Original Research Article

RP HPLC Method Development for Simultaneous Estimation of Etoricoxib and Thiocolchicoside

Abstract: A simple, specific and accurate reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Etoricoxib and Thiocolchicoside in bulk and solid dosage form. Separation was achieved by Zorbax C-18 analytical column having dimension (250mm * 4.6 mm i.d 5.0 µm) using methanol and water (60:40) as mobile phase and flow rate was 0.7ml/min. The detection was carried out at 283 nm wavelength using UV detector. The total chromatographic sample time per analysis was about 14.0 minutes with thiocolchicoside eluted at retention time 3.523 min and etoricoxib eluted at retention time 9.697 min. The method was validated for accuracy, precision, specificity, rapid, reliable and reproducible. LOD , LOQ value for etoricoxib and thiocolchicoside were found to be 0.332, 0.996 and 0976, 0.928 respectively. Regression Equation for Etoricoxib was y = 0.006x + 0.149 and regression equation for thiocolchicoside was Y = 0.030x + 0.086. As the run time was increased the retention time was decreased, so the method is simple and economical and can be adopted by regular quality control in industries and also in research laboratories.

Keywords: Etoricoxib, Thiocolchicoside , RP-HPLC, Validation

Introduction:

Etoricxoib is chemically a 5-Chloro-2-(6-Methyl Pyridine-3-yl-3-(4-methylsulfonylphenyl) pyridine. It is a non steroidal anti inflammatory drug and also used in the treatment of gout or arthritis. [1]. Gout or arthritis refers to the pain that occurs when there is too much accumulation of uric acid in the blood. It is selective COX 2 inhibitors that decrease the GI toxicity. [2] There are several methods reported for the analysis of pharmaceutical dosage form as well as biological fluids and tissues that is spectroscopic methods, biological methods, HPLC etc. [3,4] Thiocolchicoside is chemically N-[1,2-dimethoxy-10-methylsulphanyl-9-oxo-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide.

It has muscle relaxant property and is used in the symptomatic treatment of painful muscle spasm. It also has a powerful convulscent activity and thus should not be used in seizure prone individuals. [5,6,7] Literature Survey reveals [8,9] that there were no specific method reported for simultaneous estimation of etoricoxib and thiocolchicoside, hence the current method aims for Simultaneous Estimation of Etoricoxib and Thiocolchicoside by simple RP-HPLC Method. This method is validated and optimized as per ICH guidelines.

Chemicals and Reagents:

All the solvents used for method development were of HPLC grade and chemicals were of analytical grade. Methanol was obtained from Merck. HPLC water was obtained from Ambika Laboratories. All the solvents and solution were filtered through membrane filter (Millipore Millex FH, filter units, Durapore PVDF, polyethylene 0.22 µm pore size). All the solvents were degassed before use. Pure standard of Etoricoxib were received as gift sample form Medipol Pharmaceuticals Pvt. Ltd Baddi and pure standard of Thiocolchiocoside were received as a gift sample form Ashwariya Life Sciences Pvt Ltd Baddi HP.

Instrumentation and Chromatographic Condition:

Chromagraphy was performed with an Agilant Techniques 1220 compact LC (Germany) gradient pump, a variable wavelength detector and a rheodyne 9013 injector with 20 µl loop. Zorbax C-18 Column (2.5cm * 4.6 mm 5µm particles were used for chromatographic separation under suitable condition. Detection were carried out at 283nm and software used was Open Lab Software .The mobile phase was a 60:40 (v/v) mixture of methanol and water. The mobile phase was filtered through 0.45 µm membrane filter and was filtered before use. The flow rate was maintained at 0.7ml/min. The column temperature was maintained at ambient temperature. The mobile phase was used as diluent. The UV detector was made at 283nm for both drugs. The injection volume was 20µl and run time was 10 min. The peak purity was checked with PDA Detector.

Preparation of Standard Solution and Calibration:

Weighed accurately 40 mg of thiocolchicoside working standard (stock solution A) and 60mg of etoricoxib working standard (stock solution B) were transferred to 100ml volumetric flask.

Sonication was used to dissolve the content and make up the volume with diluents (methanol and water 60:40 (v/v)). Then 1ml of stock solution A and 10 ml of stock solution B were diluted to 100ml with diluents. Stock solution were diluted with mobile phase to give working standard solution containing 2 to 20 ppm of thiocolhicoside and 20 to 200 ppm of Etoricoxib. The standard solution were injected for construction of calibration plots by plotting drug peak-area ratio (y) for each of drug against concentration (x). Analysis was performed at ambient temperature. The retention time of etoricoxib and thicolchicoside under these conditions were 9.5 and 3.5 min. respectively.

Assay Procedure:

Weighed accurately 20 tablets were used to calculate the average weight. After crushing tablet powder equivalent to 500 mg of Etoricoxib and 40 mg of Thiocolhicoside were transferred to 250ml volumetric flask. About 200ml of diluent was added and was sonicated for 20 minutes with continuous shaking (maintaining the temperature of sonicator below 25°C. The volume was made upto the mark with diluents and was mixed. The solution was filtered through 0.22 µm PVDA filter, filterate was collected and first few milliliter of filtrate was discarded. Five ml of that solution was taken and was added to 100 ml diluent. A typical chromatogram is obtained as shown in figure 1.

Method Development:

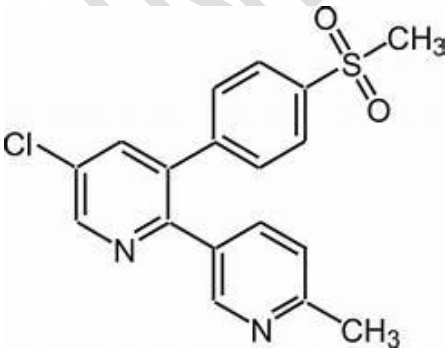
The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. The mobile phase used was a 60:40 (v/v) mixture of freshly prepared methanol and water proved to be more effective than other mixtures used for separation. Different flow rates 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml/min. The flow rate of 0.7 ml was selected because of better resolution of peaks. These chromatographic conditions were found best to provide the resolution between Etoricoxib and Thiocolhcicoside in the time of 9.6 and 3.5 min. respectively. The wavelength of detection was 283 nm and at this wavelength no interfering compound eluted at the retention time of drugs.

Method Validation: The method was validated according to International Conference of Hormonization (ICH) for validation of analytical procedure. The prarameters used to validate

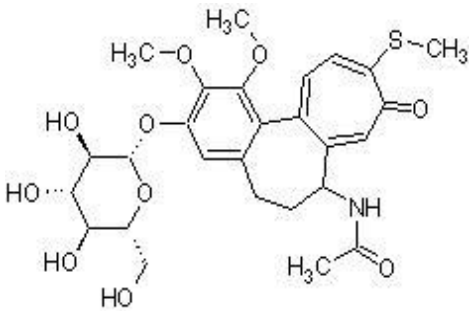
method of analysis were linearity range, accuracy, precision, limit of detection (LOD), Limit of Quantification (LOQ), specificity and robustness.

By using three series of standard solution the calibration curve was constructed. Linearity was obtained in the concentration range of 10 to 200 ppm for etoricoxib and in the range of 2 to 20 for thiocolchicoside with a correlation coefficient of 0.980 and 0.991 respectively. The equation of linear regression and statistical data is represented in table1. Linearity of calibration curve is also validated by high value of correlation coefficient. The limit of Detection (LOD) and Limit of Quantification (LOQ) is represented in table 1. Low value of LOD and LOQ means that the method is sensitive. The was no interference in peaks of drug and excipients present in the marketed formulation was also determined by the specificity. Thus the proposed method was useful to quantify the etoricoxib and thiocolchicoside in different pharmaceutical formulation.

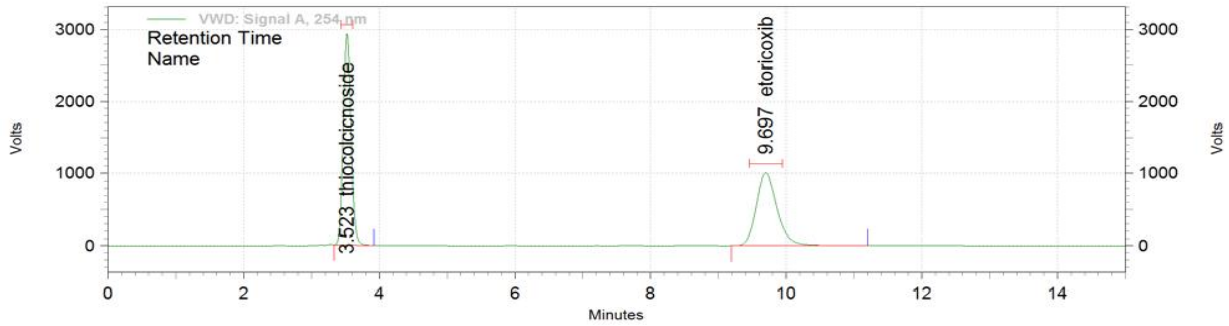
By analyzing the three concentration of bulk drug on three different ways the precision was determined. The accuracy of drug was evaluated by recovery study as evaluated in table 3. By standard addition method recovery study was also completed. A known concentration of working standard was added to fixed concentration of pre analyzed test solution. Recovery study was also very close to 100 % which proposed the suitability and accuracy of drug product.



Structure of Etoricoxib



Structure of Thiocolchicoside



VWD: Signal A, 254 nm
Results

Name	Retention Time	Area	Area %
thiocolcicnoside	3.523	392555809	52.12
etoricoxib	9.697	360678512	47.88
Totals		753234321	100.00

Fig1: Typical Chromatogram of Etroricoxib and Thiocolchicosde

Table 1: Statistical Data for Calibration of Etoricoxib and Thiocolchicoside

Parameters	Etoricoxib	Thiocolchicoside
Linearity	10 to 200	2 to 20
Regression Equation	y = 0.006x + 0.149	Y = 0.030x + 0.086
Correlation Coefficient	0.980	0.999
Slope	0.006	0.030
Intercept	0.149	0.086
Limit of Detection (µg/ml)	0.332 ppm	0.976 ppm
Limit of Quantification (µg/ml)	0.996 ppm	2.928 ppm

Table 2: Assay Data for Combined Etoricoxib and Thiocolchicoside Formulation

Tablet	Etoricoxib			Thiocolchicoside		
	Dose (mg)	Content/Tab (mg)	Label (%)	Dose (mg)	Content/tab (mg)	Label (%)

Brand 1	100	100.3	100.3	8	8.1	98.75
Brand 2	100	100.1	100.1	8	7.9	98.75
Brand 3	100	99.9	99.9	8	8	100

Table 3: Recovery Data for Standard Solution Added to Tablet Formulation:

Sample	Amount of Drug (µg) added to Powder Tablet Formulation	Amount (µg) found n = 3	Percentage of Drug Recovered	% Recovery ± SD
Etoricoxib	0.5 1.0 1.5	0.462 0.931 1.463	92.4 93.1 97.5	94.3±1.22
Thiocolchicoside	0.5 1.0 1.5	0.482 0.993 1.492	96.4 99.3 99.4	98.36±1.54

Results and Discussion:

Because of the reliability of quality control of drugs and drug product the HPLC method development has received considerable attention over the years. The purpose of this study was HPLC method development for simultaneous estimation of formulated and marketed drug product of Etoricoxib and Thiocolchicoside. The proposed method was found to be simple, statically valid and rapid for its accuracy. In the analysis of drug there were no interfering peaks observed in the chromatogram where results that tablet and excepient do not interfere with the drug. The typical chromatogram obtained in the method development is shown in figure. The retention time (RT value) for Etoricoxib and Thiocolchicoside was found to be 9.697 and 3.523 min respectively. Linearity was obtained in the calibration curve in the range of 10 to 200 for Etoricoxib and 2 to 20 for Thiocolchicoside. The Correlation coefficient for Etoricoxib was found to be 0.980 and for thiocolchicoside the correlation coefficient was found to be 0.999 respectively. The linear regression equation was y = 0.006x + 0.149 for Etoricoxib and Y =

0.030x + 0.086 for thiocolchicoside respectivelty. The mean drug content for Etoricoxib was found to be 99.9 for Etoricoxib and drug content for thiocolchicoside was found to be 99.16 respectively. The recovery test for Etoricoxib and Thiocolchicoisde was found in triplicate and the mean recovery for Etoricoxib was found to be 94.3±1.22 respectively and mean recovery for Thiocolchicoside was found to be 98.36 ± 1.54 respectively which indicates that the proposed method of analysis is highly accurate.

Conclusion:

The result obtained in the analysis has shown that the method development is simple and accurate. This method can be useful for determination of simultaneous estimation of Etoricoxib and Thiocolchicoside in pharmaceutical formulations.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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