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NEW ANALYTICAL METHOD DEVELOPMENT FOR LORNOXICAM AND THIOCOLCHICOSIDE IN SOLID DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method was developed for estimation of Lornoxicam and Thiocolchicoside by Sunfire C18 (4.6×250mm) 5µ using mobile phase Methanol: Water (65:35% v/v) pH 4.0.The flow rate was 1.0 ml/min and effluent was monitored at 295nm. The retention time is 2.0min for Thiocolchicoside and 2.4 min for Lornoxicam respectively. Proposed method was optimized.

KEYWORDS: Lornoxicam, Thiocolchicoside, HPLC.

INSTRUMENTS USED

Table: S.No	Instruments And Glassware	Model
1	HPLC	WATERS, Alliance 2695 separation module, 996 PDA detector, Software: Empower 2.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

S.No	chemical	Brand names
1	Lornoxicam and Thiocolchicoside (Pure)	SURALABS
2	Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Milli-Q water	

HPLC METHOD DEVELOPMENT

The objective of this experiment was to optimize the assay method for simultaneous estimation of Lornoxicam and Thiocolchicoside based on the literature survey made. So here the trials mentioned describes how the optimization was done.

TRAILS

Trail 1:

Column : Xterra C18 (4.6×250mm) 5µ
Column temperature : Ambient
Wavelength : 295nm
Mobile phase ratio : Methanol: Water (20:80% v/v)
Flow rate : 0.8ml/min
Injection volume : 10µl
Run time : 10minutes
Retention time : 3.8min for Thiocolchicoside and 4.9 min for Lornoxicam

Observation: From the above trail it was observed that it shows less plate count and improper baseline in the chromatogram. It's required more trails to get good peaks.

Trail 2:

Column : Xterra C18 (4.6×250mm) 5µ
Column temperature : Ambient
Wavelength : 295nm
Mobile phase ratio : Methanol: Water (15:85% v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10minutes
Retention time : 1.0min for Thiocolchicoside and 1.6 min for Lornoxicam

Observation: In this above trail one sample peak was splitted and it shows very less plate count in the chromatogram. More trails required to get perfect peaks elution.

Trail 3:

Column : Xterra C18 (4.6×250mm) 5µ
Column temperature : 27ºC
Wavelength : 295nm
Mobile phase ratio : Methanol: Water (35:65% v/v)

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 5minutes

Retention time : 1.2min for Thiocolchicoside and 1.8 min for Lornoxicam

Observation: This above trail shows very less plate count and bad peak shape in the chromatogram. More trails required to get good peaks.

Trail 4:

Column : Sunfire C18 (4.6×250mm) 5µ

Column temperature : 35ºC

Wavelength : 295nm

Mobile phase ratio : Methanol: Water (40:60% v/v) pH 3.5

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 5minutes

Retention time : 1.1min for Thiocolchicoside and 1.8 min for Lornoxicam

Observation: From the above chromatogram it was observed that it shows bad peak shape. Required more trails to obtain good peaks.

Trail 5 (Optimized chromatogram):

Column : Sunfire C18 (4.6×250mm) 5µ

Column temperature : 35ºC

Wavelength : 295nm

Mobile phase ratio : Methanol: Water (65:35% v/v) pH 4.0

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 8minutes

Retention time : 2.0min for Thiocolchicoside and 2.4 min for Lornoxicam

Observation: it show the good peaks with better resoloution

OPTIMIZED METHOD

Mobile Phase: Degassed Methanol: Water (65:35% v/v) pH 4.0.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Lornoxicam and Thiocolchicoside 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 up volume to the mark with the same Methanol.

Further pipette 0.3ml of Lornoxicam and 0.15ml of the Thiocolchicoside 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.

Preparation of Sample Solution

Take average weight of Tablet--0.3995, (10mg equivalent weight-0.1664) and crush in a mortar by using pestle and weight 10 mg equivalent weight of Lornoxicam and Thiocolchicoside sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of Lornoxicam and 0.15ml of the Thiocolchicoside stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of mobile phase

Accurately measured 650ml (65%) of HPLC Methanol and 350 ml of Water (35%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Procedure for calibration curve

The contents of the mobile phase were filtered before use through 0.45micron membrane and pumped from the respective solvent reservoirs to the column at a specified flow rate. The chromatographic separation was achieved using a mobile phase consisting of Methanol: Water (65:35% v/v) pH 4.0 the eluent was monitored using UV detector at a wavelength of 295 nm.The column was maintained an ambient temperature (25^0c) and an injection volume

of 10µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, peak area of drug was recorded graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis.

Diluent Preparation

The Mobile phase was used as the diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula.

RESULTS AND DISCUSSION

Method development

TRAIL-1

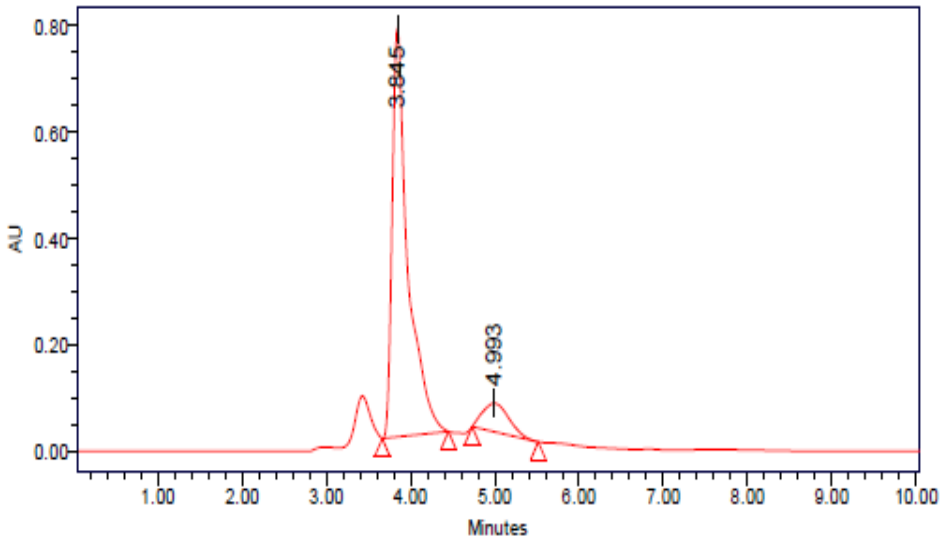
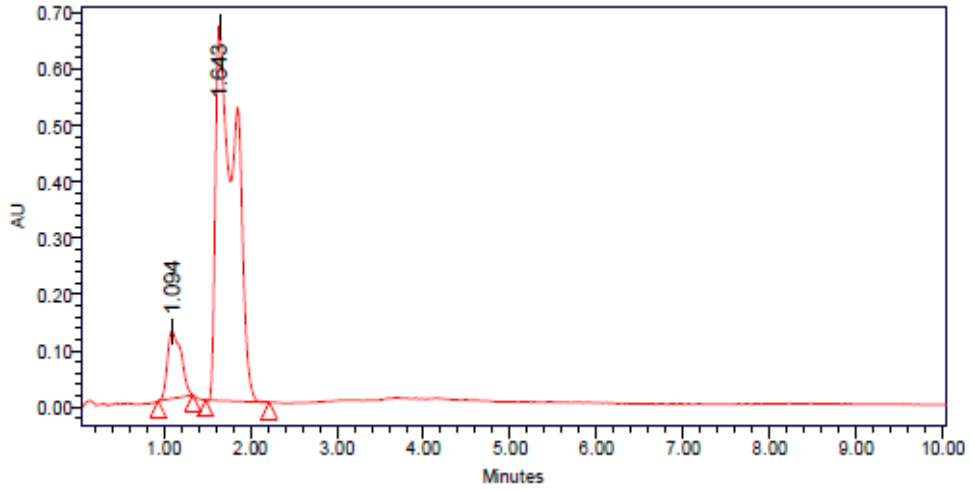


Fig 5.1.1: Chromatogram of Trial 1

Inference : From the above trail it was observed that it shows less plate count and improper baseline in the chromatogram. It's required more trails to get good peaks.

S.No	Name of the peak	Retention time(min)
1	Thiocolchicoside	3.8
2	Lornoxicam	4.9

TRAIL-2

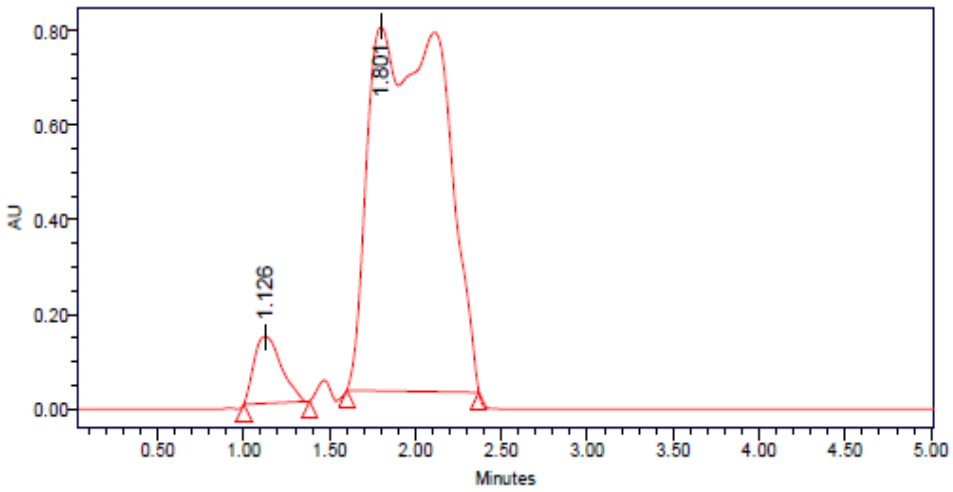


Chromatogram of Trial-2

Inference: In this above trail one sample peak was splitted and it shows very less plate count in the chromatogram. More trails required to get perfect peaks elution.

S.NO	Name of the peak	Retention time(min)
1	Thiocolchicoside	1.0min
2	Lornoxicam	1.6 min

TRAIL-3

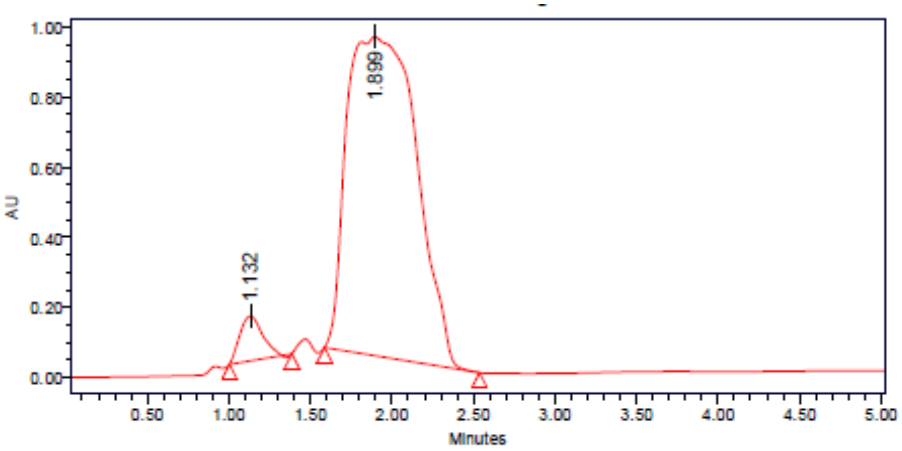


Chromatogram of Trial-3

Inference: This above trail shows very less plate count and bad peak shape in the chromatogram. More trails required to get good peaks.

S.NO	Name of the peak	Retention time(min)
1	Thiocolchicoside	1.2min
2	Lornoxicam	1.8 min

TRAIL-4

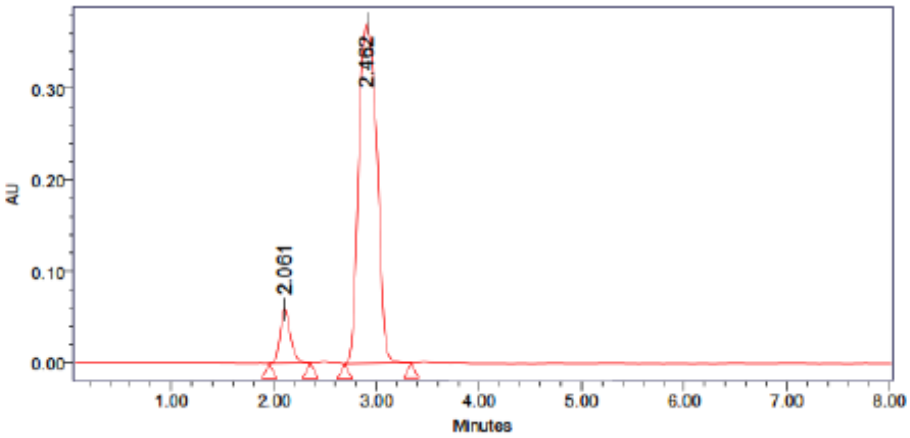


Chromatogram of Trial-4

Inference: From the above chromatogram it was observed that it shows bad peak shape. Required more trails to obtain good peaks.

S.NO	Name of the peak	Retention time(min)
1	Thiocolchicoside	1.1min
2	Lornoxicam	1.8 min

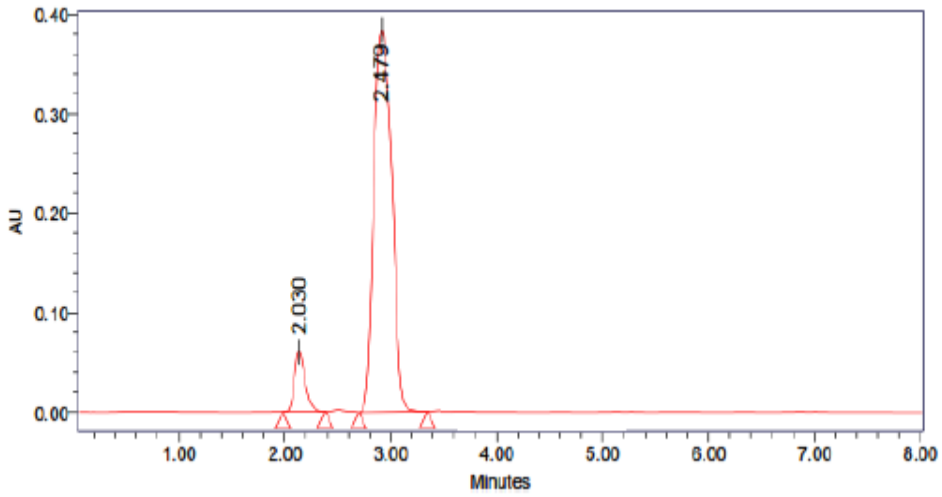
5.2 OPTIMIZED METHOD



Chromatogram of STANDARD

Inference: Got chromatogram at Rt values as of 2.0min for thiocolchicoside and 2.4 min for lornoxicam for standard.

S.NO	Name of the peak	Retention time(min)
1	Thiocolchicoside	2.0min
2	Lornoxicam	2.4 min



Chromatogram of Sample

Inference: Got same chromatogram with same RT values as of standard.

S.NO	Name of the peak	Retention time(min)
1	Thiocolchicoside	2.0min
2	Lornoxicam	2.4 min

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 295nm for Lornoxicam and Thiocolchicoside and the peaks purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Sunfire C18 (4.6×250mm) 5µ chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of Methanol: Water (65:35% v/v) pH 4.0was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

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