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Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form

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Abstract

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Lornoxicam and Thiocolchicoside from tablets by reverse phase C18 column (Inertsil ODS 3V C-18, 250 x 4.6 mm, 5 µ). The sample was analyzed using Buffer(5.7606 gm Ammonium Dihydrogen Phosphate in 2000mL of milli-Q water, adjust pH 7.3 with Tri Ethyl Amine): Methanol in the ratio of 45:55, as a mobile phase at a flow rate of 1.5 mL min^{-1} and detection at 290 nm. The retention time for Lornoxicam and Thiocolchicoside was found to be 9.40 and 2.96 min respectively. The method can be used for estimation of combination of these drugs in tablets. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 0.24 – 120 µg mL^{-1} (r^2=0.9999) for Lornoxicam and 0.235 – 120 µg mL^{-1} (r^2=0.9999) for Thiococlchicoside and recoveries from tablets were between 100 and 102%. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

Keywords: Lornoxicam, Thiocolchicoside, Validation.

Introduction

Lornoxicam (Molecular Formula: $C_{13}H_{10}C_lN_3O_4S_2$, Molecular Weight: 371.82 $gmol^{-1}$) belongs to Non-steroidal anti-inflammatory drug (NSAID) category [1]. Lornoxicam is a compound in the same chemical class as piroxicam, meloxicam and tenoxicam, with potent anti- inflammatory, antipyretic and analgesic activity [2]. It works by blocking the action of Cyclooxygenase which is

responsible for the production of Prostaglandin in the body. Lornoxicam is chemically 6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno-[2, 3-e]-1, 2-thiazine-3-carboxamide-1, 1-dioxide (Fig.1) [1].

Thiocolchicoside (Molecular Formula: $C_{27}H_{33}NO_{10}S$, Molecular Weight: 563.618 $gmol^{-1}$) is a Centrally Acting Muscle Relaxant. It is used in the treatment of Painful Muscle Spasm [3]. Thiococlchicoside is chemically N-[3-(β-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylthio)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide (Fig. 2) [4].

Literature review revealed that, both Lornoxicam and Thiocolchicoside are not official in any pharmacopoeia. On detailed literature survey, it was found that only few selected RP-HPLC, LC and LC/MS/MS methods were reported for estimation of Lornoxicam [5, 6, 7, 8, 9, 10] and Thiocolchicoside [11, 12, 13] in their individual dosage form and plasma samples. These methods are not so specific for simultaneous estimation of Lornoxicam and Thiocolchicoside in their combined dosage form. So, this is the first report of RP-HPLC method for the estimation of Lornoxicam and Thiocolchicoside in combined dosage form. The method was validated as per ICH guidelines [14].

Fig.1: Structure of Lornoxicam

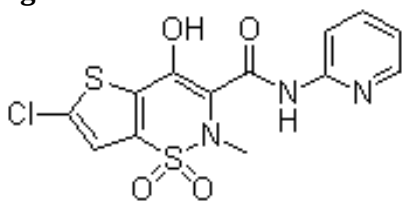
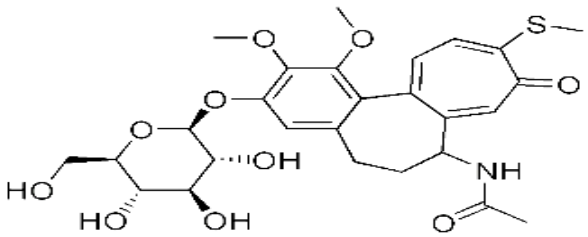


Fig. 2: Structure of Thiocolchicoside



Materials and Methods

Experimental

Lornoxicam, Active Pharmaceutical Ingredient (API) and working standard was supplied by Glenmark Generics Limited. Thiocolchicoside, Active Pharmaceutical Ingredient (API) and working standard was supplied by Alchem International Limited. Combination drug product of Lornoxicam and Thiocolchicoside was provide by Zydus Cadila Healthcare Limited (Ahmedabad, India)

Chemicals and reagents used

Ammonium Dihydrogen Phosphate: Merck Specialities PVT Ltd.

Tri Ethyl Amine: Spectrochem PVT Ltd., Mumbai.
Methanol: For HPLC, Spectrochem PVT. Ltd., India
Milli-Q Water: In-house production of company

Apparatus and equipments used
Hot air oven: Proto – Tech oven.
Analytical Balance: AX 205, METTLER TOLEDO.
pH Meter: Thermo Orion, model 420.
Sonicator: Oscar Ultra Sonics, OU- 72 (SPL).

Chromatographic condition
The HPLC system (Shimadzu Corporation, Japan), model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), low pressure gradient valve (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010C, Shimadzu), and CLASS – VP software version==SPI, binary pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and Chem station (software).

The chromatographic parameters

Column	: Inertsil ODS 3V C-18 (250 x 4.6 mm), 5 µ
Detector	: 290 nm
Injection Volume	: 10 µl
Flow Rate	: 1.5 ml/min
Temperature	: 30ºC
Run Time	: 15 minute
Mobile Phase	: Buffer: Methanol (45:55)
Diluent	: Mobile phase

Buffer preparation
Dissolve 5.7606 gm of Ammonium Dihydrogen Phosphate in 2000mL of milli-Q water and adjust pH of this buffer solution to 7.3 with Tri Ethyl Amine.

Preparation of standard solution
The standard stock solution was prepared by transferring 40 mg of Lornoxicam and 40 mg of Thiocolchicoside in a 200 mL volumetric flask. Add about 50mL of mobile phase and sonicate to dissolve. Now make volume up to mark with diluent. Dilute 10mL of this solution to 25mL with Mobile phase and mix. Final standard concentration of Lornoxicam and Thiocolchicoside is 80ppm.

Preparation of test solution
Accurately 20 intact tablets were weighed to determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to about 40mg of Lornoxicam was transferred into 200 mL volumetric flask. Then 100 mL diluent was added to flask and sonicated for 30 minutes with intermittent shaking. Make the volume up to mark with Mobile phase and mix. Solution was filtered through 0.45 µ HVLP millipore filter; collect the filtrate by discarding first

few ml of the filtrate. Dilute 10ml of this solution to 25.0mL with mobile phase and mix to obtain final concentration of 80ppm of both the drugs.

Results and Discussion

Literature review reveals that the published analytical methods for estimation of Lornoxicam and Thiocolchicoside in their formulation were not reported for the estimation in their combined dosage form.

Pure drugs chromatogram was run in different mobile phases containing methanol and different buffers in different ratios. Different columns (e.g. C8, C18, phenyle) with different dimensions were used. The retention time and tailing factor was calculated for each drugs and for each chromatogram. Finally Buffer(5.7606 gm Ammonium Dihydrogen Phosphate in 2000mL of milli-Q water, adjust pH 7.3 with Tri Ethyl Amine): Methanol as a mobile phase in the volume of ratio 45:55 v/v and Inertsil ODS 3V C-18 (250 x 4.6 mm), 5 µ analytical column was selected which gave a sharp and symmetrical peak with minimum tailing. Inertsil ODS 3V C-18 (250 x 4.6 mm), 5 µ column has an embedded polar groups. It has high carbon loads, which provide high peak purity and more retention to polar drugs.

Calibration graph was found to be linear at range 0.24 – 120 µg $mL^{-1}$and 0.235 – 120 µg mL^{-1} for Lornoxicam and Thiocolchicoside respectively. Six different concentrations of a mixture of two drugs in the range given above were prepared and 10 µL of each solution injected in HPLC. Regression analysis of the calibration data for Lornoxicam and Thiocolchicoside showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations: $y = m x + c$ was found to $y = 29738.77 x + 2700.34$ and $y = 18227.53 x -3713.71$ for Lornoxicam and Thiocolchicoside respectively.

The correlation of coefficient (r2) obtained was found to be 0.9999 for both the drugs. It was observed that the concentration range showed a good relationship. The limit of detection for both the drugs was found to be 0.8 µg mL^{-1} and the limit of quantification was found to be 0.24 µg $mL^{-1}$and 0.235 µg mL^{-1} for Lornoxicam and Thiocolchicoside respectively. It proves the sensitivity of method for the drugs. The % assay or average amount of Lornoxicam and Thiocolchicoside was found to be 96.3% and 100.2% respectively in each tablet. The average % recovery for Lornoxicam and Thiocolchicoside was found to be 100.83% and 100.53% respectively which shows that method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

The proposed method was applied for routine analysis of solid dosage form. The method was found linear, robust, specific and reproducible.

Method Validation

Validation was done as per ICH guideline Q2 (R1) [14]. The developed method was validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness and solution stability.

System suitability and system precision

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

Table 1: System suitability and system precision

Compound	Retention Time	n	R	T	k'
Thiocolchicoside	2.95 ± 0.0048	2943.34	0.00	1.11	28.65
Lornoxicam	9.39 ± 0.0074	6368.75	18.55	1.20	93.08

n= Theoretical plates; R= Resolution; T= Asymmetry, k'= Capacity Factor.

Linearity

To achieve linearity and range, stock solution containing 0.200 mg mL^{-1} Lornoxicam and 0.200 mg mL^{-1} Thiocolchicoside were diluted to yield solution in the concentration range of 0.24 – 120 µg mL^{-1} and 0.235 – 120 µg mL^{-1} for Lornoxicam and Thiocolchicoside, respectively. The solutions were prepared in triplicate and analyzed by using 10 µl into HPLC. The results related to linearity are presented in Table 2.

Table 2: Results of Linearity

Parameters	Lornoxicam	Thiocolchicoside
Linearity Range	0.24 – 120 µg mL^{-1}	0.235 – 120 µg mL^{-1}
Linearity equation	y = 29738.77 x + 2700.34	y = 18227.53 x -3713.71
Correlation coefficient	0.9999	0.9999
LOD	0.8 µg mL^{-1}	0.8 µg mL^{-1}
LOQ	0.24 µg mL^{-1}	0.23 µg mL^{-1}

LOD= Limit of detection, LOQ= Limit of quantification, RT=Retention time.

Fig. 3: Linearity and calibration curve of Lornoxicam

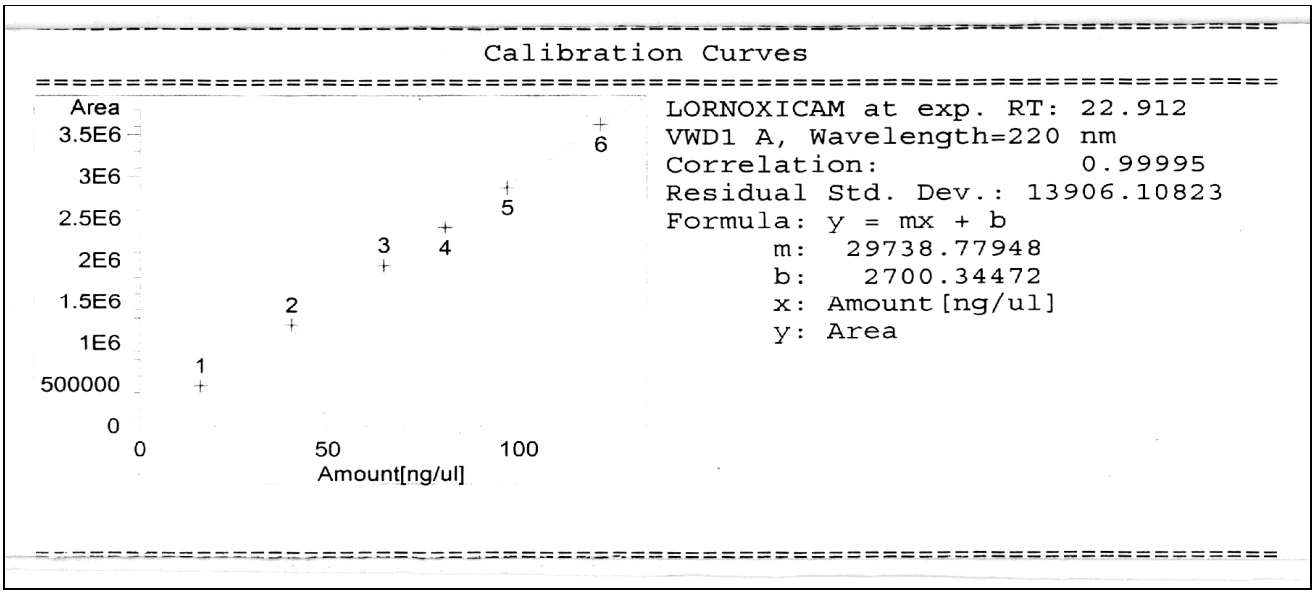
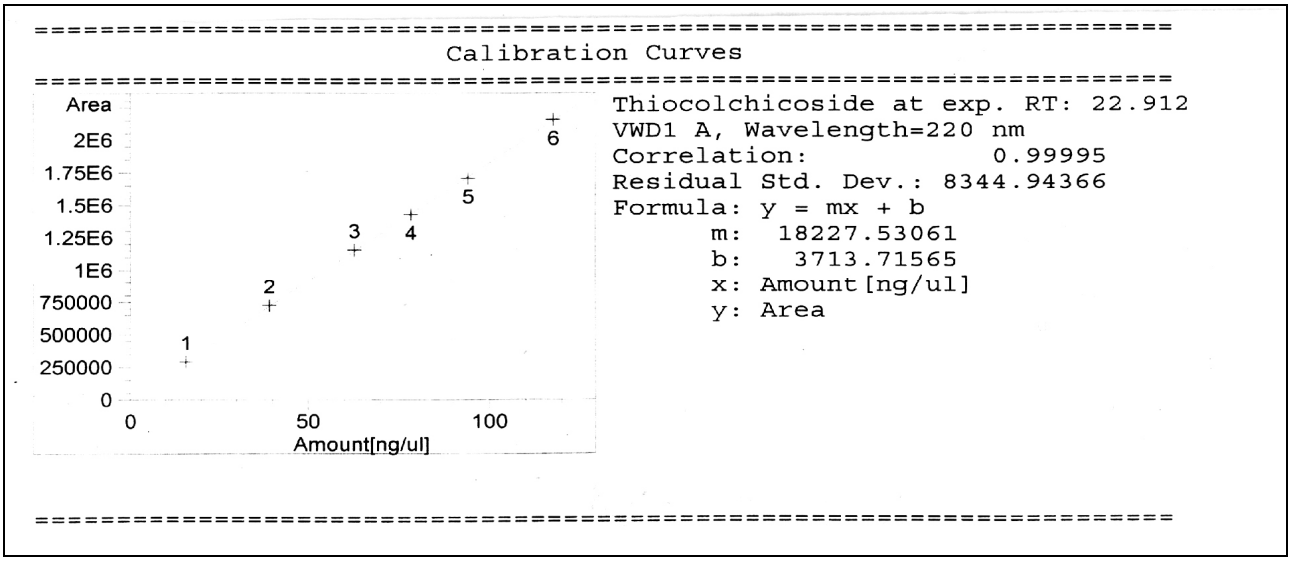


Fig. 4: Linearity and calibration curve of Thiocolchicoside



Precision

The method precision was done by preparing six different sample preparations by one analyst under the same conditions. The results are presented in Table 3. The results obtained were within 2% RSD.

Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showing ruggedness of developed analytical method. The results are presented in Table 3.

Table 3: Results of Method Precision and Ruggedness

Parameters	Lornoxicam		Thiocolchicoside	
	%Assay Mean ± SD (n=6)	% RSD	%Assay Mean ± SD (n=6)	% RSD
Method Precision	95.95 ± 0.45	0.5	100.58 ± 0.41	0.5
Ruggedness	96.28 ± 0.177	0.2	102.18 ± 0.15	0.2

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three different levels 50%, 100% and 150% of the target concentration in triplicate. The results are presented in Table 4.

Table 4: Results of Accuracy study

For Lornoxicam					
Level	Amount of Drug added (mg)	Amount of Drug recovered (mg)	Recovery (%)	Mean ± SD (%), n=3	% RSD
50 %	20.20	20.61	102.0	101.76 ± 0.169	0.2
	20.30	20.63	101.6		
	20.30	20.65	101.7		
100 %	40.40	40.40	100.0	100.23 ± 0.16	0.2
	40.40	40.55	100.4		
	40.30	40.44	100.3		
150 %	60.40	60.51	100.2	100.5 ± 0.21	0.3
	60.10	60.49	100.6		
	60.10	60.50	100.7		
For Thiocolchicoside					
Level	Amount of Drug added (mg)	Amount of Drug recovered (mg)	Recovery (%)	Mean ± SD (%), n=3	% RSD
50 %	19.96	20.32	101.8	101.26 ± 0.41	0.5
	19.96	20.20	101.2		
	20.06	20.23	100.8		
100 %	39.52	39.84	100.8	100.3 ± 0.37	0.5
	39.82	39.90	100.2		
	39.92	39.88	99.9		
150 %	59.77	59.86	100.2	99.96 ± 0.16	0.2
	59.97	59.84	99.8		
	59.87	59.79	99.9		

Table 5: Results of Peak purity in Specificity study

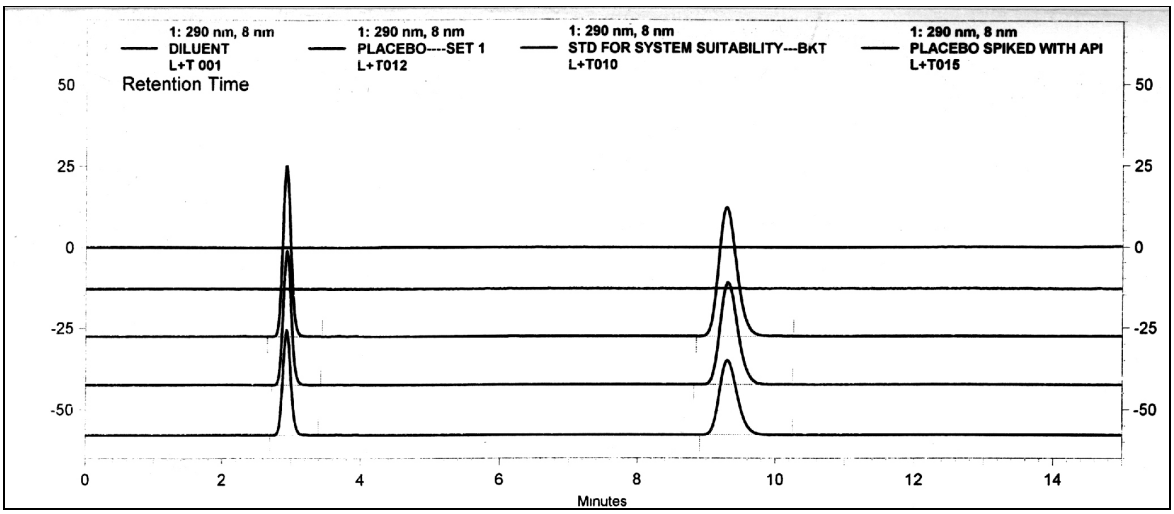
3 point purity		
	Lornoxicam	Thiocolchicoside
Standard Solution	0.9999	0.9999
Test Solution	1.0000	0.9999
Spiked Sample Solution	0.9999	0.9999

Specificity

Specificity of developed method was established by determining peak purity of active component in standard preparation, test preparation and spiked sample preparation using PDA detector. Placebo and blank preparation were also analysed for specificity. Results of peak purity in

specificity study are represented in Table 5. Typical overlay chromatograms of Diluent, Placebo, Standard, Placebo spiked with API and Test solution are shown in Fig.3.

Fig. 5: Overlay chromatogram of Diluent, Placebo, standard, placebo spiked with API and Test solution in specificity study



Robustness

Robustness of the method was carried out by deliberately made small change in the flow rate, pH, organic phase ratio and column oven temperature. Results are presented in Table 6.

Table 6: Results of Robustness study

Compound	% RSD (n=5)		
	Normal Condition	Changed Condition	
Temperature	**Normal**	**(-5°C)**	**(+5°C)**
Lornoxicam	1.2	0.9	0.9
Thiocolchicoside	1.7	0.8	1.3
pH	**Normal**	**(-0.2 unit)**	**(+0.2 unit)**
Lornoxicam	1.2	0.5	1.8
Thiocolchicoside	1.7	0.8	1.3
Flow Rate	**Normal**	**(-10%)**	**(+10%)**
Lornoxicam	1.2	1.4	0.7
Thiocolchicoside	1.7	1.2	1.2
Mobile phase ratio	***Normal***	**(-2%)**	**(+2%)**
Lornoxicam	1.2	1.7	0.3
Thiocolchicoside	1.7	1.1	1.4

Solution stability

Solution stability period for standard and sample preparation was determined by keeping the solution for 24 hours at room temperature. After 4, 8, 12, 16, 20, 24 hours the solutions were analysed. Results related to solution stability are summarized in Table 7. No significant changes

(<2%) were observed for the chromatographic responses for the solution analysed, relative to freshly prepared standard.

Table 7: Results of standard and sample solution stability

Time Hours	Standard Solution (% RSD)		Test Solution (% RSD)	
	Lornoxicam	Thiocolchicoside	Lornoxicam	Thiocolchicoside
4 hours	0.1	0.2	0.6	0.2
8 hours	0.7	0.7	0.0	-0.1
12 hours	0.1	0.1	1.4	1.3
16 hours	0.5	0.5	0.2	0.3
20 hours	0.0	0.0	0.5	0.8
24 hours	0.4	0.4	0.7	0.7

Assay

The developed validated method was successfully applied to the simultaneous estimation of Lornoxicam and Thiocolchicoside form. The results of the assay are 96.3% and 100.2% of Lornoxicam and Thiocolchicoside, respectively. The retention times of Lornoxicam and Thiocolchicoside are 9.40 and 2.96 min, respectively. The results of the assay indicated that the method is specific for the analysis of both Lornoxicam and Thiocolchicoside.

Conclusion

The high performance liquid chromatographic method for the determination of Lornoxicam and Thiocolchicoside from their fixed combined dosage form was found to be simple, rapid, accurate and precise; hence it can be used for the routine analysis in quality control department. The method was validated as per ICH guidelines**.**

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