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Original Article

Development and validation of a new stability indicating liquid chromatographic method for the simultaneous determination of thiocholchicoside and etoricoxib in combined dosage form

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ABSTRACT

A simple and sensitive stability indicating RP-HPLC method was developed and validated for the simultaneous determination of thiocholchicoside and etoricoxib in combined dosage forms. The separation of thiocholchicoside and etoricoxib was performed with the use of Hypersil BDS C18 column (250×4.6 mm;5µm particle size) and a mobile phase consisting of phosphate buffer(pH-3.4) and acetonitrile in the ratio of 35:65 v/v at a flow rate of 1.0 mL/min with UV detection at 260nm. The retention times of thiocholchicoside and etoricoxib were found to be 2.83 and 6.92min respectively. The obtained validation results and statistical parameters for thiocholchicoside and etoricoxib in formulations were satisfactory and were according to ICH guidelines.

Keywords: Thiocolchicoside, Etoricoxib, RP-HPLC and ICH guidelines.

INTRODUCTION

Thiocolchicoside **[Fig.1(a)]** [1,2] ,*N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methyl sulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo [a] heptalen -7-yl]acetamide is a muscle relaxant with anti-inflammatory and analgesic effects. It acts as competitive GABA receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. Etoricoxib**[Fig.1(b)]**[3,4],5-chloro-3-(4-methanesulfonylphenyl)-2-(6-methyl Pyridine3-yl) pyridine is a new COX-2 selective inhibitor used in the treatment of rheumatoid arthritis, osteoarthritis, spondylitis, chronic low back pain, acute pain and gout.

The combination of etoricoxib and thiocolchicoside was recently introduced to Indian market as combined lipid lowering agent can be safely used in the prevention of strokes and cardiac attacks.
Only four RP-HPLC methods [5-8] were reported so far for the simultaneously determination of both the drugs in combined dosage form. These reported methods resulted in high base noise, long tailing, long retention times and low sensitivity and this made the author to develop a validated stability indicating RP-HPLC method for the combination by degrading the drugs together under various stress conditions like acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic stress. The present paper reports the development and validation of a new stability indicating liquid Chromatographic method for the simultaneous determination of thiocholchicoside and etoricoxib in combined dosage form with better detection ranges.

EXPERIMENTAL

Instrumentation: Chromatographic assay of thiocholchicoside and etoricoxib was performed on Water's 2695 HPLC system equipped with Hypersil BDS C18 column (250×4.6 mm;5µm particle size),auto sampler and 2996 Photodiode array detector. The HPLC system and the data acquisition were performed by computer installed by Empower 2 (Waters) chromatography software. Dig sun pH meter was also used for adjusting the pH of buffer solution. Digital Balance [BL-220H; Schimadzu make was used for weighing reagent and chemicals. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase
Reagents and chemicals: Pure samples of thiocholchicoside and etoricoxib along with their analytical reports were obtained as gifted samples

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from Spectrum Pharma Research Solutions, Hyderabad. Commercial formulations (tablets) in the brand name of **NUCOXIA-MR** tablets [containing **thiocolchicoside-4.0mg** and **etoricoxib -60mg]** was procured from the local pharmacy. Analytical grade orthophosphoric acid, sodium hydroxide,hydrochloric acid and 30% hydrogen peroxide, potassium dihydrogen phosphate, Tetra ethyl amine and ortho phosphoric acid were used were purchased from Merck and Rankem Ltd. New Delhi, India.

Mobile Phase Preparation: Prepare a filtered and degassed mixture of phosphate buffer (pH-3.4) and acetonitrile in the ratio of 35:65 v/v respectively.

Buffer Preparation: Dissolve 2.40g of Potassium dihydrogen Phosphate and 0.1% Tetra ethyl amine in 1000mL of Milli-Q Water, adjust pH to 3.4 with dilute ortho phosphoric acid and Filter the solution through 0.45µm membrane filter.

Preparation of diluent: Mobile phase is used as diluent in the present assay.

Preparation of solutions:

Standard solution of thiocolchicoside & etoricoxib:

Stock solution-1: Stock solution of thiocolchicoside was prepared by transferred an accurately weighed quantity 10.0mg of thiocolchicoside reference standard in to a 100 ml volumetric flask containing 50ml of diluent and sonicate to dissolve, later make up to mark with the same diluent and mix.

Stock solution-2: Stock solution of etoricoxib was prepared by transferred an accurately weighed quantity 50.0mg of etoricoxib reference standard in to a 100 ml volumetric flask containing 50ml of diluent and sonicate to dissolve, later make up to mark with the same diluent and mix.

2.0ml solution of stock solution-1 and 5.0ml solution of stock solution-2 was together diluted with the same diluent in 100ml volumetric flask to obtain working solutions of concentrations of 20µg/mL for thiocolchicoside and 50µg/mL for etoricoxib respectively. From this working solution of concentrations in the range of 2.5 – 15µg/mL for thiocolchicoside and 5.0 to 30µg/mL for etoricoxib were prepared by proper dilutions of this stock solution with the same diluent. 20µl of the above prepared working standard solutions were injected into the prescribed column and the chromatograms were recorded.

Sample Solution: 20 tablets of the **NUCOXIA-MR [**Label claim: **thiocolchicoside-4.0mg** and **etoricoxib -60mg]** were weighed and the average weight per tablet was calculated and were crushed and ground to a fine powder. A quantity of powder equivalent to 10mg of thiocolchicoside and 50mg of etoricoxib was accurately weighed and transferred into 100ml volumetric flask containing 30ml of diluent, mixed well and filtered. Later this filtrate was diluted to the mark with the same diluent. From this solution various aliquots were pipetted into a clean and different dry 10mL volumetric flasks, and diluted up to the mark to get final concentrations that obey in the linearity range for thiocolchicoside and etoricoxib respectively. 20µL volume of these sample solutions were injected six times into the prescribed HPLC system and the peak areas were recorded.

RESULTS AND DISCUSSION

Method Development: The analytical conditions were selected after testing the different parameters that influence LC analysis, such as column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters.

Hypersil BDS C18, 250×4.6 mm column having 5µm particle size was used because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. For mobile phase selection, the preliminary trials using different compositions of mobile phases consisting of acetonitrile and water and (50:50 v/v) gave poor peak shape.

To develop good symmetrical peak, water was replaced by phosphate buffer which is adjusted to acidic pH(3.4) by ortho-phosphoric acid. Further, the mobile phase proportion was optimized to retain analytes properly that provide good resolution between thiocholchicoside and etoricoxib. The proportion of acetonitrile in the mobile phase was finalized to 65% with respect to the phosphate buffer pH(3.4). The detection at 260nm wavelength offered high response for thiocholchicoside and etoricoxib respectively. The injection volume was fixed to 20µL and the flow rate of the mobile phase is set to 1.0 ml/min at ambient temperature.

On this finalized chromatographic condition, obtained chromatograms exhibited good peak symmetry and higher theoretical plates for thiocolchicoside (**Peak-1**) and etoricoxib (**Peak-2**) with retention times 2.83 and 6.92 minutes respectively. The representative chromatogram for the same is shown as under [**Fig.2]** respectively.

Degradation Studies: To determine the stability indicating nature of the proposed method tablets containing thiocholchicoside and etoricoxib various stressed degradation studies were conducted. The degradation samples were prepared by transferring powdered tablets, equivalent to 10mg of thiocholchicoside and 50mg of etoricoxib into a 100ml round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase to attain 10 mg/ml of etoricoxib and 50mg/ml of thiocholchicoside concentration. Specific degradation conditions were described as follows.

***Acidic Condition*:** In acidic degradation the drug was heated under reflux with 1 N HCl at 80° C for 1 hr and the mixture was neutralized with 1 N NaOH solutions. thiocholchicoside and etoricoxib was found to be degrading up to 4.0 to 7.0 % in acidic condition **[Figure.3(a)]**.

***Alkaline Condition*:** In alkaline degradation the drug was treated with 0.1 N NaOH at room temperature for 100 min and the mixture was neutralized with 0.1 N HCl solutions. Major degradation was found in alkali condition that product was degraded up to 2.0-3.0% **[Figure.3(b)]**.

***Oxidative Condition*:** Oxidation degradation study was performed by taking the drug content in 3% v/v H_2O_2 at room temperature for 1 hour. In oxidative degradation, it was found that around 3.0 to 6.0 % of the drug degraded **[Figure.3(c)]**.

***Thermal Condition*:** Thermal degradation was performed by exposing solid drug at 70˚ C for 48 hour. Resultant chromatogram of thermal degradation study indicated that thiocholchicoside and etoricoxib is found to be stable under thermal degradation condition **[Figure.3(d)]**.

***Photolytic Condition*:** Photolytic degradation study was performed by exposing the drug content in UV light for 72 hrs. Very slight degradation was observed in the chromatogram of thiocholchicoside and etoricoxib in above specific photolytic condition **[Figure3(e)]**.

Method Validation: The method was validated for linearity, accuracy,intra-day and inter-day precision and robustness in accordance with ICH guidelines.

Specificity: The interference of blank and placebo was conducted for the developed method by injecting the diluent and placebo into the chromatographic system under defined chromatographic conditions and their respective chromatograms were recorded. Chromatograms of blank and placebo solutions showed no peaks at the retention time of thiocholchicoside and etoricoxib peaks indicating that the diluent and placebo solution used in standard and sample preparation did not interfered in the assay of thiocholchicoside and etoricoxib respectively.

***System Suitability*:** System suitability parameters like number of theoretical plate, retention time and peak tailing were determined for thiocholchicoside and etoricoxib by using the above defined chromatographic conditions. The values of the above described parameters were summarized in the **Table-1.**

***Linearity of detector response*:** Under the optimal experimental chromatographic conditions, linear relationship exists between the integrated peak areas and the corresponding concentrations of thiocholchicoside and etoricoxib respectively. Calibration curves for thiocholchicoside and etoricoxib were constructed by plotting peak area obtained against the corresponding concentration and regression equation was computed [**Figures.4(a)&(b)]**. Regression analysis for the calibration curves showed good linear relationships over the concentration range of 2.5 – 15µg/mL for thiocholchicoside and 5.0 - 30µg/mL for etoricoxib as judged by the correlation coefficients values $\mathbf{R^2 = 0.9985}$ for thiocholchicoside and $\mathbf{R^2 = 0.9994}$ for etoricoxib respectively. These results revealed that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above **[Table-3]**. The LOD values for thiocholchicoside and etoricoxib were found to be 0.554µg/mL and 0.741µg/mL, respectively and the LOQ values were found to be 1.86µg/mL and 2.472µg/mL for thiocholchicoside and etoricoxib revealing the good sensitivity of the proposed method.

Precision: The repeatability of the present method was evaluated by calculating the %RSD of six replicate injections of 100% concentration (10µg/ml of thiocholchicoside and 20µg/ml of etoricoxib) on the same day (Inter-day) and on different days(Intra-day). The %RSD values reported in **Table-4** for intra-day precision study and inter-day precision study was < 2.0 % for thiocholchicoside and etoricoxib confirming the high precision of the proposed RP-HPLC method.

Accuracy: In assessing the accuracy of the proposed method, freshly prepared placebo of the thiocholchicoside and etoricoxib pharmaceutical formulations were spiked with various amounts of

pure thiocholchicoside and etoricoxib at 50, 100 and 150 %. Each solution was injected in triplicate and the peak areas for three concentrations were used to calculate means recovery values (**Table-**5) and compared with those obtained with standard thiocholchicoside and etoricoxib solutions. The mean recovery of thiocholchicoside and etoricoxib was between 99.26-99.40% and 99.50-99.80% respectively which indicated the good accuracy of the proposed method..

***Robustness Studies*:** The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions flow rate (± 0.2 ml/min), the proportions of buffer: acetonitrile (33:67 and 37:63, %v/v) and changing the column temperature (35±2°C) respectively. For each different analytical condition the standard solution and test solution were prepared separately and injected into the HPLC system. The results of this study reported that system suitability parameters (e.g. Tailing factor, plate counts, resolutions etc.) were found to be within the specified limits under those deliberately varied conditions, which ensured the robustness of the proposed method for thiocholchicoside and etoricoxib. The results of this robustness study for thiocholchicoside and etoricoxib were summarized in **Table-6** respectively.

Ruggedness: The ruggedness of the proposed RP-HPLC method was evaluated by a different analyst and different analyst and different instrument in the same laboratory. The % RSD for peak areas of for thiocholchicoside and etoricoxib were calculated and the experimental results are reported in **Table-7** and these results revealed that the %RSD was within the limits indicating that the developed RP-HPLC method was found to be rugged.

Analysis of marketed formulation: Analysis of marketed tablets [**NUCOXIA-MR]** was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for thiocholchicoside and etoricoxib was found to be 99.25 and 99.98% respectively making the estimation of thiocholchicoside and etoricoxib in dosage forms was accurate within the acceptance level of 95% to 100%. The results are given in **Table-8**.

CONCLUSIONS

The proposed RP-HPLC method is simple, accurate and stability-indicating as it separates the present studied drugs from its degradation products. The method was used for the determination of thiocholchicoside and etoricoxib in combined dosage forms, without any interference from excipients and in the presence of its acidic and alkaline degradation products. The developed RP-HPLC method was found to be easily applicable and is expected to be widely used for the routine QC analysis of other related drugs in thire dosage forms in the pharmaceutical industry.

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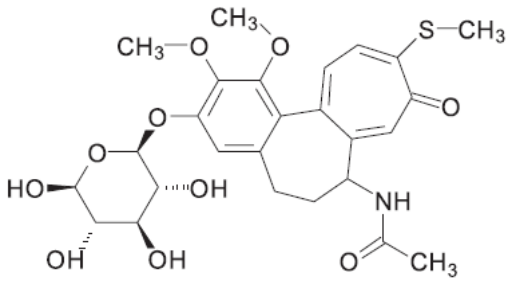

FIG.1(a).MOLECULAR STRUCTURE OF THIOCOLCHICOSIDE

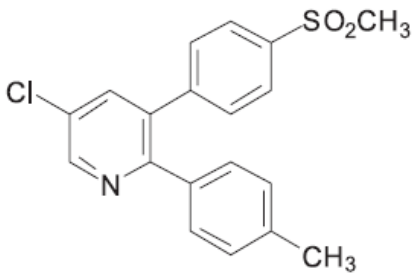

FIG.1(b). MOLECULAR STRUCTURE OF ETORICOXIB

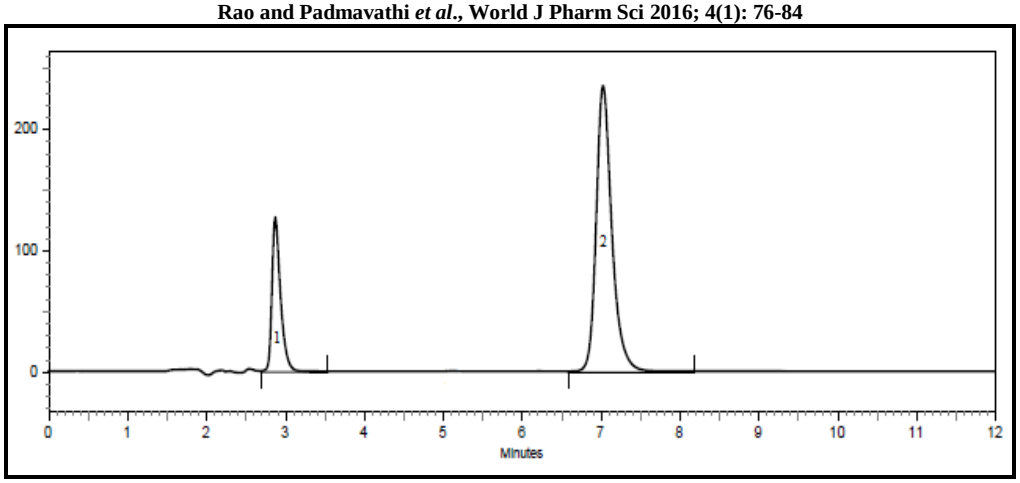

FIG. 2. CHROMATOGRAM OF ASSAY OF STANDARD SOLUTION THIOCHOLCHICOSIDE (PEAK-1) AND ETORICOXIB (PEAK -2)

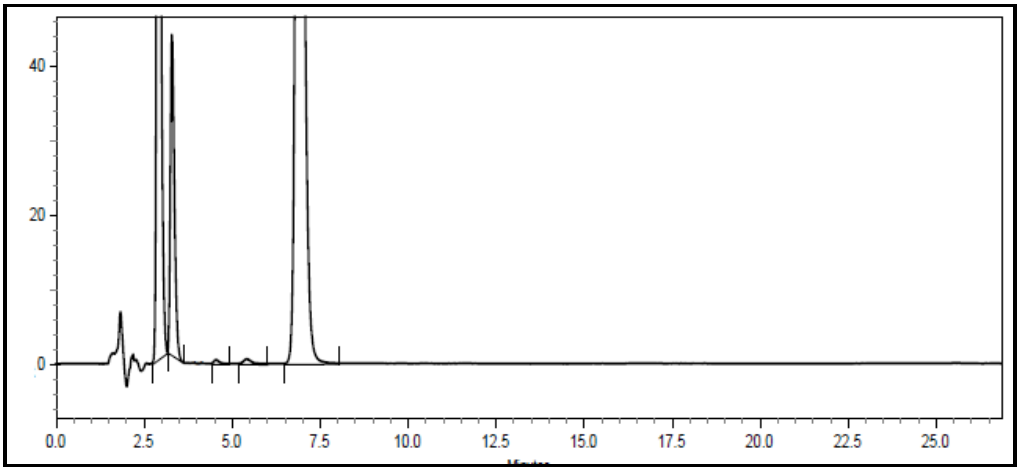

FIG. 3(a) CHROMATOGRAM OF THIOCHOLCHICOSIDE (1) AND ETORICOXIB (2) IN ACID DEGRADATION

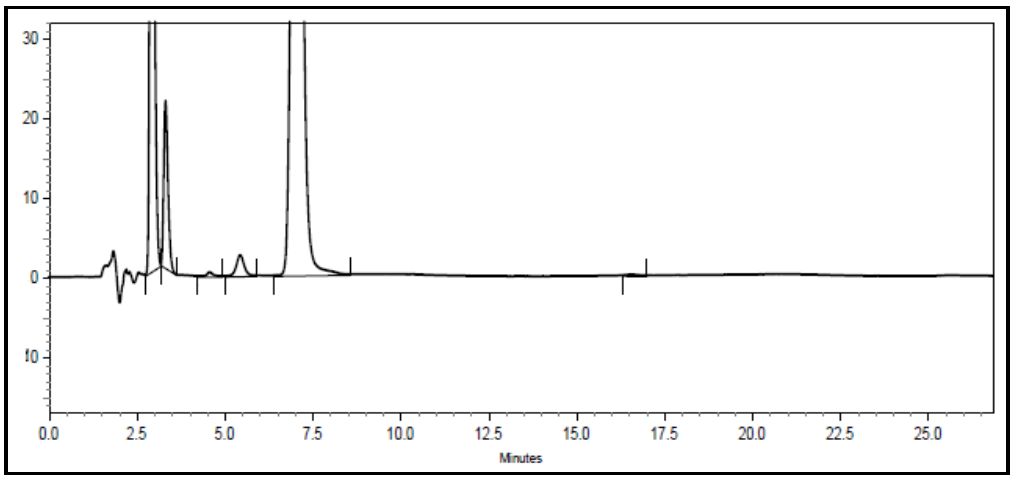

FIG.3(b) CHROMATOGRAM OF THIOCHOLCHICOSIDE (1) AND ETORICOXIB (2) IN BASE DEGRADATION

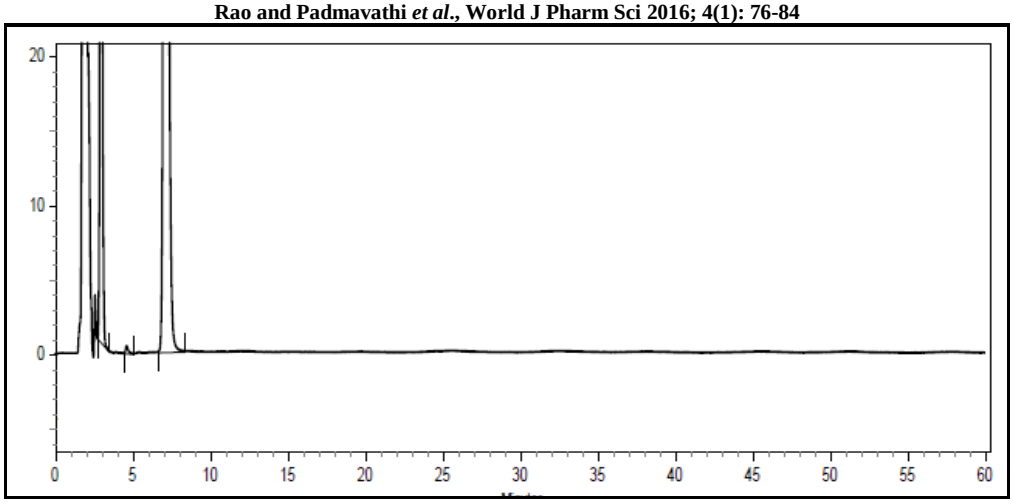

FIG.3(c) CHROMATOGRAM OF THIOCHOLCHICOSIDE (1) AND ETORICOXIB (2) IN OXIDATIVE DEGRADATION

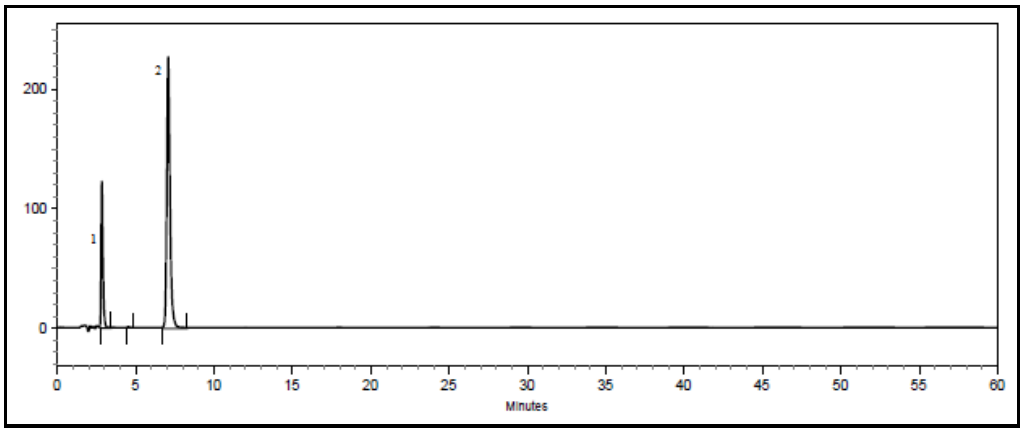

FIG. 3(d) CHROMATOGRAM OF THIOCHOLCHICOSIDE (1) AND ETORICOXIB (2) IN THERMAL DEGRADATION

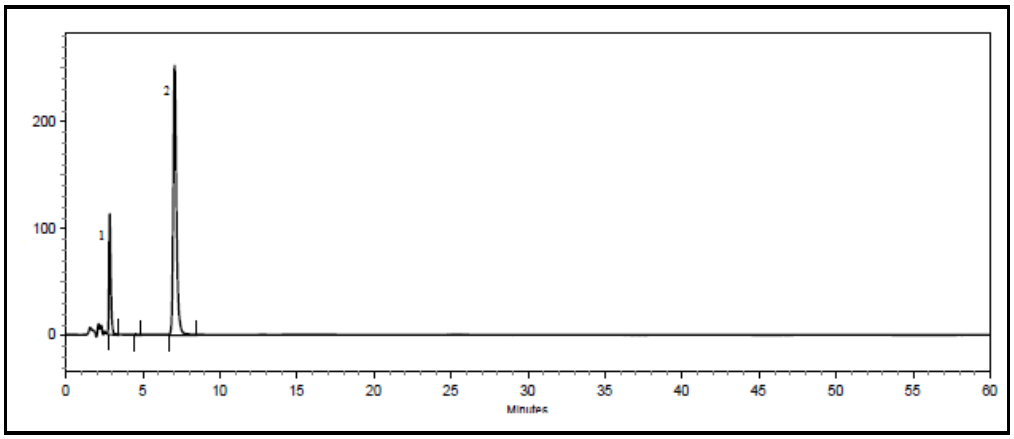

FIG. 3(e) CHROMATOGRAM OF THIOCHOLCHICOSIDE (1) AND ETORICOXIB (2) IN PHOTOLYTIC DEGRADATION

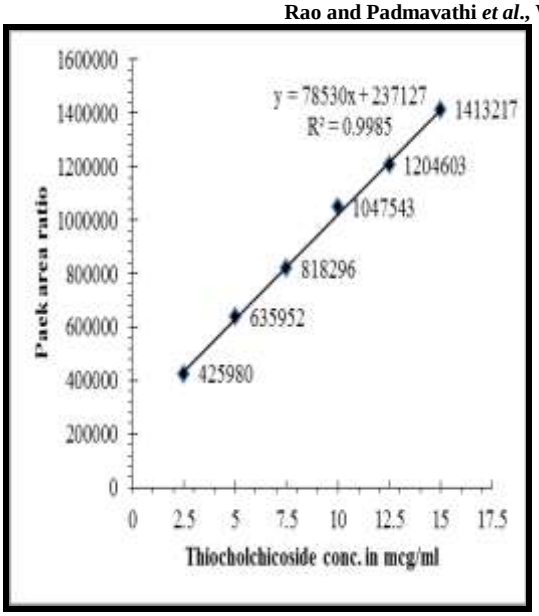

FIG. 4(a) CALIBRATION CURVE OF THIOCHOLCHICOSIDE

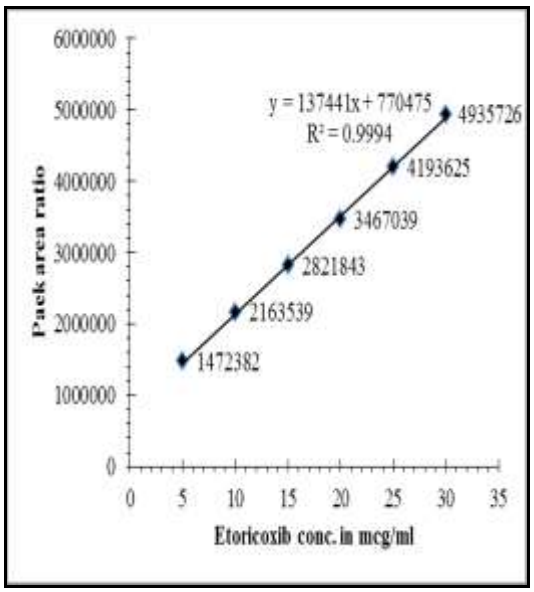

FIG.4(b) CALIBRATION CURVE OF ETORICOXIB

TABLE-1: RESULTS OF DEGRADATION STUDIES OF THIOCHOLCHICOSIDE AND ETORICOXIB

DEGRADATION PARAMETERS	%THIOCHOLCHICOSIDE Recovered	% ETORICOXIB Recovered
ACID	98.4 ± 0.7	98.5 ± 1.1
BASE	96.5 ± 1.4	96.3 ± 1.8
PEROXIDE	99.5 ± 1.4	99.7 ± 1.6
THERMAL	-----	97.6 ± 2.3
PHOTOLYTIC	99.6 ± 1.9	98.6 ± 2.1

TABLE-2: SYSTEM SUITABILITY PARAMETERS OF THIOCHOLCHICOSIDE AND ETORICOXIB

NAME OF THE COMPOUND	RETENTION TIME	THEORETICAL PLATES	ASYMMETRY
THIOCHOLCHICOSIDE	2.83	3327	1.45
ETORICOXIB	6.92	5975	1.36

TABLE-3: LINEARITY STUDIES OF THIOCHOLCHICOSIDE AND ETORICOXIB BY THE PROPOSED METHOD

LINEARITY STUDY FOR THIOCHOLCHICOSIDE			LINEARITY STUDY FOR ETORICOXIB		
% LEVEL (APPROX.)	CONC. µg/mL	AREA	% LEVEL (APPROX.)	CONC. µg/mL	AREA
25	2.5	425980	25	5	1472382
50	5.0	635952	50	10	2163539
75	7.5	818296	75	15	2821843
100	10	1047543	100	20	3467039
125	12.5	1204603	125	25	4193625
150	15	1413217	150	30	4935726
Slope		78530	**Slope**		137441
RSQ(r2)		0.9985	**RSQ(r2)**		0.9994
LOD (µg/mL)		0.554	**LOD (µg/mL)**		0.741
LOQ (µg/mL)		1.86	**LOQ (µg/mL)**		2.472

TABLE.-4: INTER AND INTRA-DAY PRECISION STUDIES FOR THIOCHOLCHICOSIDE AND ETORICOXIB

SET	THIOCHOLCHICOSIDE		ETORICOXIB	
	Intraday(n=6)	Interday(n=6)	Intraday(n=6)	Interday(n=6)
1	100.04	99.96	99.95	99.79
2	99.80	100.2	99.88	99.98
3	98.97	99.80	99.91	99.89
4	99.23	98.97	99.90	99.97
5	99.91	99.92	99.23	99.93
6	99.70	99.93	99.97	99.95
Mean	99.60	99.79	99.80	99.91
SD	0.417	0.425	0.284	0.070
%RSD	0.417	0.425	0.284	0.070

*Average of six determinations

TABLE.5: ACCURACY DATA FOR THIOCHOLCHICOSIDE AND ETORICOXIB

DRUG	%LEVEL	Theoretical Concentration (µg/ml)	*OBSERVED CONCENTRATION	%RECOVERY
THIOCHOLCHICOSIDE	%50	5.0	4.97	99.40
	%100	10	9.96	99.60
	%150	15	14.89	99.26
ETORICOXIB	%50	10	9.95	99.50
	%100	20	19.92	99.60
	%150	30	29.94	99.80

*Each value corresponds to the mean of three determinations

TABLE.6: ROBUSTNESS STUDIES OF THIOCHOLCHICOSIDE AND ETORICOXIB

ROBUST CONDITIONS		THIOCHOLCHICOSIDE		ETORICOXIB	
		Theoretical plates	Asymmetry	Theoretical plates	Asymmetry
FLOW RATE	**0.8mL/min.**	3391	1.55	6331	1.49
	ACTUAL	**3327**	**1.45**	**5975**	**1.36**
	1.2mL/min.	2907	1.49	6125	1.40
MOBILE PHASE COMPOSITION	**Buffer:CAN 33:67**	3002	1.47	5994	1.35
	ACTUAL	**3327**	**1.45**	**5975**	**1.36**
	Buffer:CAN 37:63	2985	1.64	6215	1.36
TEMPERATURE	**33ºC**	3271	1.63	6158	1.36
	ACTUAL	**3327**	**1.45**	**5975**	**1.36**
	37ºC	3182	1.65	6259	1.39

TABLE.7: RUGGEDNESS DATA FOR OF THIOCHOLCHICOSIDE AND ETORICOXIB

	THIOCHOLCHICOSIDE		ETORICOXIB	
	ANALYST-1	ANALYST-2	ANALYST-1	ANALYST-2
S.No.	Rt	Rt	Rt	Rt
1	2.834	2.725	6.923	6.845
2	2.789	2.765	6.770	7.014
3	2.878	2.674	6.884	6.954
4	2.912	2.721	6.789	6.923
5	2.897	2.789	6.885	6.876
***Average**	2.862	2.7348	6.8502	6.9224
Stdev	0.05023445	0.044252	0.066766	0.066206
%RSD	1.75	1.61	0.974	0.956

*Average of five determinations

TABLE.8: ASSAY OF THIOCHOLCHICOSIDE AND ETORICOXIB IN FORMULATIONS [NUCOXIA-MR TABLETS]

Drug name	Quantity label claim(mg)	*Quantity found ± SD	% Assay ± SD
THIOCHOLCHICOSIDE	4.0	3.97 ± 0.76	99.25± 0.72
ETORIOCOXIB	60	59.99 ± 0.95	99.98 ± 0.76

*Average of six determinations

REFERENCES:

1. Sechi G et al.Seizure 2003; 12: 50.
2. Giavina-Bianchi P et al. J. Ther.Clin. Risk Manag 2009;5:635.
3. Martindale: The Complete Drug Reference,Royal Pharmaceutical Society of Great Britain, London 2005:38.
4. Agrawal NG et al J. Clin. Pharmacol 2001;41:1106.
5. Suresh Kumar S et al.Development and validation of RP-HPLC method for simultaneous estimation of etoricoxib and thiocolchicoside in pharmaceutical dosage forms. International journal of research in pharmacy and chemistry 2011;1(3):649-53.
6. Sanjiv Kumar et al, Simultaneous estimation of etoricoxib and thiocolchicoside by RP-HPLC method in combined dosage forms, Acta Poloniae Pharmaceutica n Drug Research 2011; 68(6):845.
7. Navin Goyal et al, Method development and validation of etoricoxib and thiocolchicoside in combined pharmaceutical solid dosage form by RP-HPLC method. International Journal of Pharmaceutical Studies and Research 2011;2(1):106-109
8. Disha Palte et al, Stability Studies in Combine Dosage form of Etoricoxib and Thiocholchicoside using RP-HPLC. International Journal of Research Studies in Biosciences 2015;3(9): 163-170