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Reversed phase high performance liquid chromatography method for determination of Thiocolchicoside from active pharmaceutical dosage form

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of thiocolchicoside from active pharmaceutical ingredients. The separation of drug was achieved on Zorbax Eclipse C18 (250 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The detection was carried out at wavelength 260 nm. The mixture of buffer and acetonitrile (70:30% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze thiocolchicoside hydrochloride from active pharmaceutical ingredients.

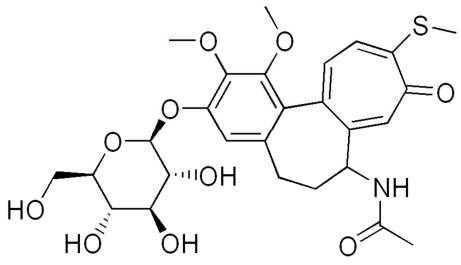
Keywords: Thiocolchicoside, Acetonitrile, tri-ethyl amine

INTRODUCTION

Thiocolchicoside, a semi synthetic derivative of naturally occurring compound of colchicoside from the seeds of various species of colchicum antumnale (autumn crocus, meadow saffron, Gloriosa upuba), chemically,*N*-[(7*S*)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide It is centrally acting muscles relaxant and it also show analgesic activity. It is used in treatment of muscular pain and gout.

In literature survey reveals, spectroflurometric [1], spectrophotometric [2], HPLC [3, 4] and HPTLC methods [5] for determination of thiocolchicoside in pharmaceutical dosage form.

Structure of thiocolchicoside



MATERIALS AND METHODS

Chemical and reagents

Reference standard of thiocolchicoside hydrochloride was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and ortho-phosphoric acid were used of analytical grade and the HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer and acetonitrile (70:30 % v/v)].

Instrumentation

The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation

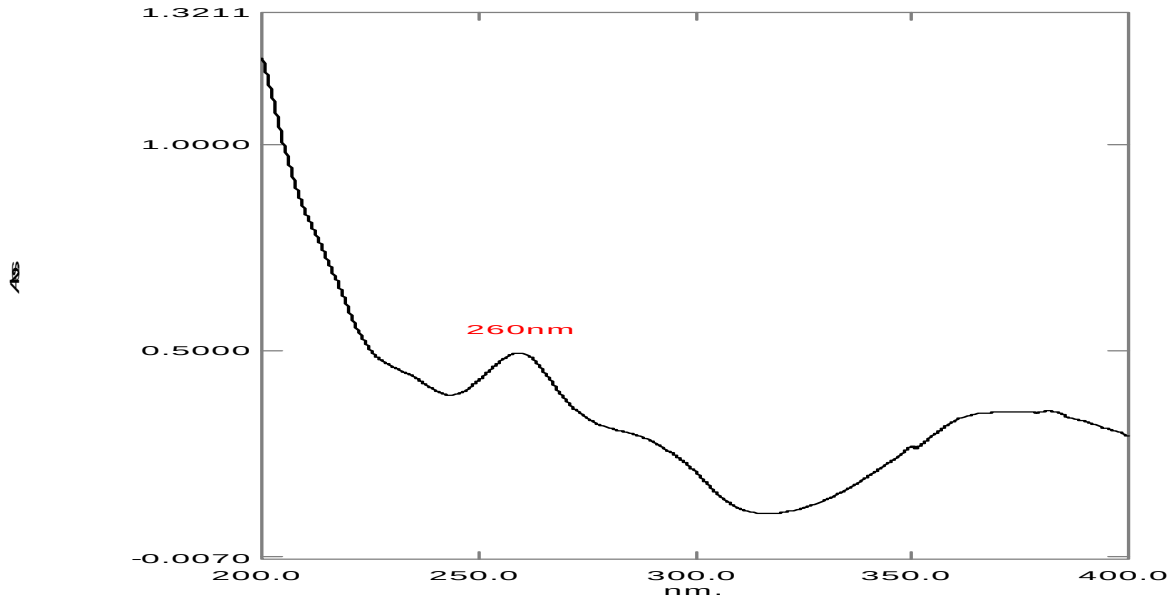
Standard solution

At 4 mg of standard thiocolchicoside was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 400 µg /ml. The working standard solution was prepared by diluting 1 ml of 400 µg /ml solution to 10 ml with diluent to get concentration 40 µg /ml.

Sample preparation

About 4 mg of thiocolchicoside sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 400 µg /ml. The sample solution was prepared by diluting 1 ml of 400 µg/ml solution to 10 ml with diluent to get concentration 40 µg /ml.

Figure 1: UV spectra of thiocolchicoside hydrochloride



Chromatographic condition

Chromatographic separation was performed at ambient temperature on a reverse phase BDS Zorbax Eclipse C18 (250 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The flow

rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 260 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, and area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution of Thiocolchicoside hydrochloride

Retention Time	Area	Area %	USP Plate Count	Symmetry
2.927	2254340	100.00	1568	1.2273

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard thiocolchicoside hydrochloride was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of thiocolchicoside hydrochloride (standard)

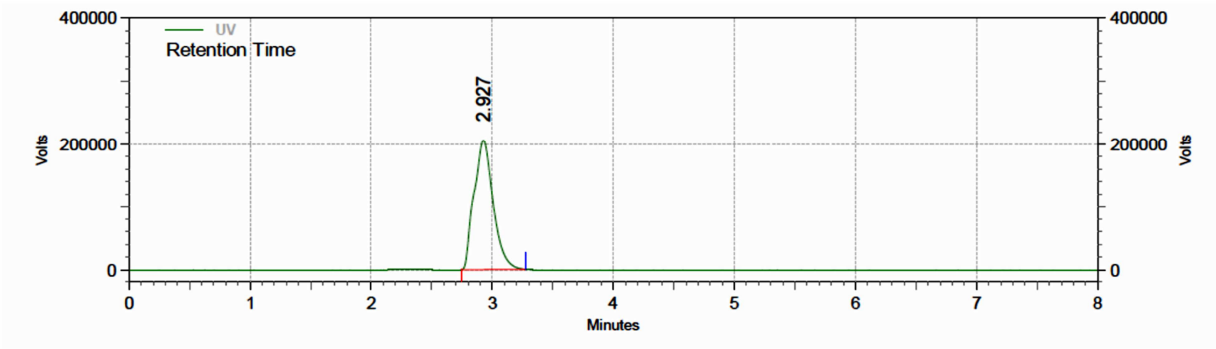
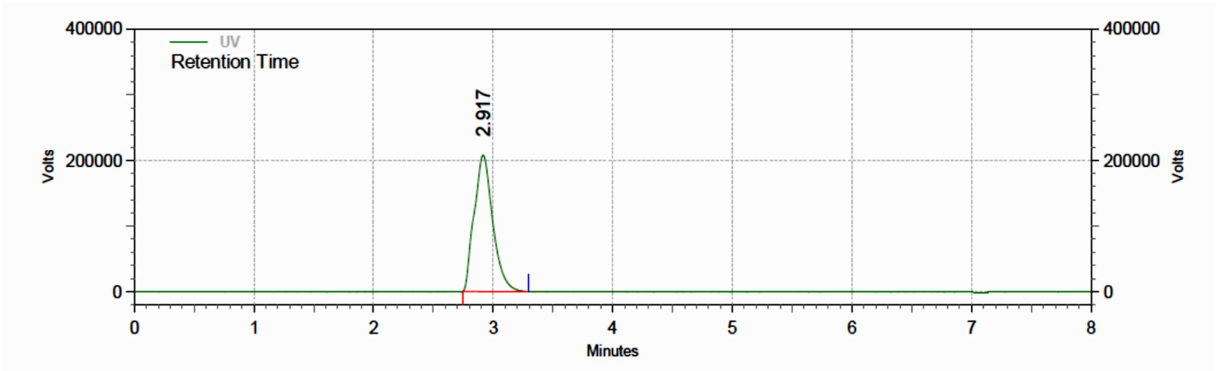


Figure 3: Typical chromatogram of thiocolchicoside hydrochloride (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Values
Correlation Coefficient (r)	0.9999
% Intercept (y)	34411
Slope (m)	52818

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 50 %, 100 % and 150 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3: Statistical evaluation of the data subjected to accuracy of thiocolchicoside hydrochloride

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg/ ml	% recovery	mean recovery
80%	1	20.71	1786328	33.12	33.01	99.66	**100.05**
	2	20.72	1799199	33.12	33.25	100.38	
	3	20.72	1794411	33.12	33.16	100.11	
100%	1	33.12	2202721	41.40	40.70	98.31	**99.29**
	2	33.17	2225336	41.40	41.12	99.32	
	3	33.18	2245379	41.40	41.49	100.22	
120%	1	49.68	2723071	49.68	50.32	101.28	**100.68**
	2	49.70	2706382	49.68	50.01	100.66	
	3	49.58	2691122	49.68	49.73	100.09	
					Mean recover of all level		**100.01**

Precision

The method precision was established by carrying out the analysis of thiocolchicoside hydrochloride. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4

Table 4: Statistical evaluation of the data subjected to method precision of thiocolchicoside hydrochloride

Test	wt of test	Area	% assay
Test-1	4.14	2235133	99.76
Test-2	4.15	2237759	100.12
Test-3	4.16	2242190	100.56
Test-4	4.16	2251409	100.97
Test-5	4.15	2249502	100.65
Test-6	4.14	2246762	100.28
	Mean Assay		**100.39**
	SD		**0.428**
	RSD		**0.426**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by $\pm$ 0.2 ml /min

Variation in mobile phase composition by $\pm$ 2 %

Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application

Twenty capsules were weighed accurately and average weight of each tablet was determined. Powder equivalent to 4 mg of thiocolchicoside sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 min to dissolve it. Further volume was made up to the mark with the diluent to give 400 µg /ml. Further the 1 ml of this solution was diluted to 10 ml with diluent to give 40 µg /ml of thiocolchicoside. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of thiocolchicoside hydrochloride in the product meets the requirement.

RESULTS AND DISCUSSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. Thus the proposed RP-HPLC method is used for estimation of thiocolchicoside from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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REFERENCES

[1] A. Suganthi, T.K. Ravi, *International Journal of ChemTech Research*, **2012**, 4,(4),1674-1680.
[2] Rachana R Joshi, Krishna R Gupta, *Der Pharma Chemica*, **2010**, 2(2), 384-391.
[3] Desai Chandni H, Henal Chorawala, Zarna R. Dedania, S. M. Vijendraswamy, *Int. J. Pharm. Sci. Rev. Res*., **2015,** 32(1), 274-278.
[4] Rachana R Joshi, Krishna R Gupta, Ketkee S Jinnawar Sudhir G Wadodkar, *American Journal of PharmTech Research*, **2012**, 2(1),589-602.
[5] Dnyansing K. Rajput, Atul A. Shirkhedkar, Jyoti K. Rajput, HarunM. Patel, Sanjay J. Surana, *Journal of Analytical Methods in Chemistry*, **2013**, Article ID 142628, 7.