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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LORNOXICAM AND THIOCOLCHICOSIDE IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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Keywords:

Lornoxicam and Thiocolchicoside, RP-HPLC, Validation, Accuracy, Robustness

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ABSTRACT: A simple, reproducible, and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Lornoxicam and Thiocolchicoside in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm × 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72 v/v) was used. The flow rate was 1.0 ml/min, and the effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Lornoxicam and Thiocolchicoside were (1.794, 3.440 min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy, and precision, robustness, and limit of detection, and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Lornoxicam and 1.28µg/ml 3.84µg/ml for Thiocolchicoside respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Lornoxicam and Thiocolchicoside in tablets.

INTRODUCTION: Lornoxicam is an orally bioavailable oxicam and non-steroidal anti-inflammatory drug (NSAID), with analgesic, anti-pyretic, anti-thrombotic, and anti-inflammatory activities. Upon oral administration, Lornoxicam [20] binds to and inhibits the activity of the cyclooxygenase enzymes (COX) type 1 (COX-1) and type 2 (COX-2). This blocks COX-mediated signaling pathways, which leads to reduced prostaglandin and thromboxane production and decreased pain, fever, and inflammation. Lornoxicam differs from other oxicam compounds in its potent inhibition of prostaglandin bio-synthesis, a property that explains the particularly pronounced efficacy of the drug.

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Lornoxicam is approved for use in Japan. The IUPAC Name [21] of Lornoxicam is 6-chloro-4-hydroxy-2-methyl-1, 1-dioxo-N-(pyridin-2-yl)-2H-1λ^6-thieno [2, 3-e] [1,2] thiazine-3-carboxamide and the chemical formula [22] is $C_{13}H_{10}ClN_3O_4S_2$. The Chemical Structure of Lornoxicam is in **Fig. 1**.

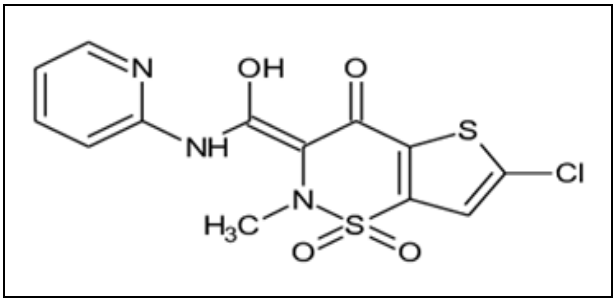

FIG. 1: CHEMICAL STRUCTURE OF LORNOXICAM

Thiocolchicoside [23] (Muscoril, Myoril and Neoflax) is a muscle relaxant with anti-inflammatory and analgesic effects. It acts as a competitive GABAA receptor antagonist and also glycine receptor antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It

has powerful convulsant activity and should not be used in seizure-prone individuals. Thiocolchicoside [24] is a semi-synthetic derivative of the colchicine, a natural anti-inflammatory glycoside which originates from the flower seeds of *Superba gloriosa*. It is a muscle relaxant with anti-inflammatory and analgesic effects. It has potent convulsant activity [25] and should not be administered to individuals prone to seizures. The IUPAC Name of Thiocolchicoside is N-[(10S)-3,4-dimethoxy- 14-(methylsulfanyl)- 13-oxo- 5-{[(2S, 3R,4S,5S,6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy}tricyclo[9.5.0.0^{2,7}]hexadeca-1(16), 2, 4, 6, 11, 14-hexaen-10-yl]acetamide and the chemical formula [25] is $C_{27}H_{33}NO_{10}S$. The chemical structure of thiocolchicoside is in **Fig. 2**.

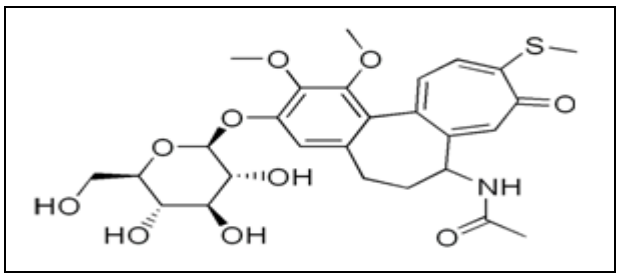

FIG. 2: CHEMICAL STRUCTURE OF THIOCOLCHICOSIDE

Literature survey [27-30] reveals that several analytical methods have been reported for Lornoxicam and Thiocolchicoside individually in bulk and in pharmaceutical dosage forms [26]. Few analytical methods using spectrophotometry, HPLC and LC-MS have been reported for the simultaneous determination of Lornoxicam and Thiocolchicoside in combined dosage forms. The objective of the present study was to develop and validate a simple, accurate and precise HPLC method for simultaneous determination of Lornoxicam and Thiocolchicoside in bulk and in pharmaceutical dosage forms.

MATERIALS AND METHODS: Lornoxicam (Pure), Thiocolchicoside (Pure) gift sample procured from Sura Labs, Dilsukhnagar, Hyderabad, Water, Methanol, Acetonitrile all are HPLC grade obtained from Merck, Orthophosphoric acid, Glacial acetic acid analytical grade obtained from Merck.

Instrumentation HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.

HPLC Method Development:

Trails:

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 remove of air completely and make volume up to the mark with the same methanol.

Further pipette 0.2ml of the above Lornoxicam and 0.3ml 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 [1] and record the chromatograms; note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [11, 12].

Mobile Phase Optimization: [2] Initially, the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Bufferin proportion 28:72 (pH-3.8) v/v, respectively.

Optimization of Column: The method was performed with various columns like the C18 column, Symmetry, and Zodiac column. Symmetry (C18) (150mm × 4.6mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution[3] at 1ml/min flow.

Optimized Chromatographic Conditions:

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	Ambient
Column	Symmetry (C18) (150mm × 4.6mm, 5µm) Column
Buffer[4]	Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication
pH	3.8
Mobile phase	Methanol: Phosphate Buffer (28:72v/v)
Flow rate	1ml/min
Wavelength	252 nm
Injection volume	20 µl
Run time	8 min

Method Validation:

Preparation of Buffer and Mobile Phase:

Preparation of Potassium Dihydrogen Phosphate (KH_2PO_4) Buffer (pH-3.8): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of Mobile Phase: Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration [5].

Diluent Preparation: The Mobile phase was used as the diluent.

Method Validation Parameters:

System Suitability: [6, 7] Accurately weigh and transfer 10 mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.2ml of the above Lornoxicam and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug: [8]

Preparation of Standard Solution: Accurately weigh and transfer 10mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.2ml of the above Lornoxicam and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution: Take average weight of one Tablet and crush in a mortar by using a pestle and weight 10 mg equivalent weight [3] 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 0.2ml of the sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. The mean and percentage relative standard deviation were calculated from the peak areas.

Procedure: Inject the three replicate injections of standard and sample solutions [9] and calculate the assay by using the formula:

% Assay = Sample area / Standard area × Weight of standard / Dilution of standard × Dilution of sample / Weight of sample × Purity / 100 × Weight of tablet / Label claim

Preparation of Drugsolutions for Linearity: Accurately weigh and transfer 10 mg of Lornoxicam & 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Preparation of Level – I (10ppm of Lornoxicam & 10ppm of Thiocolchicoside): Pipette out 0.1ml of Lornoxicam and 0.1ml of Thiocolchicoside stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (15ppm of Lornoxicam & 20ppm of Thiocolchicoside): Pipette out 0.15ml of Lornoxicam and 0.2ml of Thiocolchicoside stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Lornoxicam & 30ppm of Thiocolchicoside): Pipette out 0.2ml of Lornoxicam and 0.3ml of Thiocolchicoside stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (25ppm of Lornoxicam & 40ppm of Thiocolchicoside): Pipette out 0.25ml of Lornoxicam and 0.4ml of Thiocolchicoside stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (30ppm of Lornoxicam & 50ppm of Thiocolchicoside): Pipette out 0.3ml of Lornoxicam and 0.5ml of Thiocolchicoside stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [10].

Precision:

Repeatability:

Preparation of Lornoxicam and Thiocolchicoside Product Solution for Precision: Accurately weigh and transfer 10 mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 0.2ml of the above Lornoxicam and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC [7]. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision: To evaluate the intermediate precision [13, 14] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1: The standard solution was injected for three times and measured the area for all three injections in HPLC. The %RSD for the area of three replicate injections was found to be within the specified limits.

Day 2: The standard solution was injected three times and measured the area for all three injections in HPLC. The % RSD for the area of three replicate injections was found to be within the specified limits.

Accuracy: [15, 16]

For the preparation of 50% Standard Stock Solution: Accurately weigh and transfer 10 mg of Lornoxicamand 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 0.1ml of the above Lornoxicam and 0.15ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For Preparation of 100% Standard Stock Solution: Accurately weigh and transfer 10 mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.2ml of the above Lornoxicam and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For Preparation of 150% Standard Stock Solution: Accurately weigh and transfer 10 mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.3ml of Lornoxicam and 0.45ml of Thiocolchicoside from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the three replicate injections of individual concentrations (50%, 100%, and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for Lornoxicam and Thiocolchicoside and calculate the individual recovery and mean recovery values.

Robustness: [17, 18] The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Lornoxicam and 10mg of Thiocolchicoside working standard into a 10ml of clean, dry volumetric flasks add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 0.2ml of the above Lornoxicam and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Effect of Variation of Flow Conditions: The sample was analyzed at 0.9 ml/min, and 1.1 ml/min instead of 1ml/min; the remaining conditions [19] are the same. 20µl of the above sample was injected, and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition: The sample was analyzed by variation of the mobile phase, *i.e.*, Methanol: Phosphate Buffer was taken in the ratio and 33:64, 23:77 instead (28:72), remaining conditions are same. 20µl of the above sample was injected, and chromatograms were recorded.

RESULTS AND DISCUSSION: Method Development:

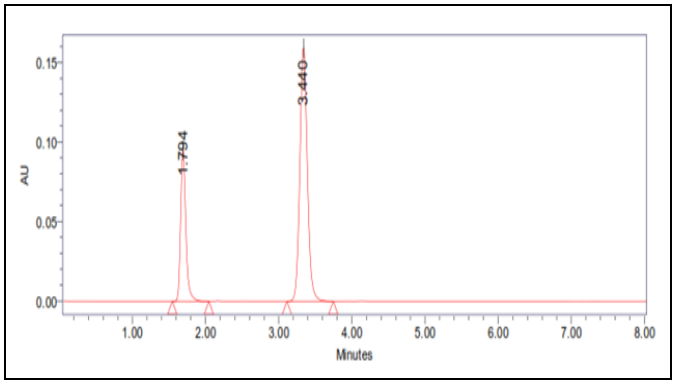

FIG. 3: OPTIMIZED CHROMATOGRAM FOR STANDARD

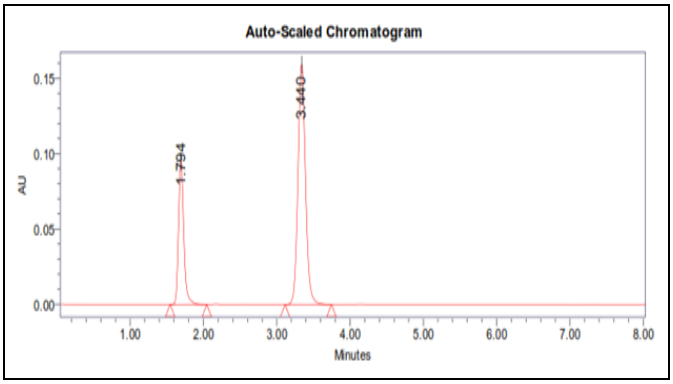

FIG. 4: OPTIMIZED CHROMATOGRAM FOR SAMPLE

METHOD VALIDATION: The proposed method was subjected to validation for various parameters like linearity and range, precision, accuracy, and robustness in accordance with International Conference on Harmonization Guidelines.

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range. Weigh accurately 10mg of Lornoxicam and 10mg of Thiocolchicoside in 10 ml of volumetric flask and dissolve in 7ml of mobile phase and make up the volume with mobile phase. This solution contains 10-30 µg/ml of Lornoxicam and 10-50 µg/ml of Thiocolchicoside.

Acceptance Criteria: The correlation coefficient should be not less than 0.999. The linearity data and respective chromatograms for Lornoxicam and Thiocolchicoside are as follows.

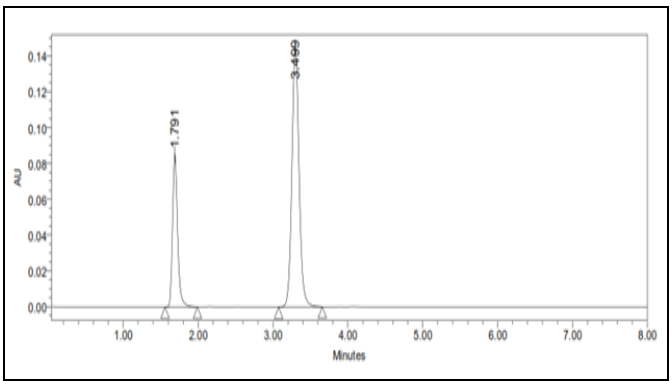

FIG. 5: CHROMATOGRAM SHOWING LINEARITY LEVEL-1

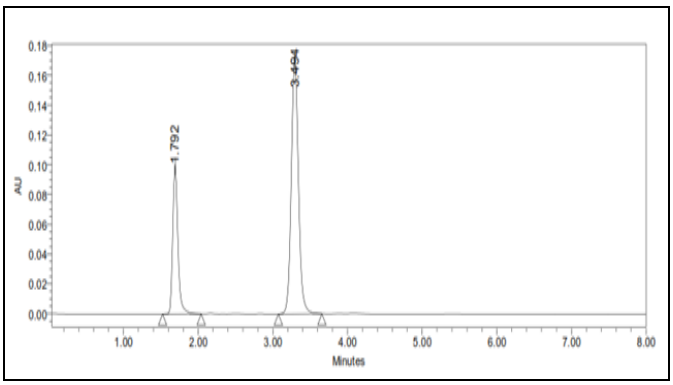

FIG. 6: CHROMATOGRAM SHOWING LINEARITY LEVEL-2

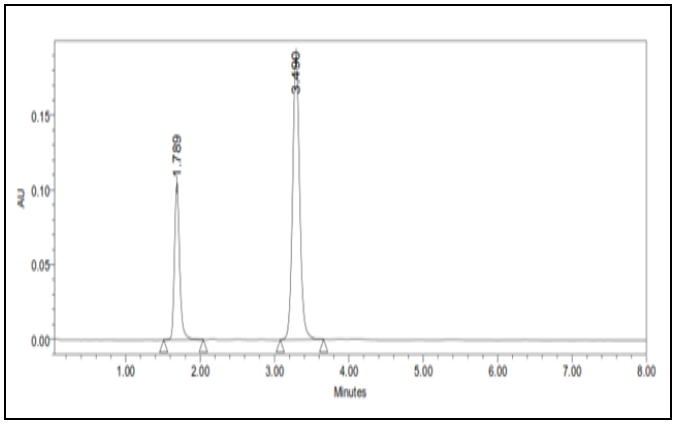

FIG. 7: CHROMATOGRAM SHOWING LINEARITY LEVEL-3

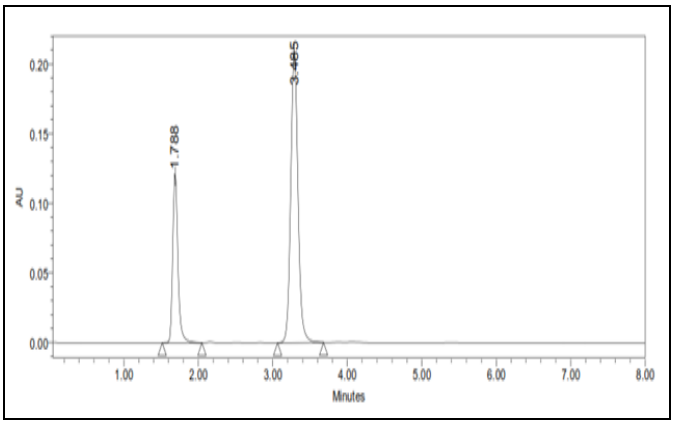

FIG. 8: CHROMATOGRAM SHOWING LINEARITY LEVEL-4

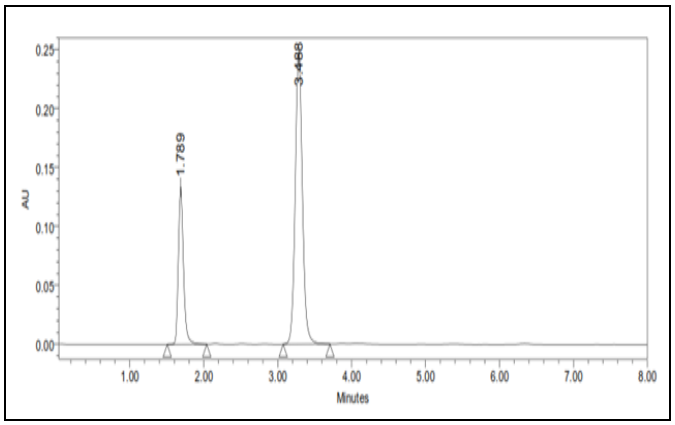

FIG. 9: CHROMATOGRAM SHOWING LINEARITY LEVEL-5

TABLE 2: CALIBRATION DATA OF LORNOXICAM

Concentration (µg/ml)	Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584

TABLE 3: CALIBRATION DATA OF THIOCOLCHICOSIDE

Concentration (µg/ml)	Average Peak Area
10	2828756
15	5485784
20	7999859
25	10656542
30	13085985

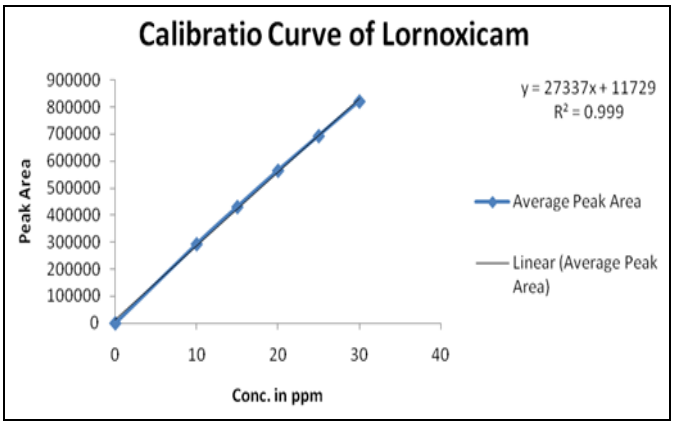

FIG. 10: CALIBRATION CURVE OF LORNOXICAM

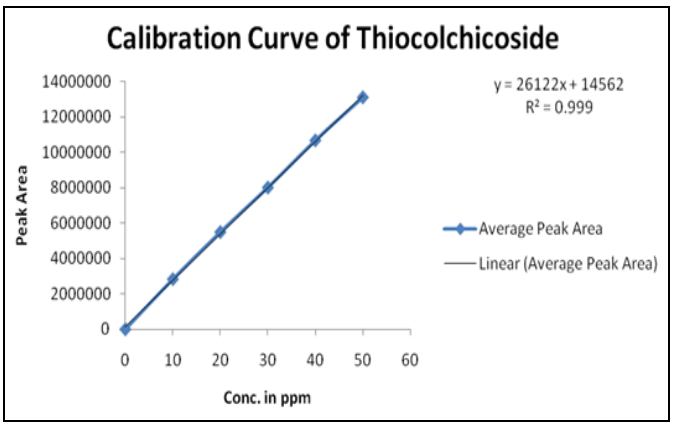

FIG. 11: CALIBRATION CURVE OF THIOCOLCHICOSIDE

Precision: Method precision, also called repeatability/Intraday precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing five test solutions at 100% concentration as per the test procedure & recording the chromatograms of five test solutions. The % RSD of peak areas of five samples was calculated. The method precision was performed on Lornoxicam and Thiocolchicoside.

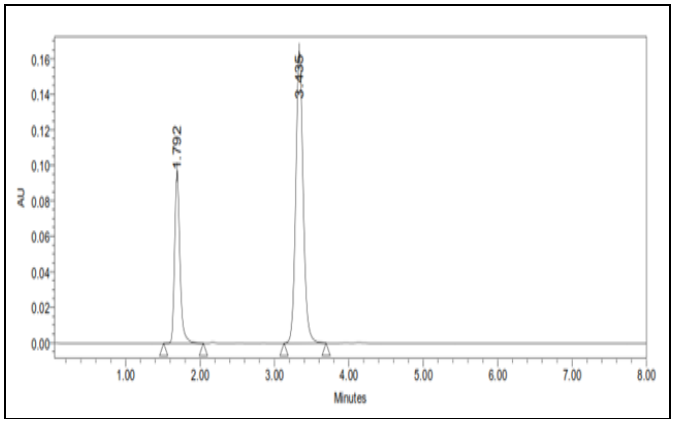

FIG. 12: CHROMATOGRAM SHOWING PRECISION INJECTION-1

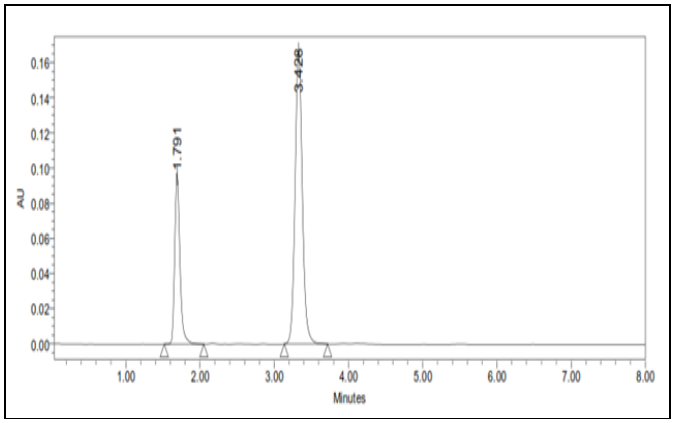

FIG. 13: CHROMATOGRAM SHOWING PRECISION INJECTION-2

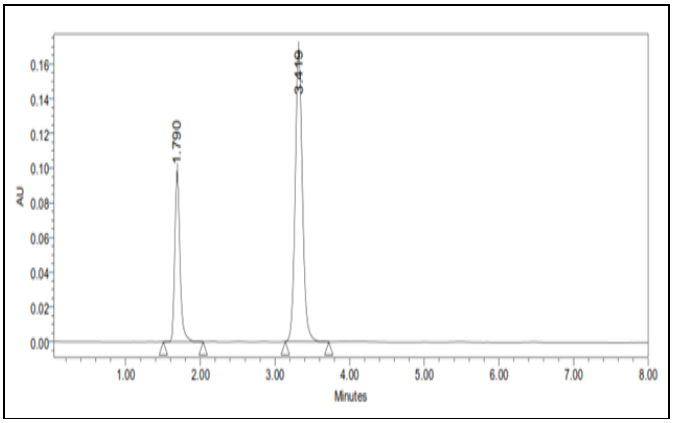

FIG. 14: CHROMATOGRAM SHOWING PRECISION INJECTION-3

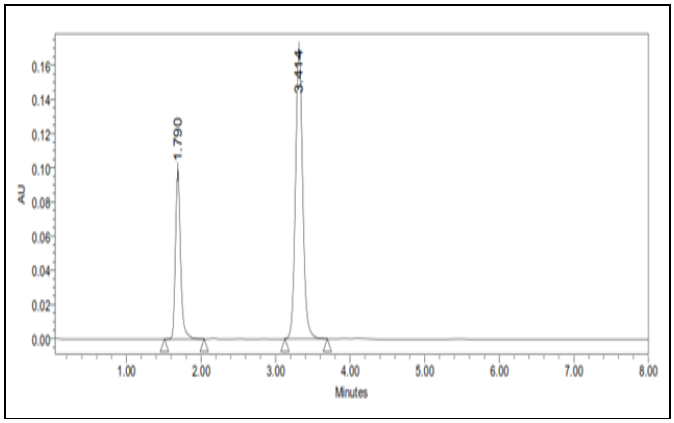

FIG. 15: CHROMATOGRAM SHOWING PRECISION INJECTION-4

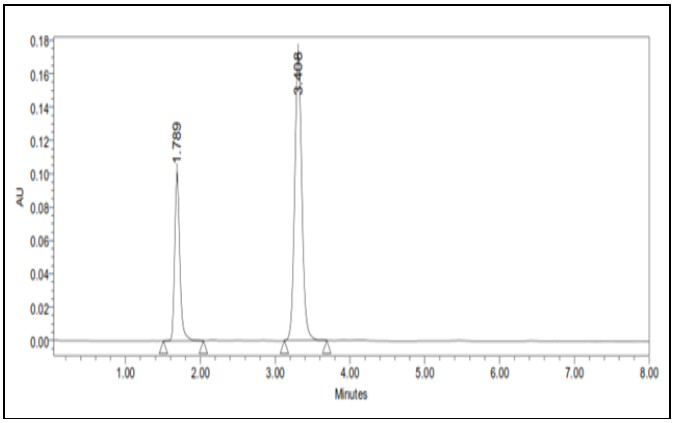

FIG. 16: CHROMATOGRAM SHOWING PRECISION INJECTION-5

TABLE 4: RESULTS OF REPEATABILITY FOR LORNOXICAM

S. no.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Lornoxicam	1.792	548698	7458	7569	1.10
2	Lornoxicam	1.791	548955	7485	7546	1.10
3	Lornoxicam	1.790	548745	7469	7592	1.09
4	Lornoxicam	1.790	549856	7463	7519	1.10
5	Lornoxicam	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std. dev			1202.217			
%RSD			0.2191554			

TABLE 5: RESULTS OF REPEATABILITY FOR THIOCOLCHICOSIDE

S. no.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Thiocolchicoside	3.435	7768958	43659	8659	1.12
2	Thiocolchicoside	3.428	7765984	43856	8647	1.13
3	Thiocolchicoside	3.419	7785469	43658	8675	1.12
4	Thiocolchicoside	3.414	7785498	43549	8652	1.12
5	Thiocolchicoside	3.408	7769852	44526	8692	1.13
Mean			**7775152**			
Std. dev			**9539.236**			
%RSD			**0.122689**			

Intermediate Precision or Ruggedness: The ruggedness of the method was verified by analyzing three samples of the same batch used for method precision as per the proposed method by the different analysts. The repeatability of sample applications and measurement of peak area was expressed in terms of %RSD since their %RSD is <2.0%, and hence, the developed method was found to be precise. Data obtained from intermediate are summarized in **Table 6, 7** and **8, 9**.

Day 1:

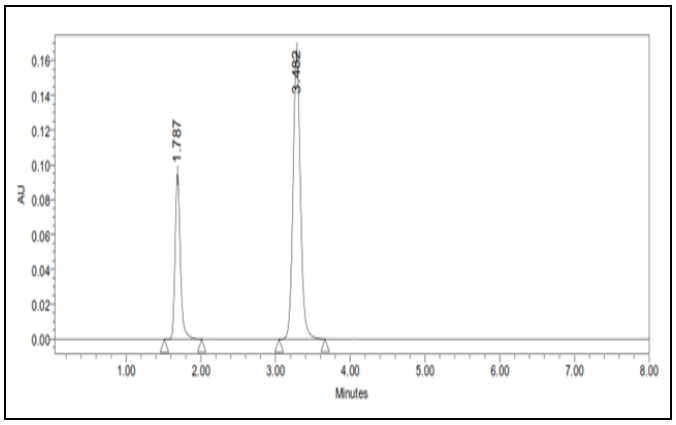

FIG. 17: CHROMATOGRAM SHOWING DAY 1 INJECTION-1

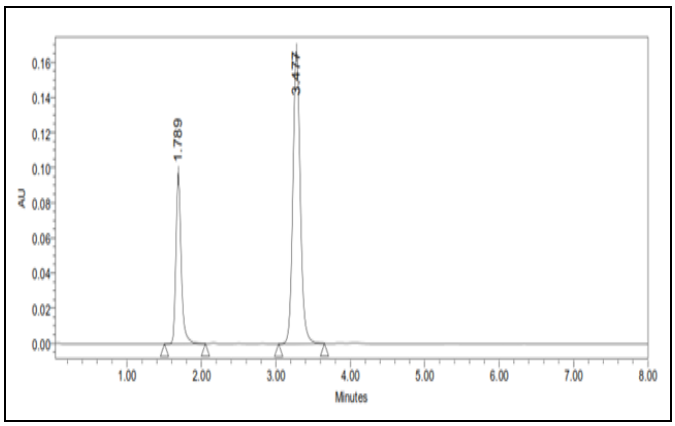

FIG. 18: CHROMATOGRAM SHOWING DAY 1 INJECTION-2

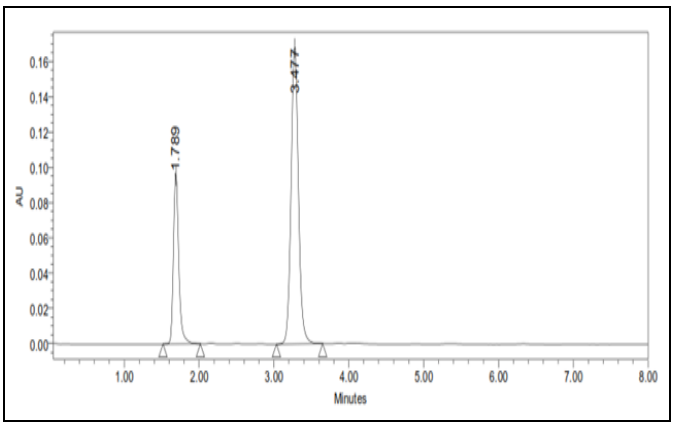

FIG. 19: CHROMATOGRAM SHOWING DAY 1 INJECTION-3

TABLE 6: RESULTS OF INTERMEDIATE PRECISION DAY1 FOR LORNOXICAM

S. no.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Lornoxicam	1.787	556985	75986	7695	1.11
2	Lornoxicam	1.789	558649	75986	7642	1.12
3	Lornoxicam	1.789	557847	75689	7683	1.12
Mean			557827			
Std. Dev.			832.1803			
%RSD			0.149183			

TABLE 7: RESULTS OF INTERMEDIATE PRECISION DAY 1 FOR THIOCOLCHICOSIDE

S. no.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Thiocolchicoside	3.482	7856982	44586	8758	1.13
2	Thiocolchicoside	3.477	7845285	44758	8769	1.14
3	Thiocolchicoside	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std. Dev.			6187.659			
%RSD			0.078801			

Day 2:

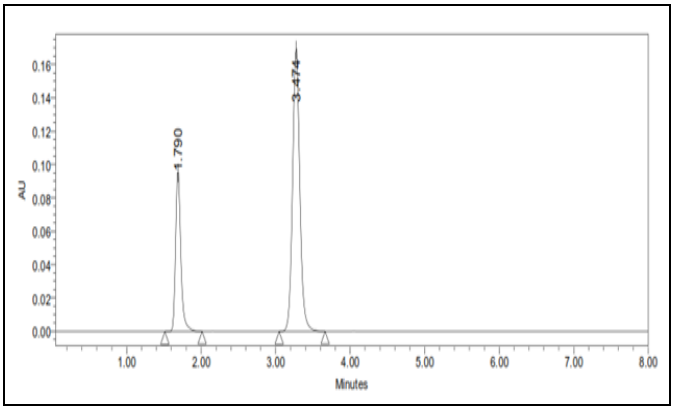

FIG. 20: CHROMATOGRAM SHOWING DAY 2 INJECTION-1

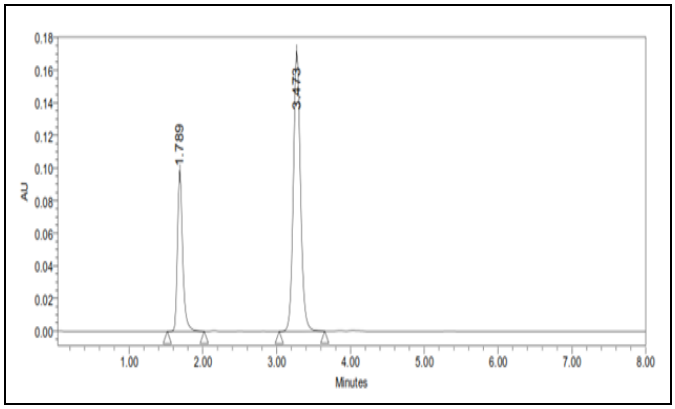

FIG. 21: CHROMATOGRAM SHOWING DAY 2 INJECTION-2

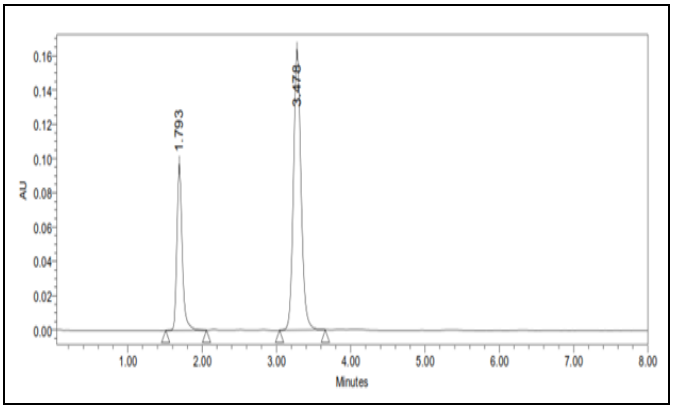

FIG. 22: CHROMATOGRAM SHOWING DAY 2 INJECTION-3

TABLE 8: RESULTS OF INTERMEDIATE PRECISION DAY 2 FOR LORNOXICAM

S. no.	Peak name	RT	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Lornoxicam	1.790	536598	7365	7459	1.08
2	Lornoxicam	1.789	534875	7358	7436	1.07
3	Lornoxicam	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std. Dev.			1049.608			
%RSD			0.196045			

TABLE 9: RESULTS OF INTERMEDIATE PRECISION DAY 2 FOR THIOCOLCHICOSIDE

S. no.	Peak name	RT	Area (µV*sec)	Height (µV)	USP plate count	USP tailing
1	Thiocolchicoside	3.474	7698521	42568	8582	1.11
2	Thiocolchicoside	3.473	7685985	42698	8546	1.10
3	Thiocolchicoside	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std. Dev.			27487.83			
%RSD			0.358064			

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 50%, 100%, and 150% and the percentage recovery was calculated and presented in **Tables 10** and **11**. Recovery was within the range of 98%-102%, which indicates the accuracy of the method.

Accuracy 50%:

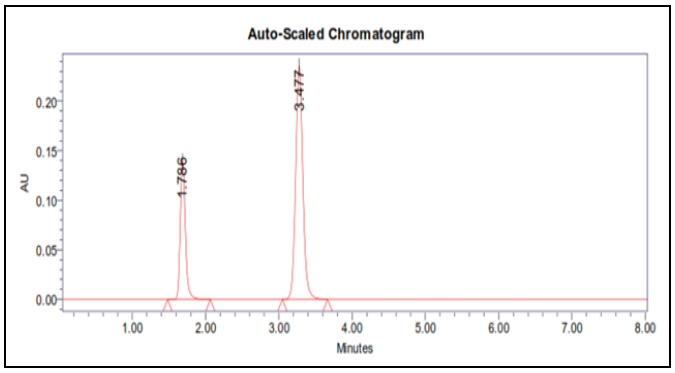

FIG. 23: CHROMATOGRAM SHOWING ACCURACY-50% INJECTION-1

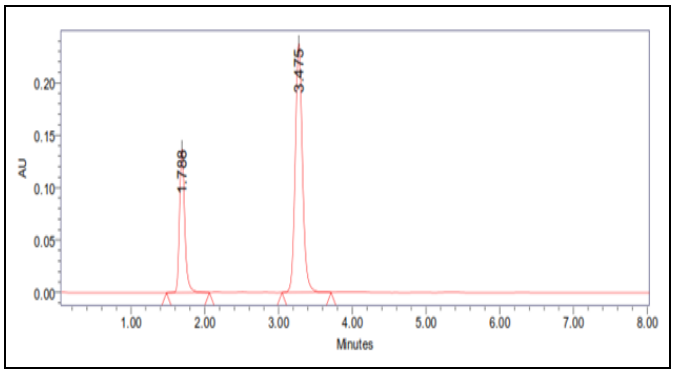

FIG. 24: CHROMATOGRAM SHOWING ACCURACY-50% INJECTION-2

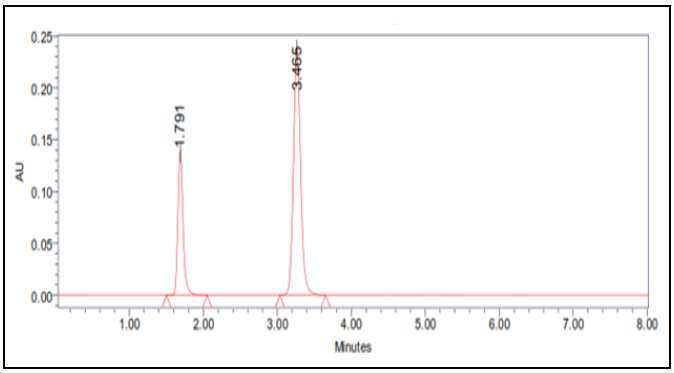

FIG. 25: CHROMATOGRAM SHOWING ACCURACY-50% INJECTION-3

Accuracy 100%:

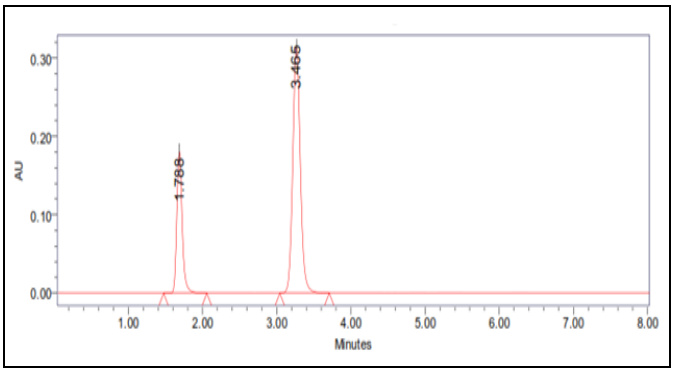

FIG. 26: CHROMATOGRAM SHOWING ACCURACY-100% INJECTION-1

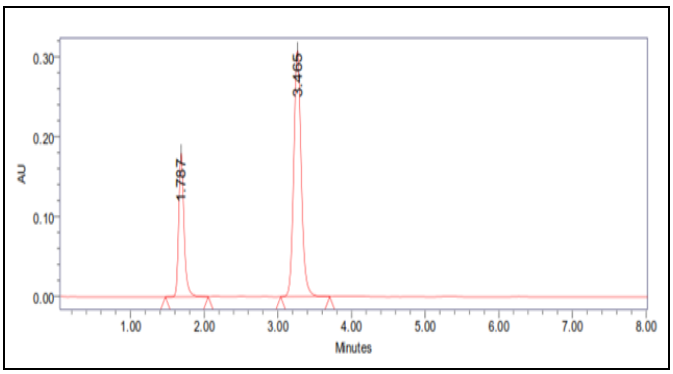

FIG. 27: CHROMATOGRAM SHOWING ACCURACY-100% INJECTION-2

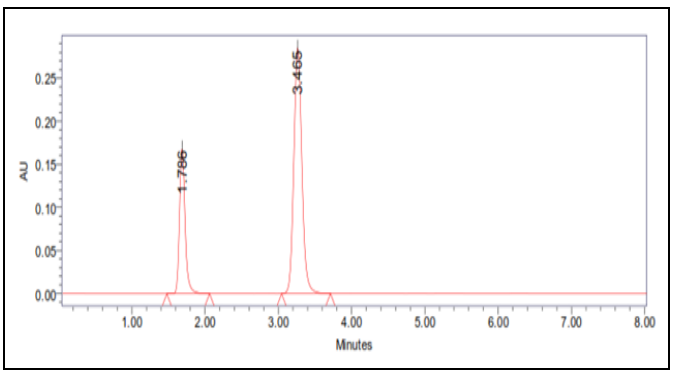

FIG. 28: CHROMATOGRAM SHOWING ACCURACY-100% INJECTION-3

Accuracy 150%:

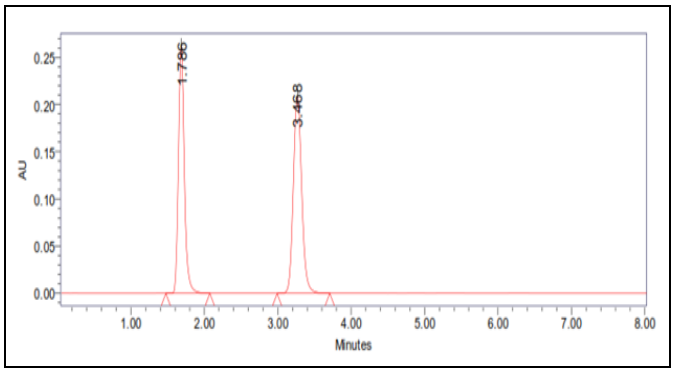

FIG. 29: CHROMATOGRAM SHOWING ACCURACY-150% INJECTION-1

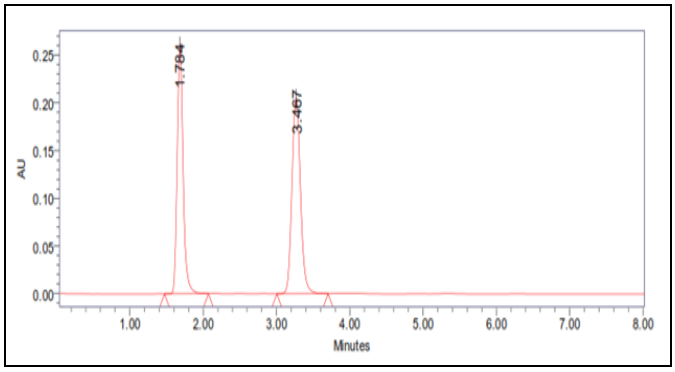

FIG. 30: CHROMATOGRAM SHOWING ACCURACY-150% INJECTION-2

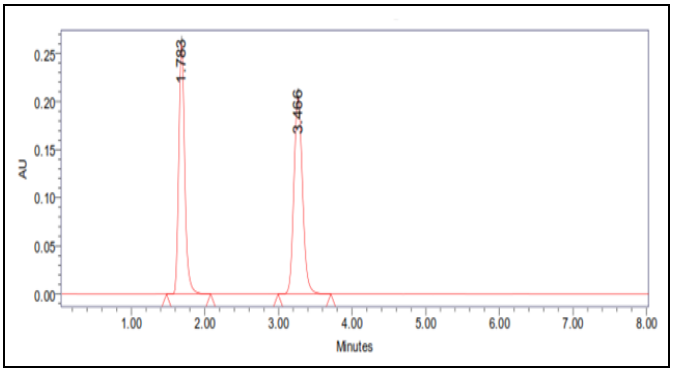

FIG. 31: CHROMATOGRAM SHOWING ACCURACY-150% INJECTION-3

TABLE 10: THE ACCURACY RESULTS FOR LORNOXICAM

% Concentration (at specification level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

TABLE 11: THE ACCURACY RESULTS FOR THIOCOLCHICOSIDE

% Concentration (at specification level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

LOD and LOQ: The LOD and LOQ were calculated using the following equation as per ICH guidelines:

$$LOD = 3.3 \times \sigma / s$$

$$LOS = 10 \times \sigma / s$$

Where σ is the standard deviation of *y*-intercepts of regression lines and *S* is the slope of the calibration curve.

The results of LOD and LOQ are summarized in **Table 12**.

TABLE 12: LOD AND LOQ VALUES OF LORNOXICAM AND THIOCOLCHICOSIDE

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Lornoxicam	0.86	1.28
Thiocolchicoside	2.58	3.84

Robustness: The robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of mobile phase ratio. The study was carried out by changing 5% of the mobile phase ratio and 0.1 mL/min of flow rate.

Variation in Flow:

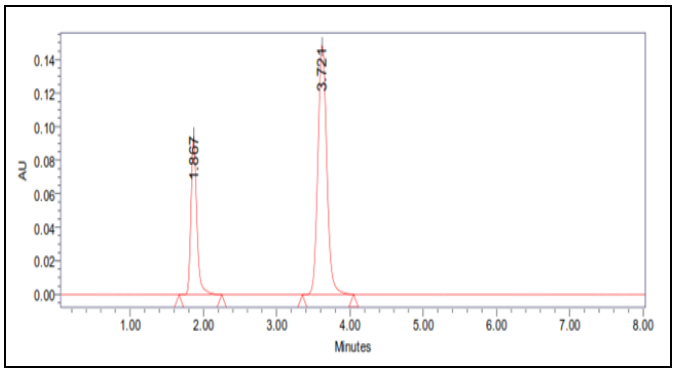

FIG. 32: CHROMATOGRAM SHOWING LESS FLOW OF 0.8ml/min

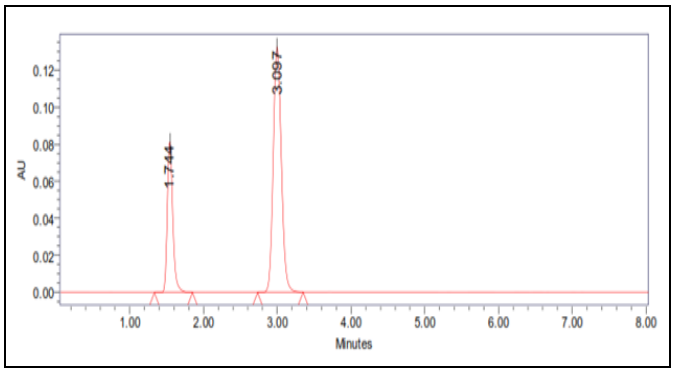

FIG. 33: CHROMATOGRAM SHOWING MORE FLOW OF 1.0ml/min

Variation of Mobile Phase Organic Composition:

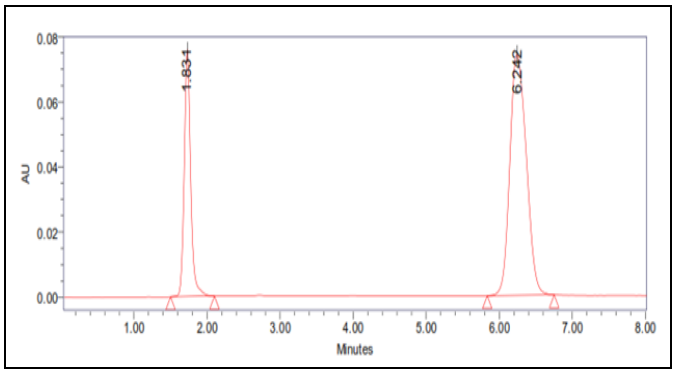

FIG. 34: CHROMATOGRAM SHOWING LESS ORGANIC COMPOSITION

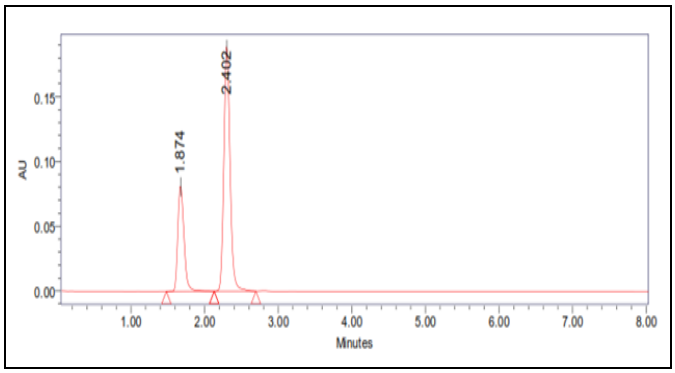

FIG. 35: CHROMATOGRAM SHOWING MORE ORGANIC COMPOSITION

TABLE 13: RESULTS FOR ROBUSTNESS–LORNOXICAM

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less flow rate of 0.8mL/min	625486	1.867	7856	1.13
More flow rate of 1.0mL/min	526548	1.744	7425	1.12
More flow rate of 0.9mL/min				
Less organic phase (about 5% decrease in Organic phase)	536548	1.831	7265	1.06
More organic phase (about 5% Increase in Organic phase)	514875	1.874	7169	1.08

TABLE 14: RESULTS FOR ROBUSTNESS–THIOCOLCHICOSIDE

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5% decrease in Organicp hase)	7685421	6.242	8365	1.10
More organic phase (about 5% Increase in Organic phase)	7569864	2.402	8254	1.09

System Suitability: A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of Lornoxicam and Thiocolchicoside to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

CONCLUSION: In the present investigation, a simple, sensitive, precise, and accurate RP-HPLC method was developed for the quantitative estimation of Lornoxicam and Thiocolchicoside in bulk drug and pharmaceutical dosage forms. Lornoxicam was found to be soluble in the organic solvents ethanol, DMSO, and dimethylformamide (DMF) and slightly soluble in water and Acetonitrile. Thiocolchicoside was found to be soluble in water, methanol, 0.1N HCl, 0.1N NaOH. Methanol: Phosphate Buffer (pH-3.8) (28:72% 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 the 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 Lornoxicam and Thiocolchicoside in bulk drug and in pharmaceutical dosage forms.

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