Analytical Method Development and Validation for the Simultaneous Estimation of Thiocolchicoside and Etodolac in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method

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***Abstract*—A simple, precise, accurate and robust Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Thiocolchicoside and Etodolac in bulk and combined pharmaceutical dosage forms. Chromatographic separation was achieved on a C18 (250 mm × 4.6 mm, 5 µm) column in isocratic mode using a mobile phase of Methanol: Acetonitrile: Water in the ratio 50:30:20 (v/v/v) at a flow rate of 1.0 mL/min, with UV detection at 226 nm. The method produced well resolved, symmetric peaks with retention times of 3.126 min for Thiocolchicoside and 5.020 min for Etodolac within a run time of 15 minutes. The method was validated as per ICH Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. Linearity was observed in the range of 24–72 µg/mL (r^2 = 0.9972) for Thiocolchicoside and 20–60 µg/mL (r^2 = 0.9981) for Etodolac. The mean percentage recovery was 100.37% for Thiocolchicoside and 100.62% for Etodolac, and the %RSD for precision was below 2% for both drugs, confirming the accuracy and reproducibility of the method. The validated method is simple, cost-effective and suitable for routine quality control analysis, stability testing and simultaneous assay of Thiocolchicoside and Etodolac in combined dosage forms.**

***Index Terms*—Etodolac; ICH Q2(R1); method validation; RP-HPLC; simultaneous estimation; Thiocolchicoside.**

I. INTRODUCTION

Pharmaceutical analysis plays a central role in ensuring the quality, safety and efficacy of drug products throughout their development and shelf life. Among the various analytical techniques used for this purpose, chromatographic methods particularly Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) are widely preferred for the quantitative estimation of active pharmaceutical ingredients, owing to their high sensitivity, specificity, reproducibility and suitability for multi-component formulations [1]-[5].

Thiocolchicoside (THC) is a semi-synthetic muscle relaxant derived from colchicine, possessing anti-inflammatory and analgesic properties; it is used in the management of painful muscle spasms associated with acute low back pain and neurological disorders. Etodolac (ETD) is a non-steroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin synthesis and is used for the relief of pain, swelling and stiffness in osteoarthritis and rheumatoid arthritis. The two drugs are frequently combined in fixed-dose oral formulations to relieve musculoskeletal pain and muscle spasm through complementary mechanisms of action, and several such combination brands are currently marketed in India.

A survey of literature shows that individual and simultaneous methods for THC and ETD have been

reported using UV-spectrophotometry, chemometric-assisted spectrophotometry and RP-HPLC, employing a range of mobile phase compositions, columns and detection wavelengths [18]-[30]. However, variability in reported retention times, run times and linearity ranges indicated scope for a further optimized, simple and rapid isocratic RP-HPLC method suitable for routine quality control. The present work was therefore undertaken to develop and validate a new RP-HPLC method for the simultaneous estimation of Thiocolchicoside and Etodolac in bulk drug and combined tablet dosage form, in accordance with International Council for Harmonisation (ICH) Q2(R1) guidelines.

The aim of the study was to develop and validate a robust, accurate and precise isocratic RP-HPLC method for the simultaneous estimation of Thiocolchicoside and Etodolac, and to apply the validated method to the assay of a combined marketed formulation. The specific objectives were: (i) to optimize chromatographic conditions capable of resolving both analytes with acceptable system suitability parameters; (ii) to validate the method for linearity, accuracy, precision, specificity, range, LOD and LOQ as per ICH Q2(R1); and (iii) to apply the method to a combined formulation and compare the assay results against the acceptance criterion of 98–102%.

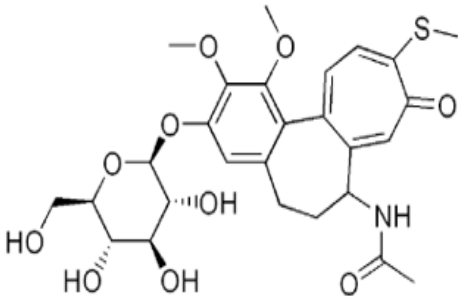


Fig. 1. Chemical structure of Thiocolchicoside.

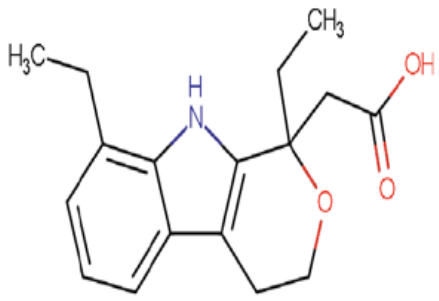


Fig. 2. Chemical structure of Etodolac.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Working standards of Thiocolchicoside and Etodolac were procured as gift samples. HPLC-grade water, acetonitrile and methanol were used throughout the study. All solvents were filtered through a 0.45 µm nylon membrane filter and degassed by sonication before use.

B. Instrumentation

Chromatographic separation was carried out on a Waters 2690 (Alliance) HPLC system. An analytical balance (Scale-tech SAB 203L), an ultrasonic bath sonicator (Digital Pro PSA-10A) and Class-A borosilicate glassware were used for solution preparation.

C. Preparation of Mobile Phase and Standard Solutions

The optimized mobile phase consisted of Methanol: Acetonitrile: Water in the ratio 50:30:20 (v/v/v), filtered and degassed prior to use; the same mobile phase was used as the diluent. Standard stock solutions were prepared by accurately weighing 60 mg of Thiocolchicoside and 50 mg of Etodolac into separate 100 mL volumetric flasks, dissolving in 60 mL of diluent with sonication for 5 minutes, and making up to volume. Aliquots of the stock solutions were further diluted to obtain a working standard solution containing 48 µg/mL Thiocolchicoside and 40 µg/mL Etodolac.

D. Method Development

The chromatographic method was optimized by evaluating different mobile phase compositions, column chemistries and flow rates. In Trial 1, using Methanol: Water (70:30 v/v) on a C18 (100 × 4.6 mm, 5 µm) column, the peaks of Thiocolchicoside and Etodolac merged. In Trial 2, a mobile phase of Methanol: Acetonitrile: Water (40:40:20 v/v/v) on a C18 (150 × 4.6 mm, 3 µm) column at 1.2 mL/min resulted in poor elution of the Etodolac peak. In Trial 3, Methanol: Acetonitrile: Water (30:50:20 v/v/v) on a C8 (250 × 4.6 mm, 5 µm) column produced splitting of the Thiocolchicoside peak. The optimized method used Methanol: Acetonitrile: Water (50:30:20 v/v/v) as the mobile phase on a C18 (250 × 4.6 mm, 5 µm) column at a flow rate of 1.0 mL/min, with UV detection at 226 nm and a 20 µL injection volume, giving well resolved, symmetric peaks for both analytes at retention times of 3.126 min (Thiocolchicoside) and 5.020 min (Etodolac) within a 15-minute run time (Table I, Fig. 3).

Table I Summary of Method Development Trials

Parameter	Trial 1	Trial 2	Trial 3	Optimized
Mobile phase	MeOH: Water 70:30	MeOH: ACN: Water 40:40:20	MeOH: ACN: Water 30:50:20	MeOH: ACN: Water 50:30:20
Column	C18, 100×4.6, 5 µm	C18, 150×4.6, 3 µm	C8, 250×4.6, 5 µm	C18, 250×4.6, 5 µm
Flow rate	1.0 mL/min	1.2 mL/min	1.0 mL/min	1.0 mL/min
RT (THC/ETD), min	3.392 / 3.662	3.188 / 4.262	2.545 / 5.026	3.126 / 5.020
Observation	Peaks merged	ETD poorly eluted	THC peak split	Well, resolved

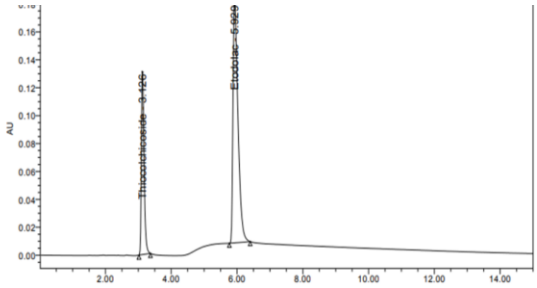


Fig. 3. Optimized chromatogram of Thiocolchicoside (RT 3.126 min) and Etodolac (RT 5.020 min).

E. Method Validation

The optimized method was validated as per ICH Q2(R1) guidelines for the following parameters:

1. System suitability: Evaluated using five replicate injections of the standard solution; acceptance criteria were theoretical plates ≥ 2000, tailing factor ≤ 2.0, and %RSD ≤ 2.0%.
2. Specificity: Assessed by comparing chromatograms of blank diluent and standard solution to confirm the absence of interfering peaks at the retention times of the analytes.
3. 3) Linearity: Determined over five concentration levels 24, 36, 48, 60 and 72 µg/mL for Thiocolchicoside and 20, 30, 40, 50 and 60 µg/mL for Etodolac by plotting peak area against concentration.
4. Accuracy: Evaluated by recovery studies at 50%, 100% and 150% of the target concentration, in triplicate, with acceptance criteria of 98–102% recovery.
5. Precision: Assessed as repeatability (six replicate injections of the sample solution) and expressed as %RSD, with an acceptance criterion of ≤ 2.0%.
6. LOD and LOQ: Calculated from the standard deviation of the response and the slope of the calibration curve using LOD = 3.3(σ/S) and LOQ = 10(σ/S).
7. Robustness: Evaluated by deliberately varying the flow rate to 0.8 and 1.2 mL/min from the optimized 1.0 mL/min and observing the effect on %RSD and tailing factor.
8. Assay: The validated method was applied to a combined marketed tablet formulation containing Thiocolchicoside and Etodolac, and the percentage assay was calculated against the label claim.

III. RESULTS AND DISCUSSION

A. System Suitability and Specificity

System suitability testing, performed using five replicate injections of the standard solution, gave a mean retention time of 3.135 min for Thiocolchicoside and 5.879 min for Etodolac, with theoretical plate counts of 6762 and 7064 respectively, tailing factors of 1.4 and 1.8, and %RSD of area within 0.2% for both analytes all within the acceptance limits (Table II). No interfering peaks were observed in the blank chromatogram at the retention times of the two analytes, confirming that the method is specific.

Table II System Suitability Parameters

Parameter	Thiocolchicoside	Etodolac
Retention time (min)	3.135	5.879
Theoretical plates	6762	7064
Tailing factor	1.4	1.8
% RSD (area, n = 5)	0.2	0.2

B. Linearity

The calibration curves for Thiocolchicoside (24–72 µg/mL) and Etodolac (20–60 µg/mL) showed excellent linearity, with regression equations of $Y = 15938x + 624.43$ ($r^2 = 0.9972$) and $Y = 47308x - 5884.9$ ($r^2 = 0.9981$) respectively (Fig. 4, Fig. 5, Table III). The correlation coefficients close to unity confirm a proportional detector response across the tested concentration range.

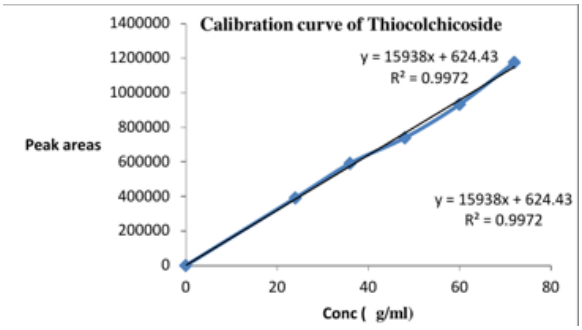


Fig. 4. Calibration curve of Thiocolchicoside.

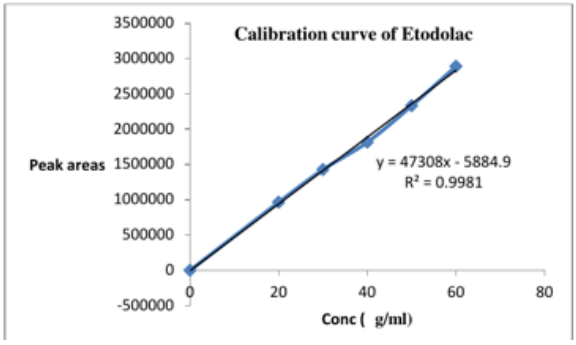


Fig. 5. Calibration curve of Etodolac.

Table III. Linearity, Lod and LOQ

Parameter	Thiocolchicoside	Etodolac
Linearity range (µg/mL)	24 – 72	20 – 60
Regression equation	Y = 15938x + 624.43	Y = 47308x − 5884.9
Correlation coefficient (r^2)	0.9972	0.9981
LOD (µg/mL)	4.067	2.756
LOQ (µg/mL)	12.324	8.353

C. Accuracy

Recovery studies conducted at 50%, 100% and 150% of the target concentration gave mean percentage recoveries of 100.37% for Thiocolchicoside and 100.62% for Etodolac (Table IV), both within the acceptance range of 98–102%, confirming the accuracy of the method.

Table IV Accuracy (Recovery) Data

Level	Thiocolchicoside % Recovery	Etodolac % Recovery
50%	100.48	100.94
100%	99.51	99.50
150%	101.13	101.44
Mean	100.37	100.62

D. Precision

Method precision, assessed from six replicate sample preparations, gave %RSD values of 0.4% for Thiocolchicoside and 0.2% for Etodolac (Table V), well within the ICH acceptance limit of 2.0%, demonstrating good repeatability of the method.

Table V Method Precision (Repeatability)

Drug	Mean % Assay (n = 6)	SD	% RSD
Thiocolchicoside	99.6	0.44	0.4
Etodolac	99.5	0.24	0.2

E. Robustness

Deliberate variation of the flow rate to 0.8 mL/min and 1.2 mL/min from the optimized 1.0 mL/min produced %RSD values below 0.4% and tailing factors within acceptable limits for both analytes (Table VI), indicating that the method is robust to small, deliberate changes in flow rate.

Table VI. Robustness Data (Effect of Flow Rate)

Flow rate (mL/min)	THC % RSD (area)	THC Tailing	ETD % RSD (area)	ETD Tailing
0.8	0.176	1.420	0.217	1.820
1.0 (optimized)	0.320	1.426	0.105	1.814
1.2	0.273	1.414	0.189	1.820

F. Assay of Marketed Formulation

The validated method was applied to the assay of a combined marketed tablet formulation. The mean percentage assay was found to be 99.65% for Thiocolchicoside and 100.15% for Etodolac (Table VII), both within the acceptance criterion of 98–102% of the label claim, confirming the suitability of the method for routine quality control analysis of the combined dosage form.

Table VII Assay of Combined Tablet Formulation

Drug	Mean % Assay	Label Claim Status
Thiocolchicoside	99.65	Within 98–102%
Etodolac	100.15	Within 98–102%

IV. CONCLUSION

A simple, accurate, precise and robust isocratic RP-HPLC method has been successfully developed and validated for the simultaneous estimation of Thiocolchicoside and Etodolac in bulk and combined pharmaceutical dosage forms, using a mobile phase of Methanol: Acetonitrile: Water (50:30:20 v/v/v) on a C18 column with UV detection at 226 nm. The method demonstrated excellent linearity, specificity, accuracy and precision, with all validation parameters meeting ICH Q2(R1) acceptance criteria. The short run time of 15 minutes and satisfactory system suitability, recovery and robustness results indicate that the method is well suited for the routine quality control, stability testing and simultaneous assay of Thiocolchicoside and Etodolac in combined tablet formulations in pharmaceutical industry laboratories.

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