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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND LORNOXICAM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Keywords:
Paracetamol, Lornoxicam, Estimation, HPLC.

ABSTRACT

A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Paracetamol and Lornoxicam in tablet dosage form. The chromatographic separation was carried out on Zorbax C18 column (150 mm x 4.6 mm I.D., 5 µm particle size) with a mixture of 20 mM ammonium acetate pH 3.2 buffer and acetonitrile in the ratio of 60:40 v/v as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 265 nm. The retention times were 2.74 minutes and 5.36 minutes for Paracetamol and Lornoxicam respectively. Calibration plots were linear (r^2=0.999 for both Paracetamol and Lornoxicam respectively) over the concentration range of 6.25-250 µg/mL for Paracetamol and 0.1-4 µg/mL for Lornoxicam. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Paracetamol and Lornoxicam in tablet dosage form. Validation studies revealed that the proposed method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Paracetamol and Lornoxicam in bulk and tablet dosage form.

INTRODUCTION

Paracetamol (Fig. 1) is a non-selective COX inhibitor and has weak activity on prostaglandin synthetase in the inflamated peripheral tissues [1]. Paracetamol is used to treat many conditions such as headache, muscle ache, arthritis, backache, toothache, cold and fever. Chemically it is N-acetyl-p-amino phenol [2].

Lornoxicam (Fig. 2) is a potent analgesic with excellent anti inflammatory properties in a range of painful and inflammatory conditions, including postoperative pain and rheumatoid arthritis [3]. Chemically it is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2*H*-thieno[2,3-e]-1,2-thiazine-3-carboxamide1,1-ioxide [4].

Literature survey reveals that few analytical methods using spectrophotometry [5--7], HPLC [8-10] and HPTLC [11-13] have been reported for the simultaneous determination of Paracetamol and Lornoxicam in combined dosage forms. Therefore, an attempt has been made to develop a novel, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Paracetamol and Lornoxicam in tablet dosage form and validated in accordance with ICH guidelines [14].

MATERIALS AND METHODS

Instrumentation

To develop a high performance liquid chromatographic method for simultaneous estimation of Paracetamol and Lornoxicam using Waters 2695 HPLC system on a Zorbax C-18 (150 mm × 4.6 mm I.D., 5 µm particle size) column was used. The instrument is equipped with pump-515,

auto sampler-2707 and UV detector-2998. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software. A Shimadzu balance was used for weighing the materials and Systronics-361 pH meter was used for pH measurements.

Chemicals and solvents

The reference samples of Paracetamol and Lornoxicam were provided as gift samples from Spectrum Pharma Research Solutions, Hyderabad, India. The marketed formulation of Paracetamol and Lornoxicam tablets (Paracetamol 500 mg and Lornoxicam 8 mg) were purchased from local market. HPLC grade water and acetonitrile were purchased from E.Merck (India) Ltd., Mumbai, India. Ammonium acetate and acetic acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

Chromatographic conditions

20 mM ammonium acetate pH 3.2 buffer and acetonitrile in the ratio of 60:40 v/v was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Paracetamol and Lornoxicam. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 265 nm. The run time was set as 8 minutes.

Preparation of mobile phase and diluent

The mobile phase was prepared by mixing 600 mL of 20 mM ammonium acetate buffer (pH was adjusted to 3.2 with acetic acid) with 400 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The solution of mobile phase was used as diluent.

Preparation of standard solution

Accurately weighed and transferred 62.5 mg of Paracetamol and 1 mg of Lornoxicam working standards into a 100 mL clean dry volumetric flask, about 10 mL of diluent was added and made volume upto the mark with the diluent. Then it was filtered through 0.45 µm filter. Further 1 mL from above stock solution was transferred into 100 mL volumetric flask and diluted upto the mark with diluent. From this serial dilutions were prepared for construct the calibration curve.

Preparation of sample solution

Twenty commercial tablets were weighed and finely powdered. An accurately weighed portion of powder equivalent to 500 mg of Paracetamol and 8 mg of Lornoxicam were transferred into a 100 mL volumetric flask, to this add 50 mL of diluent. The content of flask were sonicated for 20 min, then solution was filtered through a 0.45 µm membrane filter and then final volume of the solution was made upto 100 mL with diluent. Further 1 mL from the above stock solution was pipetted into 100 mL volumetric flask to get the concentration containing 50 µg/mL of Paracetamol and 0.8 µg/mL of Lornoxicam.

Procedure

The column was maintained at ambient temperature. The run time was set at 8 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 20 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

METHOD VALIDATION

Linearity

Several aliquots of standard solutions of Paracetamol and Lornoxicam were taken in seven different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 6.25-250 µg/mL for Paracetamol and 0.1-4 µg/mL for Lornoxicam. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 265 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regressions of the plots were computed by least square regression method.

Precision

Precision for Paracetamol and Lornoxicam was determined in terms of system precision and method precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy

The accuracy of the method was assessed by recovery studies of Paracetamol and Lornoxicam at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Paracetamol and

Lornoxicam. Each level was repeated three times. The % recovery of Paracetamol and Lornoxicam were calculated.

System suitability

The system suitability parameters like retention time, theoretical plates and tailing factor were evaluated by six replicate analysis of Paracetamol and Lornoxicam and compared with standard values. The acceptance criteria are % RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 2000 per each peak and tailing factors not more than 2.0 for Paracetamol and Lornoxicam.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Paracetamol and Lornoxicam using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness

The robustness of the method was determined by making small deliberate changes in method like variation of flow rate, mobile phase ratio and temperature.

Assay

Standard preparations are made from the bulk drug and sample preparations are made from formulation. Both standard and sample solutions were injected in six homogeneous samples. 20 µL of sample solution was injected and from the peak areas of Paracetamol and Lornoxicam, amount of each drug in the sample were computed. The results were compared with the label claim of Paracetamol and Lornoxicam in tablet dosage form. From the results the average % Assay was calculated.

RESULTS AND DISCUSSION

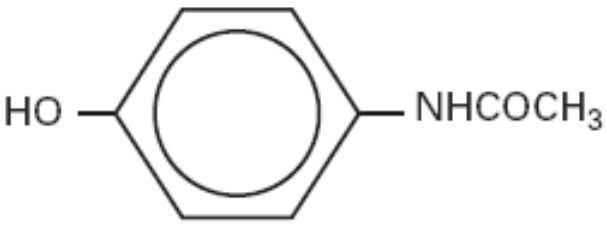

Fig. 1: Chemical structure of Paracetamol

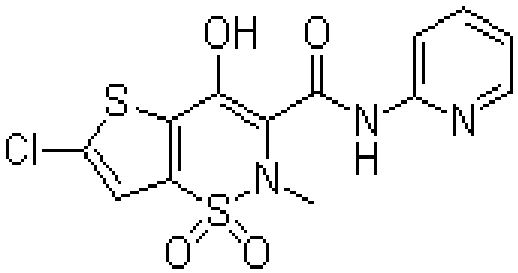

Fig. 2: Chemical structure of Lornoxicam

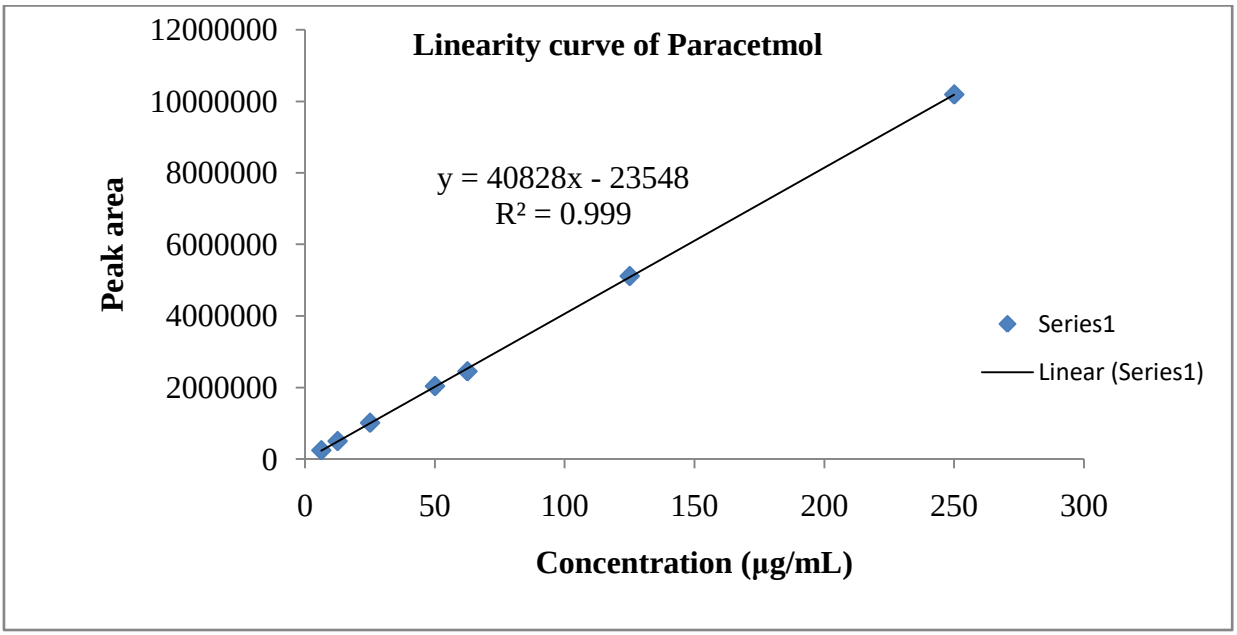

Fig. 3: Calibration curve of Paracetamol

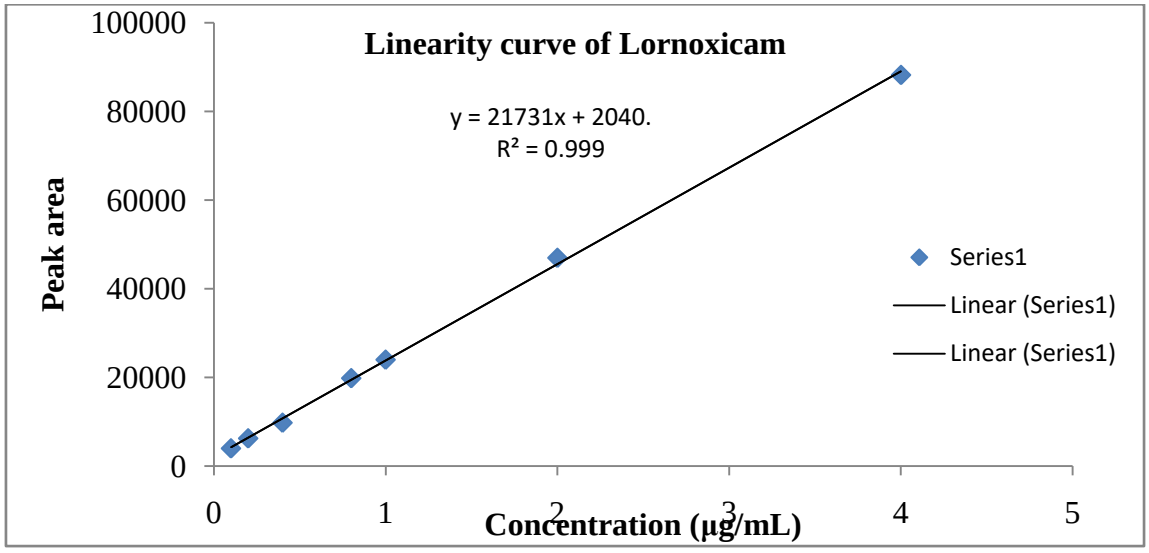

Fig. 4: Calibration curve of Lornoxicam

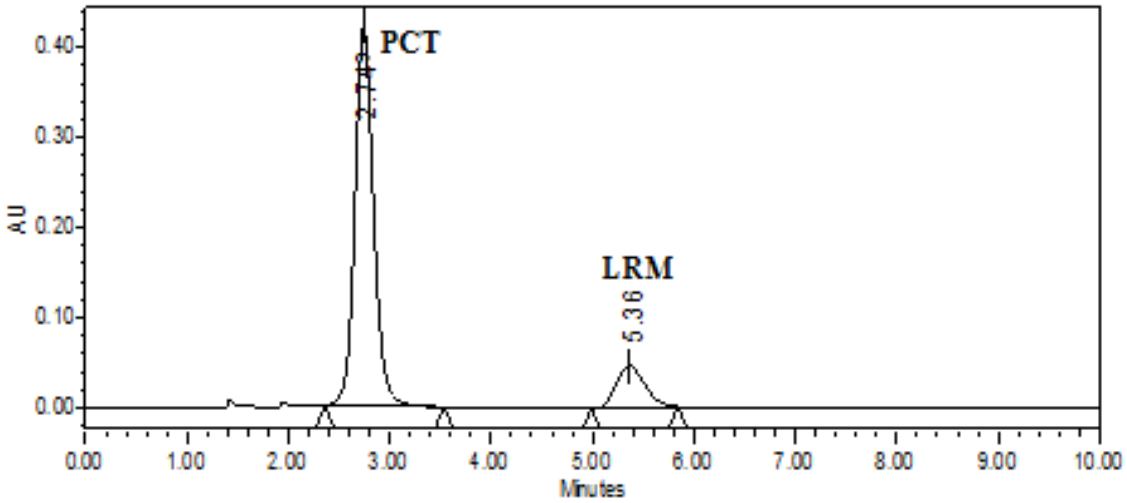

Fig. 5: Typical chromatogram of standard for Paracetamol and Lornoxicam

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	20 mM ammonium acetate:acetonitrile (60:40 v/v)
2	Diluent	20 mM ammonium acetate:acetonitrile (60:40 v/v)
3	Column	Zorbax C18 (150 mm x 4.6 mm, 5 µm)
4	Column temperature	Ambient
5	Wave length	265 nm
6	Injection volume	20 µL
7	Flow rate	1.0 mL/min.
8	Run time	8 min.

Table 2: Linearity results of Paracetamol

S. No.	Concentration (µg/mL)	Mean peak area
1	6.25	241716
2	12.5	497904
3	25	1010279
4	50	2035029
5	62.5	2447404
6	125	5109279
7	250	10183438

Table 3: Linearity results of Lornoxicam

S. No.	Concentration (µg/mL)	Mean peak area
1	0.1	3988
2	0.2	6250
3	0.4	9775
4	0.8	19824
5	1	23989
6	2	46962
7	4	88208

Table 4: Precision data of Paracetamol

S. No.	Peak area	
	System precision	Method precision
% RSD	0.29	0.44

Table 5: Precision data of Lornoxicam

S. No.	Peak area	
	System precision	Method precision
% RSD	0.28	0.28

Table 6: Accuracy studies of Paracetamol

% Concentration level	Conc. added (µg)	Conc. found (µg)	% Recovery	% Mean recovery
50%	25	24.65	98.60	99.28
100%	50	49.82	99.70	
150%	75	74.66	99.54	

Table 7: Accuracy studies of Lornoxicam

% Concentration level	Conc. added (µg)	Conc. found (µg)	% Recovery	% Mean recovery
50%	0.4	0.39	97.50	98.47
100%	0.8	0.79	98.75	
150%	1.2	1.19	99.16	

Table 8: System suitability parameters of proposed method

S. No.	Parameters	Paracetamol	Lornoxicam
1	Linearity (µg/mL)	6.25-250	0.1- 4
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	2.74	5.36
4	Tailing factor	0.85	0.95
5	Theoretical plates (N)	7445	5350
6	LOD (µg/mL)	0.10	0.05
7	LOQ (µg/mL)	0.30	0.15

Table 9: Assay results of marketed formulations

S. No.	% Assay of Paracetamol	% Assay of Lornoxicam
1	99.65	99.21
2	99.23	99.86
3	99.4	99.74
4	99.12	99.69
5	99.54	99.54

6	99.12	99.12
Mean	99.3	99.5
% RSD	0.21	0.29

The HPLC procedure was optimized with a view to develop an accurate, precise and reproducible method for simultaneous estimation of Paracetamol and Lornoxicam in tablet dosage form using Zorbax C-18 (150 mm × 4.6 mm, 5 µm) in isocratic mode with mobile phase composition of 20 mM ammonium acetate and acetonitrile in the ratio of 60:40 v/v resulted in peak with maximum separation, good shape and resolution. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time, the retention times for Paracetamol and Lornoxicam were found to be 2.74 minutes and 5.36 minutes respectively. Total run time was 8 minutes. The drug components were measured with UV detector at 265 nm. The results of optimized chromatographic conditions were shown in Table 1.

Linearity was obtained in the range of 6.25-250 µg/mL for Paracetamol and 0.1-4 µg/mL for Lornoxicam. The correlation coefficient (r^2) was found to be 0.999 for both Paracetamol and Lornoxicam respectively. The regression equation of the linearity plot of concentration of Paracetamol over its peak area was found to be y=40828x+23548, where x is the concentration of Paracetamol (µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Lornoxicam over its peak area was found to be y=21731x+2040, where x is the concentration of Lornoxicam (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and Table 3 and the calibration curves were shown in Fig. 3 and Fig. 4.

The % RSD for system precision and method precision for Paracetamol were found to be 0.29% and 0.44% respectively (limit % RSD<2.0%). The % RSD for system precision and method precision for Lornoxicam were found to be 0.28% and 0.28% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Paracetamol and Lornoxicam were furnished in Table 4 and Table 5.

The % mean recovery of the drugs Paracetamol and Lornoxicam were found to be 99.28% and 98.47% respectively and the high percentage of recovery of Paracetamol and Lornoxicam indicates that the proposed method is highly accurate. The results of accuracy studies of Paracetamol and Lornoxicam were shown in Table 6 and Table 7.

The number of theoretical plates calculated for Paracetamol and Lornoxicam was 7445 and 5350 respectively. The tailing factor for Paracetamol and Lornoxicam was 0.85 and 0.95 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Paracetamol were found to be 0.10 µg/mL and 0.30 µg/mL; 0.05 µg/mL and 0.15 µg/mL for Lornoxicam respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Paracetamol and Lornoxicam were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Paracetamol and Lornoxicam in commercial tablet dosage form. The % Assay of Paracetamol and Lornoxicam were found to be 99.30% and 99.50% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Paracetamol and Lornoxicam by the proposed HPLC method. The assay results are shown in Table 9.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Paracetamol and Lornoxicam standard were shown in Fig. 5.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the simultaneous estimation of Paracetamol and Lornoxicam and can be reliably adopted for routine quality control analysis of Paracetamol and Lornoxicam in bulk and its tablet dosage form.

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