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METHOD DEVELOPMENT AND VALIDATION SIMULTANEOUS ESTIMATION OF STABILITY SYSTEM OF LORNOXICAM AND THIOCOLCHICOSIDE BY RP-HPLC

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ABSTRACT

A new stability-indicating reversed-phase high-performance liqud chromatographic (RP-HPLC) method for the analysis of lornoxicam and thiocolchicoside was developed and validated. The column used was Inertsil –ODS C18 (250 × 4.6 mm,5µ) with flow rate of1.0ml/min using PDA detection at 239nm. The chromatograms were developed using aluminum plates pre-coated with silica gel as a stationary phase and Acetonitrile: water (55:45v/v) as a mobile phase. The described method was linear over a concentration range of 20ppm to 80ppm for the assay of lornoxicam and thiocolchicoside respectively. The retention times of lornoxicam and thiocolchicoside were found to be 2.869min and 3.942min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for lornoxicam and thiocolchicoside were found to be 1.69mg/ml and 1.74mg/ml respectively. Then the limit of detection (LOD) for lornoxicam and thiocolchicoside were found to be 0.56 mg/ml and 0.57 mg/ml respectively. The drug was exposed to acidic and alkaline hydrolysis, oxidation, photo degradation, and dry heat conditioners. The peaks of degradation products were well-resolved from the peak of the standard drug with significantly different values. Statistical analysis proved that the established RP-HPTLC method is reproducible, selective, and accurate for the determination of lornoxicam and thiocolchicoside in its formulations. The method can effectively separate the drug from its degradation products, and it can be considered as stability-indicating assay.

KEYWORDS: Lornoxicam, Thiocolchicoside, Acetonitrile.

MATERIALS AND METHOD

Chemicals and solvents

lornoxicam and thiocolchicoside as gift samples from hetero Laboratories Limited, Hyderabad, India. The commercial Pharmaceutical topical formulation of D aktacort containing 15w/w hydrocortisone, miconazole-2%W/W (manufactured by Johnson & johnson) were procured from local pharmacy. Potassium dihydrogen phosphate – AR grade (SD.Fine chem. Ltd, mumdai), Acetonitrile-HPLC grade (Merck India), Methanol – HPLC grade (Merck India).

INSTRUMENTATION

The chromatographic separations were performed using HPLC-Waters alliance (Model-2690/5) consisting of an in-built auto sampler, a column oven and Waters 996 PDA detector. The data was acquired through Empower-2-software. The column used was Inertsil ODS (250×4.6 mm, 5µ). Meltronics sonicator was used for enhancing dissolution of the compounds. Elico pH meter was used for adjusting the pH of buffer solution. All weighing was done on Sartorious balance (model AE-160).

Chromatographic conditions

The mobile phase consists of Arthophosporic acid: metanol in the ratio of 55:45 v/v. The mobile phase was pumped from solvent reservoir in the ratio of 55:45 v/v to the column in the flow rate of 1.0 ml/min whereas run time set was 10 min. The separation was performed on Inertsil ODS-3V 250mm x 4.6mm, 5µm column and the column was maintained the temperature ambient and the volume of each injection was 20µl. Prior to injection, the column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 256 nm.

OPTIMISED METHOD

Mobile Phase

Orthophosphoric acid and methanol in the ratio of (45:55)

Preparation of stock solution

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Lornoxicam 25.0 mg Thiocolchicoside l in Mobile phase, in two 100.0 mL volumetric flasks

separately and sonicate for 20min. From the above solutions take 10.0 ml from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Lornoxicam and Thiocolchicoside above, sonicated and filtered through 0.45µ membrane.

Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -BDS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile: water (55:45)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	256nm
Drug RT (min)	2.869min for Lornoxicam and 3.942for Thiocolchicoside

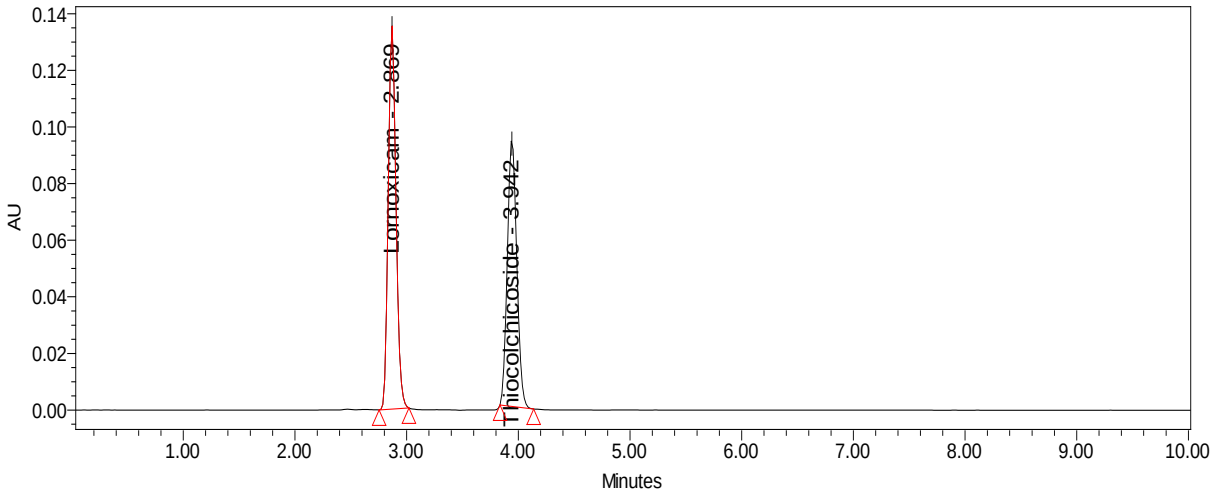


Fig 4: Chromatogram of standard

Inference

Got chromatogram at RT's of 2.869min to Lornoxicam and 3.942min to Thiocolchicoside for standard.

6.3 VALIDATION DATA

6.3.1 SYSTEM SUITABILITY

A Standard solution was prepared by using Lornoxicam and Thiocolchicoside Potassium working standards as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Lornoxicam and Thiocolchicoside**,** retention times and peak areas.

ACCEPTANCE CRITERIA

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Lornoxicam and Thiocolchicoside peaks is NLT 3000.
4. The Tailing factor (T) for the Lornoxicam and Thiocolchicoside peaks is NMT 2.0

TABLE- 1(a): Data of System Suitability for Lornoxicam andThicolchicoside

	Lornoxicam				**Thiocolchicoside**			
Injection	RT	Peak area	USP plate count	USP tailing	RT	Peak area	USP platecount	USP trailling
1	2.869	2748977	9478.317159	1.021108	3.942	729374	10953.609752	1.604407
2	2.868	2748357	9452.196217	1.080574	3.942	729587	10951.014286	1.604878
3	2.872	2748360	9569.928335	1.090824	3.944	729020	10003.278630	1.590957
4	2.868	2748206	9619.633847	1.089932	3.940	729174	10986.906427	1.584354
5	2.872	2748407	9749.907462	1.108610	3.943	729744	10946.878423	1.566451
MEAN	2.865841	2748461	9573.997	1.07821	3.9424 112	729379.8	10768.34	1.590209
SD	0.00148	297.998	…….	…….	0.0046 58	294.7104	……..	……
%RSD	0.050	0.0108	……..	…….	0.131	0.040	……..	……

OBSERVATION

The %RSD for retention times and peak areas were found to be within the limit. Refer table: 1 As shown in fig1-5.

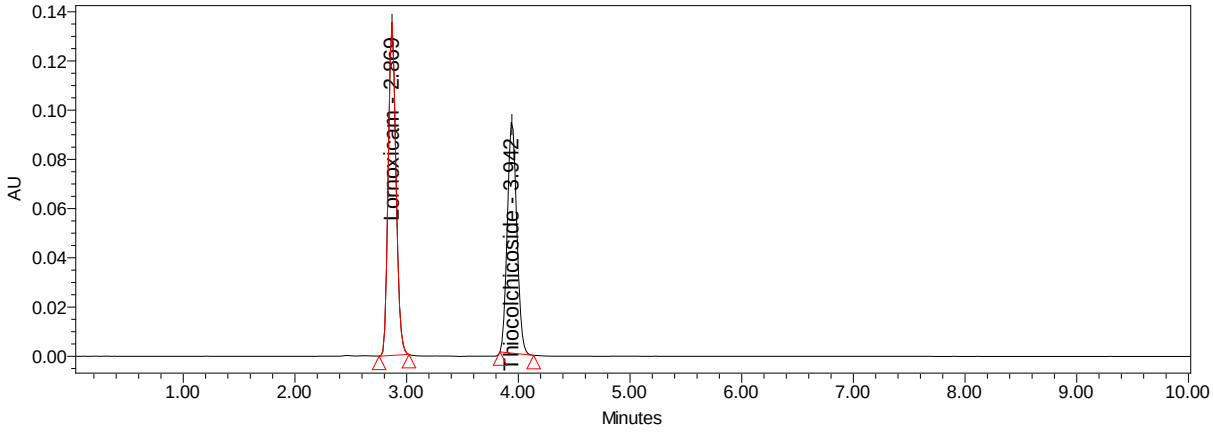
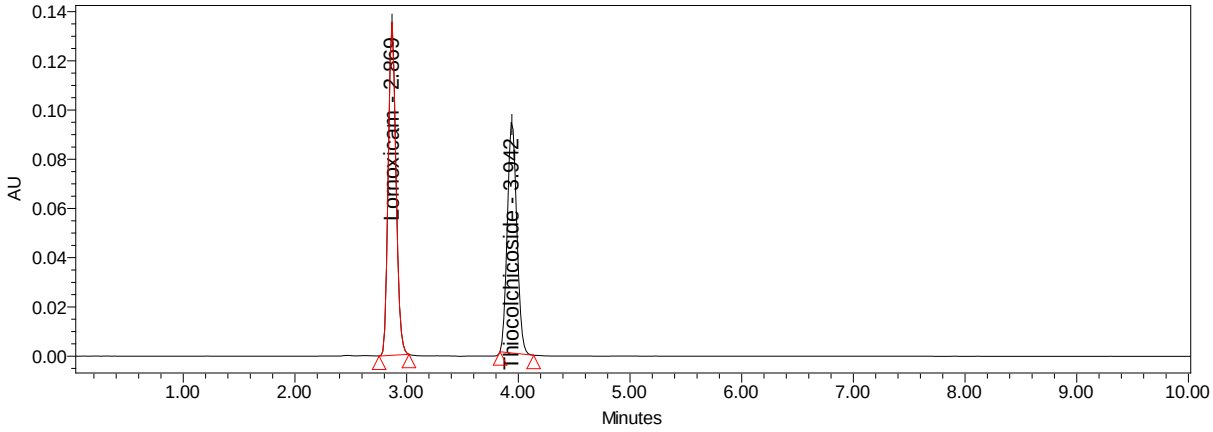
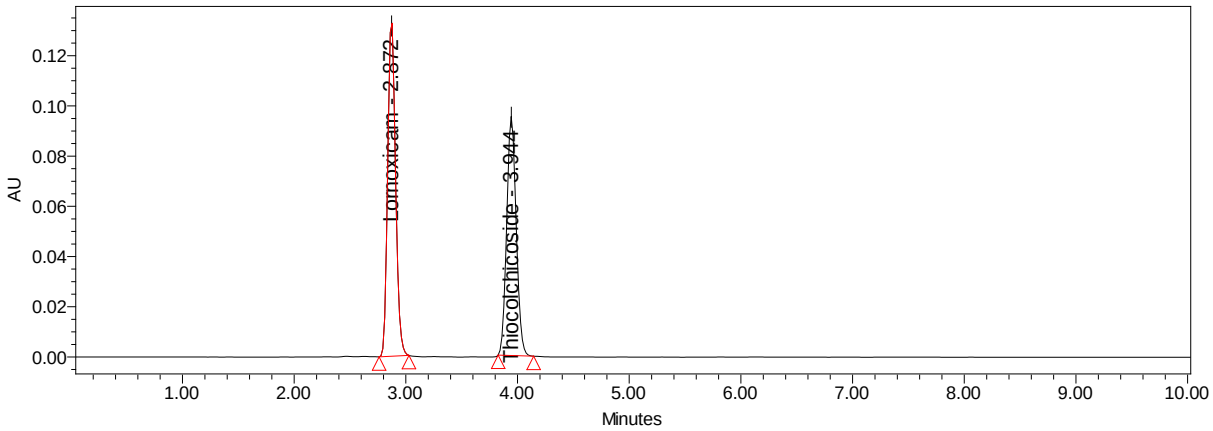


Fig: 6-10 Chromatograms of system suitability (standards 1-5)

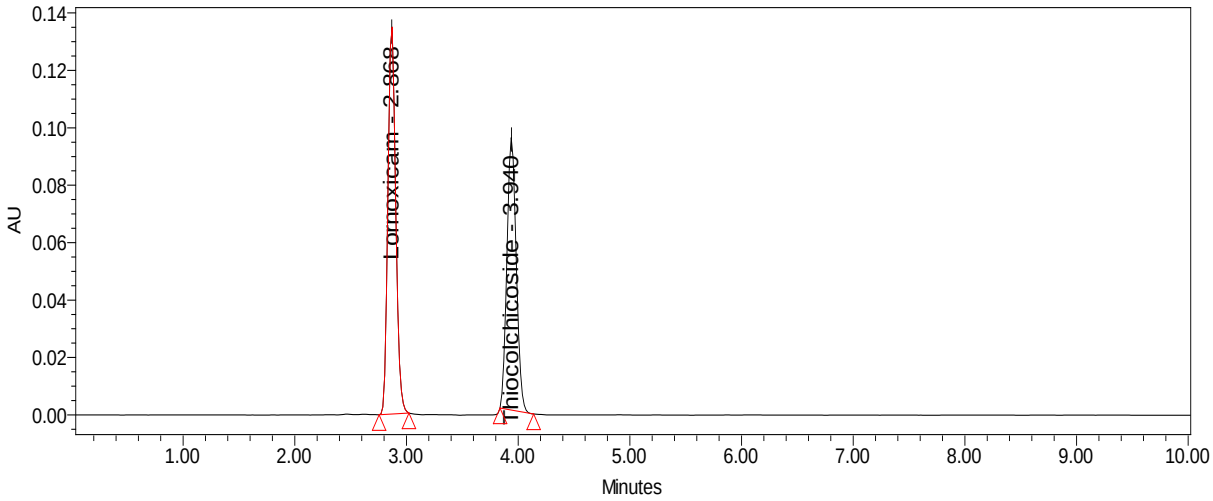
Inference: System suitability Chromatogram for standard – 1



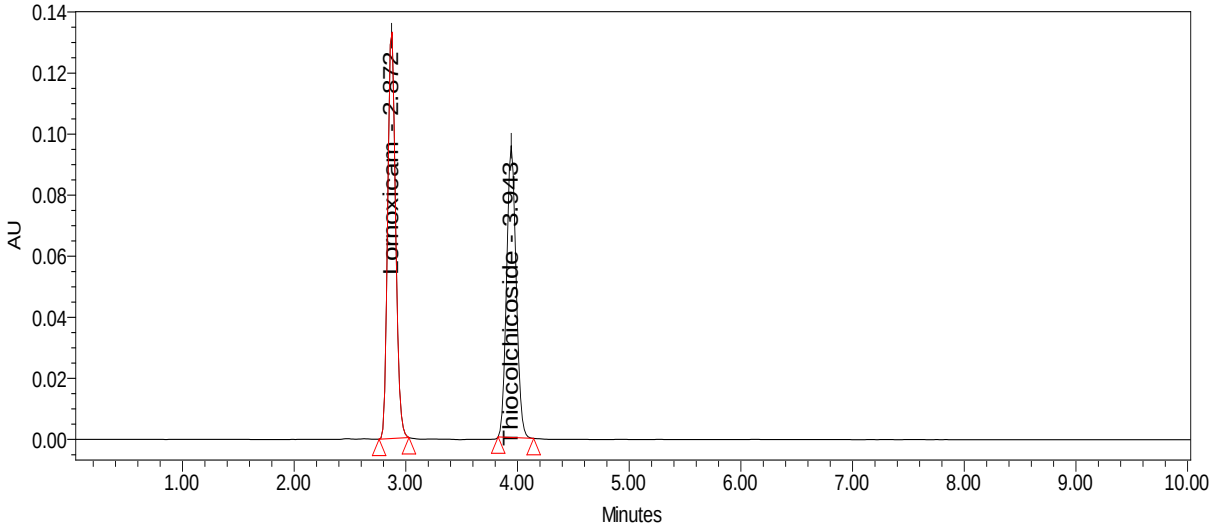
Inference: System suitability Chromatogram for standard – 2



Inference: System suitability Chromatogram for standard - 3



Inference: System suitability Chromatogram for standard - 4



Inference: System suitability Chromatogram for standard – 5

6.3.2: SPECIFICITY

Lornoxicam and Thiocolchicoside

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

ACCEPTENCE CRITERIA

Chromatograms of standard and sample should be identical with near Retention time.

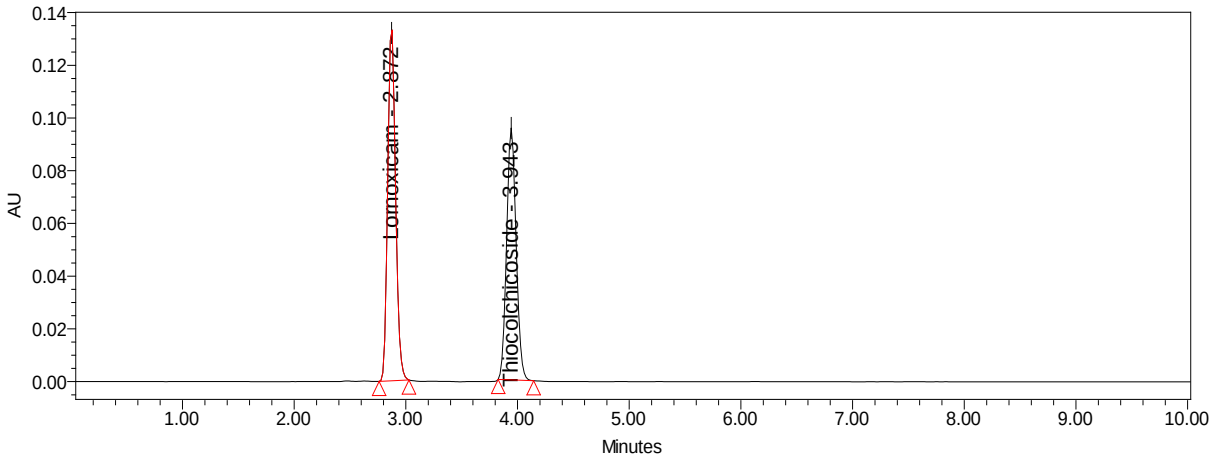


Fig 6: Chromatogram of standard

Inference: Got a peak for standard at an Rt of 2.875min for Lornoxicam and 3.943min for Thiocolchicoside

OBSERVATION

The chromatograms of Standard and Sample were same identical with same retention time. As shown in fig: 6.

6.3.3 LINEARITY

A Series of solutions are prepared using Lornoxicam and Thiocolchicoside working standards at concentration levels from 25ppm to 150 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

ACCEPTANCE CRITERIA

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

(i) Data of Linearity Lornoxicam and Thiocolchicoside

Concentration (ppm)	Average Area	Statistical Analysis of Lornoxicam		Average Area	Statistical analysis of Thiocolchicoside	
0	0	Slope	5140	0	Slope	18600
20	102965	y-Intercept	114.7	372546	y-intersept	276.2
30	154371	Correlation Coefficient	1	558296	Correlation Coefficient	1
40	205856			744400		

50	257167			930308		
60	308577			1116282		
70	359903			1302046		
80	411306			1488277		

TABLE6.3.3

OBSERVATION

The linear fit of the system was illustrated graphically. The results are presented in table6.

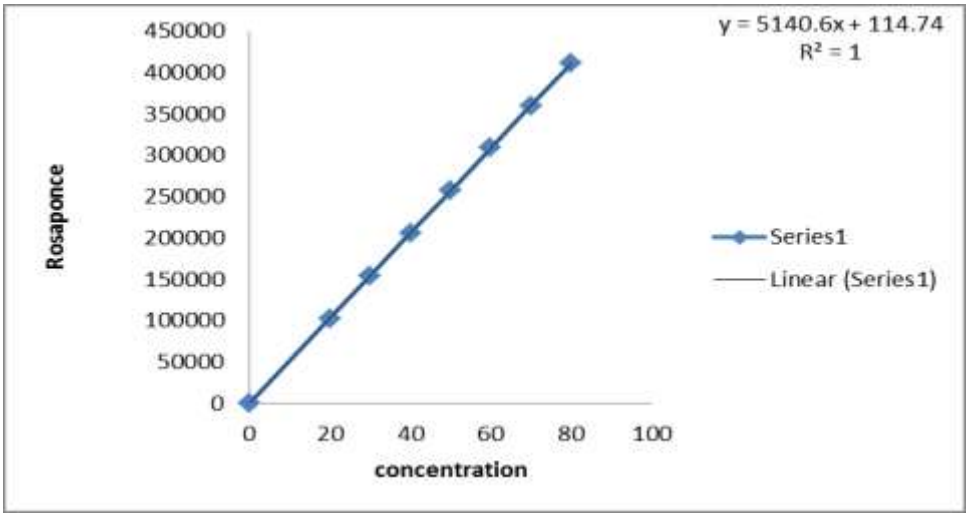


Fig: 41(a) Linearity Plot (Concentration Vs Response) of Lornoxicam

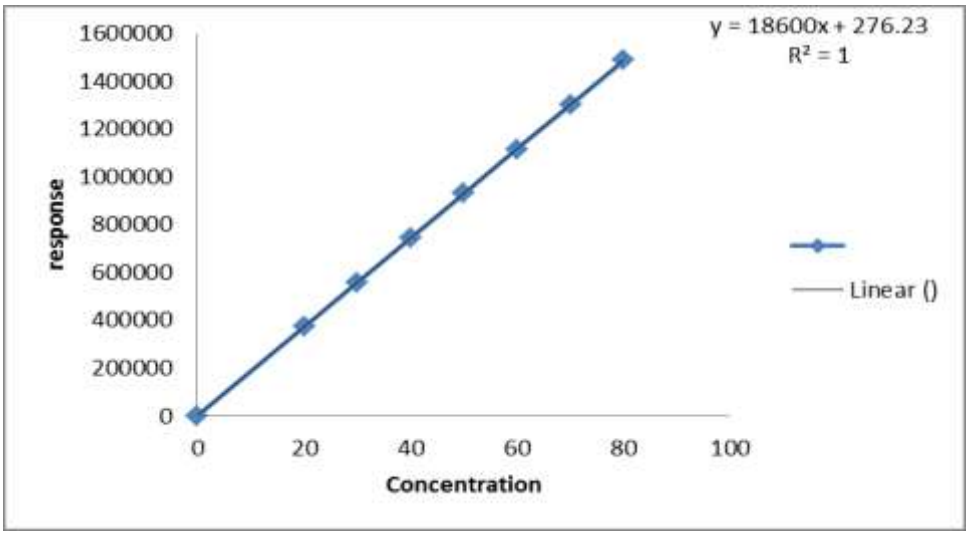


Fig: 41(b) Linearity Plot (Concentration Vs Response) of Thiocolchicoside

4: PRECISION

Repeatability

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

ACCEPTANCE CRITERIA

The % relative standard deviation of individual Lornoxicam and Thiocolchicoside, from the six units should be not more than 2.0%.

The individual assays of Lornoxicam and Thiocolchicoside should be not less than 98% and not more than 102.0%.

(a) System precision

TABLE-2(i): Data of Repeatability (System precision) for Lornoxicam and thiocolchicosid

	injection	Peak area of Lornoxicam	%Assay	Peak area of Thiocolchicoside	%Assay
Concentration 40ppm	1	205625	99.95	734360	98.66
	2	206225	100.24	739098	99.30
	3	205840	100.06	755696	101.53
	4	204283	99.30	748289	100.53
	5	205735	100.00	744147	99.98
	Mean	205541.6	99.91	744318	100.00
Statistical Analysis	SD	739.0046	0.35819	8241.164	1.107678
	%RSD	0.35	0.35	1.1	1.10

OBSERVATION

Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

(b)Method precision

TABLE-3(i): Data of Repeatability (Method precision) for Lornoxicam andthiocolchicoside

	injection	Peak area of Lornoxicam	%Assay	Peak area of Thiocolchicoside	%Assay
Concentration 40ppm	1	202110	98.6	733495	98.55
	2	203700	99.02	735992	98.88
	3	201851	98.12	739828	99.40
	4	202255	98.31	739098	99.30
	5	203283	98.81	748289	100.53
	6	202349	98.36	731322	98.28
	MEAN	202687.6	98.48	738004	99.278
Statistical Analysis	SD	771.5483	0.352647	5988.879	0.827236
	%RSD	0.38	0.35	0.81	0.83

OBSERVATION

Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

.5 Intermediate precision

A study was conducted by two analysts as per test method.

ACCEPTENCE CRITERIA

The individual assays of Lornoxicam and Thiocolchicoside should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Table4: (i) Data of Intermediate precision (Analyst 2) for Lornoxicam and Thiocolchicoside

	injection	Peak area of Lornoxicam	%Assay	Peak area of Thiocolchicoside	%Assay
Concentration 40ppm	1	205267	99.78	736792	99.99
	2	205625	99.95	734360	99.66
	3	205840	100.00	755696	101.53
	4	202735	98.55	744147	99.98
	5	208991	101.50	744127	99.97
	6	208543	101.37	752525	101.10
	MEAN	206333.5	100.19	744607.8	100.37
Statistical Analysis	SD	2572.599	1.100898	8392.59	0.753536
	%RSD	1.24	1.09	1.1	0.75

TABLE6.3.5

OBSERVATION

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 4

6 ACCURACY (RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Lornoxicam and Thiocolchicoside into each volumetric flask for each spike level to get the concentration of Lornoxicam and Thiocolchicoside equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Lornoxicam and Thiocolchicoside were calculated.

ACCEPTANCE CRITERIA

The mean % recovery of the Lornoxicam and Thiocolchicoside at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

OBSERVATION

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy. Refer table: 5

Data of Accuracy for Lornoxicam and Thiocolchicoside

	Lornoxicam					Thiocolchicoside			
Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**		**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection1	20	20.15	100.75	MEAN	99.69333	20.40	100.22	MEAN	100.06
50% Injection2	20	19.86	99.31			19.97	98.85		
50% Injection3	20	19.80	99.02	%RSD	0.92	20.02	100.11	%RSD	0.18
100% Injection1	40	39.88	99.70	MEAN	99.83333	40.01	100.02	MAEN	100.04
100% Injection2	40	40.12	100.30			40.05	100.14		
100% Injection3	40	39.80	99.50	%RSD	0.41	39.98	99.96	%RSD	0.091
150% Injection1	60	100.21	100.21	MEAN	99.97333	60.08	100.14	MEAN	100.02
150% Injection2	60	99.61	99.61			59.97	99.96		
150% Injection3	60	100.10	100.10	%RSD	0.31	59.98	99.98	%RSD	0.09

TABLE6.3.6

7 Ruggedness

a) System to System variability

ACCEPTANCE CRITERIA

The % relative standard deviation of Lornoxicam and Thiocolchicoside from the six sample preparations should be not more than 2.0%

The % assay of Lornoxicam and Thiocolchicoside should be between 98.0%-102.0%.

b) column to column variability

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analysed as per test method

ACCEPTANCE CRITERIA

The %RSD of Lornoxicam and Thiocolchicoside tablets should be NMT2.0%. The %assay of Lornoxicam and Thiocolchicoside should be between 98.0% and 102.0% for individual drugs.

Data of system to system variability (Lornoxicam and Thiocolchicoside System-2

	Lornoxicam		Thiocolchicoside	
S.NO	**Peak area**	**Assay % of Lornoxicam**	**Peak area**	**Assay % of Thiocolchicoside**
1	203625	99.98	734360	98.65
2	202225	99.30	734098	98.63
3	202840	98.60	735696	98.86
4	204283	99.30	733289	98.52
5	202735	98.55	734147	98.63
6	203110	98.73	733495	98.55
7	203136.3	99.07667	734180.8	98.64
8	0.35	0.56	0.11	0.12

TABLE6.3.7

OBSERVATION

The % RSD was found within the limit

The results obtained by comparing with both two types were within limit. Refer tables: 3 &9

8 Robustness

a) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Lornoxicam and Thiocolchicoside and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

ACCEPTANCE CRITERIA

The Tailing Factor of Lornoxicam and Thiocolchicoside standards should be NMT 2.0 for Variation in Flow.

b) Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc.

Similarly sample solution was chromatographed at 25ºC temperature. Lornoxicam and Thiocolchicoside were resolved from all other peaks and the retention times were comparable with those

ACCEPTANCE CRITERIA

The Tailing Factor of Lornoxicam and Thiocolchicoside standard and sample solutions should be NMT 2.0 for Variation in temperature.

TABLE: 10(i) Data for Effect of variation in flow rate (Lornoxicam)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	273707	1.362089	**Flow 1.0 ml**	206349	1.280574	**Flow 1.2 ml**	166195	1.285372
	273211	1.352617		205267	1.279932		165885	1.299385
	273948	1.376926		205625	1.261721		166303	1.308063
	273465	1.345752		205840	1.276089		167243	1.274662
	273862	1.374925		205735	1.250640		165762	1.267630
Avg	273638.6	1.362462	**Avg**	205763.2	1.269791	**Avg**	166277.6	1.287022
SD	301.369	0.013609	**SD**	392.1635	0.01314	**SD**	582.9758	0.016786
%RSD	0.11	0.99	**%RSD**	0.19	1.03	**%RSD**	0.35	1.3

TABLE6.3.8

OBSERVATION

The tailing factor for Lornoxicam and Thiocolchicoside was found to be within the limits. As shown in table 10.

(ii) Data for Effect of variation in flow rate (Thiocolchicoside)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1120286	1.322089	**Flow 1.0 ml**	734322	1.604878	**Flow 1.2 ml**	602077	1.285372
	1119282	1.331920		735792	1.584354		601854	1.319385
	1121337	1.296438		734360	1.543805		602403	1.292055

	1120456	1.315454		735696	1.568590		603421	1.304561
	1120765	1.326551		733147	1.559986		602465	1.294621
Avg	1120425	1.31849	**Avg**	734663.4	1.572323	**Avg**	602444	1.299199
SD	754.0018	0.013728	**SD**	1100.917	0.023367	**SD**	599.8833	0.013223
%RSD	0.06	1.04	**%RSD**	0.14	1.48	**%RSD**	0.09	1.01

TABLE6.3.8

OBSERVATION

The tailing factor for Lornoxicam and Thiocolchicoside

x is found to be within the limits. As shown in table 11.

8LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

Lornoxicam: From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3\times867.0705}{5140} = 0.56$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10\times867.0705}{5140} = 1.69$$

Thiocolchicoside

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3\times3244.904}{18600} = 0.57$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10\times3244.904}{18600} = 1.74$$

FORCED DEGRADATION STUDIES

Forced degradation studies were performed to demonstrate the optimized method is stability indicating. To prove the method which can be able to measure accurately active pharmaceutical ingredient in presence of degradants which are expected to be formed during different types of degradations applied to the drug sample.

For forced degradation analysis, aliquots of stock were separately treated with 1ml of 2N HCl (Acid stability), 1ml of 2N NaOH (Alkaline stability), 1ml of 20% H2O2 (Oxidative degradation), exposure of sample drug solution at 105ºC for 6 hrs (dry heat degradation), photo stability degradation (exposure of drug at 200 watt hours/m2) and neutral degradation (refluxing with water at 60ºC for 6 hours. Stability of these samples was compared with fresh sample on the day of analysis. The HPLC chromatograms of degraded products show no interference at the respective analyte peaks and the individual analytes peak purity values found to be within the acceptable limits, hence the method was specific and stability indicating. The chromatograms were shown in figures 22 to 27 and the results were shown in Table-8 (8A, 8B, 8C). The detailed degradation for each condition is as follows:

Oxidation

To sample stock solution of Lornoxicam, Thiocolchicoside, 1 ml of 20% hydrogen peroxide (H2O2) was added. The solution was kept for 30 min at 600C and cooled to room temperature and finally made up to volume with diluent. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To sample stock solution of Lornoxicam, Thiocolchicoside, 1mL of 2N Hydrochloric acid was added and refluxed for 30mins at 600C and cooled to room temperature and neutralized with 1 mL of 2N sodium hydroxide solution and finally made up to volume with diluent. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To sample stock solution of Lornoxicam, Thiocolchicoside, 1mL of 2N sodium hydroxide solution was added and refluxed for 30mins at 600C and cooled to room temperature and neutralized with 1 mL of 2N Hydrochloric acid solution and finally made up to volume with diluent. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The sample stock solution was placed in oven at 1050C for 6 hours to study dry heat degradation and after dry heat cooled to room temperature. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the sample stock solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the sample stock solution in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain 60µg/ml, 120µg/ml and 10µg/ml of all components and 10 µl of sample solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Frorce degradation studies result for lornoxicam

Sr. No	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Controlled sample	100.1	--	0.301	1.348	No
2	Acid Degradation	96.0	4.1	0.336	0.437	No
3	Base Degradation	97.1	3.0	0.585	1.092	No

4	Peroxide Degradation	94.4	5.7	1.604	2.194	No
5	Thermal Degradation	98.6	1.5	0.259	0.290	No
6	UV Degradation	99.5	0.6	0.308	0.329	No
7	Water Degradation	98.8	1.3	0.229	0.280	No

Table-8C: Forced Degradation study results for Thiocolchicoside

S. No	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Controlled Sample	99.9	---	0.121	0.395	No
2	Acid Degradation	95.5	4.4	0.379	0.416	No
3	Base Degradation	96.4	3.5	0.759	1.187	No
4	Peroxide Degradation	91.6	8.3	1.607	2.177	No
5	Thermal Degradation	97.9	2.0	0.381	0.425	No
6	UV Degradation	98.7	1.2	0.411	0.481	No
7	Water Degradation	98.5	1.4	0.275	0.421	No

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 363nm for Lornoxicam and 256nm for Lornoxicam.Common wavelength will be 256nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertrsil C_{18}, BDS 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 45:55 Acetonitrile: water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.56 for Lornoxicam and 0.57 for Losertan Thiocolchicoside Linearity study was, correlation coefficient and curve fitting was found to be. The analytical

method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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