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Der Pharma Chemica, 2010, 2(2): 384-391
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

UV-Spectrophotometric Determination of Thiocolchicoside in capsule

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Abstract

Simple spectrophotometric methods are described for the determination of Thiocolchicoside in bulk and in pharmaceutical formulation. Thiocolchicoside shows the maximum absorbance (λmax) at 259.8 nm in water and was utilised for its determination, Method A. Method B utilises Area under Curve (AUC) in wavelength range of 269.8-249.8 nm. Drug was found to follow the Beer-Lambert's law in the concentration range of 5-50µg/ml for both the methods. The limit of detection and limit of quantification was found to be 1.49 µg mL^{-1}& 4.51µg mL^{-1} for Method A and 1.08 µg mL^{-1} & 3.27 µg mL^{-1} for Method B. The proposed method was successfully applied to the determination of Thiocolchicoside in pharmaceutical formulations without any interference from excipients. Results of the analysis were validated statistically and found to be satisfactory.

Keywords: Thiocolchicoside, UV spectroscopy, Area under Curve, λ max.

INTRODUCTION

Thiocolchicoside is a glucoside extracted from the seeds of Colchicum. Thiocolchicoside[1] (TCD), is (s)-N-[3-(B-D-glucopyranoxyloxy)–5,6,7,9- tetrahydro–1,2 dimethoxy- 10-(methylthio)-9-oxobenzo [a]heptalen-7yl] acetamide **(Figure I)**. Literature survey reveals the determination of thiocolchicoside in its binary mixtures (thiocolchicoside/glafenine and thiocolchicoside/floctafenine by TLC / densitometry [2]. Ultraviolet absorbance detection of colchicine and related alkaloids on a capillary electrophoresis microchip[3] , The Validation of analytical procedure for the determination of thiocolchicoside drug by HPLC[4] , HPLC/UV method for quantitative analysis of Thiocolchicoside and its active metabolites in human[5] . The

aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of Thiocolchicoside in bulk drug and in formulations.

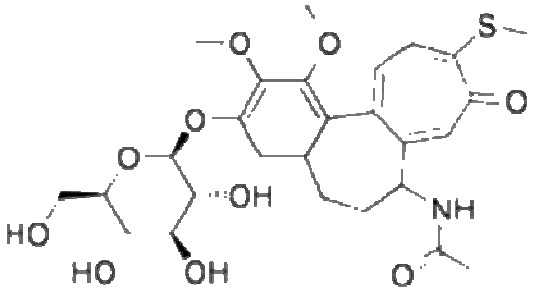

Figure I: Chemical structure of thiocolchicoside

Thiocolchicoside is an anti-inflammatory analgesic agent with muscle relaxant action. The proposed method was developed and validated according to guidelines. The developed method was applied to the determination of Thiocolchicoside in pharmaceutical formulations.

MATERIALS AND METHODS

A Shimadzu UV 1100 series Spectrophotometer was used with 1 cm matched quartz cells. UV spectra of standard and sample solutions were recorded in 1cm quartz cells at the wavelength range of 200- 400 nm.

Chemicals and reagents

Thiocolchicoside was kindly provided from Sanofi Aventis, India and it was used without further purification. Thioact capsules (4mg) were procured from market. Distilled water was used for the preparation of solutions. All the apparatus used for the experiment are graded.

Standard solutions and study of spectra

An accurately weighed quantity of TCD (~25mg) was transferred in a 25.0 mL volumetric flask, dissolved in sufficient quantity of distilled water to prepare a standard stock solution having concentration 1mg/mL. Aliquot from stock solution was diluted with water in a volumetric flask to get final concentration of 20µg/mL. The working standard solution of TCD (20µg/mL) was scanned in the range of 400-200 nm in 1.0 cm cell against solvent blank and spectra was recorded, the absorbance maxima was observed at 259.8 nm **(Figure II)** and the amount of TCD was calculated by taking A (1%, 1cm) at 259.8 nm (Method A).

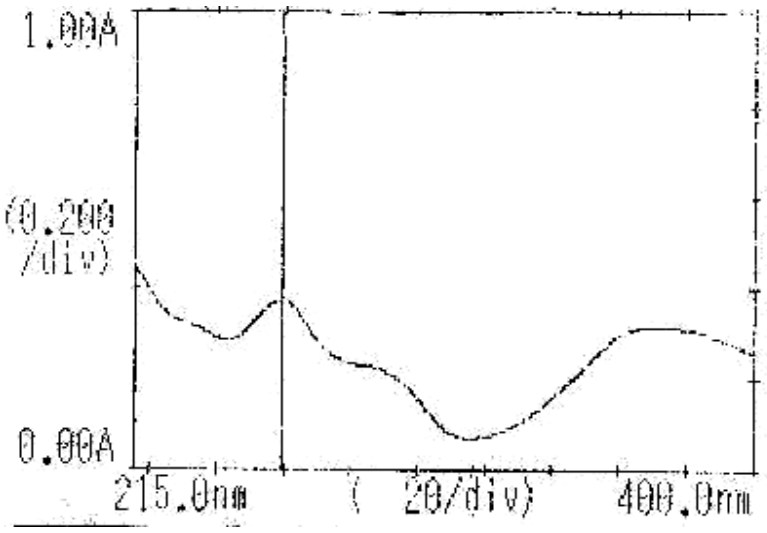

Figure II: Spectrum of Thiocolchicoside in distilled water

The AUC (Area under Curve) (method B) involves the calculation of integrated value of absorbance with respect to the wavelength λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis was selected by entering the wavelength range over which the area has to be calculated. The wavelength range from 269.8- 259.8 nm was selected which showed good linearity between area under curve and concentration **(Figure III)**.

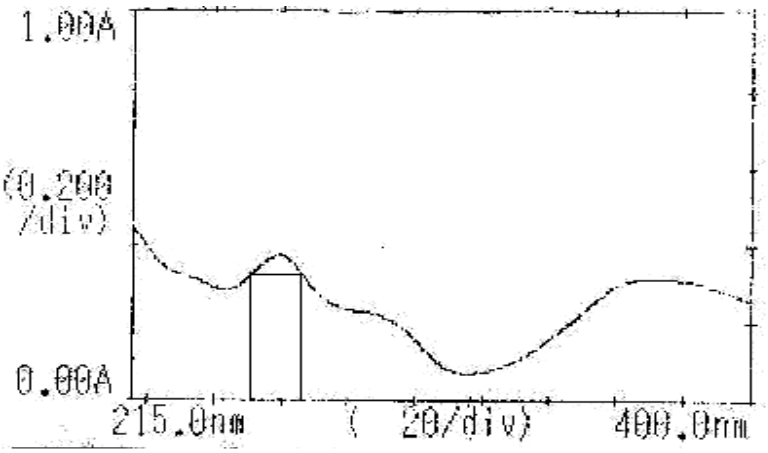

Figure III: Spectrum of Thiocolchicoside in distilled water (Area Under curve)

Preparation of calibration curve

Aliquots of standard stock solution were diluted with water to get a series of concentration of standard drug in the range of 5-50µg/mL and scanned in the spectrum mode from the wavelength range 400-200 nm. Calibration curve was plotted as absorbance Vs concentration as shown in **Figure IV** (Method A) and as area under curve against concentration as shown in **Figure V**

(Method B). The correlation coefficient was found to be 0.999 for Method A and B respectively. The optimized spectrophotometric method was applied to the direct determination of thiocolchicoside in capsule using Method A and Method B.

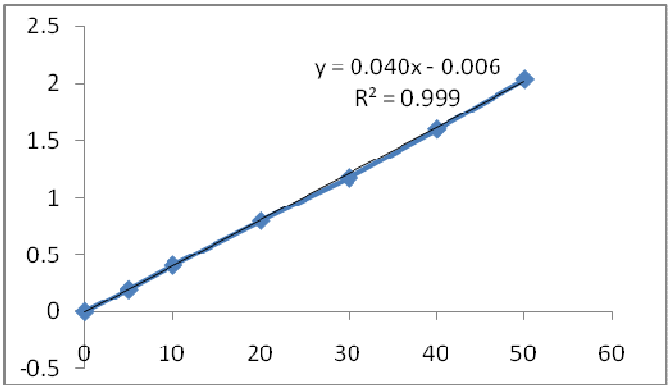

Figure IV: Calibration curve for Method A

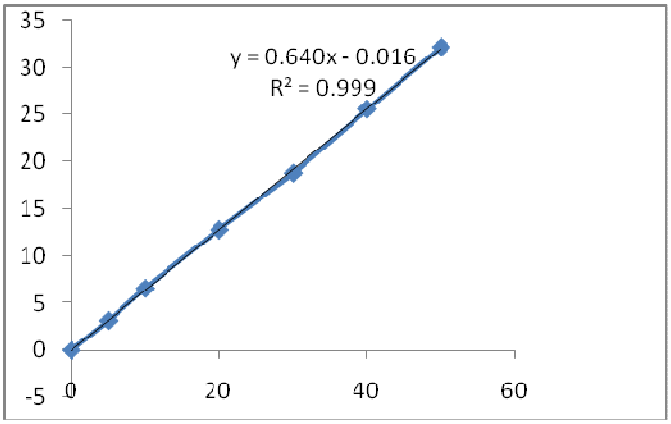

Figure V: Calibration curve for Method B

Assay

Accurately weighed quantities of capsule powder equivalent to about 5 mg thiocolchicoside was transferred to 50.0 mL volumetric flask, sonicated for 20 min with water and diluted up to the mark with water. The content of the flask was then filtered through Whatmann filter paper (No.41). A 10.0 mL of the filtrate was further diluted in a 25.0 mL volumetric flask with water to get concentration of about 40 µg/mL of thiocolchicoside (on labelled claim basis). The absorbance of the resulting solutions was read and the amount of thiocolchicoside was calculated by taking A (1% 1cm) at 259.8 nm (Method A) and by Area under Curve (AUC) (Method B) in the range of 269.8-249.8 nm. Amount of TCD was estimated by using A (1%, 1cm) value by method A, using calibration curve and regression equation by method B; Percent of label claim was estimated by using following formulae for method A and B both.

$$\text{Percent of labelled Claim} = \frac{\text{Amount of TCD estimated} \times \text{Average wt}}{\text{weight taken} \times \text{Label claim}} \times 100$$

The average amount present was determined by taking average of five replicate analysis and the mean percent estimation were found to be 99.05±0.69 % and 101.17±0.88 % for both methods respectively. The low RSD values shows that the proposed methods were successfully applied for the assay of TCD in its pharmaceutical formulations **(Table I)**

Table I: Estimation Thiocolchicoside in capsule Formulation

Method	Tablet Formulation	% Label Claim*	S. D.(±)
A	T1	99.05	0.69
B	T1	101.17	0.88

The results are the mean of five readings (n = 5) Method A is A1%, 1cm Method B is the AUC method. T1 is the brand of capsule formulations. (Thioact 4 mg)

Validation [6],[7]

All these methods were validated according to ICH guidelines for accuracy, Precision, Specificity, linearity and range, Ruggedness and Limit of detection

Recovery Studies (Accuracy)

Recovery studies were carried out by standard addition method. To previously analyzed tablet powder samples, pure drug were added at four different levels (5.1, 7.2, 9.6 and 13.4 mg). From the amount of total drug estimated, percentage recovery was calculated **(Table II)** and found to be satisfactory with the standard deviation within limits for both methods.

Table II: Recovery Study Data

Tablet Formulation	Amt of pure drug added (mg)	Amt of pure drug recovered (mg)		Percent Recovery*	
		Method A	Method B	Method A	Method B
T1	5.1	5.11	5.06	100.33	99.32
	7.2	7.24	7.15	100.61	99.31
	9.6	9.68	9.604	100.92	100.04
	13.4	13.65	13.61	101.89	101.59
%RSD				0.671	1.070

A is zero order derivative spectrum method with n = 0. B is the AUC method T1 is the brand of the capsule formulation.

The results are the mean of four readings at each level of recovery.

Precision and intermediate precision

Precision was carried out by replicate estimation of homogeneous samples of Thiocolchicoside formulation and % RSD for Method A and Method B were found to be 0.70 and 0.87 respectively. Intermediate Precision was analysed by performing inter-day and intraday precision studies. Sample was analyzed in three independent series on the same day (intra-day precision) and 5 consecutive days (inter-day precision). The precision of the analysis was determined by calculating the relative standard deviation (%RSD). The %RSD values of inter-day and intra-day studies are recorded in **(Table III).**

Ruggedness

The ruggedness of the proposed method was evaluated by applying the proposed methods to assay of 40 µg/mL of TCD using the same instrument by three different analysts under the same optimized conditions. The obtained results were found to reproducible, since there was no significant difference between results **(Table III)**.

Table III: Validation data

Parameters	Percent Label Claim	
	Method A	Method B
Intraday Precision (n=3)		
Amount found±	97.74	98.85
%RSD	1.19	1.01
Inter-day Precision (n=4)		
Amount found±	98.73	100.83
%RSD	1.12	0.29
Ruggedness (%RSD)		
Analyst to Analyst n=3	0.45	0.37

Limit of Detection and Quantitation

The limit of detection and limit of quantification were determined based on standard deviation of the response and the slope.

$$LOD = 3.3\sigma / S$$

$$LOQ = 10\sigma / S$$

Where, σ = the standard deviation of response

S = the slope of calibration curve.

RESULT AND DISCUSSION

Method A was carried out at λ max 259.8nm and Method B (Area under curve) was carried out in range of 249.8-269.8nm. Beer-Lambert's law was obeyed in the concentration range of 5-50 µg/mL for both methods (**Table IV**). The values of standard deviation were satisfactory and the

recovery studies were close to 100%. The results of obtained by method A and B for the estimation of thiocolchicoside were studied statistically by unpaired t-test with Welch correction and F-test. In the result of analysis of marketed formulations by proposed methods, t-calculated values and F- calculated values were found to be 4.20 and 1.599 for, which are less than the corresponding statistical values, indicating no significant difference in means and variances of results obtained by either of the proposed methods which are within statistical limits. The P-value was significant at 0.05.

Table IV: Optical Characteristics and other parameters

Parameters	Method A	Method B
λ_{Max} (nm) / wavelength range	259.8	269.8-249.8
Beer-Lambert's range (µg/mL)	5-50	5-50
Correlation coefficient (r)	0.999	0.999
Regression equation Y = mx + c		
a. Slope (m)	0.040	0.640
b. Intercept (c)	0.006	0.016
LOD	1.49 µg	1.08 µg
LOQ	4.51µg	3.27 µg

Where, x is concentration in µg/mL & Y is a absorbance unit, Method A is A1%, 1cm, Method B is the Area Under Curve

CONCLUSION

The results obtained by UV spectrophotometric methods are reliable, precise, accurate and are found to be statistically acceptable. Hence these methods can be useful in the routine analysis of TCD in bulk drug and formulations.

Acknowledgement
The authors are thankful to the Principal, SKB college of Pharmacy for providing necessary facilities for carrying out the work.

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