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Development and validation of HPLC analytical method with programmed wavelength UV detection for simultaneous determination of paracetamol and lornoxicam in tablet

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INTRODUCTION

Paracetamol (Acetaminophen, Figure 1) is an analgesic-antipyretic agent and is effective in treating mild to moderate pain such as headache, neuralgia and pain of musculo-skeletal origin[1]. Owing to the wide applications of paracetamol in various 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 non-steroidal anti-inflammatory drug with analgesic and antipyretic properties that belongs to the class of oxicams. It acts by non-selective inhibition of cyclo-oxygenase-1 and -2. It is prescribed for the treatment of osteoarthritis, rheumatoid arthritis, acute lumbar-sciatica conditions and for postoperative pain management[15]. In the literatures, voltammetric[16], polarograhic[17], UV spectrophotmetric[18], LC/MS/MS[19-20], TLC-densitometry[21], and HPLC[21-25] methods were reported for the analysis of lornoxicam. The combination of paracetamol and lornoxicam is intended for oral administration and is available in different strengths. This combination is mainly administered for the treatment of osteoarthritis, rheumatoid arthritis, acute sciatica, low back pain etc. Moreover, it is likely that paracetamol synergizes the efficacy of lornoxicam and along with lornoxicam is effective in the control of pain.

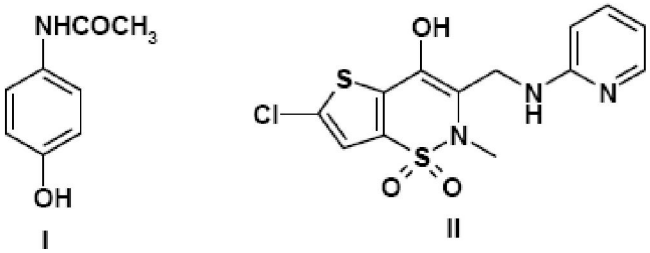

Figure 1 : Structure of paracetamol(I) and lornoxicam(II)

A comprehensive literature search revealed that UV spectrophotometric[26], HPTLC2[7] and HPLC[28] methods have been developed for the simultaneous quantitative determination of paracetamol and lornoxicam pharmaceutical dosage forms. But, more accurate, simple and widely used HPLC method is preferred for the routine analysis. The reported HPLC[28] methods is time consuming because of high retention time. The aim of this study was to develop a simple, rapid, specific and validated HPLC method for quantification and simultaneous determination of paracetamol and lornoxicam directly from the tablet formulation. However, analysis of this mixture is challenging as the concentration of the drugs differs significantly, i.e. lornoxicam is the minor component 8 mg/tablet where as paracetamol is major

component 500 mg/tablet.

EXPERIMENTAL METHODS

Chemicals

Lornoxicam was obtained as a gift from Hetero drugs (Hyderabad, India). Paracetamol was purchased from (Sigma Aldrich) were used without any purification. Methanol and formic acid used were purchased from (Sigma Aldrich, Germany). Lornasafe ™ –Plus tablets (Mankind Pharma Ltd., New Delhi, India) and Lorcam XP ® (Pharmed Ltd., Bangalore, India) containing 8 mg of lornoxicam and 500 mg of paracetamol were purchased from the Indian market.

Equipment and Chromatographic condition

Agilent 1200 (Germany), separation module appended with PDA detector system. Data acquisition was performed using powerful Chem. Station software, (version.32) of Agilent. Zorbax, XBD Eclipse RP C 18 column (150 x 4.6 mm, 5µm) was used for the validation studies. Freshly prepared mobile phase was filled in the reservoirs and run in an isocratic mode. The mobile phase was run at a flow rate of 0.8 mL/min with a run time of 5 min and the injection volume was 20 µL. Acquisition was carried out with PDA detector in the wavelength range of 210 to 400 nm; extraction of chromatograms was carried out by timed wavelength at 310 nm and 380 nm for paracetamol and lornoxicam, respectively. The intensity of paracetamol at 257 nm (its λmax), 280 nm, 290 nm and 300 nm chromatogram is so high that lornoxicam could not be identified in the chromatogram at the tablet ratio (paracetamol : lornoxicam 62.5 : 1) . Hence, Multi wave length method is selected, which increased the sensitivity of the lornoxicam (λmax in mobile phase).

The mobile phase used for the chromatographic separation comprised of methanol and 0.1% formic acid in the ratio of (80: 20 v/v). The pH was adjusted to 3 with formic acid and filtered through 0.45 µm nylon filters using Millipore filtration unit.

Standard solutions

Standard stock solutions of paracetamol (500 µg/mL) and lornoxicam (200 µg/mL) was prepared by dissolving accurately weighed 10 mg of each in methanol and 0.1M sodium hydroxide (0.1 mL, to dissolve lornoxicam). The stock solutions were further diluted serially with mobile phase to get the required concentrations.

Sample preparation

Twenty tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass equivalent to one tablet was dissolved in about 60 mL of methanol and 0.1M sodium hydroxide, sonicated for 15 min and filtered in 100 mL volumetric flask. The residue was washed three times with 10 mL methanol. The combined solution was filtered using syringe filter (0.45 µm) and then the volume was completed to the mark with same solvent. This solution was diluted with mobile phase to get the concentration in the range of Beer's law.

Linearity

Six standard concentrations of lornaxicam (0.5, 2, 5, 10, 20 and 50 µg/mL) and paracetamol (5, 10, 50, 100, 200 and 300 µg/mL) were prepared by serially diluting stock solutions of both. All the solutions were filtered through 0.45 µm nylon filters in to HPLC vials. All the injections were carried out in triplicate.

System precision and method precision

System precision was determined by analyzing six replicates of 5 µg/mL of lornoxicam and 50 µg/mL of paracetamol from the same homogeneous solutions. The %RSD was calculated for the replicates as a measure of system precision. For method precision six independent solutions, 5µg/mL of lornoxicam and 50 µg/mL of paracetamol was injected; the variation in the results was estimated in terms of %RSD of the determinations. Intermediate precision, at two levels intraday and inter day, was assessed at three concentrations (0.5, 10 and 50 µg/mL for lornoxicam and 5, 100 and 300 µg/mL for paracetamol) All the injections were carried out in triplicates.

Intraday precision was determined by analyzing three sets of freshly prepared standards in one laboratory in one day. The %RSD was calculated from the chromatographic peak area of different measurements as a measure of intraday precision. Further, inter-day precision of the method was measured as %RSD between analysis of standards on three consecutive days in the same laboratory.

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Accuracy

The accuracy was evaluated by the recovery of known amounts of the reference standard added to the sample solution contacting 1 µg/mL of lornoxicam and 62.5 µg/mL of paracetamol to obtain a final concentration of 1.5, 2 and 2.5 µg/mL of lornoxicam and 93.75, 125, and 156.25 µg/mL paracetamol corresponding to 50, 100 and 150% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

Limit of quantification (LOQ) and detection (LOD)

The LOQ and LOD were calculated from the slope and mean of standard deviation of the intercept of the mean of the three calibration curves, determined by a linear regression model as per the ICH guidelines.

Robustness

Robustness of the method was studied by changing the extraction time of paraceatmol and lornoxicam, from dosage form by 5 min, composition of mobile phase by 2% of organic phase, pH of formic acid solution by ± 0.2, flow rate by 0.1 mL/min and wavelength by 1 nm.

Analysis of the Paracetamol and lornoxicam in pharmaceutical formulation.

For the quantification of paracetamol and lornoxicam in the tablet formulations the respective stock solutions were diluted to appropriate concentration with the mobile phase, filtered, injected in triplicate and the percentage recoveries of the drugs were calculated against the reference substances.

RESULT AND DISCUSSION

To obtain the best chromatographic conditions, the mobile phase was optimized to provide adequate peak symmetry and sensitivity. Acetic acid, formic acid, potassium phosphates were tested. The use of formic acid (0.1%) in combination with methanol (20:80% v/v) at 25°C resulted in a relatively short retention time of less than 5 min, better peak symmetry (0.848 and 0.786) and simple mobile phase (without salt and buffer addition). The retention time was found to be 1.77 and 2.63 min for paracetamol and lornoxicam, respectively. The representative chromatogram is shown in Figure 2.

The UV spectra of paracetamol and lornoxicam at the tablet ratio did not give good isosbestic point to select one wave length because of very low concentration of lornoxicam when compared to paracetamol. In

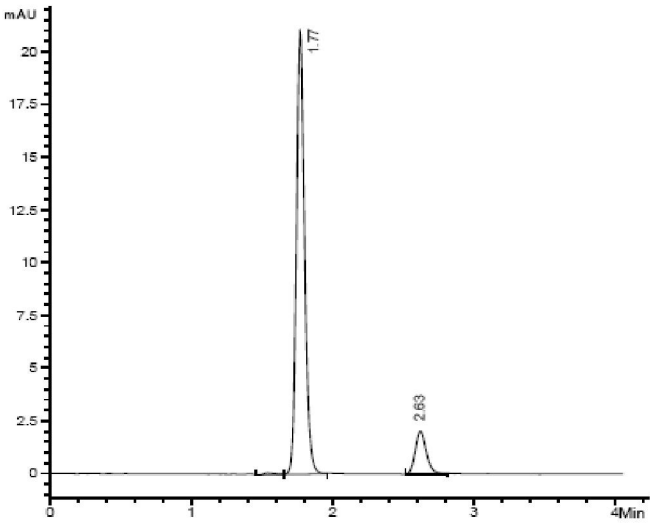

Figure 2 : Representative Chromatogram of paracetamol 125 µg mL^{-1} (Rt: 1.77 min) and lornoxicam 2 µg mL^{-1} (Rt: 2.63 min)

such cases standard addition of low concentration component method is adopted. The main objective was to develop direct quantification of paracetamol and lornoxicam in the tablet formulation. Hence for lornoxicam 380 nm (λ max) has been selected for analysis. Whereas for the paracetamol 310 nm has been selected because, at its λ max (257 nm) and 280 nm 290 nm and 300 nm the intensity of chromatogram peak was so high that lornoxicam peak was not integrated and quantified in the chromatogram. At high concentration of lornoxicam, the concentration of paracetamol was out of Beer's Law range.

Linearity and range

Linearity of HPLC detector response for determination of paracetamol and lornoxicam was evaluated by analyzing a series of standard solutions of different concentrations of each compound and calibration graphs were established. The calibration curves constructed for paracetamol and lornoxicam were found to be linear in the 5-300 µg/mL and 0.5-50 µg/mL respectively. The value of the determination coefficient calculated (PA =0.731C + 0.321, r^2 =0.9995, for paracetamol and PA=13.60C + 0.480 r^2 =0.9991 for lornoxicam, where PA is the peak area, C is the concentration in µg/mL and r is the correlation coefficient) indicated the linearity of the calibration curve for the method.

LOD and LOQ

The values calculated for LOD and LOQ were 0.79 µg/mL & 2.40 µg/mL for paracetamol and 0.13 µg/mL & 0.40 µg/mL lornoxicam respectively (TABLE 1).

Suitability of the method

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 1.6 and selectivity was above 4. Number of theoretical plates and tailing factor were observed to be satisfactory. (TABLE 1)

TABLE 1 : Linear regression data and system suitability data of paracetamol and lornoxicam analysis

Parameters	Paracetamol	Lornoxicam
Concentration range (µg/ml)	5.0 – 300.0	0.5 – 50.0
Regression equation	$y = 0.731x + 0.321$	$y = 13.60 x + 0.480$
Correlation coefficient (r^2)	0.9995	0.9991
LOD (µg/ml)	0.79	0.13
LOQ (µg /ml)	2.40	0.40
Rt±RSD	1.775*±0.017	2.62*±0.020
Tailing Factor ±RSD	0.848*±0.053	0.786*±0.095
Theoretical Plates ± RSD	4859*±0.89	5609*±0.41

RSD : Relative Standard Deviation, * Average of three readings

System precision and method 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, respectively. 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 in all the cases. 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 observed in the case of intraday measurements were found to be below 1.13 and 0.56 for paracetamol and lornoxicam, respectively. However, in the case of interday measurements, the values were found to be below 1.18 and 0.87 for paracetamol and lornoxicam, respectively (TABLE 2). Thus the observed values suggest that the proposed method is precise.

TABLE 2 : Summary of precisions

		Intra-day		Inter-day	
	Concentration (µg/ml)	Mean (n=3)	RSD (%)	Mean (n=3)	RSD (%)
Paracetamol	5	4.97	0.57	4.96	1.05
	100	100.12	1.13	100.21	1.18
	300	298.91	1.09	299.20	0.83
Lornoxicam	0.5	0.49	0.02	0.51	0.01
	10	10.13	0.14	10.18	0.29
	50	49.71	0.56	51.3	0.87

Accuracy

The accuracy was assessed from three different standard solutions containing 62.50 µg/mL of paracetamol and 1 µg/mL for lornoxicam. The highest % RSD was found to be 1.59 and 0.83 for paracetamol and lornoxicam, respectively. These values demonstrated that the proposed method was accurate within the desired range (TABLE 3).

TABLE 3 : Accuracy of the method

Drug	Amount added (µg/ml)	Amount recovered (µg/ml) (n=3)	Recovery (%)	RSD (%)
Paracetamol	31.25	31.03	99.29	1.59
	62.50	62.65	100.24	1.08
	93.75	94.84	101.16	1.45
Lornoxicam	0.5	0.51	102.00	0.70
	1.0	1.01	101.00	0.56
	1.5	1.49	99.33	0.83

Robustness

The method was found to be robust as the results were not significantly affected by minor 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 for the determination of paracetamol and lornoxicam in tablet dosage forms, without prior separation in presence of tablet excipients. The mean recovery was found to be 99.87 % for paracetamol and 101.41% for lornoxicam with RSD less than 1.29 %. These results indicate that the current method is highly accurate for simultaneous determination of the lornoxicam and paracetamol (TABLE 4).

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TABLE 4 : Results from analysis of paracetamol and lornoxicam in tablets

Paracetamol			Lornoxicam		
Label claim (mg/tab)	Found (mg/tab)	Recovery (%)	Label claim (mg/tab)	Found (mg/tab)	Recovery (%)
500.00	495.06	99.02	8.00	8.14	101.75
500.00	496.19	99.23	8.00	8.09	101.12
500.00	506.84	101.36	8.00	8.11	101.37
Mean±RSD 99.87±1.29%				101.41±0.31%	

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 standard paracetamol and lornoxicam and tablet formulations. No interference from excipients was observed in the proposed method.

CONCLUSION

The proposed method is simple, precise, accurate, specific and rapid for the simultaneous quantification of paracetamol and lornoxicam in tablet dosage forms without the interference from the excipients. The proposed method involves direct quantification of both the components within 5 min without the use of buffers in the mobile phase. Hence, it can be easily and conveniently adopted for routine quality control analysis of paracetamol and lornoxicam.

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