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Analytical Method Development & Validation for the Simultaneous Determination of Etodolac & Thiocolchicoside in Bulk & it's Pharmaceutical Dosage form by RP-HPLC

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Abstract: *The objective of the present research work was to establish a simple, reliable, and validated Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for simultaneous quantification of Etodolac and Thiocolchicoside in bulk drug and tablet dosage form. Chromatographic separation was achieved using an Inertsil ODS C18 column (4.6 mm × 150 mm, 5 µm particle size). The optimized mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 50:50 v/v with adjusted pH 4.5. The mobile phase was delivered at a flow rate of 1.0 mL/min and analyte detection was performed at 254 nm.*

The analytical procedure was validated in accordance with ICH Q2(R1) recommendations for various validation characteristics including linearity, precision, specificity, robustness, accuracy, limit of detection, and limit of quantification. The calibration plots for both analytes exhibited excellent linear response over the selected concentration range with correlation coefficients close to unity. Recovery studies confirmed satisfactory accuracy, while precision studies demonstrated low variability in results. The developed chromatographic method proved to be rapid, economical, reproducible, and suitable for routine analysis of Etodolac and Thiocolchicoside in combined pharmaceutical formulations.

Keywords: RP-HPLC, Etodolac, Thiocolchicoside, Simultaneous Estimation, Method Validation, Pharmaceutical Formulation

I. INTRODUCTION

Quality assurance is a fundamental requirement in pharmaceutical manufacturing because the therapeutic effectiveness of a drug product depends greatly on its identity, purity, and dosage accuracy. Analytical method development therefore occupies an important position in pharmaceutical research and industrial quality control. The increasing use of combined drug therapy has created the need for analytical methods capable of estimating multiple active ingredients simultaneously with high accuracy and precision.

Etodolac is a widely prescribed non-steroidal anti-inflammatory agent used in the treatment of pain, inflammation, osteoarthritis, and rheumatoid arthritis.

Its pharmacological action is mainly associated with inhibition of cyclooxygenase enzymes responsible for prostaglandin synthesis. Thiocolchicoside is a semisynthetic muscle relaxant frequently administered for painful muscular disorders and musculoskeletal spasms. The combined use of these two drugs is clinically beneficial in conditions involving inflammation accompanied by muscle stiffness and pain.

Several analytical approaches including UV spectrophotometry, HPTLC, and chromatographic methods have been employed for determination of these drugs individually or in combination with other agents. Among available techniques, Reverse Phase High Performance Liquid Chromatography remains one of the most dependable methods because of its superior resolution, sensitivity, reproducibility, and shorter analytical time.

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The present investigation was undertaken to develop and validate a rapid RP-HPLC method for simultaneous determination of Etodolac and Thiocolchicoside in pharmaceutical dosage form using optimized chromatographic conditions and validation according to internationally accepted guidelines.

II. MATERIALS & METHODS

1. Materials: The reliability and scientific validity of an analytical method depend largely on the appropriate selection of materials and the systematic application of well-defined experimental procedures. In HPLC method development, careful consideration of the quality of reagents, instrumentation, and analytical conditions is essential to ensure reproducible, accurate, and precise results. The drug used for present investigation was obtained from Micro Lab Bangalore and MG lab Hyderabad as gift sample.

- Details of Pure Drug: Drugs used were Etodolac 10g (purity- 99.4) supplied by Micro Lab India and Thiocolchicoside 10g (purity- 99.8) supplied by MG Lab India.
- Marketed Preparation: Marketed preparation brand name 'Etova-MR Forte' was used containing Etodolac & Thiocolchicoside 400mg & 8mg, respectively and was manufactured by Ipca Laboratories Ltd.
- Reagents and Chemicals:
- All reagents and chemicals used were of AR grade and HPLC grade. Chemicals used were Acetonitrile HPLC Grade, Methanol HPLC grade, Ortho-phosphoric acid, Water HPLC grade. All chemicals were manufactured by Merck Ltd., India.
- Instruments: Instruments used were UV-Visible Spectrophotometer(Mfg.- Shimadzu Model- Doublebeam-UV1900I) HPLC(Mfg.- Agilent Model- 1220 series infinity LC gradient system VL - G4288C UV Detector), PH Meter (Mfg.- Equip-tronich Model- EQ-610), Analytical column (Mfg.- Intersil ODS Model- C18,(4.6 x150 mm)) , Balance (Mfg.- Phoenix Gold Model-300 P) ,Ultrasonicator (Mfg.- Labman Model- LMUC2).

III. METHODS & PROCEDURE

- **Identification and characterization of drug:** Previous to commenced the experimental work it is necessary to determine the different physical and chemical property of the drug which provide information regarding the purity and nature of drug. This will help in selection of solvents and procedure for the robust and stable analytical method development. Etodolac & Thiocolchicoside.
- **Selection and procurement of drug:** Etodolac (ETD) & Thiocolchicoside (THC) were selected as model drug candidate for method development and validation. The drugs were kindly gifted from Pharmaceutical industry India.The procured drug was analyzed for different physical properties viz.color, odor, melting point, etc.
- **Physico-chemical characterization:** The physico-chemical characterization of drug molecule is important with regard to its purity, identification in development and validation of analytical method. The various tools used for characterization of drug molecules include melting point, UV spectroscopy, solubility study, etc.
- **Solubility Studies:** As a first step of method development solubility of both drugs was tested in different solvents to obtain a common solvent which can be used for simultaneous estimation of both drugs in a mixture.
- **Melting point range determination:** Melting point determination is an essential preformulation and physicochemical characterization parameter that supports HPLC method development. Melting point of Etodolac (ETD) & Thiocolchicoside (THC) were determined by placing small amount of sample in capillary tube closed at one end and holds the capillary on melting point apparatus. Melting point of Etodolac (ETD) & Thiocolchicoside (THC) are 146 ºC and 194 ºC, respectively.
- **UV Spectroscopy Analysis:** UV spectroscopy plays a pivotal role in the development of high-performance liquid chromatography (HPLC) methods, particularly when UV detection is employed for analyte quantification. Prior UV spectroscopic evaluation of the drug substance provides essential information

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regarding its absorption characteristics, which directly influence detector wavelength selection and overall method sensitivity.The ultraviolet absorption spectrum of ETD & THC were obtained using Shimadzu1800-UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λ max) were analyzed.

1. Selection of mobile phase:

a) Preparation of standard solutions:

ETD standard solution: Accurately weighed quantity 5mg of ETD was dissolved in methanol and volume was made up to 25 ml mark (200 mg/ml).The stock standard solution was diluted further with methanol to get final concentration of about 100 mg/ml of ETD.

THC standard solution: Accurately weighed quantity 5mg of THC was dissolved in methanol and volume was made up to 25 ml mark (200 mg/ml). The stock standard solution was diluted further with methanol to get final concentration of about 100 mg/ml of THC. As like above procedure, the standard solutions are also prepares in mobile phase.

b) Procedure: The Methanol was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing mixture of ETD and THC was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate buffer (50:50) pH 4.5 was selected, since it gives sharp reproducible retention time for ETD & THC.

- **Chromatographic conditions:** The following chromatographic conditions were established by trial and error and were kept constant throughout method.

Column	:	Intersil 4.6 (id) x 250 mm
Particle size packing	:	5 mm
Stationary phases	:	C18 Intersil
Mobile phase	:	Acetonitrile: Buffer (50:50) pH 4.5
Detection wavelength	:	255 nm
Flow rate	:	1 ml/min.
Temperature	:	Ambient
Sample size	:	20 mL

2. Preparation of Calibration Curve:

i) Preparation of standard solutions:

ACE standard stock solution: Accurately weighed quantity 10 mg of ETD was dissolved in methanol and volume was made up to 100 ml mark (100 mg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations.

THC standard stock solution: Accurately weighed quantity 10 mg of THC was dissolved in methanol and volume was made up to 100 ml mark (100 mg/ml).The stock standard solution was diluted further with mobile phase to get various concentrations.

ii) Procedure: The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 4-40 mg/ml for ETD and 1-10 mg/ml of THC solutions were injected and peak area was recorded.

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3. System suitability test: System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Preparation of standard drug solution.

ETD standard solution: Accurately weighed quantity 10 mg of ETD was dissolved in mobile phase and volume was made up to100 ml mark.The stock standard solution was diluted further with mobile phase to get final concentration of about 40 mg/ml of ETD.

THC standard solution: Accurately weighed quantity 10 mg of THC was dissolved in mobile phase and volume was made up to 100 ml mark. The stock standard solution was diluted further with mobile phase to get final concentration of about 0.8 mg/ml of THC.

A) Procedure: Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 mL std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded.

4. Application of proposed method for estimation of ETD and THC Laboratory mixture:

Preparation of laboratory mixture (standard): Accurately weighed quantity of ETD 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. And accurately weighed quantity of THC 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. The standard solution of ETD & THC were mixed and diluted with mobile phase properly to obtain laboratory mixtures containing a concentration 40 mg/ml & 0.8 mg/ml of both drug respectively.

Preparation of laboratory mixture (sample): Three different laboratory mixtures of ETD and THC were prepared by appropriately weighing the quantities of drug samples so as to get the concentration of 40 mg/ml of ETD and 0.8 mg/ml of THC. The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration.

5.**Application of proposed method for estimation of ETD and THC in formulation:** Standard stock solution:

Accurately weighed quantity of ETD 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. And accurately weighed quantity of THC 10mg was transferred to100ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. The standard solution of ETD & THC were mixed and diluted with mobile phase properly to obtain laboratory mixtures containing a concentration 40mg/ml of ETD and 0.8 mg/ml of THC.

Sample solution preparation: The quantity of drug in one ml is calculated with the help of density bottle on this the drug is taken and dilutated with mobile phase. The solution was filtered through whatman filter paper No.42. Further dilution was done with mobile phase to get concentration of 40 mg/ml of ETD and 0.8 mg/ml of THC.

Procedure: Equal volume (20 mL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of ETD and THC was calculated by comparing a sample peak with that of standard.

6. Validation parameters:

a) **Accuracy:** It was ascertained on the basis of recovery studies performed by standard addition method.

b) **Standard solution:** An accurately weighed quantity of tablet formulation was taken in 10 ml volumetric flask; to it standard solution of ETD and THC was added in different proportions. Then volume was

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adjusted up to the mark with the mobile phase. The solution was diluted with mobile phase to get final concentration & filtered through whatman filter paper No.41.

c) **Precision:** Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

d) **Ruggedness:** In the present HPLC method, ruggedness was assessed by analyzing the same homogeneous sample under different experimental conditions, including analysis by different analysts with different days while maintaining identical chromatographic parameters. The assay results obtained under these varied conditions were compared statistically.

The studies of ruggedness were carried out under two different conditions-

1) Days
2) Analyst.

i. Interday (Different days): Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated.

ii. Intraday: It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula.

iii. Different analyst: The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

e). **Specificity:** Specificity was measured as ability of the proposed method to obtain well separated peak for ETD and THC without any interference from component of matrix.

Mean retention time for–

ETD– 7.328

THC – 4.224

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

e) Linearity and range: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. ETD and THC marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

f) Robustness: Robustness and ruggedness - the ability of an analytical method to remain unaffected by small variations in method parameters and influential environmental factors and characterize its reliability during normal usage. The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate.

g) Limit of Detection (LOD) and Limit of Quantitation (LOQ): Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy.

IV. RESULT & DISCUSSION

A fixed dose combination containing Etodolac (ETD) & Thiocolchicoside (THC) is recently available in market as tablet dosage form.

Percent purity of above mentioned drugs were reported by Supplier Company as follows-

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1) Etodolac (ETD) - 99.4 %.
2) Thiocolchicoside (THC) - 99.8 %
Primary this was not analysed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

Solubility Study
a) Etodolac

Sr. No	Solvent / Medium	Solubility Observation
1	Water	Practically insoluble (~0.02 mg/mL)
2	0.1 N HCl (acidic pH)	Slightly soluble
3	Phosphate buffer pH 6.8	Moderately soluble
4	Methanol	Freely soluble
5	Ethanol	Freely soluble
6	Chloroform	Soluble
7	Acetone	Soluble

Table No 01: Result of solubility study of Etodolac.

Etodolac is a BCS Class II drug with very low water solubility, making aqueous mobile phases unsuitable for HPLC analysis. It is highly soluble in organic solvents like methanol and acetonitrile, which are commonly used for sample preparation. As a weakly acidic drug (pKa $\approx$ 4.6), its solubility improves at higher pH, so buffered mobile phases are often used to enhance ionization and peak symmetry. In RP-HPLC, mixtures of organic solvents and buffered aqueous phases provide good resolution, sharp peaks, and reliable analytical performance.

b) Thiocolchicoside

Sr. No.	Solvent	Solubility of Thiocolchicoside
1	Water	Freely soluble
2	0.1 N HCl (Acidic pH)	Soluble
3	Phosphate buffer (pH 6.8)	Soluble
4	Methanol	Freely soluble
5	Ethanol	Freely soluble
6	Chloroform	Sparingly soluble
7	Acetone	Soluble

Table No. 02: Result of solubility study of Thiocolchicoside.

Thiocolchicoside is a semi-synthetic drug with good water solubility and high solubility in polar solvents such as methanol and ethanol. Its solubility remains stable across different pH conditions, allowing easy preparation of standard and sample solutions. In RP-HPLC analysis, mixtures of aqueous buffers and organic solvents like methanol or acetonitrile are commonly used to achieve accurate, reproducible, and well-defined chromatographic peaks. These solubility properties support the development of simple and reliable HPLC methods for quantitative analysis.

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Melting point range determination

Melting point of drug was determined by placing small amount of sample in capillary tube closed at one end and holds the capillary on melting point apparatus. The melting point was noted The melting point of ETD is 146 ºC and THC is 194 ºC.

FT-IR analysis:

a) IR of Etodolac (ETD)

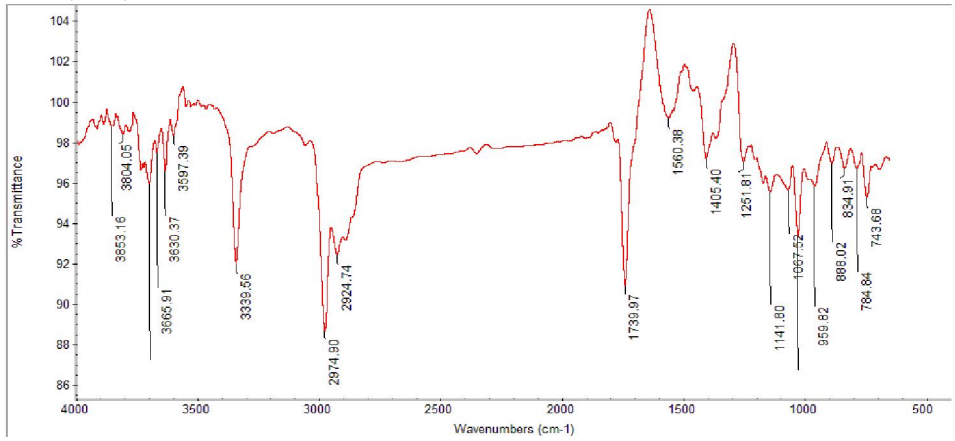

Fig No. 03: FTIR Spectra of ETD

The IR spectrum of Etodolac shows characteristic peaks confirming its functional groups and chemical structure. A broad band around 3380–3340 cm^{-1} indicates O–H stretching of the carboxylic acid group, while peaks at 3060–3000 cm^{-1} and 2920–2850 cm^{-1} correspond to aromatic and aliphatic C–H stretching vibrations. A strong peak near 1739 cm^{-1} confirms the presence of the carbonyl (C=O) group.

Peaks between 1600–1500 cm^{-1} represent aromatic C=C stretching, and bands around 1250–1050 cm^{-1} indicate C–O stretching of carboxylic acid and ether groups. Additional peaks at 900–700 cm^{-1} confirm substituted aromatic rings, supporting the identity and purity of etodolac.

b) FT-IR of Thiocolchicoside (THC)

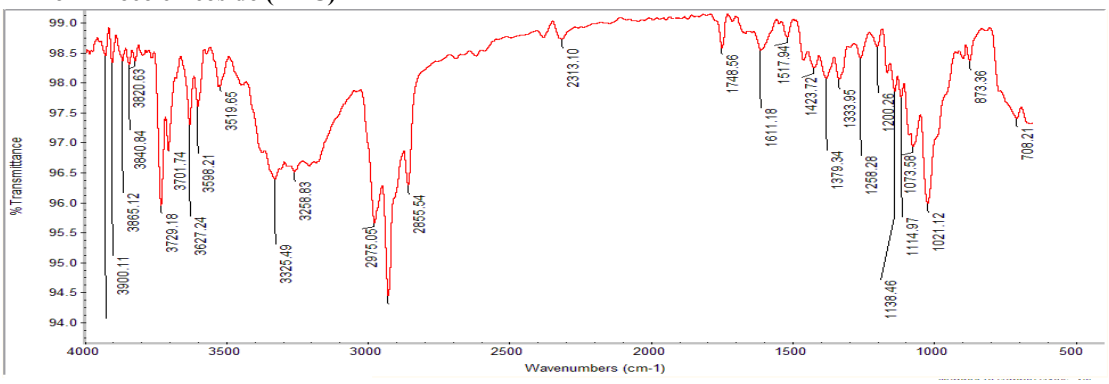

Fig No. 04: FTIR Spectra of THC

The IR absorbance spectrum of Thiocolchicoside (THC) was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1.The IR spectrum of Thiocolchicoside shows characteristic peaks confirming its functional groups and structural integrity. A broad peak at 3362.49 cm^{-1} indicates O–H stretching of hydroxyl groups, while peaks at 2975.08 cm^{-1} and 2855.54 cm^{-1} correspond to aliphatic C–H stretching vibrations.

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The strong absorption peak at 1720.65 cm^{-1} confirms carbonyl (C=O) stretching, and peaks at 1611.18 cm^{-1} and 1511.79 cm^{-1} indicate aromatic C=C stretching. Bands between 1325–1139 cm^{-1} and peaks at 1073.96 cm^{-1} and 1021.12 cm^{-1} represent C–O and C–O–C stretching of ether, glycosidic, and sugar moieties.
Additional peaks at 913.06 cm^{-1} and 708.21 cm^{-1} confirm substituted aromatic rings, supporting the identity and purity of thiocolchicoside.

UV Spectroscopy Analysis

The ultraviolet absorption spectrum of ETD & THC was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λ max) were analysed As ETD is 225 nm and THC is 259 nm.

Selection of mobile phase:

Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions.

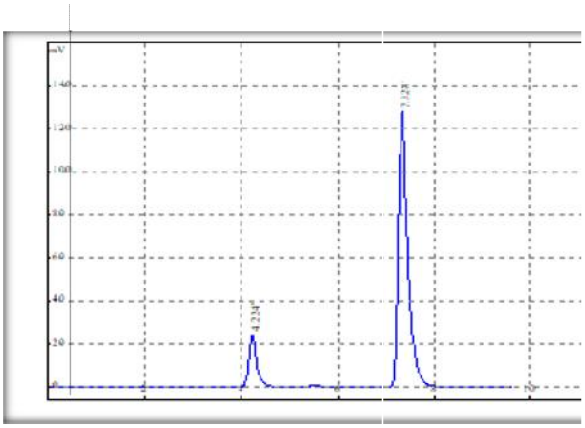

Fig 05: Final Chromatogram obtained by using Acetonitrile: Buffer (50:50) pH 4.5 as mobile phase

Preparation of calibration curve:

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The g/ml for THC□g/ml ETD and 1-10 □g/ml for 4-40 □series of concentration from drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area.

System suitability test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Sr. No	Peak area		Retention Time		Asymmetry		Efficiency	
	ETD	THC	ETD	THC	ETD	THC	ETD	THC
1	117171	8973	7.328	4.224	1.256	1.143	117515.99	67850.342
2	117150	8962	7.322	4.209	1.257	1.157	117688.12	67950.261
3	117202	8950	7.334	4.265	1.262	1.123	117423.34	67990.576
4	117109	8931	7.319	4.245	1.243	1.109	117678.1	67950.261

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5	117256	8999	7.326	4.219	1.231	1.141	117515.99	67799.111
Mean	117177.6	8963	7.3258	4.2324	1.2498	1.1346	117564.3	67908.11
± S.D	55.347086	25.446021	0.00576	0.02246	0.01263	0.01872	114.9122	79.9562
C.V	0.0004723	0.0028390	0.00078	0.00530	0.01011	0.01650	0.000986	0.001292

Fig No. 06: Result of System Suitability Study

Application of proposed method for estimation of ETD and THC Laboratory mixture:
The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration.
Application of proposed method for estimation of ETD and THC in formulation: Equal volume (20mL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of ETD and THC was calculated by comparing a sample peak with that of standard.

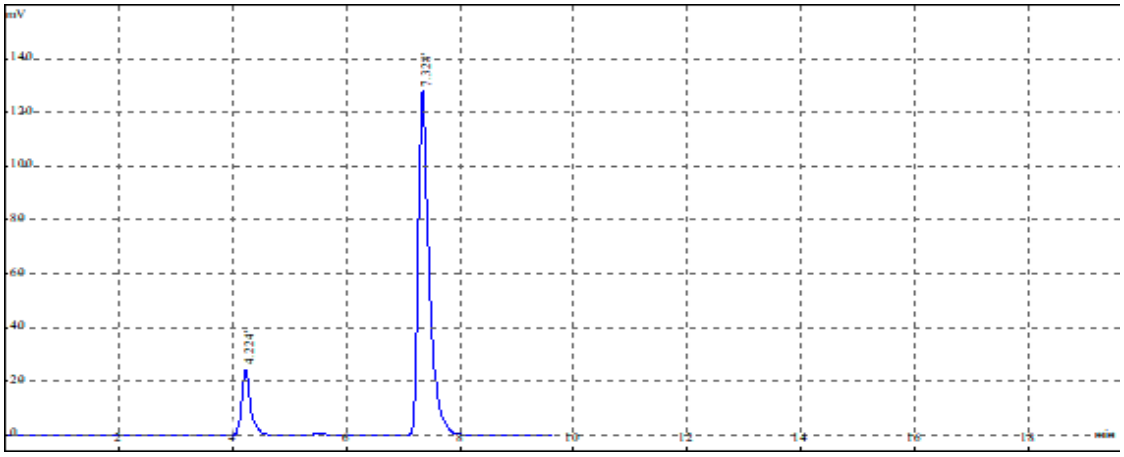

Fig. No. 07: Chromatogram obtained by formulation of ETD and THC
ETD : (RT-7.3258) and THC : (RT-4.2324)

Validation parameters:
a) Accuracy:
It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded

b) Precision:
Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

c) Ruggedness:
The studies of ruggedness were carried out under two different conditions-
1) Days
2) Analyst.

i. **Interday (Different days):** Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3.
ii. **Intraday:** It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula & Result and statistical data.

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iii. **iii) Different analyst:** The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

d) Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for ETD and THC without any interference from component of matrix. Mean retention time for – ETD – 7.328 THC – 4.224 The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

e) Linearity and range:

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. ETD and THC marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

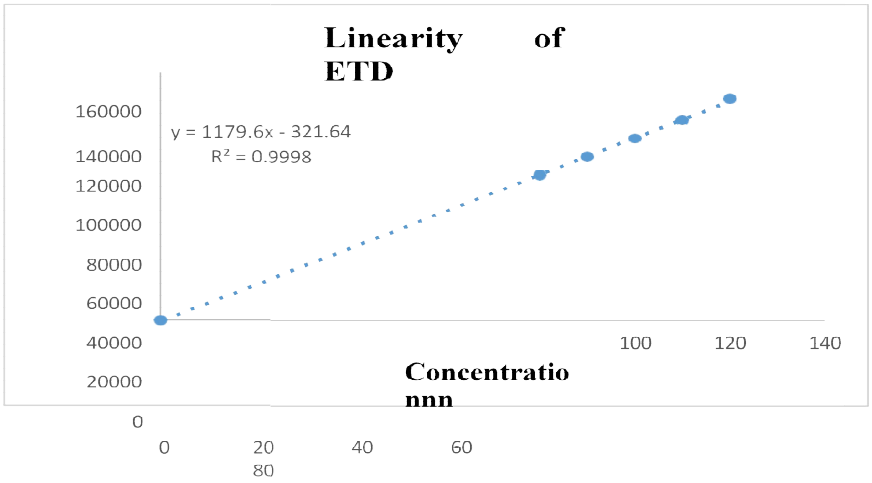

Fig No. 08: Plot of linearity and range study for ETD

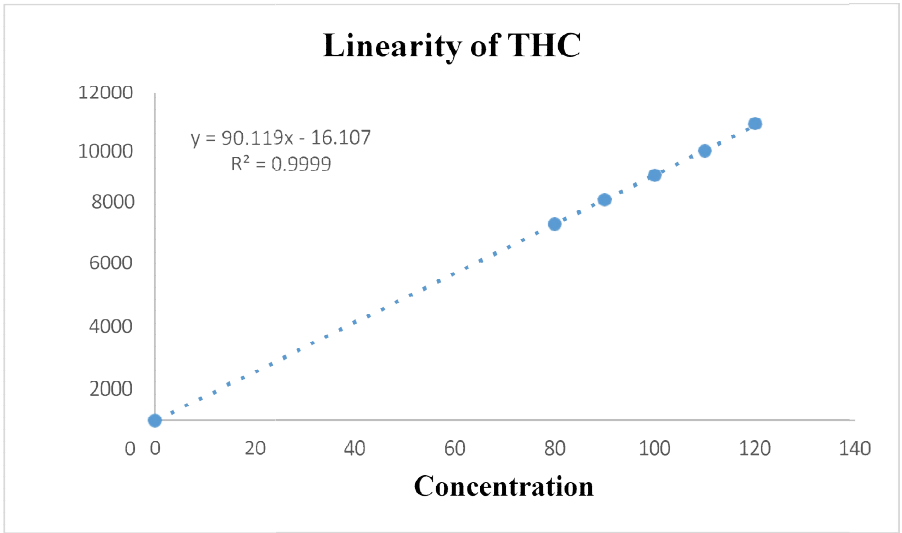

Fig. No. 09: Plot of linearity and range study for THC

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Sr. No.	%Label claim	Peak area	
		ETD	THC
1	80	93736	7178
2	90	105453	8075
3	100	117171	8973
4	110	128888	9870
5	120	142605	10867

Fig No. 10:Observations of Linearity and range study for ETD and THC

f) Robustness: -

Robustness is a measure of how well an analytical method can produce reliable results when there are small changes to the experimental conditions.

g) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD & LOQ are depicted in Table below.

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	ETD	1.116	2.32
2	THC	0.154	1.09

Table No. 11: LOD & LOQ of ETD & THC

V. SUMMARY & CONCLUSION

RP-HPLC Method Development and Validation for the Simultaneous Determination of Etodolac & Thiocolchicoside in Bulk and it's Pharmaceutical Dosage Form.

This technique was employed in the present investigation for simultaneous estimation of Etodolac (ETD) & Thiocolchicoside (THC) in tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied. HPLC with Inertsil 4.6 (id) x 250 mm column and UV detector was used for the study. The standard and sample solution of ETD and THC were prepared in mobile phase. The mobile phase that was found to be most suitable was Acetonitrile: Buffer (50:50) pH 4.5 and detection wavelength 255 nm was selected for the evaluation of the chromatogram of both drugs. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table below.

Sr. no.	Parameter	ETD	THC
1.	Peak area	117177.6	8963
2.	Retention time (min)	7.3258	4.2324
3.	Asymmetry	1.2498	1.1346
4.	Efficiency	117564.3	67908.11

Table No. 12: Summary of system suitability test results

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation. The summary of results of laboratory mixture and marketed formulation are given in the (Table. No. 13)

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Sr. no.	Sample	Statistical data	% Estimation		% Recovery	
			ETD	THC	ETD	THC
1.	Standard Laboratory mixture	Mean	99.97	100.00	-	-
		S.D.	0.321	0.361	-	-
		C.V.	0.003	0.004	-	-
2.	Etova-MR Forte	Mean	100.20	99.87	100.06	100.37
		S.D.	0.265	0.252	0.321	0.718
		C.V.	0.003	0.003	0.003	0.007

Table No. 13: Summary of laboratory mixture and marketed formulation analysis by RP- HPLC Method

The above results clearly indicate that RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their combined dosage formulation without prior separation.

The HPLC method was validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and United States Pharmacopeia guidelines to ensure accurate, precise, and reliable results.

- **Accuracy** was confirmed by recovery studies using the standard addition method.
- **Precision** showed consistent results with %SD less than 2, indicating good repeatability.
- **Specificity** studies confirmed no interference from matrix components
- **Ruggedness** testing under different analysts, days, and times showed similar results, proving the method is reliable under varied conditions.

Parameter	Statistical data	% Estimation by RP-HPLC method	
		ETD	THC
Interday	Mean	100.23	100.47
	S.D.	0.814	0.929
	C.V.	0.008	0.009
Intraday	Mean	100.13	100.93
	S.D.	0.723	0.666
	C.V.	0.007	0.007
Different analyst	Mean	100.36	100.78
	S.D.	0.795612971	0.712039325
	C.V.	0.007927590	0.007065284

Table No. 14: Summary of results of Ruggedness by RP-HPLC method

1) Linearity and Range-

ETD and THC marketed formulation was found to be linear in the range of 80% to 120

% of test concentration with R2 ≈ 0.9998 for ETD and R2 ≈ 0.9999 for THC at selected wavelength for RP-HPLC methods. Same procedure as described in USP was followed.

VI. CONCLUSION

A simple, rapid, and reliable RP-HPLC method was successfully developed for the simultaneous estimation of Etodolac and Thiocolchicoside in pharmaceutical formulations. Effective chromatographic separation was achieved using a C18 column with an optimized mobile phase composition. Both analytes produced sharp, well-resolved, and symmetrical

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peaks within a short analysis time. The method showed excellent linearity across the selected concentration range with correlation coefficients near unity. Precision studies demonstrated low %RSD values, confirming good repeatability and intermediate precision. Accuracy studies through recovery experiments provided satisfactory recovery results for both drugs. No interference from excipients or other components was observed, proving the specificity of the method. System suitability parameters such as retention time, tailing factor, and theoretical plates were within acceptable limits. The developed procedure was also found to be robust, indicating consistent performance under small experimental variations. Overall, the RP-HPLC method is economical, accurate, and suitable for routine quality control and quantitative analysis of combined dosage forms.

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