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# VALIDATED RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE & ETODOLAC IN BULK DRUG AND IN PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

*A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for simultaneous estimation of Thiocolchicoside (THC) and Etodolac (ETO) in tablet dosage form. The method was carried out on a Symmetry C $_{18}$ (150X4.6, 5µ) column with a mobile phase consisting of 0.02 M $KH_2PO_4$ and 0.003 M $K_2HPO_4$ PH adjust 3.0 with dilute $H_3PO_4$, Acetonitrile in the ratio (50:50) and flow rate of 1.0 mL min-1. The detection was carried out at 254 nm. The retention time for THC and ETO were found to be 2.638 and 4.275 min, respectively. The THC and ETO followed linearity in the concentration range of 1.0- 6.0 µg mL-1 and 100- 600 µg mL-1 with r2= 0.999 and r2=1.0 respectively. The amounts of both drugs estimated by proposed method were found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combination in tablet formulation.*

## KEYWORDS

*Thiocolchicoside; Etodolac; RP-HPLC, Validation, Symmetry C18*

## INTRODUCTION:

Thiocolchicoside is chemically N- [(7*S*)-3 -( beta-D-glucopyranosyl oxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo [a] heptalen-7-yl] acetamide [1-2] is an anti-inflammatory analgesic agent with muscle relaxant action implicated in the treatment of musculoskeletal disorders [3-4]. The drug is official in Indian Pharmacopoeia[2]. Few RP–HPLC[5-10], UV-Spectrophotometric[11] and HPTLC[12], methods have been studied for determination of THC in bulk and in pharmaceutical formulations.

Etodolac chemically is 1, 8-Diethyl-1, 3, 4, 9-tetrahydropyrano [3,4-b] indole-1-acetic acid , Belong to class of nonsteroidal anti-inflammatory drugs (NSAIDs) [1-2] is used as anti-inflammatory and analgesic agent [13]. The drug is official in Indian Pharmacopoeia [2], United State Pharmacopoeia[14] and British Pharmacopeia [15]. In literature, One LC-MS [16] method was found for determination of ETO in biological fluids. Several RP-HPLC[17-18] and UV-spectrophotometric [19] and HPTLC [20] method have been reported for estimation of ETO in combination with other drugs in bulk and in pharmaceutical formulations. However no literatures have been found for simultaneous determination of THC and ETO in pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate gradient reversed-phase HPLC method for the simultaneous determination of THC and ETO in the tablet dosage form and validation of the same as per the ICH guidelines [21-22].

## MATERIALS AND METHODS

**Chemicals:**

Thiocolchicoside obtained from Vital Lab. Pvt. ltd, Mumbai and Etodolac obtained from Inchem.lab.Pvt.Ltd, Hyderabad, as a gift samples. Potassium dihydrogen orthophosphate & Dipotassium hydrogen orthophosphate (AR Grade), ortho-phosphoric acid (AR Grade), Acetonitrile (HPLC Grade), were purchased from Merck (India) Ltd., Worli, Mumbai, India. Tablet formulation (ETOVA- MR) was purchased from Indian market, containing ETO (400 mg), THC (4 mg). Double distilled water was used throughout the experiment.

**Instrumentation:**

Analysis was performed on Waters e 2695 separation module with high pressure liquid chromatographic instrument equipped with 2489 UV-Visible detector, autoinjector, autosampler and thermostat column compartment with Empower 2 software from Waters corporation, Milford USA was employed in the study.

**Chromatographic Conditions:** A waters symmetry C-18 column (150 mm x 4.6 mm i.d., 5-µm) was used for chromatographic separation. The mobile phase composed of Acetonitrile and mixed phosphate buffer (50:50 *v/v*); pH adjusted to 3.0 with dilute ortho-phosphoric acid at a flow rate of 1.0 mL min-1 with run time of 10 min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 254 nm.

**Preparation of Standard Stock Solutions:**

Standard Stock solutions of 40 µg mL-1 of THC and 4000 µg mL-1 of ETO were prepared separately using methanol. The stock solution of THC was diluted with methanol to give working standard solutions containing 1.0 – 6.0 µg mL-1 concentrations, similarly the ETO stock solution was diluted with methanol to give working standard solutions in the range 100 – 600 µg mL-1. These standard solutions were injected into HPLC column and calibration curves were plotted by taking drug peak areas vs concentrations. A representative chromatogram presented in **Figure.1**.

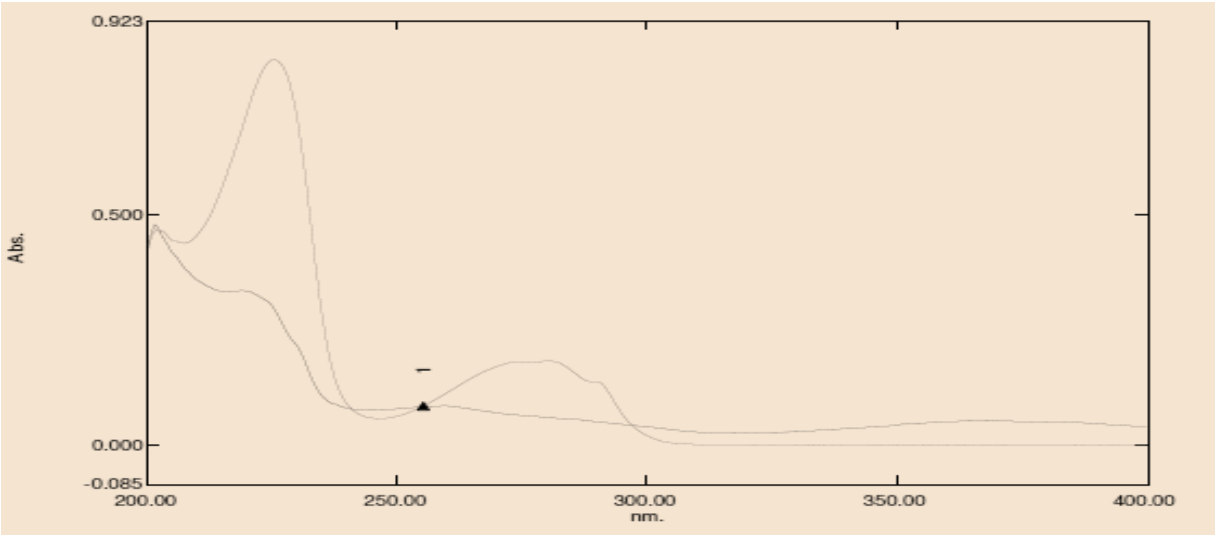



**Figure 1: A Overlay UV Spectrum of standard THC and ETO**

**Analysis of marketed tablet formulation:** To determine the content of THC and ETO in tablets (Brand name: Etova-MR, label claim:ETO 400 mg, THC 4 mg per tablet ), twenty tablets

were weighed, their mean weight determined and finally powdered. An accurately weighed tablet powder equivalent to 400 mg of ETO and 4 mg THC was transfer into 100 mL volumetric flask containing 25 mL of methanol and volume was made up to the mark with methanol, the resulting solution was filtered using 0.45 µm filter (Mill filter, Milford, MA). From filtrate, 10 mL of solution was transferred into 100 mL volumetric flask and volume was made up to mark with methanol to obtain the concentration of 400 µg mL-1 ETO and 4 µg mL-1 of THC was subjected to propose method and the amount of ETO and THC were determined.

**Validation of Method:** The HPLC method was validated in accordance with ICH guidelines.

**Precision:** The system precision of the method was verified by six replicate injections of standard solution containing ETO and THC. The method precision ws carried out the analyte six times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of ETO and THC.

**Accuracy:** Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of ETO and THC; a known amount of standard drug powder of ETO and THC were added at 50, 100 and 150 % level.

**Specificity and Selectivity:** Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

**Limit of detection and Limit of quantitation:** Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

**Robustness:** Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for ETO and THC.

**Ruggedness:** Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 40 µg mL-1 of ETO and 0.4 µg mL-1 solution of THC, respectively.

## RESULTS AND DISCUSSION:

### Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Mobile phase was optimized to separate THC and ETO using Symmetry C-18 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (ACN and phosphate buffer in 50:50 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 1.0 mL min-1. Under optimum chromatographic conditions, the retention time for THC and ETO was found to be 2.638 and 4.275 min, respectively when the detection was carried out at 255 nm. A typical chromatogram of two drugs is shown in (**Figure 1**).

**Table 1: Linearity data**

| THIOCOLCHICOSIDE | | ETODOLAC | |
|---|---|---|---|
| Conc (mcg/ml) | Mean Area | Conc (mcg/ml) | Mean Area |
| 1 | 193251 | 100 | 2792968 |
| 2 | 391607 | 200 | 5682479 |
| 3 | 592114 | 300 | 8497699 |
| 4 | 785931 | 400 | 11319348 |
| 5 | 990773 | 500 | 14091851 |
| 6 | 1193406 | 600 | 16909537 |

**Table 2: Method Precision Study**

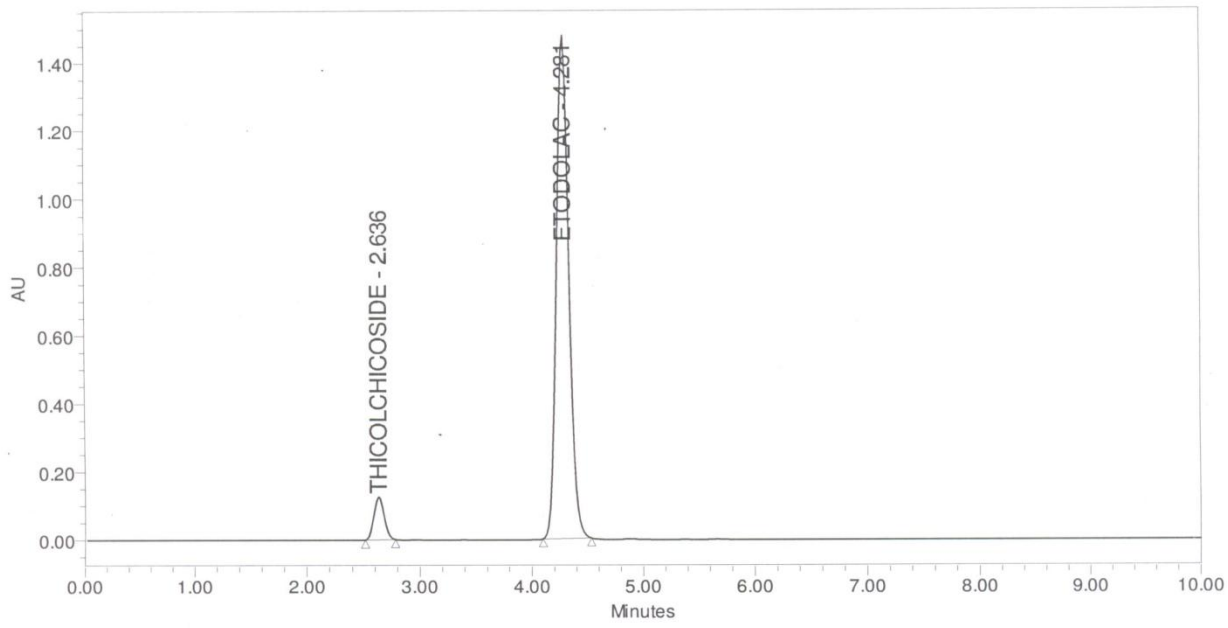
| THIOCOLCHICOSIDE | | | ETODOLAC | |
|---|---|---|---|---|
| S.No. | RT | Area | RT | Area |
| 1 | 2.637 | 793678 | 4.278 | 11445331 |
| 2 | 2.638 | 795874 | 4.28 | 11465456 |
| 3 | 2.639 | 790725 | 4.28 | 11409445 |
| 4 | 2.637 | 793122 | 4.278 | 11433976 |
| 5 | 2.637 | 790414 | 4.278 | 11386739 |
| 6 | 2.635 | 789496 | 4.276 | 11366312 |
| avg | 2.637167 | 792218.2 | 4.278333 | 11417877 |
| stdev | 0.001329 | 2416.936 | 0.001506 | 37353.14 |
| %RSD | **0.05** | **0.31** | **0.04** | **0.33** |

**Table 3: Precision Study**

| THIOCOLCHICOSIDE | | | ETODOLAC | |
|---|---|---|---|---|
| S.No. | 2.635 | 793824 | RT | Area |
| 1 | 2.636 | 792373 | 4.276 | 11462871 |
| 2 | 2.637 | 794013 | 4.277 | 11440971 |
| 3 | 2.638 | 793549 | 4.282 | 11461961 |
| 4 | 2.636 | 794550 | 4.279 | 11460446 |
| 5 | 2.636 | 794330 | 4.278 | 11466659 |
| 6 | 2.636333 | 793773.2 | 4.277 | 11463609 |
| Avg | 2.635 | 793824 | 4.278167 | 11459420 |
| Stdev | 0.001033 | 772.5742 | 0.002137 | 9270.763 |
| %RSD | **0.04** | **0.10** | **0.05** | **0.08** |

**Table 4: Accuracy Data**

| | Amount taken (µg) | Amount found (µg) | Percent Recovery | Percentage of mean recovery |
|---|---|---|---|---|
| **THIOCOLCHICOSIDE** | 2.0 | 2.01 | 100.5 | 100.5 |
| | 4.0 | 4.033 | 100.82 | 100.82 |
| | 6.0 | 6.016 | 100.27 | 100.27 |
| **ETODOLAC** | 200 | 198.16 | 99.08 | 99.08 |
| | 400 | 399.32 | 99.83 | 99.83 |
| | 600 | 596.16 | 99.36 | 99.36 |




| | Peak Name | RT | Area | Height | % Area | Resolution | Symmetry Factor |
|---|---|---|---|---|---|---|---|
| 1 | THICOLCHICOSIDE | 2.636 | 773164 | 124971 | 6.37 | | 1.09 |
| 2 | ETODOLAC | 4.281 | 11372855 | 1472539 | 93.63 | 8.99 | 1.10 |

| | USP Plate Count |
|---|---|
| 1 | 4087 |
| 2 | 6991 |

**Figure: HPLC Chromatogram of THC and ETO**

**Linearity:** The linearity was determined separately for ETO and THC. Linearity of the method was studied by injecting 6 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. The ETO and THC followed linearity in the concentration range of 100– 600 µg mL-1 and 1-6 µg mL-1; respectively. The results are shown in **Table 1**.

**Precision:** The precision study was evaluated on the basis of % RSD value was found to be in the range 0.05 - 0.08 and 0.04 – 0.1 %, respectively. As the RSD values were < 2% therefore

developed method was precise. Results of precision study are shown in **Table 2 & 3**.

**Accuracy:** The accuracy of the method studied at three different concentration levels i.e. 50 %, 100 % and 150 % showed acceptable % recoveries in the range of 99.08 – 99.36 % for ETO and 100.5 – 100.27 % for THC. The results are shown in **Table 4**.

**Sensitivity:** The LOD for THC and ETO was found to be 0.012760 and 1.08560 µg, respectively. The LOQ for THC and ETO was found to be 0.38670 and 3.28970 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

**Robustness and Ruggedness study:** Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. the low value changes of theoretical plates, tailing factor indicating robustness of the method. When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (less than 2 %) indicating ruggedness of the method.

**Analysis of marketed tablet formulation:** Six replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of ETO and THC estimated were found to 99.65 % and 99.60 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in **Table 5**.

**System Suitability Test:** The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in **Table 6**.

**Table 5: Assay Results**

| Drug | Amount present/tablet | % of Assay |
|---|---|---|
| **THIOCOLCHICOSIDE** | 3.984 mg | 99.60 |
| **ETODOLAC** | 398.59 mg | 99.65 |

**Table 6: System suitability parameters**

| Parameters | THIOCOLCHICOSIDE | ETODOLAC |
|---|---|---|
| **Tailing Factor** | 1.09 | 1.10 |
| **Theoritical plates** | 4087 | 6991 |
| **Resolution** | -- | 8.99 |

## CONCLUSION:

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of thiocolchicoside and etodolac in tablet formulation. The method was validated as per ICH guidelines.

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