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SIMUTANEOUS ESTIMATION AND METHOD DEVELOPMENT, VALIDATION OF THIOCOLCHICOSIDE AND ETODOLAC USING RP-HPLC METHOD

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ABSTRACT

Validating a method is essential to guarantee that the results it produces are accurate and consistent within the required limits. Validation serves as documented proof that the method will consistently deliver reliable results. In industrial settings, validation holds significant importance, especially with regulatory oversight. Agencies like the U.S. FDA emphasize the need for method validation as part of Good Manufacturing Practices (CGMP). The developed method was novel and simple for the simultaneous estimation of Thiocolchicoside & Etodolac by RP-HPLC. The two peaks were well resolved at 226nm in isocratic mode at retention times 3.126 and 5.929 min for Thiocolchicoside and Etodolac respectively at a run time of 15 min and flow rate 1.0 ml/min with 250mm x 4.6mm, 5µm column & Methanol: Acetonitrile: Water 30:50:20 (v/v/v) as mobile phase. % Assay values for Thiocolchicoside and Etodolac were found to be 98.99% & 99.57% respectively. Linearity was obtained in the range of 24-72 ppm & 20-60 ppm and linearity correlation coefficient was found to be 0.9910 & 0.9907 for Thiocolchicoside and Etodolac respectively. This new approach was verified using ICH guidelines and found to be specific, sensitive, precise, accurate, and linear.

KEYWORDS: Method development and validation, ICH guidelines, Thiocolchicoside, Etodolac, and RP-HPLC.

1. INTRODUCTION

1.1 DRUG PROFILE OF THIOCOLCHICOSIDE

Thiocolchicoside is a semi-synthetic compound derived from colchicine, which is a natural anti-inflammatory glycoside found in the seeds of the Superba gloriosa plant. This substance acts as a muscle relaxant and possesses both anti-inflammatory and pain-relieving properties. However, it has strong convulsant effects and is therefore contraindicated for use in individuals with a predisposition to seizures. IUPAC name of Thiocolchicoside is N-[(10S)-3,4-dimethoxy-14-(methylsulfanyl)-13-oxo-5-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy} tricyclo [9.5.0.0^{2,7}] hexadeca-1(16),2,4,6,11,14-hexaen-10-yl] acetamide and molecular formula is $C_{27}H_{33}NO_{10}S$. It has the molecuar mass of 563.620 g/mol.[1-10]

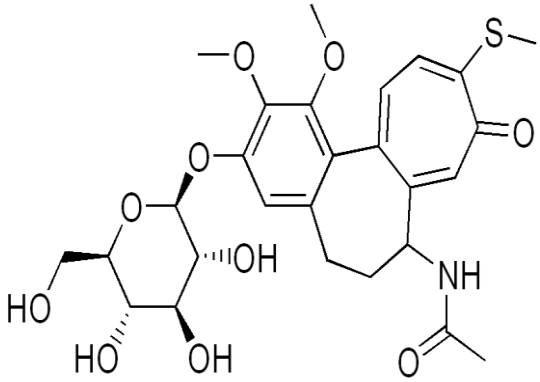


Figure 1: Chemical Structure of Thiocolchicoside.

1.2 DRUG PROFILE OF ETODOLAC

Etodolac is a non-steroidal anti-inflammatory drug (NSAID) that possesses anti-inflammatory, pain-relieving, and fever-reducing properties. Its therapeutic benefits arise from its inhibition of prostaglandin synthesis. Etodolac is commonly used to alleviate the symptoms associated with rheumatoid arthritis and osteoarthritis. The IUPAC designation for Etodolac is 2-{1,8-diethyl-1H, 3H, 4H, 9H-pyrano [3,4-b] indol-1-yl} acetic acid. Its molecular weight is 287.35 g/mol and molecular formula $C_{17}H_{21}NO_3$. It is insoluble in water but soluble in alcohols, chloroform, dimethyl sulfoxide, and aqueous polyethylene glycol. Etodolac is mainly excreted through the urine, with a smaller amount eliminated in the feces, and its clearance depends on kidney function.[11-17]

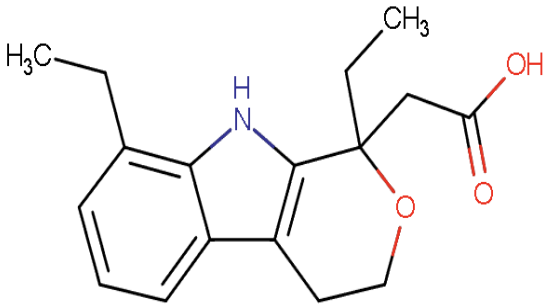


Figure 2: Chemical Structure of Etodolac.

After an exhaustive literature study[18-27], the authors concluded that a very few methods for simultaneous quantification of Thiocolchicoside and Etodolac have been assessed using spectroscopy and liquid chromatography. Therefore, there is a need to design an innovative, fast, exact, sensitive, and selective approach for simultaneous estimation of Thiocolchicoside and Etodolac by RP-HPLC in its tablet dosage forms.

2. MATERIALS AND METHODS

2.1 APPARATUS & CHEMICALS

Table 1: List of apparatus.

S.no	Name	Model	Manufacturer
1	HPLC	Waters 2690	ALLIANCE
2	Weighing Balance	SAB 203 L	Scale tech
3	Pipettes, Beakers and Burettes	NA	Borosil Class-A
4	Ultra Sonicator	PSA-10A	DIGITAL PRO

Table 2: List of chemicals.

S.no	Name	Grade	Batch No
1	Water (Milli Q / HPLC Grade water)	HPLC	P24E100596
2	Acetonitrile	HPLC	J058A24
3	Methanol	HPLC	R276G24

2.2 PREPARATION OF SOLUTIONS

Mobile phase: Methanol: Acetonitrile: Water 30:50:20 (v/v/v) was prepared and filtered through 0.45µm nylon membrane filter and degassed by sonication.

Diluent preparation: Mobile phase is used as diluent throughout the study.

Standard preparation: 60.00 mg of Thiocolchicoside and 50.00 mg of Etodolac were precisely weighed and transferred to two separate 100 ml volumetric flasks. 60 mL of diluent was added and sonicated for 5 minutes. The volume was increased to the mark using diluent. Then, 4ml of each solution was put into a 50ml volumetric flask, and the volume was adjusted to the mark using the same diluent.

Sample preparation: Equivalent powder from 20 tablets was accurately taken from FYTOLAC-T4 containing Thiocolchicoside 60mg and Etodolac 50 mg respectively and transferred to 100 ml volumetric flask. 60 mL of diluent was added and sonicated for 5 minutes. The volume was made up to the mark using diluent. Then, 4ml of solution was placed into a 50ml volumetric flask, and the final volume was produced to the mark using the same diluent.

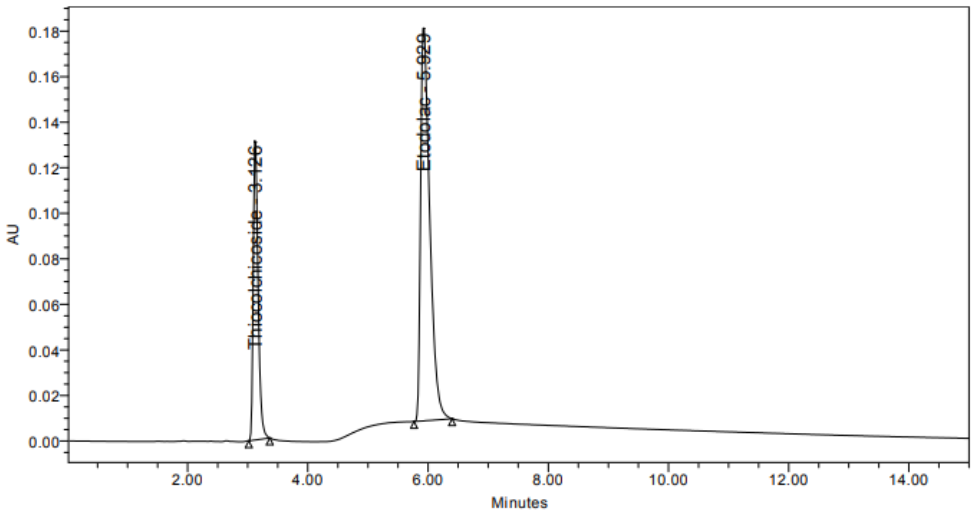
Optimized chromatographic conditions: After performing various trials in isocratic mode, the

optimized chromatogram was obtained at 226nm with 1.0 ml/min flow rate using Methanol: Acetonitrile: Water 30:50:20 (v/v/v). The Sample temperature was maintained at 25°± 5°C. Peaks were well resolved using Welchrom 250mm x 4.6mm, 5µm column at an ambient temperature for 15 min run time.

3. RESULTS AND DISCUSSION

3.1 SYSTEM SUITABILITY

System suitability in chromatography involves a set of tests designed to verify that the entire chromatographic system, encompassing equipment, electronics, analytical procedures, and samples, is functioning properly before commencing any analysis. These tests are crucial to ensure that the system's performance meets the required standards for the specific application, thereby guaranteeing the accuracy and reliability of the analytical results. Validation is performed as per the ICH validation parameters.[28]



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing	USP Resolution	K Prime
1	Thiocolchicoside	3.126	746496	28.97	132880	6762	1.4		2
2	Etodolac	5.929	1830461	71.03	172932	7064	1.8	13	5

Figure 3: System suitability for standard chromatogram.

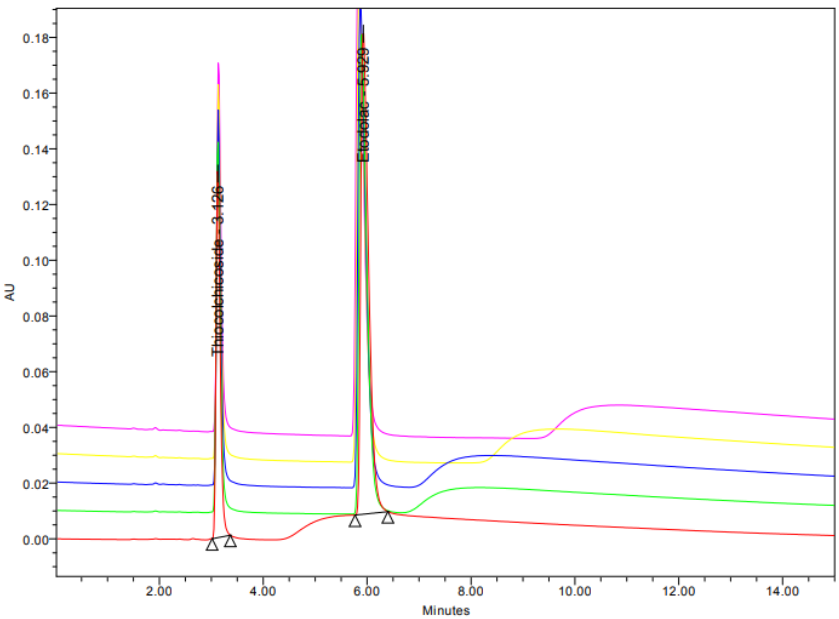


Figure 4: Overlay of System suitability for standard chromatograms.

Table 3: System suitability results.

		Thiocolchicoside		Etodolac	
		Retention Time	Peak area	Retention Time	Peak area
1	Mean*	3.135	745776.2	5.879	1824993.7
2	Std. Dev	0.006	1318.7	0.036	3952.8
3	% RSD	0.20	0.2	0.61	0.2

***** Average of five replicate injections

Discussion: The developed method successfully passed the system suitability criteria, as evidenced by a theoretical plate value exceeding 2000, tailing factor not exceeding 2.0, and % RSD remaining below 2.0%.

3.2 SPECIFICITY

Specificity in chromatography refers to the ability of a method to clearly identify and measure the target analyte, even when other components such as impurities, degradation products, matrix elements, or other analytes are present. High specificity ensures that the chromatographic method can accurately and precisely separate and detect the target compound without interference from other substances in the sample.

Blank

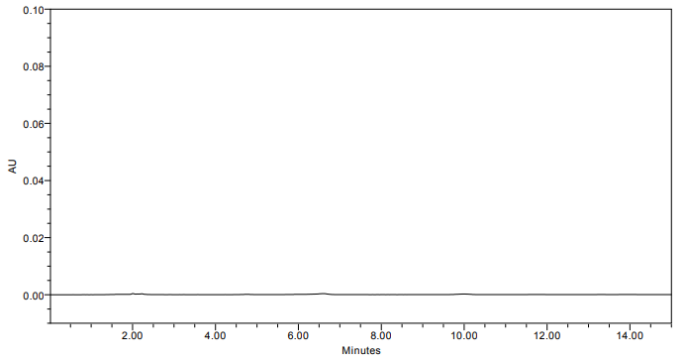


Figure 5: Blank chromatogram.

Discussion: The specificity chromatogram for the blank showed no interference with the primary peak, indicating that the technique is specific.

3.3 ACCURACY

Accuracy in an analytical method is defined by how closely the test results match the true or accepted value. To evaluate accuracy, sample solutions are prepared at different concentrations, typically 50%, 100%, and 150% of the target analyte's expected concentration. These samples are then injected into the system, and the percentage recovery is determined by comparing the measured values to the expected ones.

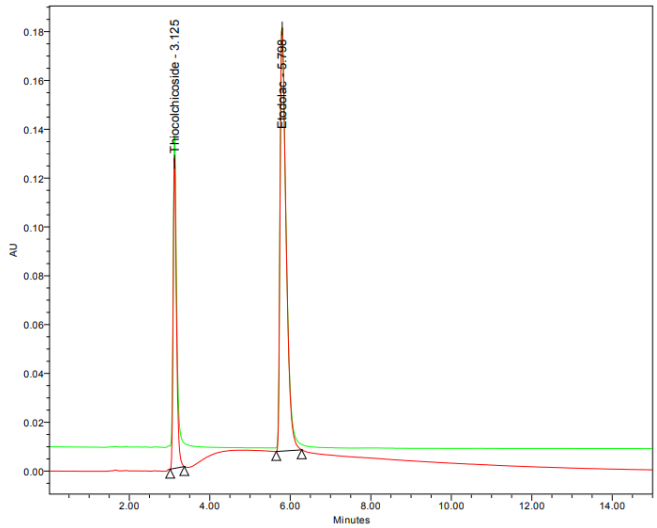


Figure 6: Sample solution chromatogram.

Table 4: Results for Accuracy.

S.No	Sample solution concentration*	Thiocolchicoside		Etodolac	
		% RSD	Recovery %	% RSD	Recovery %
1	50%	0.67	99.65%	0.15	100.15%
2	100%	0.45		0.31	
3	150%	0.22		0.14	

***** Average of three replicate injections

Discussion: The RSD percentage does not exceed 2.0%. The approach is considered accurate because the percentage recovery acceptability criterion for Thiocolchicoside and Etodolac ranges between 98.0% and 102.0%.

3.4 PRECISION

Consistency and reproducibility involve obtaining identical results when the same sample is analysed multiple times under the same conditions. Precision is used to quantify the degree of variation or dispersion in these results and is usually represented by statistical metrics like standard deviation, relative standard deviation (RSD), or coefficient of variation.

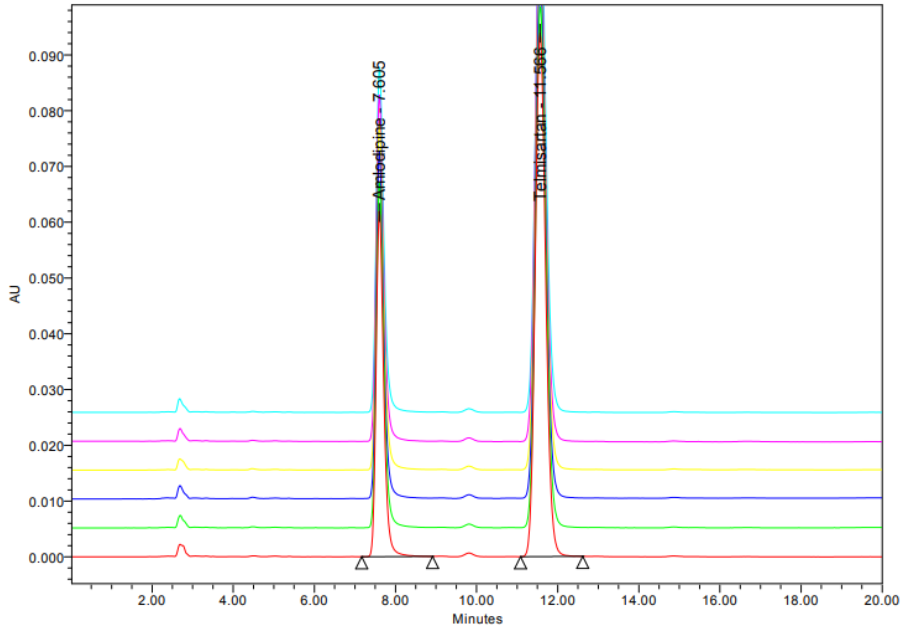


Figure 6: Precision chromatograms overlay of Thiocolchicoside and Etodolac.

Table 5: Method precision results for Thiocolchicoside and Etodolac.

S. No	Peak Name*	Average	SD	% RSD
1	Thiocolchicoside	732349	0.44	0.4
2	Etodolac	1810975	0.24	0.2

***** Average of six replicate injections

Discussion: The RSD percentage does not exceed 2.0%. The approach is regarded as precise since the precision values are within the acceptable range for Thiocolchicoside and Etodolac.

3.5 LINEARITY

Linearity in chromatography refers to the relationship between the concentration or amount of analyte injected and the detector response. It indicates how well the detector response correlates with changes in analyte concentration across a defined range. A chromatogram demonstrating linearity would show that as the analyte concentration increases or decreases, the detector response (peak area or height) changes proportionally. This characteristic is essential for precise and reliable quantification of substances in samples using chromatographic methods. Thiocolchicoside and Etodolac were made in five concentrations, and each concentration was injected three times to test linearity. A graph was plotted taking concentration of analyte on x-axis and peak area on y-axis.

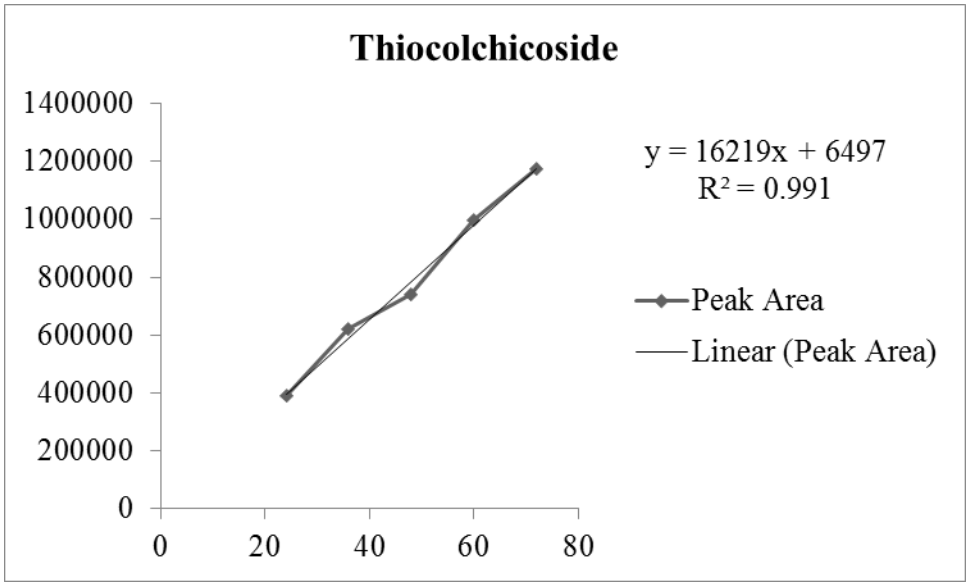


Figure 7: Linearity graph for Thiocolchicoside.

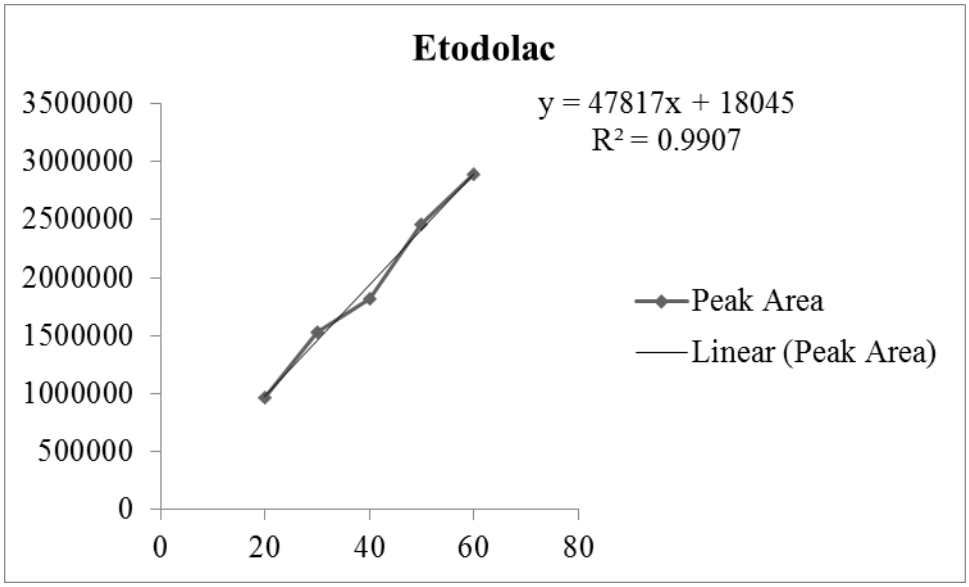


Figure 8: Linearity graph for Etodolac.

Table 6: Results for linearity.

THIOCOLCHICOSIDE		ETODOLAC	
Conc. in PPM*	**Peak Area**	**Conc. in PPM***	**Peak Area**
24	390989	20	963809
36	621137	30	1526790
48	738983	40	1815648
60	998588	50	2458788
72	1175422	60	2888684
Regression Equation	y = 16219x + 6497	Regression Equation	y = 47817x + 18045
Linearity Correlation Coefficient (R^2)	0.9910	Linearity Correlation Coefficient (R^2)	0.9907

***** Average of three replicate injections

Discussion: The R^2 values are within the acceptability standards, i.e. NLT 0.99 for Thiocolchicoside and Etodolac, indicating that the method is linear.

3.6 RANGE

The range is the interval between the highest and lowest analyte concentrations in the sample across which the technique has been demonstrated to be exact, accurate, and linear.

Table 7: Range values for Thiocolchicoside & Etodolac.

Percentage of solution	% RSD for Thiocolchicoside	% RSD for Etodolac
50%	0.08%	0.19%
100%	0.20%	0.04%
150%	0.36%	0.11%

Bracketing standard

Bracketing is an analytical approach in which samples are evaluated at the higher and lower limits of a specified range to ensure accuracy and precision over the whole range.

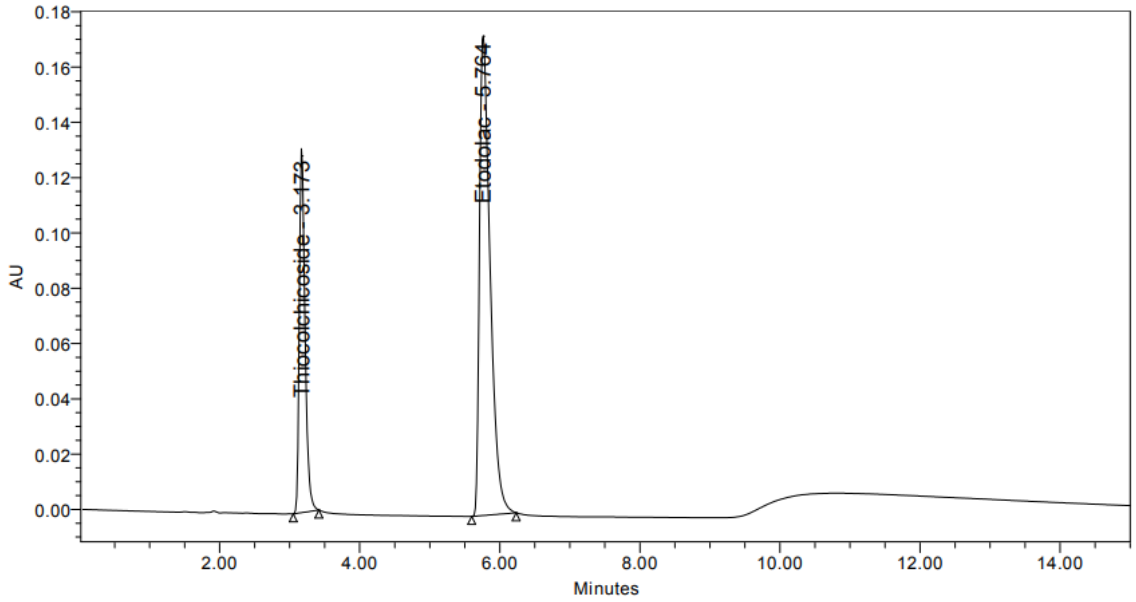


Figure 9: Sample solution Bracketing Standard chromatograms.

Method application to the analysis of Thiocolchicoside and Etodolac

The proposed and verified technique was used to simultaneously determine Thiocolchicoside and Etodolac in commercially available tablet dosage form FYTOLAC-T4. The assay findings are reported in the table below. It was discovered that no dosage form excipients interfered with their analysis, indicating that the approach is suitable for routine quality control work.

Table 8: %Assay of Thiocolchicoside and Etodolac.

FYTOLAC-T4 Tablets		
Name of the drug	**Labeled claim (mg)**	**%Assay***
Thiocolchicoside	4	98.99
Etodolac	300	99.57

***** Average of six replicate injections

SUMMARY AND CONCLUSION

The study successfully established and validated a particular, new, and accurate RP-HPLC technique for simultaneously estimating Thiocolchicoside and Etodolac in Tablet Dosage Forms.

Parameters	Thiocolchicoside	Etodolac
% Recovery in Accuracy	99.65%	100.15%
% RSD in Precision	0.40%	0.20%
Linearity Correlation coefficient	0.9910	0.9907
% Assay	98.99%	99.57%

The study focused on the development and validation of a reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Thiocolchicoside and Etodolac in tablet dosage form. This method was optimized to ensure effective separation and

quantification of tablet dosage form. The use of a mobile phase consisting of methanol, acetonitrile, and water in the ratio of 30:50:20 (v/v/v) allowed for efficient chromatographic separation with a flow rate of 1 mL/min. The detection was performed using a UV detector set at 226 nm, which provided clear and distinct peaks for both Thiocolchicoside and Etodolac. Parameters such as theoretical plates, tailing factor, and % RSD were all within acceptable limits, ensuring that the system was functioning optimally.

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