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A New RP-HPLC Method development and validation for Etodolac and Thiocolchicoside

Chanukya Senapathi[1], Alekhya Somepalli[2], Nanda Gopala Krishna Gona[3], Bhargavi pacchipala[4], Sony Priyanka Arava[5]

[1]Assistant Professor, Pharmaceutical Analysis, NRK&KSR Gupta College of pharmacy
[2]Associate Professor, Pharmaceutical Chemistry, ASN Pharmacy College
[3]Associate Professor, Pharmaceutical Analysis, ASN Pharmacy College
[4]Assistant Professor, Pharmaceutical Analysis, ASN Pharmacy College
[5]Assistant Professor, Pharmacy practice, ASN Pharmacy College

Abstract
The aim of this work is to develop and validate a simple, sensitive, rapid and accurate and less time consuming validated RP-HPLC method for Etodolac and Thiocolchicoside in pharmaceutical dosage form. The method was developed and validated for various parameters as per ICH guidelines. The results obtained were within the acceptance criteria. The proposed method was applied for the determination of Etodolac and Thiocolchicoside in marketed formulation. The assay results confirm with the label claim of formulation.. Hence, the proposed method was found to be satisfactory and could be used for the routine analysis of Etodolac and Thiocolchicoside in combined tablet dosage forms. The retention time were found to be was 2.777 and 2.380 min for Etodolac and Thiocolchicoside respectively. The quantitative estimation gave a satisfactory result for Etodolac (99.66 % w/w) and Thiocolchicoside (99.3% w/w) respectively. The regression values over its peak areas were found to y = 17537x + 361.5 and y = 17769x + 1505 respectively and correlation coefficient found to be about 0.9999 and 0.9998 for Etodolac and Thiocolchicoside respectively. The percentage recovery for Etodolac and Thiocolchicoside were found to be 99.99% and 101.2 % respectively

Keywords: Etodolac, Thiocolchicoside,

1. Introduction

1.1 Etodolac: It is a Non-steriodal anti- inflammatory agent.[12] The common side effects of Etodolac are Dyspepsia, abdominal pain, Diarrhoea, Flatulence, Nausea, Constipation, Gastritis, melena, vomiting. Similar to other NSAIDS, the inflammatory effects of Etodolac results from inhibition of the enzyme cyclooxygenase (COX).[13],[14] This decreases the synthesis of peripheral prostaglandins involved in mediating inflammation. Etodolac binds to the upper portion of the COX inhibitor, but it is now known to be 5-50 times more selective for COX-2 than COX-1. Bioavailability : 80%, Protein binding : >99% bound to plasma proteins, Halflife**:** 6.4 hours. The systemic bioavailability of Etodolac is 100% as compared to solution and at least 80% as determined from mass balance studies.

Etodolac is well absorbed and had a relative bioavailability of 100% when 200 mg capsules were compared with a solution of Etodolac. Etodolac is extensively metabolized in the liver. The role, if any, of a specific cytochrome P450 system in the metabolism of Etodolac is unknown. Several Etodolac metabolites have been identified in human plasma and urine. The mean oral clearance of Etodolac following oral dosing is 49 (±16) mL/h/kg. Approximately 1% of an Etodolac dose is excreted unchanged in the urine with 72% of the dose excreted into urine as parent drug plus metabolite. Although renal elimination is a significant pathway of excretion for Etodolac metabolites, no dosing adjustment in patients with mild to moderate renal dysfunction is generally necessary. The terminal half-life (t½) of Etodolac is 6.4 hours (22% CV). Fecal excretion accounted for 16% of the dose.[17],[19]

1.2 Thiocolchicoside: It is a Non-steroidal anti- inflammatory agent.[26] Thiocolchicoside exhibits a selective affinity for the inhibitory gamma-amino butyric acid and glycinergic receptors. It has an agonistic action at the spinal-strychnine-sensitive receptors that could mediate its myorelaxant effect. However, experimental and clinical evidence strongly suggest a proconvulsant action for thiocolchicoside. Interaction with glycine receptors does not explain the convulsant action of the molecule.[27] It has been suggested that thiocolchicoside might preferentially interact with a cortical subtype of the gamma-amino butyric acid type A (GABAA) receptor that expresses low-affinity binding sites for GABA. The low-affinity recognition site seems to be an antagonist-binding site. This explains the proconvulsant effect of thiocolchicoside. [28] This is in contrast to earlier studies that suggested a GABA mimetic effect which would explain its muscle relaxant property. Oral bioavailability is ~25%, The binding of thiocolchicoside to serum proteins is 12.8 +/- 5.3%, predominantly albumin. Thiocolchicoside bind erythrocytes in whole blood at 3.4 +/- 0.8%, Following oral administration the half lives of 3-O-glucurono-demethylcolchicine and 3-demethylcolchicine are 3.2-7 hours and 0.8 hours, respectively. Following IM injection the half lives of thiocolchicoside and 3-O-glucurono-demthylcolchicine are 1.5-1.9 hours and 9 hours, respectively. Centrally acting muscle relaxants have been used in the treatment of painful muscle spasm associated with local tissue trauma or muscle strains, neurological disorders such as multiple sclerosis, cerebral palsy and stroke. Thiocolchicoside is indicated for the symptomatic treatment of painful muscle spasms. However, with the recent evidence of development of aneuploidy, the European Medicines Agency's Committee on Human Medicinal Products has recommended that the authorized uses for thiocolchicoside containing medicines for use by mouth or injection should be restricted.[29],[31].s

2. Materials And Methods

The various materials and equipment's used for the present study are summarized as follows:

Table 1: List of various materials used

S.No	Materials	Grade	Company
1	Water	HPLC	Fischer Scientific
2.	Acetonitrile	HPLC	Fischer Scientific
3.	Methanol	AR	Sd-fine chemicals

Table 2: Working Standard / Reference Standard/ API

S.No	Working standard	Company
1.	Etodolac	Yarrow chem products, Hyderabad
2.	Thiocolchicoside	Yarrow chem products, Hyderabad

Table 3: Test Sample

S.NO	Brand Name	Composition
1	Etova-MR	Etodolac-400 mg & Thiocolchicoside-4 mg
2	Etogesic- MR	Etodolac-400 mg & Thiocolchicoside-4 mg

Table 4: List of various Equipment used

INSTRUMENT	SPECIFICATION
HPLC instrument	WatersHPLC2695 series with Quaternary pumps, Photo Diode array detector
Injector	Auto sampler(robotic injector) integrated with empower 2software
Column	BDS 250mm x 4.6 mm, 5µ particle size.
UV spectrophotometer	Lab India UV double beam spectrophotometer with UVwin5.
pH meter	Lab India
Electronic balance	Denver
Ultra sonicator	Lab India
Pipettes, burettes, beakers	Borosil

3. Method Development

3.1 Instrument and materials: A Cary 60 UV-Visible double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of HPLC grade from SD fine- chem limited (SDFCL).

3.2 Solvent Selection: In order to select suitable solvent for simultaneous estimation of Etodolac and Thiocholchicoside in various solvents were selected for the solubility studies and it was found that they were freely soluble in water and Methanol. Hence in the present work, drugs were dissolved in methanol and make up with water was used for all the dilutions.

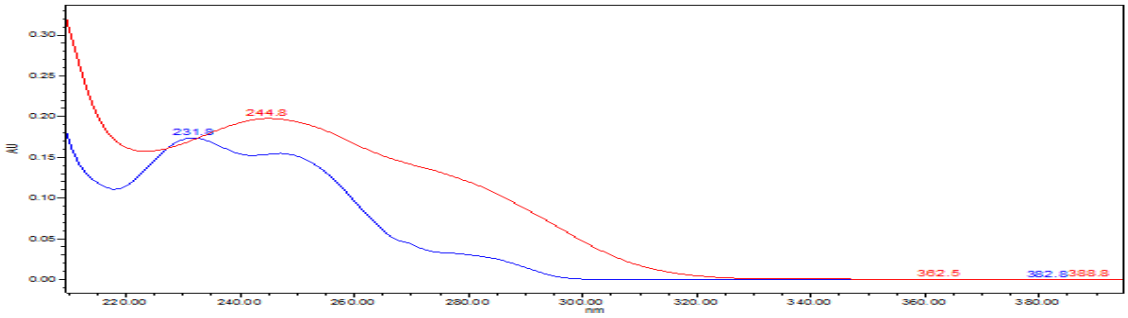
3.3 Preparation of Standard Stock Solution of Etodolac: Accurately weighed and transferred 2.5 mg of Etodolac working standard into a 100 ml clean dry volumetric flask and it was dissolved in methanol and the volume was made up with water to get a concentration of 1000 µg /ml solution. 2.5 ml of the above standard stock solution was pipetted into 25 ml volumetric flask and diluted up to the mark with water to get concentration of 100µg/ml solution.

3.4 Preparation of Standard Stock Solution of Thiocholchicoside: Accurately weighed and transferred 2.5 mg of Thiocholchicoside working standard into a 100 ml clean dry volumetric flask and it was dissolved in methanol and the volume was made up with water to get a concentration of 1000

µg/ml solution. 2.5 ml of the above standard stock solution was pipetted into 25 ml volumetric flask and diluted up to the mark with water to get concentration of 100µg/ml solution.

Optimization of UV- conditions Initially method development work was done in UV by taking UV-Visible spectra from the wavelength range of 200-400 nm for Etodolac and Thiocholchicoside .Results revealed the isobestic point of Etodolac and Thiocholchicoside standard was and nm. The isobestic point of two drugs shows maximum absorbance at 232 nm. Hence this λ_{Max} 232 was utilized for HPLC method development.

Figure.1 Isobestic point of Etodolac And Thiocholchicoside



3.5 HPLC Method Development:

3.5.1 Instrument: An Agilent model - 1220 Infinity LC – HPLC system with Agilent openLAB CDS (EZ Chrome) software "version A.04.05" equipped with a variable wavelength detector (VWD) and a manual injector was used. It was manufactured by Agilent Technologies, USA. An Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µm particle size) was used for the analytical separation and quantification of the mixtures. An ELICO (LI 120) pH meter was used for adjusting the pH of the buffer.

3.5.2: Reagents

1. Methanol : HPLC grade
2. Millipore water : HPLC grade
3. Acetonitrile : HPLC grade
4. Phosphate buffer : AR grade
5. Ortho phosphoric acid s : AR grade

3.5.3 Trials: The present study describes the development and validation of simultaneous estimation of Etodolac and Thiocholchicoside combined tablet dosage forms by RP-HPLC. During development of analytical method, methanol: 0.01 M phosphate buffer pH adjusted to 6.8 with Ortho phosphoric acid (50:50), methanol: acetonitrile (80:20), methanol: water (70:30) and acetonitrile: 0.01 M Phosphate buffer (60:40) were tried but both the drugs were found to be soluble in methanol : water (70:30) which were used for the preparation of standard stock solutions. Then chromatographic Trials are performed on Eclipse XDB plus C18 column by changing chromatographic parameters like mobile phase composition and flow rate.

3.5.4 Preparation of Buffer: Accurately weighed 2.72gm of sodium dihydrogen phosphate in a 1000ml of Volumetric flask, add about 900ml of Millipore water and sonicate to degas and finally make up the volume with water. Then pH was adjusted to 3 with dilute phosphoric acid solution.

3.5.5 Chromatographic Conditions:

Mobile phase : Methanol: Phosphate Buffer (85:15)

Column : Eclipse XDB Plus C_{18} column (150×4.6 mm; 5µ)

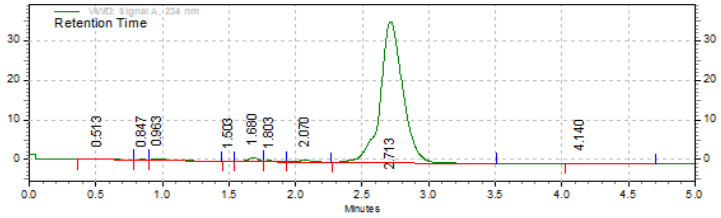
Detector wave length : 232 nm
Injection volume : 20µl
Runtime : 6 min

Table. 5: List of chromatographic trials

S. NO	TRIALS	MOBILE PHASE	FLOW RATE	OBSERVATION
1	Trial-1	Methanol : phosphate buffer (85:15)	1ml/min	Irregular base line was observed
2	Trial-2	Methanol : phosphate buffer (85:15)	0.8ml/min	Irregular base line was observed
3	Trial-3	Methanol : phosphate buffer (85:15)	0.9ml/min	Multiple peaks were observed
4	Trial-4	Methanol : phosphate buffer (85:15	0.6ml/min	Optimized peak was observed

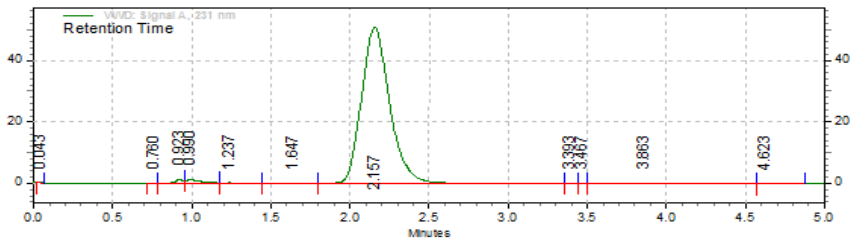
3.5.6 Standard solution of Etodolac: Accurately weighed and transferred 492 mg of Etodolac in to a 500 ml volumetric flasks . Add 3/4ml of diluent (Methanol: phosphate buffer 85:15), sonicate for 15 minutes and cooled to room temperature, makeup the final volume with diluents to get 1000 µg/ml. From the above solution 10 was taken and made upto 100 ml with mobile phase to get 100 µg/ml, from that 50 µg/ml was prepared and run through RP-HPLC.

Figure 2: Typical chromatogram for Etodolac Standard Solution



3.5.7 Standard solution of Thiocolchicoside: Accurately weighed and transferred 5 mg of Thiocolchicoside in to a 10 ml volumetric flasks . Add 3/4ml of diluent (Methanol: phosphate buffer 85:15), sonicate for 15 minutes and cooled to room temperature, makeup the final volume with diluents to get 1000 µg/ml. From the above solution 10 was taken and made upto 100 ml with mobile phase to get 100 µg/ml, from that 50 µg/ml was prepared and run through RP-HPLC.

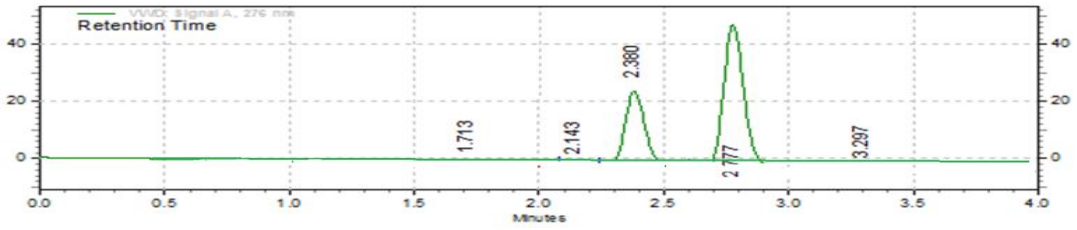
Figure 3: Typical chromatogram for Thiocolchicoside Standard Solution



3.5.8 Preparation of mixed standard solution: About 492 mg of Etodolac and 5 mg of Thiocholchicoside were weighed accurately and each was transferred in to a 500 ml volumetric flask.

After adding 300 ml of diluent to each flask, the contents were subjected to sonication for 10 min. The rest of volume was made up with diluent, to get a concentration of 50 µg/ml of Etodolac and 50µg/ml of Thiocholchicoside solutions.

Figure 4: Typical chromatogram for mixed standard solutions



3.5.9 Optimized Chromatographic Conditions:

Flow rate : 0.6 ml/min
Column : Eclipse XDB Plus C_{18} column (150×4.6 mm; 5µ)
Detector wave length : 232 nm
Column temperature : 30±2 ^{0}C
Injection volume : 20µl
Runtime : 6 min
Retention time : 2.777 min (Etodolac) & 2.380 min (Thiocolchicoside)

3.6 Estimation Of Etodolac And Thiocholchicoside: From the working mixed standard solution, dilutions ranging from 10-60 µg/ml of Etodolac and Thiocholchicoside were prepared in 10 ml volumetric flasks with the mobile phase. 20 µl of the solution was injected into the column and corresponding chromatograms were obtained. From these chromatograms retention times and the area under the peak of the drugs for each dilution were calculated. A relevant calibration curve was constructed with concentration on x-axis and area under the peak on y-axis. The linearity range was found to be 10-50 µg/ml for Etodolac and Thiocholchicoside. The regression equations of the curve were computed. The linearity range and calibration curves were shown in Table 6 & 7 and Fig.2 & 3 respectively. A typical chromatogram of mixed standard solution is shown in Fig. 4.

Table. 6. Linearty of Etodolac

S. No	Concentration (µg/ml)	Peak area (Mv*min)
1.	10	410209
2.	20	896414
3.	30	1424627
4.	40	2011046
5.	50	2613679
(Correlation coefficient) R2		0.998

Figure 5: Calibration curve of Etodolac

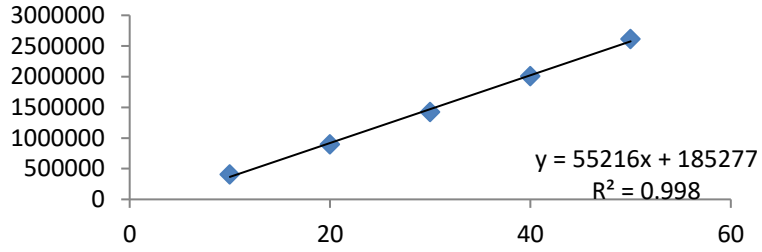
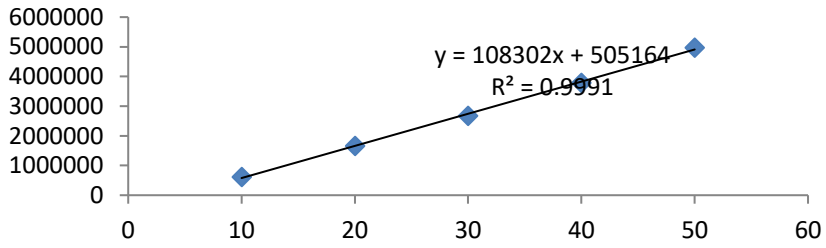


Table. 7. Linearty of Thiocholchicoside

S. No	Concentration (µg/ml)	Peak area (Mv*min)
1.	10	618214
2.	20	1662329
3.	30	2678815
4.	40	3791243
5.	50	4968847
(Correlation coefficient) R2		0.9991

Figure 6: Calibration curve of Thiocholchicoside



4. Method Validation

Validation is a "process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce meeting, its predetermined specifications and quality attributes". The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Validation parameters

a. System suitability
2. Linearity
3. Accuracy
4. Precision
5. Limit of Detection

6. Limit of Quantification
7. Specificity
8. Ruggedness
9. Robustness

4.1 System Suitability: Five replicates of working mixed standard solution were injected and the parameters like theoretical plate number (N), tailing factor (K) and resolution are calculated to check the system suitability. The theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The chromatograms (Fig. 7) were recorded and the results are shown in Table 8.

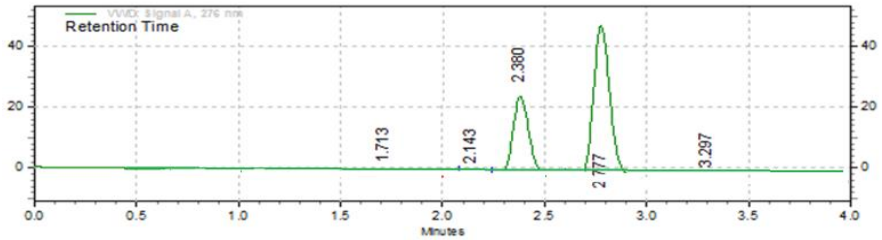
Acceptance criteria:

1. The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution should be not more than 2.0%.
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Etodolac and Thiocolchicoside peaks is NLT 2000.
4. The tailing factor (T) for the Etodolac and Thiocolchicoside peaks is NMT 2.0.

Table 8: System suitability of the proposed method

System suitability parameters	Results	
	Etodolac	Thiocolchicoside
Tailing factor	1.12	1.37
No. of theoretical plates	3489	4108
Resolution	5.2	

Figure 7: Chromatogram showing System suitability



4.2 Linearity: The calibration curves constructed by analyzing a series of concentrations of each drug ranging from 10-50 µg/ml for Etodolac and 10-50 µg/ml for Thiocolchicoside showed good linearity. The calibration curve was constructed for the standard solutions by plotting their concentrations against their respective peak areas. Regression equation was obtained and the values of slope-a, intercept-b, and correlation coefficient (R2) were determined as shown in Fig. 8&9 and the results are tabulated in Table 9.

Table 9: Linearity results of proposed method

S. No	Etodolac		Thiocholchicoside	
	Concentration (µg/ml)	Peak area (mV*min)	Concentration (µg/mL)	Peak area (mV*min)
1	10	410209	10	618214

2.	20	896414	20	1662329
3.	30	1424627	30	2678815
4.	40	2011046	40	3791243
5.	50	2613679	50	4968847

Figure 8: Linearity curve of Etodolac

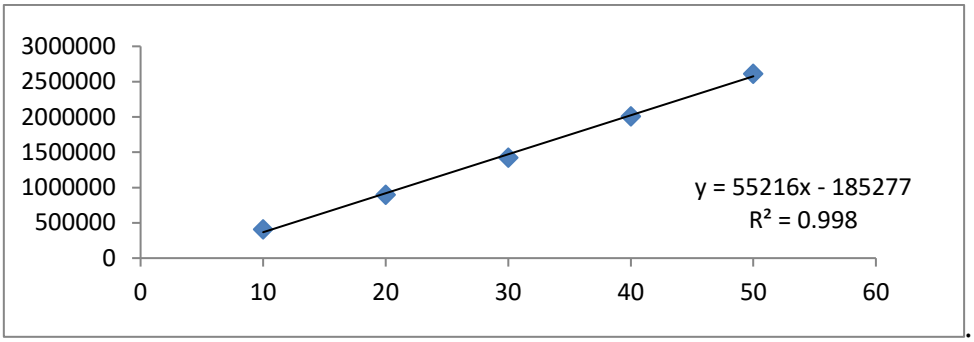
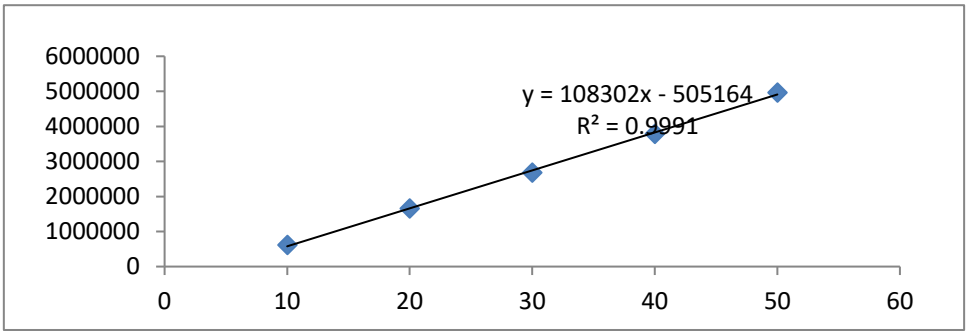


Figure 9: Linearity curve of Thiocholchicoside



4.3 Accuracy: To check the accuracy of the method, solutions containing 10, 20, 30, 40, 50 µg/ml of Etodolac and 10, 20, 30, 40, 50 µg/ml of Thiocholchicoside were subjected to the proposed HPLC method of analysis and the recoveries obtained were noted. Two, three, four ml of mixed sample solution was taken in 3 different volumetric flasks and mixed with mobile phase to obtain concentration of10, 20, 30, 40, 50 µg/ml of Etodolac and, 10, 20, 30, 40, 50 µg/ml Thiocholchicoside that gives 80 %, 100 % and 120 % of the analytical method target concentrations. A known amount of standard Etodolac and Thiocholchicoside was added to target concentrations. The results are presented in Table 10 &11.

Table 10: Accuracy result for Etodolac

Sample taken (µg/ml)	Spiked level	Active drug added (mg/ml)	Recovered amount(mg/ml)	% Recovery	% RSD	Mean% Recovery
50	80%	40	89.96 89.97 89.98	99.96	0.0088	99.94
	100%	50	99.98 99.87	99.93	0.055	

			99.95			
	120%	60	109.98 109.97 109.95	99.93	0.0513	
100	80%	80	179.88 179.99 179.97	99.96	0.021	99.96
	100%	100	199.87 199.98 199.96	99.96	0.0295	
	120%	180	219.87 219.99 219.98	99.97	0.0016	

Table 11: Recovery data of Thiocolchicoside

Sample taken (µg/ml)	Spiked level	Active drug added (mg/ml)	Recovered amount (mg/ml)	% Recovery	% RSD	Mean% Recovery
50	80%	40	89.95 89.96 89.99	99.95	0.0022	99.96
	100%	50	99.98 99.99 99.95	99.97	0.020	
	120%	60	109.98 109.96 109.97	99.96	0.0009	
70	80%	56	125.98 125.94 125.97	99.96	0.016	99.96
	100%	70	139.99 139.92 139.97	99.97	0.025	
	120%	84	153.94 153.98 153.96	99.97	0.012	

Acceptance criteria: The % recovery of Etodolac and Thiocolchicoside was found to be 99.94 % and 99.8 % respectively. (NLT 98 % and NMT 102 %).

4.4 Precision:

1. System precision
2. Method precision

4.4.1 System Precision: Standard stock solutions were diluted to 10 ml to get a concentration of (50µg/ml) Etodolac and (50µg/ml) Thiocolchicoside. The corresponding peak areas for 5 replicate injections were measured and % RSD calculated. The results are tabulated in Table 12.

Table 12: System precision of proposed method

Concentration (µg/ml)	Injection (n)	Peak areas (mV*min)	
		Etodolac	Thiocolchicoside
Mixed standard solution	1	410209	618214
	2	896414	1662329
	3	1424627	2678815
	4	2011046	3791243
	5	2613679	4968847
Statistical analysis	Mean	1471195	2743889.6
	SD	3292.4	32537.3
	% RSD	0.223	1.18

4.4.2 Method Precision: It was performed for five replicate sample preparations of marketed formulation of (50 µg/ml) Etodolac and (50µg/ml) Thiocolchicoside. The corresponding peak areas were measured and % RSD calculated. The results are exhibited in Table 13.

Table 13: Method precision of proposed method

Drug	Sample taken	%RSD	
		Interday	Intraday
Etodolac	50	0.03	0.09
	60	0.053	0.04
	70	0.028	0.037
Thiocolchicoside	40	0.042	0.125
	50	0.042	0.114
	60	0.043	0.08

Acceptance criteria: The % relative standard deviation of Etodolac and Thiocolchicoside peak areas from the five replicates should be not more than 2.0 %. Test results are showing that the method is quite precise i.e., 0.223–1.18 (System precision) and 0.42-0.106 for THC & 0.037- 0.056 for ETC (Method Precision).

4.5 Limit Of Detection (LOD): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. The detection limit can be calculated based on the Standard Deviation of the Response and the Slope of the regression equation.

LOD = 3.3 F/S

Where,

F = Residual Standard deviation of the response = 15063.89(Etodolac)
= 50701.16 (Thiocolchicoside)

S = Slope of the calibration curve = 55216 (Etodolac)
= 108302 (Thiocolchicoside)

The LOD for this method was found to be 0.816µg/mL and 1.404µg/mL for Etodolac and Thiocolchicoside

4.6 Limit Of Quantitation (LOQ): The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit can be calculated based on the Standard Deviation of the Response and the slope of the regression equation.

LOQ = 10 F/S

Where,

F = Residual Standard deviation of the response = 4287.60(Etodolac)
= 50701.1(Thiocolchicoside)

S = Slope of the calibration curve= 55216 (Etodolac)
= 1496.25 (Thiocolchicoside)

The LOQ for this method was found to be 0.77µg/mL and 0.24µg/mL for Etodolac and Thiocolchicoside.

4.7 Specificity: The specificity was determined to check whether there is any interference due to presence of excipients, impurities or other components with the retention time of analytical peaks which may affect the specificity of the analytical method. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges. The results are shown in Table 14.

Acceptance criteria: The specificity test was performed for Etodolac and Thiocholchicoside. It was found that there was no interference of impurities in retention time of analytical peak. The method showed excellent specificity with Etodolac and Thiocholchicoside eluting at retention time of 2.777 and 2.380 minutes respectively.

Figure 10: Chromatogram of Blank

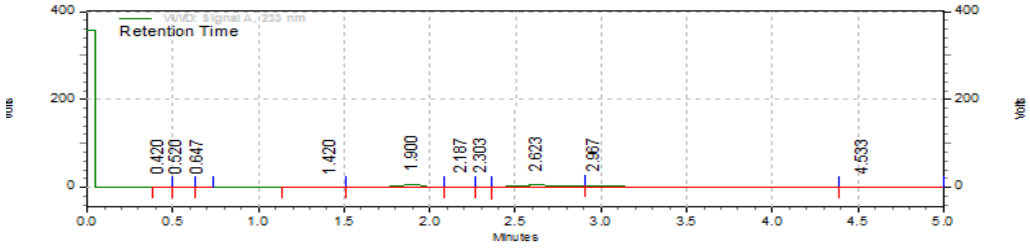


Figure 11: Standard Chromatogram for Specificity of Etodolac and Thiocholchicoside

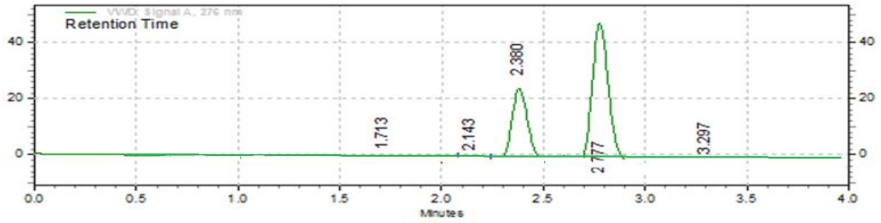


Figure 12: Tablet Chromatogram for Specificity of Etodolac and Thiocholchicoside

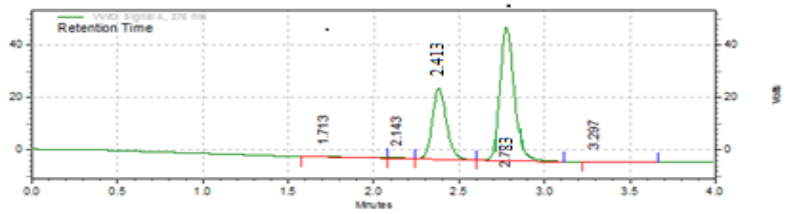


Table 14: Specificity of Proposed method

Sample	Area obtained (mV*min)		% Content of drug (w/w)	
	Etodolac	Thiocholchicoside	Etodolac	Thiocholchicoside
Standard	7018640	2358694	99.17	99.04%
Standard +Placebo	7018486	2357532	98.72	99.28%
Placebo	0	0	0	0

4.8 Ruggedness: Ruggedness was determined by injecting the standard and sample solutions by two different analysts to check the reproducibility of the present analytical method. The retention time and peak areas were obtained. % of the Assay was calculated and the results are presented in Table 15.

Table 15: Ruggedness report of proposed method

S.no	Parameter	Etodolac	Thiocholchicoside
1	Analyst – 01	99.98% w/w	100.43% w/w
2	Analyst – 02	99.94% w/w	99.72% w/w
3	Acceptance criteria	98-102 % w/w	98-102 % w/w

Acceptance criteria: The assay results of Etodolac and Thiocholchicoside should be between 98-102 %

4.9 Robustness: Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate from 0.8 ml/min by ±0.1, change in wavelength by ±5 nm and change in the mobile phase B ratio ± 5 %. The chromatograms were recorded and the results are shown in Table 16 & 17. w/w

Table 16: Robustness report of Etodolac

S.NO	Parameter	Etodolac		
		Retention time(min)	Peak area (mV*min)	Tailing factor
1	Standard	2.777	4968847	1.18
2	Flow rate (1.1 ml/min)	2.713	7018640	1.50
3	Flow rate (1 ml/min)	3.000	5999725	1.43
4	Mobile phase (65:45 % v/v)	1.505	1168.357	1.35
5	Mobile phase (75:25 % v/v)	1.532	1236.365	1.23

6	Wavelength (277 nm)	1.540	1501.225	1.45
7	Wavelength (273 nm)	1.510	1660.305	1.52

Table 17: Robustness report of Thiocholchicoside

S. No	Parameter	Thiocholchicoside		
		Retention time(min)	Peak area (mV*min)	Tailing factor
1	Standard	2.380	1224.790	1.39
2	Flow rate (1.2 ml/min	4.549	1315.725	1.49
3	Flow rate (0.8 ml/min)	4.520	1346.260	1.44
4	Mobile phase (65:45 % v/v)	4.430	1295.760	1.38
5	Mobile phase (75:25 % v/v)	5.012	1315.260	1.27
6	Wavelength (277 nm)	4.559	1360.520	1.50
7	Wavelength (273 nm)	4.535	1325.026	1.41

Acceptance criteria: It was found that the system suitability parameters were within the limits at all variable conditions. The analytical method was found to be robust with respect to small variations in flow rate, mobile phase composition and wavelength.

5. Estimation Of Etodolac And Thiocholchicoside In Tablet Dosage Form

5.1 Preparation of mixed sample solution: 20 tablets were weighed accurately and crushed to fine powder. Each tablet contained 400 mg of Etodolac and 4 mg of Thiocholchicoside. The quantity of powder equivalent to 25 mg of Etodolac was weighed and dissolved in sufficient quantity of mobile phase in a 25 ml volumetric flask and finally made up to volume with the mobile phase. The solution was filtered through 0.45 µ nylon membrane filter paper. The amount of Etodolac and Thiocholchicoside present in tablet formulation was calculated by comparing the peak area of the standard with that of sample. From the working mixed sample solution dilutions ranging from 10-50 µg/ml of Etodolac and 10-50 µg/ml of Thiocholchicoside were prepared in 10 ml volumetric flasks with the mobile phase. 20 µl of the solution was injected into the column and corresponding chromatograms were obtained. From these chromatograms retention times and the area under the peak of the drugs for each dilution were calculated.

5.2 Assay: The amount of Etodolac and Thiocholchicoside present in tablet formulation was calculated by comparing the peak area of the standard with that of sample. The amount of drugs in tablet was calculated by using the given formula:

$$\%\ \textbf{Assay} = \frac{\textbf{sample Avg peak area}}{\textbf{Std avg peak area}} \times \frac{\textbf{Wt of drug(mg)}}{\textbf{dilution of std}} \times \frac{\textbf{dilution of tab solution}}{\textbf{Wt of sample}} \times \frac{\textbf{\%purity}}{\textbf{100}} \times \frac{\textbf{Avg. Wt}}{\textbf{lable claim}} \times \textbf{100}$$

Calculations

% Assay of Etodolac

% Assay =298624/1471195x 5/100 x 100/152 x 99.8/100 x 618.5/4 x100

=0.202 x 0.05 x 0.65 x 0.998 x 154.6 x 100

= 101.2%

% Assay of Thiocholchicoside

% Assay = 549298/2743889.6x 492/100 x 100/152 x 99.8/100 x 618.5/400 x100

= 0.200 x4.92 x 0.65 x 0.998 x 1.546 x 100

= 98.6%

Acceptance criteria: The percentage purity of Etodolac and Thiocholchicoside was found to be 101.2% and 98.6 % respectively (NLT 98 % and NMT 102 %). The same procedure and calculation followed for other formulation (Etogesic- MR).

Figure 13: Typical Chromatogram for Tablet Solution (Etova-MR)

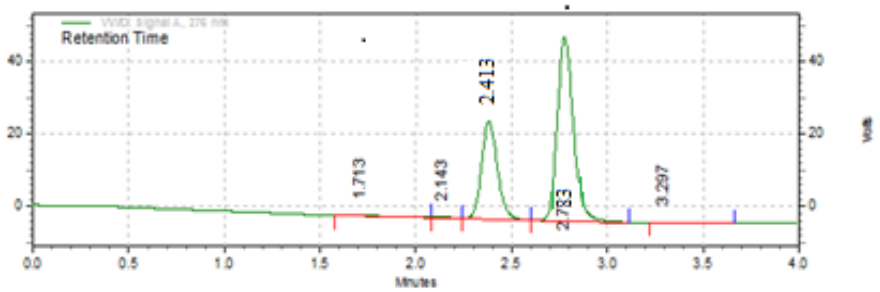


Figure 14: Typical Chromatogram for Tablet Solution(Etogesic-MR)

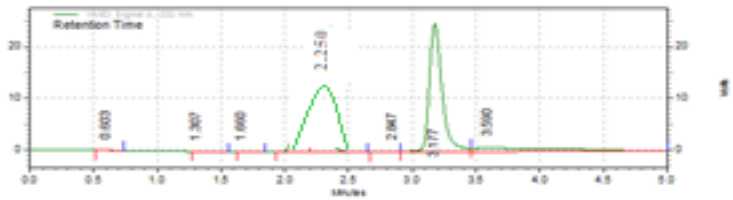


Table 18: Assay results for commercial formulation

S. No	Brand name	% Assay	
		Etodolac	Thiocolchicoside
1	Etova- MR	101.2%	98.6%
2	Etogesic- MR	100.4%	99.1%

6. Results And Discussion:

The present study was aimed to develop a more sensitive, precise and accurate method for simultaneous estimation of Etodolac and Thiocolchicoside in tablet dosage forms by RP-HPLC.To ascertain the maximum wavelength, λ_{max} of the drug, the drug solution of Etodolac and Thiocolchicoside were

scanned between the wavelength ranges of 200-400 nm. The λ_{max} was found to be 244.8nm for Etodolac and 231.8 nm for Thiocolchicoside. Initially various λ_{max} were tried but the λ_{max} (isobestic point) at 232 nm was found to be most appropriate chromatogram for the combination with good peak area and less tailing factor. This λ_{max} 232 nm was utilized for HPLC method development. Octa decyl siliane c18 (250mm x 4.6 mm, 5□.) column was chosen as the stationary phase for the separation and determination of Etodolac and Thiocolchicoside. For the optimization of the mobile phase, various mixtures consisting of methanol and phosphate buffer were examined at different ratios. The choice of the optimum composition is based on chromatographic response factor. The injection volume was set to 10 µL and the Variable Wavelength Detector was set at 232 nm. The run time was 6 min. A flow rate of 1.0 ml/min was found to be optimum from the studied range 0.8-1.2 ml/min, which gave optimum retention time, base line stability and noise. The retention time were found to be was 2.777 and 2.380 min for Etodolac and Thiocolchicoside respectively. The chromatograms for the validation studies were recorded and shown in fig. The quantitative estimation gave a satisfactory result for Etodolac (99.66 % w/w) and Thiocolchicoside (99.3% w/w) respectively.

The linear dynamic range was 10, 20, 30, 40, 50 µg/ml for both Etodolac and Thiocolchicoside. The regression values over its peak areas were found to y = 17537x + 361.5 and y = 17769x + 1505 respectively and correlation coefficient found to be about 0.9999 and 0.9998 for Etodolac and Thiocolchicoside respectively.

The recovery study was performed on 50 %, 100 % and 150 % of the target concentration of Etodolac and Thiocolchicoside sample preparation. The percentage recovery for Etodolac and Thiocolchicoside were found to be 99.99% and 101.2 % respectively .

The Robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and wavelength which may differ but the responses were still within the specified limits of the assay.

6.1 Effect of variation of flow rate: A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates 0.8 ml / min, 1.0 ml/min and 1.2 ml / min. The effect of variation of flow rate was evaluated. The results were discussed in their respective Table. 10

6.2 Effect of variation of Mobile phase:

A study was conducted to determine the effect of variation in Mobile phase. Standard solution was prepared and injected in to the HPLC system with mobile phase taken in the ratio of 40:60 and 30:70. The retention time values were measured. The results were discussed in their respective Table. 10

Acceptance criteria

1. The tailing factor of standard should be not more than 2.0 for Variation in flow.
2. The % RSD of Asymmetry and retention time of standard should be not more than 2.0 % for variation in flow.

Table 19: Results of the Propsed method

PARAMETERS	OBTAINED RESULTS		ACCEPTED LIMITS
	Etodolac	Thiocolchicoside	
Retention Time	2.777min	2.380 min	-

Accuracy	99.94%	99.96%	**98%-102%**
%RSD	0.046	0.21	**≤ 2**
Linearity(r^2)	0.998	0.9991	**0.998-1**
LOD	0.816µg/mL	1.404µg/Ml	**-**
LOQ	0.77µg/mL	0.13µg/mL	**-**
Assay	101.2%	98.6%	**98%-102%**

Table 20: Overall Summary of proposed method

S. No	Parameter	RESULTS	
		Etodolac	**Thiocolchicoside**
1	System suitability	Theoretical plates = 3489 Tailing factor = 1.12	Theoretical plates = 4108 Tailing factor = 1.37
		Resolution between two peaks was found to be 5.2	
2	Linearity	10-50 µg /mL Correlation coefficient = 0.998	10-50 µg /mL Correlation coefficient = 0.9991
3	Method precision	%RSD = 0.71	%RSD = 0.74
4	Accuracy	Mean recovery = 99.95%	Mean recovery = 99.96%,
5	Robustness	The system suitability parameters are within the limits	The system suitability parameters are within the limits
6	Ruggedness	The % RSD are within limits for both analysts	The % RSD are within limits for both analysts
7	Specificity	No interference with placebo or impurities in standard and sample chromatograms	No interference with placebo or impurities in standard and sample chromatograms
8	LOD	0.816µg/mL	1.404µg/mL

9	LOQ	0.77µg/mL	0.13µg/mL

7. Conclusion:

Pharmaceutical analysis simply means analysis of pharmaceuticals. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe, efficacious product that fulfill unmet medical needs. The pharmaceutical analyst plays a major role in assuring identify, safety, efficacy, purity and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications and method validation. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being developed with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, precision and accuracy in estimation of drugs. The method was developed and validated for various parameters as per ICH guidelines. The results obtained were within the acceptance criteria. The proposed method was applied for the determination of Etodolac and Thiocolchicoside in marketed formulation. The assay results confirm with the label claim of Sformulation. Hence, the proposed method was found to be satisfactory and could be used for the routine analysis of Etodolac and Thiocolchicoside in combined tablet dosage forms.

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