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# Stability Indicating HPTLC Method For Determination of Lornoxicam and Thiocolchicoside in the Pharmaceutical Dosage Form

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**ABSTRACT**
*A simple, sensitive and rapid high performance thin layer chromatographic (HPTLC) method has been developed and validated for quantitative determination of Lornoxicam (LOR) and Thiocolchicoside (THIO) in bulk and formulations. The chromatographic development was carried out on HPTLC plates precoated with Silica gel $GF_{254}$ pre-coated on aluminum sheet (10 cm × 10 cm) of 0.20 mm layer thickness as a stationary phase and Chloroform: Hexane: Methanol: Glacial Acetic Acid (2: 3.5: 2.5:0.2 v/v/v/v). Detection was carried out densitometrically at 286 nm (Absorbance mode). where Lornoxicam and Thiocolchicoside have significant absorbance. The retardation factor ($R_f$) values was found to be 0.17 ± 0.01 for LOR and 0.75 ± 0.01 for THIO. Peak Purity was found to be 0.998 and 0.997 respectuvely for LOR and THIO. The method was validated as per ICH guideline with respect to linearity, accuracy, precision, robustness etc. The method has demonstrated high sensitivity and specificity. The method was applied for Forced degradation studies also. The method is new, simple, economic and stability indicating for routine estimation of LOR and THIO in bulk, preformulation studies and pharmaceutical formulation to help the industries as well as researchers for their sensitive determination LOR and THIO rapidly at low cost in routine analysis.*
***Key words:*** *Lornoxicam,Thiocolchicoside, HPTLC,Stress degradation*

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## INTRODUCTION

Currently, HPTLC has become a usual analytical practice due to its advantages of consistency in quantitation of analytes at micro and nanogram levels and due to its cost efficiency. The main benefit of HPTLC is that a number of samples can be analyzed concurrently using a small amount of mobile phase unlike HPLC. This trim down the time and cost of analysis. As well it reduces revelation risks and appreciably reduces disposal problems of lethal organic effluents, in that way reducing possibilities of environment pollution. HPTLC also facilitates repeated detection (scanning) of the chromatogram with similar or dissimilar parameters. Simultaneous assay of several components in a multi component formulation is possible. The aim of the present study was to develop and validate a new, simple, accurate, specific and reproducible HPTLC method for determination of LOR and THIO.

Lornoxicam 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 (Fugure.1) is a non-steroidal anti-inflammatory drug of the oxicam class with analgesic (pain relieving), anti-inflammatory and antipyretic properties. Thiocholchicoside is chemically N-[(7S)-3-(beta-D-glucopyranosy-loxy)-1,2-dimethoxy -10-(methylsulfanyl)- 9- oxo-5, 6, 7, 9-benzo [a] heptalen- 7-etrahydroyl] acetamide. Thiocolchicoside (Figure.2) 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 [1-5].

The literature survey reveals that there are analytical methods available for determination of Lornoxicam and Thiocolchicoside from biological matrices, bulk drug and dosage forms by RP-HPLC/MS methods. The review of literature also reveal that no satisfactory HPTLC method available for determination of this combination. [6-13].

## MATERIAL AND METHOD

Pure Lornoxicam was kind gifts from Sun Pharmaceuticals, India and Thiocolchoside from Glenmark Pharmaceuticals, India. Commercial tablets (LORCHEK tab) containing Lornoxicam (LOR) and Thiocolchicoside (THIO) were used for the study. Chloroform, hexane, Methanol, Acetonitrile and Glacial Acetic Acid used were of analytical grade (E. Merck, Mumbai, India). Hydrochloric acid, sodium hydroxide, and hydrogen peroxide used for stress degradation studies were of analytical reagent grade, CDH Chemicals, Delhi, India. De-ionized water prepared using Milli-Q plus purification system, Millipore (Bradford, USA) was used throughout the study. All the other chemicals used were also of analytical grade (E. Merck, India).

### Instrumentation:

HPTLC plates pre-coated with silica gel GF aluminium TLC plate, (10 cm x 10 cm) were from Merck. Densitometry was carried out with a CAMAG TLC Scanner III, with a win CATS 1.3.4 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V, and developed in a CAMAG 10 cm x 10 cm twin trough chambers.

### Preparation of solutions:

The 100 mg of LOR were accurately weighed and transferred to 100 ml volumetric flask containing few ml (10 ml) of methanol. The flasks were sonicated for 2 minutes to dissolve the solids and volume was made up to the mark with methanol to obtain a standard solution containing 1000µg/ml LOR. Further 1ml of standard stock solution was transferred to 10 ml volumetric flask and solution was diluted up to the mark with methanol to get 100 ng/µl of LOR.

The 100 mg of THIO were accurately weighed and transferred to separate 100 ml volumetric flask containing few ml (10 ml) of methanol. The flasks were sonicated for 2 minutes to dissolve the solids and volume was made up to the mark with methanol to obtain a standard solution containing 1000µg/ml THIO. Further 1ml of standard stock solution was transferred to 10 ml volumetric flask and solution was diluted up to the mark with methanol to get 100 ng/µl of THIO.

### Chromatographic conditions

The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. Samples and standards were applied to the plate as 8mm band under a flow of $N_2$ gas, 11.6 mm apart, 15 mm from the bottom edge, and starting 15 mm from the edge of the HPTLC plate with Linomat V applicator. The constant sample-application rate was 100nL/s. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 400nm. The scanning speed is 20mm/sec was employed. The Slit dimension was 6 × 0.45 millimetres. The Spotting parameter is a) Band width: 8 millimetre b) Syringe size: 100µl. Spotting of samples was done by using Hamilton microliter syringe.

### Method validation

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to linearity and range, specificity, precision, accuracy, limit of detection and limit of quantification. [14,15]

### System suitability

System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard preparation (200ng/band for LOR and 200ng/band for THIO) and one injection of a check standard were made. The parameters measured were retardation factor, peak purity and peak area LOR and THIO.

### Stress Study

To evaluate the stability indicating properties of the developed HPTLC method, forced degradation studies were carried out in accordance to the ICH guidelines. The stress studies were carried out under the conditions of hydrolysis, photolysis, oxidation and dry heat, as defined in the ICH guideline Q1A (R2) (ICH, 2002)

Stock solutions were prepared by accurately weighing 25 mg of THIO and 25 mg of LOR and transferring to three separate 25 ml volumetric flasks containing few ml of methanol. The flasks were swirled to dissolve the solids and diluted up to the mark with methanol. These stock solutions were used for forced degradation studies including Alkali hydrolysis, Acid hydrolysis, Neutral hydrolysis, Oxidative stress degradation, Dry heat degradation, Wet heat degradation and Photo degradation study. The results are indicated in Table 1.

### Linearity and Range

Semi automatic spotter was used for spotting the sample on HPTLC plate. Appropriate aliquots of LOR working standard solution were taken in 10 ml volumetric flasks to obtain final concentration of 50 µg/ml of LOR. Appropriate aliquots of THIO working standard solution were taken in 10 ml volumetric flasks to obtain final concentration of 50µg/ml THIO. The solution having concentration of 50 µg/ml of LOR and 50 µg/ml THIO was filled in the syringe and under nitrogen stream, it was applied in form of

band of desired concentration range for each of drug on a single plate having concentration of 50 to 500ng/band for LOR and 50 to 500ng/band for THIO. Triplicate 1 – 5µL portions of each standard solution were spotted as bands on a TLC plate. The plate was then developed using the previously described mobile phase and scanned under above established densitometry condition. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves for each compound.

The regression equation was found to be y = 17.02 x - 77.52 and correlation coefficient was found to be 0.997 for LOR. The regression equation was found to be y = 35.73 x - 27.35 and correlation coefficient was found to be 0.998 for THIO. The results are indicated in Table 2.

**Precision**

The repeatability of developed method was determined by analyzing 200ng/band LOR solution six times on the same day. The percentage RSD was found to be 0.032. The repeatability of developed method was determined by analyzing 200ng/band THIO solution six times on the same day. The percentage RSD was found to be 0.047. The results of repeatability data are shown in Table 5.5.7.

The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 5.5.8 for LOR. Replicate analyses of three different concentrations 50, 200 and 400 ng/band of LOR solutions showed good reproducibility. The percentages RSD of intraday and interday studies were found to be 0.023–0.035% and 0.031–0.15% respectively for LOR.

The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 5.5.9 for THIO. Replicate analyses of three different concentrations 50, 200 and 400 ng/band of THIO solutions showed good reproducibility. The percentages RSD of intraday and interday studies was found to be 0.025–0.04% and 0.028–0.434% respectively for THIO.

**Accuracy**

The recovery of the method was carried out by the standard addition to the preanalysed test sample at three different concentration levels 80%, 100% and 120%. Triplicate determinations were made at each concentration level. Known amount of standards of THIO (0, 160, 200 and 240 ng per band) and LOR (0, 80, 100 and 120 ng per band) were spiked to a pre-quantified sample (200 ng per spot) of THIO and (100 ng per spot) of LOR from tablet dosage form and the mixtures were analyzed by proposed HPTLC method. The percentage recovery of LOR and THIO was determined by measuring the peak areas and fitting these values into the regression equation of the calibration plot. The recoveries were found to be 100.21 - 100.64 and 99.04- 100.21 for THIO and LOR, respectively. The values indicate that the method is accurate (table 3).

**Limit of detection and Limit of quantification**

The detection limits for LOR and THIO were found to be 0.65 ng/band and 1.8 ng/band, respectively, while quantitation limits were found to be 1.95 ng/band and 5.78 ng/band, respectively. The above data shows that a microgram quantity of both the drugs can be accurately and precisely determined. The values of LOD and LOQ of LOR and THIO respectively indicate the sensitivity of proposed method.

**Specificity and Selectivity**

The peak purity index and HPLC chromatogram showed peaks for both the drugs (LOR and THIO) without any interfering peak and the estimation of both the drugs were found to be satisfactory. Test solution is prepared by mixing of LOR and THIO with the tablet powder excipients. 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 shown in figure 4 at peak start, peak apex and peak end positions of the spot. Specificity is proven by comparing the chromatogram of Diluent, standard solution and test preparation solution and by peak purity index to show that there was no any interference of excipients with the peak of LOR and THIO.

Good correlation was obtained between standard and sample spectra of LOR and THIO. The comparative UV spectrum of standard and sample is found to be same. Also the results of comparison between peaks start, maximum, and end indicate the closeness in these positions between dosage forms and standards. The appearance of LOR and THIO spot at specific Rf different from its degrades indicates the specificity of the proposed method.

**Robustness Study**

The optimize densitometry condition of mobile phase is Chloroform: Hexane: Methanol: Glacial Acetic Acid (4: 3.5: 2.5:0.2 v/v/v/v), chamber saturation time is 30min and solvent migration distance is 80mm. The small changes chamber saturation time is 25 min and 35 min, and solvent migration distance is 70mm and 90mm, and mobile phase composition is evaluated. After small changes in this parameter effect on the peak area of LOR and THIO was determined. The low value of the RSD was found to indicating that the proposed method was robust, as small but deliberate changes in method parameters

have no detrimental effect on the method performance. The low value of percentage relative standard deviation indicates that the method is robust.

## RESULT AND DISCUSSION

The summary of method validation parameter and their result are shown in Table 4 indicating that the developed is validated as per ICH guidelines and result are within the ICH guidelines values.

Figure 1. Chemical Structure of Lornoxicam

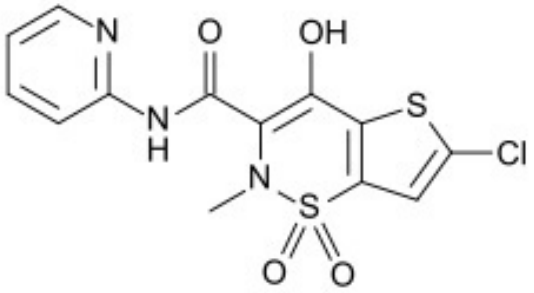


Figure 2. Chemical Structure of Thiocolchicoside

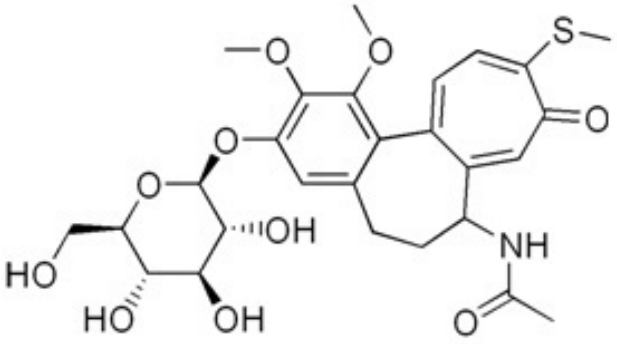


Figure 3. Typical Densitogram of Lornoxicam (LOR) and Thiocolchicoside (THIO) in marketed formulation

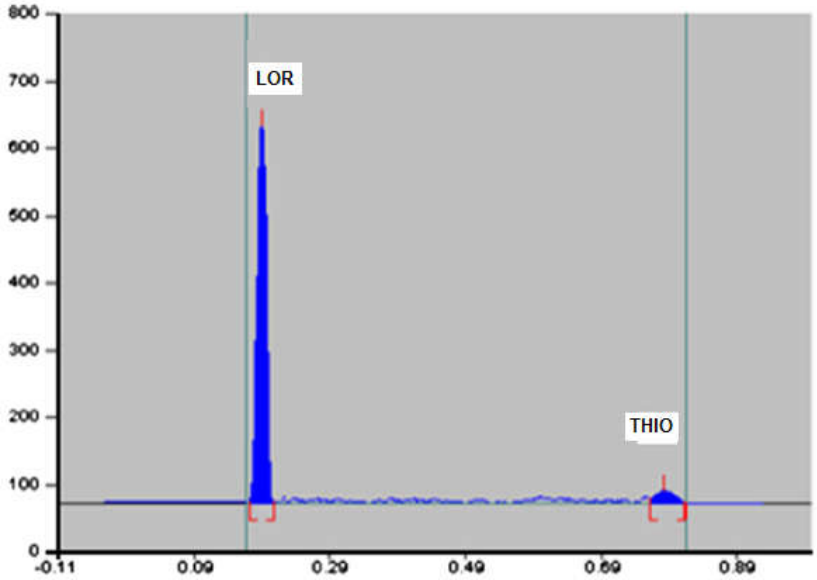

Figure 4. Purity spectra of LOR and THIO

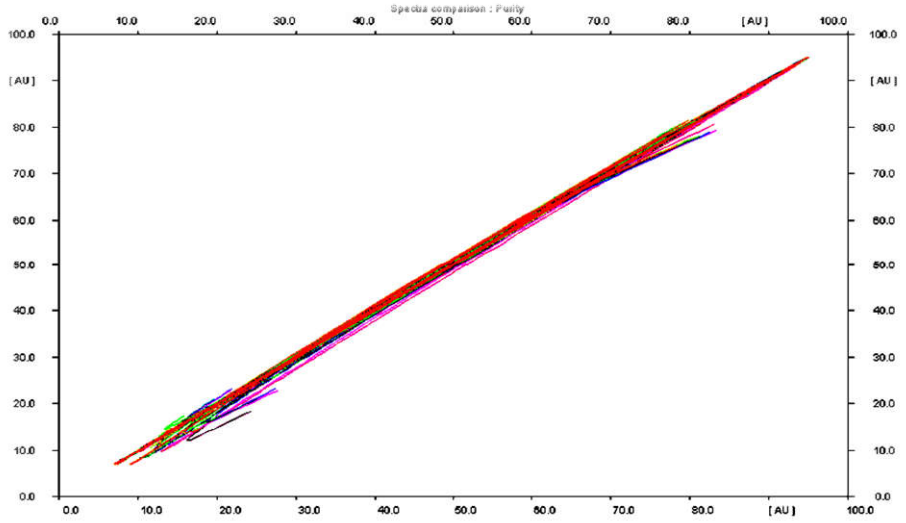


Table 1: Stress Study by Proposed HPTLC Method

| Stress condition | Time | % Degradation | |
|---|---|---|---|
| | | THIO | LOR |
| Alkaline hydrolysis (1N NaOH) | 2 hr | 16.23 | 13.5 |
| Acidic hydrolysis (0.1N HCl) | 4 hr | 12.23 | 7.5 |
| Neutral Hydrolysis | 4 hr | 1.2 | 1.5 |
| Oxidative Degradation (6% $H_2O_2$) | 4 hr | 19.23 | 12.5 |
| Dry heat (70 °C) | 4 hr | 1.23 | 9.5 |
| Wet Heat (Boiling Water bath) | 4 hr | 0.93 | 6.75 |
| Sun light | 72 hr | 1.03 | 1.75 |
| UV radiation (254nm) | 4hr | 2.3 | 2.35 |
| UV radiation (365nm) | 4hr | 1.6 | 1.15 |

Table 2: Statistical analysis data of calibration curve

| Parameters | THIO | LOR |
|---|---|---|
| Linear Range | 50- 500 ng/band | 50- 500 ng/band |
| Slope | 35.73 | 17.02 |
| Intercept | 27.35 | 77.52 |
| Regression Coefficient ($r^2$) | 0.997 | 0.996 |
| Standard deviation of slope | 139.88 | 63.05 |
| Standard deviation of intercept | 287.24 | 85.76 |
| LOD (ng/band) | 0.65 | 1.8 |
| LOQ (ng/band) | 1.95 | 5.78 |

Table 3: Accuracy study

| Level | Drug added (ng/band) | Drug Recovered (ng/band) [a] | % Drug Recovered ± SD |
|---|---|---|---|
| For LOR | | | |
| 80 | 80 | 80.15 | 100.18 ± 0.95 |
| 100 | 100 | 100.21 | 100.21 ± 1.40 |
| 120 | 120 | 118.85 | 99.04 ± 1.62 |
| For THIO | | | |
| 80 | 160 | 160.15 | 100.19 ± 1.98 |
| 100 | 200 | 201.31 | 100.65 ± 1.51 |
| 120 | 240 | 242.02 | 100.84 ± 1.41 |

***a=Average of Three determination***

Table 4: Summary of validation parameters

| Parameters | LOR | THIO |
|---|---|---|
| Linear Range | 50- 500 ng/band | 50- 500 ng/band |
| Regression Coefficient | 0.997 | 0.998 |
| Regression equation | y = 17.02 x - 77.52 | y = 35.73 x - 27.35 |
| Recovery % | 97.39 – 99.52 | 97.67 – 99.59 |
| Repeatability (RSD, n=6) | 0.032 | 0.047 |
| Precision (RSD)<br>Intra - day (n=3)<br>Inter - day (n=3) | <br>0.023 – 0.035<br>0.031 - 0.15 | <br>0.025 – 0.04<br>0.028 – 0.434 |
| Limit of Detection (ng/spot) | 1.8 | 0.65 |
| Limit of Quantitation (ng/spot) | 5.78 | 1.95 |
| Robustness | Robust | Robust |
| Solvent stability | Stable for 48hrs | Stable for 48hrs |
| Specificity | Specific | Specific |
| Peak Purity | 0.998 | 0.997 |

## CONCLUSION

A quick, precise and accurate method based on normal-phase HPTLC has been developed for routine analysis of metformin Lornoxicam (LOR) and Thiocolchicoside (THIO) in fixed-dose combination tablets. The method was validated for linearity, precision, accuracy and specificity. Forced degradation studies were carried out and degradation product peaks were well resolved from drug peaks. It has the advantage over HPLC methods in general. It consumed less than 35mL of mobile phase per run (8 samples per plate), whereas HPLC methods would consume more than 50mL per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method took an average of 1 h, whereas HPLC methods would generally take more than 2 h for the same number of samples. It is cheap, quick and does not use chloroform, therefore suitable for routine analysis Lornoxicam (LOR) and Thiocolchicoside (THIO) in fixed-dose combination tablets. When compared with the reported HPLC method, the developed HPTLC method is both time and cost effective for the determination Lornoxicam (LOR) and Thiocolchicoside (THIO) mixtures.

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