www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Development and Validation of a RP-HPLC-PDA method for Simultaneous Determination of Lornoxicam and Thiocolchicoside in Pharmaceutical dosage form and its Application for Dissolution study

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ABSTRACT

A simple, selective, rapid, and precise RP-HPLC-PDA method has been developed for the simultaneous estimation of Lornoxicam (LOR) and Thiocolchicoside (THIO) in pharmaceutical dosage form by reverse phase liquid chromatography using Waters Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) column. The mobile phase consisting of methanol: THF: acetate buffer (60: 10: 30 v/v); pH adjusted to 5.5 with glacial acetic acid at a flow rate of 0.75 mL min^{-1} and column was maintained at 50 ^{0}C with detection at 382 nm. The retention time of Thiocolchicoside and Lornoxicam was 3.36 and 4.08 minutes, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness, limit of detection and limit of quantification. Linearity of Lornoxicam and Thiocolchicoside were in the range of 0.2 to 80 µg mL^{-1} and 0.1 to 40 µg mL^{-1}, respectively and its percentage recovery were found to be 100.37 % and 100.51 %, respectively. The proposed method is suitable for simultaneous determination of Lornoxicam and Thiocolchicoside in pharmaceutical dosage form. Method was successfully applied for dissolution study of tablet formulation.

Keywords: Lornoxicam; Thiocolchicoside; RP-HPLC-PDA; Method validation; Column Liquid Chromatography; Dissolution.

INTRODUCTION

Lornoxicam (LOR, 6-chloro-4-hydroxy-2-methyl- *N*-2-pyridyl-2H-thieno [2, 3-e]-1, 2-thiazine-3-carbox- amide-1, 1-dioxide) is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties [The Merck Index 13th, 2001]. LOR, belongs to the chemical class oxicams, this includes piroxicam, tenoxicam, and meloxicam [S. Radhofer-Welte et al., 1998]. Its principal mode of action is by inhibition of the enzyme cyclooxygenase and thus of prostaglandin synthesis from arachidonic acid. [Kiran R. Patil et al., 2009].

Thiocolchicoside is (THIO, (s)-N- [3-(B-D- glucopyran oxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10- (methylthio) -9-oxobenzo [a] heptalen-7yl] acetamide [Matrindale 3th, 2009; N.A. El-Ragehy et al., 2003]. Thiocolchicoside is a synthetic sulphur derivative of colchicoside. Thiocolchicoside has a selective affinity for γ-amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA-nergic inhibitory pathways thereby acting as a potent muscle relaxant [F.C.W. Sutherland et al., 2002].

Literature survey reveals that few HPLC and UV Spectroscopic methods are reported for the estimation of LOR and THIO individually as bulk and in pharmaceutical formulations [NEMUTLU E. et al., 2005; Young Hoon Kim et al., 2007; Devanand B. Shinde et al., 2009; Suwa, T. et al., 1993; Jadon R.S. et al., 2009; Artusi M et al., 2003]. Authors have developed Ratio Derivative and Absorption corrected method for its estimation in the combination in the same laboratory. The review of the literature revealed that there is no RP-HPLC method available for determination of this combination. Therefore aim of the present work was to develop simple, precise and accurate RP- HPLC-PDA method for simultaneous determination of LOR and THIO in pharmaceutical dosage form and application of the method for dissolution study. The method was validated according to ICH guidelines.

EXPERIMENTAL

Instrumentation

Waters HPLC system, Milford USA consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) and Kromasil C18 (250 mm × 4.6

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Received on: 14-05-2010
Revised on: 27-10-2010
Accepted on: 11-11-2010

mm, 5.0 µ) column. The column was supported with waters symmetry C18, (waters C18, 20 x 3.9 mm, 5 µ) guard column. The peak purity was checked with the photodiode array detector. A calibrated dissolution apparatus (USP II) was used with paddles for dissolution studies. Shimadzu analytical weighing balance - Model AUW220D and Equip-Tronics micro controller pH Meter Model EQ-621 was used for study.

Materials and Reagents

HPLC grade methanol and THF was purchased from Merck (Mumbai, India). Analytical reagent grade glacial acetic acid was purchased from Analab lab fine chemicals (Mumbai, India). Pure drug sample of LOR, % purity 98.80 and THIO, % purity 99.92 was kindly supplied as a gift sample by Glenmark pharmaceuticals Ltd. Baddi and Medley Pharmaceuticals Ltd. Baddi, respectively. These samples were used without further purification. Two tablet formulations (Lot 302 and 304) ,supplied by JPLC Pharma ltd., Jalgaon were used for analysis containing LOR 8 mg and THIO 4 mg per tablet.

Chromatographic Conditions

The isocratic elution with Methanol: THF: Acetate buffer (pH 5.5) (60: 10: 30 v/v) mobile phase at the flow rate of 0.75 mL min^{-1} was carried out. The run time was set at 5 min and temperature was maintained at 50 ^{0}C. The volume of injection was 20 µL, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. Detector signal was monitored at a wavelength of 382 nm.

Standard Solutions and Calibrations Graphs

Standard stock solution of LOR and THIO (100µg mL^{-1}) were prepared separately in methanol. To study the linearity range of each component, serial dilutions of LOR and THIO were made from 0.2 - 80 µg mL^{-1} and 0.1 - 40 µg mL^{-1}, respectively in mobile phase and injected on to the column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to the column. The system suitability test was performed from six replicate injections of mixed standard solution. A typical chromatogram obtained from a standard solution is shown in Fig.1.

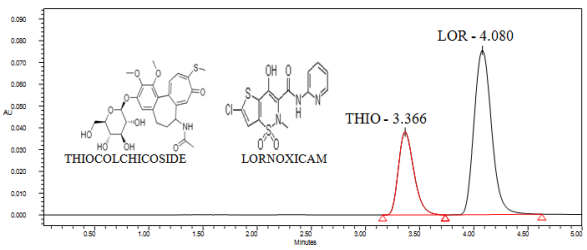

Figure 1: Typical chromatogram of THIO (20 µg mL-1) and LOR (40 µg mL-1) in combination along with structures of analytes

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 8 mg of LOR and 4 mg of THIO was weighed and transferred to a 100 mL volumetric flask containing about 70 mL of methanol, ultrasonicated for 5 min and volume was made up to the mark with the methanol and suitably diluted to get solutions of concentrations of 40 µg mL^{-1} of LOR and 20 µg mL^{-1} of THIO in mobile phase. The sample solution was then filtered using 0.45 µ nylon filter and 20 µL of the test solution was injected, chromatogram was recorded and the amounts of the drugs were calculated.

Validation Procedure

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted and were analyzed using the proposed HPLC method. Linearity test solutions were prepared. The Values of LOD and LOQ were calculated using σ (standard deviation of response) and b (slope of the calibration curve) and using equations, LOD = (3.3 x σ)/ b and LOQ = (10 x σ)/ b. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.5 ml/min, the percentage of me-OH&THF was varied by (±) 1%, column temperature was varied by (±) 2 ^{0}C, pH of mobile phase was varied by (±) 0.1, salt concentration of buffer was varied by (±) 1 the column was changed from different manufacturer and wavelength of measurement was changed by (±) 1. The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing at 4^{0}C for 30 days. Auto-sampler stability was determined by storing the samples for 24 h in the auto-sampler. For method development and optimization, retention factor (*k*) was calculated using the equation: $k = (t_R - t_M)/ t_M$. Where, t_R = retention time, t_M = is the elution time of the solvent front.

Dissolution Study

A calibrated dissolution apparatus (USP II) was used with paddles at 50 rpm and bath temperature maintained at 37±1° C. Nine hundred milliliter freshly prepared and degassed 0.1 N HCl solutions were used as the dissolution medium. Six tablets were evaluated for each drug product tested. Dissolution samples were collected at 5, 10, 15, 20, 25, 30, 35, 40 and 45 min for the tablet drug products. At each time point, a 5 mL sample was removed from each vessel sample, filtered

through a nylon filter (0.45µm, 25 mm). 2.5 mL of filtrate was diluted to 5 mL with mobile phase and analyzed proposed method. The amount of LOR and THIO in the test samples was calculated, as percentage dissolved, from the measured peak area for the test samples by using equation 1 and alternatively by using peak areas of sample (S_1) and Standard(S_2) using equation 2.

Dissolved (%) = (Conc. estimated by the method× 900×2×100)/ (1000×DL) … (1)

Dissolved (%) = (900/DL) × (Peak Area (S_1)/Peak Area (S_2))×Conc. (std.)×100….(2)

Where, DL- is drug load, which are 8 mg and 4 mg for LOR and THIO, respectively.

RESULTS AND DISCUSSION

Optimization of HPLC method

In order to achieve simultaneous elution of the two components, different chromatographic conditions were attempted. Stationary phases like C8 (Qualisil), C18 (kromasil and symmetry) were used .The experimental studies revealed that the C 18 (Symmetry, 5 micron 4.6×250mm) column was the most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity. Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the strength of the Acetate buffer and the flow rate. Acetonitrile and methanol individually and Methanol, Acetonitrile, THF in mixture are used for the study but it did not give good resolved peaks. The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 40-60% methanol and 5%-20% THF. To minimize the peak tailing, THF (15%) was added as an organic modifier. Therefore Methanol and THF were the organic modifier of choice giving symmetrical narrow peaks. Ratio less than 70% of organic resulted in peaks with more tailing, whereas ratios higher than 70% resulted in decreased resolution. The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 4.0 - 6.5 (Fig.2 (A)). Peak tailing for Lornoxicam was found to be well within the limit of 2 at pH 5.5. Thus pH of 5.5 was the most appropriate one giving well-resolved, symmetrical peaks and highest no. of theoretical plates. The effect of changing the concentration of acetate buffer on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentration of 10, 20, 30, 40 and 50 milimoles of acetate buffer (Fig.2 (B)). Table.1 shows that 25 milimoles acetate buffer was found to be the most suitable giving best resolution and highest number of theoretical plates.

The effect of flow rate on the formation and separation of peaks of the studied compounds was studied and a flow rate of 0.75 mL min^{-1} was optional for good separation in a reasonable time. The tailing factors were < 1.5 for both the peaks. The elution order was THIO (t_r = 3.36 min) and LOR (t_r = 4.08 min). The UV detector response of LOR and THIO was studied and the best wavelength was found to be 382 nm showing highest sensitivity.

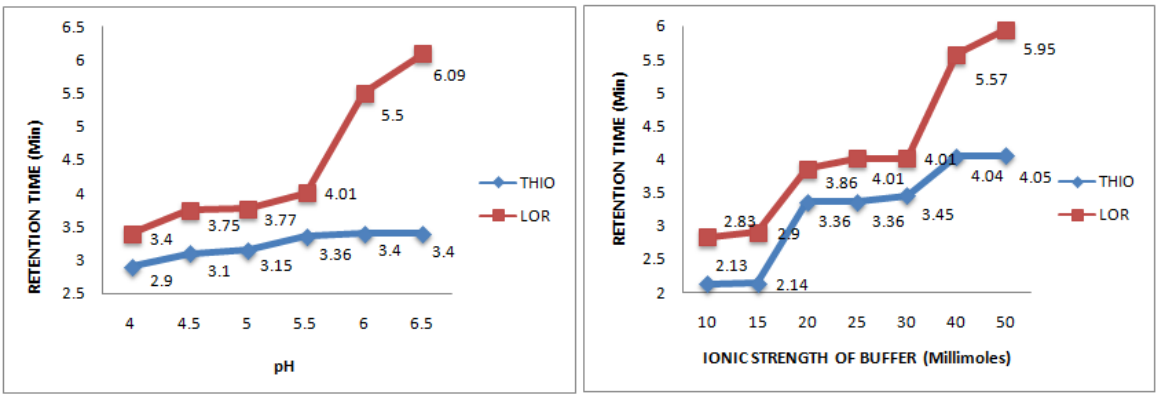

Figure 2: Effect of pH (A) and ionic strength (B) of Acetate Buffer in mobile phase on Retention times of LOR and THIO

Method development

Methanol, THF (15%) and Acetate buffer (pH adjusted up to 5.5) in the ratio of 60:10:30 v/v were employed as a mobile phase. The present RP-HPLC-PDA method for the quantification of THIO and LOR in bulk and pharmaceutical combined dosage forms, revealed as simple, accurate and precise method with significant shorter retention time of 3.36 and 4.08 min, respectively.

Method Validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness [ICH-Q2B, 2006]. System suitability was established by injecting standard solution and results are given in Table 1.

Table 1: System suitability parameters and precision study

Compound	System Suitability		Precision of the Method [b] (n=5)		
	Parameter	Value	Actual Conc. (µg mL^{-1})	Measured conc. (µg/mL), % R.S.D	
				Intra-day	Inter-day
THIO	Theoretical plates[a]	3308	10	10.03, 0.60	9.93, 0.59
	USP resolution[a]	-	20	19.05, 0.76	20.01, 0.70
	Peak Tailing[a]	1.18	30	30.01, 0.87	29.02, 0.29
	% R.S.D.	0.98	-	-	-
LOR	Theoretical plates[a]	4872	5	5.03, 1.25	5.04, 1.02
	USP resolution[a]	2.75	10	9.95, 0.99	9.52, 1.15
	Peak Tailing[a]	1.30	15	14.99,1.09	14.55, 1.05
	% R.S.D.	1.35	-	-	-

[a]USP-NF 29 section 621, pp. 2135. [b] Data expressed as mean for "measured concentration" values

Table 2: Results of Tablet analysis and accuracy studies

Compound (Label Claim)	Formulation Study (n=6)		Recovery (accuracy) Study	
	Batch No.	%Assay Found, % RSD	Recovery Level	% Recovery, % RSD (n=3)
LOR (8mg)	Batch I	99.89, 0.97	50	99.86, 0.80
	Batch II	100.08, 1.09	100	100.11, 0.89
			150	100.16, 0.77
THIO (4mg)	Batch I	100.24, 1.03	50	99.46, 0.30
	Batch II	100.01, 0.99	100	100.02, 0.84
			150	101.83, 1.03

The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found. Peak purity was verified by confirming homogeneous spectral data for LOR and THIO.

Linearity

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for LOR in the range of 0.2-80 µg mL^{-1} and for THIO 0.1- 40 µg mL^{-1}. The correlation coefficient ('r') values were >0.999 (n = 6). The regression equations for the calibration curve was found to be y = 568.79 x+300.63.34 for LOR, y=478.37 x+242.82 for THIO.

Estimation in tablet formulation

The assay for the marketed tablets was established with present chromatographic condition was found to be more accurate and reliable. The average drug content was found to be 99.98 % for LOR and 100.12 % for THIO of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results are given in Table .2

Specificity

The specificity of the HPLC method is illustrated in Fig. 3, where complete separation of LOR and THIO was noticed in presence of tablet placebo. In addition there was no any interference at the retention time of LOR and THIO in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes.

System suitability

To know reproducibility of the method, system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values. The overlain chromatogram and result is shown in Fig.3 & Table 1.

Precision

The precision of repeatability was studied by replicate (n=5) analysis of tablet solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table 1. The result revealed the precision with %RSD (0.74% and 0.52%for THIO) and (1.11% and 1.07% for LOR), respectively for intraday and inter day precision is shown in Table 1.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ values were found to be 0.008 µg mL^{-1}, 0.024 µg mL^{-1} and 0.007 µg mL^{-1}, 0.021 µg mL^{-1} for LOR and THIO, respectively.

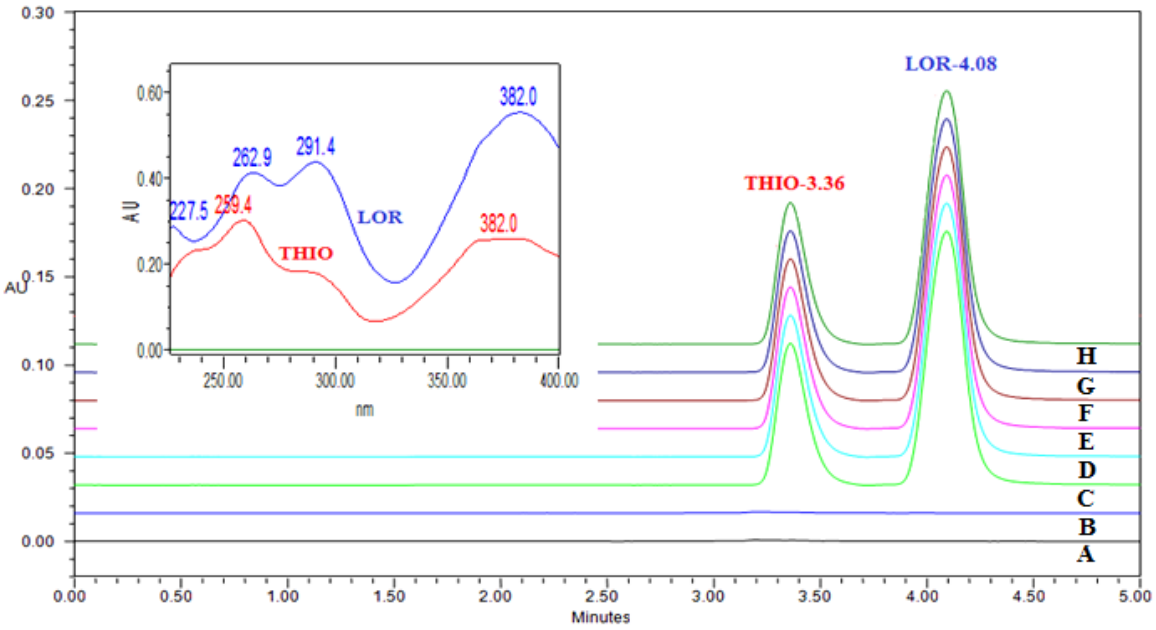

Figure 3: Specificity Chromatogram consists of A) Mobile Phase, B) Placebo, C) formulation, D-H) system suitability standards of THIO (20 µg mL-1) and LOR (40 µg mL-1) and online overlain PDA spectra of analytes

Table 3: Result of robustness study

Parameter	Variation	System suitability					
		Theoretical plates		Tailing		%RSD	
		LOR	THIO	LOR	THIO	LOR	THIO
Flow rate (mL min^{-1})	50:21	4754	3170	1.29	1.19	0.56	0.78
	56:23	4727	3287	1.26	1.16	0.67	0.87
Temp. (°C)	48^0c	4705	3364	1.29	1.19	0.55	0.63
	52^0c	4805	3279	1.21	1.17	1.05	0.78
Measurement Wave-length(nm)	383	4869	3021	1.29	1.14	1.05	0.53
	381	4745	3101	1.23	1.20	1.03	0.88
Composition (me-OH:THF)	84:16	4815	3234	1.31	1.11	0.65	0.93
	86:14	4791	3272	1.22	1.21	1.07	0.72
pH	5.6	4809	3164	1.23	1.13	0.85	0.73
	5.4	4815	3309	1.25	1.15	1.20	0.71
Salt Conc. (Milimoles)	26MM	4785	3264	1.21	1.21	0.57	0.53
	24MM	4834	3299	1.24	1.14	1.09	0.77

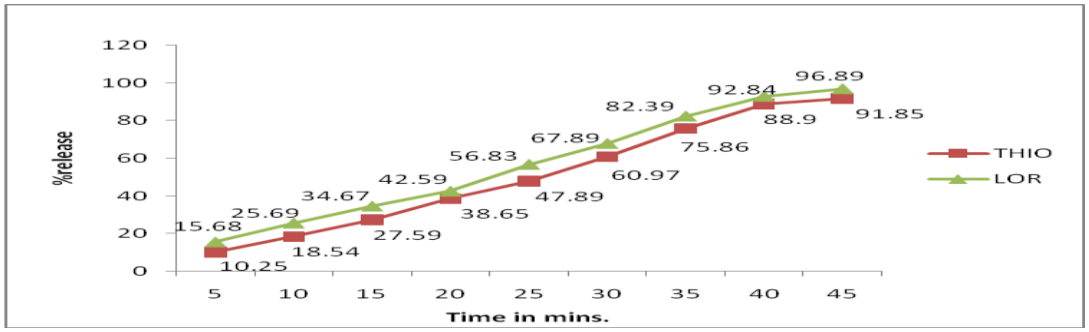

Figure 4: Dissolution profile of analytes from tablet formulation by the proposed method

Solution Stability

Stability as described in method development under experimental section was studied. Result of short-term, long-term and the auto sampler stability of the LOR and THIO solutions were calculated from nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (98–102%) and the retention time , peak area of LOR and THIO remained almost unchanged (% R.S.D. less than 2.0) indicating no significant degradation within the

indicated period, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness

Ruggedness of the method was estimated by preparing six dilutions of the LOR and THIO as per the proposed method and each dilution injected in duplicate using different column and analyst on different days.

Robustness of the method was determined by making slight changes in the chromatographic conditions.

It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, and System suitability parameters were found to be within acceptable limits (Table. 3), indicating that the method robustness under all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

DISSOLUTION

Analytical RP-HPLC method was developed and validated for quantitative determination of LOR and THIO from two tablet formulations. The manuscript describes, first time this type of method was reported for this combination. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. The method is very simple, specific, reliable, rapid and economic. As the peaks are well separated and there is no interference by excipients peaks with total runtime of 5 min, which makes it especially suitable for routine quality control analysis work. Dissolution studies were also carried out to know the percentage release from the drug combination and we found that there is a release of 91.85 & 96.89% for LOR and THIO (Fig. 4).

CONCLUSION

The proposed method gives good resolution between LOR and THIO within short analysis time (< 5 min). The method is very simple, rapid, and economic and no complicated sample preparation is needed. High percent of recovery shows the method is free from interference of excipients present in the formulations.

The proposed LC chromatographic method is rapid, accurate and precise for the simultaneous determination of LOR and THIO in combined dosage forms and is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination as per ICH guidelines. The method is also successfully applied for dissolution study. Method can be applied for estimation of analytes in plasma samples and can be adopted for LC- MS study as the mobile phase volatile.

ACKNOWLEDGEMENT

The authors are thankful to m/s Glenmark Pharmaceuticals Ltd. Baddi, India and Medley Pharmaceuticals Ltd., Baddi, India for providing gift samples of Lornoxicam and Thiocolchicoside, respectively. The authors are thankful to Management of MAEER's Maharashtra Institute of Pharmacy, Pune for providing necessary facility for the work.

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