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Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ETORICOXIB AND THIOCOLCHICOSIDE IN COMBINED DOSAGE FORM

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Abstract

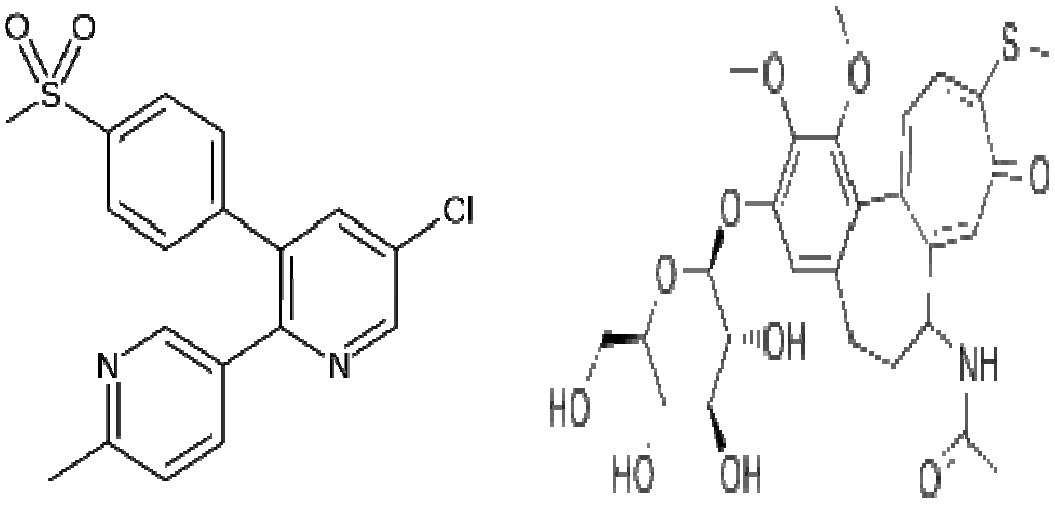
Objective: To develop a new stability indicating reversed phase high-performance liquid chromatographic method and to validate for the simultaneous determination of two Non Steroidal Anti-Inflammatory Drugs (NSAIDs) viz. Etoricoxib and Thiocolchicoside. Methods: Chromatography was carried out on a reversed-phase Hypersil BDS C_{18} (250 x 4.6 mm, 5µ.) with mobile phase mixture of Buffer and Acetonitrile taken in the ratio 60:40 and the pH was adjusted to 3.1, at a flow rate of 1.2 mL/min. The UV range was detected at 258 nm for Etoricoxib and Thiocolchicoside. The stability-indicating capability of the method was demonstrated through adequate separation of aged and stress degraded Etoricoxib and Thiocolchicoside stability samples. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) and robustness were determined according to International Conference on Harmonization (ICH Q2B) guidelines. Results: The linearity of the calibration curves for each analyte in the desired concentration range is good (r^2 >0.999). The mean recoveries of the method were 99.75% and 100.24 % for Etoricoxib and Thiocolchicoside respectively. Conclusion: The proposed stability indicating method is rapid, easy, highly sensitive, precise and accurate and it successfully applied to estimate the amount of Etoricoxib and Thiocolchicoside in the formulations by easily available low cost materials.

Key words: RP-HPLC, Etoricoxib and Thiocolchicoside, Stability indicating Assay, method development, validation.

Introduction: Etoricoxib is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic and antipyretic activities [1-4]. Etoricoxib is a potent, orally active, highly selective cyclooxygenase-2 (COX-2) inhibitor. This drug is used for treatment in rheumatoid arthritis, osteoarthritis and pain [5-8]. It is chemically 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine. The structure is given in figure 1.

Thiocolchicoside is a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, which has been found to displace both [3H] gamma amino butyric acid ([3H] GABA) and [3H] strychnine binding, suggesting an interaction with both GABA and strychnine - sensitive glycine receptors. T HC is potent competitive antagonist of GABA function, there by acting as potent muscle relaxant and displays anti – inflammatory and analgesic properties [9].

It is chemically described as N-[(7S)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-l] acetamide, the structure is illustrated in figure 1.



Etoricoxib **Thiocolchicoside**

Fig-1. Structures of Etoricoxib and Thiocolchicoside

Literature survey suggests that a variety of spectrophotometric and chromatographic methods including UV, HPLC methods have been reported for determination Etoricoxib and Thiocolchicoside either single or in combination with other drugs[10-17]. Whereas no stability indicating HPLC method has been reported for simultaneous quantitative determination of Etoricoxib and Thiocolchicoside in the combined dosage form.

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[18] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated. The aim of the present study was to demonstrate inherent stability of Etoricoxib and Thiocolchicoside through stress studies under a variety of ICH recommended test conditions[19] and to develop and validate a rapid stability-indicating reverse phase assay method[20–22].

Materials and Methods

I. **Materials and Reagents:** Etoricoxib and Thiocolchicoside were supplied by Clearsynth Labs, Mumbai. Other reagents such as Acetonitrile, Methanol used were of HPLC and water of milli-Q grade. Potassium dihydrogen Orthophosphate, Ortho phosphoric acid, Triethylamine used were of AR grade.

II. **Chromatography conditions:** Chromatographic separation was performed on a HPLC (Alliance with PDA detector) at the wavelength of 258 nm. A reverse phase Hypersil BDS C_{18} (250 x 4.6 mm, 5µ.) column was used with mobile phase mixture of Buffer and Acetonitrile taken in the ratio 60:40 and the pH was adjusted into 3.1, at a flow rate of 1.2 mL/min. and injection volume was 10µl and the chromatographic runtime of 10 min was used.

III. **Preparation of buffer solution:** Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask added about 900ml of milli-Q water and added 1ml of triethylamine and degassed to sonicate and finally made up the volume with water, then pH adjusted to 3.1 with dilute OPA solution.

IV. **Preparation of mobile phase:** 1000 mL of mobile phase was prepared by mixing 600ml of buffer and 400ml of Acetonitrile.

V. **Preparation of Standard solution:**

Accurately Weighed and transferred 60mg&10mg of Etoricoxib and Thiocolchicoside working Standards into a 10 ml and 25ml clean dry volumetric flask, add 7ml of diluent (Acetonitrile : water:: 50:50), sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1 ml was pippeted out in to a 10ml volumetric flask and then make up to the final volume with diluent (600µg/ml Etoricoxib & 40µg/ml Thiocolchicoside).

VI. **Preparation of Sample solution:** five tablets were weighed and calculated the average weight then the weight equivalent to one tablet was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 2 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

VII. **Method validation:** Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy, Limit of detection and Limit of quantification.

i. **Stability studies:** The stability studies were carried out by attempting deliberate degradation of the sample with exposure to stress conditions like acidic (2N HCl), alkaline (2N NaOH), 105ºC dry heat, oxidizing agents (H_2O_2) and Photo Stability.

Oxidation: To 1 ml of stock solution of Etoricoxib and Thiocolchicoside, 1 ml of 20% hydrogen peroxide (H_2O_2) was added. The solution was kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 600µg/ml & 40µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock solution Etoricoxib and Thiocolchicoside, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 600µg/ml & 40µg/ml solution and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Etoricoxib and Thiocolchicoside, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 600µg/ml & 40µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105^0C for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to 600µg/ml & 40µg/ml solution and 10µl was injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the drug stock solution to UV Light by keeping the beaker in UV Chamber for 6 hours. For HPLC study, the resultant solution was diluted to obtain 600µg/ml & 40µg/ml solutions and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

ii. **Precision:** The precision of the method was evaluated by carrying out six independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated

(% RSD should not be more than 2%).

iii. **Accuracy:** Accuracy for the assay of Etoricoxib and Thiocolchicoside determined by applying the method in triplicate samples to which known amount of Etoricoxib and Thiocolchicoside standard was added at different levels (50%, 100%, and 150%). Each solution was injected thrice (n=3) into HPLC system and the percent recoveries were

calculated from the peak areas and average recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

iv. **Linearity:** The Linearity of detector response was established by plotting a graph of concentration versus area of Etoricoxib and Thiocolchicoside standard and determining the correlation coefficient. A series of solution of Etoricoxib and Thiocolchicoside standard solution in the concentration ranging from about 150 – 900 ppm of Etoricoxib and 10 – 60 ppm of Thiocolchicoside respective levels of the target concentration were prepared and injected into the HPLC system.(Correlation coefficient should be not less than 0.999.)

v. **Limit of Detection (LOD) Limit of Quantification (LOQ):** LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

Results and Discussion

I. Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, and pH of mobile phase were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, peak tailing, theoretical plates, and run time were the major tasks while developing the method. Buffers like sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate did not yield the desired results. At Acetonitrile: Buffer (40:60v/v) of pH 3.1 with flow rate of 1.2ml/min and detection at 258nm of runtime of 10min, a perfect chromatogram was eluted. The typical chromatogram obtained from final HPLC conditions are depicted in Figure 2.

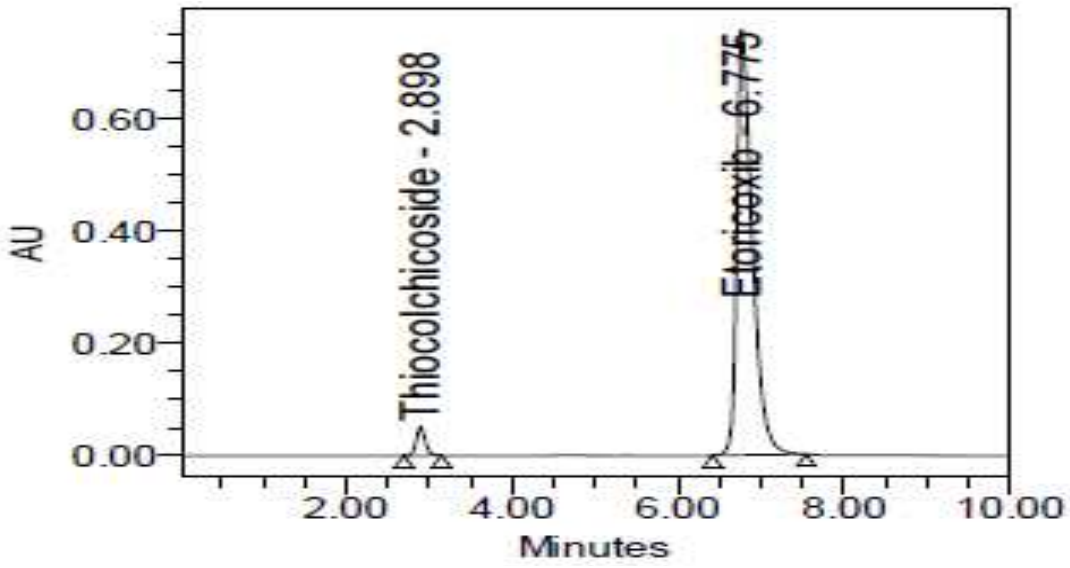


Figure 2: Typical chromatogram of Thiocolchicoside and Etoricoxib by proposed method

II. Method validation: Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to specificity, system suitability, linearity, accuracy, precision, LOD and LOQ as follows.

(i) Stability studies:

Oxidation: For hydrogen peroxide-induced degradation, The peroxide induced placebo and sample spiked chromatograms were given Fig.3 & 4 respectively. The figure shows the major degradation found at RT 2.575 and RT 3.435. All the major and minor degradation products were well separated from Thiocolchicoside and Etoricoxib peaks. The peak purity is checked for Thiocolchicoside and Etoricoxib and the results are summarized in Table 1.

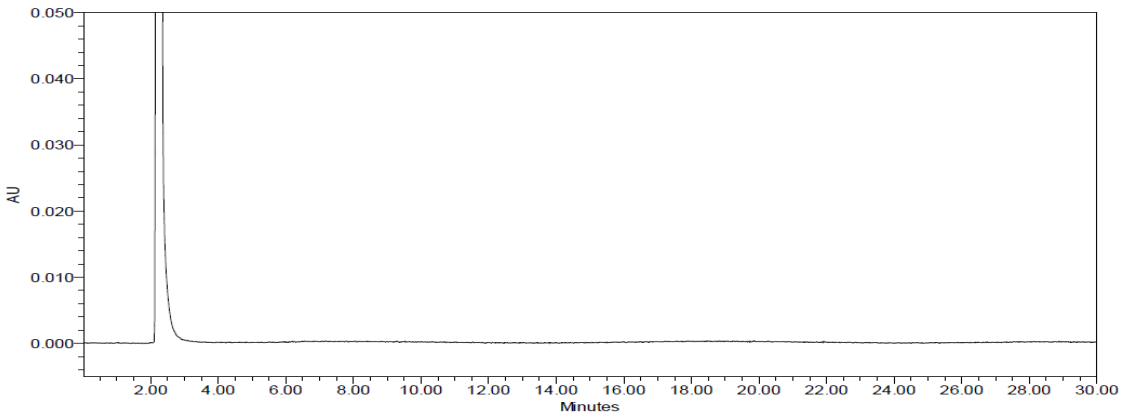


Figure 3: Peroxide induced degradation placebo chromatogram

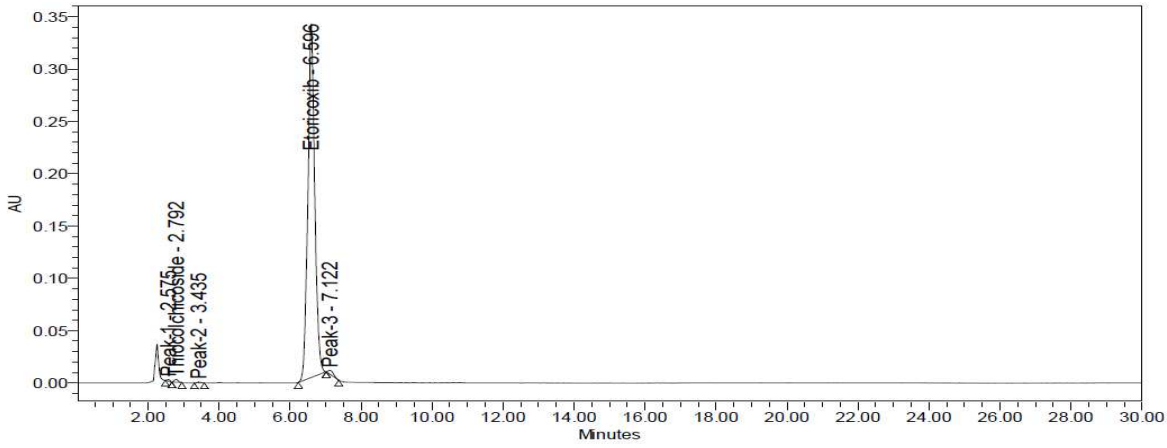


Figure 4: Peroxide induced degradation sample spiked chromatogram

Acid Degradation Studies:

For acid induced degradation, the acid induced placebo and sample spiked chromatograms were given Fig.5 & 6 respectively. The figure shows the major degradation found at RT 2.660 and RT 4.1. All the major and minor degradation products were well separated from Thiocolchicoside and Etoricoxib peaks. The peak purity is checked for Thiocolchicoside and Etoricoxib and the results are summarized in Table 1.

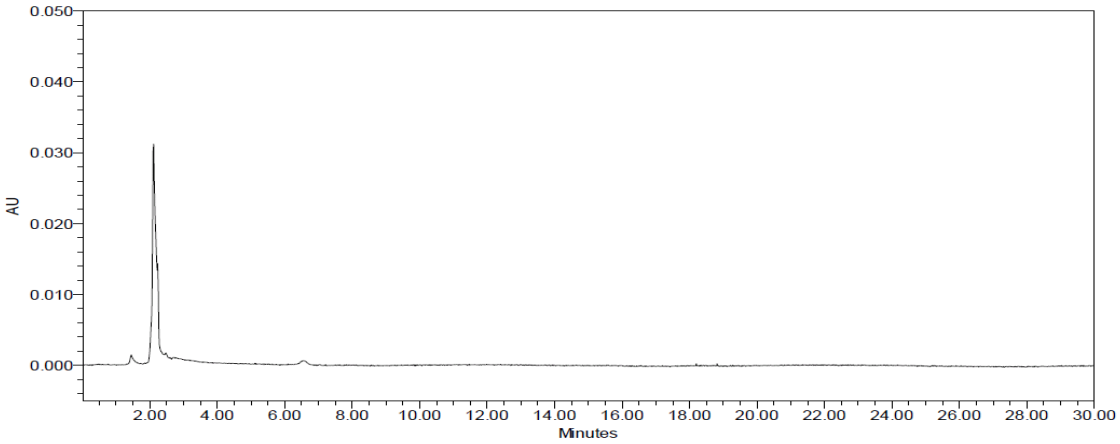


Figure 5: Acid induced degradation placebo chromatogram

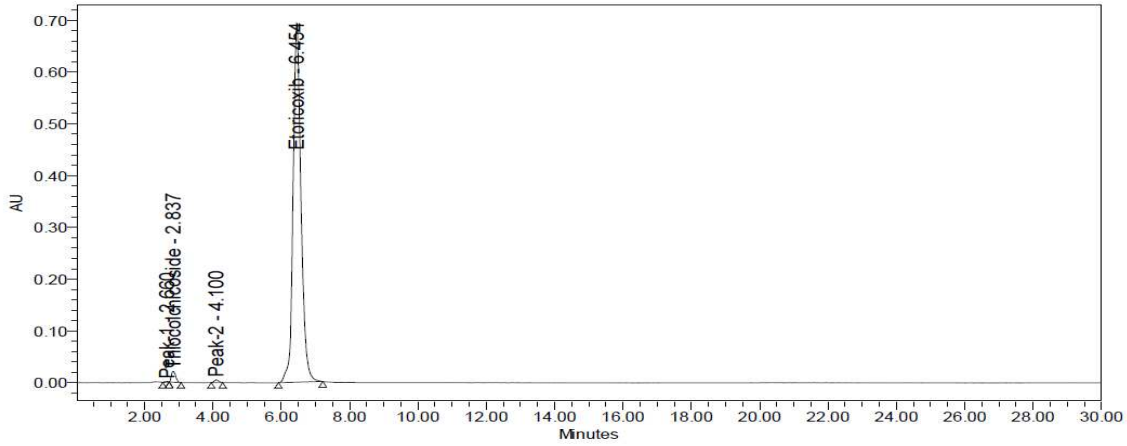


Figure 6: Acid induced degradation sample spiked chromatogram

Alkali Degradation Studies: For base induced degradation, the base induced placebo and sample spiked chromatograms were given Fig.7 & 8 respectively. The figure shows the major degradation found at RT 2.687 and RT 3.181. All the major and minor degradation products were well separated from Thiocolchicoside and Etoricoxib peaks. The peak purity is checked for Thiocolchicoside and Etoricoxib and the results are summarized in Table 1.

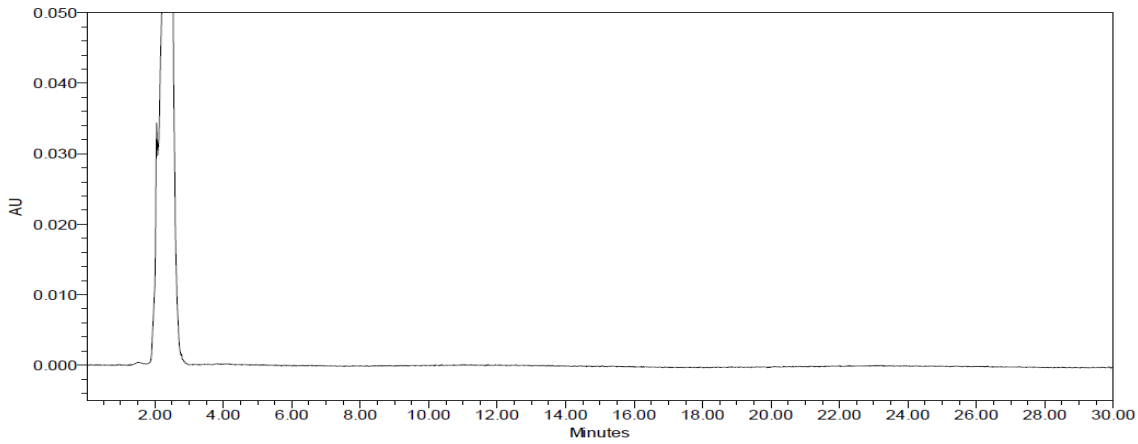


Figure 7: Alkali induced degradation placebo chromatogram

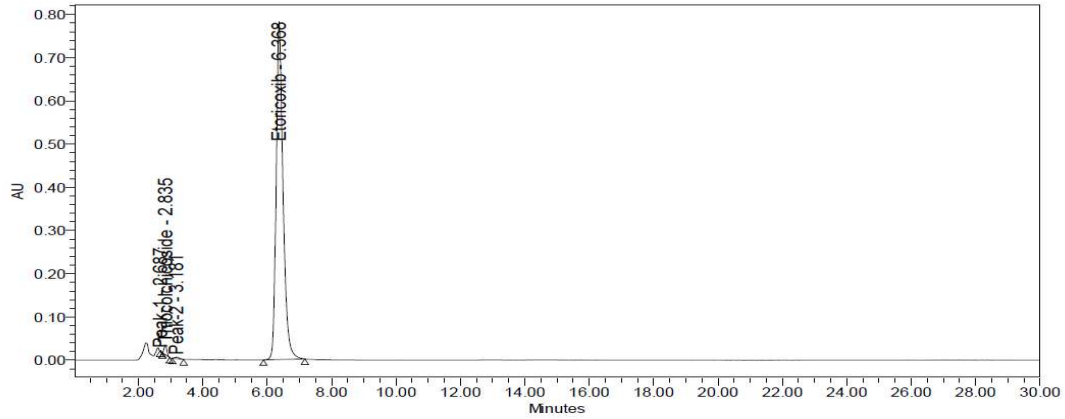


Figure 8: Alkali induced degradation sample spiked chromatogram

Dry Heat Degradation Studies:

For heat induced degradation, the figure 9 shows the major degradation found at RT 2.660 and RT 3.4 All the major and minor degradation products were well separated from Thiocolchicoside and Etoricoxib peaks. The peak purity is checked for Thiocolchicoside and Etoricoxib and the results are summarized in Table 1.

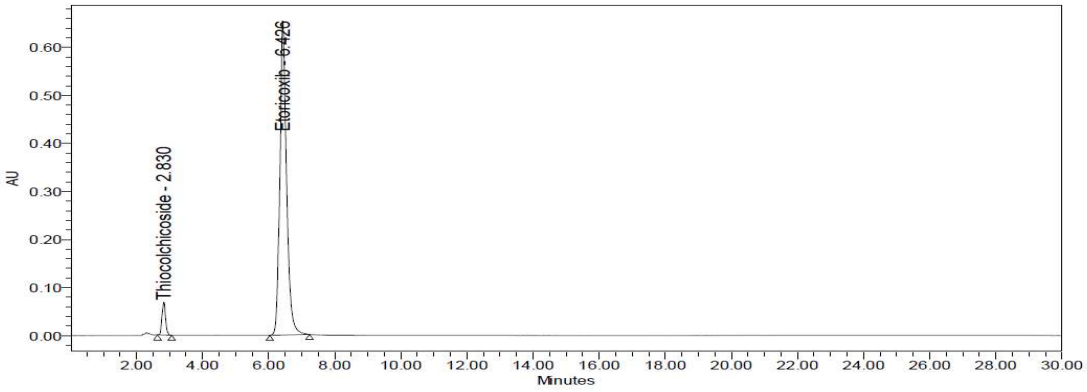


Figure 9: Dry heat induced degradation sample spiked chromatogram

Photo Stability studies: The drugs Thiocolchicoside and Etoricoxib are stable under photolytic conditions and the corresponding chromatogram depicted in fig.10. The peak purity is checked for Thiocolchicoside and Etoricoxib and the results are summarized in Table 1.

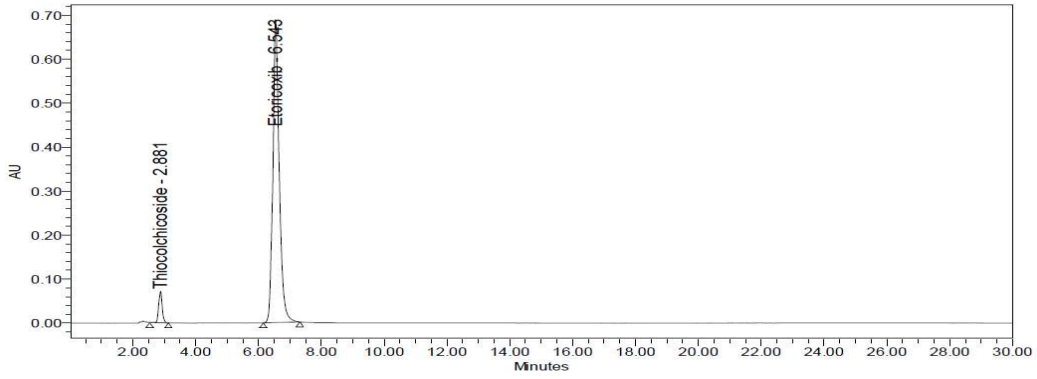


Figure 10: Photolytic degradation sample spiked chromatogram

Table 1: Peak purity results of Thiocolchicoside and Etoricoxib

Study	Purity Angle		Purity Threshold	
	Thiocolchicoside	Etoricoxib	Thiocolchicoside	Etoricoxib
Oxidation	0.218	0.282	2.044	2.263
Acid Degradation Studies	0.456	0.230	0.622	0.361
Alkali Degradation Studies	0.537	0.081	1.583	0.163
Dry Heat Degradation Studies	0.203	0.050	0.349	0.334
Photo Stability studies	0.172	0.055	0.377	0.359

(II) Precision: The % R.S.D. of Thiocolchicoside and Etoricoxib assay during the method precision was found to be 1.3% and 1.04% respectively, indicating excellent precision of the method. The results are summarized in table 2.

Table 2- Results of precision of Thiocolchicoside and Etoricoxib

Injection	Thiocolchicoside Peak area	Etoricoxib Peak area
1	380781	9804879
2	380484	9767009
3	381335	9803152
4	390529	9980633
5	388331	9981501
6	377735	9768434
AVG	383199	9850935
SD	5032.2	102101.3
%RSD	1.3	1.04

(III) Accuracy: Percent recovery of Thiocolchicoside samples ranged from 99.07% to 101.82%, and the Percent recovery of Etoricoxib samples ranged from 99.05% to 100.91% showing the good accuracy of the method. The results are shown in Table 3 and Table 4.

Table 3- Summary results of Accuracy for Thiocolchicoside

Thiocolchicoside	Concentration added in PPM	Area	%Recovery	%Mean Recovery
Accuracy 50%	20	578199	99.93	99.52
		576567	99.07	
		578670	100.17	
Accuracy 100%	40	772510	100.64	100.13
		772379	100.61	
		769646	99.90	
Accuracy 150%	60	964944	100.56	100.1
		972226	101.82	
		958850	99.50	
		Avg	100.24	

		SD	0.79	
		RSD	0.79	

Table 4- Summary results of Accuracy for Etoricoxib

Etoricoxib	Concentration added in PPM	Area	%Recovery	%Mean Recovery
Accuracy 50%	300	15219341	99.17	100.25
		15296097	100.68	
		15308058	100.91	
Accuracy 100%	600	20357028	100.12	99.49
		20248292	99.05	
		20274187	99.30	
Accuracy 150%	900	25379664	99.68	99.50
		25320833	99.30	
		25356467	99.53	
		Avg	99.75	
		SD	0.67	
		RSD	0.68	

(IV) Limit of Detection (LOD) Limit of Quantification (LOQ): The LOD of Thiocolchicoside and Etoricoxib were found to be 0.423 µg/ml and 0.140µg/ml respectively. The LOQ was 1.282 µg/ml and 0.425µg/ml for Thiocolchicoside and Etoricoxib respectively. Since the LOQ and LOD values of Thiocolchicoside and Etoricoxib achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

(V) Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 10-60 ppm for Thiocolchicoside and 150-900 ppm for Etoricoxib three times, and the correlation coefficient obtained was 0.999 for both the drugs, thus indicating excellent correlation between peak areas and concentrations of the analytes.

Conclusion

The developed RP-HPLC method proves to be simple, linear, precise, accurate and specific. The total runtime was 10 minutes within which two drugs and their degradation products were separated. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs Thiocolchicoside and Etoricoxib in presence of degradation products by the industry. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with other available HPLC methods. The shorter run time allows the analysis of a

large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control method of Thiocolchicoside and Etoricoxib in combined dosage forms and as well as for single drug analysis.

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