Original Article

A validated high-performance thin layer chromatography method for estimation of lornoxicam and paracetamol in their combined tablet dosage form

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Abstract

Introduction: Lornoxicam (LORN) a nonsteroidal antiinflammatory drug of oxicam class is marketed in combination with Paracetamol, a common analgesic for acute inflammatory disease of joints. **Materials and Methods:** LORN and Paracetamol (PCM) were estimated at 280 nm by densitometry using silica gel 60 F_{254} as stationary phase and a premix of toluene: chloroform: methanol: formic acid (3:5:1.5:0.2 *v/v/v/v*) as mobile phase. The method was found linear in a range of 160–560 nanograms/spot for LORN and 10 000–35 000 nanograms/spot for PCM with a correlation coefficient >0.99 for both. **Result:** PCM and LORN were well resolved with Rf 0.57 ± 0.02 and 0.75 ± 0.02, respectively. **Conclusions:** The developed high-performance thin layer chromatography method was found to be simple, specific, precise, and reproducible and can be used for the routine estimation of LORN and PCM in the combined tablet dosage form, available in market.

Key words: High-performance thin layer chromatography; lornoxicam and paracetamol; simultaneous validation

INTRODUCTION

Lornoxicam (LORN) is a nonsteroidal anti-inflammatory drug of the oxicam class with the analgesic, anti-inflammatory and antipyretic properties having chemical name (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide. It has been found to be effective in Inflammatory diseases of the joints, osteoarthritis, pain following surgery, and sciatica.[1] Unlike other oxicam, it has shorter elimination half-life of 3–5 h.[2] Paracetamol (PCM) common analgesic have chemical name *N*-(4-hydroxyphenyl)acetamide. PCM/acetaminophen is used for relief in fevers, aches, and pains associated with many parts of the body. It has weak antiinflammatory properties. It is combined with LORN in the tablet dosage form.[3-6]

Analytical techniques such as spectrophotometry,[7,8] high-performance liquid chromatography (HPLC),[9-11] high-performance thin layer chromatography (HPTLC),[12] LC/MS/MS,[13] etc. are reported for the detection of LORN and PCM individually in plasma and bulk pharmaceutical formulation alone, and also some spectrophotometry[14,15] and HPTLC[16] methods for the estimation of LORN and PCM in their combination have been reported. Hence, our aim is to develop a new accurate simple and rapid HPTLC method for the estimation of LORN and PCM in the combined tablet dosage form.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation of drugs were performed on Merck TLC plates

precoated with silica gel 60 F_{254} (20×10 cm with 250 mm layer thickness, E. Merck, Germany). The samples were applied onto the plates as a band with the width of 4 mm using Camag 100 µl sample syringe (Hamilton, Switzerland) with an applicator (AS-30, Desaga, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20×10 cm). Densitometric scanning was performed using the TLC scanner (CD 60, Desaga, Switzerland) and operated by software (proquant). Electronic balance (ACCULAB Model ALC-210.4 Huntington valley, PA), Sonicator (EN 30 US, Entertech Fastclean, Mumbai, India).

Materials

LORN and PCM working standards were obtained as a gift samples from Cirex pharmaceutical Ltd (Gundla, Mandal district, A.P., India) and Cadila Pharmaceutical Ltd (Dholka, Ahmedabad, Gujarat, India), respectively. LORN and PCM combined tablet (Claiming 8 mg of LORN and 500 mg of PCM per tablet) of two different brands Lornasafe-plus (B.No. LFS045, Mankind Pharma Ltd., New Delhi) and Lorsaid-P (B.No. LOS10001, Piramal Healthcare Ltd., Mumbai) were collected from market and analyzed for the LORN and PCM content by the proposed method. All the other chemicals and reagents were of analytical grade.

Selection of detection wavelength

The wavelength was selected at 280 nm, at which, LORN shows high absorbance. This could be used to compensate for relatively low concentration of LORN compared to PCM in the marketed formulation. In the tablet dosage form PCM and LORN were found in the ratio of 500:8 mg/tab. Hence, the selected wavelength was convenient to obtain good response peaks for both the drugs.

Preparation of solution

Standard stock solution of both drugs were prepared by dissolving 1000 mg of PCM and 16 mg of LORN in Acetonitrile to obtain 100 ml stock solution (10 000:160 µg/ml) and further diluted to get final linear concentrations.

Chromatographic condition

A premix of toluene: chloroform: methanol: formic acid (3:5:1.5:0.2 *v*/*v*/*v*/*v*), respectively was optimized for thin layer chromatography plate development. The chamber was saturated with the mobile phase at room temperature for 30 min. A run distance was kept about 67 mm and 10 ml of the mobile phase was used for single development. The dosing speed of nitrogen applicator was kept 150 nl/sec with a predosage volume of 5 µl. Samples were applied as bands of 4 mm width with the gaps of 10 mm in between. Developed plates were dried at room temperature for 5 min. Detection was done at 280 nm using the deuterium lamp in the absorption reemission mode. The slit dimension of detection was kept to be 0.4 mm × 0.02 mm.

Method validation

This optimized HPTLC method was then validated for the parameters listed below as per International Conference on Harmonisation (ICH) guidelines.[17]

Linearity

Different concentrations of LORN (160 to 560 ng/band) and PCM (10 000 to 35 000 ng/band) were applied on the TLC plate and peak area were measured in densitometer. The calibration curves were constructed by plotting the peak areas *vs* concentrations for both drugs and the regression equation was calculated. Each response was an average of three determinations.

Precision

Precision of the method was determined in the terms of intraday and interday variation (%RSD). Intraday precision (%RSD) was assessed by analyzing standard drug solutions (240:15 000 ng/spot, 320:20 000 ng/spot and 400:25 000 ng/spot of LORN:PCM) within the calibration range, three times on the same day. Interday precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample solution at three different levels 50%, 100%, and 150 %. Mean percentage recovery was determined.

Specificity

The absence of any secondary spot having spectra different from LORN and PCM in the typical constituted placebo chromatogram of the tablet preparation, which may interfere with LORN and PCM peak, indicates the specificity of the analytical method.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were obtained by calculating using the standard formula as per the ICH guidelines,

LOD=3.3×(σ/S),LOQ=10×(σ/S).

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Formulation analysis

Ten combined tablets of two different LORN and PCM tablet samples Lornasafe-plus and Lorsaid-P were finely powdered and powder equivalent to 1000 mg of PCM and 16 mg of LORN was dissolved in acetonitrile to obtain 100 ml stock solution (10 000:160 µg/ml). It was sonicated and filtered through the milipore filter and further diluted with acetonitrile to get final concentration.

RESULTS AND DISCUSSION

Development of the optimum mobile phase

Different mobile phases were tried to resolve LORN and PCM. The optimum results were obtained with mobile phase consisting of toluene: chloroform: methanol: formic acid (3:5:1.5:0.2 *v/v/v/v*). The Rf values of LORN and PCM peak were observed about 0.75 ± 0.02 and 0.57 ± 0.02, respectively. The representative densitogram is given in [Figure 1].

Validation of the developed stability-indicating method

Linearity

The response for the drugs was found to be linear in the concentration range 160–560 ng/band for LORN and 10 000–35 000 ng/band for PCM with correlation coefficient of 0.995 and 0.997, respectively. The linear regression equation obtained are $y = 0.901(x)$ + 335.4 and $y = 0.062(x)$ + 1023 for LORN and PCM, respectively [Table 1].

Precision

The % RSD values for intraday precision study were found to be not more than 1.95% and 1.44% for LORN and PCM, respectively and for interday precision were found to be not more than 1.98% and 1.95% for LORN and PCM, respectively, thus confirming precision of the method [Table 2].

Accuracy

Excellent recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80%, 100%, 150% level) indicated the mean recovery for LORN 98.67–99.06% and for PCM 99.14–101.77% [Table 3].

Limit of detection

The LOD as calculated by standard formula as given in ICH guidelines was found to be 63 ng/band and 1478 ng/band for LORN and PCM, respectively.

Limit of quantitation

The LOQ as calculated by standard formula as given in ICH guidelines was found to be 192 ng/band and 4480 ng/band for LORN and PCM, respectively.

Table 1: Calibration data for linearity

Amount in nanogram/spot		AUC[a] ± %RSD[b] at 280 nm (n=3)	
LORN	PCM	LORN	PCM
160	10 000	490 ± 2.00	1622 ± 1.74
240	15 000	544 ± 1.95	1951 ± 1.44
320	20 000	628 ± 1.69	2347 ± 1.43
400	25 000	704 ± 1.94	2600 ± 1.18
480	30 000	795 ± 1.97	2923 ± 1.07
560	35 000	844 ± 1.72	3165 ± 1.15

[a] AUC: Area under the curve. [b] %RSD- % relative standard deviation.
LORN: Lornoxicam, PCM: Paracetamol

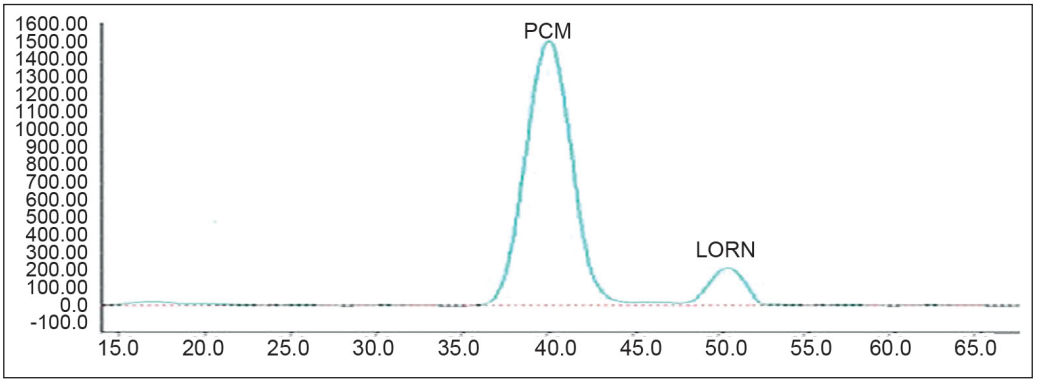

Figure 1: HPTLC densitogram of Paracetamol (PCM) and Lornoxicam (LORN).

Table 2: System precision of the analytical method

Amount in nanogram/spot		AUC ± %RSD (*n*=3) intraday		AUC ± %RSD (*n*=3) interday	
LORN	PCM	LORN	PCM	LORN	PCM
240	15 000	557 ± 1.95	1986 ± 1.44	558 ± 1.98	1963 ± 1.81
320	20 000	641 ± 1.69	2391 ± 1.43	614 ± 1.82	2366 ± 1.95
400	25 000	722 ± 1.94	2562 ± 1.18	689 ± 1.97	2605 ± 1.90

LORN: Lornoxicam, PCM: Paracetamol

Table 3: Recovery study of the analytical method

Test concentration		Amount spiked		%Recovery ± %RSD	
LORN	PCM	LORN	PCM	LORN	PCM
160	10 000	80	15 000	98.78 ± 1.57	99.14 ± 1.22
160	10 000	160	20 000	99.06 ± 1.27	101.77 ± 1.43
160	10 000	240	25 000	98.67 ± 1.82	99.61 ± 1.36

LORN: Lornoxicam, PCM: Paracetamol

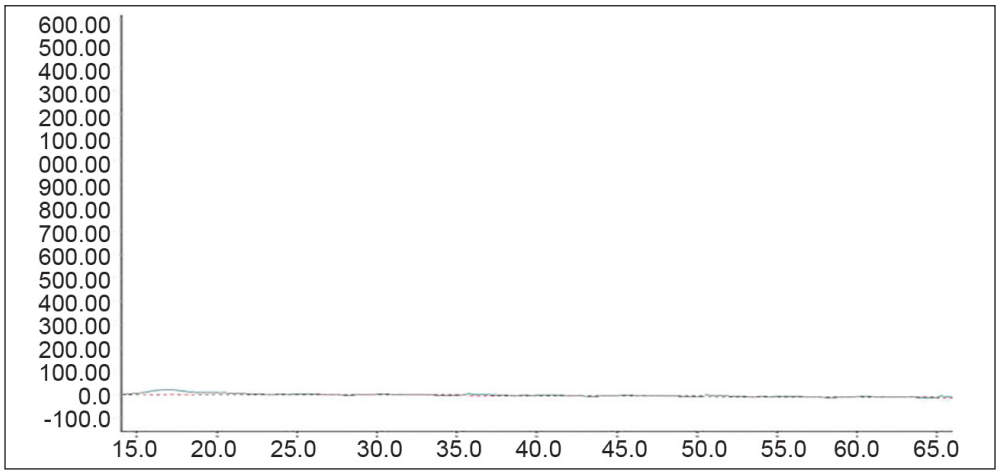

Figure 2: Chromatogram of constituted tablet placebo

Table 4: Summary of validation

Validation parameter	LORN	PCM
Regression equation	*y*=0.901(*x*) + 335.4	*y*=0.062(*x*) + 1023
Linearity	0.995	0.997
Precision	1.69–1.95	1.18–1.44
Recovery	98.67–99.06 %	99.14–101.77 %
LOD	63 ng/band	1478 ng/band
LOQ	192 ng/band	4480 ng/band
Specificity	Specific	Specific
%Assay (lorsaid-P)	98.83%	100.18%
(lornasafe-plus)	98.23%	99.45%

LORN: Lornoxicam, PCM: Paracetamol, LOD: Limit of detection , LOQ: Limit of quantitation

Formulation analysis

The percentage Assay of LORN and PCM in two different tablet samples Lornasafe-plus and Lorsaid-P were calculated and recorded in Table 4.

Specificity

The specificity of the method was ascertained by the absence of any other peak of placebo [Figure 2].The validation summary is given in Table 4.

CONCLUSION

The developed HPTLC method was found to be simple, specific, precise, accurate, and reproducible and can be used for the routine estimation of LORN and PCM in the combined tablets dosage form available in market. The developed method is found to be less sensitive but more accurate and specific than the previously published method.

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