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Development and Validation of Analytical Method for Estimation of Paracetamol, Lornoxicam and Serratiopeptidase in their Combined Dosage Form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Paracetamol, Lornoxicam and Serratiopeptidase in Tablet dosage form. Chromatography was carried on C18 (25cm x 0.46 cm) Hypersil BDS analytical column using mobile phase Buffer (ammonium acetate pH5): Methanol (60:40) at a flow rate of 01.0 ml/min. The detection was carried out at 215 nm. The retention time of Gresiofulvin is found to be 13min. Correlation co-efficient for PCM, LOR and SER was found to be 0.999, 0.998 and 0.998 respectively. Assay result of marketed formulation of PCM, LOR and SER was found to be in 98.67%, 98.51% and 98.668% respectively. The proposed method was validated with respect to linearity, accuracy, precision, selectivity, and robustness. Recovery PCM, LOR and SER was found in the range of 99.47% - 100.89%, 99.81% - 100.47%, 100.23% - 100.71% respectively. Statistical Analysis proves that the developed methods were successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEY WORDS: Paracetamol, Lornoxicam, Serratiopeptidase, RP-HPLC, Mobile phase, Validation

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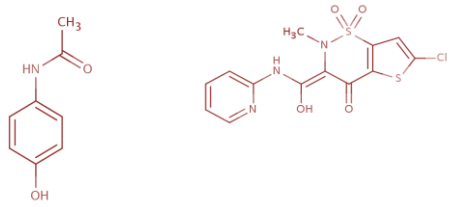
INTRODUCTION:

The IUPAC name of the Paracetamol and Lornoxicam is N-(4-hydroxyphenyl)acetamide and (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide respectively, with molecular formula $C_8H_9NO_2$ and $C_{13}H_{10}ClN_3O_4S_2$ respectively and molecular weight 151.1626 and 371.819 respectively. The molecular structure of the drug is given in Fig.1.

This combinational drug is used as analgesic as well as anti-inflammatory.Paracetamol and Lornoxicam gives relief in pain and fever whereas Serriopeptidase acts as anti-inflammatory. This immunologically active enzyme is completely bound to the alpha 2 macroglobulin in biological fluids. Serrapeptase digests non-living tissue, blood clots, cysts and arterial plaque and inflammation in all forms. This combination is used in rheumatoid arthritis, swollen joints, pain reliever.

Paracetamol is Official in Indian Pharmacopoeia (2007), US Pharmacopoeia 37 (NF 32) and Serratiopeptidase is official in Indian Pharmacopoeia (2010). However no analytical method has been reported till date for the estimation of Paracetamol, Lornoxicam and Serratiopeptidase using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Paracetamol, Lornoxicam and Serratiopeptidase in Pharmaceutical dosage form using RP-HPLC. The proposed method are optimized and validated as per ICH guidelines.



Paracetamol Lornoxicam

Figure 1: Chemical structure

Materials and methods

Materials

HPLC Thermo separation Product TSP UV 2000.Gresiofulvin was purchased from GITAR LABORATORY. The commercial fixed dose LOROX-SP (250 mg) was procured from local market. All solvents (HPLC grade) were obtained from Merck Chemicals.

Working Standard preparation:

Preparation of standard solution of mixtures of SER (5 µg/mL) PCM (32.5 µg/mL) and LOR (0.8 µg/mL)

Take 1 mL from SER stock solution,1 mL from PCM stock solution and 1ml from LOR stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by Borate Buffer which was used in particular trials.

Procedure for derivatization:

- Sodium borate buffer (200mM NaOH, pH ~8.2)

a. Add 8g NaOH (MW=40), 47g boric acid (MW=61.83) to 900ml distilled water, make sure all powers dissolve completely.

b. Make final volume to 1L by adding water.

c. Use 0.2 micron filter membrane to filter. pH should be around 8.2.

- FMOC-Cl Solution preparation: 500mg of FMOC-cl into a 100ml volumetric flask

Stock soln: 5mg/ml in acetonitrile

Glycine stock soln: 10mg/ml in water

- Std stock soln of PCM: 32.5mg-100ml with methanol (325mcg/ml)

- Std stock soln of LORN:8mg-100ml with methanol (80mcg/ml)

- Std stock soln of SER: 50mg100ml with water (500mcg/ml)

Take 0.1ml of working std (SER+PCM+LOR) to a 10ml volumetric flask. Add 0.5ml FMOC-cl solution and mix for 20 second. Incubate this solution at 50C for 15 minutes in a waterbath.

In order to terminate the reaction,0.1ml glycine solution was added to the solution and mixture was vortexed for 10 seconds. Inject above Solution 20 µl.

Sample preparation:

Working Sample Preparation (SER 5 µg/mL, PCM 32.5 µg/mL and LOR 0.8 µg/mL):

Take 1 mL from this and transferred to 10 ml volumetric flask and made up volume up to the mark with Borate Buffer. Use This Solution for Derivatization as Mention Below.

Take 0.1ml of working sam (SER+PCM+LOR) to a 10ml volumetric flask. Add 0.5ml FMOC-cl solution and mix for 20 second. Incubate this solution at 50C for 15 minutes in a waterbath.

In order to terminate the reaction,0.1ml glycine solution was added to the solution and mixture was vortexed for 10 seconds. Inject above Solution 20 µl for analysis.

Method validation:

Chromatographic conditions and System Suitability Parameters:

1. **Pumps:**

Mode of chromatography: Reversed Phase Chromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

2. Oven:

Oven Temperature: 30° ± 2°C

3. Detector:

Type: DAD detector

Lamp: D2 lamp

Wavelength: 215 nm

4. Auto sampler Configuration:

Rinsing Volume: 1000 µl

Sampling speed: 20 µl/sec

5. Other parameters:

Column: C18 (25cm x 0.46 cm) Hypersil BDS
Sample Volume: 20 µl

Run time: 13 min

Mobile Phase: Buffer(ammonium acetate pH5) : Methanol (60:40)

Diluent: Methanol

6. System Suitability Parameters:

Retention time: PCM(3.393), LOR(4.170), SER(11.49)

Asymmetry: PCM(1.435), LOR(1.370), SER(1.544)

Theoretical plates: PCM(5974), LOR(7078), SER(5977)

Linearity and Range (n=3):

The linearity for PCM, LOR and SER were assessed by analysis of combined standard solution in range of 16.25-18.75 µg/ml, 0.4-1.2 µg/ml and 2.5-7.5 µg/ml respectively,
5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of PCM(325 µg/ml), LOR (8 µg/ml) and SER (50 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 16.25,24.375,32.5.40.625 and 48.75 µg/ml, 0.4,0.6,0.8,1, and 1.2 µg/ml and 2.5,3.75,5,6.25 and 7.5 µg/ml for PCM, LOR and SER respectively.

The plot of peak area against concentration was plotted. Correlation coefficient and regression line equations were calculated.

Linearity range was established through consideration of required practical range and according to each drug concentration present in the pharmaceutical product, to give accurate, precise and linear results**.**

Precision

Repeatability

The data for repeatability of peak area measurement for PCM (32.5 µg/ml), LOR (0.8 µg/ml) and SER (5 µg/ml), based on six measurements of same solution of PCM (32.5 µg/ml), LOR (0.8 µg/ml) and SER (5 µg/ml).The % RSD for PCM, LOR and SER was found to be 0.844,0.875 and 0.504 respectively.

Intraday Precision

Standard solution containing (16.25,32.5,48.75 µg/ml) of PCM and (0.4,0.8,1.2µg/ml) of LOR and (2.5,5,7.5 µg/ml) of SER were analyzed three times on the same day and % R.S.D was calculated**.**

Interday Precision:

The inter-day precision of the proposed method was determined by measuring the corresponding responses on 3 different days over a period of 1 week for 3 different concentration of Standard solution containing (16.25,32.5,48.75 µg/ml) of PCM and (0.4,0.8,1.2µg/ml) of LOR and (2.5,5,7.5 µg/ml) of SER were analyzed three times on the same day and % R.S.D was calculated.

Accuracy (% Recovery)

- ✓ The accuracy of the method was determined by calculating recovery of Paracetamol, Lornoxicam and Serratiopeptidase by the Standard addition method.
- ✓ **For PCM**

  16.25 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of PCM was calculated at each level and % recoveries were computed.
- ✓ **For LOR**

  0.4 µg/ml drug solution was taken in

three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of LOR was calculated at each level and % recoveries were computed.

- ✓ **For SER**

2.5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of SER was calculated at each level and % recoveries were computed.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.

LOD = 3.3 × σ /s ,

LOQ = 10 × σ /s

Robustness

The robustness was studied by analyzing the sample of Paracetamol, Lornoxicam and Serratiopeptidase by deliberate variation in the method parameters. The change in the response was noted. Robustness of the method was studied by changing different experimental conditions like temperature of column by ± 2°C, Flow rate by ±0.2 ml/min, Mobile phase by ± 2 %.

RESULT AND DISCUSSION:

Validation parameters:

Linearity:

The linearity for PCM, LOR and SER were assessed by analysis of combined standard solution in range of 16.25-18.75 µg/ml, 0.4-1.2 µg/ml and 2.5-7.5 µg/ml respectively,

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of PCM(325 µg/ml), LOR (8 µg/ml) and SER (50 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 16.25,24.375,32.5.40.625 and 48.75 µg/ml, 0.4,0.6,0.8,1, and 1.2 µg/ml and 2.5,3.75,5,6.25 and 7.5 µg/ml for PCM, LOR and SER respectively

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Correlation co-efficient for calibration curve PCM, LOR and SER was found to be 0.999,0.998 and 0.998 respectively.

The regression line equation for PCM, LOR and SER are as following:

For PCM: **y = 73.50x + 60.54**

For LOR: **y = 284.5x + 13.67**

For SER **y = 78.53x + 20.29**

Table 1: Linearity data for PCM.

Sr.No	Concentration (µg/ml)	Area
1	16.25	1238.628
2	24.375	1876.785
3	32.5	2457.175
4	40.625	3022.707
5	48.75	3651.753

Table 2: Linearity data for LOR.

Sr.No	Concentration (µg/ml)	Area
1	0.4	123.275
2	0.6	187.157
3	0.8	243.52
4	1	302.451
5	1.2	350.179

Table 3 : Linearity data for SER

Sr.No	Concentration (µg/ml)	Area
1	2.5	208.575
2	3.75	319.724
3	5	419.61
4	6.25	515.148
5	7.5	601.678

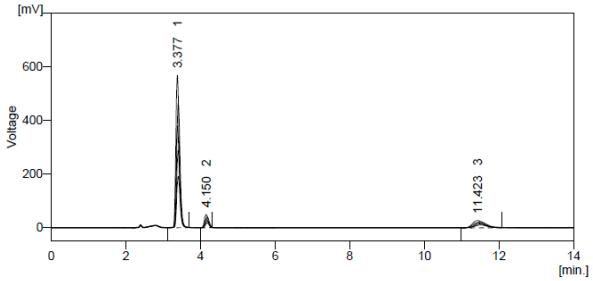


Figure 2: Overlay chromatogram of different concentrations of mixtures of PCM ,LOR and SER

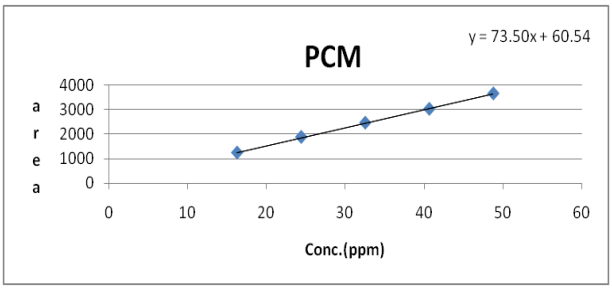


Figure 3.: Calibration Curve of PCM (16.25-48.75 µg/ml).

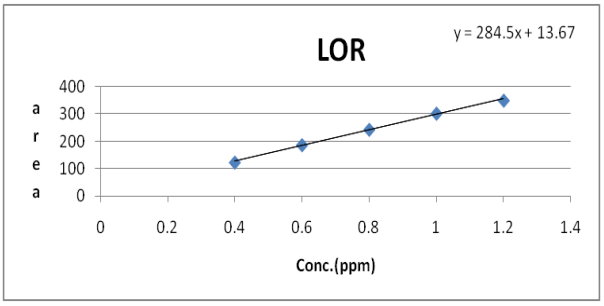


Figure 4.: Calibration Curve of LOR (0.4-1.2 µg/ml).

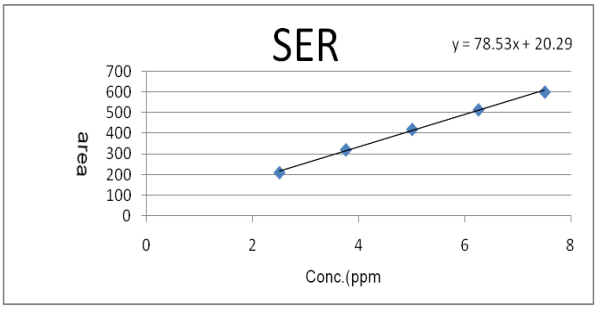


Figure 5.: Calibration Curve of SER (2.5-7.5 µg/ml).

Precision

I. Repeatability

The data for repeatability of peak area measurement for PCM (32.5 µg/ml), LOR (0.8 µg/ml) and SER (5 µg/ml), based on six measurements of same solution of PCM (32.5 µg/ml), LOR (0.8 µg/ml) and SER (5 µg/ml).The % RSD for PCM, LOR and SER was found to be 0.844,0.875 and 0.504 respectively.

Table 4: Repeatability data for PCM.

		PCM		
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
		2424.566		
		2451.261		
		2453.665		
1.	32.5	2488.101	2451.654±20.689	0.844
		2443.545		
		2448.789		

Table 5: repeatability data for LOR.

		LOR		
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
		242.185		
		244.842		
		245.095		
1.	0.8	248.535	244.765	0.875
		244.563	±2.143	
		243.374		

Table 6: repeatability data for SER

		SER		
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
		414.657		
		419.226		
		419.638		
1.	5	420.466	418.823	0.504
		420.007	±2.112	
		418.944		

II. Intraday precision

Standard solution containing (16.25,32.5,48.75 µg/ml) of PCM and (0.4,0.8,1.2µg/ml) of LOR and (2.5,5,7.5 µg/ml) of SER were analyzed three times on the same day and % R.S.D was calculated**.**

Table 7: Intraday precision data for estimation of PCM

	PCM		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	16.25	1227.763 ± 3.658	0.298
2	32.5	2417.821± 14.232	0.588
3	48.75	3649.414± 9.853	0.269

Table 8: Intraday precision data for estimation of LOR

	LOR		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	0.4	122.316 ± 0.568	0.464
2	0.8	241.042± 2.196	0.911
3	1.2	364.244 ± 1.228	0.337

Table 9: Intraday precision data for estimation of LOR

	SER		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2.5	207.008 ± 1.732	0.836
2	5	413.565± 2.353	0.569
3	7.5	621.323 ± 3.967	0.638

III. Interday precision

Standard solution containing (16.25,32.5,48.75 µg/ml) of PCM and (0.4,0.8,1.2µg/ml) of LOR and (2.5,5,7.5 µg/ml) of SER were analyzed three times on the different day and % R.S.D was calculated.

Table 10: Interday precision data for estimation of PCM.

	PCM		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	16.25	1222.3.15 ± 11.519	0.942
2	32.5	2430.983± 18.703	0.769
3	48.75	3674.560± 26.192	0.713

Table 11 : Interday precision data for estimation of LOR.

	LOR		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	0.4	121.894 ± 1.154	0.946
2	0.8	242.902± 1.771	0.729
3	1.2	366.667 ± 3.303	0.901

Table 12: Interday precision data for estimation of SER.

	SER		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2.5	204.668 ± 3.889	1.900
2	5	413.011± 7.480	1.811
3	7.5	626.649 ± 6.029	0.962

2.1.3 Accuracy:

- ✓ **For PCM**

16.25 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of PCM was calculated at each level and % recoveries were computed.

- ✓ **For LOR**

0.4 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of LOR was calculated at each level and % recoveries were computed.

- ✓ **For SER**

2.5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of SER was calculated at each level and % recoveries were computed.

Table 13: Recovery data for PCM.

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1		16.25	13	13.146	101.126	
2	80 %	16.25	13	13.205	101.577	100.897 ± 0.818
3		16.25	13	12.999	99.989	
4		16.25	16.25	16.393	100.883	
5	100 %	16.25	16.25	16.328	100.480	99.932 ± 1.313
6		16.25	16.25	15.995	98.434	
7		16.25	19.5	19.660	100.819	
8	120 %	16.25	19.5	19.308	99.015	99.478 ± 1.180
9		16.25	19.5	19.227	98.599	

Table 15: Recovery data for LOR

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	0.4	0.32	0.322	100.519	99.871 ± 1.345
2		0.4	0.32	0.315	98.324	

3		0.4	0.32	0.322	100.769	
4		0.4	0.4	0.406	101.533	
5	100 %	0.4	0.4	0.401	100.160	100.472 ± 0.945
6		0.4	0.4	0.399	99.722	
7		0.4	0.48	0.487	101.406	
8	120 %	0.4	0.48	0.478	99.573	99.818 ± 1.481
9		0.4	0.48	0.473	98.475	

Table16: Recovery data for SER

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1		2.5	2	2.035	101.761	
2	80 %	2.5	2	1.994	99.720	100.711 ± 1.022
3		2.5	2	2.013	100.652	
4		2.5	2.5	2.528	101.132	
5	100 %	2.5	2.5	2.497	99.863	100.445 ± 0.641
6		2.5	2.5	2.509	100.341	
7		2.5	3	3.039	101.291	
8	120 %	2.5	3	3.008	100.259	100.235 ± 1.067
9		2.5	3	2.975	99.157	

2.1.4 LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Limit of Detection :

Table 17: Limit of Detection data for PCM and LOR and SER.

PCM	LOR	SER
LOD = 3.3 x (SD / Slope) = 3.3 x (22.854/73.5) = 1.026 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (4.921/284.5) = 0.057 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (8.317/78.53) = 0.349 µg/ml

Limit of Quantitation :

Table 18: Limit of Quantitation data for PCM and LOR and SER.

PCM	LOR	SER
LOQ = 10 x (SD / Slope) = 10 x (22.854/73.5) = 3.110 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (4.921/284.5) = 0.173 µg/ml	LOQ = 10 x (SD / Slope) = 3.3 x (8.317/78.53) = 1.059 µg/ml

2.1.5 Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 5.2 and 4.8.
3.Ratio of Mobile phase was changed(±2) Buffer : Methanol (62:38) and Buffer : Methanol (58:42)

Table 19: Robustness data for PCM.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	2680.590	2195.333	2278.577	2598.571	2634.485	2244.109
2	2650.124	2212.550	2303.676	2601.503	2663.532	2266.586
3	2683.523	2201.830	2303.604	2604.458	2644.869	2290.562
%R.S.D	0.692	0.395	0.630	0.113	0.556	1.025

Table 20: Robustness data for LOR

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	219.337	267.998	227.628	259.806	263.396	224.191
2	220.458	263.094	230.137	259.949	266.297	226.447
3	219.972	267.528	229.311	260.381	264.027	226.928
% R.S.D	1.016	0.256	0.558	0.115	0.577	0.647

Table 21: Robustness data for SER.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	457.516	375.650	389.719	413.558	449.678	383.975
2	448.749	378.703	382.958	445.451	443.180	387.816
3	458.339	376.820	394.049	440.967	451.440	392.041
%R.S.D	1.168	0.408	1.437	0.907	0.971	1.040

2.1.6: Analysis of marketed formulation by developed method

Sample Stock Solution (SER 50 µg/mL, PCM 325 µg/mL and LOR 8 µg/mL):

Take Crushed Tablet powder equivalent to 32.5 mg of PCM, 0.8 mg LOR and 5 mg of SER was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (SER 5 µg/mL, PCM 32.5 µg/mL and LOR 0.8 µg/mL):

Take 1 mL from this and transferred to 10 ml volumetric flask and made up volume up to the mark with Borate Buffer. Use This Solution for Derivatization as Mention Below.

Take 0.1ml of **working sample (SER+PCM+LOR)** to a 10ml volumetric flask. Add 0.5ml FMOC-Cl solution and mix for 20 second. Incubate this solution at 50C for 15 minutes in a waterbath.

In order to terminate the reaction,0.1ml glycine solution was added to the solution and mixture was vortexed for 10 seconds. Inject above Solution 20 µl for Assay Analysis.

Table 22: Analysis on marketed formulation

Tablet	LOROX-SP		
mg/Tablet powder Assay (% of label claim*) Mean ± S. D.	PCM (325 mg)	LOR (8 mg)	SER (50 mg)
	98.678±0.412	98.516±0.302	98.668±0.319

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

4. CONCLUSION

In Estimation of Paracetamol, Lornoxicam and Serratiopeptidase in their combined tablet dosage form, separation was achieved on C18 (25cm x 0.46 cm) Hypersil BDS at 30°C temperature by using a mobile phase Buffer(ammonium acetate pH5) : Methanol (60:40) at a flow rate of 1.0 ml/min and UV detection for Paracetamol, Lornoxicam and Serratiopeptidase was carried out at 215 nm. Data suggests that the results obtained were found within the acceptance criteria.

Results of the validation for Paracetamol, Lornoxicam and Serratiopeptidase of the above method were linear in the range of 16.25-18.75 µg/ml, 0.4-1.2 µg/ml and 2.5-7.5 µg/ml respectively. The % recovery was found to be 99.47% - 100.89%, 99.81% - 100.47%, 100.23% - 100.71% respectively. The results of the precision study indicate that the proposed method shown good repeatability with a % RSD of 0.844,0.875 and 0.504 respectively. Similarly %RSD from the intraday precision data was found to be 0.269% - 0.588%, 0.337% - 0.911%, 0.569% - 0.836% respectively and %RSD from the Interday precision data were found to be 0.713% - 0.942%, 0.729% - 0.946%, 0.962% - 1.900% respectively. Absolute difference between mean assay values of method precision and intermediate precision was found to be less than 2.0 %. Robustness is performed by making changes in flow rate,

Mobile phase composition and temperature. The assay obtained after proposed changes compared with the assay obtained in normal conditions. According to the acceptance criteria difference in the assay should not be more than 2%. The results obtained are well within the acceptance criteria. The % assay results of 98.67%, 98.51% and 98.668% respectively indicates that the proposed method was successfully utilized for the estimation Gresiofulvin in Tablet dosage forms.

Hence, the method can be termed as robust. Since the results are well within the limit of acceptance criteria for all validation parameters, therefore the method can be considered as validated and suitable for intended use. So, the proposed RP-HPLC assay method can be successfully applied for the estimation of Paracetamol, Loroxicam and Serratiopeptidase in in their combined tablet dosage form.

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