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Development and Validation of Stability indicating NP- HPLC Method for Determination of Thiocolchicoside in Capsule Dosage Formulation

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ABSTRACT

A Stability indicating Normal Phase 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. The stability indicating capability was established by forced degradation experiments. The drug was subjected to Acid, Alkali, Oxidation and Thermal Degradation. The degradation studies indicated Thiocolchicoside to be susceptible to Acid, Alkaline condition. The degdration products of Thiocolchicoside were well resolved from pure drugs.

Keywords: *Thiocolchicoside, NP-HPLC method, Stability indicating, 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].

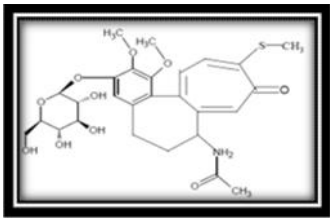

Fig. 1: Thiocolchicoside Structure

A Single dose Formulation is available for the treatment of Muscle Relexant. 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-7 yl] 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].

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 [5-10]. Extensive Literature survey revealed that no stability indicating NP-HPLC method has been reported for the determination of Thiocolchicoside in Capsule dosage form. 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].

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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 Chemiline, 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).**

Analysis of Marketed Formulation:

Table No. 1: Marketed Formulation

Dosage Form	Brand Name	Label Claim	Manufacturing Company
Thiocolchicoside- Capsule	Myoril (4 mg)	Thiocolchicoside - 4 mg	Sanofi Aventis Ltd.

Force Degradation Studies: [5]

The ICH guidelines indicate that stress testing is designed to help determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used.

Preparation of Standard solution:

The stock solution (100µg/ml) of Thiocolchicoside was prepared by dissolving accurately weighed 10 mg of the drug, transferred into 100 mL volumetric flask, dissolved and made up to the volume by using Methanol. 10µg/ml of Thiocolchicoside Stock solution was prepared by diluting 1 ml stock solution to 10 ml dilute with methanol. (10 µg/ml).

Preparation of Sample solution:

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). Forced degradation was performed for Thiocolchicoside in acidic, alkali oxidative, thermal, conditions.

Alkali degradation:

First 0.1N NaOH was taken in a 10ml volumetric flask then add 2ml of Thiocolchicoside from stock solution (10 µg/ml) was transferred to 10 ml volumetric flask and made up to the mark with methanol. Then this solution is reflux for 1 hour at 60°C and chromatogram was recorded. Then sample was withdrawn for 2 hr.

Acid Degradation:

First 0.1N HCL was taken in a 10ml volumetric flask then add 2ml of Thiocolchicoside from stock solution (10 µg/ml) was transferred to 10 ml volumetric flask and made up to the mark with methanol. Then this solution is reflux for 1 hour at 60°C and chromatogram was recorded. Then sample was withdrawn for 2 hr.

Oxidation Degradation:

3% H_2O_2 was taken in a 10ml volumetric flask then add 2ml of Thiocolchicoside from stock solution (10 µg/ml) was transferred to 10 ml volumetric flask and made up to the mark with methanol. Then this solution is kept reflux for 1 hour at 60°C and chromatogram was recorded. Then sample was withdrawn for 2 hr.

Thermal Degradation:

Pure Thiocolchicoside when subjected to dry heat at 105°C for 5 hours. To 10 ml of stock solution of 100 µg /ml, to study the degradation under thermal conditions.

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).

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.

Precision:

A. Repeatability:

Measure area of standard mixed solutions containing Thiocolchicoside 10 µg/ml at 360 nm. The area of solution was measured 6 times and %C.V. was calculated.

B. 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.

C. 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%.

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.

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 quantitated 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.

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 AND DISCUSSION

Validation Parameters:

A simple, economic, precise, accurate method for estimation of Thiocolchicoside was developed. This developed method was validated according to ICH guidelines.

Table No. 2: 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

Linearity and Range:

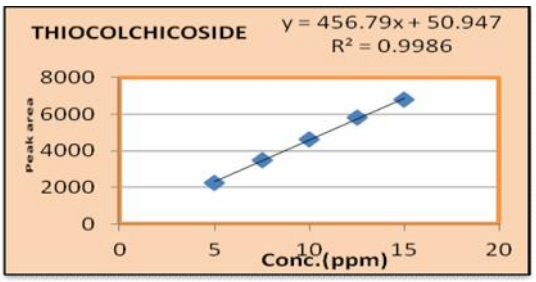

Fig. 2: Calibration Curve of THIO

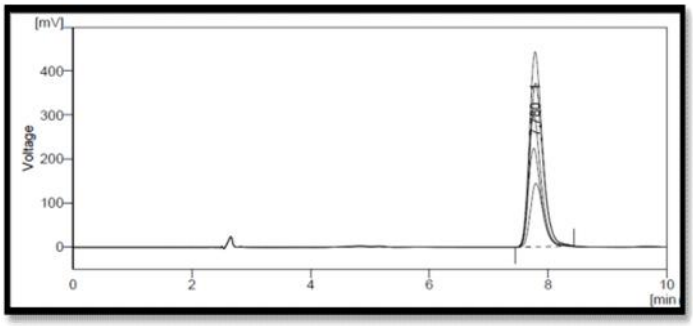

Fig. 3: Chromatogram of standard solution of THIO

Precision:

Repeatability:

Table No. 4: Data for Repeatability

Con.(µg/ml)	Area	Mean	S.D	%RSD
10	4647.32	4647.81	32.14	0.69
10	4637.96			
10	4696.24			
10	4642.25			
10	4688.67			
10	4674.42			

Table No. 5: Intraday and Interday 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

LOD and LOQ:

Table No. 6: LOD and LOQ data of THIO

Parameter	THIO
LOD (µg/ml)	0.15
LOQ (µg/ml)	0.46

Table No. 7: Result of Analysis of Capsule Formulation

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

Accuracy:

Table No. 8: 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	
80%	5	4	9.03	100.89	0.79
80%	5	4	9.01	100.45	
100%	5	5	9.99	99.84	
100%	5	5	10.06	101.21	0.89
100%	5	5	9.97	99.54	
120%	5	6	11.01	11.19	
120%	5	6	11.07	101.27	0.54
120%	5	6	11.03	100.60	

Robustness:

Table No. 9: 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

System Suitability Test Results:

Table No. 10: System Suitability Test Parameters

Sr No	System Suitability Parameters	Thiocolchicoside	IP'2010 specification
1.	Number of Theoretical Plates (N)	5185	>2000
2.	Tailing Factor	1.12	<1.5
3.	Retention Time	7.78	-
4.	Peak Area	4365.97	-
5.	Assymetry	1.51	< 2

Table No. 11: Summary of Parameters

PARAMETER	THIOCOLCHICOSIDE
LINEARITY AND RANGE	5-15 µg/ml
r^2 DATA	0.9986
REGRESSION EQUATION	y=456.79x + 50.95
REPEATABILITY	0.69
INTRADAY PRECISION	0.25-0.35
INTERDAY PRECISION	0.77-1.38
ACCURACY	98-102%
LOD µg/ml	0.15
LOQ µg/ml	0.46
ROBUSTNESS	0.29 - 0.66
ASSAY	100.018 ±0.61

Table No.12: Summary of Force Degradation Study

Stress Condition		% Degradation
		Thiocolchicoside
Acid	0.1N HCL (1 hr Reflux at 60°c)	15.70
	0.1N HCL (2 hr Reflux at 60°c)	18.76
Alkali	0.1N NAOH (1 hr Reflux at 60°c)	12.42
	0.1N NAOH (2 hr Reflux at 60°c)	23.48
Oxidation	3% H2O2 (5 hr at 60°C)	15.37
	3% H2O2 (5 hr 60°C)	27.30
Thermal	24 hr at 105°c	15.65

Standard Chromatograms of Thiocolchicoside:

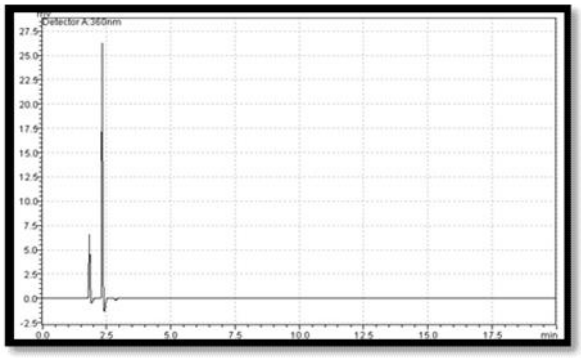

Fig. 4: Blank Chromatogram

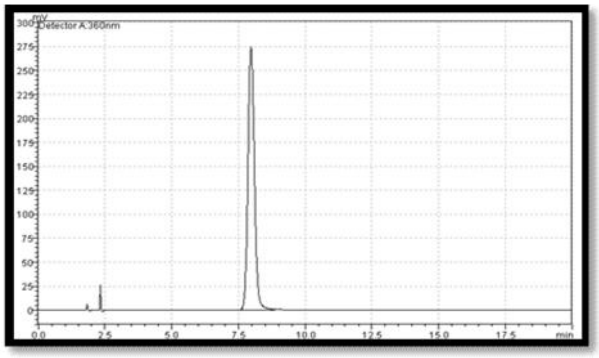

Fig. 5: Chromatogram of Drug Sample

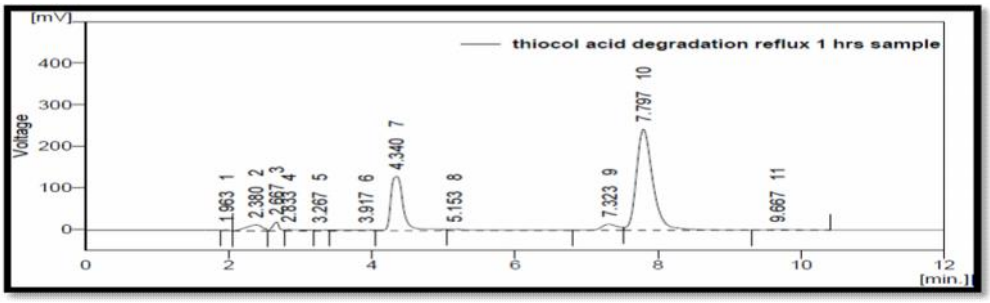

Fig. 6: Chromatogram of Acid Reflux (0.1 N HCL 1 hr at 60°c)

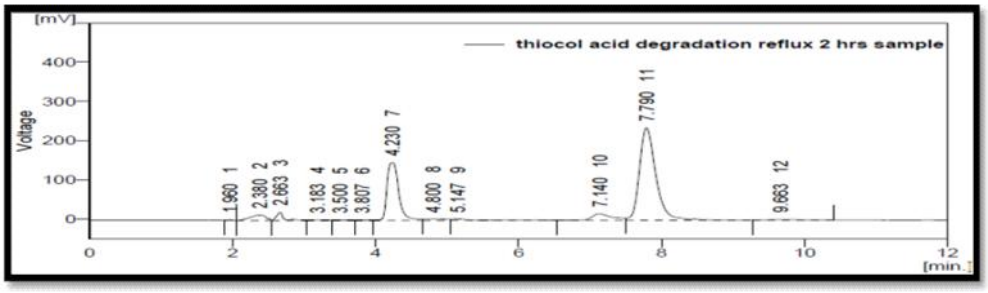

Fig. 7: Chromatogram of Acid Reflux (0.1 N HCL 2 hr at 60°c)

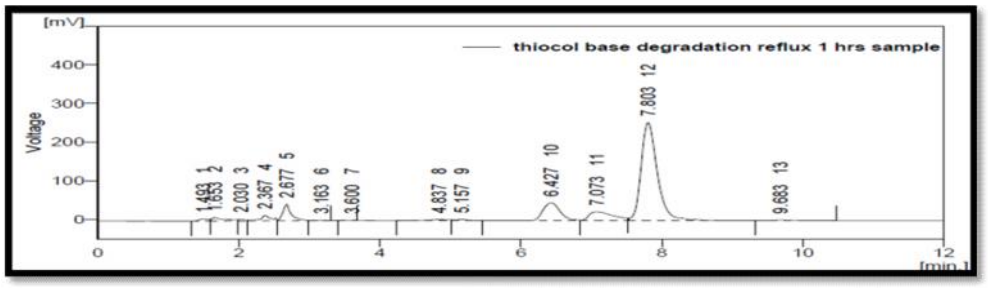

Fig. 8: Chromatogram of Alkali Reflux (0.1 NAOH 1 hr at 60°c)

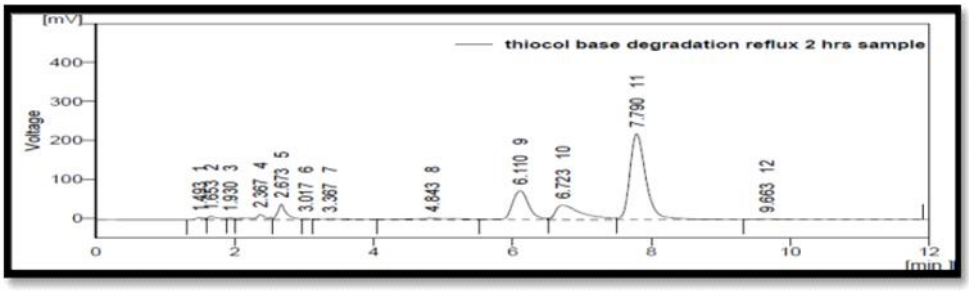

Fig. 7: Chromatogram of Alkali Reflux (0.1 N NAOH 2 hr at 60°c)

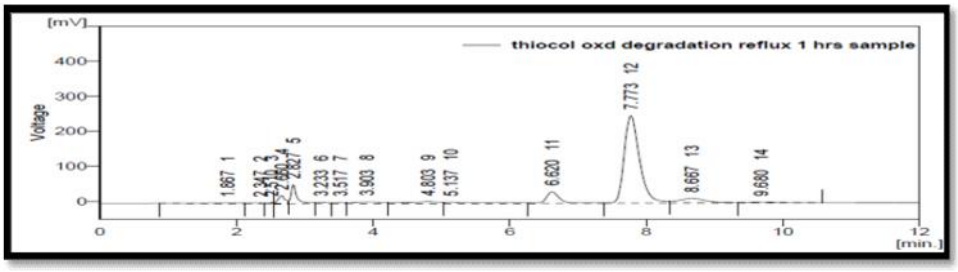

Fig. 8: Chromatogram of Oxidation Reflux (3% H_2O_2 1 hr at 60°c)

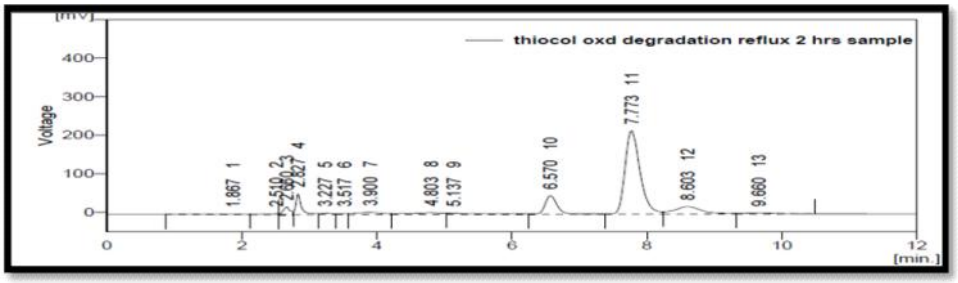

Fig. 9: Chromatogram of Oxidation Reflux ((3% H_2O_2 2 hr at 60°c)

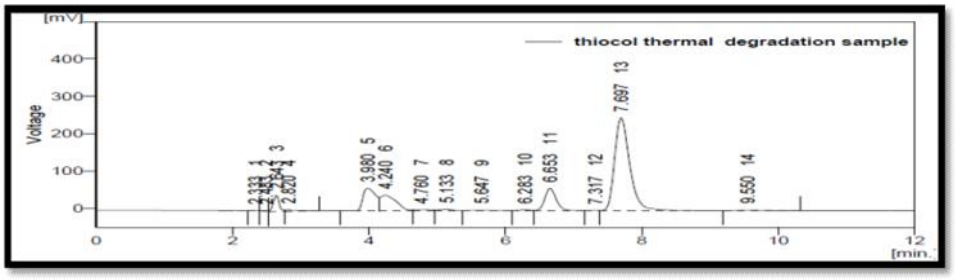

Fig. 10: Chromatogram of Thermal at 24hr 105 °c)

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, precision, Repeatability, LOD, LOQ and Robustness. Force degradation study was also found to be satisfactory.

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REFERENCES:

1. Shethi PD. HPLC-Quantitative analysis of pharmaceutical formulations; 11th edn; CBS publishers & distributors, **2001**; p. 3-141.
2. Snyder LR. And Kirkland JL. Glajch JL. Practical HPLC Method Development; Wiley, New York: **1997**; p. 613.
3. Vaidya MG. Ayurvedic Pharmacognogy and Therapeutic Uses of Medicinal plants; 3rd Edn; p 712.
4. Dr. Sardana S and Dr.Sharma OP. Fundamentals of Pharmacognosy; Birla Publications PVT.LTD, **2009-2010**; p 269.
5. ICH QIA (R2): "Stability Testing of New Drug Substances and Products." **2005**.
6. Rajput D, Shirkhedkar A, Rajput J, Patel H and Surana S. *J. of Analytical Chem.,* **2013**; pp 143-152.
7. Karbhari P, Joshi S, and Bhoi S. *J. of phar. and bioallied sci.,* **2014**; 6(4): pp 246-252.
8. Balan P, Nagappan K. *Int. Current Pharmaceutical J.,* **2014**; 3(7): pp 296-300.
9. Kumar P, Shukla S, Subudhi S, Ganure A. *Int. j. of phar. and pharmaceutical sci.* **2012**; 4(3): pp 252-259.
10. Dhiware D, Gandhi S, Deshpande S. *Int.j. of phar. and pharmaceutical sci.*, **2012**; 4(4): pp 214-216.
11. Sahoo M, Syal M, Lngale S. *Int. J. of Research in Pharmaceutical Sci.,* **2011;** pp 32-37.
12. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Geneva, Nov **2005**.
13. Indian Pharmacopoeia, Government of India Ministry of Health and Family Welfare, Ghaziabad, India: The Indian Pharmacopoeia Commission, **2010**; vol. II: pp 2216.

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