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Simultaneous RP-HPLC Method for Determination of Lornoxicam and Thiocholchicoside in Pharmaceutical Dosage Form

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Abstract

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Lornoxicam and Thiocholchicoside in pharmaceutical dosage form. Chromatography was performed on a Varian C_{18} column (250 mm×4.6 mm i.d., 5 µm particle size), column with mobile phase containing Methanol, Acetate buffer (pH-4.6) and THF in the ratio of 50:35:15 v/v. The flow rate was 0.5 ml/min and the eluent was monitored at 375 nm. The selected chromatographic conditions were found to effectively separate Lornoxicam (RT- 6.400 min) and Thiocholchicoside (RT- 2.923 min). Linearity for Lornoxicam and Thiocholchicoside were found in the range of 4-100 µg/ml and 2-50 µg/ml. The values obtained of LODs were 0.29 and 0.14 µg/ml, LOQs were 0.89 and 0.44 µg/ml for Lornoxicam and Thiocholchicoside, respectively. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis of Lornoxicam and Thiocholchicoside in combined pharmaceutical formulations.

Keywords: Lornoxicam; Thiocholchicoside; Reversed-phase HPLC; Tetrahydrofuran (THF)

Introduction

Lornoxicam (LOR) is chemically ((3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one1,1-dioxide), Lornoxicam is a non-steroidal anti-inflammatory drug of the oxicam class with analgesic (pain relieving), anti-inflammatory and antipyretic properties [1-3] (Figure 1). Literature survey revealed that very few analytical methods have been reported for the estimation of Lornoxicam (LOR) which includes HPLC [4-9], Stability Indicating RP-HPLC [10]. Second drug, Thiocholchicoside (THIO) (Figure 2) is chemically N-[(7S)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-benzo[a]heptalen-7-etrahydroyl]acetamide. Thiocolchicoside is a muscle relaxant with anti-inflammatory and analgesic effects. It acts as a competitive $GABA_A$ receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. The individual determination of Thiocholchicoside (THIO) has been carried out in formulations by HPLC [6], HPTLC [11]. Literature review did not reveal any method for simultaneous determination of LOR and THIO in combined pharmaceutical dosage form. So, we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged and economic method for simultaneous determination of these drugs in combined dosage forms. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in combined dosage form.

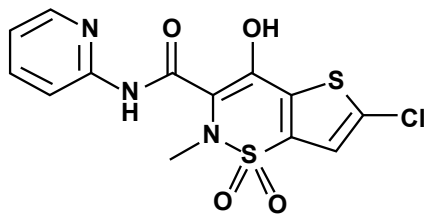

Figure 1: Structure of Lornoxicam

Experimental

Apparatus

A Younglin RP-HPLC instrument (YL 9100 HPLC System) equipped with an UV-Visible detector, manual injector with 20 µl loop, and Varian C_{18} (250 mm×4.6 mm i.d., 5 µm particle size), and YL- Clarity software were used. AX 200 Electronic Balance (Shimadzu), and Ultrasonic bath (Life care Fast Clean ultrasonic cleaning system, Mumbai, India) were used during the study.

Reagents and materials

The drug samples of Lornoxicam (LOR) and Thiocholchicoside (THIO) were kindly supplied by Glanmark Pharmaceuticals Ltd, Baddi

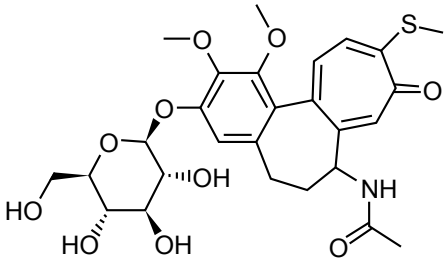

Figure 2: Structure of Thiocholchicoside

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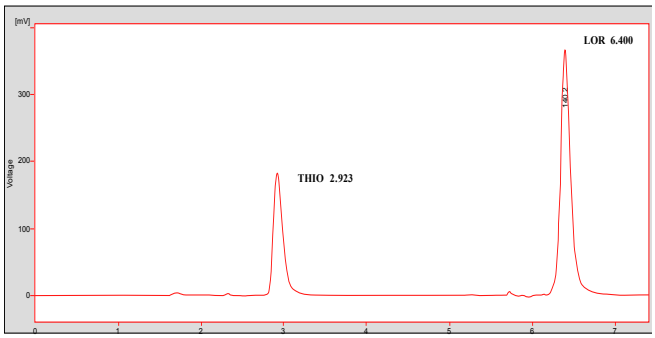

Figure 3: HPLC Chromatogram of LOR (40 µg/ml) and THIO (20 µg/ml)

and Alchem International Ltd., Vapi. Methanol (HPLC grade, S.D. Fine Chemicals Ltd., Mumbai), Water (HPLC grade Finar Chemicals Ltd., Ahmedabad.)

Tetrahydrofuran, (Merck, Mumbai, India) Sodium acetate and Glacial acetic acid (AR grade, S.D. Fine Chemicals Ltd., Mumbai), 0.22 µm filter (Millipore, Bangalore). Pharmaceutical formulation of LOR and THIO were purchased from local pharmacy.

Chromatographic conditions

Varian C_{18} column (250 mm×4.6 mm i.d., 5 µm particle size), The mobile phase was Methanol, acetate buffer (pH4.6) and THF in the ratio of 50:35:15 v/v and Flow rate 0.5 ml/min, Pressure was 1370 PSI, filter through Nylon 0.22 µm membrane filter, Mobile Phase was degassed before use, detection wavelength was 375 nm, The injection volume: 20 µl, and Temperature: 25 ± 3°C.

Preparation of mobile phase

Accurately weighed Sodium acetate (5.4 gm) in 50 ml water, 2.4 ml glacial acetic acid was added and is diluted with 100 ml water.

The mobile phase was Methanol, Acetate buffer (pH-4.6) and THF in the ratio of 50:35:15 v/v. The mobile phase was filtered through nylon 0.22 µm membrane filter and was degassed within the instrument before use.

Standard stock solution (500 µg/ml)

Accurately weighed LOR (50 mg) and THIO (50 mg) were transferred to two separate 100 ml volumetric flask. 50 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 500 µg/ml stock solution of both drugs.

Working standard solution (100 µg/ml)

From the above stock solution (500 µg/ml) of both drugs, transfer an accurately measured 20 ml volume of the stock solution into a 100 ml volumetric flask and make the final volume with methanol to prepare 100 µg/ml working solutions.

Sample solution

The powder from 20 tablets are weighed and collected. Accurately a quantity of the powder is weighed equivalent to about 8 mg of LOR and 4 mg of THIO in a 10 ml measuring flask and sonicate in Acetonitrile for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residues were washed thoroughly with acetonitrile. The filtrate and washings were combined in a 10 ml volumetric flask and diluted to the mark with methanol. Transfer 1 ml of extract into 10 ml volumetric flask and dilute to the mark with methanol to get a final concentration of 80 µg/ml of LOR and 40 µg/ml THIO.

Determination of wavelength of maximum absorbance:

The standard solution of LOR and THIO were scanned in the range of 200-400 nm against mobile phase as a blank. LOR and THIO showed maximum absorbance at 375 nm. So the wavelength selected for the determination of LOR and THIO was 375 nm.

Method Validation [12-16]

Calibration curve (Linearity)

A calibration curves were plotted over a concentration range of 4-100 µg/ml for LOR and 2-50 µg/ml for THIO. Accurately measured standard stock solutions of LOR (0.4, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, and 10.0 ml) and THIO (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0 and 5.0 ml) were transferred to a series of 10 ml corning volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solution was injected into the column and the peak area obtained at retention time 6.400 and 2.923 minute and flow rate 0.5 ml/min were measured at 375 nm for LOR and THIO respectively. Calibration curves were constructed for LOR and THIO by plotting peak area versus concentration at 375 nm. Each reading was average of three determinations.

Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets. (LOR 40 µg/ml and THIO 20 µg/ml) with three different concentrations of standards (LOR 10, 20, 30 µg/ml and THIO 10, 15, 20 µg/ml). The good recoveries with the standard addition method (Table 3) prove the good accuracy of the proposed method.

Method precision

It is a precision under a same condition (same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of LOR (40 µg/ml) and THIO (20 µg/ml) for six times and analyzed as per the proposed method. Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of LOR and THIO in triplicate at three different concentrations. Inter-day precision was evaluated by using freshly prepared solutions of LOR and THIO in triplicates at three different days. The coefficients of variation (CV) values at these concentration levels were calculated. Relative standard deviation of all the parameters was less than 2%, which indicates that the proposed method is repeatable.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for both drugs were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) and using following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD=3.3\times\sigma/S$

$LOQ=10\times\sigma/S$

Where σ=the standard deviation of the responses and S=Slope of calibration curve.

Specificity

The ICH document that defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

Analysis LOR and THIO in combined dosage forms

Pharmaceutical formulation of LOR and THIO was purchased from local pharmacy. The responses of formulations were measured at 375 nm for quantification of LOR and THIO by using RP-HPLC. The amounts of LOR and THIO present in sample solution were determined by fitting the responses into the regression equation for LOR and THIO. Results are given in table 4.

Result and Discussion

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Methanol, Acetate buffer (pH-4.6) and THF in the ratio of 50:35:15 v/v at 0.5 ml/min flow rate. The optimum wavelength for detection was set at 375 nm at which much better detector responses for both drugs were obtained. As it was shown in figure 3, the retention times were 6.400 min for LOR and 2.923 min for THIO. The calibration graphs for LOR and THIO were constructed by plotting the peak area versus their corresponding concentrations, respectively; good linearity for LOR and THIO were found over the range 4-100 and 2-50 µg/ml. Results obtained by applying the RP-HPLC method showed that the concentrations of LOR and THIO could be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of LOR and THIO in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives present do not interfere with analysis of the studied mixtures. System suitability test parameters for LOR and THIO for the RP-HPLC method are reported in table 1. The optical and regression characteristics and validation parameters are reported in table 2.

Parameters	LOR	THIO
Retention Time	6.400	2.923
Tailing factor	1.202	1.221
Asymmetry factor	1.286	1.368
Theoretical plate	14918	3288

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

Parameters	LOR	THIO
Calibration range	4-100 µg/ml	2-50 µg/ml
Detection limit	0.29 µg/ml	0.14 µg/ml
Quantitation limit	0.89 µg/ml	0.44 µg/ml
Slope	32.0940	30.5329
Intercept	8.4416	8.9551
Mean	99.66	101.00
Standard deviation	0.2539	0.294
Coefficient of variance	0.2547	0.291
Correlation coefficient	0.9998	0.9995
Intraday RSD, %	0.092-0.79	0.088-1.39
Interday RSD, %	0.017-0.43	0.142-0.751

Table 2: Assay parameters and method validation obtained by applying the proposed methods for determination of LOR and THIO in binary mixtures.

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
LOR	10	10	19.53	97.69 ± 0.343
	10	20	30.18	100.62 ± 0.127
	10	30	39.79	99.48 ± 0.173
THIO	5	10	15.32	102.14 ± 0.121
	5	15	20.21	101.07 ± 0.220
	5	20	24.52	98.11 ± 0.115

Table 3: Data of Recovery study for LOR and THIO by HPLC method.

LOR			THIO		
Amount taken (µg/ml)	Amount found (µg/ml)	% Amount found S.D. (n=3)	Amount taken (µg/ml)	Amount found (µg/ml)	% Amount found S.D. (n=3)
40	39.82	99.56 ± 0.14	20	20.20	101.04 ± 0.34
80	79.66	99.58 ± 0.094	40	40.19	100.47 ± 0.35
100	100.29	100.29 ± 0.057	50	50.55	101.10 ± 0.11

Table 4: Application of the proposed method to the pharmaceutical dosage forms.

Conclusion

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of LOR and THIO in pharmaceutical formulations without interference and with good sensitivity.

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