

Accurate estimation of some pharmaceutical compounds using HPLC technology

ABSTRACT

For the assessment of different chemical components in pharmaceutical and biological samples, High Performance Liquid Chromatography (HPLC) can be defined as a commonly utilized analytical method in pharmaceutical samples. HPLC offers high sensitivity, specificity, and accuracy, making it a preferred method for the analysis of complex samples. In this technique, the sample is prepared by extracting, purifying, and concentrating the target analyte from the matrix. Mobile and stationary phases are after that used in chromatographic separation of the prepared sample. Several detectors, including fluorescence, UV-vis, and mass spectrometry, are used to identify and measure the eluted compounds. HPLC is capable of estimating a wide range of elements, including small molecules, peptides, and proteins, making it an essential tool in pharmaceutical research and development. The theoretical plates, peak tailing, and % assay have not been significantly impacted by the deliberate alterations made to the method. This implies that the existing technique is robust. The low values of LOQ and LOD show how sensitive the suggested approach is. The current research used a standard drug solution with six replicates to examine the system suitability parameters. It was discovered that the computed parameters fell under the acceptable criteria. The theoretical plate count, tailing factor, and HETP parameters are all within acceptable ranges. The results of this work are compared with those of earlier research. Upon analyzing the chart, it can be inferred that the proposed method presents certain benefits. The investigation employed a Kromasil C18 column. The obtained retention time is comparatively lower than that of all other methodologies. The maximum quantifiable threshold is 600 µg/mL, surpassing or equating all of the aforementioned techniques. The duration of the execution is also minimal. Therefore, the author draws the conclusion that the HPLC method proposed exhibits sensitivity and reproducibility in analysis of etodolac and thiocolchicoside in combined tablet dosage form, while also requiring a short analysis time.

Keywords: HPLC, drugs, accuracy, etodolac, thiocolchicoside

I. INTRODUCTION

Etodolac can be defined a non-steroidal drug that has analgesic, antipyretic, and

anti-inflammatory effects 1. It is utilized to manage acute pain as well as treat osteoarthritis and rheumatoid arthritis. Because it can inhibit cyclo-oxygenase enzyme, which in turn inhibits prostaglandin synthesis, it has therapeutic effects 2. Etodolac is official in I.[1]

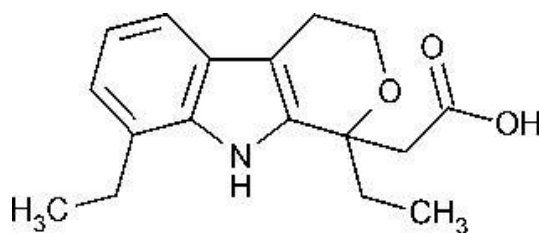


Figure 1. Chemical structure of etodolac

1.1 Mechanism of Action

Etodolac functions as a prostaglandin synthesis inhibitor, specifically targeting those involved in the physiological responses of fever, swelling, pain, and inflammation. The administration of Etodolac is in the form of a racemate. Like other nonsteroidal anti-inflammatory drugs (NSAIDs), the S- enantiomer is pharmacologically active, whereas R- enantiomer is pharmacologically inactive. Both enantiomers exhibit stability and there is no observed in vivo conversion from R- to S-. Etodolac functions as a COX enzyme inhibitor, resulting in a reduction in the production of peripheral prostaglandins that play a role in the mediation of inflammation, similar to other nonsteroidal anti-inflammatory drugs. Etodolac inhibits the COX enzyme through binding to its upper active site as well as obstructing the entry of its substrate, arachidonic acid. This mechanism of action has been documented in literature. The hypothalamus plays a pivotal role in inducing antipyresis, which leads to peripheral vasodilation, augmented cutaneous blood flow, and consequent dissipation of heat[1,2]

1.2 Pharmacokinetics

Etodolac exhibits high bioavailability upon oral administration. Mass balance studies indicate that the tablet or capsule formulation of etodolac exhibits a systemic availability of no less than 80%. Upon oral administration, Etodolac experiences negligible first-pass metabolism. The range of mean peak plasma concentrations falls between 14 ± 4 to 37 ± 9 $\mu\text{g/mL}$ following single doses of 200 to 600 mg, with attainment occurring in 80 ± 30 minutes. The bioavailability of etodolac remains unaffected by food intake. The plasma protein binding of Etodolac is predominantly to albumin, with a binding rate exceeding 99%. The primary site of metabolism for Etodolac is the liver.[3] Considerable amounts of etodolac metabolites have been found in human subjects' plasma and urine. Etodolac metabolites include etodolac glucuronide and 6-, 7-, and 8-hydroxylated etodolac. When etodolac metabolites undergo hydroxylation, they are after that glucuronidated, excreted by the kidneys, and partially eliminated by feces. Seventy-two percent of the dose is excreted in urine as a mixture of the parent drug and its metabolites, with the remaining about 1% of administered dose being removed in the urine without any changes.[4]

Thiocolchicoside is a naturally occurring derivative of colchicine that has been further modified through semi-synthesis, resulting in the creation of a new compound known as a semisynthetic derivative of colchicoside. This substance functions as a muscle relaxant that acts centrally. Furthermore, it exhibits anti-inflammatory and analgesic properties. The utilization of thiocolchicoside-containing formulations has been prevalent in European nations for a considerable period. These formulations were initially approved for the treatment of symptomatic spasms and contractures associated with muscular, rheumatic, and neurologic illnesses. This is attributed to the comparatively lower sedative effects of thiocolchicoside in comparison to other centrally acting muscle relaxants. Thiocolchicoside has been recognized as an official substance in the n pharmacopoeia of 2010.[5]

Thiocolchicoside exhibits preferential binding to gamma-aminobutyric acid and glycinergic receptors. Empirical and medical data indicate that the epileptogenic effect of the compound cannot be accounted for by its association with glycine receptors Thiocolchicoside exhibits a preference for interacting with a specific cortical subtype of gamma- Aminobutyric Acid Type A (Gaba_A) Receptor[6]

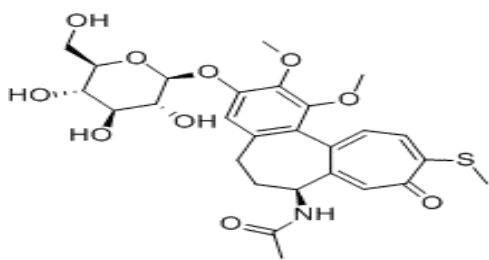


Figure 2. Chemical structure of thiocolchicoside *Mechanism of action*

This particular receptor subtype expresses low-affinity binding sites for gaba, which can account for the proconvulsant effect of thiocolchicoside. This finding contradicts previous research that proposed a gaba mimetic mechanism as an explanation for its muscle relaxant characteristic. The muscle relaxation action of thiocolchicoside is not significantly influenced by gabab receptors. The precise mechanism underlying muscle relaxation remains unknown. However, based on the currently available evidence, it is plausible that inhibition of glycine receptors may serve as a potential mechanism.[7]

Pharmacokinetics upon oral administration, the presence of thiocolchicoside in plasma is not detected. Two metabolites, namely s118.0740 and s159.0955, have been detected. The maximum concentration (c_{max}) of thiocolchicoside is achieved within 30 minutes following intramuscular administration. For a dose of 8 mg and 4 mg, c_{max} values are 175 ng/ml and 113 ng/ml, respectively. When 8 mg of thiocolchicoside is taken orally in one dose, auc and c_{max} of s118.0740 are about 130 ng.h/ml and 60 ng/ml, respectively. S159.0955's data show a notable decline, with a c_{max} of about 13 ng/ml and an auc that ranges from 15.5 ng.h/ml (within 3 hrs) to 39.7 ng.h/ml (within 24 hrs). Following an im dose of 8 mg, the apparent volume of distribution of thiocolchicoside is calculated to be about 42.7 [8]. Following injection, the thiocolchicoside's apparent t_{1/2} is 1.5 hours, and the plasma clearance is 19.2 l/h. Feces or urine do not contain any unaltered thiocolchicoside following oral dosing. Demethylthiocolchicine, also known as s159.0955 or m2, is a compound that can damage cells that are dividing and result in harmful outcomes, like changes to neoplastic growths, toxicity to developing embryos, and reduced male fertility. Consequently, it is suggested that the indicated oral dose must not be used for longer than seven days, and the intramuscular dose should not be taken for longer than five days. Topical skin preparations have reduced levels of toxicity.[9]

Table 1. List of Representative Combined Dosage Forms of Etodolac and Thiocolchicoside

S. N	Trade name	Formulation	Strength	Manufacturer
1.	Etogesic-MR	Tablet	400 mg +4 mg, 400	Cadila Healthcare, Ahmeda
2.	Proxy m	Tablet	200 mg +4mg	Emcure Pharmaceuticals
3.	Etova - Forte	Tablet	400 mg+8 mg	Ipca Laboratories.
4.	EtornextT H	Tablet	400 mg+4 mg	<u>Alembic Pharmaceuticals.</u>
5.	Etosma MR	Tablet	400mg +8mg	<u>Molekul e</u> , Mumbai

Just a few analytical techniques using HPLC and HPTLC were reported for the simultaneous quantification regarding etodolac and thiocolchicoside in both bulk form as well as formulations, according to the findings of a review of the relevant literature.[10] HPTLC and HPLC are two examples of such techniques. Kiran Rathod and colleagues (2012) created a technique called reversed-phase high-performance liquid chromatography (RP-HPLC) as part of their research. This method was created for simultaneous analysis of thiocolchicoside and etodolac in a combined tablet formulation. The approach involved use a Phenomenex C-18 column in conjunction with a mobile phase comprising a blend of methanol and phosphate buffer at a volume-to-volume ratio of 85:15. The volumetric flow rate was determined to be 0.8 milliliters per minute by the use of a measuring device. The 259 nm wavelength was applied for the detecting process. According to the results of the research, the amount of time that it takes for etodolac and thiocolchicoside to be retained by the body is 4.39 minutes and 3.52 minutes, respectively[11]. According to the findings of the research, linearity could be identified anywhere between concentration range of 4-24 g/ml for thiocolchicoside and 16- 96 g/ml for etodolac.

Raghavender et al. (13) provided an explanation of HPLC approach for concurrent measurement of thiocolchicoside and etodolac in a tablet formulation that was mixed. In order to carry out chromatography, the experiment required the use of a Symmetry C 18 column. At pH 3.0, acetonitrile and K₂HPO₄ in a 50:50 ratio made up the mobile phase. The concentrations of K₂HPO₄ were 0.02 M and 0.003 M, respectively. In the course of the investigation, a wavelength of 254 nanometers was used. Both thiocolchicoside and etodolac had different levels of retention, with thiocolchicoside having a time of 2.638 minutes and etodolac having a duration of 4.275 minutes. Within concentration ranges of 1.0-6.0 and 100-600 g/mL, respectively, linearity was seen with thiocolchicoside and etodolac, and their respective coefficients of determination (r²) were 0.999 and 1.0.[12]

In the research that Alagar Raja and colleagues (2014) conducted, they used a Symmetry C18 column to carry out simultaneous determinations of etodolac and thiocolchicoside in a tablet combination that included both substances. The mobile phase that was employed in this investigation was a combination of acetonitrile as well as potassium dihydrogen phosphate buffer that had a pH of 3.0. The ratio of the two components was 50:50 (v/v). The measurement was taken at a wavelength of 255 nanometers when the detection was carried out. The action of moving something from one location to another or transferring something from one location to another.

The volume that passed through each minute was one milliliter. According to the results of the research, the amount of time that it takes for etodolac and thiocolchicoside to be retained by the body is 4.27 and 2.63 minutes, respectively. The research's conclusions indicate that linearity was demonstrated to exist between 100 and 600 g/mL for etodolac and between 1-6 g/mL for thiocolchicoside, with correlation coefficients of 1.0 and 0.999, respectively. This was the case for both compounds. It was established that the retention times for etodolac were three minutes and twelfths of an hour, respectively. The pharmaceutical ingredients were observed using a wavelength of 260 nanometers in order to conduct the test.[13]

Isolating thiocolchicoside and etodolac in a combination tablet formulation was the topic of a research that Patel and colleagues conducted in 2016. In that work, they presented a method. This was accomplished by using a reverse phase C18 column in conjunction with a mobile phase that was constituted of acetonitrile and phosphate buffer with a pH of 5.0 in the ratio of 60:40 by volume. The volumetric flow rate was determined to be one milliliter per minute based on the measurement taken. The value 259 was used in the measurement that was performed.[14]

The research's conclusions showed that linearity was present in the concentration ranges of 50–250 g/mL and 10–50 g/mL for etodolac and thiocolchicoside, respectively, with r^2 values of 0.9993 and 0.9999. According to the findings of the research, the retention time for thiocolchicoside was found to be 2.45 mins, while the retention time for etodolac was found to be 7.10 minutes, and the resolution was found to be 16.55. An HPTLC method was devised by Swapnil et al. (17) to simultaneously determine the amounts of etodolac and thiocolchicoside in bulk as well as tablet formulations. In order to carry out the chromatographic separation of the medicines, aluminum plates were employed

The stationary phase in such experiment was silica gel 60 F254, and the solvent system was a mixture of methanol, toluene, and chloroform with a volumetric ratio of 6:2:3. At a wavelength of 238 nm, a densitometric assessment was carried out on the zones that had been separated. The R_f values of 0.20 and 0.50, respectively, proved to be optimal for achieving a clean separation of etodolac and thiocolchicoside.[15]

In light of the aforementioned results, an effort was made to develop an HPLC method with improved accuracy and specificity for the analysis of etodolac and thiocolchicoside in unprocessed drug specimens (API) as well as in amalgamated tablet formulations.[16,17]

The technique that was developed went through the appropriate validation procedures in accordance with guideline 18 of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals intended for Human Use (ICH).[18]

II. MATERIALS AND METHODS

A. Instrumentation

The study utilized a waters alliance liquid chromatograph (model 2695) equipped with kromasil c18 column (150 x 4.6 mm, 5m) and a diode array detector (model 2996). The

injections were performed utilizing an automated injector. The empower2 software was utilized for the purpose of managing data. The solubility of the substances was increased through the utilization of an ultrasonicator (ultrasonics 3.51) for sonication. The substances were weighed using a sartorius balance, specifically model cpa225d.

B. Drugs, chemicals and solvents

The reference compounds utilized in this study were the pure samples of etodolac and thiocolchicoside, which were procured from sl drugs & pharmaceuticals located in hyderabad, andhrapradesh. The study employed the utilization of the commercial tablet formulation of etodolac (400mg) and thiocolchicoside (4mg), which is known as "etova - mr" and is produced by ravenbhel healthcare pvt. Ltd. Located in amritsar, punjab. The chemicals utilized in this study, namely ammonium acetate, orthophosphoric acid, and HPLC grade methanol, were procured from rankem fine chemicals ltd. Located in mumbai. Hplc-grade water was generated through utilization of the millipore milli-q system

C. Preparation of acetate buffer solution (PH 3.7)

A quantity of 0.77 grams of ammonium acetate was introduced into a beaker with a volume of 1000 milliliters, which already contained approximately 800 milliliters of water. The contents were thoroughly homogenized and the final volume was adjusted to 1000 ml using distilled water. Subsequently, the ph value of the solution was modified to 3.7 by utilizing ortho phosphoric acid. Subsequently, the solution underwent filtration utilizing a membrane filter with a pore size of 0.45 μ .

D. Preparation of the mobile phase

A solution was prepared by combining 200 ml of acetate buffer (pH 3.7) with 800 ml of methanol in a one-liter flask, resulting in a mixture with a volume ratio of 20:80 v/v. The contents underwent degassing in an ultrasonic bath for a duration of 5 minutes, followed by filtration through a 0.45 μ membrane filter. The aforementioned solution was employed as the mobile phase in the process of chromatography.

2.1 Stock and working standard solutions The reference compounds of etodolac and thiocolchicoside, weighing 400 mg and 4 mg respectively, were carefully measured and subsequently transferred into a volumetric flask with a capacity of 100 ml. Subsequently, a total of 60 ml of diluent was introduced, followed by a 5-minute sonication process, and a subsequent addition of diluent to achieve the final volume. As the standard solution, the previously specified one was applied. A 10 ml volumetric flask was filled with 1.0 ml of stock solution, and the mobile phase was then diluted to volume to create the working standard solution. The present study employed a working standard solution of the combination, consisting of 400 μ g/ml and 4 μ g/ml of etodolac and thiocolchicoside, respectively.

2.2 Optimization of chromatographic conditions and method development

Throughout technique optimization research, the current work explored several solvent as well as buffer proportions and combinations on a Kromasil C18 (150 x 4.6 mm, 5 μ m) column to get the best resolution of thiocolchicoside and etodolac. Using isocratic elution with a mobile phase of acetate buffer (pH 3.7) and methanol (20:80 v/v) at a flow rate of 0.8 mL/min, the drugs have been satisfactorily separated in 10 mins. At a wavelength of 242 nanometers, the compounds of interest in extracted solution were detected and quantified. Under ideal circumstances, the retention durations for thiocolchicoside and etodolac were

found to be 2.285 and 3.176 minutes, respectively. Before the drug solution was injected, the mobile phase was pumped through the column to equilibrate it for at least 20 mins. A standard chromatogram of a mixed medication solution is shown in Figure 3.

Table 2. Optimized Chromatographic Conditions of the Proposed Method

Stationary phase	Kromasil C18(150 X 4.6 mm,um)
Mobile phase	Acetate buffer (pH 3.7) – methanol (20:80 v/v)
Flow rate	0.8 mL/min
Column temperature	30 ⁰ C
Injection volume	10 uL
Detection wavelength	242 nm
Run time	10 min

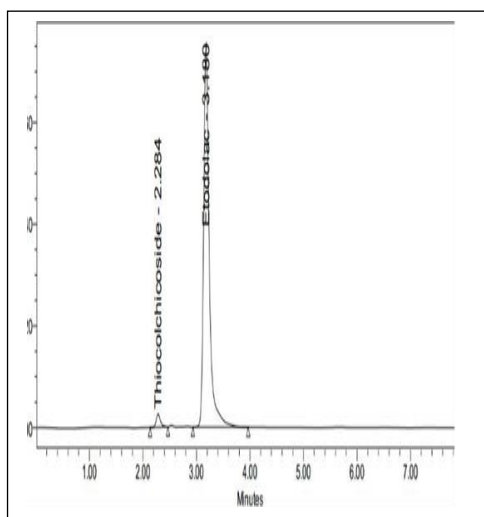


Figure 3. A representative chromatogram of the mixed working standard solution

2.3 Validation of the Proposed Method

A. Linearity

Different concentrations of etodolac and thiocolchicoside including the established standard working concentration, were formulated in the solvent. The column was subjected to three injections of 10 μ L for each concentration and the sample size was n=3. The wavelength of 242 nm was utilized to measure the absorbance, and the resulting chromatograms were duly recorded. The computation of mean peak areas was performed on chromatograms at different concentration levels, and linearity plots were produced for each drug through the graphical representation of the concentration versus the mean peak area.

Table 3. Linearity data for etodolac

Concentration of etodolac (µg/mL)	Peak area	Mean peak area	SD	% RSD
100	241056	241590	809.59	0.35
	242558			
	243007			
200	491432	490879	370.39	0.1
	490776			
	492551			
300	730882	736411	1884.83	0.23
	731556			
	732091			
400	961332	969502	153.44	0.19
	960775			
	962551			
500	121664	1219541	458.09	0.05
	121998			
	1212853			
600	146133	1466470	466.72	0.04
	1462152			
	1464011			

Tables 2 and 3 present the linearity data pertaining to etodolac and thicolchicoside, respectively. The linearity plots for etodolac and thicolchicoside are depicted in Figures 4 and 5, respectively. The chromatograms obtained from the linearity solutions are typically represented in figures 6 to 11

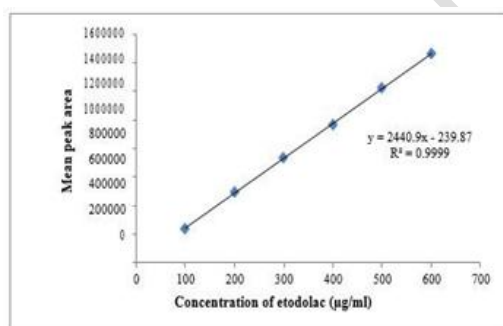


Figure 4. Linearity plot for etodolac

Table 4. Linearity data for thicolchicoside

Concentration of etodolac µg/L	Peak area	Mean peak area	SD	%RSD
1	208456	20738	133.23	0.64
	207568			
	203327			
2	371462	38864	150.20	0.39
	380783			
	372471			
3	530522	58701	323.23	0.55
	536156			
	533791			
4	761353	67664	165.17	0.27
	760732			
	762533			

5	916649	76341	224.68	0.23
	919984			
	921285			
6	116133	96952	243.12	0.47
	116215			
	116401			

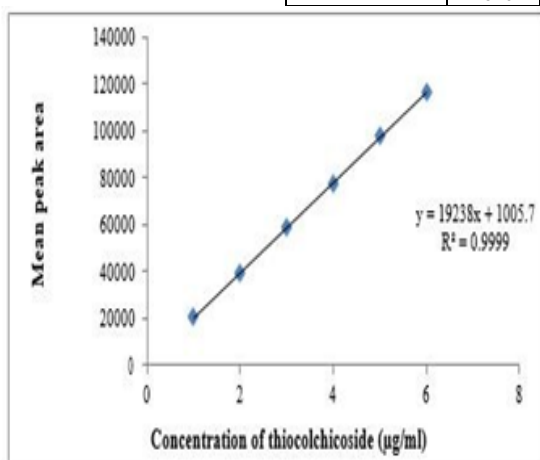


Figure 5. Linearity plot for thiocolchicoside

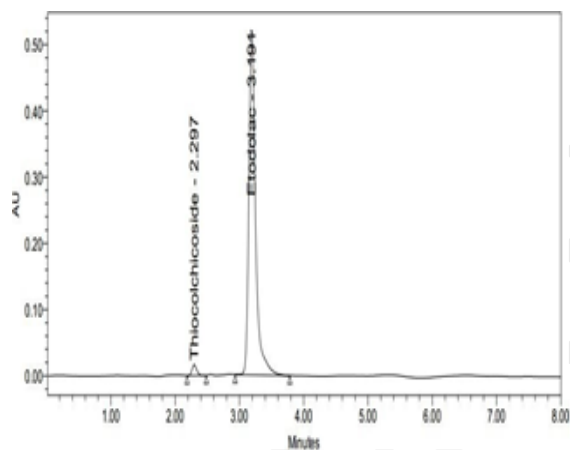


Figure 6. A representative chromatogram of 25% concentration level of etodolac and thiocolchicoside

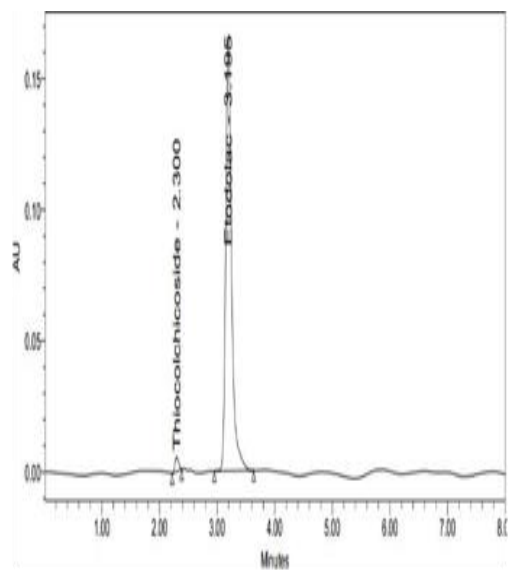


Figure 7. A representative chromatogram of 50% concentration level of etodolac and thiocolchicoside

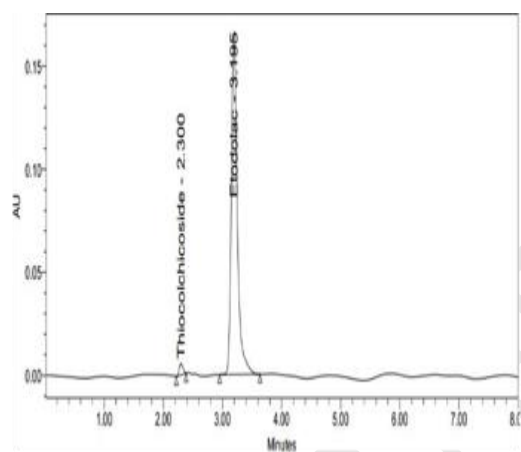


Figure 8. A representative chromatogram of 75% concentration level of etodolac and thiocolchicoside

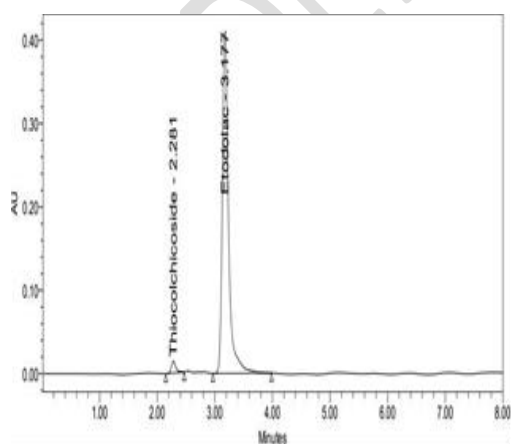


Figure 9. A representative chromatogram of 100% concentration level of etodolac and thiocolchicoside

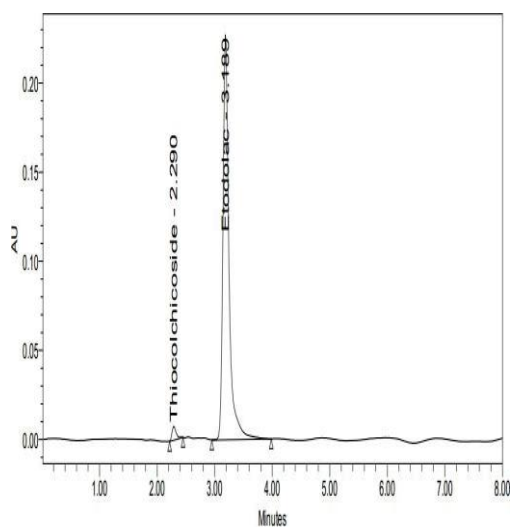


Figure 10. A representative chromatogram of 125% concentration level of etodolac and thiocolchicoside

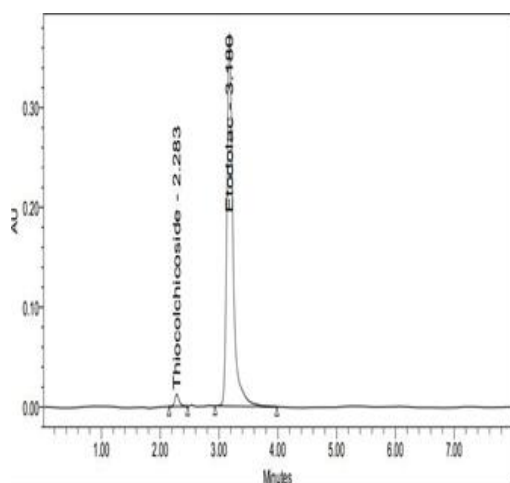


Figure 11. A representative chromatogram of 150% concentration level of etodolac and thiocolchicoside

B. Precision

Analyzing standard solutions of etodolac and thiocolchicoside on the same day (n=6) and on two consecutive days, respectively, allowed for the assessment of repeatability and intermediate precision. The findings of the investigations into repeatability and intermediate precision are shown in Tables 5 and 6, respectively

Table 5. Repeatability data

s.No.	Peak areas of etodolac	Peak areas of thiocolchicoside
1	969579	77513
2	968112	77553
3	969414	76996

4	970135	77374
5	969958	77294
6	970066	77579
Average	969544.0	77384.8
SD	756.56	219.98
%RSD	0.078	0.284

Table 6. Intermediate precision data

Day	Average peak areas of etodolac (n=6)	Average peak areas of thiocolchicoside (n=6)
1	968532	77405
2	969421	77490
Over all average	969043	77471
SD	1305.88	309.97
%RSD	0.144	0.3994

Table 7. Recovery data of etodolac

Concentration (µg/mL)	Peak area	Recovery	Mean recovery	SD of recoveries	% recovery
200	491874	201.7	201	0.29	100.84
	490456	201.1			100.55
	491012	201.3			100.67
400	970415	397.8	97.8	0.31	99.45
	971284	398.2			99.54
	969784	397.6			99.39
600	1459781	598.4	600.1	1.47	99.73
	1465416	600.7			100.11
	1466451	601.1			100.18

Table 8. Recovery data of thiocolchicoside

Concentration (µg/mL)	Peak area	Recovery	Mean recovery	SD of recoveries	% recovery
2	37578	2.0		0.02	100.28
	38033	2.0			101.46
	37164	2.0			99.20
4	77018	4.1		0.01	101.39
	76476	4.0			100.69
	76865	4.0			101.19
6	114145	6.0		0.05	99.76
	115875	6.1			101.26
	115165	6.0			100.64

D. Limit of detection (LD) and limit of quantitation (LOQ)

The LOQ and LOD were computed by making use of the residual standard deviation of the answer as well as the slope of the regression line. It was discovered that the LOD and LOQ for etodolac and thiocolchicoside are 6.092 and 18.46 g/mL and 0.074 and 0.225 g/mL respectively.

In accordance with the ICH requirements, the method's dependability was tested across a wide range of situations, including changes to the flow rate, temperature of the column, and chemical make-up of the mobile phase. The following is a summary of the findings that were achieved by purposefully altering the settings of the procedure and the data.

Table 9. Robustness data for etodolac

Chromatographic condition	Value	Retention time(min)	Tailing factor	Number of theoretical plates
Flow rate (mL/min)	0.7	3.196	1.40	5483
	0.8 (O)	3.171	1.47	5155
	0.9	2.846	1.36	5213
Temperature (°C)	25	3.121	1.36	5419
	30 (O)	3.171	1.47	5155
	35	3.090	1.36	5459
Mobile phase (Buffer: Methanol)	18:82	3.068	1.36	5586
	20:80 (O)	3.171	1.47	5155
	22:78	3.235	1.36	5548

Table 10. Robustness data for thiocolchicoside

Chromatographic condition	Value	Retention time(min)	Tailing factor	Number of theoretical plates
Flow rate (mL/min)	0.7	2.292	1.09	5187
	0.8(O)	2.284	1.20	3995
	0.9	2.049	1.31	4030
Temperature (°C)	25	2.268	1.04	4972
	30(O)	2.284	1.20	3995
	35	2.255	1.03	4933
Mobile phase (Buffer: Methanol)	18:82	2.266	1.11	5194
	20:80(O)	2.284	1.20	3995
	22:78	2.267	1.01	3965

(O) – Optimised value

2.4 Specificity of the Proposed Method

The approach' specificity has been assessed in relation to potential interference arising from the inclusion of excipients in tablet formulation. No interfering peaks have been observed within retention time ranges of the drug matrix as indicated by the HPLC chromatograms. Figures 2 and 12 depict the chromatograms that were obtained during the analysis of the working standard solution and the formulation sample solution, respectively. The presented data indicates a distinct separation of the chosen pharmaceuticals..

A. System suitability

In order to determine whether or not the system is suitable, six iterations of the working standard sample were carried out, and the parameters that resulted from these and the parameters that resulted from these iterations were acquired. These parameters included the peak retention duration, the number of theoretical plates (n), and the tailing factor. The aforementioned discoveries are presented in tabular form for your perusal

Table 11. System suitability parameters for the proposed method

S	Parameter	Result for etodolac	Result for thiocolchicoside
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No.		(n=6)	e (n=6)
1	Retention time(min)	3.171	2.284
2	Retention time(min)	3.171	2.284
3	Number of theoretical plates	5155	3995

2.5 Applicability of the method for estimation of the drug in tablet dosage form

A. Estimation of the drugs from tablet dosage forms

Ten tablets of "Etova - MR" comprising of etodolac (400 mg) and thiocolchicoside (4 mg) were pulverized into a fine powder. A 100 mL volumetric flask was utilized to transfer a quantity of powder that is equal to 400 mg of etodolac. A volume of 80 milliliters of the diluent was introduced and subjected to sonication for a duration of 30 minutes. The diluent was added to the volume, and subsequently, the contents were thoroughly mixed. The mixture underwent filtration using a 0.45 μ membrane filter, with initial few mL of filtrate being discarded. A volume of 1.0 mL was extracted from the filtered solution and subsequently added to a 10 mL volumetric flask. The flask was then filled to the brim with the diluent

Table 12. Recovery of the drugs from the tablet dosage form "Etova- MR"

Drug	Labeled amount (mg)	Amount recovered (mg)(n=6)	%Recovery
Etodolac	400	400.23	100.1
Thiocolchicoside	4	4.01	100.3

Table 13. A comparison chart of the proposed (current) method with the reported methods for determination of etodolac and thiocolchicoside

Method	Column	Mobile phase	Flow rate	Retention times	Linearity range	Run time
Proposed method	Kromasil C18 (150 x 4.6 mm, 5 μ m)	acetate buffer (pH 3.7) and methanol (20:80 v/v)	0.8 mL/min	etodolac 3.171 min. thiocolchicoside 2.284 min.	100 – 600 μ g/mL for etodolac and 1 – 6 μ g/mL for thiocolchicoside.	10 min
Kiran Rathod <i>et al</i> ¹²	Phenomenex C-18 (250 x 4.6 mm, 5 μ)	methanol and phosphate buffer pH 6.0 (85:15v/v)	0.8 mL/min	etodolac 4.39 min. thiocolchicoside 3.52 min.	16-96 μ g/ml for etodolac and 4-24 μ g/ml for thiocolchicoside.	-
Raghavender <i>et al</i> ¹³	Symmetry C 18 (150 x 4.6 mm, 5 μ)	0.02 MH ₂ PO ₄ , 0.003 M K ₂ HPO ₄ (pH 3.0) and acetonitrile (50:50)	1.0 mL/min	etodolac 4.275 min. thiocolchicoside 2.638 min.	100 – 600 μ g/mL for etodolac and 1 – 6 μ g/mL for thiocolchicoside.	-
Alagar Raja <i>et al</i> ¹⁴	Symmetry C 18 (150 x 4.6 x 5 μ)	acetonitrile and: potassium dihydrogen phosphate buffer (pH 3.0) (50:50v/v)	1.0 mL/min	etodolac 4.27 min. thiocolchicoside 2.63 min.	100 – 600 μ g/mL for etodolac and 1 – 6 μ g/mL for thiocolchicoside.	-
Phawade Pradnya <i>et al</i> ¹⁵	Hypersil C18 (250 x 4.6mm, 5 μ m)	phosphate buffer (pH 6.8)and acetonitrile (70:30 v/v)	1.0 mL/min	etodolac 12 min. thiocolchicoside 3min	-	-
Patel <i>et al</i> ¹⁶	reverse phase C18 (250x4.6 mm, 5 μ)	acetonitrile: and phosphate buffer (pH 5.0) (60:40 v/v)	1.0 mL/min	etodolac 7.10 min. thiocolchicoside 2.45 min.	50-250 μ g/mL for etodolac and 10-50 μ g/mL for thiocolchicoside.	-

The previously described solution was then subjected to six rounds of chromatography. Through examining the acquired chromatograms, the average drug content in formulation was ascertained. A representative chromatogram of the tablet analysis of thiocolchicoside and etodolac is shown in Figure 12.

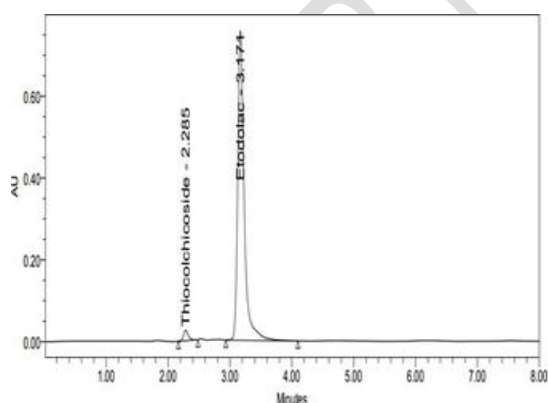


Figure 12 Representative chromatogram obtained from analysis of formulation sample solution

B. Method Suitability

The proposed method was utilized to analyze the commercial tablet formulation known as "Etova - MR," which contains etodolac (400 mg) and thiocolchicoside (4 mg). The findings of this analysis are presented in Table 12. The method proposed yielded a recovery that was observed to be in concurrence with the labeled quantity of the drugs. This substantiates the

appropriateness of the approach for the assessment of thicolchicoside and etodolac in tablet formulations

CONCLUSION

The author endeavored to establish a meticulous, precise, and responsive HPLC technique for the evaluation of thicolchicoside and etodolac in both bulk drug and combined dosage form. In order to examine the constituent peaks, various blends of methanol and acetate buffer were utilized as the mobile phase on a Kromasil C18 (150 x 4.6 mm, 5 m) stationary phase. The most optimal combination among all the attempted mixtures was found to be an 80:20 v/v binary mixture of methanol and acetate buffer (PH 3.7). This was determined based on the improved definition and resolution of chromatographic peaks, which were observed to be almost completely free from tailing. The retention times of etodolac and thicolchicoside were determined to be 3.171 min and 2.284 min, respectively. All samples underwent six injections, and consistent retention times were observed across all instances. The reproducibility of the peak areas of etodolac and thicolchicoside was demonstrated by the low coefficient of variation. The study observed a strong linear correlation ($r=0.999$) between the concentrations of etodolac (ranging from 1 to 6 $\mu\text{g/mL}$) and thicolchicoside (ranging from 100 to 600 $\mu\text{g/mL}$) and their corresponding peak areas. The regression curves for etodolac and thicolchicoside were generated through linear regression analysis, resulting in the mathematical expressions $Y = 2440X - 239.8$ and $Y = 19238X + 1005$, respectively. In these expressions, Y represents peak area and X represents drug concentration. Table 3 and Table 4 present the regression characteristics. The proposed method was utilized to determine the intra and inter-day variations of solutions containing etodolac and thicolchicoside. The results indicated a low coefficient of variation. The high recovery values from the different dosage forms show how accurate the suggested approach is. The absence of additional peaks indicates that the usual excipients used in the tablets do not interfere. The amount of drug contained in the tablets was measured using the suggested analytical technique. According to the research, the tablets contained, on average, 100.3% and 100.1% of thicolchicoside and etodolac listed on the label. The low coefficient of variation in the thicolchicoside and etodolac assay indicates a good degree of repeatability in dose formulations.

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