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SIMULTANEOUS ESTIMATION OF ETORICOXIB AND THIOCOLCHICOSIDE BY RP-HPLC METHOD IN COMBINED DOSAGE FORMS

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Abstract: A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of etoricoxib and thiocolchicoside in combined tablet dosage form. Formulation containing etoricoxib and thiocolchicoside is used as analgesic. Chromatography was performed on a 250 mm × 4.6 mm, 5-µm particle size, BDS Hypersil C-18 column with trifluoroacetic acid buffer (pH 2.6) and acetonitrile (75:25, v/v) as a mobile phase. The detection of the combined dosage form was carried out at 220 nm and a flow rate employed was 1.5 mL/min. The retention times were 6.6 and 3.1 min for etoricoxib and thiocolchicoside, respectively. Linearity was obtained in the concentration range 20 to 160 ppm for etoricoxib and in the range 2 to 16 ppm for thiocolchicoside with a correlation coefficient of 0.9918 and 0.9994, respectively.. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

Keywords: etoricoxib, thiocolchicoside, RP-HPLC, simultaneous estimation, tablet formulation

Etoricoxib is 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl)pyridine. It is used as a non-steroidal anti-inflammatory agent (1). It is selective inhibitor of COX-2 that decreases GI toxicity and is without effects on platelet function (2). Several methods have been reported for the analysis of etoricoxib in pharmaceutical dosage form as well as in the biological fluids and tissues, i.e., spectrophotometric methods (3, 4) and chromatographic methods: HPLC (5, 6) and LC/mass spectrophotometry (7–9). Thiocolchicoside, chemically N-[1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide, is a muscle relaxant, which has been claimed to possess GABA mimetic and glycinergic actions. It is used in the symptomatic treatment of painful muscle spasm (10). It has powerful convulsant activity and should not be used in seizure-prone individuals (11, 12).

Thiocolchicoside is not official in any pharmacopoeia. On detailed literature survey, it was found that thiocolchicoside can be estimated by spectrophotometry (13, 14), HPLC (15, 16) and by HPTLC (17) methods individually or in combination with other drugs (18–22).

Since no HPLC methods are reported for the simultaneous estimation of etoricoxib and thiocolchicoside in combination, therefore in the present work, a successful attempt has been made to estimate both these drugs simultaneously by a simple RP-HPLC method. The proposed methods were optimized and validated as per ICH guidelines. The structures of etoricoxib and thiocolchicoside are given in Figure 1.

EXPERIMENTAL

Chemicals and reagents

All solvents were of HPLC grade and reagents were of analytical grade. Acetonitrile and trifluoroacetic acid were obtained from Merck. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore Millex-FH, filter units, Durapore-PVDF, polyethylene, 0.22 µm pore size) and degassed before use. Pure standards of etoricoxib and thiocolchicoside were obtained as gift samples from Zydus Cadila (Ahamedabad, India).

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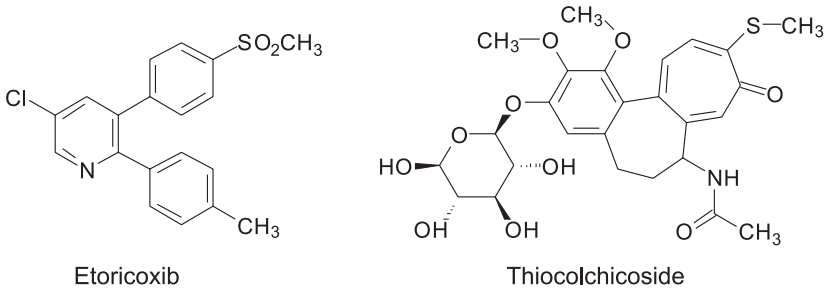
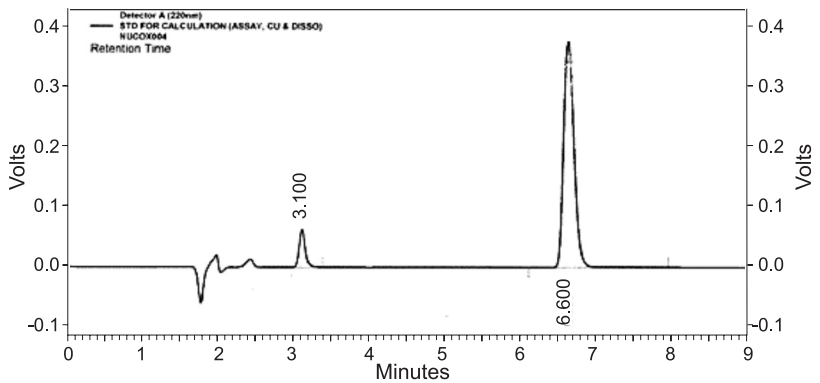


Figure 1. Structure of etoricoxib and thiocolchicoside



Detector A (220 nm)

Pk #	Name	Retention Time	Area
1	THIOCOLCHICOSIDE	3.10	341168
2	ETORICOXIB	6.60	3547727
Totals			3888895

Figure 2. Typical chromatogram obtained from etoricoxib and thiocolchicoside

Instrumentation and chromatographic conditions

Chromatography was performed with an Agilent Technologies 1020 compact LC (Germany) gradient pump, an variable wavelength detector and a rheodyne 9013 injector with 20-µL loop. LiChrospher RP-18 column (250 × 4.6 mm., 5-µm particles) was used for chromatographic separation under suitable conditions. Detection was carried out at 220 nm and the software used was EZChrom Elite version 3.3. The mobile phase was a 75:25 (v/v) mixture of freshly prepared buffer (trifluoroacetic acid) and acetonitrile. The mobile phase was filtered through a 0.45 µm membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.5 mL/min. The column temperature was maintained at ambient temperature. The mobile phase was used as a diluent. The UV detection was made at 220 nm for both drugs. The injection volume was 20 µL and total run time was 10 min.

Preparation of standard solution for calibration plots

Weighed accurately 40 mg of thiocolchicoside working standard (stock solution A) and 60 mg of etoricoxib (stock solution B) were transferred to a 100 mL volumetric flask. Sonication was used to dissolve the content and make up the volume with diluents (acetonitrile and water 50:50 (v/v)). Then, 1 mL of stock solution A and 10 mL of stock solution B were diluted to 100 mL with diluents. Stock solution was diluted with mobile phase to give working standard solution containing 2 to 16 ppm of thiocolchicoside and 20 to 160 ppm of etoricoxib. These standard solutions were injected for construction of calibration plots by plotting drug peak-area ratio (y) for each of the drug against concentration (x). Analysis was performed at ambient temperature. The retention times of etoricoxib and thiocolchicoside under these conditions were 6.6 and 3.1 min. respectively.

Assay procedure

Weighed accurately 20 tablets were used to calculate the average weight. After crushing, tablet powder equivalent to 300 mg of etoricoxib and 20 mg of thiocolchicoside was transferred in 250 mL volumetric flask. About 100 ml of diluent was added and sonicated for 30 min with continuous shaking (maintaining the temperature of sonication below 20°C). The volume was made up to the mark with diluent and mixed. The solution was filtered through 0.45 µm PVDF filter; the filtrate was collected and the first few milliliters of the filtrate were discarded. Five mL of that solution was taken and again added in 100 mL of diluents. A typical chromatogram obtained from a sample solution is shown in Figure 2.

Method development

The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. The mobile phase used was a 75:25 (v/v) mixture of freshly prepared buffer (trifluoroacetic acid) and acetonitrile, proved to be more effective mixture than the other mixtures used for the separation. Then, the flow rate tested were 0.4, 0.8, 1.0, 1.2 and 1.5 mL/min. This last was selected for the assay because of better resolution of the peaks.

The mentioned chromatographic conditions were the best to provide resolution between etoricoxib and thiocolchicoside in a reasonable time of 6.60 and 3.10 min, respectively. The optimum wavelength for detection was 220 nm and no indigenous interfering compounds eluted at the retention times of the drugs.

Method validation

The method was validated according to the guidelines set by the International Conference on

Table 1. Statistical data for calibration curve of etoricoxib and thiocolchicoside.

Parameters	Etoricoxib	Thiocolchicoside
Linearity	20 to 160 ppm	2 to 16 ppm
Regression equation	y = 6911x – 79095	y = 8762x – 13621
Correlation coefficient	0.9918	0.9994
Slope	6911	8762
Intercept	–79095	–13621
Limit of detection	1.1067 ppm	0.1531ppm
Limit of quantitation	3.3537 ppm	0.4639 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	60	60.2	100.3	4	3.9	97.5
Brand 2	60	58.8	98.0	4	4.1	102.5
Brand 3	60	59.3	98.8	4	3.9	97.5

Table 3. Recovery data for standard solutions added to tablet formulations.

Sample	Amount of drug added (µg) to powdered tablet formulation	Amount (µg) found n = 3	Percentage of drug recovered	% Recovery ± SD
Etoricoxib	0.5 1.0 1.5	0.481 0.987 1.489	96.2 98.7 99.3	98.06 ± 1.64
Thiocolchicoside	0.5 1.0 1.5	0.479 0.991 1.478	95.8 99.1 98.5	97.8 ± 1.75

Harmonisation (ICH) for the validation of analytical procedures. The parameters which were used to validate the method of analysis were linearity range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity and robustness.

Calibration curves were constructed using three series of standard solutions. Linearity was obtained in the concentration range 20 to 160 ppm for etoricoxib and in the range 2 to 16 ppm for thiocolchicoside with a correlation coefficient of 0.9918 and 0.9994, respectively. The equations of linear regression and statistical data are presented in Table 1. The linearity of calibration curve was validated by high value of correlation coefficient. The limit of quantitation (LOQ) and the limit of detection (LOD) are shown in Table 1. Low value of LOD and LOQ indicate the method to be sensitive. The specificity of the proposed method demonstrated that the excipients present in market formulation do not interfere with the drug peaks. Furthermore, well resolved peaks indicate the specificity of the method (Fig. 2). Thus the proposed method is useful to quantify etoricoxib and thiocolchicoside in different pharmaceutical formulations.

The precision was determined by analyzing three different concentrations of the bulk drug on three different days. The accuracy was evaluated by recovery studies shown in Table 3. Recovery studies were performed by standard addition method. A known concentration of working standard was added to a fixed concentration of the pre-analyzed test solution. Recovery results are very close to 100%, which prove the suitability and accuracy of the proposed method.

RESULTS

Typical chromatograms obtained are shown in Figure 2. The retention time (RT) of etoricoxib and thiocolchicoside were 6.6 and 3.1 min, respectively. The calibration curve showed linearity over a concentration range of 20 to 160 ppm and 2 to 16 ppm for etoricoxib and thiocolchicoside with correlation coefficients of 0.9918 and 0.9994, respectively; and representative linear regression equations were $y = 69115x - 79095$ and $y = 87620x - 13621$, respectively. The assay results are given in Table 2. The mean drug content was found to be 99.03 ± 1.12% for etoricoxib and 99.17 ± 0.87% for thiocolchicoside. The recovery test, which was performed in triplicate, indicates that the mean recovery was 99.07 ± 1.64 and 97.80 ± 1.75 % for etoricoxib and thiocolchicoside, respectively (Table 3).

DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention over the years because of their reliability in the quality control of drugs and drug products. The goal of this study was to develop a rapid HPLC method for the analysis of etoricoxib and thiocolchicoside in a finished tablet formulation using a commonly employed reversed phase C-18 column with UV detector. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in the analysis of drugs. The calibration curve showed linearity and was linear with a correlation coefficient of 0.9918 and 0.9994 for etoricoxib and thiocolchicoside, respectively. Recovery test, which was performed in triplicate, averaged 99.07 ± 1.64% and 97.80 ± 1.74% for etoricoxib and thiocolchicoside, respectively, indicating that the proposed method for the analysis of drugs is highly accurate.

CONCLUSION

The results of this study showed that the developed method is simple and accurate. It should be useful for the simultaneous determination of etoricoxib and thiocolchicoside in pharmaceutical formulations.

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