REVERSED PHASE-HPLC METHOD FOR SIMULTANEOUS ESTIMATION AND VALIDATION OF ETORICOXIB AND THIOCOLCHICOSIDE IN TABLET DOSAGE FORM

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1. Abstract:
Etoricoxib is a type of nonsteroidal anti-inflammatory drug (NSAID) known as a COX-2 inhibitor. Thiocolchicoside is an anti-inflammatory and analgesic muscle relaxant. Combination of Etoricoxib and Thiocolchicoside is used in the management of pain of muscle spasm. Simultaneous estimation of Etoricoxib and Thiocolchicoside in combined pharmaceutical dosage forms, a cost-effective RP-HPLC method using a PDA detector at 245 nm wavelength has been developed. The method was validated in accordance with ICH guidelines for Etoricoxib and Thiocolchicoside respectively, over concentration ranges of 20–160 ppm and 1–10 ppm. Analyzer column temperature of 25°C +/- 0.5°C was used with Puritas Eximius C18, 250 X 4.6 mm, five microns. Mobile phase used was acetonitrile and 0.1% acetic acid in water were mixed in a 70:30 volume-to-volume composition and flow rate of 1.0 mL/min was used. Retention times of 4.2 and 2.1 min were obtained for Etoricoxib and Thiocolchicoside respectively. Etoricoxib and Thiocolchicoside have percentage recoveries of 98.28% and 102.1%, respectively. Every time, the relative standard deviations are under 2%.

Keywords: Etoricoxib, Thiocolchicoside, Simultaneous analysis, RP-HPLC, Tablets.

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2. Introduction:

Etoricoxib is prescribed to treat rheumatoid arthritis, psoriatic arthritis, osteoarthritis, chronic low back pain, ankylosing spondylitis, acute pain, and gout. Different countries have certified different indications [1]. A Cochrane systematic review assessed the effectiveness of a single dose of Etoricoxib in reducing immediate postoperative pain in adults. In comparison to a placebo, oral Etoricoxib reduces post-operative pain by four times while having a same amount of negative side effects [2]. At a dosage of 120 mg, Etoricoxib is as effective as or more effective than other commonly prescribed analgesics [3].

2.1. Etoricoxib

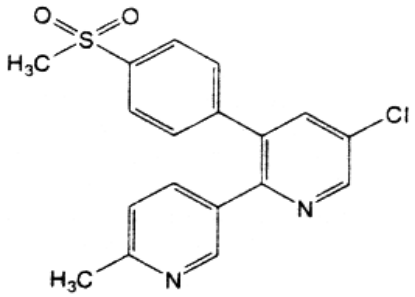

Figure 1. 5-Chloro-6'-methyl-3-[4-(methyl sulfonyl) phenyl]-2,3'-bipyridine

In order to treat a variety of musculoskeletal problems, usually NSAIDs are combined with the centrally acting muscle relaxant Thiocolchicoside. Because it has a lower sedative effect than other centrally acting muscle relaxants, it is usually suggested for ailments including low back pain (LBP), orthopaedic, traumatic, and rheumatologic illnesses. Muscle relaxant Thiocolchicoside (Muscoril, Myoril, Neoflax) has anti-inflammatory and analgesic properties. It is a competitive GABA-A receptor antagonist, a glycine receptor antagonist with comparable activity, and a nicotinic acetylcholine receptor antagonist to a much lesser extent. It should not be taken in those who are prone to seizures because it has strong convulsant activity [4].

2.2. Thiocolchicoside

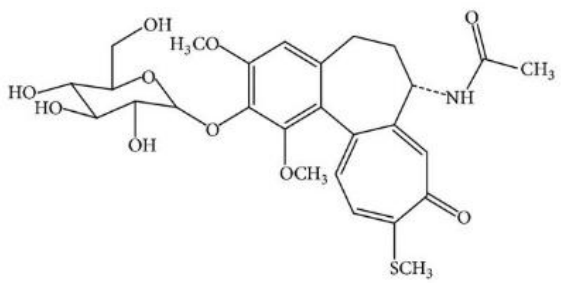

Figure 2. N-[(7S)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] ethanamide

2.3. Mechanism of action

Etoricoxib selectively inhibits isoforms of COX-2, just comparable to any other COX-2 inhibitor ("coxib") (COX-2). It favours COX-2 inhibition over COX-1 by a factor of about 106. Because of this, arachidonic acid produces fewer prostaglandins (PGs) [5]. Among the several roles played by PGs, their contribution to the chain of events leading to inflammation should be emphasized. When traditional nonsteroidal anti-inflammatory drugs are compared to selective COX-2 inhibitors, the former exhibit lower COX-1 activity (NSAID). Multiple clinical trials with various Coxibs have demonstrated that the decreased activity is due to the reduced gastrointestinal adverse effects [6].

Benzodiazepine-free, centrally acting muscle relaxant called Thiocolchicoside [7]. It has a negligible postsynaptic GABA agonist effect. The molecular targets and mechanisms of action of Thiocolchicoside, which has been used in clinical for over 35 years as a muscle relaxant, anti-inflammatory, and analgesic medication, are still being studied. It has been demonstrated that this compound prevents [3H] GABA (g-aminobutyric acid) or [3H] strychnine from attaching to the membranes of the rat cerebro-cortical or spinal cord, respectively. *In-vitro* as well as to corresponding auto radiographic sections *invivo*. It was also discovered that Thiocolchicoside interacts preferentially with a subset of GABA-ARs that have GABA binding sites with low affinities. Thiocolchicoside has been hypothesized to act, despite the lack of knowledge regarding its exact methods of action.

A thorough literature review led to the discovery of spectrophotometric methodology [8,9]. HPLC methods [10,11,12,13,14]. Stability indicating methods [15,16] and Plasma extraction methods [17,18]. is a GABA-AR agonist that causes myorelaxation by depressing the central nervous system. These medications, either alone or in combination with other medications, were reported for analysis in pharmaceutical dosage forms. A few HPLC methods are available with the combination of the above medications. A cost-effective RP-HPLC test technique for estimating Etoricoxib and Thiocolchicoside from formulated dosage forms has been developed and validated which has wider linearity range or longer

retention durations. The new approach is validated in accordance with ICH guideline and all pertinent criteria and has superior retention times and shorter run times than other currently available methods. It is also compatible with LCMS [19,20].

3.MATERIAL AND METHODS:

3.1. Reagents and Chemicals

Etoricoxib and Thiocolchicoside working standards were obtained as gift samples from Bangalore. Water of HPLC grade, Acetonitrile and Acetic acid were purchased from S.D. Fine Chemical in Bangalore. The drug store provided tablets containing 4 mg of Thiocolchicoside and 60 mg of Etoricoxib.

3.2. Instrumentation and Chromatographic Conditions

Hamilton syringe and auto sampler are chosen for chromatography, While the 2695 series of Waters HPLC is connected to the 2996 series of Waters Photodiode array detector. Additionally, the system has a degasser to get rid of dissolved air and a column oven to keep things at the right temperature. Mobile phase with a composition of Acetonitrile: 0.1% Acetic acid in water:70:30 v/v with 1.0ml flow rate Puritas Eximius C18, 250 × 4.6 mm, the chromatographic conditions were chosen with a stationary phase of 5µ and an injection volume of 5µL. Fixed at 245 nm was the detector wavelength.

3.3. Working (Operational) Standard Stock Solution Preparation.

Working standards weighing 10 mg of Etoricoxib and 9.7 mg of Thiocolchicoside were precisely weighed before being transferred to corresponding 25mL volumetric flasks containing diluent. Further 2.5ml of standard stock solution of Thiocolchicoside was diluted to 50ml. Prior to being diluted to the desired volume, the solutions were sonicated for 5 minutes to hasten dissolution. The stock solution's concentration and dilutions are shown in the table below.

3.4. Calibration curve standards preparation.

According to Table-1, calibration curve spiking solutions for Etoricoxib and Thiocolchicoside were made from corresponding stock solutions that ranged from 20 to 160 ppm and 1 to 10 ppm, respectively.

3.5. Tablet Solution (Sample) Preparation

Ten Nucoxia pills containing 60mg Etoricoxib and 4mg Thiocolchicoside were weighed and pulverized into a fine powder. The powder equal to 60mg Etoricoxib and 4mg Thiocolchicoside was precisely weighed and transferred to 50ml volumetric flask containing a few mL of diluent (mobile phase). After 30 minutes of rigorous mixing and sonication, the volume was adjusted. 5 mL of this solution was transferred to 100 mL volumetric flask and diluted to the required volume. A 0.45-micron syringe filter is used to filter this solution.

Table 1. Calibration curve standards Preparation.

Conc. of Std. stock solution (ppm) Etoricoxib	Vol. taken (mL)	Final volume	Conc. of Etoricoxib (µg/mL)	Conc.of Std.stock solution (ppm) Thiocolchicoside	Vol. taken (mL)	Final volume	Conc. Of Thiocolchicoside (µg/mL)
400	0.5	10	20	19.4	0.5	10	0.97
	1.0		40		1.0		1.94
	1.5		60		1.5		3.88
	2		80		2		5.82
	3		120		3		7.76
	4		160		4		9.7

4.RESULTS AND DISCUSSION:

4.1. Method development

Following the choice of the drug combination, both medications were dissolved in the appropriate diluent to produce a clear solution. Reverse phase chromatography was chosen as the best method for chromatography separation based on the literature. By adjusting different mixtures of buffers and organic solvents, the mobile phase was improved. With the mobile phase composition of acetonitrile: 0.1% acetic acid in water 70:30 v/v at a flow rate of 1 mL/min and measured at 245 nm, the resolution and the peak shape of both drugs were determined to be

significant. The retention time observed (4.2min for Etoricoxib and 2.1 for Thiocolchicoside) allows a rapid determination of these drugs. A typical chromatogram is shown below in Figure-3.

4.2. Method Validation

4.2.1. System Suitability Test

Six replicate injections of 100% target solution of Etoricoxib and Thiocolchicoside were used to test the suitability of the system. All of the metrics, including the number of theoretical plates, area, and peak tailing, were calculated and found to be within acceptable ranges. Results were shown in Table-2.

Figure 3. Typical chromatogram of Etoricoxib and Thiocolchicoside

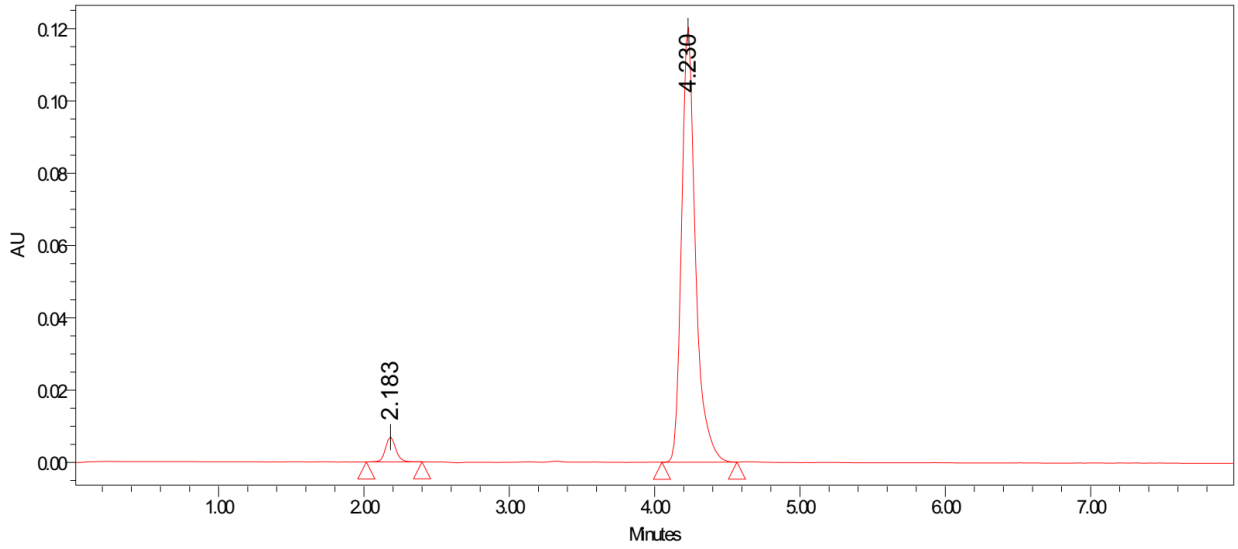

Table 2. System Suitability data of Etoricoxib and Thiocolchicoside

Parameters	Etoricoxib	Thiocolchicoside
USP Tailing	1.34	1.1
Theoretical plates	4485	10237

4.2.2. Specificity

By injecting samples of the mobile phase, a placebo, the sample solution, an unspiked sample, and a spiked sample, a specificity experiment was carried out. The outcomes revealed no interaction between Etoricoxib and Thiocolchicoside during their retention times.

4.2.3. Linearity

The chromatographic conditions mentioned above were used to produce and inject standard solutions of Etoricoxib (20-160ppm) and Thiocolchicoside (1-10ppm). The drug concentration was plotted against the corresponding peak regions at 245 nm to generate calibration curves. According to the findings, there is a strong relationship between detector response and the concentration of each drug within the concentration range.

4.2.4. Accuracy

The tablet solutions of Etoricoxib and Thiocolchicoside had known additions of reference solution to Etoricoxib and Thiocolchicoside equal to 50%, 100%, and 150% of the label claim. These results are summarized in Table 4.

Both the drugs showed a linear response and the equation Y= (mx+c) was used to represent the linearity as follows:

Y (ET) = 13059.x +7680.2 and Y (TH) = 8610.7.x+518.7

The results are given in Table 3 and the resulted chromatograms are shown in Figures 4 and 5

Table 3. Results of chromatograms

Parameters	Etoricoxib	Thiocolchicoside
Linearity	20 to 160 ppm	2 to 16 ppm
Regression equation	Y (ET) = 13059.x +7680.2	Y (TH) = 8610.7.x+518.7
Correlation coefficient	0.9999	0.9998
Slope	13059.04	8610.714

Limit of detection	0.0857	0.1026
Limit of quantitation	0.2597	0.3111

4.2.5. Repeatability

The new method's intraday and interday precisions—referred to as intermediate precisions—were evaluated. This was done by varying the analyst and HPLC column. The % RSD for Etoricoxib and Thiocolchicoside were calculated, which is found to be within the acceptable limits (RSD < 2) and resented in Table 5.

Table 4. Accuracy and Repeatability data of Etoricoxib and Thiocolchicoside

Level	Area		%Recovery	
	Etoricoxib	Thiocolchicoside	Etoricoxib	Thiocolchicoside
50%	367224	19587	98.45	98.70
	368828	19753	98.88	98.80
	368001	19460	98.66	99.36
100%	740951	36928	98.72	98.45
	741950	36653	98.85	98.80
	745782	36559	99.36	98.60
150%	1124447	54580	99.88	99.88
	1133086	55240	100.64	98.86
	1130273	54879	100.39	99.30
Avg			99.32	98.97
Std Dev			0.803987	0.452554
RSD			0.809519	0.457252

Table 5. Precision data for Thiocolchicoside and Etoricoxib

Validation Parameter	Intra-Day		Inter-Day	
	Etoricoxib	Thiocolchicoside	Etoricoxib	Thiocolchicoside
%Mean	100.37	99.62	98.324202	98.99
SD	0.88	0.03	0.4690131	0.87
%RSD	0.88	0.03	0.4770067	0.88

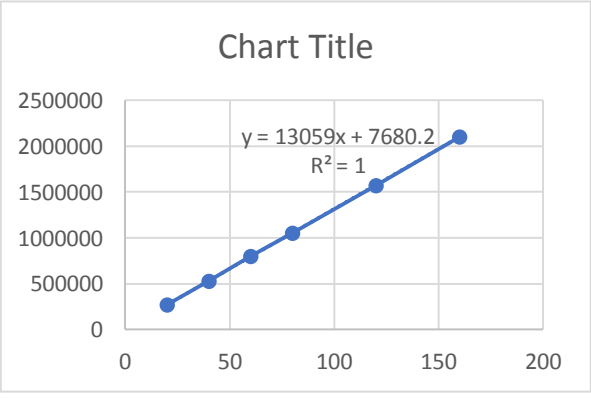

Figure 4. Calibration curve of Etoricoxib

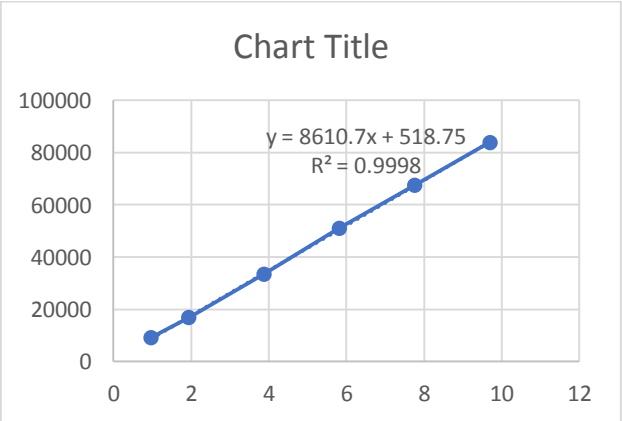

Figure 5. Calibration curve of Thiocolchicoside

4.2.6. Robustness

By making small adjustments to the flow rate, column temperature, and mobile phase concentration, robustness is achieved. The changes and the results were tabulated in Table 6.

Table 6. Robustness data of Etoricoxib and Thiocolchicoside

	Changed value	Retention time		% Assay	
		Etoricoxib	Thiocolchicoside	Etoricoxib	Thiocolchicoside
Column Temperature	20°C	4.01	2.14	98.9	98.0
	25°C	4.06	2.15	98.4	99.6
	30°C	4.13	2.20	98.9	100.4
Flow Rate	0.8ml/min	5.34	2.83	98.7	98.8
	1.0ml/min	4.45	2.36	101.2	100.5
	1.2ml/min	3.93	2.09	100.2	100.8
Mobile phase	25:75	4.91	2.79	98.3	99.2
	30:70	5.23	2.78	99.6	99.8
	35:65	5.49	2.70	99.8	98.8
Average				99.32	98.97
STDEV				0.803987	0.452554
%RSD				0.809519	0.457252

4.2.7. LOD and LOQ

The LOD and LOQ of Etoricoxib and Thiocolchicoside were calculated in the current chapter using the linearity curve approach. LOD and LOQ were determined by using the equations- LOD = 3.3 σ /S and LOQ= 10 σ /S

Where, "σ"is the standard deviation of the response, and "s" is the slope of the linearity curve. The LOD values were 0.08µg/mL and 0.10µg/mL for Etoricoxib and Thiocolchicoside respectively. The LOQ values were 0.25µg/ml and 0.31µg/ml for Etoricoxib and Thiocolchicoside respectively.

4.2.8. Stability of Sample Solution

After 24 hours at room temperature, the stability investigations were conducted in the diluent under the aforementioned chromatographic conditions. These investigations showed that Etoricoxib and Thiocolchicoside remained stable in the diluent for at least 24 hours, demonstrating the accuracy of the analysis in the suggested method. Results are shown in Table-7

Table 7. Stability data of Etoricoxib and Thiocolchicoside.

	Percentage of Assay	
Drug	%Assay at 0hr	%Assay at 24hr
Etoricoxib	98.28	99.58
Thiocolchicoside	98.19	98.38

5. CONCLUSION:

For the simultaneous measurement of Etoricoxib and Thiocolchicoside in tablet dosage form, a new RP-HPLC method was developed and validated. The calibration curve was found to be linear for the concentration ranges of Etoricoxib (20-160 g/mL) and Thiocolchicoside (1-10 g/mL), respectively. To get the best match for the concentration vs. detector response, a linear equation was developed. When the data is validated, the "r2" value is equal to 0.99. The acquired% RSD value of 2 demonstrates the effectiveness of the suggested method's precision. The suggested RP-HPLC approach may be easily used for the regular quality control analysis of combination formulations including these drugs because the separation of the analytes only took 8 minutes.

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7. CONFLICT OF INTEREST: The authors declare no conflict of interest.

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