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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RELATED COMPOUNDS IN THIOCOLCHICOSIDE FOR INJECTION

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ABSTRACT

A novel stability-indicating reverse phase liquid chromatographic (NP-LC) method was developed for the Estimation of Related Compounds in Thiocolchicoside for injection the following parameters give optimised results using columm Cosmicill Adore Ph 150 X 3.9 mm, 3µm with mobile phases Mobile phase A (20Mm of octane-1-sulfonic acid sodium salt solution), Mobile phase-B: (100% Acetonitrile), Mobile phase-C(100% Tetrahydrofuran(THF)) with the gradient program with flow rate of 0.8 mL/min, wave length 360 nM, injection volume 10µL, Run time 45 minutes. The linearity equation were found to be for thiocolchicoside, Colchicoside, N-Deacetyl-N-formyl Thiocolchicoside. Colchicine Y= 19828X+415.6, Y=16424X+1324, Y=4626X+319.8, Y=26173+2468 respectively and LOD and LOD 0.17 and 0.51, 0.18 and 0.55 and 0.19 AND 0.58, 0.17 and 0.51 respectively, for related compound the relative retention time and relative response factor were found to be 0.49 & 0.83, 1.05 &0.23, 1.55&1.32 respectively. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating power of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and system suitability.

KEYWORDS: Thiocolchicoside, Development, Reverse phase liquid chromatography, Stability-indicating.

INTRODUCTION

Thiocolchicoside chemically called as *N*-[(7*S*)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5*H*-benzo[a]heptalen-7-yl]acetamide and molecular formula $C_{27}H_{33}NO_{10}S$.This compound has been shown to inhibit the binding of [3H] GABA or [3H] strychnine of cerebrocortical or spinal cord membranes. [3H] TCC was also displaced in a concentration –dependent manner by GABA or by several agonists or antagonists of the type A receptor for GABA (GABAAR). TCC has been thought to act as a GABAAR agonist that induces depression of the central nervous system[1] and, in turn, myorelxation. Moreover, TCC possesses a molecular structure similar to that of colchicines, a plant alkaloid that binds to tubulin and induces the depolymerisation of microtubules, disrupts axonal transport[2,3] and inhibits mitosis. Following oral administration, TCC is well absorbed from the gastrointestinal tract. It shows its effect in 1-2 hours after oral administration and peak plasma level is achieved in about 0.7 hours and elimination half-life is about 2.5 to 5 hours. Its effect is continued for 24 hours.

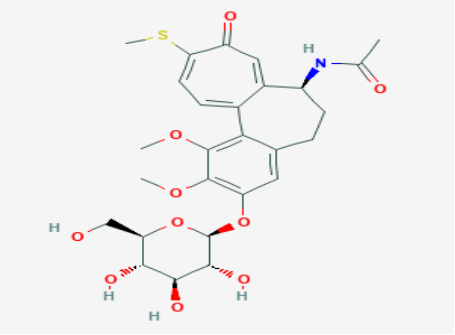


Figure 1: Structure of Thiocolchicoside.

High performance liquid chromatography (HPLC) is playing a more and more important role for the resolution of drugs enantiomers in the field of pharmaceutical industry.[4] However, the development of the methods for the quantitative analysis of chiral compounds along with other process related substances is extremely challenging.[5] In the literature there were limited LC methods have been reported for determination of Thiocolchicoside and related compounds in parenteral preparations. In this work plan to stability indicative method for development of estimation of Thiocolchicoside and related compounds in

parental dosage form using the RP-HPLC method. The related compounds present in Thiocolchicoside were colchicine, colchicoside, N-deacetyl N-formy thiocolchicoside respectively.

EXPERIMENTAL WORK

Materials methods: Thiocolchicoside were colchicine, colchicoside, N-deacetyl N-formy thiocolchicoside working standard, octane-1-sulfonic acid sodium salt solution, Acetonitrile. Tetrahydrofuran(THF) HPLC grade were purchased from Merck. All the instruments and glassware were used in this work calibrated.

Chromatographic conditions

The method was developed using the Column Cosmicill Adore Ph 150 X 3.9 mm, 3µm with mobile phases Mobile phase A (20Mm of octane-1-sulfonic acid sodium salt solution), Mobile phase-B: (100% Acetonitrile), Mobile phase-C(100% Tetrahydrofuran(THF)) with the gradient program that can be tabulated in table1:, with flow rate of 0.8 mL/minm wave length 360 nM ,injection volume 10µL, Run time 45 minutes.

Table 1: gradient program.

Time (in min)	Mobile phase A(%v/v)	Mobile phase B(%v/v)	Mobile phase C(%v/v)
0.00	95.5	0.5	4.0
3.00	95.5	0.5	4.0
7.00	90.0	4.0	6.0
10.00	88.5	5.5	6.0
13.00	88.5	5.5	6.0
18.00	86.0	8.0	6.0
20.00	86.0	8.0	6.0
29.00	80.0	14.0	6.0
36.00	80.0	14.0	6.0
40.00	95.5	0.5	4.0
45.00	95.5	0.5	4.0

Preparation of standard solution

Standard was prepared 2.0 µg/mL.

Preparation of Spiked solution

Prepared 400 µg/mL test solution spiked 0.5% of each impurity in Methanol.

Analytical Method validation

The proposed method was validated as per ICH guidelines.[6]

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its related compounds and impurities.

Precision

The precision of the related substances method verified by repeatability and by intermediate

Precision

Limit of Detection (LOD) and Limit of Qualification (LOQ)

LOD and LOQ of thiocolchicoside were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations, Precision study was also carried out at LOQ level by injecting six individual preparations of impurities and calculating the % RSD of the area.

Linearity and Range

Linearity test solutions for the assay method were prepared from thiocolchicoside stock solution at five different concentrations. The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the related substance method were prepared by diluting stock solution to the required concentrations.

Linearity (Concentrations)

Name	Concentrations in PPM					
	LOQ	50%	75 %	100%	150%	200%
Colchicoside	0.10	1.04	1.50	2.08	3.16	4.20
N-deacetyl N-formyl Thiocolchicoside	0.20	2.04	3.02	4.10	6.25	8.11
Colchicine	0.10	1.02	1.56	2.12	3.10	4.15

Accuracy

Accuracy of the assay method was evaluated in triplicate using three concentration levels 50, 100 and 200 µg/ml on sample. Standard addition and recovery experiments were conducted on sample to determine accuracy of the related substance method. Study was carried out in triplicate using four concentration levels LOQ, the percentages of recoveries for Thiocolchicoside related compounds were calculated.

Name	Accuracy and LOQ Concentrations in PPM				
	LOQ	50%	100%	150%	200%
Colchicoside	0.10	1.04	2.08	3.10	4.16
N-deacetyl N-formyl Thiocolchicoside	0.20	2.04	4.10	6.25	8.11
Colchicine	0.10	1.02	2.11	3.02	4.20

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the Relative retention time and Relative Response Factor Thiocolchicoside related compounds were recorded.

RESULTS AND DISCUSSION

System suitability

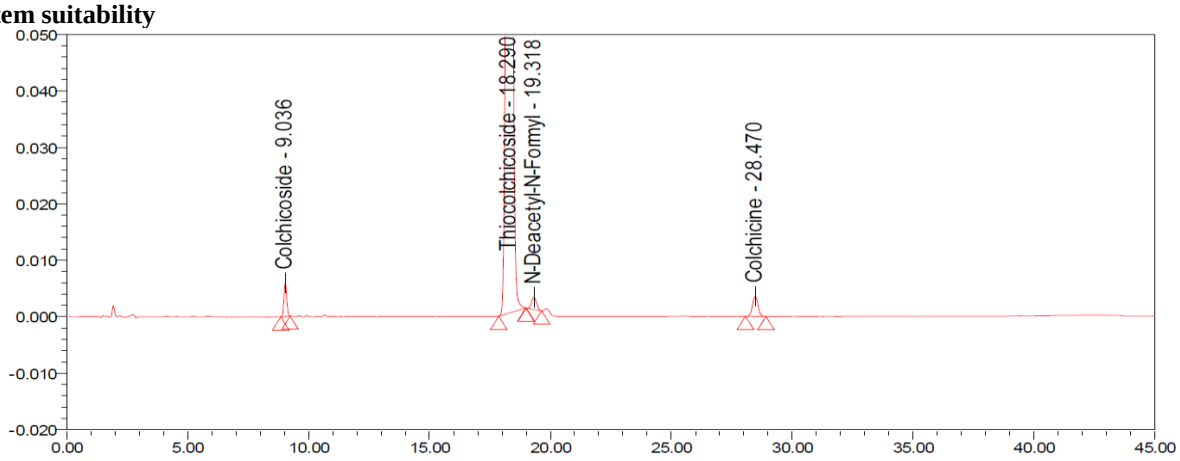
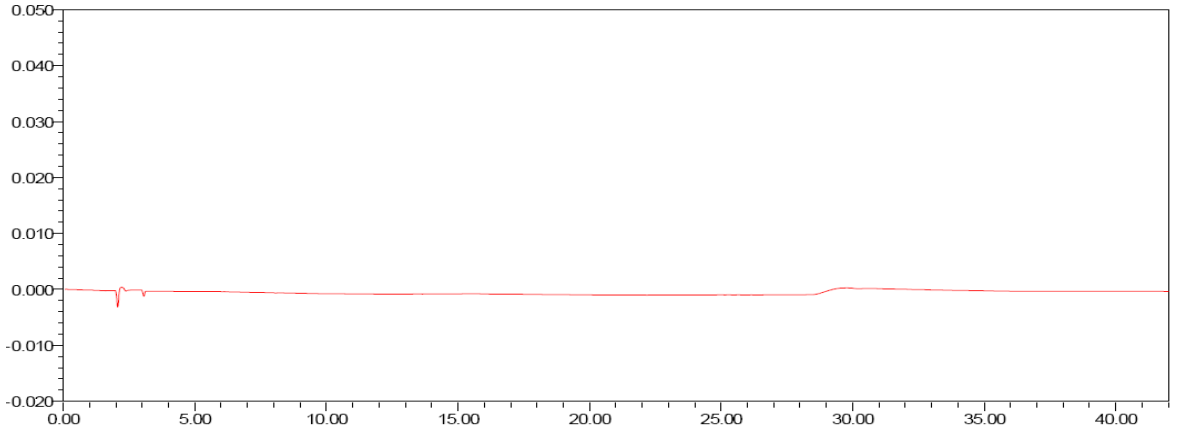


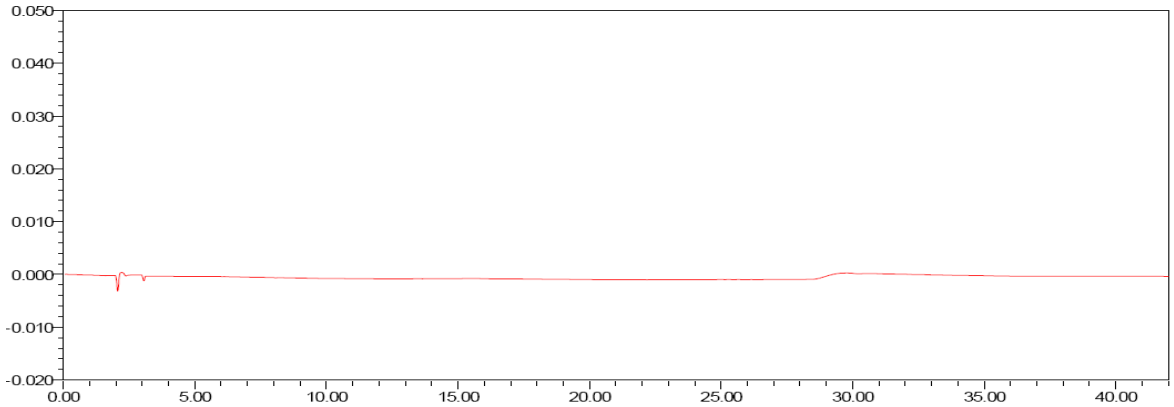
Fig: Chromatogram for system suitability.

Table 2: System suitability values.

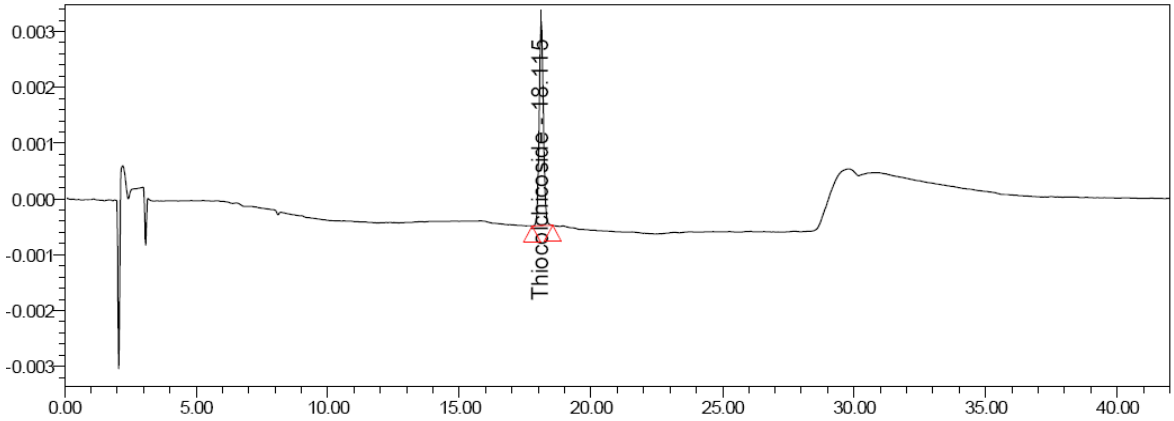
	Injection	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	1	Thiocolchicoside	18.382	70999	5449	45926	1.02
2	2	Thiocolchicoside	18.380	70864	5392	44909	1.00
3	3	Thiocolchicoside	18.379	71183	5453	45568	1.00
4	4	Thiocolchicoside	18.379	71596	5473	45904	1.01
5	5	Thiocolchicoside	18.386	70911	5498	46320	1.01
6	6	Thiocolchicoside	18.356	71580	5449	45484	1.02
Mean				71188.7			
Std. Dev.				327.6			
% RSD				0.5			



Typical chromatogram of blank.



Typical chromatogram of Placebo.



Typical chromatogram of standard.

Validation parameters

Method Precision

Method was precisized the % RSD OF the individual impurities was below

Table3: Method Precision.

S.no	Name	%RSD
1	colchicine	1.20
2	colchicoside	1.46
3	N-deacetyl N-formy thiocolchicoside	0.84

LOQ And LOD

Table 4: LOD and LOQ values.

S.no	Name	LOD in µg	LOQ in µg
1	colchicine	0.17	0.51
2	colchicoside	0.18	0.55
3	N-deacetyl N-formy thiocolchicoside	0.19	0.58
4	Thiocolchicoside	0.17	0.50

Specificity

Table5: specificity values.

S.No	Name of the component	Retention time
1	Colchicoside	9.032
2	Thiocolchicoside	18.382
3	N-Deactyl-N-Formyl Thiocolchicoside	19.368
4	Colchicine	28.545

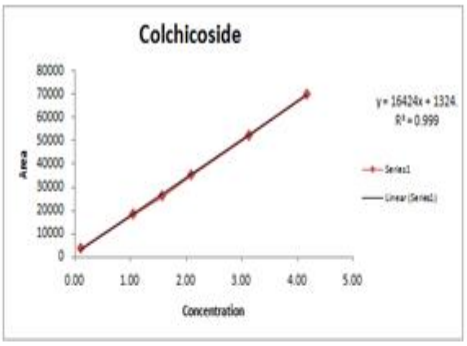
Accuracy

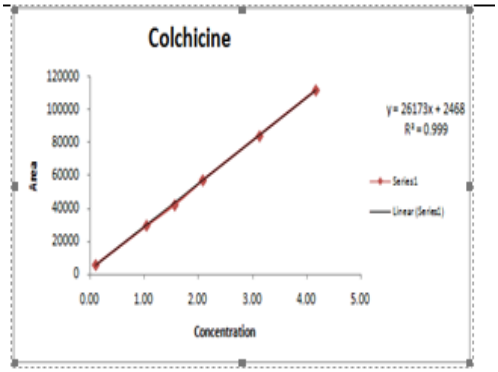
Table 6: Accuracy values.

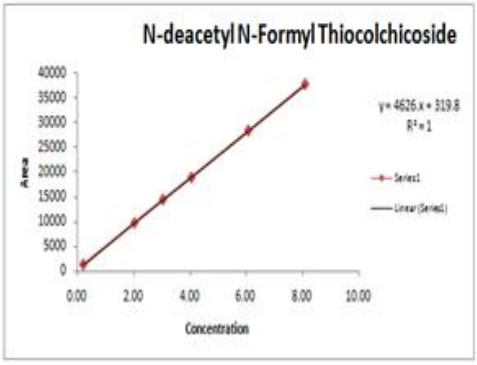
Name	Values at different Concentrations in PPM				
	LOQ	50%	100%	150%	200%
Colchicoside	106.2	102.0	101.0	100.01	99.98
N-deacetyl N-formyl Thiocolchicoside	105.89	101.2	100.6	100.8	100.89
Colchicine	107.2	102.1	103.6	101.2	99.75

Linearity

RRT and RRF of Impurites







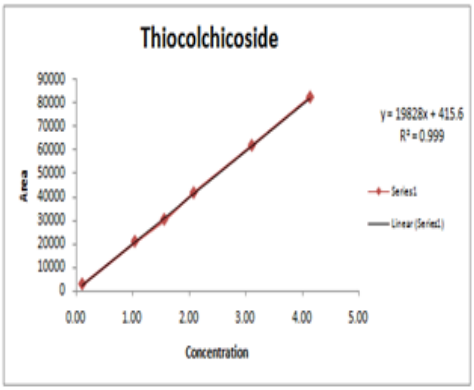


Table 7:

S. No.	Name of the impurity	RRT	RRF
1	Colchicoside	0.49	0.83
2	N-Deacetyl-N-formyl Thiocolchicoside	1.05	0.23
3	Colchicine	1.55	1.32

Stability studies

Table8: stability studies values.

Name	condition	Colchicoside%	N-Deactyl N-formyl Thiocolchicosode %	Coclchicne %	Max. Unknown %	Net Degradation %
Control sample	-	0.09%	0.99%	-	0.22%	1.44%
Sample (1.0 N HCl)	60°C/ 2 Hrs/2mL	0.09%	0.05%	-	6.87%	9.87%
Sample (0.1 N NaOH)	60°C/ 1Hr/2mL	0.02%	0.75%	-	0.27%	1.32%
Sample (Thermal)	120°C/ 24 Hrs/2mL	0.21%	0.92%	-	0.51%	2.02%
Sample (3% H_2O_2)	60°C/ 30min/2mL	0.23%	0.74%	-	4.63%	9.72%

Sample (Photo stability)	Sun Light /5hrs/2mL	0.02%	0.90%	-	0.37%	1.86%
Sample (Neutral)	60°C/ 1 Hrs/2mL	0.22%	0.65%	-	2.77%	6.07%

Name	Purity Angel	Purity Threshold
Thiocolchicoside	0.05	0.589
Colchicoside	0.231	0.985
N-Deactyl N-formyl Thiocolchicosoide	0.390	0.897
Coclchicne	0.256	0.568

DISCUSSION

The system suitability parameters were passed for the method with column Cosmicill Adore Ph 150 X 3.9 mm, 3µm with mobile phases Mobile phase A (20Mm of octane-1-sulfonic acid sodium salt solution), Mobile phase-B: (100% Acetonitrile), Mobile phase-C(100% Tetrahydrofuran(THF)) with the gradient program with flow rate of 0.8 mL/min, wave length 360 nM, injection volume 10µL, Run time 45 minutes. The linearity equation were found to be for thiocolchicoside, Colchicoside, N-Deacetyl-N-formyl Thiocolchicoside. Colchicine Y= 19828X+415.6, Y=16424X+1324, Y=4626X+319.8, Y=26173+2468 respectively and LOD and LOD 0.17 and 0.51, 0.18 and 0.55 and 0.19 AND 0.58, 0.17 and 0.51 respectively, for related compound the relative retention time and relative response factor were found to be 0.49 & 0.83, 1.05 &0.23, 1.55&1.32 respectively. The stability parameters were tabulated in table.

CONCLUSION

A simple, accurate, precise, and linear stability-indicating HPLC method has been developed and validated for Thiocolchicoside related compounds, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Thiocolchicoside related compounds. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

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