IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2021 Vol.:20, Issue:3

Development and Validation of Analytical Method for Simultaneous Estimation of Paracetamol, Lornoxicam and Tramadol HCL in Tablets

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Milan Nayakpara[1], Dhara Amitkumar Chavda*[2], Malaykumar Bhavanbhai Chotaliya[3], Reena Govindbhai Korat[3], Anjali Pratik Sangani[3], Mina Jaysukhbhai Sinhar[3]

[1]Shree H.N Shukla Institute of Pharmaceutica Education and research, Rajkot, India [2]Head, Shree H.N.Shukla Institute of Pharmaceutical Education & Research-Rajkot B/H lalpari lake, Marketing Yard (Bhichri), Rajkot-360005, Gujarat (India). [3]Assistant Professors, Shree H.N.Shukla Institute of Pharmaceutical Education & Research-Rajkot, B/H lalpari lake, Marketing Yard (Bhichri), Rajkot-360005. India.

Submitted: 10 January 2021
Revised: 30 January 2021
Accepted: 19 February 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Paracetamol (PCM), Lornoxicam (LOR), Tramadol (TRM), (RP-HPLC)

ABSTRACT

An accurate, simple, precise, rapid, selective, and economic reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of Paracetamol, Lornoxicam and Tramadol HCl was developed and validated. Paracetamol, Lornoxicam and Tramadol HCl were separated using a C_{18} (25cm x 0.46 cm) Hypersil BDS chromatographic column by isocratic elution with a flow rate of 1ml/min. The mobile phase composition was 0.05 M Sodium dihydrogen phosphate Buffer (pH 5.0): Methanol (80:20) and wavelength was selected at 222 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The retention time for PCM, LOR and TMD were 3.394 min, 5.151 min and 7.808 min respectively. The calibration curve for PCM, LOR and TMD was found to be linear in the range of 32.5-97.5µg/ml, 0.8-2.4µg/ml and 3.75-11.25µg/ml with a correlation coefficient of 0.9997, 0.9996 and 0.9996. The detection limits for PCM, LOR and TMD were 1.659µg/ml, 0.046µg/ml, 0.212µg/ml respectively, while quantitation limits were 5.026µg/ml, 0.139µg/ml, and 0.643µg/ml respectively. The method was shown to be linear, reproducible, specific, sensitive, and rugged. It is a user-friendly and importance tool for analysis of combined tablet dosage forms.

INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs. Paracetamol is available in different dosage forms tablet, capsules, drops, elixirs, suspensions, and suppositories. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias [1,2,3].

Lornoxicam (LORN) Chemically (3E)-6-chloro-3-[hydroxy(pyridine-2-ylamino) methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one1,1-dioxide is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties.

IUPAC name of Tramadol Hydrochloride is (lRS, 2RS)-2-[(dimethylamino)methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride. Tramadol is a synthetic codeine analogue that is a weak μ-opioid receptor agonist. It is used as an oral non-steroidal anti-inflammatory drug with good analgesic and tolerability profile in various painful conditions [4].

The proposed method was optimized and validated in accordance with international Conference on Harmonization (ICH) guidelines [5-8]. Extensive Literature survey reveals High Performance Liquid Chromatographic (HPLC) [9,10], Spectrophotometric [11-14], High Performance Thin Layer Chromatographic (HPTLC) [15-17] methods for determination of PARA either in single or in combination with other drugs. Analytical methods have been reported for the determination of LORN includes HPLC [18-20], Spectrophotometric [21], and Polarographic[22] as single component or in combination with other drugs. Literature survey also reveals RP-HPLC methods [23-31] for analysis of Lornoxicam from bulk drug and marketed formulation. To the best of our knowledge, no RP-HPLC method of analysis has yet been reported for simultaneous analysis of PCM, LOR, and TRM in combination. This paper describes simple, accurate, and precise RP-HPLC method for simultaneous determination of PCM, LOR and TRM in combined tablet dosage form.

MATERIALS AND METHOD

Equipment and Chromatographic Conditions

LC System used consists of Shimadzu LC-20AT. The detection carried by using UV detector (SPD-20AT) at 222 nm. Data acquisition was performed by using LC solution software. Chromatographic separation was carried out at room temperature with C18 (25 cm x 0.46 cm)

Hypersil BDS column by use of a mobile phase consisting of 0.05 M Sodium dihydrogen phosphate Buffer (pH 5.0): Methanol (80:20). The mobile phase was filtered through a 0.45 µm membrane filter and degassed for 10 minutes on sonicator. The injection volumes for samples and standards were 20µl and eluted at a flow rate of 1mL/min at 25±5°C. The eluents were monitored at 222 nm. In order to obtain a satisfactory and full detection for this new method, UV-VIS Spectra of Standard Paracetamol, Lornoxicam and Tramadol was chosen for detection of this new HPLC Method at which the best detector responses for all substances were obtained and the Chromatogram is monitored and extracted at 222 nm.

Preparation of Mobile Phase

0.05 M Sodium dihydrogen phosphate Buffer (pH 5.0): Methanol (80:20) sonicated to degas. The solution was filtered through 0.45µm membrane filter.

Standard Solution of Lornoxicam (A) 1.6ppm

Dissolve 16mg of Lornoxicam working standard in 100 mL of volumetric flask and dilute up to 100 mL with methanol. (160µg/ml in methanol) Dilute 1ml of Lornoxicam standard stock solution to 100ml and make up with methanol (1.6µg/ml in methanol).

Standard Solution of Tramadol (B) 7.5 ppm

Dissolve 75mg of Tramadol HCl working standard in 100mL of volumetric flask and dilute up to 100ml with Methanol. (750 µg/ml in Methanol), Dilute 1mL of Tramadol HCl standard stock solution to 100ml and make up with Ethanol. (7.5 µg/ml in Methanol).

Standard Solution of Paracetamol (C) 65 ppm

Dissolve 65mg of paracetamol working standard in 100mL of volumetric flask and dilute up to 100mL with methanol. (650 µg/ml in Methanol) Dilute 1mL of paracetamol standard stock solution to a 10mL and make up with methanol (65 µg/ml in Methanol).

Preparation of Mixture of Standard Solution of Paracetamol (65 µg/mL), Lornoxicam (1.6µg/mL) and Tramadol HCl (7.5 µg/mL) (D)

Take 1 mL from paracetamol stock solution (650ppm), 1 ml from lornoxicam stock solution (16ppm) and 1ml from Tramadol HCl stock solution (75ppm) in 10 ml volumetric flask and make up the volume to the mark with Mobile phase.

Preparation of Sample Solution

Sample Stock Solution (Tramadol HCl 75 µg/mL, Paracetamol 650 µg/mL and Lornoxicam 16 µg/mL):

Take 20 Tablets and weight it accurately and powdered it than take a powder equivalent to 65mg of paracetamol, 1.6 mg Lornoxicam and 7.5mg of tramadol HCl was transferred to a 100ml of volumetric flask, add 60 ml of mobile phase and shake for 15 min and make up the volume to the mark with mobile phase. The solution was filtered through Whatman filter paper no.42.

Working Sample Preparation (Tramadol HCl 7.5µg/mL, Paracetamol 65µg/mL and Lornoxicam 1.6µg/mL):

Pipette out 1 mL of the sample stock solution and transfer it to 10 ml of volumetric flask and make up the volume up to the mark with Mobile phase. 20 µl of above solution was injected to obtain chromatogram.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could not be achieved. A satisfactory separation and good peak symmetry for PCM, LOR and TRM were obtained with a mobile phase 0.05 M Sodium dihydrogen phosphate Buffer (pH 5.0): Methanol (80:20) in purified water at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was carried out at 222 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained Figure 1. System suitability test parameters for Paracetamol, Lornoxicam and Tramadol for the proposed method are reported in Table 1. The Chromatogram of Paracetamol, Lornoxicam and Tramadol is extracted at 222 nm. The standard and test solution is scanned between 200nm-400nm with UV-detector. The UV Chart of Paracetamol, Lornoxicam and Tramadol is shown in Figure 2.

Table No. 1: System Suitability Parameters

Parameters (units)	Paracetamol	Lornoxicam	Tramadol
Retention time(min)	3.394	5.151	7.808
Tailing Factor	1.363	1.315	1.265
Theoretical Plates	3165	8663	3537
Resolution	PCM and LOR	LOR and TRM	
	7.534	7.157	

PCM=Paracetamol, LORN=Lornoxicam and TRAM=Tramadol

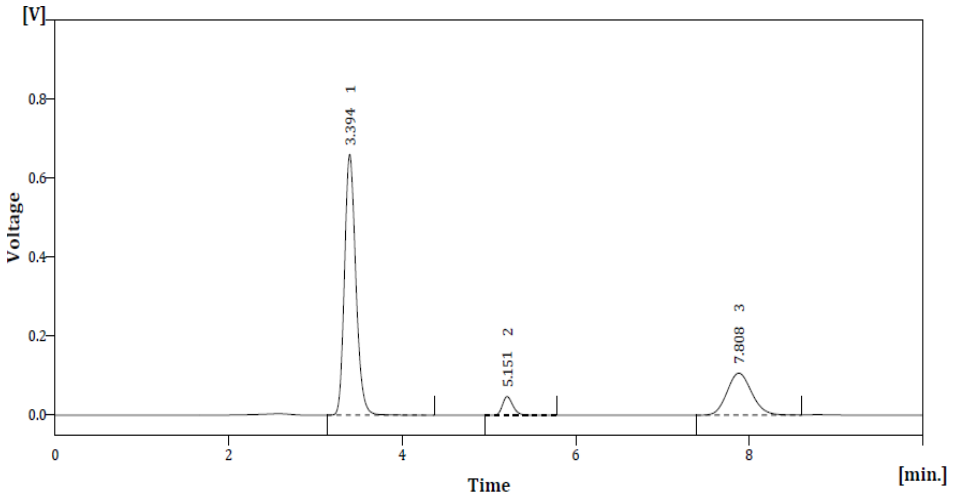


Figure. No. 1: HPLC Chromatogram of PCM 65ppm, LOR 1.6ppm, and TRM 7.5ppm in Buffer (pH 5): Methanol (80:20)

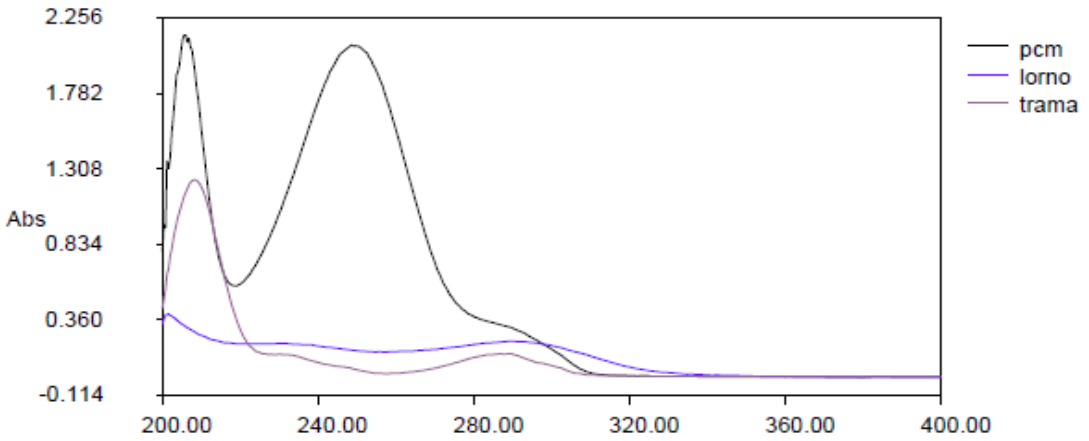


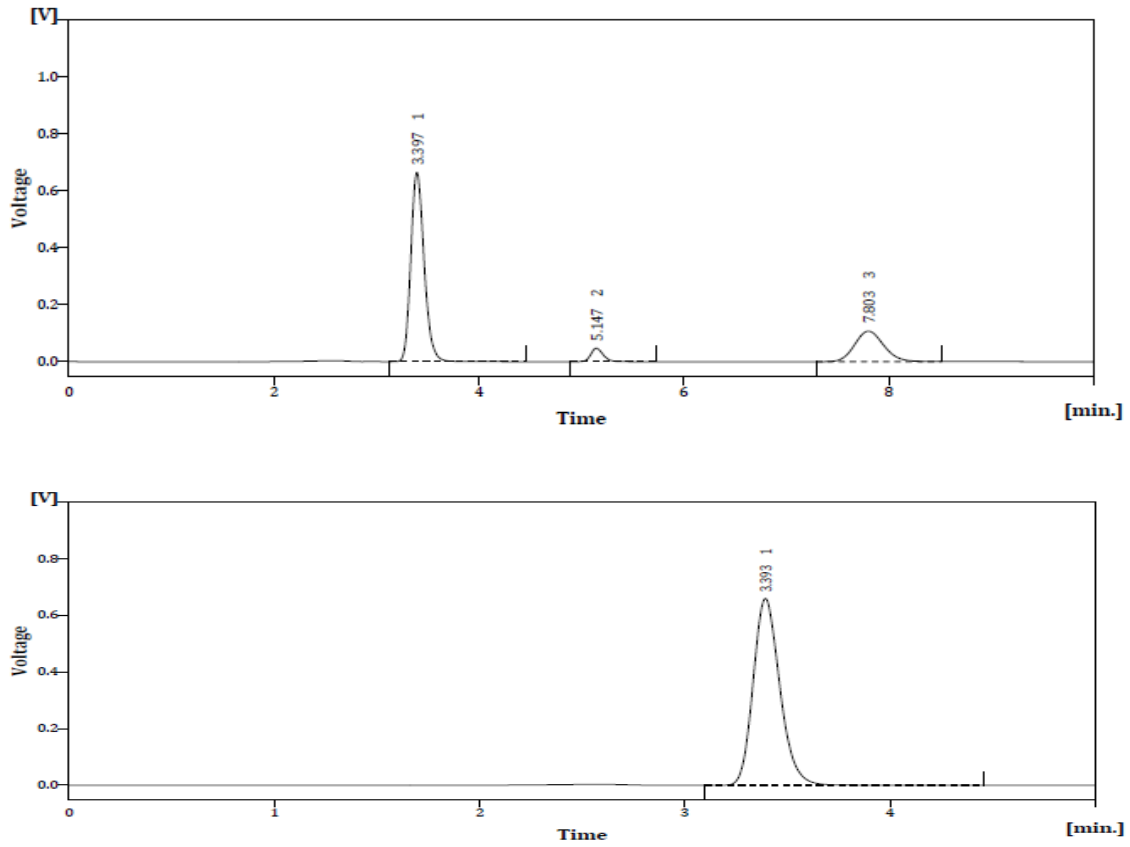
Figure. No. 2: UV Spectra of Paracetamol, Lornoxicam, Tramadol

Method Validation

Specificity

The specificity of the HPLC method was shown by the complete separation of Paracetamol, Lornoxicam and Tramadol in Figure 3. Resolution factor values were always >1.9, which ensured the complete separation of Paracetamol, Lornoxicam and Tramadol.

Studies using UV detection to determine the purity of Paracetamol, Lornoxicam and Tramadol the Purity angle is less than the calculated Threshold Angle, within the noise of the system the peak is spectrally homogenous, thereby indicating that the Paracetamol, Lornoxicam and Tramadol peaks were free from any coeluting peaks.



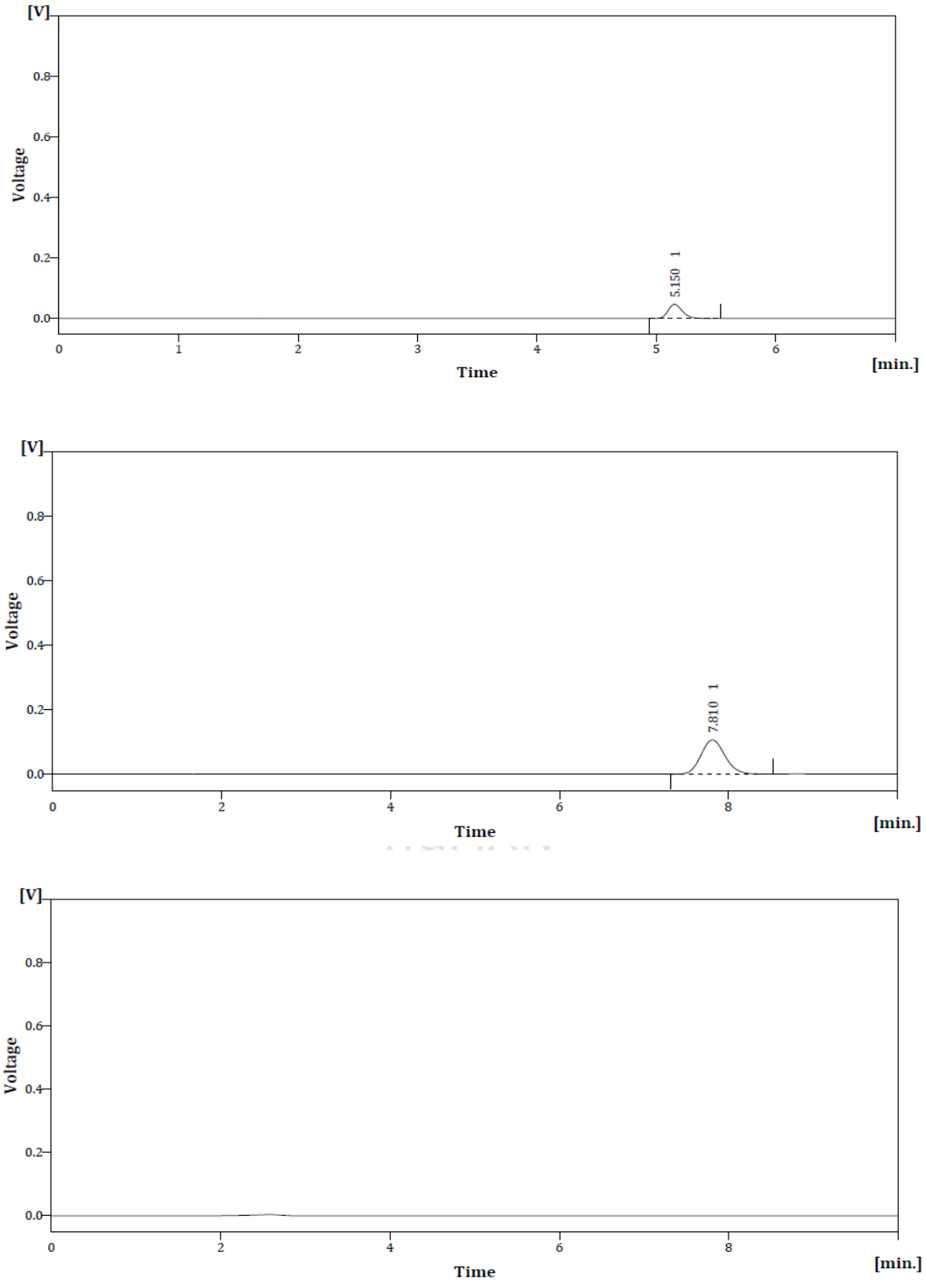
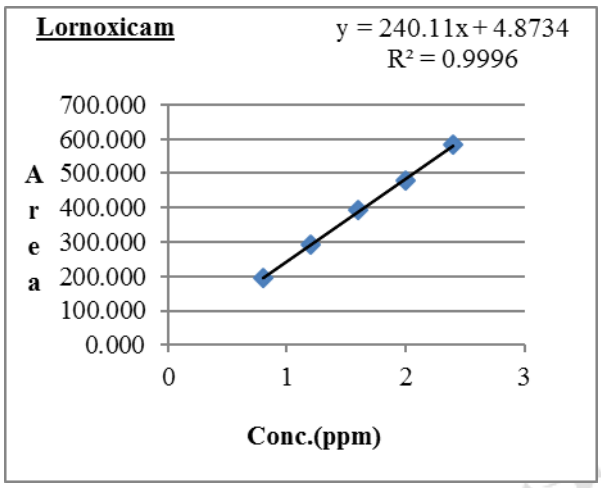


Figure No. 3: Spectra of Mix standard, Paracetamol, Lornoxicam, Tramadol HCl and Blank

LINEARITY

Under the experimental conditions described above, linear calibration curves for all three drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the three drugs i.e., Area (y) v/s concentration (x). The regression analysis data obtained is tabulated in Table 2. The linear ranges were 32.5-97.5µg/ml of Paracetamol Fig.4, 0.8-2.4µg/ml of Lornoxicam Figure 5, 3.75-11.25 µg/ml of Tramadol Figure 6.



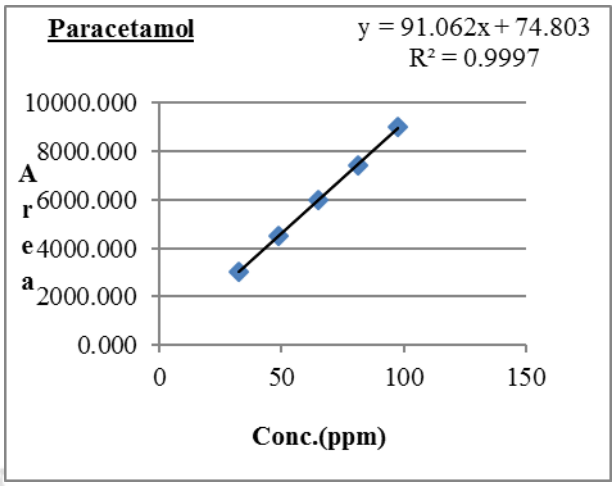


Figure. No. 4: Calibration Curve of PCM (32.597.5µg/ml) Figure. No. 5: Calibration Curve of LOR (0.8-2.4µg/ml)

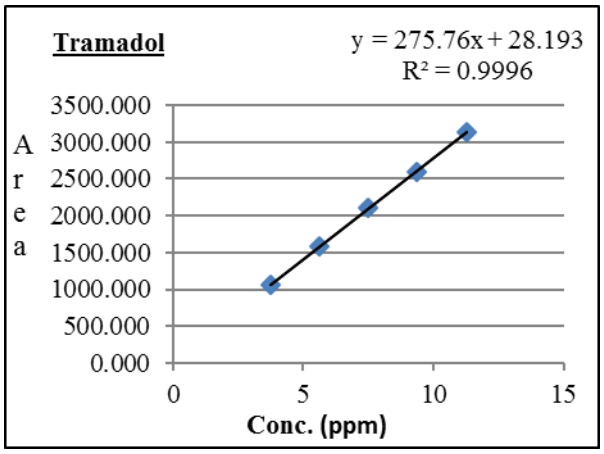


Figure No. 6: Calibration Curve of TRM (3.75-11.25µg/ml)

Table No. 2: The regression analysis data

Parameters (Units)	RP-HPLC Method		
	PCM	LOR	TRM
Concentration Range(µg/ml)	32.5-97.5	0.8-2.4	3.75-11.25
Regression Equation (Y=mx + C)	Y=91.062x+74.803	Y=240.11x+4.8734	Y=275.76x+28.193
Slope	91.062	240.11	275.76
Intercept	74.803	4.8734	28.193
Correlation Coefficient	0.9997	0.9996	0.9996
LOD(µg/ml)	1.659	0.046	0.212
LOQ(µg/ml)	5.026	0.139	0.643

ACCURACY

To an aliquot of the analyzed formulation contains 37.5mg Tramadol, 325mg Paracetamol and 8mg of Lornoxicam a known concentration of standard solution was added. Accuracy studies were performed at 80%, 100%, 120% spiked sample. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis for PCM, LOR and TRM are enclosed under Table 3, Table 4 and Table 5 respectively.

Table No. 3: Recovery data for Paracetamol

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added(µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	32.5	26	26.282	101.085	101.569 ± 0.750
2		32.5	26	26.632	102.432	
3		32.5	26	26.309	101.188	
4	100 %	32.5	32.5	32.633	100.410	99.942 ± 0.802
5		32.5	32.5	32.180	99.016	
6		32.5	32.5	32.630	100.400	
7	120 %	32.5	39	38.984	99.958	99.775 ± 0.481
8		32.5	39	38.700	99.230	
9		32.5	39	39.054	100.138	

Table No. 4: Recovery data for Lornoxicam

SR. NO.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80%	0.8	0.64	0.647	101.131	101.646 ±0.730
2		0.8	0.64	0.656	102.482	
3		0.8	0.64	0.648	101.324	
4	100%	0.8	0.8	0.789	98.681	99.400 ± 0.936
5		0.8	0.8	0.792	99.062	
6		0.8	0.8	0.804	100.458	
7	120%	0.8	0.96	0.960	100.027	99.824 ± 0.508
8		0.8	0.96	0.953	99.246	
9		0.8	0.96	0.962	100.197	

Table No. 5: Recovery data for Tramadol

SR. NO.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	3.75	3	3.032	101.073	101.269 ± 1.065
2		3.75	3	3.073	102.419	
3		3.75	3	3.009	100.316	
4	100 %	3.75	3.75	3.707	98.865	99.415 ± 0.841
5		3.75	3.75	3.712	98.997	
6		3.75	3.75	3.764	100.383	
7	120 %	3.75	4.5	4.496	99.908	99.436 ± 0.975
8		3.75	4.5	4.424	98.316	
9		3.75	4.5	4.504	100.086	

PRECISION

The method precision was established by carrying out the analysis of tablet powder blend containing three drugs. The assay was carried out of the three drugs using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limit for Paracetamol, Lornoxicam and Tramadol indicating the sample repeatability of the method. The results obtained are in Table 6.

Table No. 6: Precision of the Proposed Method

Drug	Concentration (ppm)	Area Mean ± S.D. (n=3)		(±) RSD (%)	
		Intra Day	Inter Day	Intra Day	Inter Day
Paracetamol	32.5	3036.384 ±13.255	3045.948± 11.577	0.437	0.380
	65	6002.678± 39.212	6020.735± 44.952	0.653	0.747
	97.5	9016.341± 40.474	8980.191± 36.428	0.449	0.406
Drug	**Concentrations (ppm)**	**Area Mean ± S.D. (n=3)**		**(±) RSD (%)**	
		Intra Day	**Inter Day**	**Intra Day**	**Inter Day**
Lornoxicam	0.8	196.320± 1.423	197.517±0.912	0.725	0.462
	1.6	388.906± 2.976	390.658±3.338	0.765	0.854
	2.4	584.579± 2.013	582.236± 2.931	0.344	0.503
Drug	**Concentrations (ppm)**	**Area Mean ± S.D. (n=3)**		**(±) RSD (%)**	
		Intra Day	**Inter Day**	**Intra Day**	**Inter Day**
Tramadol	3.75	1053.419 ± 15.695	1064.705±4.559	1.490	0.428
	7.5	2098.918± 11.750	2104.971±17.404	0.560	0.827
	11.25	3149.853 ± 13.025	3127.595±29.077	0.413	0.930

ROBUSTNESS

The robustness of the method is determined as a measure of the analytical methods capability to be unaffected by small variation in method parameters.

The different variations are as given below:

Variation in flow rate by ± 0.2 ml /min.

Variation in pH by ± 0.2 units.

Variation in Mobile Phase ratio by ± 0.2 Units.

ANALYSIS OF PHARMACEUTICAL DOSAGE FORM (TABLETS)

The values of analysis of tablets obtained by the proposed method were between 99.81%, 99.29% and 99.25% which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%. Refer Table 7.

Table No. 7: Results of analysis of marketed formulation

Drug	Quantity Claimed	Quantity Found	% Label Claim ±SD, N = 6
Tramadol	37.5	37.43	99.81±0.038
Paracetamol	325	322.71	99.29±0.88
Lornoxicam	8	7.94	99.25±0.025

CONCLUSION

In conclusion, the chromatographic separation of Paracetamol, Lornoxicam and Tramadol in our study was characterized with good accuracy and precision. The new HPLC method developed and validated for simultaneous determination of Paracetamol, Lornoxicam and Tramadol in combined pharmaceutical dosage form. The method was found to be simple, accurate, economical, and rapid. The proposed methods are used for the routine analysis of the drugs in the quality control.

REFERENCES

1. Reynolds JEF. The Extra Pharmacopoeia. 31sted. Pharmaceutical Press, London; 1996.
2. Rockville. The United States Pharmacopoeia. 24th revision, U.S.Pharmacopeial Convention; 2000.
3. Wikipedia, Last edited 20 dec 2020.
Available from: http://en.wikipedia.org/wiki/Lornoxicam.
4. Goodman G. The pharmacological basis of therapeutics. 10thed.McGraw Hill; 2001.
5. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceutical for Human use. Stability Testing of New Drug Substance and Products ICH Q1A (R2). 2003.
6. Chandran S, Singh RSP. Comparison of various international guidelines for analytical method validation. Pharmazie. 2007;62:4-14.
7. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedure: Text and Methodology ICH Q2 (R1). 2005.
8. ICH QIA (R2). Stability Testing of New Drug Substances and Products. 2003.

9. Momin M Y, Yeole PG, M. Puranik M P, Wadher S J. Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. Ind J Pharm Sci. 2006; 68: 387-389.
10. Pattan SR, Jamdar SG, Godge R K, Dighe N S, Daithankar A V. RP-HPLC method for simultaneous estimation of paracetamol and etoricoxib from bulk and tablets. J of Chem and Pharm Res. 2009;1:329-335.
11. Shrestha B R, Pradhananga R R. Spectrophotometric method for the determination of Paracetamol. J. Nepal Chem Soc. 2009; 24: 39- 44.
12. Nagulwar V, Dhurvey YR, Deshpande S, Upadhye K, Bakhle S, Wadetwar R. UV spectrophotometric simultaneous estimation of valdecoxib and paracetamol in combined tablet dosage form. Ind J Pharm Sci. 2006; 68:639-640.
13. Dudhane NP, Vidhate SS, Borkar BH, Lohiya RT, Umekar MJ. Simultaneous UV Spectrophotometric estimation of metoclopramide hydrochloride and paracetamol in solid dosage form. J Pharm Sci Res. 2010; 2:48-52.
14. Likhar AD, Gupta KR, Wadodkar SG. Spectrophotometric methods for the simultaneous estimation of paracetamol and etoricoxib in tablet dosage forms. Int J of Pharm and Pharm Sci. 2010; 2: 156 –161.
15. Titus J, Thenmozhi A, Sridharan D, Palanivelu M. HPTLC method for simultaneous estimation of tramadol hydrochloride and paracetamol in combined tablet dosage form. Int J of Pharma Recent Res. 2009; 1:22-26.
16. Yadav A, Singh RM, Mathur SC, Saini PK, Singh G N. A simple for simultaneous analysis of domperidone and paracetamol in tablet dosage forms. J of Planar Chromatography. 2009; 22:421-424.
17. Dighe V V, Sane R T, Menon S N, Tambe H N, Pillai S, Gokarn V N. Simultaneous determination of diclofenac sodium and paracetamol in a pharmaceutical preparation and in bulk drug powder by high performance thin layer chromatography. J of Planar Chromatography. 2006; 19:443-448.
18. Kim Y H, Ji H Y, Park E S. Liquid chromatography electrospray ionization and mass spectrometric determination of lornoxicam in human plasma. Arch Pharmacal Res. 2007; 30:905-910.
19. Zeng Y L, Chen X Y, Zhang Y F. Determination of lornoxicam in human plasma by LC/MS/MS. YaoXueXueBao. 2004;39:132-135.
20. Patil K R, Rane V P, Sangshetti J N, Shinde D B.Stability indicating LC method for analysis of lornoxicam in the dosage form, J of chromatographia. 2009; 69: 1001.
21. Sivasubramanian L, Lakshmi KS, Tintu T. Simultaneous spectrophotometric estimation of paracetamol and lornoxicam in tablet dosage form. Int J of Pharm and Pharm Sci. 2010; 2:166-168.
22. Brahim C, Kocak N, Aycan S. Polar graphic determination of lornoxicam in pharmaceutical formulations. J Sci.2009; 5:11-18.
23. Jain D K, Dubey N, Sharma Y. Development, and validation of a stability indicating HPTLC method and HPLC method for simultaneous estimation of Lornoxicam and Paracetamol in combined tablet dosage forms. Int J of Pharm and Biomed Sci. 2011;2(2):55-60.
24. Jagtap AS, Yadav SS, Rao JR. Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation. Int J of Comprehensive Pharmacy. 2011;9(4):1-4.
25. Bhavsar SM, Patel DM, Khandhar AP and Patel CN. Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form. J of Chem and Pharm Res. 2010;2(2):563-572.
26. Attimarad M. Rapid RP-HPLC method for quantitative determination of Lornoxicam in tablets. Journal of basic and clinical pharm. 2011;2(1):61-66.
27. Shah D A, Patel N J, Baldania S L, Chhalotiya U K and Bhatt K K. Stability Indicating LC- Method for estimation of Paracetamol a n d Lornoxicam in Combined Dosage Form. Scientia Pharmaceutica. 2011; 79(1):113–122.
28. Burugu R. Stability- indicating LC method for analysis of Lornoxicam in the finished dosage form. 2011;3(4):318-322.
29. Amin NM, Sen DB, Khandhar AP and Seth AK. Development and validation of stability indicating assay method for Lornoxicam & Tramadol in tablet dosage form by RP-HPLC. An Int J of Pharm Sci.2011;11-29.
30. Sharma S and Sharma MC. Isocratic RP HPLC Method for Simultaneous Estimation of Paracetamol and Lornoxicam in combined tablet Dosage Form and its Dissolution Assessment. International Journal of Chem Tech Research.2011; 3(2):997-1002.
31. Patel. Development and Validation of Analytical Methods for the Simultaneous Estimation of Lornoxicam and Paracetamol from their Pharmaceutical Dosage Forms. Current Pharm Res. 2012;3:364-366.