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Stability Indicating RP-HPLC Method for Estimation of Thiocolchicoside in Capsule Dosage Forms

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ABSTRACT

A rapid, simple and precise stability indicating RP-HPLC method for the determination of Thiocolchicoside on C18 column (250mm × 4mm, 5µm) has been developed. A mobile phase consisting of acetonitrile: water (70:30) was used. The flow rate was 1.0 mL min-1 with UV detection at 286 nm.The specificity of the method was ascertained by forced degradation studies by acid and alkali degradation, oxidation and photolysis. Beer Law is obeyed over a concentration range of 0-10µg/ml and correlation coefficient as 0.9996. This method was also successfully applied for routine analysis of Thiocolchicoside in capsule dosage forms.

Keywords: Thiocolchicoside, Forced degradation, RP-HPLC

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INTRODUCTION

Thiocolchicoside Chemically it is N-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo [*a*] heptalen-7-yl] acetamide[1]. And has the empirical formula C10H21NO7. THC is a muscle relaxant. Its mode of action includes modulation of chemokine and prostanoid production and inhibition of neutrophil and endothelial cell adhesion molecules by which it interferes with the initiation and amplification of the joint inflammation[2,3].

Extensive literature review reveals that several spectrophotometric methods have been reported so far for determination of Thiocolchicoside.But no stability indicating RP-HPLC method has been reported so far for the estimation of Thiocolchicoside In Capsule Dosage. In view of these points an attempt was made to develop a simple, accurate and validated stability indicating RPHPLC method for estimation of Thiocolchicoside in Capsule Dosage Form [4, 5, 6, 7, 8].

METHODS & MATERIALS

Instrumentation

Quantitative HPLC was performed on Agilent 1120 -technologies ltd variable wavelength programmable UV/Visible detector SPD-20A and Redone (7725 i) with 20µL fixed loop and data analyzed by using Azihrome Elite software.

Reagents and Chemicals Used

Pure sample of Thiocolchicoside was kindly supplied by the Ajanta Pharmaceutical Ltd. Mumbai. Methanol, acetonitrile and water used were of HPLC grade. All other reagents used in this study were of AR grade.

Method Optimization

To ascertain the detection lmax of the proposed method, the drug solution (10 mg mL-1) was scanned between the wavelength ranges of 200 – 380 nm and was found to be 286 nm. To develop a suitable and robust HPLC method for the determination of Thiocolchicoside different mobile phases like, Acetonitrile: water were tried in different compositions of mobile phases (30:70, 40:60, 50:50, 70:30,80:20) at different flow rates (0.5,0.75,1.0, 1.2, 1.5, mL min-1). The mobile phase acetonitrile: i water in the ratio of 70:30%v/v at a flow rate of 1.0 mL min-1 gave peaks with symmetric shape and good resolution for Thiocolchicoside Drugs were eluted at retention times of 3.3.20min The Optimized chromatographic conditions and System Suitability parameters are shown in Table 1.

Table No 1: Optimized Chromatographic Conditions and System Suitability Parameters

Parameters	Method
Optimized Chromatographic Conditions	
Column	Intersil 250 mm x 4.6 mm (lot20081120)
Mobile Phase	Acetonitrile: Water (70:30)
Flow rate (mL min-1)	01
Run time (minutes)	6.0
Column temperature (°C)	Ambient
Volume of injection loop (mL)	20
Detection wavelength (nm)	286
Drug RT (minutes)	2.208
System Suitability	
Theoretical Plates(N)	8148
Resolution(Rs)	3.334
Tailing Factor(T)	1.25

Preparation of Mobile Phase

Acetonitrile : Water. Then it was ultrasonicated for 30 min.Also acetonitrile HPLC grade Both the components were then filtered through a 0.45µm membrane filter. Method was developed using Metaxalone as the internal standard.

Preparation of Standard Stock Solution and Calibration Curve

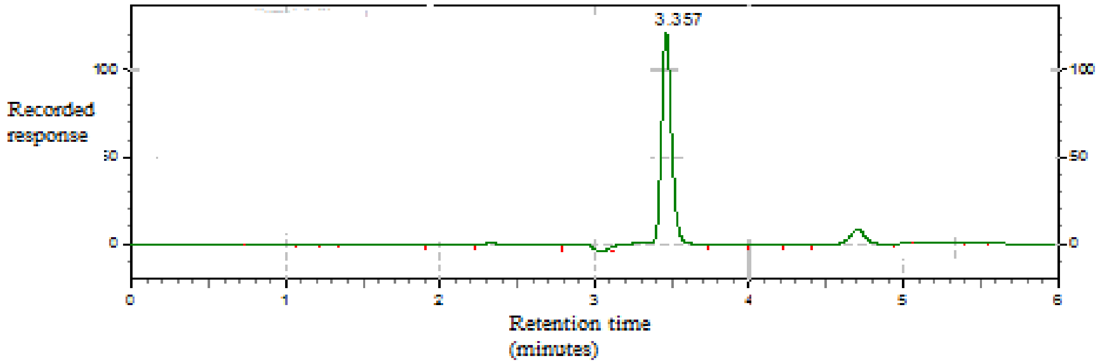

Fig. No 1 Representative Chromatogram of Thiocolchicoside (10µg /ml) (pure drug)

10 mg of drug and internal standard were weighed accurately and transferred to a 25mL volumetric flasks .The drug was then dissolved in mobile phase,ultrasonicated and finally volume was made up to mark to get a concentration of 1000µg mL-1.Both solutions were filtered through a 0.45µm membrane filter. Appropriate aliquots of the standard stock solution of Thiocolchicoside (1000µg mL-1) were transferred into a series of 10ml volumetric flasks spiked with 20µg mL-1of internal standard. Each solution was made up to the mark by mobile phase and injected. A representative chromatogram of standard drug is shown in Figure 1.The

Calibration Curve was plotted taking peak area ratio on y-axis against concentration of drug on x-axis.

Preparation of Sample Solution

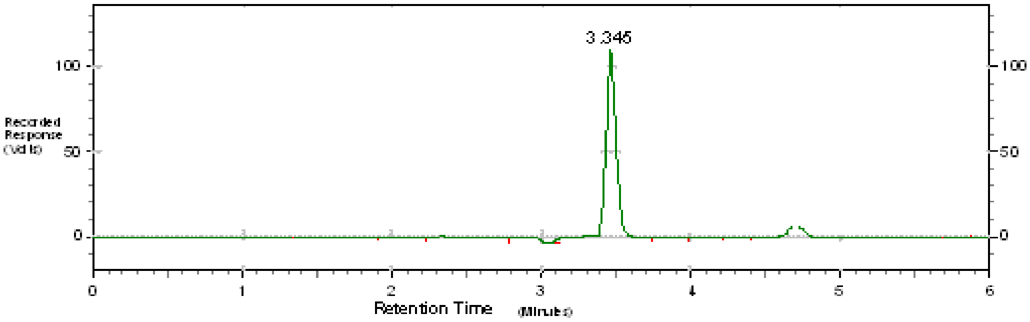

Fig. No 2 Representative Chromatogram of Thiocolchicoside (10µg /ml) (Formulation)

20 capsule were weighed accurately and finely powdered. Powder equivalent to 25mg of Thiocolchicoside taken in a 25mL volumetric flask, dissolved in mobile phase and ultrasonicated for 30 minutes. Then the volume was made up to the mark with the mobile phase and filtered through 0.45µm membrane filter. Sample solutions were prepared from this filtered solution and spiked with internal standard of concentration 20µg mL-1.Sample solutions were injected into HPLC and chromatograms were obtained. A representative chromatogram of Thiocolchicoside in capsule dosage form has been given in Figure 2.The amount of drug present in the sample solution was determined using the calibration curve of standard drug.

Intra-day and Inter-day Precision

The intra-day and inter-day precision of the method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard on same day and on different days. The percent relative standard deviation was calculated.

Recovery Studies

To check the accuracy of the proposed method, recovery studies were carried out at 80,100 and 120% of the test concentration as per ICH guidelines [16].The recovery study was performed 3 times at each level.

Specificity

The specificity of the HPLC method was determined by complete separation of Thiocolchicoside in presence of its degradation products.

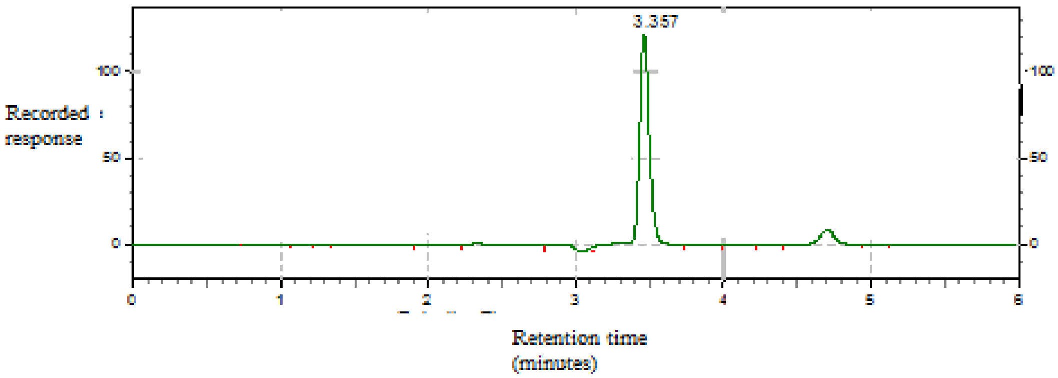

Fig. No 3 Representative Chromatogram of Thiocolchicoside (10µg/ mL)-1 in 0.1N HCl

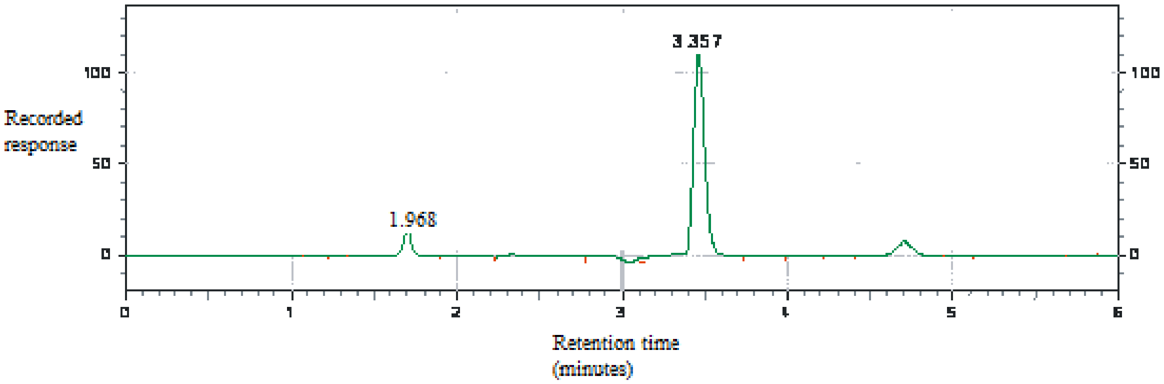

Fig. No.4 Representative Chromatogram of Thiocolchicoside (10µg/ mL)-1 in 0.1N NaOH

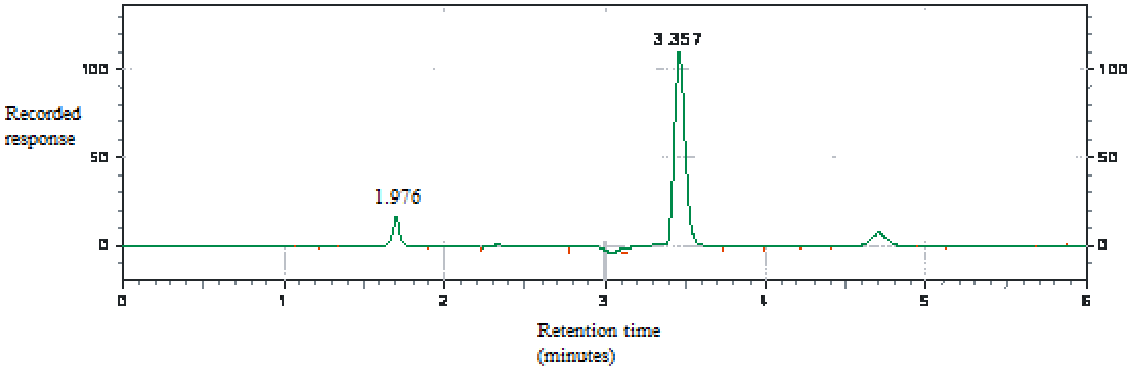

Fig. No 5 Representative Chromatogram of Thiocolchicoside (10µg/ mL)-1 in 3% H2O2

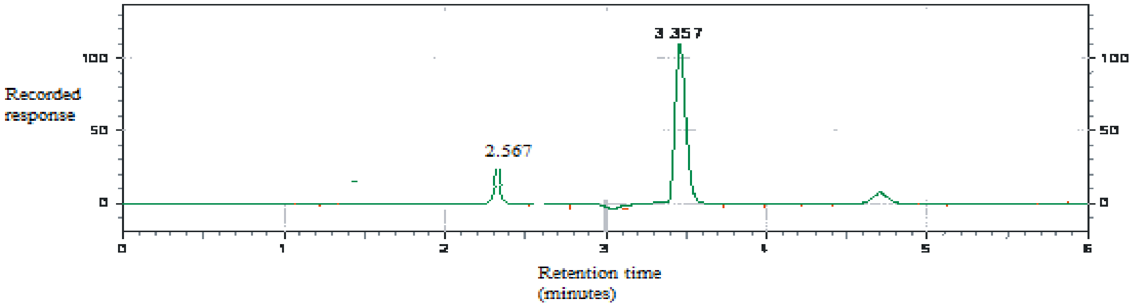

Fig. No 6 Representative Chromatogram of Thiocolchicoside (10µg/mL)-1 after Photolysis

Table No 2. Degradation data

Conditions	Time in hrs.	% Recovery	Retention time of Drug
0.1N HCl.	½ hr	92.79	2.125
0.1N NaOH	½ hr.	63.64	2.125
H2O2(3% v/v solution)	½ hr	87.10	2.125
Photolysis	8 hr	85.37	2.125

The representative chromatograms of drug with degraded products in 0.1N HCl, 0.1N NaOH, 3% H2O2 degradation and Photolysis are given in Figure 3, 4,5and 6 respectively. The degradation data along with percent recovery of the drug by the proposed method are given in Table 2.

LOD And LOQ

The LOD and LOQ were separately determined based on the S/N Ratio. For LOD the S/N ratio is 3:1 and for LOQ the ratio is 10:1.

RESULTS AND DISCUSSIONS

Table No 3. Method Validation

Parameters	Obtained Values
Linearity(µg mL-1)	0.05-200
Intraday Precision	0.17%RSD
Interday Precision	0.63%RSD
Recovery Studie	98.8-100.81%
LOD(µg mL-1)	0.03
LOQ(µg mL-1)	0.042
Name of Dosage Form	**Mean Drug Estimated(mg)±S.D.**
Thiorelax -4 mg	296.1 ± 0.4242

A critical evaluation of the method was performed. The different method validation parameters were validated and the results are shown in Table 3.The good % recovery in tablet formulation suggests that the excipients present in the formulation have no interference in the estimation. The %RSD was also less than 2% showing high degree of precision of the proposed method. The developed method was also specific as it was capable of determining Thiocolchicoside in presence of its degradation products. The forced degradation study of Thiocolchicoside shows that; the drug degrades in the order of 0.1N NaOH > Photolysis >3% H2O2 >0.1N HCl.So the developed method can be accepted as a novel stability indicating ionpairing RP-HPLC method which uses acetonitrile: water as the mobile phase which has been not used previously as per literature.

CONCLUSION

It can be concluded that the developed RP-HPLC method is stability indicating, rapid, simple, specific, accurate, and precise and can be employed successfully for the determination of Thiocolchicoside in capsule dosage form.

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