Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation

Abstract

Aim: The objective of the study was to develop simple RP-HPLC method for the simultaneous determination of paracetamol and lornoxicam without prior separation. **Materials and Methods:** In this method, Kromasil C8 (250 mm, 4.6 mm, 5 µm) column was used. The mobile phase used was methanol:phosphate buffer (60:40, v/v, pH 6.4), at flow rate of 1 ml min⁻¹. UV detection was monitored at 302 nm. **Results:** Calibration graphs were established in the range of 1-150 µg ml⁻¹ and 0.5-100 µg ml⁻¹ for paracetamol and lornoxicam, respectively. The average retention time for paracetamol and lornoxicam was found to be 3.15 ± 0.03 min and 5.25 ± 0.06 min, respectively. The detection limit and quantitation limit for paracetamol are 0.19 µg ml⁻¹ and 0.59 µg ml⁻¹ and for lornoxicam 0.10 µg ml⁻¹ and 0.31 µg mL⁻¹, respectively. The intraday and interday precision expressed as percent relative standard deviation were below 2%. The mean recovery of paracetamol and lornoxicam was found to be in the range of 99.03-101.2%. **Conclusion:** The validated HPLC method was found to be rapid, precise and accurate and can be readily utilized for analysis of paracetamol and lornoxicam in bulk and in pharmaceutical formulations.

Key words: HPLC, lornoxicam, paracetamol, simultaneous determination, validation

INTRODUCTION

Paracetamol [acetaminophen, Figure 1] is an analgesic-antipyretic agent. It is effective in treating mild-to-moderate pain such as headache, neuralgia, and pain of musculo-skeletal origin.[1] Owing to widespread use of paracetamol in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of paracetamol individual and in combination are being investigated. The most recent methods for determination of paracetamol include chromatographic,[2-5] electrochemical,[6-9] spectrophotometric,[10-13] and fluorescence spectroscopic[14] techniques.

Lornoxicam (6-chloro-4-hydroxy-2-methyl-*N*-2-pyridinyl-2H-thieno[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide, $C_{13}H_{10}N_3O_4S_2Cl$, Figure 1) is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties that belongs to the class of oxicams. It acts by nonselective inhibition of cyclo-oxygenase-1 and -2. It is prescribed for osteoarthritis, rheumatoid arthritis, acute lumbar-sciatica conditions, and for postoperative pain management.[15] In the literatures, a voltammetric,[16] polarograhic,[17] UV spectrophotmetric,[18] LC/MS/MS,[19,20] TLC-densitometry,[21] and high performance liquid chromatographic (HPLC)[21-26] methods were reported for the analysis of lornoxicam.

Many HPLC methods have been developed for quantitative determination of paracetamol and lornoxicam in various pharmaceutical dosage forms. Spectrophotometric[27] and HPTLC[28] methods are reported for simultaneous estimation of paracetamol and lornoxicam in formulations. But, more accurate, simple, and widely used HPLC method has been not reported for the simultaneous estimation of paracetamol and lornoxicam in combination

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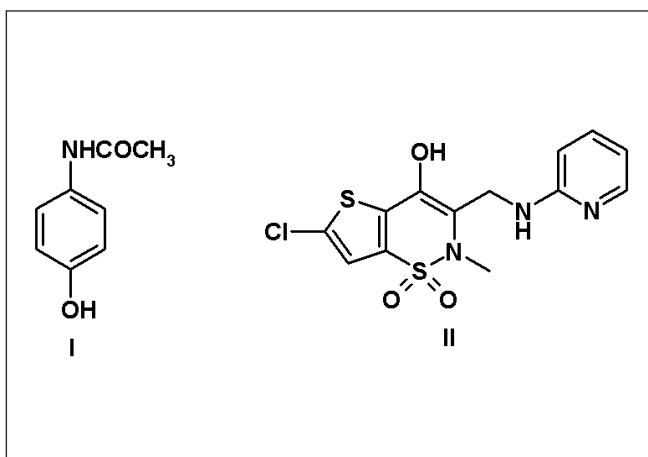
Figure 1: Structure of paracetamol (I) and lornoxicam (II)

formulation. Analysis of this mixture is challenging because of huge difference in the concentration, i.e., lornoxicam is the minor component 8 mg/tablet whereas paracetamol is major component 500 mg/tablet. Generally in the simultaneous estimation in the HPLC isosbestic point of UV scan is selected. But, in this combined formulation the concentration of both the components did not give easy selection of this point. Therefore, the goal of this research is to develop and validate a simple, rapid, accurate, sensitive and precise RP-HPLC method for the simultaneous estimation of paracetamol and lornoxicam in marketed pharmaceutical dosage form.

MATERIALS AND METHODS

The HPLC system (Shimadzu Prominence Liquid chromatography), consisted of 20AT pump, CTO-20A column oven, SPD-20A UV visible absorbance detector, a manual injector, with CMB-20A data module. Paracetamol and lornoxicam powder with 99.71% and 99.80% pure, respectively were used as standard. Tablet dosage form (paracetamol 500 mg and lornoxicam 8 mg per tablet) of Lornasafe Plus (Mankind Pharma Ltd., Mumbai, India) were used for the analysis. HPLC grade methanol and formic acid were purchased from Sigma-Aldrich (Germany). The water for LC was prepared by double distillation and filtered through a nylon 0.45 μm membrane filter (Millipore, Bedford, MA, USA).

Chromatographic condition
Analytical conditions were standardized through the LC system using Kromasil C 8 column (250 × 4.6 mm, 5 μm). The mobile phase used was methanol:0.01M phosphate buffer (60:40, v/v, pH 6.4), at a flow rate

of 1 ml min^{-1}. UV detection was made at 302 nm. The volume of injection was fixed at 20 μl. All analyses were done at temperature 30°C. The mobile phase was prepared fresh each day, vacuum-filtered through 0.45 μm Millipore nylon filters.

Validation of the method
The developed method was validated as per ICH guidelines[29] in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability. The accuracy was expressed in terms of percent recovery of the known amount of the active pharmaceutical ingredient in presence of excipients. The precision (%relative standard deviation, %RSD) was expressed with respect to the intraday and interday variation in the expected drug concentrations. After validation, the developed method was applied to pharmaceutical dosage forms containing paracetamol and lornoxicam.

System suitability
The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (N), resolution (R), and tailing factors (T).

Linearity and range
Stock solution was prepared by dissolving 10 mg each of paracetamol and lornoxicam in 50 ml volumetric flask with methanol. From the above stock solutions, dilutions were made to get the concentration in the range of 1-150 μg/ml of paracetamol and 0.5-100 μg/ml of lornoxicam. A volume of 20 μl of each sample was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration. The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision
The precision of the proposed method was assessed as repeatability and intermediate precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated over a 2-day period to evaluate day-to-day variability (intermediate precision).

Accuracy

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 50, 75 and 100% level. The basic concentration levels of sample solution selected for spiking of the standard drugs solution were 62.5 µg/ml of paracetamol and 1 µg/ml of lornoxicam. Percentage recovery and relative standard deviation were then calculated.

Assay procedure for dosage forms

To determine the amount of paracetamol and lornoxicam in the tablet (Claim paracetamol 500 mg and lornoxicam 8 mg), twenty tablets were weighed, average weight was determined and powdered. An amount of powder equivalent to 125 mg of paracetamol and 2 mg of lornoxicam was weighed and dissolved in 40 ml of methanol and sonicated for 15 min to ensure the complete dissolution of both the drug. After filtration the volume was made up to 50 ml with methanol. Further diluted with mobile phase to get 62.5 µg/ml of paracetamol and 1 µg/ml of lornoxicam, which was subjected to the above method and amount of paracetamol and lornoxicam were determined.

RESULTS AND DISCUSSION

HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of paracetamol and lornoxicam in bulk and tablet dosage forms.

Optimization of the chromatographic conditions

Initially, the mobile phase used was methanol: phosphate buffer (80: 20%) than ratio of the solvents were varied, at 70:30 % there was no good separation and at 50:50% the retention time of lornoxicam was too high. Good resolution of both the components was obtained with methanol: phosphate buffer at ratio 60:40% v/v. Different values of pH of phosphate buffer were tried. At lower pH the intensity of UV absorption by lornoxicam was less and at pH 6.4 it showed good absorption and peak shapes of both components were also good, hence, phosphate buffer with pH 6.4 has been selected for analysis. The flow rate of 1.0 ml/min, was optimum. From the UV spectra of lornoxicam and paracetamol isosbestic point cannot be used for the analysis because of very low concentration of lornoxicam when compared to paracetamol. At isosbestic wavelength the intensity of paracetamol chromatogram peak was so high that

lornoxicam peak was not integrated and quantified in the chromatogram. Whereas at high concentration of lornoxicam, the concentration of paracetamol was out of Beer's Law range. At 302 nm paracetamol is having low UV absorbance whereas lornoxicam has high UV absorbance with good Beer's law range. Also at this wavelength both paracetamol and lornoxicam can be quantified at tablet concentration ratio. Hence, 302 nm determined empirically has been found to be optimum. The average retention times for paraceatmol and lornoxicam was found to be 3.15 ± 0.03 and 5.25 ± 0.06 min, respectively. A typical HPLC chromatogram is shown in Figures 2, 3.

Linearity, limit of detection, and limit of quantification

The calibration graph was constructed for the proposed method from the data points over the concentration range cited in Table 1. The linearity of the calibration graph and conformity of the
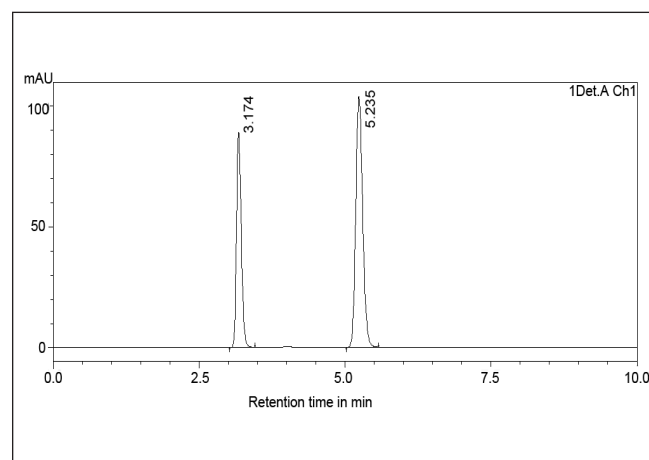
Figure 2: Representative chromatogram of standard paracetamol and lornoxicam (100 µg/ml each)
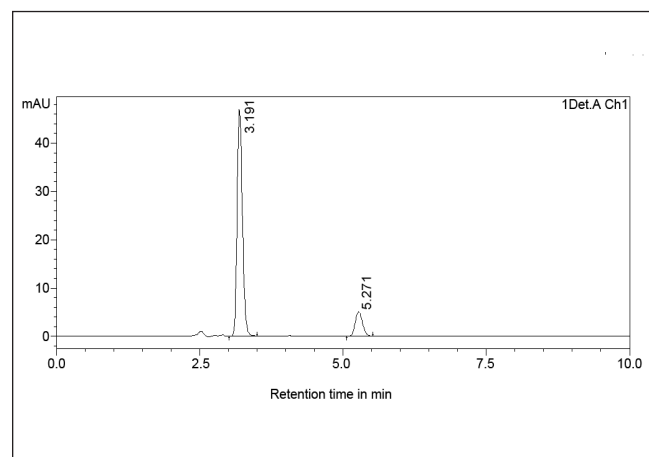
Figure 3: Chromatogram of paracetamol (62.5 µg/ml) and lornoxicam (1 µg/ml) from tablet.

HPLC method proved by the high values of the correlation coefficients (*r*) of the regression equation. According to ICH recommendations the approach based on the SD of the response and the slope was used for determining the detection and quantitation limits. The detection limit and quantitation limit of paracetamol were found to be 0.19 μg/ml and 0.59 μg/ml and lornoxicam 0.10 μg/ml and 0.31 μg/ml respectively.

Suitability of the method

According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1. Chromatographic parameters such as resolution, selectivity and peak symmetry were satisfactory for both the compounds. The calculated resolution between paracetamol and lornoxicam was not less than 2.5 and selectivity was above 4. Number of theoretical plates and tailing factor were observed to be satisfactory.

Precision

The precision evaluated as the repeatability resulted in a %RSD value of 0.59 (*n* = 6) for paracetamol and 0.47% (*n* = 6) for lornoxicam. Method precision measures the closeness of analytical results when six separately prepared standards are injected. The %RSD was found to be less than 1.55 for both the drugs. Intermediate precision was assessed by analyzing three samples over period of time in terms of intraday and interday precision. Concentrations were deduced from the linearity plots using chromatographic peak areas. The %RSD valves obtained were below 1.13 and 1.83 for paracetamol and lornoxicam, respectively, for intraday measurements, while it was found to be below 1.34 and 1.29 for paracetamol and lornoxicam, respectively, for interday measurements [Table 2]. The values indicate that the method is precise.

Accuracy

The accuracy was assessed from three different added standard solutions containing 62.50 μg ml^{-1} of paracetamol and 1 μg ml^{-1} for lornoxicam. The highest %RSD was found to be 1.21 and 1.61 in HPLC method for paracetamol and lornoxicam, respectively, demonstrated that the method was accurate within the desired range. Table 3 gives the detailed results of the accuracy.

Table 1: System suitability data of paracetamol and lornoxicam analysis

Parameters	Paracetamol	Lornoxicam
Concentration range (μg/ml)	1–150	0.5–100
Intercept	2953.36	4001.03
Slope	4944.97	43290.66
Correlation coefficient (*r*)	0.9997	0.9999
LOD (μg/ml)	0.19	0.10
LOQ (μg/ml)	0.59	0.31
t_R ± SD	3.17 ± 0.03	5.26 ± 0.04
Tailing ± %RSD	0.648 ± 0.053	0.986 ± 0.095
Theoretical plates ± %RSD	8859 ± 0.89	4609 ± 0.41

t_R= retention time, SD =Standard deviation, %RSD = Percent relative standard deviation.

Table 2: Summary of precisions

	Concentration (μg/ml)	% Mean Intraday	Recovery ± RSD[a] Interday
Paracetamol	5	99.38 ± 0.57	101.47 ± 1.05
	50	100.29 ± 1.13	99.41 ± 1.34
	100	100.91 ± 1.09	101.62 ± 1.23
Lornoxicam	1	100.49 ± 0.97	99.51 ± 1.01
	50	100.83 ± 1.48	101.53 ± 1.29
	100	101.77 ± 1.83	100.22 ± 1.08

[a]Mean of six determinations.

Table 3: Accuracy of the method

	Amount (μg/ml)		
	Added	Found	%Recovery ± RSD[a] (%)
Paracetamol	30	29.80	99.33 ± 0.92
	45	45.41	100.91 ± 1.09
	60	59.42	99.03 ± 1.21
Lornoxicam	0.50	0.496	99.20 ± 1.51
	0.75	0.743	99.06 ± 1.61
	1.00	1.012	101.20 ± 1.14

[a]Mean of three measurements.

Robustness

The HPLC method was found to be robust as the results were not significantly affected by slight variation in the extraction time, composition of mobile phase, flow rate and wavelength.

Analysis of tablets

The rapid RP HPLC method developed in the present study was applied to bulk drug mixture and two different batches of commercial formulations. A summary of the results are shown in Table 4. The

Table 4: Assay of paracetamol and lornoxicam in bulk and in tablets

Sample	% Assay (mean ± RSD)[a]	
	Paracetamol	Lornoxicam
Lab mixture[b]	99.25 ± 0.96	100.28 ± 1.17
Batch no. 1[c]	99.52 ± 1.23	98.90 ± 0.93
Batch no. 2[c]	101.36 ± 0.89	101.12 ± 1.61

[a]Mean and relative standard deviation for six determinations; percentage recovery from the lable clame amount. [b]Mixture of standard paracetamol (500 mg) and lornoxicam (8 mg) powder. [c]Commercial tablets are the product of Mankind Pharm Ltd., India Each per tablet was labeled to containe 500 and 8 mg of paracetamol and lornoxicam, respectively.

mean recovery is 99.25 % for paracetamol and 100.28% for lornoxicam from the laboratory mixture, and it is from 98.90% to 101.36% from tablet formulations. The results indicate the method is highly accurate for simultaneous determination of the paracetamol and lornoxicam.

Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all sample components (excipients). The results were compared with the analysis of a standard paracetamol and lornoxicam and tablet formulations [Table 4]. Comparison of standard drugs and tablet chromatograms [Figures 2 and 3] showed no interference from excipients by the proposed method.

CONCLUSION

The proposed method is accurate, simple, economical, rapid and selective for the simultaneous estimation of paracetamol and lornoxicam in bulk and in tablet dosage form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. By HPLC method analysis can be done within 6 min with the use of simple solvents. Hence, developed HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulations.

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