Original Article

Development and validation of a HPTLC method for simultaneous estimation of lornoxicam and thiocolchicoside in combined dosage form

Abstract

Aim: To develop a simple, precise, rapid and accurate HPTLC method for the simultaneous estimation of Lornoxicam (LOR) and Thiocolchicoside (THIO) in bulk and pharmaceutical dosage forms. **Materials and Methods:** The separation of the active compounds from pharmaceutical dosage form was carried out using methanol:chloroform:water (9.6:0.2:0.2 v/v/v) as the mobile phase and no immiscibility issues were found. The densitometric scanning was carried out at 377 nm. The method was validated for linearity, accuracy, precision, LOD (Limit of Detection), LOQ (Limit of Quantification), robustness and specificity. **Results:** The R_f values (±SD) were found to be 0.84 ± 0.05 for LOR and 0.58 ± 0.05 for THIO. Linearity was obtained in the range of 60–360 ng/band for LOR and 30–180 ng/band for THIO with correlation coefficients $r^2 = 0.998$ and 0.999, respectively. The percentage recovery for both the analytes was in the range of 98.7–101.2 %. **Conclusion:** The proposed method was optimized and validated as per the ICH guidelines.

Key words: Densiotometry, HPTLC, lornoxicam, method validation, thiocolchicoside

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INTRODUCTION

Thiocolchicoside (THIO), (s)-N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1,2-dimethoxy-10-(methylthio)-9-oxobenzo[a]heptalen-7yl] acetamide [Figure 1], a semi-synthetic derivative of the naturally occurring compound colchicoside, has a relaxant effect on skeletal muscle, with a potent competitive antagonist of GABA function. It is used as a muscle relaxant and displays anti-inflammatory and analgesic properties with strong epileptogenic and convulsing activity. Lornoxicam (LOR, 6-chloro-4-hydroxy-2-methyl- *N*-2-pyridyl-2H-thieno [2, 3-e]-1, 2-thiazine-3-carbox- amide-1, 1-dioxide) [Figure 2] is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. LOR belongs to the chemical class oxicams, which includes piroxicam, tenoxicam and meloxicam.[1-5]

Literature survey reveals that few high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopic methods are reported for the estimation of LOR and THIO individually as bulk and in pharmaceutical formulations, and authors have developed RP-HPLC-PDA and Ratio Derivative and Absorption-corrected spectrophotometric methods for its estimation in the combination in the same laboratory. The review of the literature also revealed that there is no HPTLC method available for determination of this combination.[6-14] Therefore, the aim of the present work was to develop a simple, precise and accurate HPTLC method for simultaneous determination of LOR and THIO in the pharmaceutical dosage form. The method was validated according to ICH guidelines.[15]

MATERIALS AND METHODS

Reagents and Materials

A pure drug sample of LOR, % purity 98.80 and THIO, % purity 99.92 was kindly supplied as a gift sample by Glenmark Pharmaceuticals Ltd., Baddi, India and Medley Pharmaceuticals Ltd., Baddi, India, respectively. These samples were used without further purification. Two tablet formulations (Lot 302 and 304) were supplied by JCPL Pharma Ltd., Jalgaon, India, and were used for analysis containing LOR 8 mg and THIO 4 mg per tablet. TLC, aluminum plates pre-coated with silica gel $60F_{254}$, (10 cm × 10 cm) with 250-µm thickness were from Merck, Germany. Analytical grade methanol, chloroform and ammonia were procured from Merck Chemicals, Mumbai, India.

Instrumentation and Chromatographic Conditions

The standard solution ranging from 60 to 360 ng/band for LOR and 30 to 180 ng/band for THIO were applied on pre-coated silica gel aluminum $60F_{254}$ plates (10 cm × 10 cm with 250-µm thickness; E. Merck, Damstadt, Germany) using a Camag Linomat V sample applicator. The plates were pre-washed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 150 nL/s was used and the space between two bands was 12 mm. The slit dimension was kept at 6 mm × 0.30 mm and the scanning speed was 20 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. It was developed in a 10 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated pad, which was previously soaked in mobile phase. The mobile phase consisted of methanol:chloroform:water (9.6:0.2:0.2 v/v/v) and 10 ml of mobile phase was used per chromatogram run, and the length of each chromatogram run was 8 cm. After development, the plate was immediately dried and was observed under the CAMAG TLC visualizer. The air flow in the laboratory was maintained unidirectional. The well-resolved bands of drugs were scanned at 377 nm with a CAMAG TLC scanner III densitometer controlled by WINCAT's software (version V 1.4.4, Camag). The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm [Figure 3].

Methods

Preparation of standard solutions and calibration curve

Accurately, about 50 mg of each drug, LOR and THIO, were weighed separately and dissolved in 20 ml of analytical grade methanol. To this, 0.5 ml of ammonia solution was added and the volume was made up to 50 ml with methanol so as to get a concentration of 1000 µg/mL. From each of these solutions, 1 ml of the solution was pipette out and transferred to 10 ml volumetric flasks and the volume was made up to the mark using methanol so as to get the concentration of 100 µg/mL. It was observed that both the drugs show

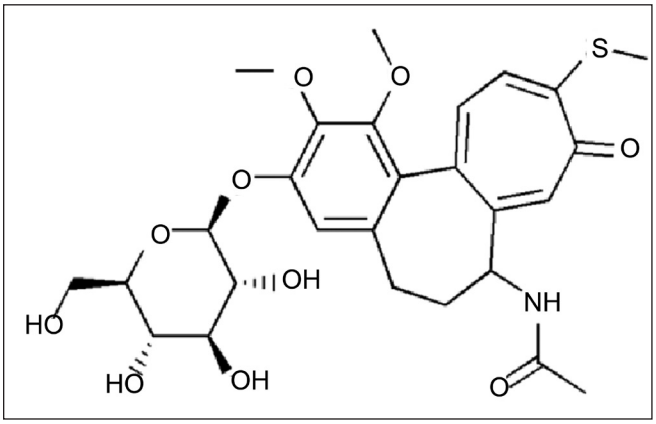

Figure 1: Structure of thiocolchicoside

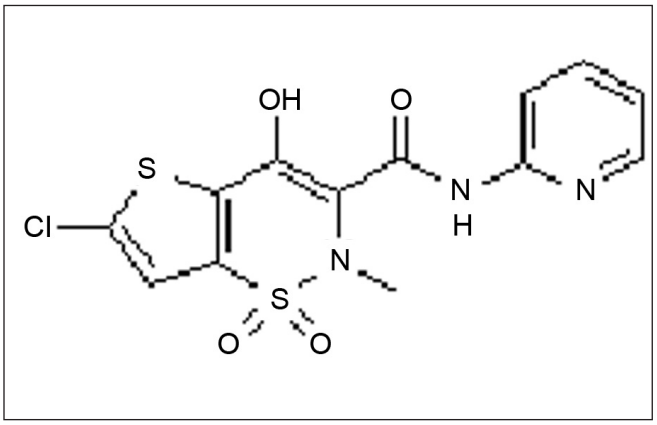

Figure 2: Structure of lornoxicam

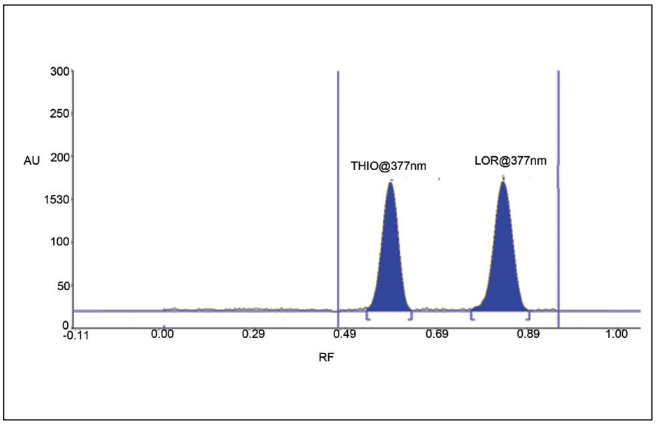

Figure 3: Densitogram of lornoxicam (8 µg/ml) and thiocolchicoside (4 µg/ml)

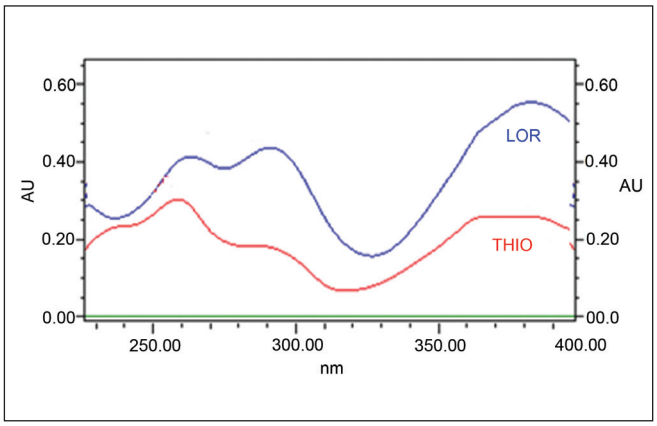

Figure 4: Overlain spectra of lornoxicam (8 µg/ml) and thiocolchicoside (4 µg/ml)

considerable absorbance at 377 nm [Figure 4], and peak area has a linear response in the concentration range of 60–360 ng/band and 30–180 ng/band for LOR and THIO, with correlation coefficients $r^2 = 0.998$ and 0.999, respectively.

Analysis of tablet formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 8 mg of LOR and 4 mg of THIO label claim was weighed and dissolved in 40 ml of methanol with the aid of ultrasonication for 10 min, and the solution was filtered through Whatman paper No. 41 into a 50 ml volumetric flask. The filter paper was washed with methanol, adding washings to the volumetric flask, and the volume was made up to the mark with methanol. From the filtrate, an appropriate dilution was prepared in the mobile phase to get a solution of 40 µg/mL of LOR and 20 µg/mL of THIO, and amount of solutions corresponding to 200 and 400 ng/band, respectively, were applied on the plates and the plates were developed by using optimized chromatographic conditions. These solutions were estimated according to the procedure given above.

Method validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and specificity.

Linearity

Linearity of the method was studied by spotting six concentrations of the drug prepared in the mobile phase in the range of 60–360 ng/band for LOR and 30–180 ng/band for THIO, and noting the peak areas. Peak areas of developed spots were measured and used to plot the calibration curve.

Accuracy

For accuracy of the method, a recovery study was carried out by applying the method to drug samples to which a known amount of LOR and THIO were added at the level of 50%, 100% and 150% of the label claim. At each level of the amount, three determinations were performed and the results obtained were compared with the expected results.

Precision

The precision of the method was demonstrated by repeatability, intra-day and inter-day variation studies at three different concentration level of analytes covering the concentration range. In the intra-day studies, three repeated measurements of standard and sample solutions were made in a day and three repeated measurements of standard and sample solutions were made on three consecutive days for inter-day variation studies. Percentages Relative Standard Deviation (RSD) were calculated for intra-day and inter day variation.

Limit of detection and limit of quantification

LOD and LOQ were calculated using σ (standard deviation of the response) and b (slope of the calibration curve) using the following formulae: LOD = (3.3 x σ) / b and LOQ = (10 x σ) / b.

Robustness

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like methanol:chloroform:water (9.6:0.2:0.2 v/v/v), (9.6:0.4:0.2 v/v/v), (9.4:0.2:0.2 v/v/v), (9.6:0.2:0.3 v/v/v) and (9.6:0.5:0.2 v/v/v) were tried and chromatograms were run. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 min. The amount of mobile phase was varied by ±5%; development distance was varied by ±5 mm. Robustness of the method was done at three different concentration levels: 60, 240, 360 ng per band and 30, 120, 180 ng per band for LOR and THIO, respectively. Time from spotting to chromatography and from chromatography to scanning was varied by +10 min; ultrasonication time of tablet extraction was varied by +2 min. Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and ambient temperature were altered, and the changes on the R_f values were noted.

Specificity

The peak purity of both drugs was assessed by comparing the respective spectra of standard drugs

and samples at peak start, peak apex and peak end positions of the spot. A blend of commonly used tablet excipients was treated as per the developed procedure and the densitogram showed no inferring peaks at the retention factor of the two drugs.

RESULT AND DISCUSSION

Optimization of solvent system and chromatographic conditions

Chromatographic separation studies were carried out on the stock solution of LOR and THIO. Initially, on the plates, 10 µL of stock solution was applied as a band of 8 mm width. Initially, plates were developed by using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. without chamber saturation. Based on the initial observation, solvent systems like methanol:water:toluene, chloroform:acetone:acetonitrile etc. were tried. On the basis of this observation, the methanol:water:chloroform system was tried in various ratios. After several trials, the mixture of methanol:chloroform:water (9.6:0.2:0.2 v/v/v) was chosen as the mobile phase for analysis and no immiscibility issues were found with the selected mobile phase combination. Other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks and detection wavelength were optimized to give reproducible R_f values, better resolution and symmetrical peak shape for the two drugs. Densitometry scanning was performed at 377 nm for the detection of LOR and THIO with R_f values of 0.84 and 0.58, respectively. Well-defined bands of standards were obtained in the chamber (Camag)-saturated pad that was previously soaked in mobile phase.

Linearity

Linearity of the method was studied by spotting six concentrations of the drug prepared in the mobile phase in the range of 60–360 ng/band for LOR and 30–180 ng/band for THIO. The correlation coefficient (r) values were >0.999 (n = 6). Typically, the regression equations for the calibration curve were found to be Y = 1550 + 19.46 * X for LOR and Y = 654.5 + 21.01 * X for THIO.

Table 1: Regression analysis of calibration curves, sensitivity and formulation analysis

Parameters		LOR	THIO
Det ection wavelength (nm)		377	377
Linearity range (ng/spot)		60–360	30–180
Regression equation (y = bx + a)	Slope (b)	19.56	21.01
	Intercept (a)	1550	654.5
Correlation coefficient (r^2)		0.998	0.999
Limit of detection (ng/spot)		20	10
Limit of quantitation (ng/spot)		60	30
Formulation analyzed (assay, % RSD)		100.32, 0.62	100.48, 1.05

Analysis of tablet formulation

The proposed method was also evaluated in terms of assay of commercially available tablets containing LOR and THIO. Three replicate determinations were performed on accurately weighed amounts of the tablets [Table 1].

Precision and accuracy

The method was found to be precise, as indicated by % RSD not more than 2. Intra-day and inter-day studies [Table 2] support the precision of the method. The proposed method when used for estimation of THIO and ACE from the pharmaceutical dosage form after spiking with the working standard afforded recovery of 99–101% [Table 3].

Sensitivity of the ethod (LOD and LOQ)

The limit of detection was found to be 20 ng/spot and 10 ng/spot, while the limit of quantitation was found to be 60 ng/spot and 30 ng/spot for LOR and THIO, respectively. The low value of LOD and LOQ indicates that the method is sensitive.

Robustness

The standard deviation of the peak areas was calculated for each parameter change and the % RSD was found to be less than 2%. The low values of the % RSD [Table 4] indicated robustness of the method.

Specificity

The method was found to be specific as no interfering spots were seen when R_f values of the standard and sample were compared. There is no difference in the

Table 2: Intra-day and inter-day precision (n = 3)

Drug name	Conc. (ng/spot)	Measured concentration (ng/spot), %RSD	
		Intra-day	Inter-day
LOR	60	60.07, 0.62	59.81, 0.97
	120	119.91, 0.73	120.04, 0.46
	180	179.91, 1.21	180.14, 0.54
THIO	30	29.88, 0.89	30.05, 0.36
	60	59.86, 1.10	59.13, 1.51
	90	90.14, 1.19	90.01, 0.45

Table 3: Results of the accuracy study

Parameters	Densitometric peak area, *n* = 3					
Level of recovery	50%		100%		150%	
Analyte name	LOR	THIO	LOR	THIO	LOR	THIO
Concentration (ng/spot)	60	30	120	60	180	90
Mean conc. found (ng/ml)	59.12	30.09	120.28	59.19	180.01	89.51
Mean % recovery	99.78	100.04	99.98	99.79	100.05	100.01
% RSD	0.84	1.04	0.98	0.97	0.53	1.25

Table 4: Robustness study of lornoxicam and thiocolchicoside (n = 3)

Parameter	SD of peak area		% RSD	
	LOR	THIO	LOR	THIO
Mobile phase composition	6.74	4.97	0.76	0.34
Amount of mobile phase	19.87	6.98	1.01	0.98
Time from spotting to chromatography	14.27	16.03	0.37	1.13
Time from chromatography to scanning	5.89	21.11	0.97	0.47
Plate from different lot no.	2.90	9.69	0.76	1.45

spectra of the sample and standard solution, which indicate the specificity of the method. The peak purity of both drugs was assessed by comparing the respective spectra of standard drugs and samples at peak start, peak apex and peak end positions of the spot.

CONCLUSION

HPTLC, with its advantage of low-operating cost, high-sample thought and minimum sample preparation need, is now-a-days preferred as a routine analytical technique for control and assurance. The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive and, thus, can be used for routine analysis of LOR and THIO in tablet dosage form to determine uniformity of contents for both the analytes in tablet in a short time.

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