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STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF EPROSARTAN IN PURE AND PHARMACEUTICAL FORMULATION

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Abstract

A Stability indicating Reverse-Phase liquid chromatographic method for the simultaneous estimation of RPS and LSP was developed. The chromatographic assay involves the use of C_{18} Column (150 × 4.6mm, with particle size 5µm) with a simple mobile phase composition (Phosphate Buffer pH-3.3 and Methanol 55:45 v/v) at a flow rate of 1mL/min with U.V detection at wavelength of 230 nm. The method showed good linearity in the concentration range of 90.0-210.0 µg/mL for LSP and 24.0–56.0 µg/mL for RPS. The proposed method was also successfully applied to 20 tablets of marketed formulation (Neopride). The developed method was successfully validated as per the ICH guidelines for following parameters. Accuracy, precision, repeatability, ruggedness, robustness, system suitability tests, etc. The RSD for Intra-day and Inter-day precision was found to be 1.02-1.83, 0.96-1.42 For LSP and 0.55-0.59, 0.75-0.63 for RPS. Average Percent recovery was found to be 98±0.2, 100.57±0.2, 99.80±0.2 for LSP and 101.38±0.2, 98.67±0.2, 99.53±0.2 for RPS which was a good agreement with labeled amount of pharmaceutical formulation. The stability indicating capacity was tested by accelerated degradation of marketed formulation in acidic (0.1 N HCl), basic (0.1 N NaOH), Neutral (water), Oxidative (3% H_2O_2), Thermal (60^0C), Sunlight exposure.

Keywords: RPS, LSP, Stability Indicating, RP-HPLC, Force degradation, Validation, Assay Method, Study.

1. Introduction

The technique HPLC is so called because of its improved performance over the classical column chromatography. The technique basically involves the use of porous material as a stationary phase and the liquid mobile phase is pumped into the column under high pressure. The development of this technique is attributed to the small particle size of stationary phase. As the particle size is small the resistance to the flow of mobile phase is very high that is the reason why the high pressure is recommended.[9,5] The stability indicating assays are defined as validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. Stress testing is the main tool that is use to predict stability problems, develop analytical methods, and identify degradation product and pathways. Stress testing is likely to be carried out on single batch of the drug substance. It should include the effect of temperature in 10ºC increments (Eg.50ºC, 60ºC etc). Above that for accelerated testing, humidity (Eg. 75% RH or greater) where appropriate oxidation and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension[13,21]. Photostability testing should be an integral part of stress testing. The literature survey showed that the very few stability indicating assay methods for the above combination has been reported so the present study was undertaken to develop economical, simple, accurate, precise and reproducible stability indicating RP-HPLC analytical method for estimation of these drugs in their combined dosage form[3-8, 14-17]. Rabeprazole sodium (RPS) [Figure1] chemically is 2-({[4-(3-methoxypropoxy) methyl2pyridinyl] methyl} sulphenyl)-1*H*-benzimidazole sodium. And it is used in the states of Gastro-oesophageal reflux disease, Peptic ulcer. It is white to light yellow, crystalline powder and hygroscopic. It is freely soluble in water, chloroform, ethyl acetate, Methanol. It is proton pump inhibitor. While the Levosulpiride (LSP) [Figure 2] chemically is *N*-[[(2*S*)-1-Ethylpyrrolidin-2-yl] methyl]-2-methoxy-5 sulfamoylbenzamide and it is used in anxiety, Depression, schizophrenia. It is almost white and it is practically insoluble in water and sparingly soluble in methanol. It is a D_2 Receptor agonist[18-20].

Figure 1: Structure of RPS.

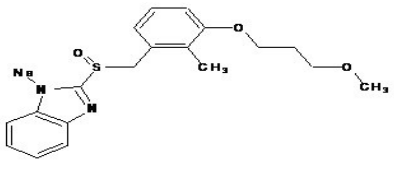
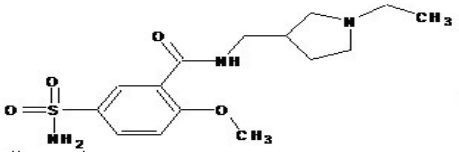


Figure 2: Structure of LSP.



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2. Experimental

2.1 Reagents and Chemicals: Standard samples of RPS and LSP were received as a gift samples from Baroque Pharma, Khambhat (Gujarat) and Symed Labs Limited, Hyderabad (AP). The marketed formulation Neopride (INTAS Pharmaceutical) was purchased from the local market. While the methanol, acetonitrile, water, triethylamine and ortho-phosphoric acid of HPLC Grade were used. and the sodium di hydrogen phosphate and bisodium hydrogen phosphate used in buffer preparation were of AR Grade. The mobile phase is degassed and filtered through 0.45µ filter before use.

2.2 Instrument: Waters 600 system with 996 PDA detector & software empower program having Kinetex, RP C-18, Column (150 × 4.6mm), with particle size 5µm was used for the study. An isocratic elution is performed using Methanol and Phosphate buffer pH 3 (55:45 v/v) as a mobile phase at a flow rate of 1 mL/min and the detection is carried out at 230 nm.

2.3 Preparation of Mobile Phase: Initially different Mobile phase trails were undertaken at different flow rate and pH values but the most optimized chromatogram was obtained at Methanol and Phosphate buffer pH 3.3 (55:45) composition. The optimized chromatogram for the above mobile phase is shown in Figure 4.

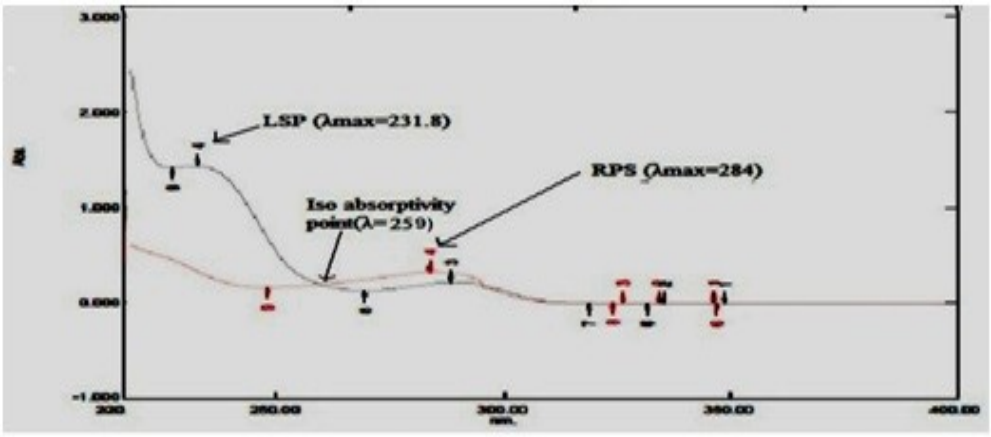
2.4 Preparation of Phosphate buffer: 13.8 g of Monobasic Dihydrogen Phosphate in 1000 mL volumetric flask (0.1mM), 945.0 mL of this solution was mixed with 45.0 mL of 0.1 mM ortho phosphoric acid and pH-3.2 buffer was prepared then by addition of 0.1mM Monobasic Dihydrogen Phosphate solution pH-3.3 was adjusted.

2.5 Preparation of Stock Standard Solution (Solution A): Standard stock solution was prepared by dissolving 75.0 mg of LSP and 20.0 mg of RPS in 10.0 mL of methanol that give concentration 7500 and 2000 µg/mL for LSP and RPS respectively.

2.6 Preparation of Working Standard Solution (Solution B): From the standard stock solution, the mixed standard solutions were prepared using methanol to contain 150.0 µg/mL of LSP and 40.0 µg/mL of RPS.

2.7 Selection of analytical wavelength: From the overlain spectra of both the drugs the analytical wavelength selected was 230 nm.

Figure 3: Overlain spectra of LSP & RPS.



2.8 Linearity: From the stock standard solution, aliquots portions (0.9 – 2.1 mL) were transferred into a series of 10.0 mL volumetric flasks and diluted up to the mark with mobile phase to obtained final concentration in the range of 90.0-210.0 µg/mL for LSP and 24.0 – 56.0 µg/mL for RPS. A constant volume of 20.0 mL of each sample was injected with the help of auto sampler. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* the drug concentration, the calibration curve for both drugs is shown in Figure 5 and Figure 6.

2.9 Analysis of Marketed Formulation: Accurately weighed quantity equivalent to 75.0 mg of LSP and 20.0 mg RPS was transferred to 50.0 mL of volumetric flask containing methanol and volume was adjusted to mark with methanol, and filtered through Whatman filter paper. An appropriate volume, 4.0 mL was diluted to 10.0 mL with methanol. The resulting solution (20.0 µl) was injected into the system and chromatogram was recorded. The concentration was determined by using linear regression equation. Calculate the amount of LSP/RPS in mg per capsule using following formula:

$$\text{mg/capsule} = \frac{AT_1 \times WS_1 \times Ds \times P_1}{AS_1 \times W_T \times D_t} \times \text{Avg.wt}$$

Further calculate the amount of LSP/RPS present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/capsule)} \times 100}{\text{Label claim of LSP/RPS}}$$

2.10 Method Validation[12]: The proposed method was validated as per ICH guidelines. The solutions of

the drugs were prepared as per the earlier adopted procedure given in the experiment.

2.10.1. Accuracy: It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of LSP and RPS standard was added to preanalysed sample (150.0 µg/mL of LSP and 40.0 µg/mL of RPS) and subjected to the proposed HPLC method. . The percent recovery was then calculated by using following formula

$$\% \text{ Recovery} = \frac{E_w - B}{C} \times 100$$

Where, E_w = Total drug estimated (mg)

B= Amount of drug contributed by preanalyzed capsule powder (mg)

C= Weight of pure drug added (mg)

2.10.2. Precision: Intraday precision was determined by analyzing, the three different concentration 120.0 µg/mL, 150.0 µg/mL and 180.0 µg/mL of LSP and 32.0 µg/mL, 40.0µg/mL and 48.0 µg/mL of RPS respectively, for three times in the same day. Interday variability was assessed using above mentioned three concentration analysed on three different days, over a period of one week. This result shows reproducibility of the assay.

2.10.3. Repeatability: Repeatability experiment was performed by injecting sample 150.0 µg/mL of LSP and 40.0 µg/mL of RPS into the system and measuring the peak area.

2.10.4. Ruggedness: Ruggedness of the method was studied by two different analysts using same operational and environmental conditions. An appropriate concentration 150.0 µg/mL of LSP and 40.0 µg/mL of RPS was analysed and concentration were determined. The procedure were repeated for six times.

2.10.5. Robustness: Robustness of the method was studied by making deliberate variation in parameters such as flow rate (± 0.1mL), % of methanol in the mobile phase composition (± 10%), and change in detection wavelength (±2 nm) and the effect on the results were examined. It was performed using 150.0 µg/mL and 40.0 µg/mL solution of LSP and RPS in triplicate respectively.

2.10.6. System Suitability Test: According to USP, system suitability test are integral part of liquid chromatography methods. System suitability testing is essential for the assurance of the quality performance of the chromatographic condition were tested for system suitability testing.

2.11. Force degradation studies[20,13]:

2.11.1. Acid Degradation: Accurately weight capsule equivalent to 75.0 mg of LSP & 20.0 mg of RPS were dissolved in 5.0 mL of aqueous 0.1N HCl in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1 hr.

2.11.2. Alkali Degradation: Accurately weight capsule equivalent to 75.0 mg of LSP & 20.0 mg of RPS were dissolved in 5.0 mL of aqueous 0.1N sodium hydroxide in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr.

2.11.3. Neutral Degradation: Accurately weight capsule equivalent to 75.0 mg of LSP & 20.0 mg of RPS were dissolved in 10.0 mL of water in a separate volumetric flask and kept at room temperature for 1hr.

2.11.4. Oxidative Degradation: Accurately weight capsule equivalent to 75.0 mg of LSP & 20.0 mg of RPS were dissolved in 10.0 mL of 3% H_2O_2 in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr.

2.11.5. Photo Degradation: Accurately weight capsule equivalent to 75.0 mg of LSP & 20.0 mg of RPS were uniformly spread as thin layer in a separate covered petri-dish which were then kept in sunlight for 3 days. Finally, the prepared stressed samples were injected in equilibrated HPLC column and chromatograms were recorded using optimized mobile phase and chromatographic conditions.

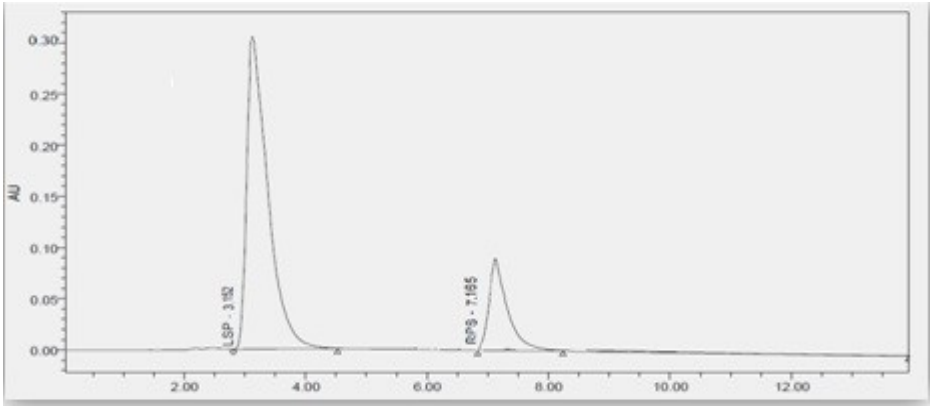
3. Results and Discussion

RP-HPLC stability indicating assay method was developed. Both of these drugs are well resolved on Kinetex, RP C-18,(150 × 4.6mm), with particle size 5µm using Methanol and Phosphate buffer pH 3 (55:45) as a mobile phase. The flow rate 1ml/min and the detection is carried out at 230 nm.

3.1 HPLC method development and Optimization: Initially several mobile phase trials were undertaken at different flow rate and pH values. The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So, numbers of trials were taken for the selection of mobile phase as shown in. After number of trials Phosphate Buffer pH-3.3 and Methanol (55:45 v/v) was selected. The selection of analytical wavelength was truly based on the overlain spectra of both drugs in methanol as solvent as decipted in Figure 3. So the final optimized chromatographic condition is

Chromatographic Mode	:Chromatographic Condition
HPLC System	:Waters 996 system
Detector	:PDA detector
Data processor	:Empower
Stationary phase	:Kinetex, RP C-18, (150 × 4.6mm, 5µm)
Mobile phase	:Buffer:Methanol (55:45, v/v)
Detection wavelength	:230 nm
Flow rate	:1 mL/min
Sample size	:20 µL

Figure 4: An optimized chromatogram Methanol and Phosphate buffer pH3 (55:45)



3.2 Analysis of Marketed formulation: The marketed formulation used was Neopride (INTAS Pharmaceuticals). All the dilutions were carried out in methanol. An appropriate volume, 4.0 mL was diluted to 10.0 mL with methanol. The resulting solution (20.0 µl) was injected into the system and chromatogram was recorded. The concentration was determined by using linear regression equation. The % RSD is 0.57 equal for both LSP and RPS.

Table no 1: Summary of Marketed formulation Analysis

Sr. No.	Weight of std.(mg)		Weight of sample	Peak area of std		Peak area of sample		% Label claim	
	LSP	RPS	(mg)	LSP	RPS	LSP	RPS	LSP	RPS
1			336			4792004.4	907576.52	101.14	102.41
2			334.2			4738450.5	897433.71	100.01	101.27
3	75	20	335.8	4738096	886199	4774520.7	904265.15	100.77	102.04
Mean								100.64	101.91
±SD n=3								0.58	0.58
%RSD								0.57	0.57

3.3 Method Validation[12]:

3.3.1. Accuracy: The accuracy was ascertained by the recovery studies in which known amount of standard drugs were added to preanalysed solutions at a level of 80%, 100%, 120% and then subjected to proposed HPLC method. Average Percent recoveries were found to be 98±0.2, 100.57±0.2, 99.80±0.2 for LSP sand 101.38±0.2, 98.67±0.2, 99.53±0.2 for RPS which was in good agreement with labeled amount of Pharmaceutical formulation.

Table no 2: Percentage recovery for RPS.

Label claim (mg/Cap)	Amount Added (mg)	Total Amount	Amount Recovered (mg)	% RSD (n=3)	% Recovery
75.00	60(80%)	135.00	133.45	1.75	98.82±0.2
75.00	75(100%)	150.00	151.20	1.39	100.57±0.2
75.00	90(120%)	165.00	164.30	0.90	99.80±0.2

Table no 3: Percentage recovery for LSP.

Label claim (mg/Tab)	Amount Added(mg)	Total Amount	Amount Recovered (mg)	% RSD (n=3)	% Recovery
20.00	16 (80%)	36.00	36.32	1.63	101.38±0.2
20.00	20 (100%)	40.00	39.45	0.78	98.67±0.2
20.00	24 (120%)	44.00	43.76	1.34	99.53±0.2

3.4 Linearity: It was ascertained by Transferring aliquots portions (0.9 – 2.1 mL) into a series of 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of 90.0-210.0 µg/mL for LSP and 24.0 – 56.0 µg/mL RPS. A constant volume of 20.0 mL of each sample was injected with the help of auto sampler. The calibration curve is decipted in figure

Figure 5: Calibration curve of LSP.

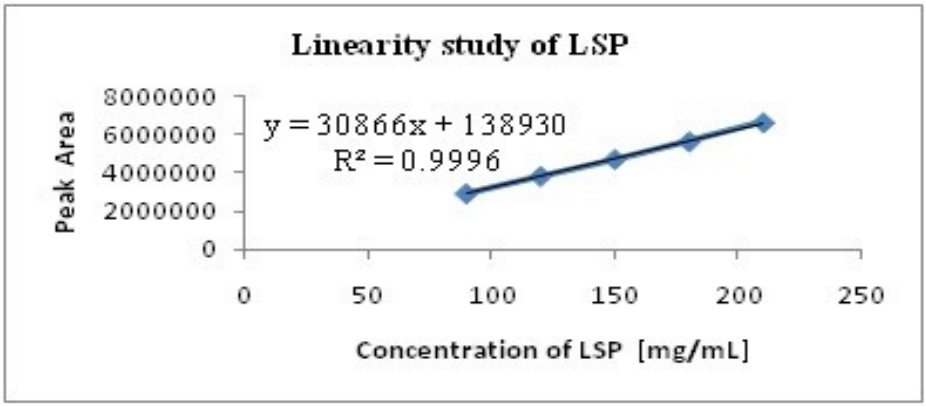


Figure 6: Calibration curve of RPS.

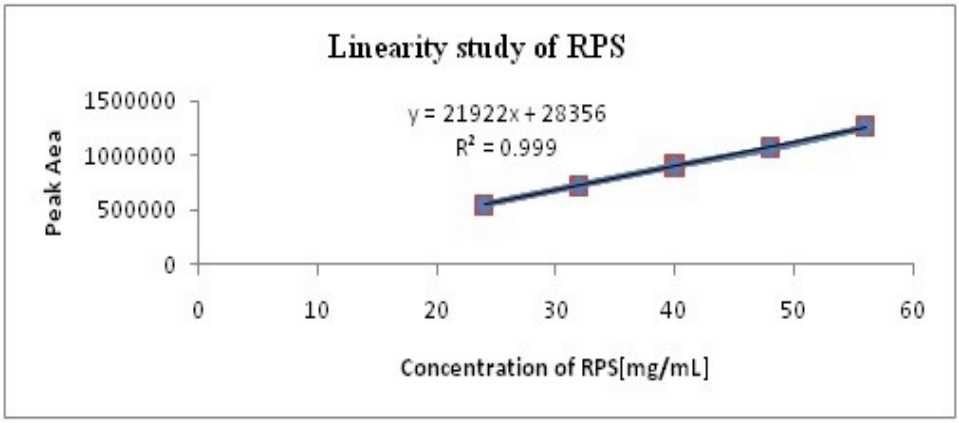


Table no 4: Linearity data for RPS.

Sr. No.	Concentration of RPS [ug /mL]	Peak area [Mean ± SD; n = 5]	% RSD
1	24	555799 ± 6421.96	1.15544
2	32	729075 ± 8380.05	1.14941
3	40	907587 ± 9524.47	0.61346
4	48	1072974 ± 6582.24	0.83938
5	56	1260726 ± 10582.24	1.04944

Table no 5: Linearity Data for LSP.

Sr. No	**Concentration of LSP [ug/mL]**	**Peak area [Mean ± SD; n=]**	**% RSD**
1	90	2934623 ± 15117.97	1.16
2	120	3849516.7 ± 17471.28	1.15
3	150	4738096 ± 14940.48	0.61
4	180	5665302. ± 18587.87	0.84
5	210	6656635 ± 20123.54	0.20

3.5. Precision: Intraday precision was determined by analyzing, the three different concentration 120.0 µg/mL, 150.0 µg/mL and 180.0 µg/mL of LSP and 32.0 µg/mL, 40.0µg/mL and 48.0 µg/mL of RPS respectively, for three times in the same day. Interday variability was assessed using above mentioned three concentration analysed on three different days, over a period of one week. This result shows reproducibility of the assay.

Table no 6: Precision data for LSP.

Conc. [µg/mL]	Intraday Amount found [µg/mL]			Interday Amount found [µg/mL]		
	Mean	±SD n=3	% RSD	Mean	±SD n=3	% RSD
120	120.45	8380.05	1.02	119.11	8470.05	0.96
150	148.21	9524.47	0.47	151.42	9844.47	1.18
180	178.02	6582.24	1.83	177.98	7452.24	1.42

Table no 7: Precision Data for RPS.

Conc. [µg/mL]	Intraday Amount found [µg/mL]			Interday Amount found [µg/mL]		
	Mean	±SD n=3	% RSD	Mean	±SD n=3	% RSD
32	32.47	8390.05	0.55	32.27	8460.05	0.75
40	40.5	9594.47	0.52	40.35	9624.47	0.49
48	48.51	6752.24	0.59	48.41	67888.24	0.63

3.7. Repeatability: Repeatability experiment was performed by injecting sample 150.0 µg/mL of LSP and 40.0 µg/mL of RPS into the system and measuring the peak area. The RSD was found to be 0.45 for LSP and 0.82 for RPS.

Table no 8: Showing repeatability data for LSP and RPS

Concentration of LSP [µg/mL]	Peak Area	Concentration of RPS [µg/mL]	Peak Area
150	4799004.0	40.00	906452.5
150	4748450.0	40.00	896452.2
150	4779520.0	40.00	904542.1
150	4785002.0	40.00	890525.2
Mean	4777994.0		899493.0
± SD	21336.27		7384.58
%RSD	0.45		0.82

3.8. Ruggedness: Ruggedness of the method was studied by two different analysts using same operational and environmental condition. An appropriate concentration 150.0 µg/mL of LSP and 40.0 µg/mL of RPS was analysed and concentration were determined. The % RSD for LSP was found to be 0.81-0.64 and the % RSD for RPS was found to be 0.73-0.83 for RPS.

Table no 9: Ruggedness for LSP

Condition	Mean	± SD (n=3)	%RSD
Analyst I	4780020	38371.75	0.81
Analyst II	4782788	30715.5	0.64

Table no 10: Ruggedness for RPS

Condition	Mean	± SD (n=3)	%RSD
Analyst I	898547	6587.25	0.733100216
Analyst II	896521	7458.85	0.831977165

3.7. Robustness: Robustness of the method was studied by making deliberate variation in parameters such as flow rate (± 0.1mL), % of Methanol in the mobile phase composition (± 10%), and change in detection wavelength (±2 nm) and the effect on the results were examined. It was performed using 150.0 µg/mL and 40.0 µg/mL solution of LSP and RPS in triplicate. The % RSD was found to be 0.76-0.74 for LSP and %RSD for RPS was found to be 1.71-1.06.

Table no 11: Robustness for LSP.

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	4783522	36492.14	0.76
Change in detection wavelength (± 2 nm)	4794517	35715.5	0.74

Table no 12: Robustness for RPS.

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	907455	15474.49	1.71
Change in detection wavelength (± 2 nm)	897556	9498.56	1.06

3.9. System suitability Test:

Table no 13: Showing system suitability data.

System Suitability Parameter	Standard	Proposed Method of LSP	Proposed Method of RPS
Retention time (tR) [min]	5-10 min	3.152± 0.02 min	7.165± 0.02 min
Resolution	Should be > 2	3.32	
Theoretical plate (N)	More than 2000	3592	3325

3.10. Force degradation Studies[21,13]:

Table no 14: Summary of Force degradation Studies.

Condition	%Assay LSP	%Degradation LSP	%Assay RPS	%Degradation RPS
Initial sample	99.74	-	98.95	-
0.1N HCL	98.50	1.24	98.10	0.90
0.1N NaOH	98.78	0.21	98.88	0.86
3% H2O2	97.44	1.70	96.90	2.05
Neutral	99.72	-	98.25	0.57
Sun	96.92	1.92	95.57	4.48

Figure 7: Chromatogram of Acid degradation (0.1 N HCL).

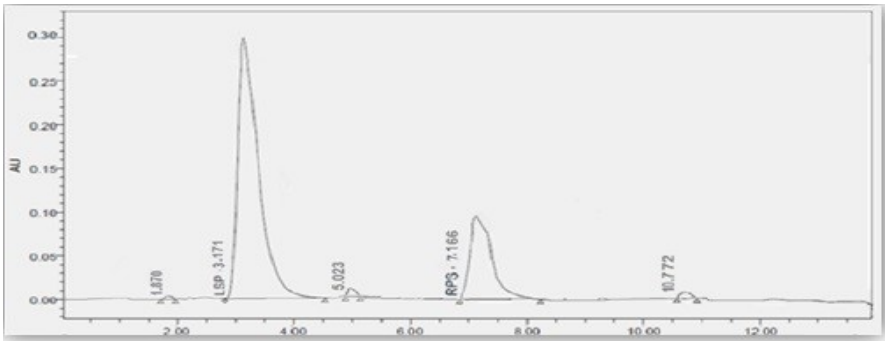


Figure 8: Chromatogram of Basic Degradation (0.1 N NaOH).

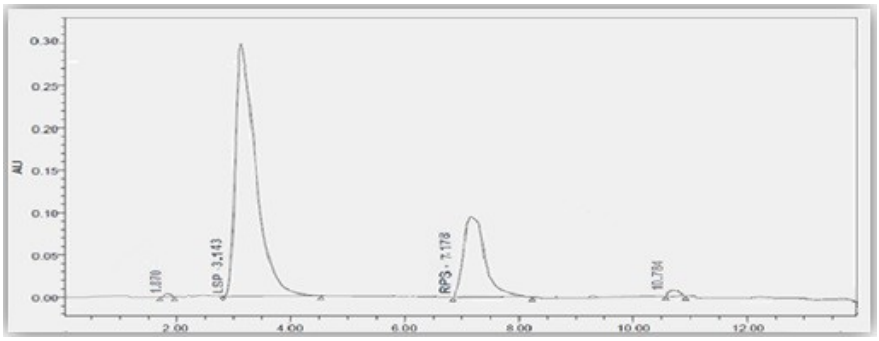


Figure 9: Chromatogram of Neutral Degradation.

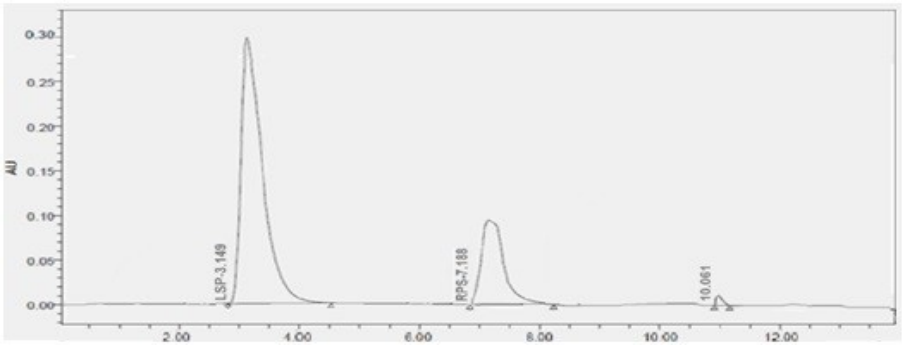


Figure 10: Chromatogram of Oxidative degradation.

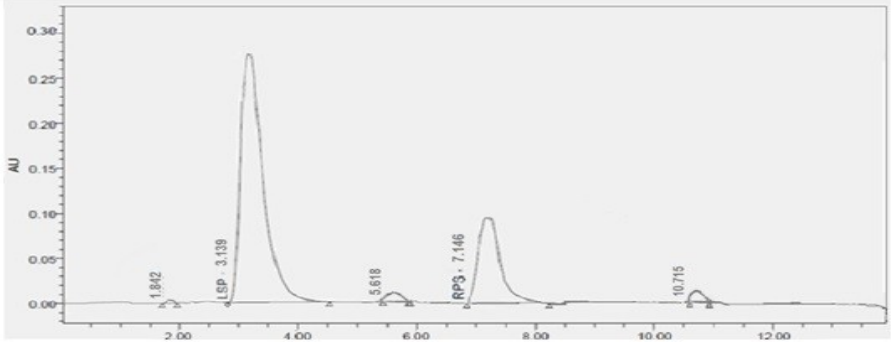
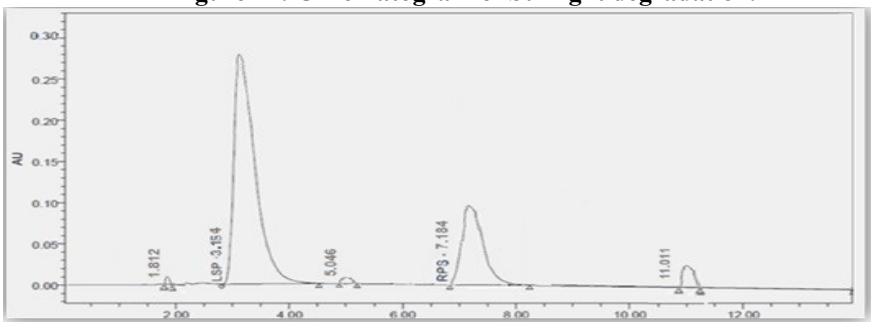


Figure 11: Chromatogram of Sunlight degradation.



4. Conclusion

The proposed method was validated as Per the ICH Guidelines. The proposed method also showed the good resolution between RSP and LSP with run time of 13.5 min. The method is very simple and rapid and no where involves complicated sample preparation and mobile phase preparation. Also the proposed method showed good specificity and selectivity in order to determine RPS and LSP in the presence of their degradation products. The linearity and reproducibility data of the drugs carried out by this method showed that no major interference is caused in the estimation of the drugs. Therefore the method can be use for routine quality control of these drugs.

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