Research Article

Development and Validation of Normal Phase HPLC Method for Estimation of Thiocolchicoside in Capsule Dosage Formulation.

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ABSTRACT

A simple and sensitive high performance liquid chromatographic method has been developed and validated for the estimation of the Thiocolchicoside in Capsule dosage forms. The stationary phase used was Thermo Hypersil Silica 5 µ, (250mm x 4.6mm). The mobile phase used was a mixture of N-Heptane: Methanol: Chloroform: Acetic Acid (70: 20: 10: 0.2 %v/v). The Flow rate was 1 ml/min with UV Detection at 360 nm. The Retention Time of Thiocolchicoside was found to be 7.787. The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and Robustness. The calibration curve was found to be linear between (5-15 µg/ml) with significantly high value of correlation coefficient (r^2 > 0.99). The limits of detection and Quantitation were found to be 0.15 and 0.46 respectively. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 98-102%. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % RSD value less than 2 indicate that the method is precise.

Keywords: Thiocolchicoside, NP-HPLC method, Validation.

INTRODUCTION

HPLC is a physical separation technique conducted in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column).

An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, polymers, and other organic compounds.[1,2]

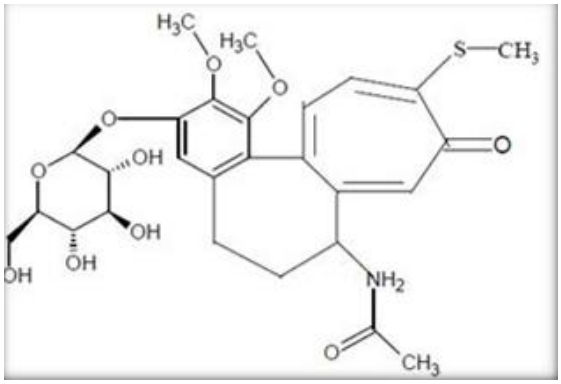
A single dose formulation is available for the treatment of Muscle Relaxant. Chemically THIO known as Thiocolchicoside (THC) chemically, N-[(7S)-3-(beta-D glucopyranosyloxy)-1,2-dimethoxy-10-(methyl sulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7yl] acetamide. It is a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, has been found to displace both [3H] gamma-amino butyric acid ([3H] GABA) and [3H] strychnine binding, suggesting an interaction with both GABA and strychnine sensitive glycine receptors.

THC is potent competitive antagonist of GABA function, thereby acting as potent muscle relaxant and displays anti-inflammatory and Analgesic properties.[3,4]

Thiocolchicoside

A capsule formulation containing Thiocolchicoside 4 mg has been introduced in to clinical practice. The literature survey reveals that various methods for the determination of Thiocolchicoside are reported.

Among this liquid chromatography, RP-HPLC, RP-UPLC methods are for Thiocolchicoside. A survey of literature revealed that there is no NP-HPLC method is reported for determination of Thiocolchicoside Capsule Dosage Form.[5-15] The present work describes the simple, precise and accurate NP-HPLC method for determination of Thiocolchicoside in Capsule Dosage form. It is validated by ICH guidelines.[12]



MATERIALS AND METHODS

Materials

Pharmaceutical grade of Thiocolchicoside was obtained as generous gift samples from Micro Labs, Mumbai, India. It was used without further purification and a commercial Capsule Myoril 4mg was purchased from local market. Methanol, Chloroform, Acetic Acid used was of HPLC grade and was purchased from Merck, India. The HPLC Instrument SPD-20AT, Shimadzu, which consisted of following components: a binary pump SPD-20AT, variable wavelength programmable PDA detector with auto sampler system was employed for the present study.

Instrumentation and Chromatographic Conditions

The chromatographic analysis was performed using Spinchrom software on a Thermo Hypersil Silica 5 µ, (250mm x 4.6mm) column.

In addition, an electronic balance (Shimadzu. Elec. balance AX-200), a pH meter Hemline, a sonicator (Leclasonic ultrasonic cleaner), were used in the study. Separation was achieved using a mobile phase consisting of N-Heptane: Methanol: Chloroform: Acetic Acid (70: 20: 10: 0.2% v/v). The Flow rate was 1 ml/min with PDA Detection at 360 nm. The column was maintained at ambient temperature and injection volume of 20 µl was used.

Preparation of Standard Solution

A standard Stock solution of Thiocolchicoside (100µg/ml) was prepared by dissolving 10 mg in100 ml conical Flask with 50 ml methanol. Ultrasonic till completely dissolved and make up the volume with methanol. (100µg/ml).

Preparation of Working Standard Solution of Thiocolchicoside

10µg/ml of Thiocolchicoside Stock solution was prepared by diluting 1 ml stock solution to 10 ml dilute with methanol. (10 µg/ml).

Determination of Wavelength for Measurement

From the 10µg/ml stock solution take 1 ml solution and diluted up to 10 ml with methanol.

Solution was scanned within the range of 400-200nm.

Calibration curve of Thiocolchicoside

Weigh accurately about 10 mg of Thiocolchicoside and dissolve it in 100 ml methanol. (100µg/ml). Take 1 ml from the above stock solution and diluted up to 10 ml. (10µg/ml).

From the above working standard solution, the solutions of Thiocolchicoside ranging from 5-15(µg/ml) were prepared by pipetting out 5, 7.5, 10, 12.5, 15 ml of stock solution of Thiocolchicoside (10µg/ml) in to a series of 10 ml volumetric flasks. The absorbance of the solutions was measured at 360 nm. The calibration curve was plotted at Area *vs.* Conc.

Analysis of Marketed Formulation

Thiocolchicoside

Brand Name: Myoril (4mg)

Manufacturing Company: Sanofi Aventis

Ten Capsules were weighed and powdered. The capsule powder equivalent to 10 mg of Thiocolchicoside was transferred to 100ml conical flask, dissolved and sonicated for 20 min and diluted up to mark with methanol. The solution was filtered through 0.45 µ filter paper and first few ml of filtrate were discarded. From this solution take 1 ml and diluted up to 10 ml (10µg/ml).

Method validation[11]

Linearity

The linearity of Thiocolchicoside was assessed in the range of (5-15 µg/ml). The absorbance values were plotted against the respective concentrations of drug to get the analytical curve.

The results were subjected to regression analysis by the least squares method to calculate the slope (m), intercept (c) and regression coefficient (R2). The results are shown in below Table 1.

Linearity and Range

Table 1: Linearity Data of THIO

Sr. No.	Conc. (µg/ml)	Mean Area ± S.D (n=3)
1.	5	2274.01 ± 25.05
2.	7.5	3509.329 ± 67.50
3.	10	4653.991 ± 37.48
4.	12.5	5836.379 ± 15.05
5.	15	6820.304 ± 13.14

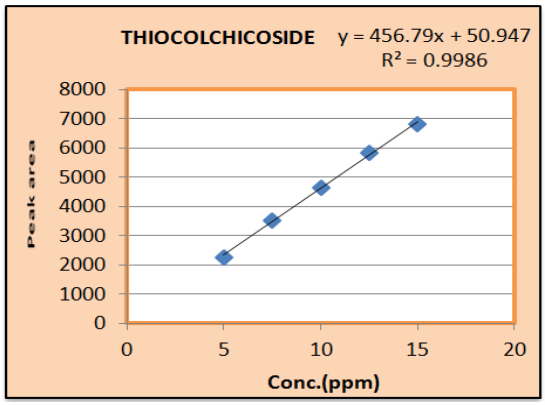


Figure 1: Calibration Curve of Thiocolchicoside

Precision

Intra-day precision

Intra-day precision was determined by analyzing Thiocolchicoside 5, 10, 15 µg/ml concentrations were determined 3 times a day interval of 1 hour, simultaneously and %RSD was calculated. % RSD should be less than 2.

Inter-day precision

Inter-day precision was determined by analyzing Thiocolchicoside 5, 10, 15 µg/ml concentrations were determined daily for 3 days and %RSD was calculated. % RSD should be less 2%. Results are shown in Table 2.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage Recovery of Thiocolchicoside was found out. Recovery between 98-102% justify the Accuracy method. Results are shown in Table 3.

Table 2: Precision Data

Condition	Con. (µg/ml)	Area 1	Area 2	Area 3	Mean	SD	% RSD
Intraday	5	2280.79	2271.63	2287.53	2279.99	7.98	0.35
	10	4668.29	4656.43	4686.42	4671.05	14.19	0.30
	15	6925.86	6891.26	6905.79	6907.63	17.37	0.25
Interday	5	2299.05	2276.11	2237.34	2270.86	31.19	1.38
	10	4705.63	4682	4634.81	4674.15	36.06	0.77
	15	6926.03	6843.01	6790.22	6853.09	68.46	0.99

Table 3: Recovery Study Data

Assay level	Conc. of THIO from Capsule (µg/ml)	Amount of Standard added (µg/ml)	Total amount of drug recovered(µg/ml)	% Recovery	% RSD
Blank	5	-	4.89	0	0
	5	-	4.87	0	0
	5	-	4.92	0	0
80%	5	4	8.97	99.35	0.79
80%	5	4	9.03	100.89	
80%	5	4	9.01	100.45	
100%	5	5	9.99	99.84	0.89
100%	5	5	10.06	101.21	
100%	5	5	9.97	99.54	
120%	5	6	11.01	11.19	0.54
120%	5	6	11.07	101.27	
120%	5	6	11.03	100.60	

Table 4: Data for Robustness

No.	Factor	Level	Peak area* ± SD	%RSD
Thiocolchicoside (10 µg/ml)				
1.	Change in Mobile Phase Ratio	72:18	4154.08 ± 18.62	0.44
		68:22	5065.98 ± 14.70	0.29
2.	Change in the Flow Rate (ml/min)	1.2	3939.08 ± 19.95	0.50
		0.8	5307.17 ± 35.49	0.66

Table 5: Result of Analysis of Capsule Formulation

Formulation	Capsule content taken (µg/ml)	Amount found (µg/ml)	Assay % estimated (n=3 MEAN ± SD)	LOD	LOQ
Myoril	**10**	**9.98**	**100.18 ± 0.61**	**0.15**	**0.46**

Standard Chromatograms of Thiocolchicoside

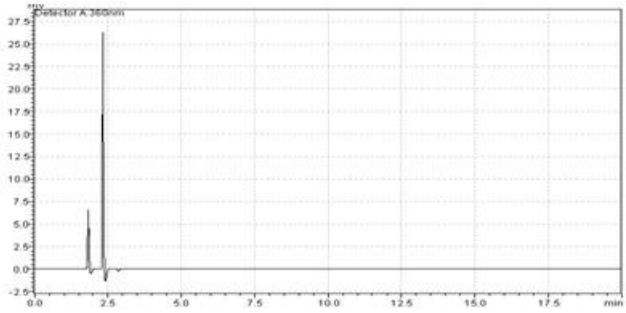


Figure 2: Blank Chromatogram

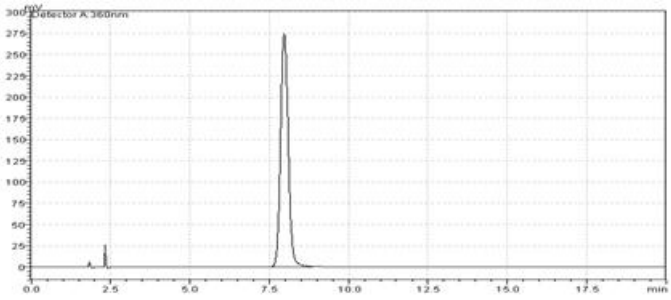


Figure 3: Chromatogram of Drug Sample

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value particularly important for limit tests.

$$\text{LOD: } 3.3\,\sigma/S$$

Where,

σ = standard deviation of intercept and it was calculated from the equation,

S= Slope obtained from calibration curve

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$$\text{LOQ: } 10\,\sigma/S$$

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. Robustness of the method was determined by subjecting the method to slight change in the method condition, individually, the Pump flow rate, Mobile phase ratio. Three replicates were made for the same concentration 10µg/ml of Thiocolchicoside. % RSD was calculated. Results are shown in Table 4.

System Suitability Studies

The system suitability was evaluated by five replicate analyses of THIO. The column efficiency and peak asymmetry were calculated for the standard solutions. Results are shown above in Fig. 2 and 3.

RESULTS AND DISCUSSION

A simple, economic, precise, accurate method for estimation of Thiocolchicoside was developed. This developed method was validated according to ICH guidelines. From the proposed research, it was found that Thiocolchicoside obeys linearity within the concentration range 5-15µg/ml. Accuracy of proposed methods was ascertained by recovery studies & results are expressed as % recovery. Percent recovery for Thiocolchicoside was found in range of 98 to 102%. Interday and Intraday Precision data were found to be less than 2, so the method is precise.

CONCLUSION

From the above results it can be concluded that the NP-HPLC method for Thiocolchicoside is simple, rapid, accurate, precise and economical. Hence the method can be applied for quantitative analysis of Thiocolchicoside in bulk and pharmaceutical Capsule dosage forms. The proposed NP-HPLC method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, and precision, Repeatability, LOD, LOQ and Robustness.

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