RESEARCH ARTICLE

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 relavent substances in bulk and pharmaceutical dosage forms. The chromatographic technic 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 a Agilent Eclipse XDB-C18, 150x4.6 mm, 3.5 µ column, using gradient elution with mobile phase A consisting of mixture of 0.1% orthophosphoric acid and water and acetonitrile as mobile phase B. The instrumental settings included a flow rate of 1.0 mL/min for both related substances and assay, a detector wavelength of 225 nm, by using a Photodiode Array Detector (PDA) detector.The established method was validated according to the current International Conference on Harmonization (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 R2 > 0.999, indicating the method's linearity was within 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 to calculate both active ingredients and their impurities in tablet dosage forms.

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

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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 product, characterized by HPLC analytical data. The structures are shown in Figure 1.

L-ornithine-L-aspartate (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 hyperammonemic and cirrhotic patients.[8,9] LOLA has direct hepatoprotective effect, exerts via an antioxidative mechanism, by stabilization of antioxidant balance of liver cells.[10,11]

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Silymarin(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 SL complex component is silybin (50 to 60% of SL), which is the major bioactive component of SL.[15,16] The SL supports healthy DNA function and is able to modulate the immune system. As a result of pharmacological activities of SL including hepatoprotection 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

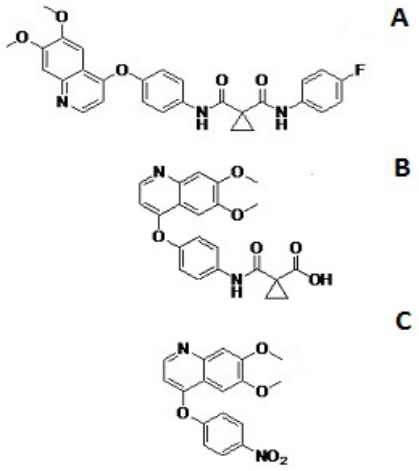

Figure 1: Chemical structures of LOLA (A), LOLA imp-1 (B), and LOLA imp-2 (C)

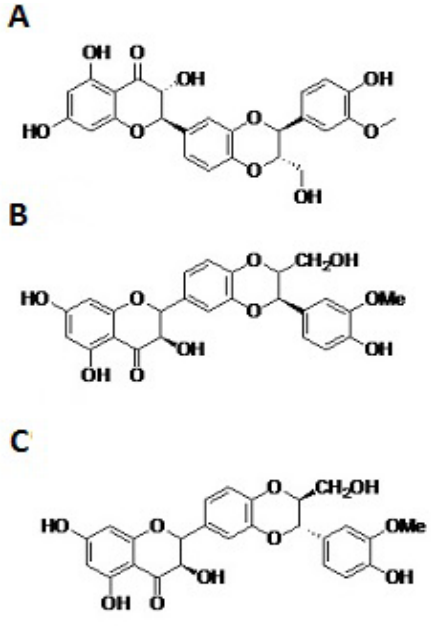

Figure 2: Chemical structures of SL (A), silybin-A (B), silybin-B (C)

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 literature review 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. Therefore, it was 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 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 ICH guidelines.[22-25]

EXPERIMENTAL

Chemicals and Reagents

Acetonitrile, orthophosphoric acid and HPLC grade water were purchased from Merk (India) Ltd. Worli, Mumbai, India. L-Ornithine l-aspartate and Silymarin (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 quartenary pump and PDA detector 2996. 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 an Agilent, Eclipse XDB-C18 column (150 × 4.6 mm, 3.5 µm) with flow rate of 1.0 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 200–400 nm inder to raise the method's sensitivity 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 1lt water. It is degassed and filtered by using 0.45 µ membrane filter.

The 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

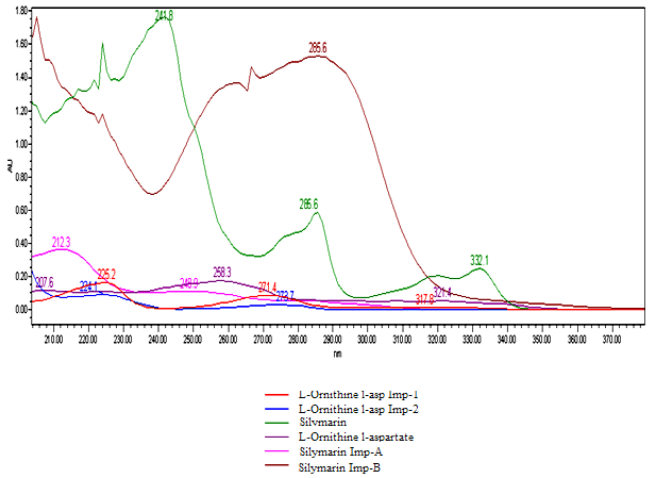

Figure 3: PDA spectra for L-Ornithine l-aspartate and silymarin

retention time, for that reason it was selected for further method development.

The Gradient program, 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 Related Substances

Ten tablets were weighed and triturated in a mortar. The weight equivalent to one tablet (350 mg of the sample) was then 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-1of 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 7.0 mg of SL (working standards), and it was transferred to 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 diluent solution, and it was filtered through 0.45 µ nylon syringe filter.

Table 1: Gradient program for LOLA and SL

Time (min)	*Mobile Phase-A*	*Mobile Phase-B*
0.00	80	20
5	50	50
10	30	70
12	80	20
17	80	20

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 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 occational shaking to dissolve and made the solution upto the mark. Further, 5ml 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 a good performance, the chromatographic conditions were optimized.

Method Optimization

Development trails were performed to obtain good resolution between LOLA and SLand 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 was 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 method's optimization, 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, 150x4.6 mm, 3.5 µ column connected to PDA detector. The flow rate of the mobile phase was 1.0 mL/min. UV detection has been done at 225 nm 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 injected. The retention times of LOLA and SL were about 4.16 and 7.0 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 and10.93 respectively. The number of theoretical plates for LOLA and SL peak were about 3098 and 47312, which indicates the efficient performance of the column. % RSD for six replicate injections was around 0.94

%, indicates that the proposed method is highly accurate.The method developed was validated as per ICH guidelines with all of the results within limits, so that the method was selected for purity of LOLA and SL. Moreover, under the optimized chromatographic conditions and using the drugs' solubility data, 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 was 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 below figures. The figures shows 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 with the analyte concentration of the sample and was determined by computing correlation coefficient from calibration curves.

The are opf the linearity of peak verses different concentrations has been evaluated for LOLA and SL and their relavent substances. The test solutions awere prepared for related substance method from impurity stock solution at various concentration levels. The linearity range was found to be 2.65 to 39.75 µg/mL for LOLA, 0.5 to 7.5 µg/mL for LOLA impurity-1, 0.25 to 3.75 µg/mL for LOLA impurity-2 and 0.74 to 11.1 µg/mL for SL, 0.55 to 8.25 µg/mL for SL impurity-A, 0.55 to 8.25 µg/mL SL impurity-B. Under optimum chromatographic conditions, we got linear relationship between the peak areas and the corresponding concentrations, Figure 6. Slopes, correlation coefficients, and

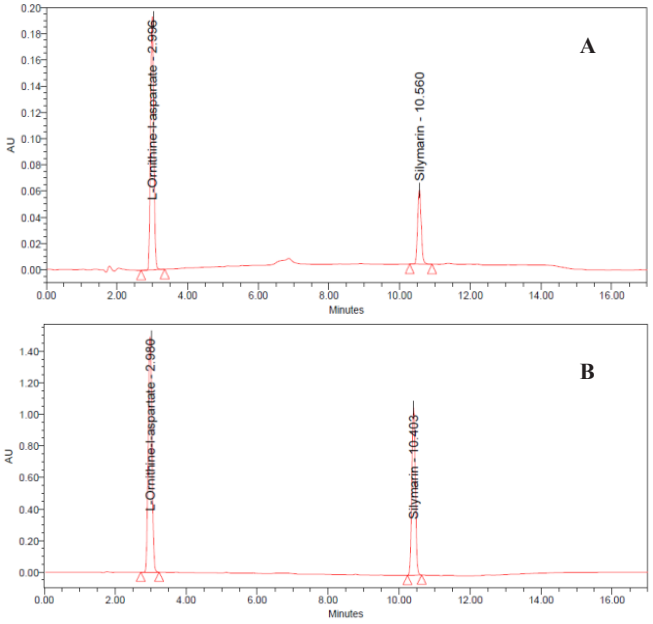

Figure 4: Chromatograms for SST (A) and RS (B)

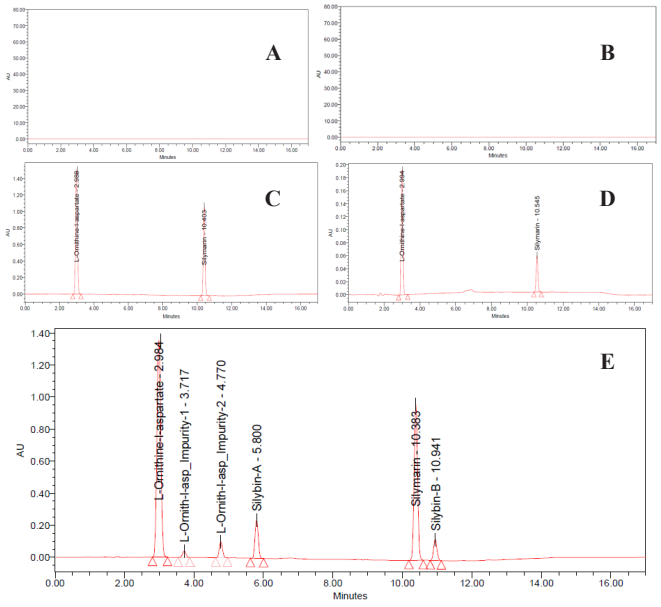

Figure 5: Chromatograms for Blank (A), Placebo (B), Standard solution (C), Sample Solution (D), and spiked sample solution (E)

Table 2: System suitability data

		Drug name			
		LOLA		*SL*	
System Suitability parameter	*Acceptance criteria*	*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	3474	4280	47609	49059

y-intercepts were determined. Correlation coefficients for all the components were under limit. The calibration curves were plotted for all the related substances and mentioned in the figures. 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 10-150 % with respect to an analyte concentration of 25 ppm (LOLA) and 7 ppm (SL). The linearity range was found to be 2.5 µg/mL to 37.5 µg/mL for LOLA and 0.7 µg/mL to 10.5 µg/mL for SL.Under optimum chromatographic conditions, linear relationships between the peak areas and the corresponding concentrationswere obtained. Correlation coefficients, slopes and y-intercepts were calculated. Correlation coefficients for all the components were under the limit. Calibration curves were plotted for all the related substances and mentioned in the figures. The results demonstrate that there was an excellent correlation existed between the peak area and the corresponding concentrations, Figure 8.

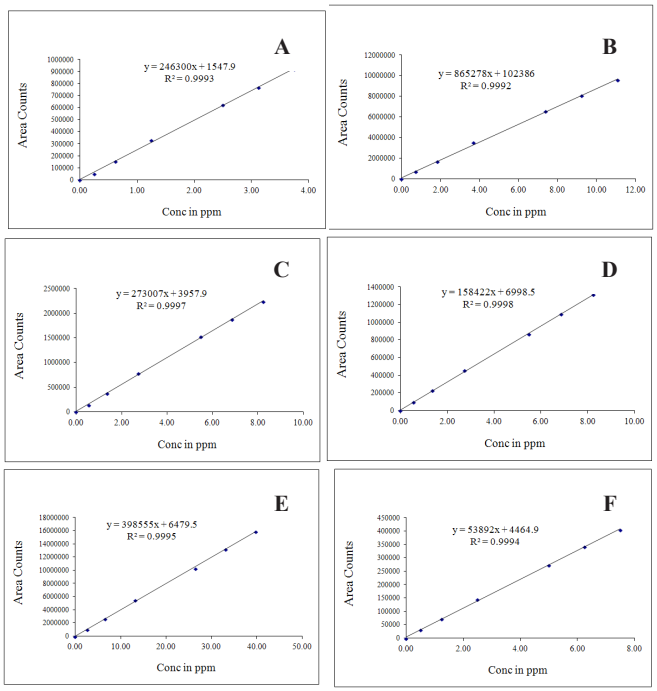

Figure 6: RS Linearity Plot for LOLA (A), LOLA imp-1 (B), LOLA imp-2 (C), SL (D), SL imp-A (E) and SLimp-B (F).

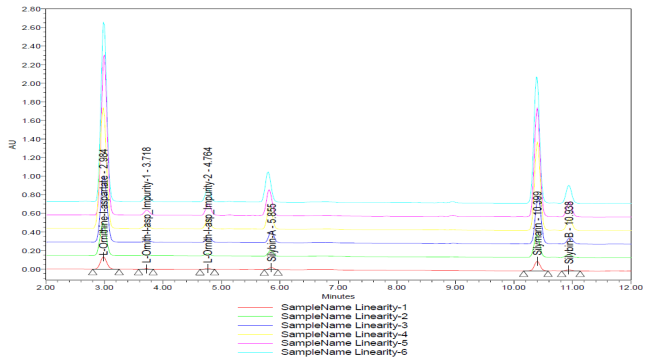

Figure 7: Overlay chromatogram for RS-Linearity

Accuracy

It is the closeness of the measurements to a specific value with a found value.Recovery experiments have been carried out to determine the accuracy of the related substance technique for the quantification of all 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 calculated and those are ranging from 99.8 to 100.9%. Good percentage recoveries were obtained and tabulated, Table 3.

The assay technique's accuracy 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 was injected three times for each

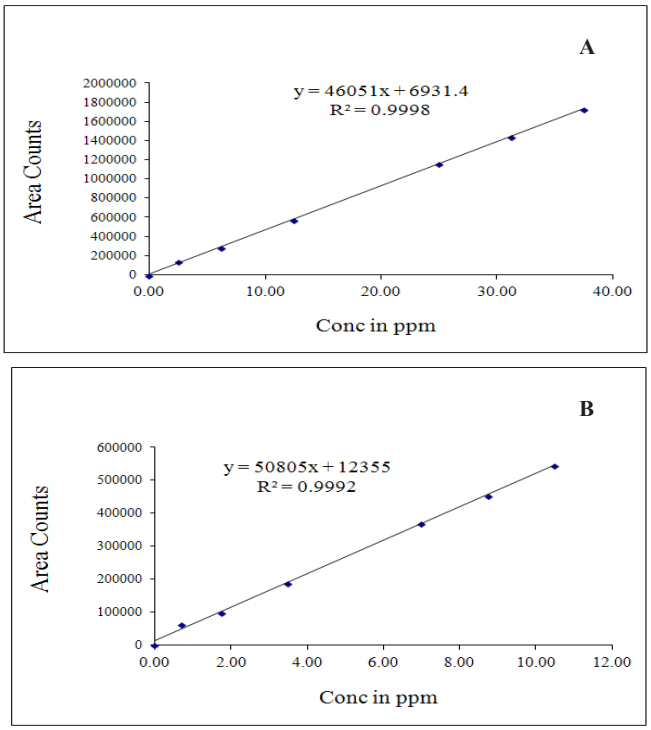

Figure 8: Assay Linearity Plot for LOLA (A) and SL (B)

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	100.0	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			
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-total Imp.)*	*% Assay*
1	2.16	0.47	99.53	100.20
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	0.450	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			
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-Total Imp.)*	*% Assay*
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

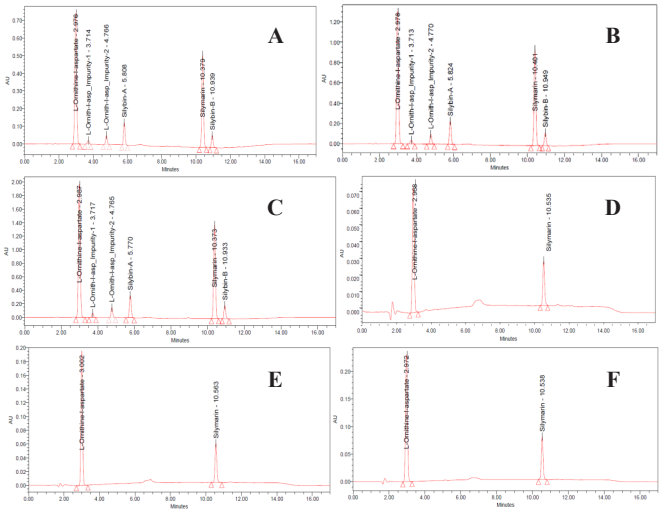

Figure 9: Chromatograms for RS-Acc 50 % (A), RS-Acc 100 % (B), RS-Acc150 % (C), Assay-Acc 50 % (D), Assay-Acc 100 % (E), Assay-Acc 150 % (F)

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

Precision

An analytical technique's precision 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 individual 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 specifed 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 makeing the same laboratory. Results are furnished in Tables 5-8.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of detection is the lowest amount of analyte in the sample that gives the measurable response (3.3 σ/S) and limit of quantification is the lowest amount of analyte that gives accurately quantified response (10 σ/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 the lower concentrations of the standard solutions progressively. The concentrations LoD of LOLA and their impurities-1,2 are 0.265,0.05,0.025 µg/ml and their s/n values are 7,5,3 and SL and their impurities-A,B are 0.074,0.055,0.055 µg/ml their s/n values are 6,4,4. The LoQ concentration for LOLA and their impurities-1,2 are 2.65, 0.5, 0.25 µg/ml their

Table 8: Intermediate precision results for SL

	% of related substances			
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-Total imp.)*	*% Assay*
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

Table 7: Assay Results for intermediateprecision of LOLA

	% of Related substances			
Sample No.	*Spiked impurities*	*Total impurities*	*% Purity (100-total imp.)*	*% Assay*
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 9: Robustness datafor LOLA and SL

	% RSD			
	LOLA		*SL*	
Parameter name	*RS*	*Assay*	*RS*	*Assay*
Flow (0.8 ml/min)	0.64	0.52	0.72	0.34
Flow (1.2ml/min)	0.38	0.26	0.68	0.92
Organic solvent (+10%)	059	0.84	0.29	0.85
Organic solvent (-10%)	0.52	0.56	0.64	0.46
Wave length (+5nm)	0.75	0.38	0.58	0.28
Wave length (-5nm)	0.77	0.17	0.49	0.56

s/n values are 27,25,23 and SL and their impurities-A,B are 0.74, 0.55, 0.55 µg/mL their s/n values are 26, 24, 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. To measure the robustness of a developed method, the experiment's conditions 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 effected 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 upto 24 hr at room temperature and at 2–8°C. These solutions were then injected into the system and calculated the percentage 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 hr during the analysis. Results are furnished in Table 10.

Table 10: Results for solution stability of LOLA and SL

Stability	*Purity of LOLA*	*% of deviation*	*Purity of SL*	*% of deviation*
Initial	99.82	0.00	99.64	0.00
6Hr	99.74	0.08	99.55	0.09
12Hr	99.71	0.11	99.49	0.15
18Hr	99.65	0.17	99.32	0.32
24Hr	99.52	0.30	99.27	0.37

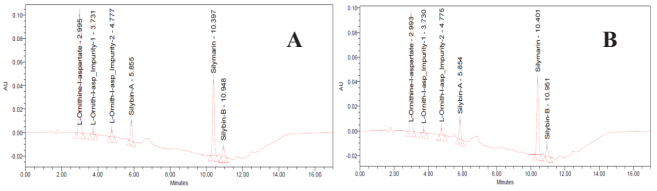

Figure 10: Chromatogram for LoD (A), LoQ (B)

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

Table 11: Results for forced degradation

	LOLA		*SL*	
Degradation Condition	*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

Table 12: Results for dissolution study for LOLA and SL

	% Drug release							
	LOLA				*SL*			
Tablet	*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	0.00	1.79	0.85	0.4	2.12	1.59	0.78	0.37

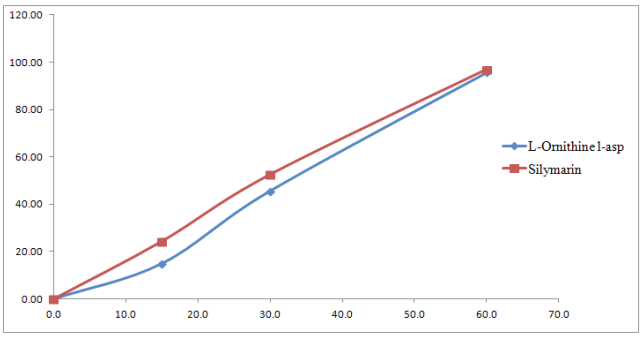

Figure 11: Dissolution profile for LOLA and SL

hydrolysis in acedic medium (0.1N HCl at 60ºC for 15 mins), hydrolysis under basic conditions (0.1 N NaOH at 60ºC for 15 mins), peroxide degradation (10% H_2O_2 at 30 minutes), reduction degradation (10 % $NaHCO_3$ at 60ºC for 15 minutes). There is no interference between the peaks and were well separated with a resolution of atleast 1.0. 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. 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. We 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 0.45 µ syringe filter after the end of each test time. The samples were prepared according to test concentration and equal volumes of these test solutions were injected into the chromatographic and peak areas were measured, Figure 11. The percentage content was calculated. Results are furnished in Table 12.

CONCLUSION

In this paper, we present a simple, selective, validated and well-defined stability-indicating gradient RP-HPLC mehod for the quantitative determination of LOLA and SL and their chromatographic impurities 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 an 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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