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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND LORNOXICAM IN TABLET DOSAGE FORM

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ABSTRACT

A simple and cost effective reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Thiocolchicoside and Lornoxicam in tablet dosage form. In this mehod, Chromatography was carried out on an Hypersil BDS, C-18 column (4.6 x 250 mm, 5 µ particle size) with a mobile phase composed of acetonitrile -phosphate buffer pH-3 in Gradient technique at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 260 nm. Validation parameters were studied as per ICH guidelines. The retention times for Thiocolchicoside and Lornoxicam are 2.3 min and 6.1 min, respectively. The linearity range for Thiocolchicoside and Lornoxicam are 10-60 µg/mL and 20-120 µg/Ml, respectively. The percentage recoveries of Thiocolchicoside and Lornoxicam are 99.96% and 100.65%, respectively. The correlation coefficients for both components are close to 1. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

Key words: RP-HPLC, Thiocolchicoside, Lornoxicam, Simultaneous estimation.

INTRODUCTION

Thiocolchicoside, is a synthetic sulphur derivative of colchicoside, a naturally occurring glucosidecontained in the Colchicum autumnale plant. Thiocolchicoside has a selective affinity for g-amino-butyric acid (GABA) receptors and acts on themuscular contracture by activating the GABA-nergicinhibitory pathways thereby acting as a potent muscle relaxant Thiocolchicoside (Muscoril, Myoril, Neoflax) is a muscle relaxantwith anti-inflammatory and analgesic effects. It acts as a competitive GABA. A receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic

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acetylcholine receptors to a much lesser extent. It has powerful convulsant activity and should not be used in seizure-prone individuals[1-2]. Lornoxicam is a member of the oxicam group of nonsteroidal antiinflammatory drugs, producing analgesic and antipyretic effects through the non-selective inhibition of cyclo-oxygenase-1 and -2. Besides its inhibitory effect on COX-1 and COX-2 peripheral receptors, is also increases endogenous dinorphin and beta-endorphin levels promoting central analgesic and anti-inflammatory effects. Lornoxicam is completely absorbed after oral administration, reaching peak plasma concentrations of 280 mg/L within 2.5 hrs aftera 4 mg dose. After intramuscular injection maximum plasma concentrations are achieved after approximately 20-25 mins. Lornoxicam is extensively metabolished in liver by cytochrome P4502DC9 to inactive metabolite 5'-hydroxy-lornoxicam. The mean elimination half life is 3 to 4 hrs. There is plenty of literature available on the effect of lornoxicam on chronic and acute pain management[3]. Various HPLC assay methods are reported in the literature for the estimation of Thiocolchicoside and Lornoxicam individually and in-combination with other drugs[4-9]. According to literature survey there is no official method for the simultaneous estimation of Thiocolchicoside and Lornoxicam by RP-HPLC in combined tablet dosage forms. In this study, an HPLC method was optimized and validated for simultaneous estimation and validation of Thiocolchicoside and Lornoxicam in tablet formulation in accordance with the ICH guidelines[10-11].

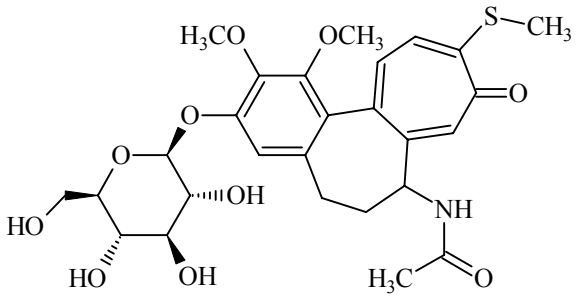

Fig. 1: Structure of Thiocolchicoside

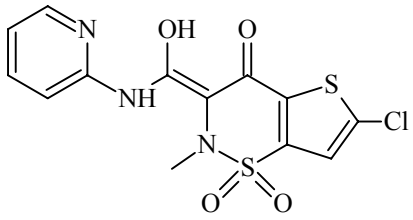

Fig. 2: Structure of Lornoxicam

EXPERIMENTAL

Materials and methods

Instrumentation

Chromatography was performed with water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower 2 (Waters) chromatography software.

Reagents and chemicals

Pharmaceutically pure sample of Thiocolchicoside and Lornoxicam were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck Chemical division, Mumbai and Commercial tablets of Thiocolchicoside (4 mg), and Lornoxicam (8 mg); FLEXISPAZ was procured from the local drug market.

Chromatographic condition

The isocratic mobile phase consisted of acetonitrile: phosphate buffer (pH-3.1) in the gradient ratio at a flow rate of 1.0 mL min^{-1}. Hypersil BDS C-18 column (4.6 x 250 mm, 5 µ particle size) was used as the stationary phase. Although the Thiocolchicoside and Lornoxicam have different λ_{max}, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 382 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 40 mg of Thiocolchicoside drug and 80 mg of Lornoxicam into a clean and dry 100 mL volumetric flask, 70 mL of diluent was added, sonicated for 5 mins and volume was made upto 100 mL with diluent to get stock solution.

Preparation of working standard solutions

Aliquot of 0.25 mL, 0.5 mL, 0.75 mL, 1.0 mL, 1.25 mL and 1.5 mL were pipetted out from stock into 10 mL volumetric flask separately and volume was made upto 10 mL

with diluent. This gives the solutions of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL and 60 µg/mL, respectively for Thiocolchicoside, and 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL and 120 µg/mL, respectively for Lornoxicam.

Sample preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 50 mL volumetric flask, 30 mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1 mL was pipeted out into a 10 mL volumetric flask and made upto 10 mL with diluent.

Method validation

Parameters like system suitability, linearity, accuracy, precision, LOD, LOQ, solution stability and robustness were estimated as per ICH guidelines.

RESULTS AND DISCUSSION

Method development

Various mobile phase combinations were tried initially to separate thiocolchicoside and Lornoxicam on C18 column. Preliminary experiments indicated that using different concentrations of acetonitrile or methanol with water was not able to separate the peaks of Thiocolchicoside and Lornoxicam or to obtain suitable retention and peak shape.

In order to achieve acceptable peak shapes and perform the separation on a suitable run time, various buffer systems were tried systematically. Thereafter, buffer: acetonitrile were taken in gradient: T (min)/%buffer/% acetonitrile: 0.01/82/18, 2.5/82/18, 6/15/85, 7/15/85 and 10/82/18 with flow rate of 1.0 mL/min was employed. Hypersil BDS C-18 column (4.6 x 250 mm, 5 µ particle size) was used as the stationary phase was selected to improve resolution, short run time and the tailing of both peaks were reduced close to 1. To analyze both drugs detection were tried at various wavelengths from 215 nm to 400 nm. The wavelength at which both Thiocolchicoside and Lornoxicam showed maximum absorption at 382 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.3 min and 6.1 min for Thiocolchicoside and Lornoxicam, respectively. The chromatogram obtained was shown in the Fig. 3.

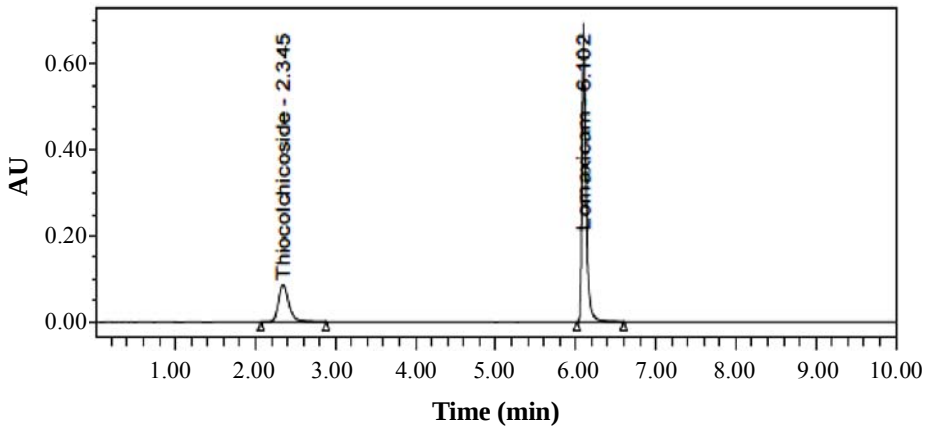

Fig. 3: Representative chromatogram of Thiocolchicoside and Lornoxicam

System suitability tests

To ensure the validity of the analytical procedure, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions of Thiocolchicoside and Lornoxicam were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time. The results obtained are shown in Table 1.

Table 1: System suitability of Thiocolchicoside and Lornoxicam

Parameters	Thiocolchicoside	Lornoxicam
No of theoretical plates	4772	71233
Tailing factor	1.28	1.4
Area	805145	2424867
% RSD	1.3	0.8

Linearity

The solutions for linearity were prepared at six concentration levels ranging from 25 to 150% of the target concentration. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentrations and the correlation coefficients, slopes and Y-intercepts of the calibration curve were determined. These results were summarized Figs. 4 and 5.

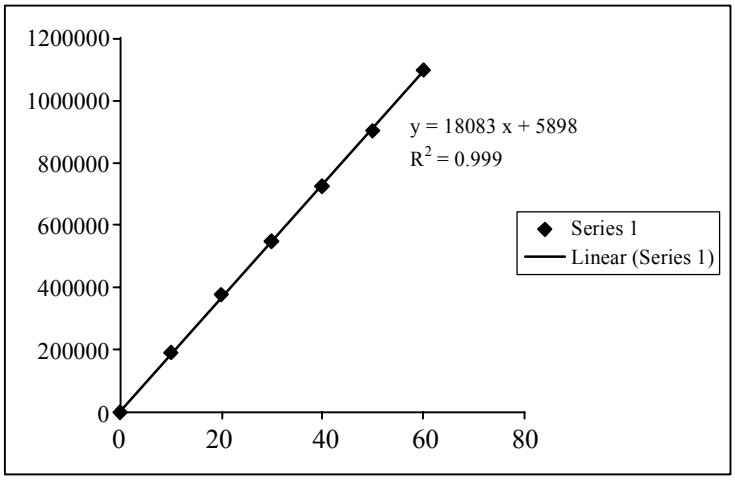

Fig. 4: Calibration curve for Thiocolchicoside

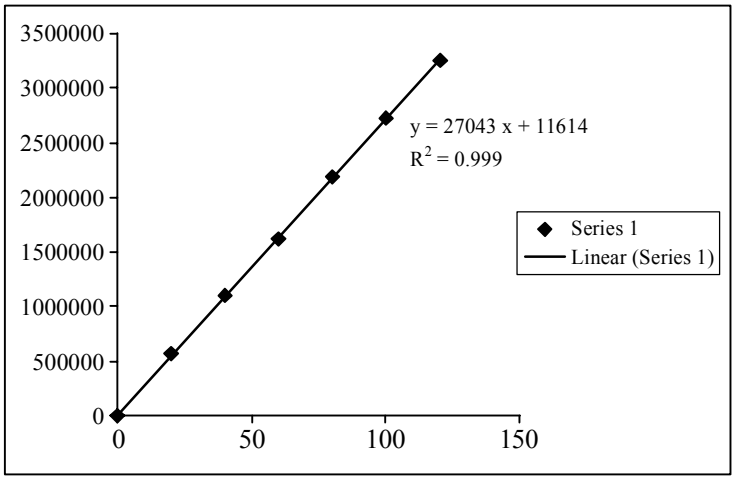

Fig. 5: Calibration curve for Lornoxicam

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Thiocolchicoside and Lornoxicam to which known amounts of standard Thiocolchicoside and Lornoxicam corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method. These results are summarized in Table 2.

Table 2: Results of recovery experiments of Thiocolchicoside and Lornoxicam

Preanalysed sample solution conc. (ug/mL)		Standard drug conc. (ug/mL)		% Recovered	
TCD	**LXM**	**TCD**	**LXM**	**TCD**	**LXM**
20	40	40	80	100.51	101.93
20	40	40	80	100.98	100.43
20	40	40	80	101.36	101.29
40	80	80	80	100.21	100.51
40	80	80	80	99.376	99.22
40	80	80	80	100.30	99.26
60	120	120	80	99.55	100.71
60	120	120	80	100.51	99.97
60	120	120	80	100.99	100.40
		Mean		100.42	100.41
		SD		0.655	0.874
		% RSD		0.65	0.87

Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of Thiocolchicoside and Lornoxicam. Determinations were performed on the same day as well as well as on consequent days. Hence no significant difference is observed in the precision results carried out on two consecutive days.

Robustness

The change was made in the ratio of column temperature was changed to 25 & 30^{o}C, flow rate was changed to 0.9 & 1.1 mL/min and pH of buffer was changed to 3.0 & 3.2. All the results were found within the limits.

Stability of sample solution

The sample solution injected after 24 hr did not show any appreciable change. Results are shown in Table 3.

Table 3: Stability data of Thiocolchicoside and Lornoxicam

Drug	% Assay at 0 hr	% Assay at 24 hr	% Deviation
TCD	99.65	101.59	1.94
LXM	99	100.03	1.03

LOD and LOQ

LOD and LOQ of Thiocolchicoside and Lornoxicam were determined by calibration curve method. Solutions of both Thiocolchicoside and Lornoxicam were prepared in the linear range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 × Syx)/b, LOQ = (10.0 × Syx)/b. Where Syx is residual variance due to regression; b is slope. LOD and LOQ for Thiocolchicoside were 0.40 and 0.123 µg/mL respectively and for Lornoxicam were 0.27 and 0.84 µg/mL, respectively.

Tablet analysis

Content of Thiocolchicoside and Lornoxicam found in the tablets by the proposed method are shown in Table 4 and Fig. 6.

Table 4: Results of HPLC analysis of tablets

Formulation	Label claim		Amount found		% Assay	
	TCD	LXM	TCD	LXM	TCD	LXM
FLEXISPAZ	4 mg	8 mg	4.01 mg	7.98 mg	99.85	100.6

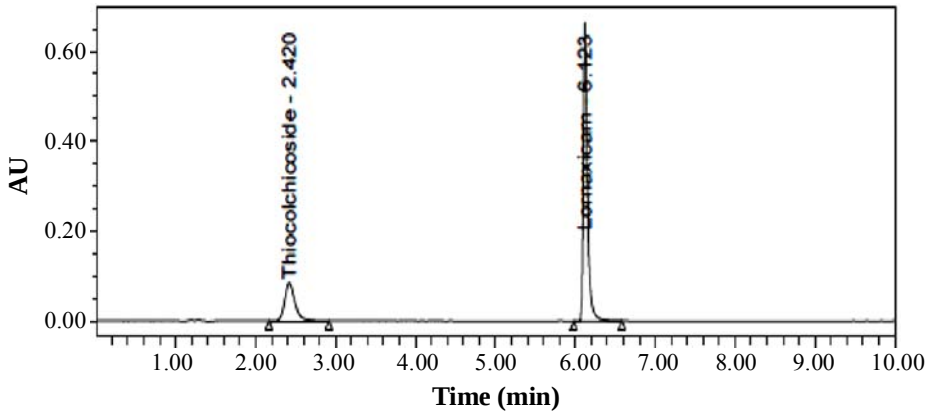

Fig. 6: Chromatogram of sample

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of Thiocolchicoside and Lornoxicam in tablet dosage form. The resolution between two peaks was always more than 2. The system suitability tests revealed that numbers of theoretical plates were above 2000 and tailing factor is less than 2. Thiocolchicoside and Lornoxicam showed a linearity of response between 10-60 µg/mL and 20-120 µg/mL. The regression value is 0.999 for both drugs and the response is linear. Repeatability and intermediate precision values were within the acceptable limits. This indicates that the method is precise. Selectivity experiment shows that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of the drugs. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 hours. Change in flow rate, temperature and mobile phase composition doesn't cause any significant change in results shows stability of the development method.The percentage RSD for precision is < 2, which confirms that method is sufficiently precise. The total run time required for the method is only 10 minutes for eluting both Thiocolchicoside and Lornoxicam. So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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REFERENCES

1. A. R. Umarkar, S. R. Bavaskar and P. N. Yewale, Int. J. Pharma. Bio. Sci., **1(3)**, 364-371 (2011).
2. F. Tüzün, H. Unalan, N. Oner, et al., Joint, Bone, Spine: Revue Du Rhumatisme, **70(5)**, 356-361 (2003).
3. G. Sushila, R. K. Srivastava, G. Rajesh and B. Garima, J. Drug Del Ther. **3(2)**, 145-148 (2013).
4. A. R. Umarkar, N. S. Rewatkar, M. S. Charde, R. M. Charde and A. V. Kasture, J. Pharm. Res., **4(5)**,1307-1308 (2011).
5. S. Kumar, A. Joshi, R. Thakur, A. K. Pathak and K. Shah, Acta Pol. Pharm. **68(6)**, 839-845 (2011).

6. K. R. Gupta and R. R. Joshi, Pharm. Sin., **1(2)**, 44-51 (2010).

7. S. R. Dhaneshwar, V. K. Bhusari and S. T. Patil, Int. J. Pharma. Bio. Sci., **2(2)**, 482-490 (2011).

8. E. Nemutlu, S. Demircan and S. Kır. Pharmazie, **60(6)**, 421-425 (2005).

9. Y. H. Kim, H. Y. Ji, E. S. Park, S. W. Chae and H. S. Lee, Arch. Pharm. Res., **30(7)**, 905-910 (2007).

10. International Conference on Harmonisation, Guidelines for the Photostability Testing of New Drug Substances and Products, Step 4, Q1B (1996).

11. International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology, in ICH Harmonized Tripartite Guidelines Q2 (R1) (2005).

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