Original Article

Development and validation of reversed phase–high-performance liquid chromatography method for determination of paracetamol and lornoxicam in tablet dosage form

Abstract

A simple, precise, reliable, rapid and reproducible reversed phase–high-performance liquid chromatography method was developed and validated for the simultaneous estimation of Paracetamol (PCM) and Lornoxicam (LOX) present in tablet dosage forms. Chromatographic separation achieved isocratically on Luna C_{18} column (5 µm, 150 × 4.60 mm) and methanol/phosphate buffer (60:40, v/v, pH 7.0) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 260 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for PCM and LOX was found to be 2.06±0.013and 4.38±0.07 min, respectively. Linearity for PCM and LOX was in the range of 10-50 µg/ml and 8-40 µg/ml, respectively. The mean recoveries obtained for LOX and PCM were 100±0.16 and 99.50±0.43%, respectively, and relative standard deviation (RSD) was less than 2. The correlation coefficients for all components are close to 1. The RSDs for three replicate measurements in three concentrations of samples in tablets are always less than 2%. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of PCM and LOX in tablets.

Key words: Lornoxicam, paracetamol, RP-HPLC, simultaneous estimation

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INTRODUCTION

Paracetamol, (PCM) chemically, (N-(4-hydroxyphenyl) acetamide) [Figure 1a] has analgesic and antipyretic activity and is used for the treatment of pain such as headache, toothache, rheumatism and neuralgia.[1] The mechanism of action of PCM is due to its inhibition of the cyclooxygenase enzyme and the prostaglandin synthesis in the central nervous system[2] and its direct activity on the centre for the body temperature regulation in the hypothalamus.[3] Lornoxicam (LOX) chemically, (6-chloro-4-hydroxy-2-methyl- N-2-pyridyl-2H-thieno [2, 3-*e*]-1, 2-thiazine-3-carbox- amide-1, 1-dioxide) [Figure 1b] is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties.[4] LOX is a yellow crystalline substance with a pKa of 4.7 and a partition coefficient of 1.8 determined in octanol–phosphate pH 7.4. PCM alone or in combination with other drugs is reported to be estimated by spectrophotometric method[5,6] high-performance liquid chromatography (HPLC),[7] TLC,[8] HPTLC,[9] LC-MS,[10] FT-IR,[11] amperometric determination,[12] Fluorimetry[13] and Micellar electrokinetic chromatographic method.[14] Few analytical methods for determination of LOX using a voltametric,[15] polarographic,[16] UV spectrophotmetric,[17] LC/MS/MS[18,19] and HPLC[20-23] in plasma and pharmaceutical formulation have been reported.

Extensive literature survey reveals that no reversed-phase (RP)-HPLC method is reported for simultaneous determination of LOX and PCM in tablet dosage form. Fixed dose combination containing PCM (500 mg) and LOX (8 mg) is available in tablet form in the market. Therefore, an attempt was made to develop a new, rapid and sensitive RP-HPLC method for the simultaneous

determination of PCM and LOX in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines,[24,25] which is mandatory also.

EXPERIMENTAL

Instrumentation

Liquid chromatographic system from Shimadzu (LC-20AT) comprising of manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery and Photodiode array detector SPD-M20A connected to software LC solution for controlling the instrumentation as well as processing the data generated was used.

Reagents and chemicals

Analytically pure sample of LOX and PCM was kindly supplied by Lupin Laboratories Mumbai, India. Methanol, potassium dihydrogen phosphate, disodium hydrogen phosphate was of HPLC grade supplied by Merck Ltd., India. The pharmaceutical dosage form used in this study was a Neucam-P (Lupin (maxter) Laboratories, Mumbai, India) and Lornicam plus 8 (Aristo Pharmaceutical Pvt. Ltd, Delhi, India) tablets containing 500 mg PCM and 8 mg LOX were purchased from the local drug market. Triple distilled water was generated in house.

Chromatographic condition

The isocratic mobile phase consisted of methanol–phosphate buffer (pH 7.0) in the ratio of (60:40 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. A Luna C_{18} column (5 μm, 150mm x 4.60mm) was used as the stationary phase. Although the PCM and LOX have different λmax viz 248 and 380, 290, 261 nm, respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 260 nm was selected as the detection wavelength for UV-PDA detector.

Standard preparation

Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of methanol and phosphate buffer in the ratio of 60:40 (pH 7.0) to get concentration of 1000 μg/ ml.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 10-50, 8-40 μg/ml for PCM and LOX, respectively.

Sample preparation

Twenty tablets of each brad Neucam-P and Lornicam plus 8 were weighed individually and ground to a fine powder. An accurately weighed powder sample equivalent to 8 mg of LOX and 500 mg PCM were transferred to 100 ml of volumetric flask. Drug was extracted with three 20 ml quantities of mixture of diluent. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark and filtered through Whatman filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of methanol/ phosphate buffer (60:40, v/v, pH 7.0) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a RP C18 column, the retention times for PCM and LOX were observed to be 2.06 and 4.38 min, respectively. Total time of analysis was less than 5 min. The maximum absorption of PCM and LOX together as detected at 260 nm and this wavelength was chosen for the analysis [Figure 2].

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for PCM and LOX were 2581 and 3728, respectively.

Linearity

PCM and LOX showed a linearity of response between 10-50 and 8-40 μg/ml, respectively. The linearity

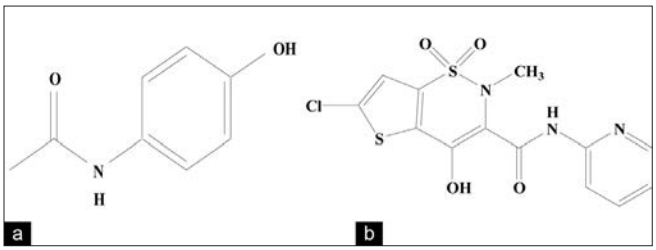

Figure 1: Chemical structures of (a) Paracetamol (b) Lornoxicam

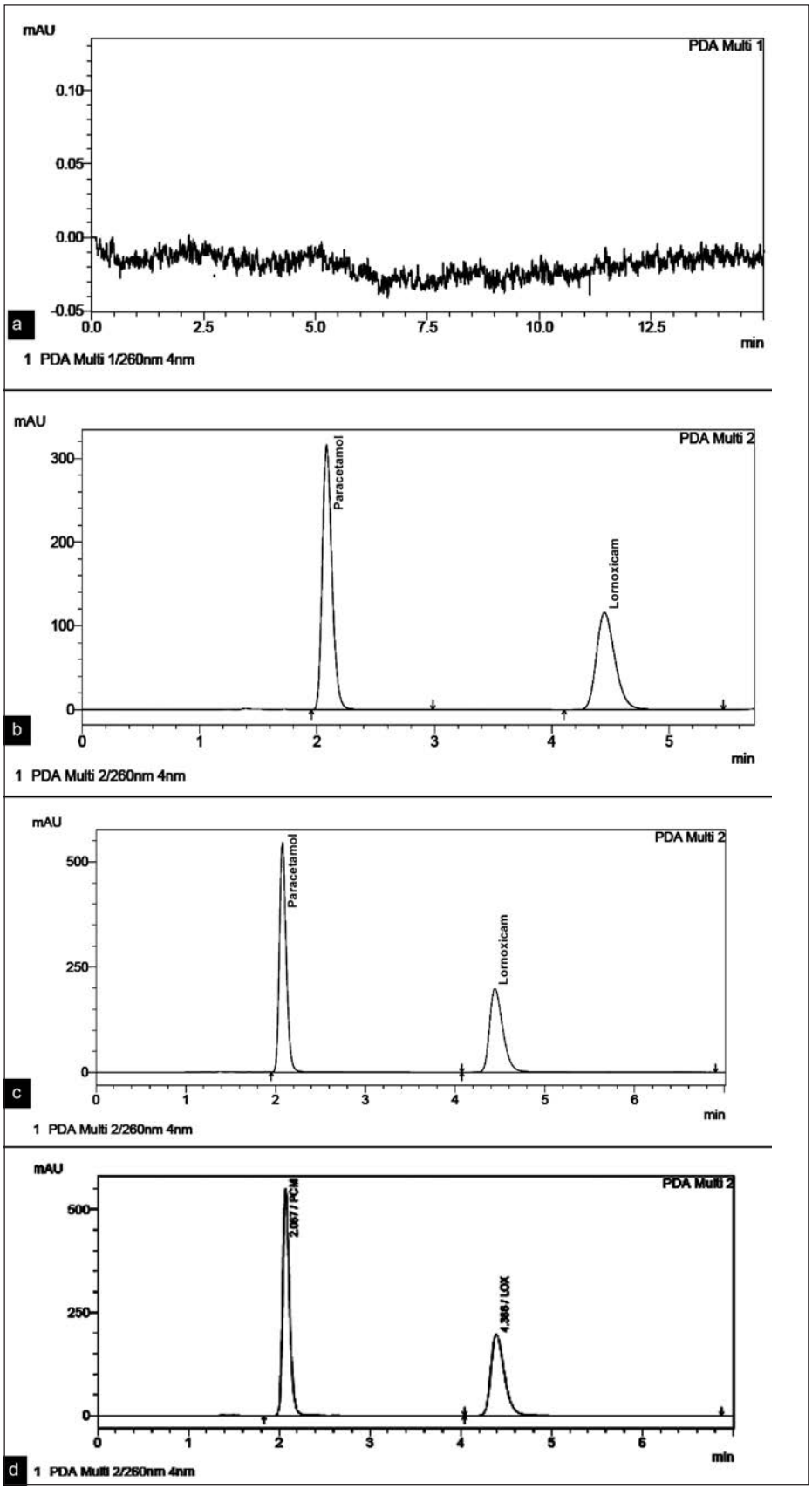

Figure 2: Chromatograph resulting from (a) mobile phase (b) standard paracetamol (30 µg) and lornoxicam (24 µg) (c) standard paracetamol (50 µg) and lornoxicam (40 µg) with Rf min 2.06±0.013 and 4.38±0.07 min, respectively (d) tablet sample paracetamol (50 µg) and lornoxicam (40 µg).

was represented by a linear regression equation as follows. The results of statistical analysis were shown in Table 2.

Y (PCM)= 57965.56 conc. + 32783.18 (r^2=0.9994)
Y (LOX)= 51745.89 conc. + 2703.4 (r^2=0.9995)

where Y is area under curve and r^2 is correlation coefficient.

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method Table 3. The mean percentage recoveries obtained for LOX and PCM were 100±0.16 and 99.50±0.43%, respectively.

Table 1: System suitability parameters

Parameter	Paracetamol	Lornoxicam
Retention time*	2.06±0.013	4.38±0.07
No. of theoretical plate*	2581.00±36.469	3728.50±61.45
Tailing factor*	1.33±0.010	1.26±0.02
HETP*	0.10±0.001	0.07±0.001
Linearity range	10-50 mg/ml	8-40 mg/ml

*Each value is the Mean ± S.D of six determinations

Repeatability

Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits (relative standard deviation, RSD ‹ 2) as shown in Table 4.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD ‹ 2) as shown in Table 4.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of methanol:phosphate buffer (pH 7.0) (60:40v/v), methanol:phosphate buffer (pH 7.0) (55:45 v/v), was used as a mobile phase. Results of analysis were summarized in Table 5.

Stability of sample solution

The sample solution injected after 12 hr do not show any appreciable change. Results are shown in Table 6.

Table 2: Statistical analysis for the calibration curves of paracetamol and lornoxicam

Serial no.	Parameter	Mean±SD*		RSD	
		PCM	LOX	PCM	LOX
1	Linearity	10-50 μg/ml	8-40 μg/ml		
2	Correlation coefficient	0.9994 ± 0.0001	0.9995 ± 0.0001	0.0001	0.0001
3	Slope	57965.56 ± 285.39	51745.89 ± 311.02	0.0049	0.0060
4	Intercept	32783.18 ± 3742.5	12810.88 ± 2703.4	0.1141	0.2110

*Each value is the Mean ± S.D of six determinations

Table 3: Result of recovery studies with statically evaluation

Serial no.	Conc. of drug in preanalyzed samples (μg/ml)		Std. drug sol. Added (μg/ml)		Recovered amount* (μg/ml)		% Recovered	
	PCM	LOX	PCM	LOX	PCM	LOX	PCM	LOX
1	10	8	20	8	19.89	7.91	99.45 ± 0.03	99.19 ± 0.62
2	20	16	20	16	19.82	15.87	99.1 ± 0.26	99.63 ± 0.51
3	30	24	20	24	19.99	23.92	99.95 ± 0.12	99.72 ± 0.22
						Mean	99.50 ± 0.43	99.51 ± 0.16
						%R.S.D	0.429	0.160

*Mean of nine determinations (3 replicates at 3 concentration level)

Table 4: Result of precision

Validation parameter	Percentage Mean ± S.D.* (n=15)		Percentage RSD*	
	PCM	LOX	PCM	LOX
Repeatability	100.05 ± 0.06	99.56 ± 0.12	0.25	0.37
Intermediate precision				
Day-to-Day	99.90 ± 0.155	99.61 ± 0.08	0.69	0.327
Analyst-to-Analyst	98.56 ± 0.01	99.50 ± 0.05	0.04	0.16

*Mean of 15 determinations (3 replicates at 5 concentration level)

Table 5: Results of robustness

Validation parameter	% Mean*		S.D.		% R.S.D.	
	PCM	LOX	PCM	LOX	PCM	LOX
Robustness	98.87	100.22	0.64	1.30	0.64	1.29

*Mean of six determinations

Table 6: Stability data of PCM and LOX

Hours	PCM 10 μg/ml	LOX 8 μg/ml
0	634366 ± 0.31	351220 ± 0.53
6	634455 ± 0.42	351160 ± 0.68
12	634656 ± 0.35	351090 ± 0.93

Tablet 7: Result of marketed tablet analysis

Parameter	Neucam-P		Lornicam Plus 8	
	PCM	LOX	PCM	LOX
Mean % estimated	99.87	99.06	99.28	99.69
Standard deviation (S.D.)	0.089	1.069	0.59	1.59
% Coefficient of variation	0.089	1.07	0.60	1.60
*Standard error (SEσ)	0.021	0.252	0.139	0.375

*Mean of 15 determinations (3 replicates at 5 concentration level)

Tablet analysis

Content of PCM and LOX found in the tablets by the proposed method are shown in Table 7. The low values of RSD indicate that the method is precise and accurate.

CONCLUSIONS

RP-HPLC method was developed and validated for simultaneous estimation of PCM and LOX in tablet dosage form. The developed method is suitable for the identification and quantification of binary combination of PCM and LOX. A high percentage of recovery shows that the method can be successfully used on a routine basis. Proposed method is simple, fast, accurate, precise and sensitive and could be applied for quality and stability monitoring of PCM and LOX combination.

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