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Stability Indicating HPLC Method for Simultaneous Determination of Thiocolchicoside and Lornoxicam

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of thiocolchicoside and lornoxicam, using a RP-18 column and a mobile phase composed of 10mM ammonium acetate : methanol(50:50), pH7 adjusted with 1% triethyl amine. The retention time of thiocolchicoside and lornoxicam were found to be 4.6 and 10.2 min, respectively. Linearity was established for both thiocolchicoside and lornoxicam in the range of 1-10 µg/ml. The percentage recoveries of thiocolchicoside and lornoxicam were found to be 100.45±0.4489 and 100.70±0.5111, respectively. Both the drugs were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, and photolytic degradation. The degradation studies indicated thiocolchicoside to be susceptible to acid, alkaline and neutral hydrolysis while lornoxicam showed degradation under acid and alkali. The degradation products of thiocolchicoside and lornoxicam were well resolved from the pure drugs with significant differences in the retention time values. This method can be successfully employed for simultaneous quantitative analysis of thiocolchicoside and lornoxicam in bulk drugs and formulations.

Keywords: Thiocolchicoside , lornoxicam, degradation products, stability-indicating, HPLC

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INTRODUCTION

Thiocolchicoside (THIO) is an antispasmodic drug. Chemically it is N-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo [*a*] heptalen-7-yl] acetamide[1].(Fig-1) It is used as muscle relaxant, anti-inflammatory and analgesic. The antispasmodic activity is mainly due to the activation of GABA- inhibitory pathways. Thiocolchicoside is not official in any pharmacopoeia. Literature survey reveals that thiocolchicoside can be assayed by UV spectrophotometric[2], HPLC[3] and HPTLC[4] methods individually or in combination with other drugs.

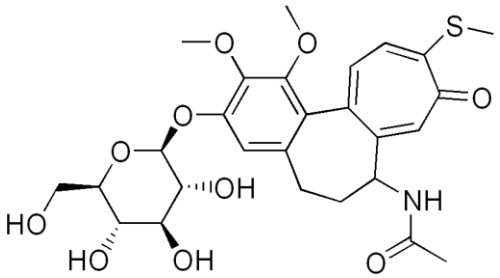

Figure 1: Chemical structure of thiocolchicoside

Lornoxicam (LOR) is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. It is under the same chemical class as piroxicam, meloxicam and tenoxicam[1]. It works by blocking the action of cyclo oxygenase which is responsible for the production of prostaglandin in the body. Chemically it is 6-Chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H- thieno[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide. LOR is not official in any pharmacopoeia(Fig-2). Literature survey reveals that LOR can be assayed by UV spectrophotometric[5] and HPLC[6] methods individually or in combination with other drugs[7,8]. Extensive literature survey reveals that no stability indicating chromatographic method has been reported for simultaneous determination of thiocolchicoside and LOR in tablet dosage form.

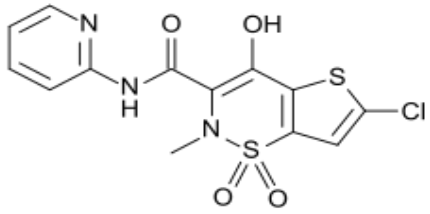

Figure 2: Chemical structure of lornoxicam

The International Conference on Harmonization (ICH) guideline entitled 'Stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances [9, 10]. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the

implementation of an analytical methodology to determine THIO and LOR simultaneously, in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought necessary to study the stability of THIO and LOR under acidic, alkaline, neutral hydrolysis, oxidative, dry heat, and photolytic conditions. This paper reports validated stability-indicating HPLC method for simultaneous estimation of THIO and LOR in presence of their degradation products.

MATERIALS AND METHODS

Pharmaceutical grade of thiocolchicoside and lornoxicam (purity > 99%) were used without further purification and a commercial Capsule Flexispaz 8 was purchased from local market. Methanol used was of HPLC grade and was purchased from Merck, India. The liquid chromatograph mass spectrometer Shimadzu LCMS-2010EV, which consisted of following components: a binary gradient pump, variable wavelength programmable PDA detector with auto sampler system was employed for the present study.

Instrumentation and chromatographic conditions

The chromatographic analysis was performed using Compaq Intel Core-2 DUO HP W/907 software on a pre-packed RP-18 column (250×4.6 mm, 5 µm particle size). In addition, an electronic balance (Shimadzu. Elec. balance BL-220H), a pH meter (Eli co L127), a sonicator (Leclasonic ultrasonic cleaner), a hot air oven (Inlab equipments Ltd) were used in the study. Separation was achieved using a mobile phase consisting of 10mM ammonium acetate (pH 7): methanol(50:50 v/v) , at a flow rate of 1ml/min and the eluent was monitored using PDA detector at 288nm. The column was maintained at ambient temperature and injection volume of 20 µl was used.

Preparation of standard sample solutions

Standard Preparation

Standard stock solutions containing 100 µg /ml of THIO and LOR were prepared in separate 100 ml volumetric flasks using methanol. A stock solution containing mixture of THIO and LOR in the ratio of 1:1 was also prepared using methanol. Working solutions were prepared by diluting the stock solutions with mobile phase to contain 1-10 µg/ml of both THIO and LOR. These solutions were used to obtain the calibration graph by plotting peak area versus concentrations and regression equations were computed for both the drugs.

Sample preparation

Twenty capsules (Flexispaz 8), each containing 8 mg of THIO and 8 mg of LOR were weighed,

and powder equivalent to 8 mg of THIO was weighed accurately and taken into 100ml volumetric flask. The drugs were extracted into methanol, volume was adjusted to 100ml, vortexed and then filtered through a 0.22µm nylon filter. From this solution, further dilutions were made using mobile phase to get a final concentration 8 µg/ml of both THIO and LOR. Twenty microlitres of solution was injected into HPLC system to obtain chromatogram for standard drug solution (6 replicates) and sample solution (6 replicates). Concentrations of THIO and LOR in the formulation were calculated by comparing the peak area of sample with that of standard.

Forced Degradation Studies

Forced degradation studies like acid/base/neutral hydrolysis, oxidation, dry heat and photolysis of both the drugs were carried out. Dry heat and photolytic degradation of drug product were carried out in solid state. For each study, four samples were prepared: the blank solution stored under normal condition, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which was stored under normal conditions and the drug solution subjected to stress treatment.

Forced degradation studies were conducted separately and for the combination for THIO and LOR at a concentration 80 µg/ml. Then the study was extended for the formulation. Forced degradation with acidic media was performed by heating the drug under reflux with 0.1M hydrochloric acid for half an hour. Base hydrolysis included heating the drug solution under reflux with 1M NaOH for half an hour. To study neutral hydrolysis, the drug was dissolved in methanol and heated under reflux with water for 12 hours. Degradation with hydrogen peroxide was performed by treating the drug solution with 10% H_2O_2 (v/v) for 24 hours at room temperature. For thermal degradation, solid drugs were kept in petri dish in oven at 80°C for 12 hours. The photolytic degradation study was also performed by exposing the drug to sunlight for 12 hours.

The solutions were then left to equilibrate to room temperature and an aliquot of sample was withdrawn and diluted with mobile phase to get the concentration equivalent to 8 µg/ml of THIO and LOR. Then 20 µl solutions was injected into the HPLC system and analyzed under the chromatographic condition described earlier.

Validation

Specificity

To assure specificity of the developed method, a blank matrix consisting of excipients was extracted with methanol and the filtrate was injected under fixed chromatographic condition. The

chromatogram was observed for any additional peaks, particularly at the retention time of the analytes. Subsequently, all the potentially interfering compounds were injected into the HPLC system along with the active ingredients to demonstrate their separation from the peaks of interest with a specified resolution and peak purity index using photodiode array detector at dual wavelength.

Linearity

Linearity was determined by preparing standard stock solutions containing 100µg /ml of THIO and LOR separately in 100 ml volumetric flasks using methanol. A stock solution containing mixture of THIO and LOR in the ratio of 1:1 was also prepared using methanol. Working solutions were prepared by diluting the stock solutions with mobile phase to contain 1-10 µg/ml of both THIO and LOR. These solutions were then analyzed in triplicate. Calibration curves were then generated by plotting peak area versus concentrations and regression equations were computed for both the drugs.

Accuracy and precision

Accuracy of the method was evaluated through recovery experiments by standard addition method. Known amounts of analyte were spiked at different levels into a sample matrix that already contains preanalyzed sample. In this 50, 100, and 150% of the expected analyte concentration was added to the matrix. Repeatability of measurements, intraday and interday precision studies were conducted for three different concentrations (4&4, 5&5 and 6&6 µg/ml) of THIO and LOR respectively and assayed under same experimental conditions. The percentage assay was calculated using calibration curves. The unspiked and spiked samples were analyzed. Signal-to-noise ratios were used to estimate limits of detection (3:1) and limits of quantitation (10:1).

Robustness

To demonstrate robustness of the method, experimental conditions were purposely altered. To study the effect of flow rate it was changed to 0.1 units from 0.8 to 0.9 ml/min and 0.7 ml/min while keeping other parameters constant. Effect of pH was studied by altering the pH of ammonium acetate buffer by ±0.1 unit. The effect of mobile phase composition was studied by using 49:51 and 51:49 v/v of methanol and 10 mM ammonium acetate.

Stability

The stability of the standard and test solution was determined during the course of experimentation on the same day and also after storage of the drug solution for 48 hours under laboratory bench conditions (32 ± 1°C) and refrigeration (8 ± 0.5°C). The responses from the

aged solutions were compared with those from freshly prepared standard solution.

System suitability

System suitability parameters like plate number (N), tailing factor, k, α, resolution, and relative standard deviation of peak area of repetitive injection were carried out prior to the analysis of samples each day to ensure that the method could generate results of acceptable accuracy and precision.

RESULTS AND DISCUSSIONS

Selected wavelength and mobile phase

Different mobile phases were employed and proposed chromatographic condition was found to be appropriate for the quantitative determination of thiocochicoside and lornoxicam in presence of their degradation products. The optimum mobile phase consisted of 10mM ammonium acetate: methanol (50:50), pH7 adjusted with 1%triethyl amine, selected because it was found to ideally resolve the peaks of THIO (R_t 4.6 min) and LOR (R_t 10.2 min), with adequate separation in presence of their degradants at a flow rate of 0.8 ml/min. UV detection wavelength at 288 nm, injection volume 20µl, ambient temperature for column and HPLC system was found to best for analysis.

Table 1: Summary of degradation studies for THIO and LOR

Degradation condition	Time (h/day)	%THIO	%LOR	t_R(min) of DP	
				THIO	LOR
Acid, 0.1 M HCl (reflux at 70°c)	1/2h	78.25	73.9	11.8	2.4
Base , 1 M NaOH (reflux at 70°c)	1/2h	82.66	60.65	6.9,11.8	2.4
Neutral , water (reflux at 90°c)	12h	85.87	98.32	-	-
Oxidative,10%v/v H_2O_2(ambient, in dark)	12h	95.36	98.15	-	-
Dry heat (80°c)	12h	98.23	99.18	-	-
Direct sun light (photolysis)	12h	97.54	-	-	-

t_R - retention time, ND - no degradation observed, DP – degradation product

Forced degradation studies

ICH guidelines recommend 10-20% degradation for establishing stability indicating nature of the assay method[9] while Singh and Bakshi, in their article on stress testing suggested a target degradation of 20-80%[10]. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 10-80%, this could not be achieved in some cases even after exposure for prolonged duration (12 hours). Table 1 indicates the extent of THIO and LOR degradation under various stress condition. Figure. 3 show normal chromatogram of bulk while figure.4 and 5 shows the chromatogram of forced degraded samples. The degradation study

indicated that THIO was susceptible to acid hydrolysis more than alkaline and neutral hydrolysis whereas LOR was susceptible to acid hydrolysis more than alkaline hydrolysis and both were stable to H_2O_2, thermal and direct sunlight. Specificity of the method for the simultaneous estimation of THIO and LOR in presence of their degradants was demonstrated by the absence of co-eluting peaks with the main peaks.

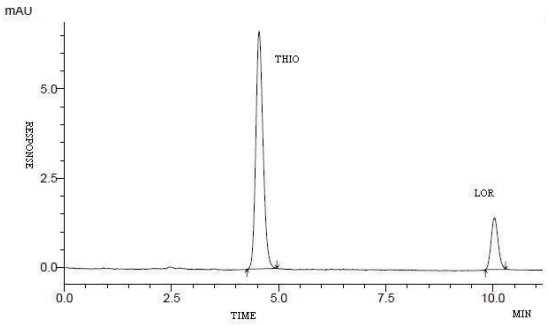

Figure 3: Chromatogram of mixture of untreated THIO and LOR (bulk)

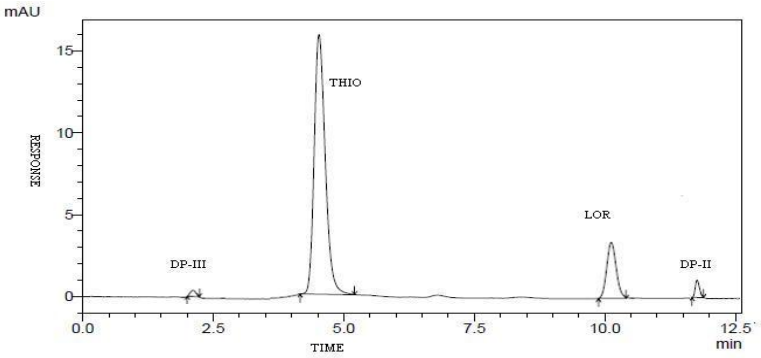

Figure 4: Chromatogram of mixture of THIO and LOR degradated with 0.1M hydrochloric acid

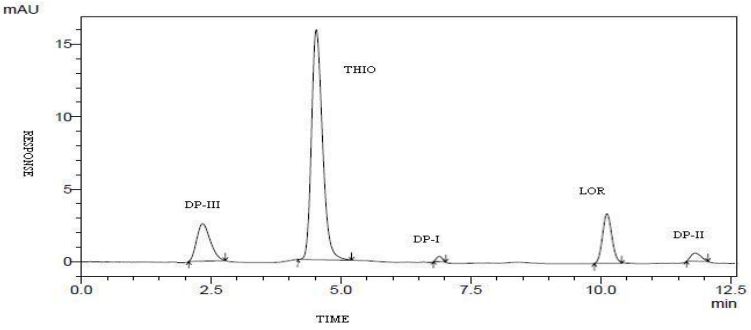

Figure 5: Chromatogram of mixture of THIO and LOR degradated with 0.01M sodium hydroxide

The UV overlay spectra of pure THIO and LOR was compared with the spectrum of the drugs subjected to different forced conditions; Changes in the spectra were observed for all degradants of THIO while LOR showed spectral changes only on acid and base hydrolysis. Comparative UV spectra between THIO, LOR and degradation products are shown in Fig 6 Purity of all degradation products was confirmed by comparing their spectra with the spectrum of THIO and

LOR.

Validation

The described method has been validated for specificity, linearity, LOD, LOQ, accuracy, precision, robustness and system suitability[11].

Specificity

There was no interference of excipients and degradation products. Resolution between closely eluting components were greater than 2.0, indicating selectivity of the method. Peak purity test suggested that the peaks of THIO, LOR, and degradation products were pure for all the stressed samples analyzed. The UV spectrum of pure THIO and LOR was compared with the spectrum of the drugs subjected to the different forced conditions. All the spectra showed changes in the absorption pattern. Purity of all degradation products was confirmed by comparing their spectra with the spectrum of THIO and LOR (Figure 6).

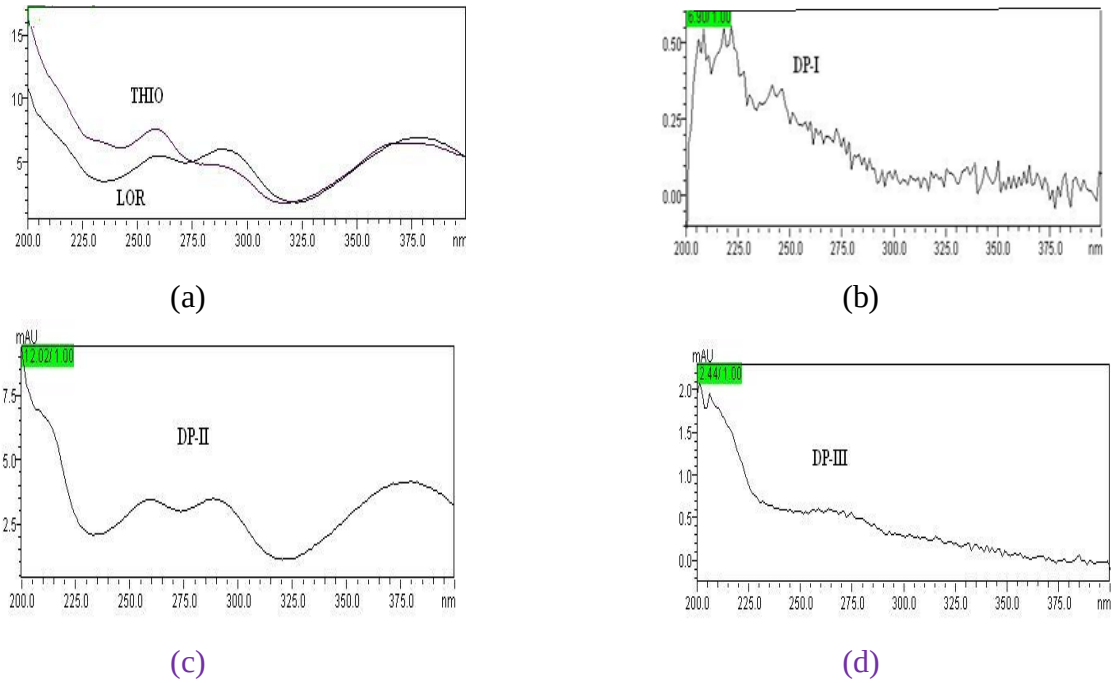

Figure 6: Comparative UV spectra between THIO, LOR and degradation products. (a) overlay spectra of THIO and LOR (b) DP-I (c) DP-II (d) DP-III.

Linearity

The standard solutions for linearity were prepared at ten different concentration levels. The calibration curve for THIO and LOR was found to be linear over the range of 1-10 µg/ml respectively.

LOD and LOQ

LOD and LOQ were calculated at a signal-to-noise ratio of 3:1 and 10:1 respectively. LOD was

found to be 0.3359 and 0.3931 µg/ml for THIO and LOR and LOQ were found to be 1.01 and 1.19 µg/ml for THIO and LOR respectively.

Accuracy and precision

Accuracy of the method was carried out by recovery studies using standard addition method at three different concentration levels. Summary of validation and system suitability test parameters are given in table 2. The intra- and inter-day variation of the method was carried out for one concentration level. The low % CV values of within a day, day to day variations and analyst to analyst variation for THIO and LOR revealed that the proposed method is precise.

Table 2:Summary of validation and system suitability test parameters.

Parameter (units)	THIO	LOR
Linearity range (µg/ml)	1-10	1-10
Correlation Coefficient(±SD*)	0.9997±0.0011	0.9997±0.0012
LOD (µg /ml)	0.3359	0.3931
LOQ (µg/ml)	1.01	1.19
Recovery (%±RSD*)	100.45±0.4489	100.70±0.5111
Interday precision (%RSD*)	0.4363	0.6046
Intraday Precision (%RSD*)	0.5623	0.4856
Robustness	Robust	Robust
Retention Time (min)	4.6	10.2
Tailing Factor	1.2	1.3
Theoretical plate	187856	61786
Resolution	-------	10.88

*mean of six determination

Table 3: Robustness evaluation of the method

Factor	Level	Asymmetric Factor		Number of Theoretical Plates		Resolution
		THIO	LOR	THIO	LOR	
Flow Rate (mL min^{-1})						
0.7	-0.1	1.23	1.25	186862	60523	11.2
0.8	0	1.2	1.3	187856	61786	10.88
0.9	+0.1	1.19	1.20	181823	60378	9.6
%B of mobile phase						
49	-1	1.22	1.21	185926	61539	10.4
50	0	1.2	1.31	187856	61786	10.88
51	+1	1.18	1.20	184266	57509	11.23
pH of mobile phase						
6.9	-0.1	1.2	1.2	181138	60759	10.63
7	0	1.2	1.31	187856	61786	10.88
7.1	+0.1	1.23	1.22	189272	61086	10.82

Robustness

For robustness evaluation of both the drugs, few parameters like flow rate, percentage of

methanol in mobile phase and pH of mobile phase were deliberately changed. Table 3 shows the robustness evaluation of the method. Each factor selected was changed at three levels with respect to the optimized parameters. Robustness of the method was done at the concentration of 5 µg/ml for both THIO and LOR and the method was found to be robust.

Assay of THIO and LOR from its tablet dosage form

The assay results of THIO and LOR in tablet dosage forms were comparable with the values of labeled claim. The results presented in table 4 indicate the suitability of the method for routine analysis of THIO and LOR from their combined tablet dosage form.

Table 4: Assay of THIO-LOR in formulation

Drug	Label Claim(mg/cap)	Estimated	% Amount	±%RSD*
THIO	8	7.9872	99.84	0.1161
LOR	8	7.9856	99.82	0.08145

*Average of 6 determination

CONCLUSION

The study presents a simple stability-indicating HPLC method for the simultaneous estimation of THIO and LOR in presence of their degradation products and validated as per ICH guidelines. Statistical analysis proved that the method developed was accurate, precise and repeatable. The method was successfully used for the estimation of drugs in pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.84±0.1161 and 99.82±0.0815, for THIO and LOR respectively. There was no interference observed due to excipients or other components present in the tablet dosage form. The results indicated the suitability of the method to study stability of THIO and LOR under various forced degradation condition viz. acid, base, neutral, oxidative, dry heat and photolytic degradation. It can be concluded that the developed method may be employed for analysis of stability samples of THIO and LOR since the method could separate the drugs from their degradation products.

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