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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF ETODOLAC AND THIOCOLCHICOSIDE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC" METHOD

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ABSTRACT

An RP-HPLC method was developed and validated for the simultaneous determination of Etodolac and Thiocholchicoside. The chromatographic system was equipped with Hypersil BDS, C_{18} column (4.5mm × 250mm) and PDA detector set at 260 nm, in conjugation with a mobile phase of potassium dihydrogen phosphate buffer (pH 6.8) and Acetonitrile in the ratio of 70:30 % v/v, at a flow rate of 1ml/min. The retention time of Thiocholchicoside and Etodolac were found to be 3.05 and 12.10 minutes respectively. Linearity was observed in the concentration range of 4.012 to 12.03 µg/ml for thiocholchicoside and 249.09 to 747.29 µg/ml for etodolac, with good linearity response of 0.999 and 0.998 respectively. Percentage recoveries obtained for thiocholchichoside and etodolac were 98-102% and 98-102% respectively. The proposed method is precise, accurate, selective and rapid for simultaneous determination of thiocholchicoside ans etodolac.

This method can be used to estimate either of these drugs individually when present separately in formulation or in combination.

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KEYWORDS: *Etodolac, Thiocholchicoside, RP-HPLC method.*

INTRODUCTION

Etodolac is one of the most potent inhibitors of prostaglandin synthesis. Chemicaly, it is *(S+)*-2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid, clinically used in the treatment of painful musculoskeletal condition such as pain caused by osteoarthritis and rheumatoid arthritis.[6,7] Literature survey reveals that etodolac can be estimated Spectrophotometric method[9,10] and RP-HPLC[11,12] method.

Thiocolchicoside acts as a competitive $GABA_A$ receptor antagonist and also inhibit glycine receptors with similar potency and nicotinic acetylcholine receptor to a much lesser extent.

Chemicaly, it is *N*-[(7S)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide. It is used in treatment of painful musculoskeletal condition such as pain caused by osteoarthritis and rheumatoid arthritis.[6,7] Literature survey reveals that thiocolchicoside can also be estimated by RP-HPLC[13,14,16,17], HPTLC[18], and Spectroscopic method.[15]

MATERIALS AND METHODS:

Chemicals and reagents:

Pure sample of Etodolac and Thiocolchicoside were supplied by the Emcure Pharmaceuticals Ltd.(Pune, India). Methanol, Acetonitrile were used of HPLC grade. Ortho-Phosphoric Acid, Sodium Hydroxide, Hydrochloric acid, Triethyl amine, Hydrogen Peroxide reagents used in this study were of AR grade.

Instruments used:

Chromatographic separation was performed on a WATER'S 2695 HPLC System chromatographic system equipped with a quaternary, low pressure mixing pump and inline vacuum degassing. Flow rates 50 ml/min to 1 ml/min can be generated for with 2.1 mm ID columns and larger. The detector is photodiode array (model 2996) with wavelength range of 190 – 800 nm sensitivity setting from 0.0001 – 2.000 absorbance unit. All components of the HPLC are controlled through Water Empower software. Analytical balance and pH meter, UV-visible spectrophotometer 1600, Shimadzu.

Optimized chromatographic conditions:

Mobile phase consisting of a mixture of potassium dihydrogen phosphate buffer (pH 6.8) and Acetonitrile in the ratio of 70 : 30 %v/v was delivered at a flow rate of 1ml/min. The Hypersil BDS, C_{18} (4.5mm × 250mm) column used. Detection wavelength is 260 nm and column temperature is 35 $^{\circ}$ C with sample size 20µl and run time is 15 minutes.

Preparation of Standard Stock Solution of Etodolac

Transfer 75mg of Etodolac working standard in 50 ml volumetric flask. Add about 30 ml of mobile phase and dissolve. Make volume up to the mark with mobile phase and mix.

Preparation of Standard Stock Solution of Thiocolchicoside

Transfer 20 mg of thiocolchicoside working standard in 200 ml volumetric flask. Add 100 ml of mobile phase and sonicate to dissolve. Make volume up to the mark with mobile phase and mix.

Preparation of Mix Standard Solution

Pippet out 5 ml of stock solution of Etodolac and 2 ml of stock solution of Thiocolchicoside in 25 ml volumetric flask and add 15 ml mobile phase to mix. Make volume up to mark with mobile phase to obtain final standard concentration of Thiocolchicoside and Etodolac 8 µg/ml and 300 µg/ml, respectively.

Preparation of Test Sample Solution of Etodolac and Thiocolchicoside

Accurately 20 intact tablet were weighted and average weight was calculated. Tablets were crushed into a fine powder. Transfer an accurately weighed quantity of powder equivalent to 300 mg of Etodolac in to a 100 ml volumetric flask. Then 70 ml of mobile phase added to flask and sonicated for 15 minute with intermittent shaking. Make up volume to the mark with mobile phase and mix. Solution thereafter was filtered through Whatman 41 filter. 5.0 ml of filtrate was transferred into 50 ml volumetric flask and diluent was added up to mark to get final concentration of 8 µg/ml of thiocolchicoside and 300 µg/ml of etodolac.

METHOD VALIDATION:

HPLC method for the estimation of Etodolac and Thiocolchicoside in tablet formulation has been developed and validated as per principles of ICH guidelines.

Selectivity/ Specificity:

A method is said to be specific when it produces a response for no interference from blank and placebo. The specificity of the method was determined by checking the interference with the components. No interference was observed for any of the components like excipients of drug. The retention time observed at 3.05 min for Thiocochicoside and 12.10 min for Etodolac.

System Suitability Parametres:

The system suitability studies were carried out to determine theoretical plates, resolution and tailing factors. The results were given in Table 2. The values obtained demonstrated the suitability of the system for the analysis of investigated drug combination, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Linearity and calibration curves:

The linearity of the method was studied by injecting 20 μL of working standard solutions of concentration ranging from 4 to 12 μg/mL for thiocolchicoside and 150 to 451 µg/ml for etodolac into the column, and linearity report was obtained with regression of 0.999. A calibration curve were constructed by plotting concentration against peak area for Thiocolchicoside and Etodolac in Figure 2 and Figure 3 respectively.

Precision:

Precision of a method was expressed in terms of statistical parameters such as standard deviation and % RSD. The % RSD was calculated for six replicate measurements and found to be less than 2.0.

Accuracy (Recovery):

The accuracy of the method was determined from recovery experiments. The recovery studies were carried out at three different concentration levels (50%, 100%, and 150% of target concentration). The percentage recovery of the drug at three different concentration levels is presented in Table 4 and Table 5.

Robustness:

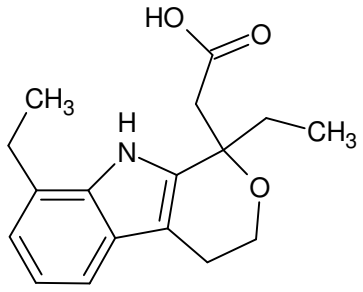
Robustness of the proposed method is checked by making slight deliberate change in the experimental procedures. In the present method, a deliberate change in the Flow rate and mobile phase composition is made to evaluate the impact on the method. Chromatographic characteristics were evaluated by calculating % RSD. The results were found to be within the limits as per ICH Guidelines.

RESULTS AND DISCUSSION:

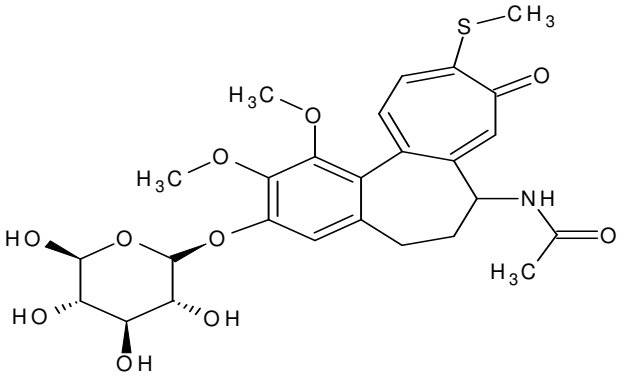
The goal of this study was to develop a rapid and sensitive HPLC method for the analysis of Etodolac and Thiocolchicoside in bulk drug samples and its formulation using the most commonly employed RP-C18 column with PDA detection. The mobile phase consisted of potassium dihydrogen phosphate buffer (pH 6.8) and Acetonitrile in the ratio of 70 : 30 %v/v. The retention time of Thiocholchicoside and Etodolac were found to be 3.05 and 12.10 minutes respectively Figure 1. The peak areas of the drug was reproducible as indicated by RSD values which are less than 2%. The results of the formulation analysis, recovery studies and its statistical validation data indicate high degree of precision and accuracy of the proposed method. Hence it can be concluded that the developed RP-HPLC method can be employed successfully for the estimation of Etodolac and Thiocolchicoside in both bulk and drug formulation.

CONCLUSION:

RP-HPLC method was developed for Etodolac and Thiocolchicoside and was validated as per ICH guidelines. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of said drug in bulk as well as its tablet dosage form.



Structure of Etodolac



Structure of Thiocolchicoside

Table1: Assay of Thiocolchicoside and Etodolac

Drug	Labled claim (mg)	Amount found (mg)	% Assay
Thiocolchicoside	8	8.07	100.92
Etodolac	300	300.75	100.25

Table 2: System suitability test parameters

Parameters	Thiocolchocoside	Etodolac
Area	330833	6403901
Theoretical plates	7697	6235
Retention time	3.05 min	12.10 min
Asymmetry	1.31	1.49

Table 3: Regression analysis data and validation data

Parameters	Thiocolchicoside	Etodolac
Detection wave length (nm)	260	260
Linearity range(µg/ml)	4.012 – 12.03	249.09 – 747.29
r^2	0.999	0.998
Precision(%RSD)	0.36	0.40
Mean Recovery	98.06 %	99.96 %

Table 4: Recovery study for Thiocolchicoside

Level of % Recovery	Area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
50	199808	4.73	4.72	99.85
100	363943	8.52	8.60	100.97
150	513621	12.03	12.07	100.37

Table 5: Recovery study for Etodolac

Level of % Recovery	Area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
50	3311740	153	153.66	100.43
100	6630337	306.46	307.64	100.39
150	9777773	454.05	454.19	100.03

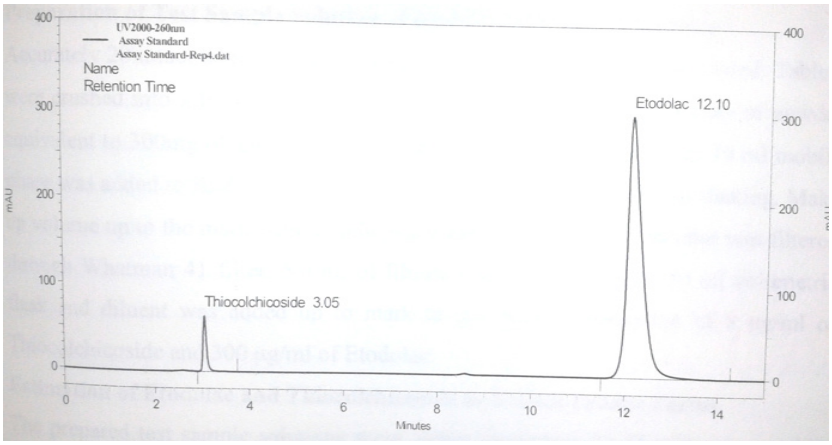


Figure 1: Representative HPLC chromatogram of Thiocolchicoside and Etodolac

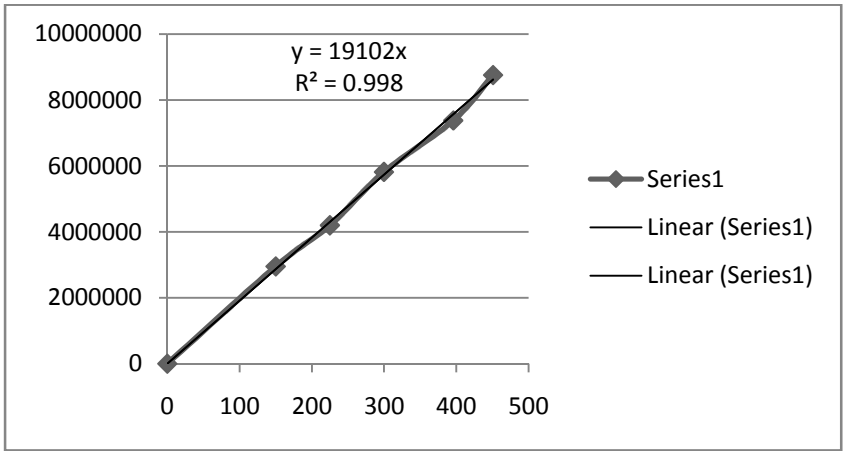


Figure 2: Linearity graph of Thiocolchicoside

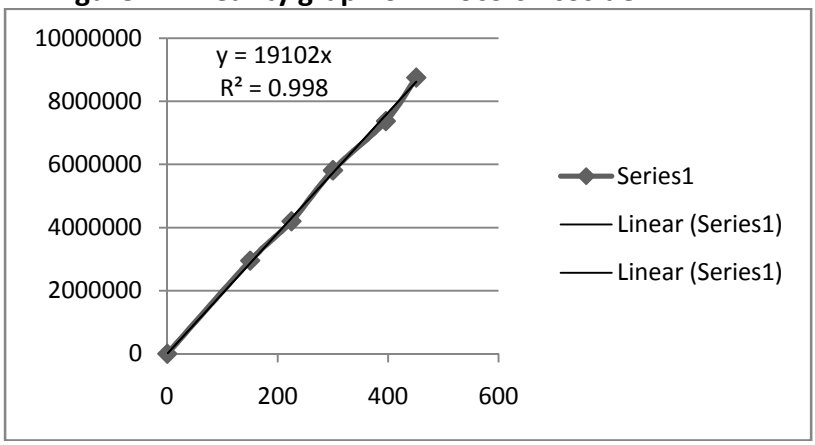


Figure 3: Linearity graph of Etodolac

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