**International Journal of Pharmacy and Industrial Research (IJPIR)**

***IJPIR |Vol.13 | Issue 1 | Jan - Mar -2024***

**www.ijpir.com**

DOI : https://doi.org/10.61096/ijpir.v13.iss1.2024.63-72

**ISSN: 2231-3656**

**Print: 2231-3648**

**Research**

# Simultaneous Estimation Of Lornoxicam And Thiocolchicoside In Pure And Pharmaceutical Dosage Form By Using Rp-Hplc Method.

**J. Suchitha*[1], R. Mounika[1], K. Chaitanyaprasad[1], P. Vedavahini[1]**

[1]*Department of Pharmaceutical Analysis, Samskruti College of Pharmacy in Ghatkesar, Telangana. 501301, India.*

*Author for Correspondence: J. Suchitha
Email: jsuchitha2000@gmail.com

Published on:15 Feb 2024

Published by:
DrSriram Publications



## Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Thiocolchicoside and Lornoxicam, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (5:95% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 285nm. The retention time of the Thiocolchicoside and Lornoxicam was 2.088, 6.068 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Thiocolchicoside and 20-100mg/ml of Lornoxicam. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

**Keywords:** Thiocolchicoside, Lornoxicam, RP-HPLC, Validation.

## INTRODUCTION

**Analytical chemistry[1]**

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.

- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

**Analytical methods**

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

**Method development** forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

**Chromatography**[2]

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

*Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system.*

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process. Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC).

**Chromatographic Process**

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

## MATERIALS AND METHODS

Thiocolchicoside from Sura labs, Lornoxicam from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck.

**HPLC METHOD DEVELOPMENT**

**TRAILS**

**Preparation of standard solution**

Accurately weigh and transfer 10 mg of Thiocolchicoside and Lornoxicam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1.2ml of the Lornoxicam and 0.6ml of the Thiocolchicoside from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure**

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

**Mobile Phase Optimization**

Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Buffer: Methanol: ACN in proportion 65:25:10v/v respectively.

**Optimization of Column**

The method was performed with various columns like C18 column, ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**OPTIMIZED CHROMATOGRAPHIC CONDITIONs**

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : 35ºc
Column : Altima C18 (4.6×150mm, 5µ)
Mobile phase : Methanol: Water (5:95 % v/v)
Flow rate : 1ml/min
Wavelength : 285 nm
Injection volume : 10 µl
Run time : 14 min

**VALIDATION**

**PREPARATION OF MOBILE PHASE**

**Preparation of mobile phase**

Accurately measured 950ml (95%) of hplc Water and 5ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation**

The Mobile phase was used as the diluent.

## RESULTS AND DISCUSSION

**Optimized Chromatogram (Standard)**

Mobile phase : Methanol: Water (5:95% v/v)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 285 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 14 minutes

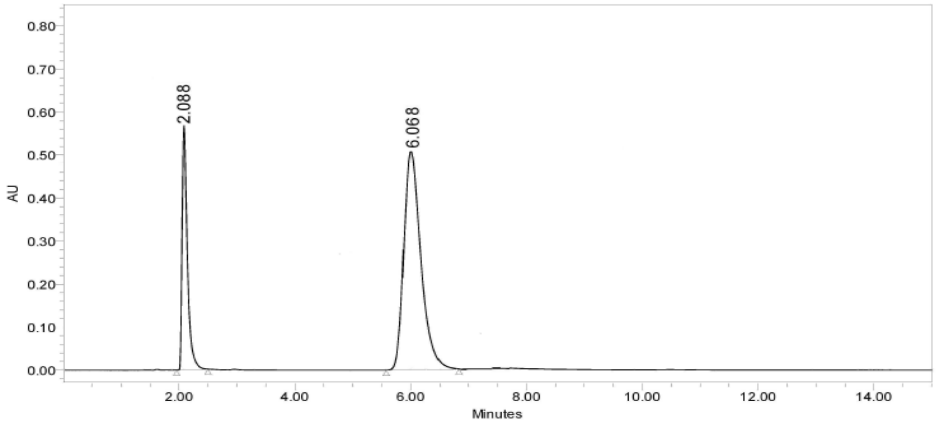


**Fig 1: Optimized Chromatogram**

**Table 1: Peak results For Optimized Chromatogram**

| S. No | Peak name | $R_t$ | Area | Height | USP Resolution | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.088 | 3425413 | 567933 | | 1.0 | 5565.5 |
| 2 | Thiocolchicoside | 6.068 | 1629854 | 517733 | 2.5 | 1.1 | 5355.2 |

From the above chromatogram it was observed that the Lornoxicam and Thiocolchicoside peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

**Optimized Chromatogram (Sample)**

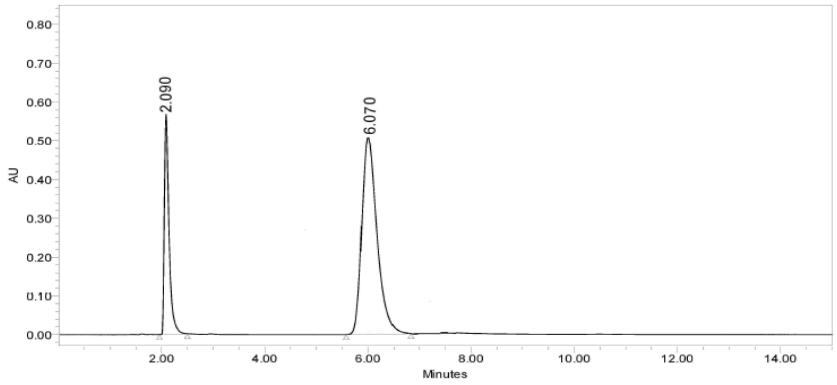


**Fig 2: Optimized Chromatogram (Sample)**

**Table 2: Optimized Chromatogram (Sample)**

| S.No | Name | Retention time(min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.090 | 3468547 | 567933 | | 1.0 | 5565.5 |
| 2 | Thiocolchicoside | 6.070 | 16289441 | 517733 | 2.5 | 1.1 | 5355.2 |

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

**System suitability**

**Table 3: Results of system suitability for Lornoxicam**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.080 | 3569412 | 567917 | 5568.0 | 1.0 |
| 2 | Lornoxicam | 2.080 | 3465125 | 517719 | 6359.2 | 1.1 |
| 3 | Lornoxicam | 2.080 | 3598154 | 567933 | 5565.5 | 1.0 |
| 4 | Lornoxicam | 2.081 | 3586491 | 517733 | 5355.2 | 1.1 |
| 5 | Lornoxicam | 2.081 | 3582694 | 567917 | 6348.0 | 1.0 |
| Mean | | | 3560375 | | | |
| Std. Dev | | | 54225.61 | | | |
| % RSD | | | 1.523031 | | | |

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

**Table 4: Results of method precession for Thiocolchicoside**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Thiocolchicoside | 6.056 | 3582264 | 567917 | 5568.0 | 1.0 | 2.5 |
| 2 | Thiocolchicoside | 6.056 | 3586491 | 517719 | 5359.2 | 1.1 | 2.5 |
| 3 | Thiocolchicoside | 6.056 | 3598154 | 567933 | 5565.5 | 1.0 | 2.5 |
| 4 | Thiocolchicoside | 6.057 | 3564125 | 517733 | 5355.2 | 1.1 | 2.5 |
| 5 | Thiocolchicoside | 6.057 | 3569412 | 562173 | 5568.0 | 1.0 | 2.5 |
| Mean | | | 3580089 | | | | |
| Std. Dev | | | 13609.81 | | | | |
| % RSD | | | 0.380153 | | | | |

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

**Assay (Standard)**

**Table 5: Peak results for assay standard**

| S.No | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.087 | 3425681 | 567917 | | 1.0 | 5568.0 | 1 |
| 2 | Thiocolchicoside | 6.067 | 16235984 | 517719 | 2.5 | 1.1 | 5359.2 | 1 |
| 3 | Lornoxicam | 2.088 | 3425413 | 567933 | | 1.0 | 5565.5 | 2 |
| 4 | Thiocolchicoside | 6.068 | 16298543 | 517733 | 2.5 | 1.1 | 5355.2 | 2 |
| 5 | Lornoxicam | 2.088 | 3465423 | 567933 | | 1.0 | 5545.5 | 3 |
| 6 | Thiocolchicoside | 6.068 | 16265213 | 517733 | 2.5 | 1.1 | 5352.1 | 3 |

**Assay (Sample)**

**Table 6: Peak results for Assay sample**

| S.No | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.089 | 3469821 | 567917 | | 1.0 | 6568.0 | 1 |
| 2 | Thiocolchicoside | 6.069 | 16259845 | 517719 | 2.5 | 1.1 | 5359.2 | 1 |
| 3 | Lornoxicam | 2.090 | 3468547 | 567933 | | 1.0 | 5565.5 | 2 |
| 4 | Thiocolchicoside | 6.070 | 16287531 | 517733 | 2.5 | 1.1 | 5355.2 | 2 |
| 5 | Lornoxicam | 2.090 | 3468143 | 567813 | | 1.0 | 5391.1 | 3 |
| 6 | Thiocolchicoside | 6.070 | 16282431 | 517623 | 2.5 | 1.1 | 5564.0 | 3 |

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=16276602 / 16266580 × 10/60 × 60/0.1766 × 99.6/100 × 0.212/12 × 100
= 100.1%
The % purity of Lornoxicam and Thiocolchicoside in pharmaceutical dosage form was found to be %.

**LINEARITY: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY**
**Lornoxicam**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 33.3 | 20 | 1010252 |
| 66.6 | 40 | 2049374 |
| 100 | 60 | 3072706 |
| 133.3 | 80 | 3921068 |
| 166.6 | 100 | 4952813 |

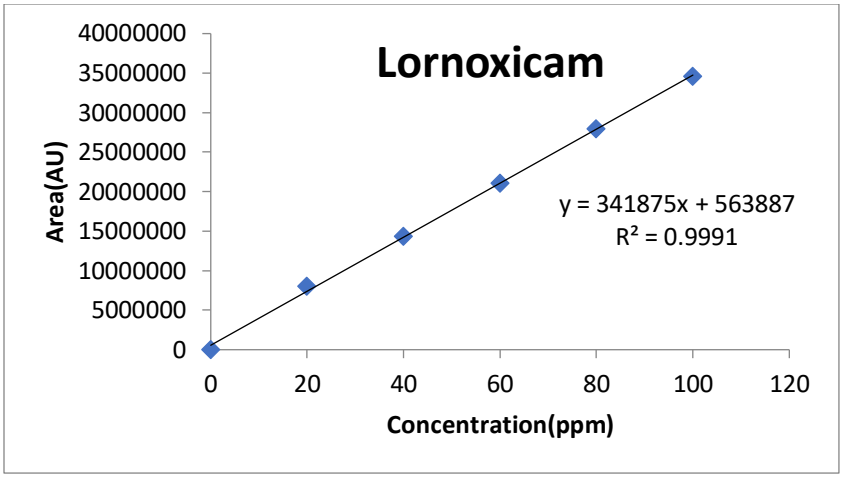


**Fig 3: Calibration graph for Lornoxicam**

**Thiocolchicoside**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 33 | 10 | 8040807 |
| 66 | 20 | 14318417 |
| 100 | 30 | 21087985 |
| 133 | 40 | 27913928 |
| 166 | 50 | 34584741 |

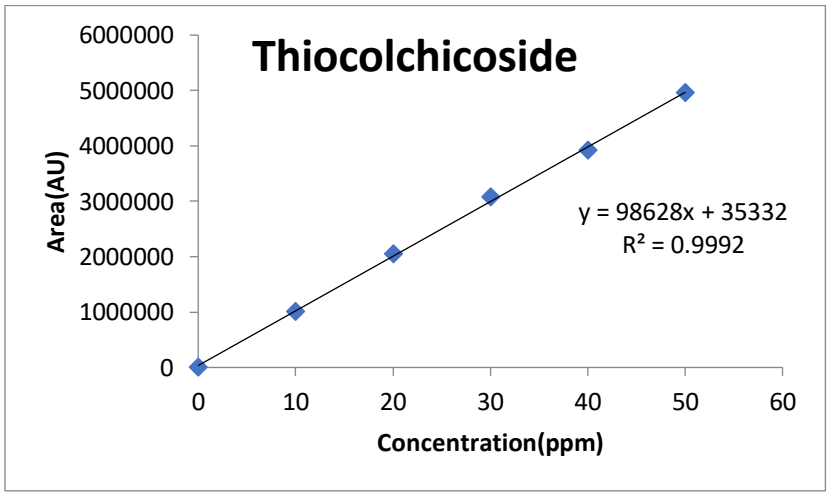


**Fig 4: calibration graph for Thiocolchicoside**

**REPEATABILITY**

**Table 7: Results of repeatability for Lornoxicam**

| Sno | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.084 | 3569412 | 567917 | 5568.0 | 1.0 |
| 2 | Lornoxicam | 2.083 | 3465125 | 517719 | 5359.2 | 1.1 |
| 3 | Lornoxicam | 2.082 | 3598154 | 567933 | 5565.5 | 1.0 |
| 4 | Lornoxicam | 2.081 | 3586491 | 517733 | 5355.2 | 1.1 |
| 5 | Lornoxicam | 2.080 | 3582694 | 567917 | 5568.0 | 1.0 |

| | |
|---|---|
| Mean | 3560375 |
| Std. Dev | 54225.61 |
| % RSD | 1.523031 |

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

**Table 8: Results of method precession for Thiocolchicoside**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Thiocolchicoside | 6.056 | 3582264 | 567917 | 5568.0 | 1.0 | 2.5 |
| 2 | Thiocolchicoside | 6.057 | 3586491 | 517719 | 5359.2 | 1.1 | 2.5 |
| 3 | Thiocolchicoside | 6.058 | 3598154 | 567933 | 5565.5 | 1.0 | 2.5 |
| 4 | Thiocolchicoside | 6.059 | 3564125 | 517733 | 5355.2 | 1.1 | 2.5 |
| 5 | Thiocolchicoside | 6.060 | 3569412 | 562173 | 5568.0 | 1.0 | 2.5 |
| Mean | | | 3580089 | | | | |
| Std. Dev | | | 13609.81 | | | | |
| % RSD | | | 0.380153 | | | | |

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

**Intermediate precision**

**Table 9: Results of Intermediate precision for Lornoxicam**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.081 | 3481579 | 567917 | 5568.0 | 1.0 |
| 2 | Lornoxicam | 2.082 | 3458121 | 517719 | 5359.2 | 1.1 |
| 3 | Lornoxicam | 2.083 | 3426581 | 567933 | 5565.5 | 1.0 |
| 4 | Lornoxicam | 2.084 | 3465712 | 517733 | 5355.2 | 1.1 |
| 5 | Lornoxicam | 2.085 | 3451476 | 567917 | 5568.0 | 1.0 |
| 6 | Lornoxicam | 2.085 | 3452106 | 567514 | 5359.2 | 1.1 |
| Mean | | | 3455929 | | | |
| Std. Dev | | | 18188.92 | | | |
| % RSD | | | 0.5 | | | |

- %RSD of six different sample solutions should not more than 2

**Table 10: Results of Intermediate precision for Thiocolchicoside**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Thiocolchicoside | 6.061 | 15481579 | 567917 | 5568.0 | 1.0 | 2.5 |
| 2 | Thiocolchicoside | 6.062 | 15369852 | 517719 | 5359.2 | 1.1 | 2.5 |
| 3 | Thiocolchicoside | 6.063 | 15248454 | 567933 | 5565.5 | 1.0 | 2.5 |
| 4 | Thiocolchicoside | 6.064 | 15874692 | 517733 | 5355.2 | 1.1 | 2.5 |
| 5 | Thiocolchicoside | 6.064 | 15236547 | 567933 | 5568.0 | 1.0 | 2.5 |
| 6 | Thiocolchicoside | 6.064 | 15217547 | 567133 | 5359.2 | 1.1 | 2.5 |
| Mean | | | 15404779 | | | | |
| Std. Dev | | | 251289.4 | | | | |
| % RSD | | | 1.6 | | | | |

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

**Table 11: Results of Intermediate precision Day 2 for Lornoxicam**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lornoxicam | 2.081 | 3481579 | 567917 | 5568.0 | 1.0 |
| 2 | Lornoxicam | 2.082 | 3458121 | 517719 | 5359.2 | 1.1 |
| 3 | Lornoxicam | 2.083 | 3426581 | 567933 | 5565.5 | 1.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Lornoxicam | 2.084 | 3465712 | 517733 | 5355.2 | 1.1 |
| 5 | Lornoxicam | 2.085 | 3451476 | 567917 | 5568.0 | 1.0 |
| 6 | Lornoxicam | 2.085 | 3452106 | 567514 | 5359.2 | 1.1 |
| Mean | | | 3455929 | | | |
| Std. Dev | | | 18188.92 | | | |
| % RSD | | | 0.5 | | | |

- %RSD of six different sample solutions should not more than 2

**Table 12: Results of Intermediate precision for Thiocolchicoside**

| S.No | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Thiocolchicoside | 6.061 | 15481579 | 567917 | 5568.0 | 1.0 | 2.5 |
| 2 | Thiocolchicoside | 6.062 | 15369852 | 517719 | 5359.2 | 1.1 | 2.5 |
| 3 | Thiocolchicoside | 6.063 | 15248454 | 567933 | 5565.5 | 1.0 | 2.5 |
| 4 | Thiocolchicoside | 6.064 | 15874692 | 517733 | 5355.2 | 1.1 | 2.5 |
| 5 | Thiocolchicoside | 6.064 | 15236547 | 567933 | 5568.0 | 1.0 | 2.5 |
| 6 | Thiocolchicoside | 6.064 | 15217547 | 567133 | 5359.2 | 1.1 | 2.5 |
| Mean | | | 15404779 | | | | |
| Std. Dev | | | 251289.4 | | | | |
| % RSD | | | 1.6 | | | | |

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

**ACCURACY**

**The accuracy results for Lornoxicam**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1543793 | 15 | 15.2 | 101.9 | 100.9% |
| 100% | 3035883 | 30 | 30.4 | 101.4 | |
| 150% | 4451005 | 45 | 44.7 | 99.4 | |

**The accuracy results for Thiocolchicoside**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1084420 | 30 | 30.07 | 100.2 | 99.6% |
| 100% | 2096069 | 60 | 59.6 | 99.4 | |
| 150% | 3112684 | 90 | 89.3 | 99.3 | |

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

**Robustness**

**Lornoxicam**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Flow rate of 1.0 mL/min | 3425413 | 2.088 | 5568.2 | 1.0 |
| Flow rate of 0.9 mL/min | 3425282 | 3.111 | 5922.2 | 1.2 |
| Flow rate of 1.1 mL/min | 3517879 | 1.880 | 5868.8 | 1.2 |
| Less aqueous phase | 3175485 | 3.101 | 5836.2 | 1.2 |
| More aqueous phase | 3365431 | 1.881 | 5282.6 | 1.1 |

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**Thiocolchicoside**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Flow rate of 1.0 mL/min | 2029854 | 6.068 | 5359.2 | 1.1 |
| Flow rate of 0.9 mL/min | 1738319 | 7.101 | 5999.1 | 1.2 |

| | | | | |
|---|---|---|---|---|
| Flow rate of 1.1 mL/min | 1638304 | 5.007 | 5989.2 | 1.1 |
| Less aqueous phase | 1973724 | 7.108 | 5387.2 | 1.1 |
| More aqueous phase | 2102838 | 5.008 | 5938.1 | 1.1 |

## CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Thiocolchicoside and Lornoxicam in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Thiocolchicoside and Lornoxicam was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol and Water (5:95% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Thiocolchicoside and Lornoxicam in bulk drug and in Pharmaceutical dosage forms.

**ACKNOWLEDGEMENT**

The Authors are thankful to the Management and Principal, Department of Pharmacy, Samskruti college of pharmacy in Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

## BIBLIOGRAPHY

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, 2002, PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, 1996, Pp 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st ed, Academic press, 1997, PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, 2007, PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, 2004, PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online),URL:http:// amitpatel745.topcities.com/index_files/study/column care.pdf
9. Detectors used in HPLC (online )URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL:http:// hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL:http:// www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http:// www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http:// www.chem.agilent.com/Library/applications/59643559.pdf
14. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
15. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, 1995, PP 1126.
16. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996, PP 1- 8.
17. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
18. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
19. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, 1997, PP 180-182.
20. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. 1994, PP 1-5.