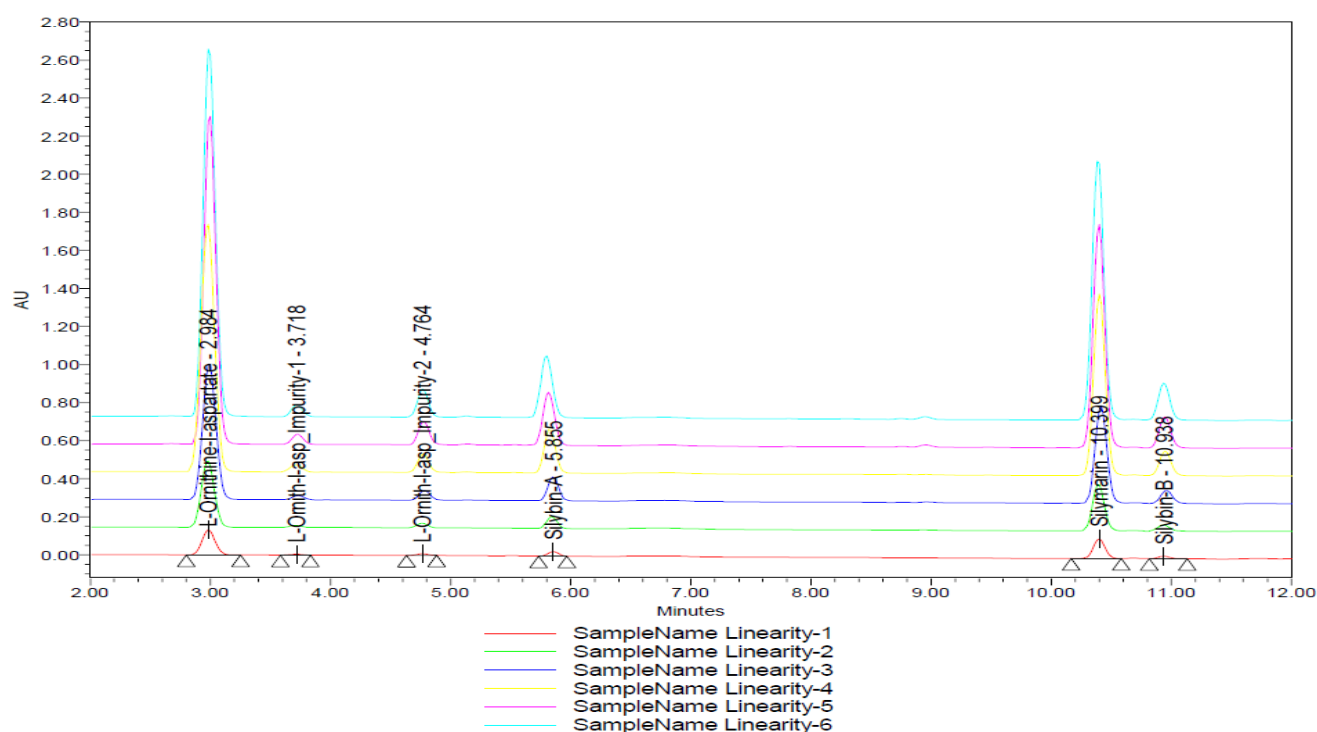


Figure 7: Overlay chromatogram for RS-linearity

determined. Correlation coefficients for all the components were under the limit. The calibration curves were plotted for all the related substances and mentioned in Figures 7. The % RSD was found to be within the acceptable theoretical limits (Figure 7).

The linearity test solutions were prepared for the assay method by diluting the stock solution to the desired concentrations and these solutions were prepared from 10 to 150% with respect to an analyte concentration of 25 ppm (LOLA) and 7 ppm (SL). The linearity range was found to be 2.5 to 37.5 µg/mL for LOLA and 0.7 to 10.5 µg/mL for SL. Under optimum chromatographic conditions, linear relationships between the peak areas and the corresponding concentrations were obtained. Correlation coefficients, slopes, and y-intercepts were calculated. Correlation coefficients

for all the components were under limit. Calibration curves were plotted for all the related substances and mentioned in Figures 7. The results demonstrate that there was an excellent correlation existed between the peak area and the corresponding concentrations (Figure 8).

Accuracy

It is the closeness of the measurements to a specific value with found value. Recovery experiments have been carried out to determine the accuracy of the related substance technique for the quantification of all the four impurities. The study was conducted in triplicate by analyzing active pharma ingredients (LOLA and SL) sample solution spiked with known amounts of all the related impurities at three kinds of concentration levels of 50, 100, and 150% of each at a specified limit. The percentage recoveries for all impurities were

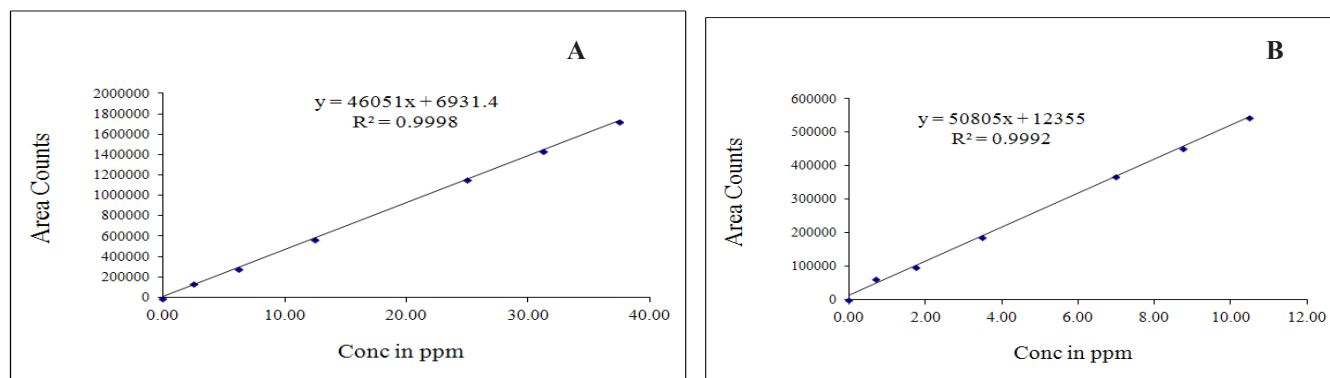


Figure 8: Assay linearity plot for LOLA (A); SL (B)

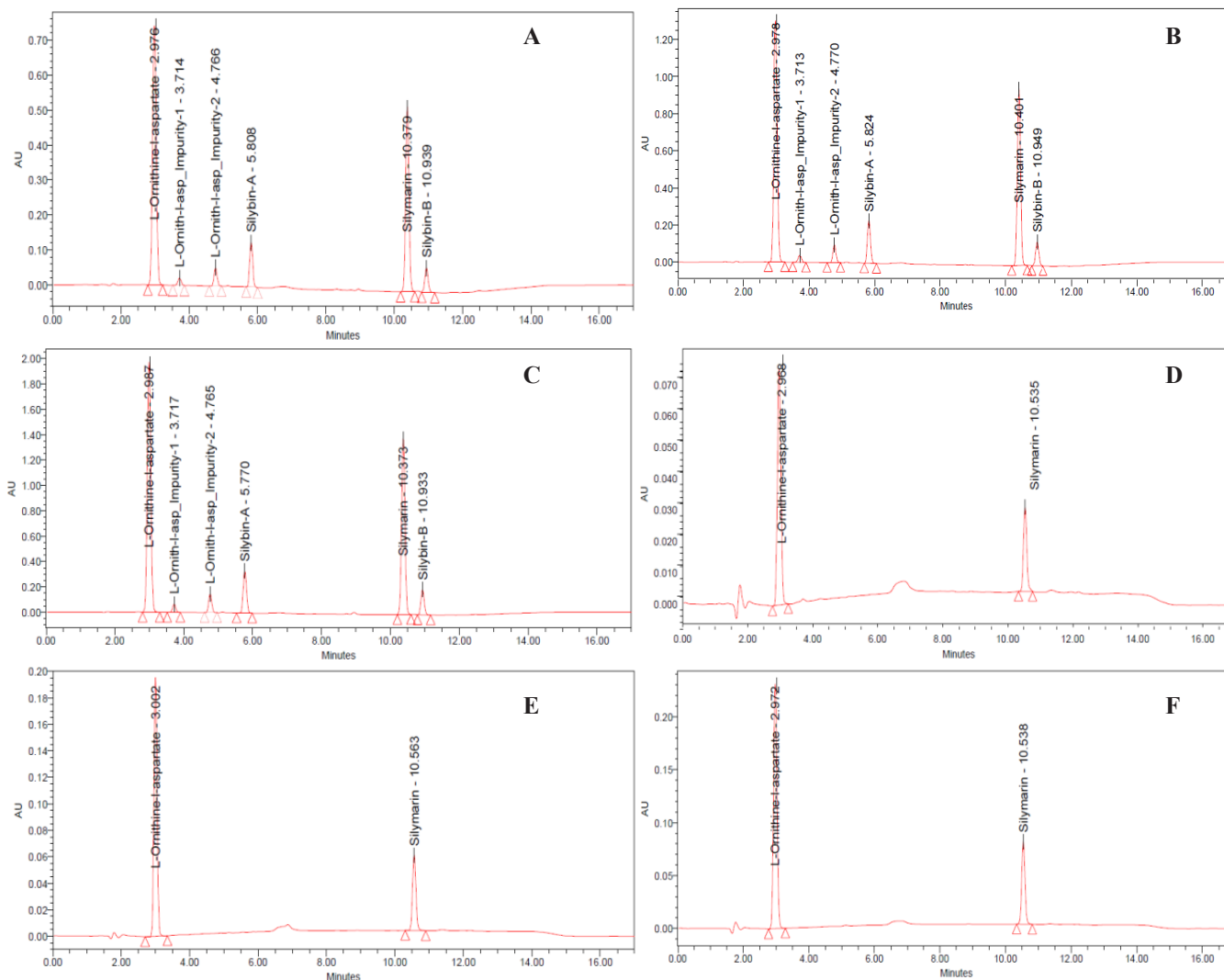


Figure 9: Chromatograms for RS-Acc 50% (A); RS-Acc 100% (B); RS-Acc 150% (C); Assay-Acc 50% (D); Assay-Acc 100% (E); Assay-Acc 150% (F)

calculated and those are ranging from 99.8 to 100.9%. Good percentage recoveries were obtained and tabulated (Table 3).

The accuracy of the assay technique was evaluated by selecting three kinds of concentrations, such as, 12.5, 25, and 37.5 µg/mL of LOLA; 3.5, 7, and 10.5 µg/mL of SL (50, 100, and 150%) were prepared. The test solution has injected three

times for each spike level and according to the test method the assay was carried out (Figure 9). The percentage recovery and the related standard deviations were determined. These results show that the values of percentage recovery were close to 100% and also the RSD values were less than ±2%. Results are furnished in Table 4.

Precision

The precision of an analytical technique is the degree of closeness of series of measurements derived from multiple samplings of a homogeneous mixture. The precision of related substance method was performed by injecting six individuals determinations of LOLA (26.5 ppm) and SL (7.4 ppm) spiked with that of 5% of impurity-1 and 2.5% of impurity-2 of LOLA, and each 5% level of impurity-A and impurity-B of SL and the % RSD was determined for each impurity and these results showed that the method is precise under the specified experimental conditions.

The precision of the assay method was examined by analyzing six replicates of sample solutions having of LOLA (25 ppm) and SL (7 ppm) were prepared and % RSD was calculated and chromatograms were recorded. As per the

procedure, the intermediate precision was also performed by analyzing six replicates of standard and sample solution on a different day, by a different analyst and using an instrument of different make in the same laboratory. Results are furnished in Tables 5 to 8.

Limit of detection (LoD) and Limit of quantitation (LoQ)

The limit of detection is the lowest amount of analyte in the sample that gives the measurable response ($3.3 \sigma/S$) and the limit of quantification is the lowest amount of analyte that gives an accurately quantified response ($10 \sigma/S$), where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot.

LoD and LoQ (25) of the compounds have been carried out by injecting progressively the lower concentrations of the standard solutions. The concentrations LoD of LOLA and their

Table 3: Accuracy results for LOLA

S. No.	% level	LOLA RS Avg. % recovery	LOLA assay Avg. % recovery
1	50	100.1	100.31
2	100	100.1	100.14
3	150	100	100.25

Table 4: Accuracy result for SL

S. No.	% level	SL RS Avg. % recovery	SL assay Avg. % recovery
1	50	99.9	100.42
2	100	100.2	99.95
3	150	99.9	100.26

Table 5: RS-results for method precision of LOLA

% of related substances				% assay
Sample no.	Spiked impurities	Total impurities	% purity (100-total imp.)	
1	2.16	0.47	99.53	100.2
2	2.14	0.45	99.55	100.45
3	2.13	0.42	99.58	99.92
4	2.18	0.51	99.49	100.01
5	2.12	0.39	99.61	100.24
6	2.15	0.46	99.54	100.58
Average	2.147	0.45	99.55	100.23
% RSD	1.01	9.22	0.04	0.25

Table 6: RS-results for method precision of SL

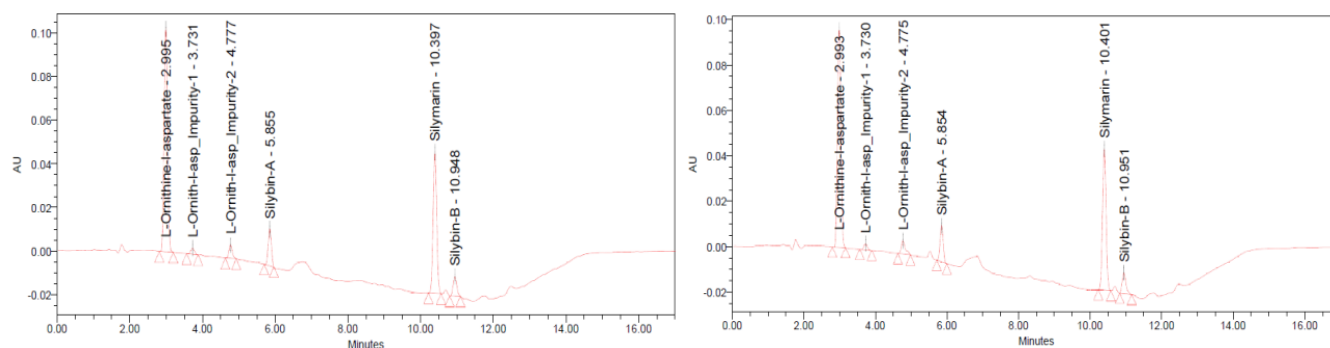
% of related substances				% assay
Sample no.	Spiked impurities	Total impurities	% purity (100-total imp.)	
1	2.72	0.64	99.36	100.46
2	2.76	0.68	99.32	100.15
3	2.75	0.66	99.34	100.75
4	2.74	0.65	99.35	100.62
5	2.73	0.62	99.38	100.08
6	2.71	0.61	99.39	100.32
Average	2.74	0.64	99.36	100.41
% RSD	0.68	4.01	0.03	0.26

Table 7: Assay results for intermediate precision of LOLA

Sample no.	% of related substances		% purity (100-total imp.)	% assay
	Spiked impurities	Total impurities		
1	2.24	0.63	99.37	100.12
2	2.26	0.66	99.34	99.94
3	2.21	0.62	99.38	100.42
4	2.16	0.53	99.47	100.63
5	2.18	0.59	99.41	100.11
6	2.17	0.58	99.42	100.03
Average	2.21	0.61	99.41	100.21
% RSD	1.83	7.54	0.05	0.26

Table 8: Intermediate precision results for SL

Sample no.	% of related substances		% purity (100-total imp.)	% assay
	Spiked impurities	Total impurities		
1	2.55	0.75	99.25	99.88
2	2.54	0.76	99.24	100.24
3	2.53	0.72	99.28	100.62
4	2.57	0.74	99.26	100.08
5	2.59	0.78	99.22	99.89
6	2.58	0.77	99.23	100.25
Average	2.56	0.75	99.25	100.16
% RSD	0.92	2.87	0.02	0.28

**Figure 10:** Chromatogram for LoD (A); LoQ (B)

impurities-1, 2 are 0.265, 0.05, and 0.025 $\mu\text{g/mL}$ and their s/n values are 7, 5, and 3, and SL and their impurities-A, B are 0.074, 0.055, and 0.055 $\mu\text{g/mL}$ their s/n values are 6, 4, and 4. The LOQ concentration for LOLA and their impurities-1, 2 are 2.65, 0.5, and 0.25 $\mu\text{g/mL}$ their s/n values are 27, 25, and 23, and SL and their impurities-A, B are 0.74, 0.55, and 0.55 $\mu\text{g/mL}$ their s/n values are 26, 24, and 24 (Figure 10).

Robustness

The robustness of a method indicated its ability to remain unaffected by a small but deliberate variation in parameters of the method. In order to measure the robustness of a developed method, the conditions of the experiment were deliberately changed, such as, flow rate, organic percentage of the mobile phase, and wavelength. In all these varied conditions, the

resolution between active pharma ingredients from impurities was not significantly affected and there was no significant influence on the time of retention, plate count, and tailing factor. Hence, this method was robust. The results obtained are recorded in Table 9.

Stability

The standard and the sample solutions were kept up to 24 hours at room temperature, and 2-8°C. These solutions were then injected into the system and calculated the % of deviation from initial to 24 hours. There was no significant degradation observed within the period which is sufficient for carrying the analytical process and confirmed that solutions were stable up to 24 hours during the analysis. The results are furnished in Table 10.

Table 9: Robustness data for LOLA and SL

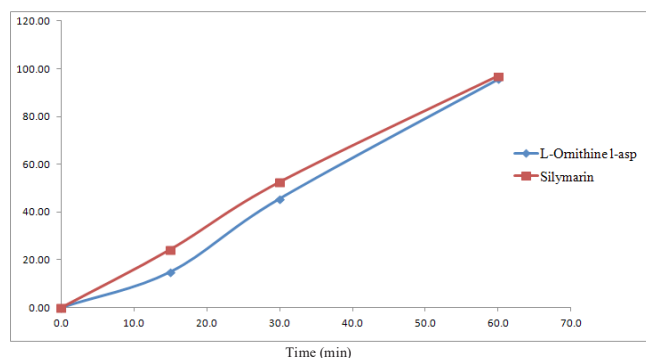
Parameter name	% RSD			
	LOLA		SL	
	RS	Assay	RS	Assay
Flow (0.8 mL/min)	0.64	0.52	0.72	0.34
Flow (1.2 mL/min)	0.38	0.26	0.68	0.92
Organic solvent (+10%)	0.59	0.84	0.29	0.85
Organic solvent (-10%)	0.52	0.56	0.64	0.46
Wave length (+5 nm)	0.75	0.38	0.58	0.28
Wave length (-5 nm)	0.77	0.17	0.49	0.56

Table 10: Results for solution stability of LOLA and SL

Stability	Purity of LOLA	% of deviation	Purity of SL	% of deviation
Initial	99.82	0	99.64	0
6 hr	99.74	0.08	99.55	0.09
12 hr	99.71	0.11	99.49	0.15
18 hr	99.65	0.17	99.32	0.32
24 hr	99.52	0.3	99.27	0.37

Table 11: Results for forced degradation

Degradation condition	LOLA		SL	
	Purity	Total impurities	Purity	Total impurities
Acid degradation	97.54	2.46	96.45	3.55
Alkali degradation	97.62	2.38	96.53	3.47
Peroxide degradation	97.56	2.44	96.27	3.73
Reduction degradation	97.25	2.75	97.58	2.42
Thermal degradation	97.43	2.57	96.32	3.68
Photolytic degradation	97.64	2.36	97.56	2.44

**Figure 11:** Dissolution profile for LOLA and SL

Degradation Studies

LOLA and SL were stable under stress conditions, such as, photolytic stress, acid, base hydrolysis, peroxide, reduction, and thermal conditions. Forced degradation samples under various conditions were analyzed at an initial concentration of 26.5 ppm of LOLA and 7.4 ppm of SL by spiking with known impurities using a PDA detector to ensure homogeneity of LOLA and SL peaks. The degradation studies were performed as follows. The hydrolysis in acidic medium (0.1N HCl at 60°C for 15 minutes), hydrolysis under basic conditions (0.1 N NaOH at 60°C for 15 minutes), peroxide degradation (10% H₂O₂ at 30 minutes), reduction degradation (10% NaHCO₃ at 60°C for

15 minutes). There is no interference between the peaks and were well separated with the resolution at least one. Hence, the test results of the peak purity obtained by using a PDA detector confirmed that LOLA and SL peaks are homogeneous and pure in all the stress samples analyzed. The results are furnished in Table 11.

Dissolution Testing

Dissolution Medium

Water

Procedure

The dissolution studies of the drug were conducted with the paddle method at 50 rpm, and the dissolution medium was 900 mL of water buffer. The temperature of the cell was maintained at 37 ± 5°C. Weighed and dropped one tablet in each of the six dissolution vessels containing dissolution media for the respective drugs under analysis. The aliquots of samples were withdrawn at 15, 30, and 60 minutes, and immediately replaced with an equal volume of fresh medium and these samples were filtered through a 0.45 μ syringe filter after the end of each test time. The samples have been prepared according to test concentration and equal volumes of these test solutions were injected into the chromatographic system and peak areas were measured (Figure 11). The percentage

Table 12: Results of dissolution study for LOLA and SL

Tablet	% drug release							
	LOLA				SL			
	15 min	30 min	60 min	REC	15 min	30 min	60 min	REC
1	15	45	95	99.25	24	52	98	99.64
2	15	46	96	99.87	25	53	96	99.53
3	15	45	95	99.34	24	54	97	99.85
4	15	45	95	99.37	24	52	97	99.57
5	15	46	97	100.2	25	52	97	100.5
6	15	47	96	99.25	24	52	96	99.64
Avg.	15	45.67	95.67	99.55	24.33	52.5	96.83	99.79
RSD	0	1.79	0.85	0.4	2.12	1.59	0.78	0.37

of content was calculated. The results are furnished in Table 12.

CONCLUSION

We present in this paper a simple, selective, validated, and well-defined stability-indicating gradient RP-HPLC method for the quantitative determination of LOLA and SL, as well as, their chromatographic impurities was described and also dissolution studies were well established. All the products of degradation formed during the stress conditions and the related impurities of active pharma ingredients are well separated and peaks were well resolved from each other and separate with appropriate retention time, indicating that the proposed method demonstrated to be fast, simple, feasible, and affordable when challenged for robustness either in RS, assay, and dissolution conditions. Therefore, the developed method can be used for routine analysis of production samples and to check the quality of drug samples during stability studies.

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