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Simultaneous Determination of Paracetamol and Lornoxicam in Tablets by Thin Layer Chromatography Combined with Densitometry

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Abstract: A simple, rapid and accurate High-performance thin-layer chromatography (HPTLC) method has been established and validated for the simultaneous determination of paracetamol and lornoxicam in tablets. The method is based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 270 nm. The separation was carried out on Merck TLC aluminium sheets of silica gel 60F-254 using ethyl acetate: methanol: toluene: glacial acetic acid (7:2.5:1:0.5, v/v/v/v) as a mobile phase. Calibration curves were linear in range of 200- 1200 ng/spot and 100 - 600 ng/spot for paracetamol and lornoxicam, respectively. Method was successively applied to tablet formulation. No chromatographic interferences from the tablet excipients were found. The method was validated in accordance with the requirements of ICH guidelines.

Key words: Paracetamol; Lornoxicam; High-performance thin-layer chromatography; Validation.

Introduction

Paracetamol (PARA), chemically 4-hydroxy acetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic[1-3]. Literature survey reveals, there are UV, HPLC and HPTLC methods reported for the estimation of PARA in Pharmaceutical formulations [4-9].

Lornoxicam (LOX) is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2- thiazine-3-carboxamide 1,1-dioxide; is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. LOX belongs to the chemical class oxicams, which includes lornoxicam, tenoxicam and meloxicam. LOX, which is commercially available as an 8-mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, pain after surgery, and sciatica[10]. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body[11-13].

Extensive literature survey reveals, only one method is available that is based on estimation of Paracetamol and Lornoxicam by simultaneous equation method[14]. The present paper describes a reliable, rapid and accurate HPTLC method for determination of paracetamol and lornoxicam using HPTLC densitometry. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[15]

2. Experimental

2.1 Materials and Reagents

Paracetamol and Lornoxicam were kindly supplied as a gift sample by Sun Pharmaceuticals Ltd., Vadodara. Toluene, methanol, ethyl acetate and glacial acetic acid were used as solvents to prepare the mobile phase. All the reagents used were of analytical reagent grade (S.D. Fine Chemicals, Mumbai, India) and used without further purification.

2.2 Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width 6 mm with 100 µL sample syringe on precoated silica gel aluminium plate 60F-254 (20 cm x 10 cm) with 250 µm thickness; (E MERCK, Darmstadt, Germany) using a Camag Linomat V (Switzerland). The plates were prewashed with methanol and activated at 1100C for 5 min, prior to chromatography. A constant application rate of 150 nL /sec was employed and space between two bands was 11.6 mm. The slit dimension was kept at 6 mm x 0.45 mm. The mobile phase consists ethyl acetate: methanol: toluene: glacial acetic acid (7:2.5:1:0.5, *v/v/v/v*). Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 35 min, at temperature (25^0C . 2); the length of chromatogram run was 8 cm and TLC plates were air dried. Densitometric scanning was performed on Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 270nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using linear regression analysis *via* peak areas.

2.3 Standard solutions and calibration graphs

Combined standard stock solution containing 0.2g/L of paracetamol and 0.1 g/L of lornoxicam was prepared in methanol. Calibration was done by applying mix standard solutions ranging from 1.0 – 6.0 µL by Hamilton syringe with the help of automatic sample applicator Linomat V on TLC plate that gave concentration 200-1200 ng/spot of paracetamol and 100 - 600 ng/spot of lornoxicam, respectively. Each concentration was spotted six times on the TLC plates. The plates were developed using previously described mobile phase. The calibration graph was plotted as peak areas *versus* corresponding concentrations.

2.4 Method Validation

2.4.1 Optimization of HPTLC method

Initially, ethyl acetate and methanol in the ratio of 1:1 (*v/v*) was tried for both drugs simultaneously. The spots were not developed properly and dragging was observed. Then, toluene, methanol and ethyl acetate in the ratio of 1:2.5:7 (*v/v/v*) was tried. The developed spots were diffused. To the above mobile phase, 0.2 mL glacial acetic acid was added. Both the peaks were symmetrical in nature and tailing was observed. To improve resolution, the volume of glacial acetic acid was increased to 0.5 mL. Ultimately, mobile phase consisting of ethyl acetate:methanol:toluene:glacial acetic acid (7:2.5:1:0.5 v*/v/v/v*) gave good resolution. Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 270 nm. The chamber was saturated with the mobile phase for 35 min at room temperature and plates were activated at 110 ^{o}C for 5 min to obtain well-defined spots.

2.4.2 Linearity

Linearity responses for paracetamol and lornoxicam were assessed in the concentration range 200-1200 ng/spot and 100-600 ng/spot, respectively. The linear equations for the calibration plots were Y = 2.553 X - 251.63 and Y = 11.015 X + 44.17, with correlation coefficient (r) being 0.9990 and 0.9998 for paracetamol and lornoxicam, respectively. Range was established with five replicate readings of each concentration.

2.4.3 Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week. The results are shown in Table 1.

Table 1: Result of Precision Study

Drug	Amount Applied ng/spot	Intraday Precision [%RSD, n=3]	Interday Precision [%RSD, n=3]
PARA	200	0.022	0.011
	400	0.269	0.007
	600	0.055	0.062
LOX	100	0.089	0.038
	200	0.021	0.045
	300	0.065	0.025

Table 2: Results of Recovery Studies

Component	Label claim (mg/tablet)	Amount of Standard Drug added (%)	% Drug Recovered *	% RSD
Paracetamol	500	50 100 150	99.79 99.00 99.66	0.53 0.69 1.21
Lornoxicam	8	50 100 150	100.84 99.77 100.98	0.70 0.83 0.81

* mean of three estimations at each level

2.4.4 Sensitivity

The sensitivity of measurement of paracetamol and lornoxicam by the use of proposed method was estimated in terms of Limit of Quantitation (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated by visual detection. The LOD and LOQ value are 50.0 and 200.0 ng/spot for PARA; 80.0 and 200.0 ng/spot for LOX.

2.4.5 Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (paracetamol and lornoxicam) was added at three different levels. These solutions were subjected to re-analysis by the proposed method; results of the same are shown in Table 2.

2.4.6 Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Fig.1. The spot for paracetamol and lornoxicam was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 270 nm for detecting peak purity of paracetamol and lornoxicam was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

2.4.7 Repeatability

Repeatability of sample application was assessed by spotting (0.1 µg µL-1) of drug solution seven times on a TLC, followed by development of plate and recording the peak area for seven spots. The % RSD for peak area values of paracetamol and lornoxicam was found to be 0.23 and 0.56, respectively.

2.5 Analysis of paracetamol and lornoxicam in marketed formulation

To determine the content of paracetamol and lornoxicam simultaneously in conventional tablets (label claim 500 mg paracetamol and 8 mg lornoxicam); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 2 mg (lornoxicam) and 125 mg (paracetamol) was transferred into 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to mark with same solvent. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). 1µL of the above solution applied on TLC plate followed by development and scanning as described in section 2.2.The analysis was repeated for six times. Paracetamol and lornoxicam gave sharp and well defined peaks at R_f 0.72 and 0.39, respectively, when scanned at 270 nm. The results are shown in Table 3 indicate that there was no interferences from the excipients commonly present in the tablets.

Table 3: Assay of Tablet Formulation

Component	Label Claim (mg)	% Label Claim*	% RSD
Paracetamol	500	99.90	0.23
Lornoxicam	8	98.75	0.14

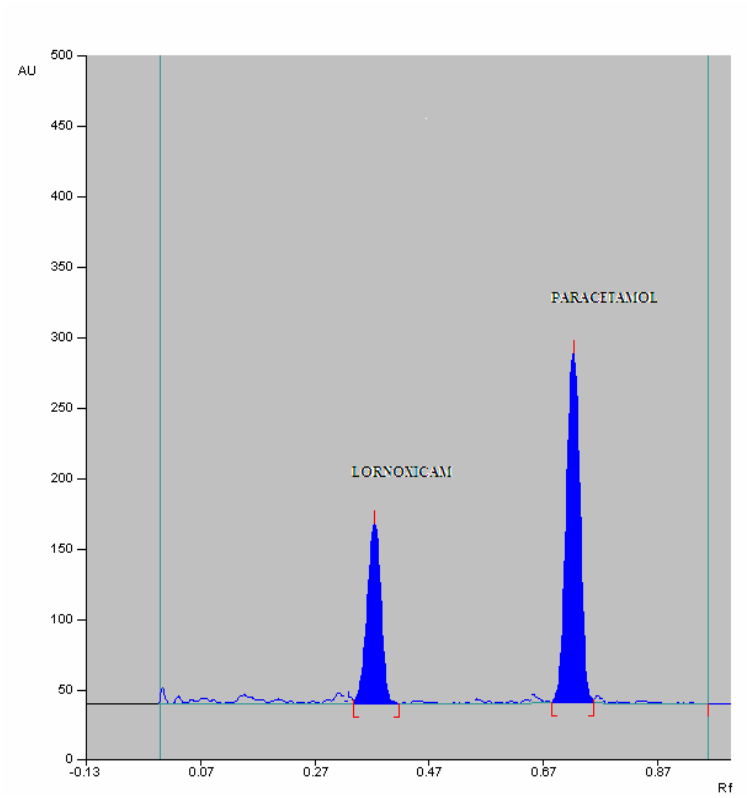

FIG 1: A TYPICAL DENSITOGRAM OF PARACETAMOL AND LORNOXICAM

3. Conclusion

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of paracetamol and lornoxicam in tablets. The method was validated as per ICH guidelines.

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