ORIGINAL ARTICLE

New derivative spectrophotometric methods for the determination of thiocolchicoside – A semisynthetic derivative of colchicoside

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Abstract

Introduction: Thiocolchicoside is a semisynthetic derivative of colchicoside. It is clinically as anti-inflammatory, analgesic, and muscle relaxant. New spectrophotometric techniques have been proposed for the determination of thiocolchicoside in hydrochloric acid, sodium acetate buffer, and phosphate buffer (pH 7.0). **Materials and Methods:** Shimadzu double beam UV-VIS spectrophotometer (UV-1800 Model) was used for the present study. Zero-order and first derivative spectrophotometric techniques have been developed for the determination of thiocolchicoside in hydrochloric acid, sodium acetate buffer, and phosphate buffer (pH 7.0). **Results and Discussion:** Thiocolchicoside has shown absorption maxima at 259 nm in all the methods, and linearity was observed 1.0–70, 0.1–2, and 0.1–6 µg/ml in hydrochloric acid, sodium acetate buffer, and phosphate buffer, respectively, and all the methods were validated as per ICH guidelines. **Conclusions:** These methods are simple and economical and can be successfully applied for the estimation of thiocolchicoside in pharmaceutical dosage forms.

Key words: First-order derivative spectroscopy (D_1), ICH guidelines, thiocolchicoside, zero order (D_0), validation

INTRODUCTION

Thiocolchicoside is a colchicoside derivative, obtained from the seeds of various species of colchicum antumnale (autumn crocus, meadow saffron, Gloriosaupuba). Thiocolchicoside is chemically, N-[(7S)-3-(β-D-Glucopyranosyl oxy)-1,2-dimethoxy-10-(methyl sulfanyl)-9-oxo-5,6,7,9-tetrahydro benzo[a]heptalen-7-yl] acetamide.[1] It is used clinically for its muscle relaxant, anti-inflammatory and analgesic properties.[2] It is also used in the treatment of muscular pain and gout. Thiocolchicoside is rapidly absorbed after oral administration and metabolized into three main metabolites and two of them are thiocolchicoside aglycon and the glucuronidated derivative of thiocolchicoside. Del *et al.* reported the degradation products and their characterization from the forced degradation studies.[3] Thiocolchicoside was quantified by different analytical techniques such as spectrophotometry,[4-8] high-performance liquid chromatography,[9-10] high-performance thin-layer chromatography,[11] and liquid chromatography–mass spectrometry[12] for the assessment of bioequivalence after oral administration of the drug in human plasma, and the authors have proposed three new spectrophotometric techniques for the assay of thiocolchicoside in capsules and validated as per ICH guidelines [Figure 1].[13]

MATERIALS AND METHODS

Model No. UV-1800 double beam UV-VIS spectrophotometer (Shimadzu) with quartz cells is used for the entire study, and all the solutions were scanned 200–400 nm.

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Preparation of Solutions

Buffer solutions such as sodium acetate (pH 4) and phosphate buffer pH 7.0 were prepared as per IP 2010. Stock solution of thiocolchicoside was prepared by dissolving 25 mg of thiocolchicoside in 25 ml volumetric flask with methanol (1000 µg/ml) and further working standard solutions (100 µg/ml) were prepared by diluting the stock solution with respective buffers as per the requirement for the proposed methods. Thiocolchicoside is available as capsules with brand name Colchico (Label claim: 4 mg; 8 mg), Thiocheck (Future Pharma Pvt. Ltd.), Thiopace (Minova Life Sciences Pvt. Ltd.), Thioquest (Alchem International Ltd.), and Zyplex (Zydus Cadila Healthcare Ltd.) (Capsule and Injection), etc., in India.

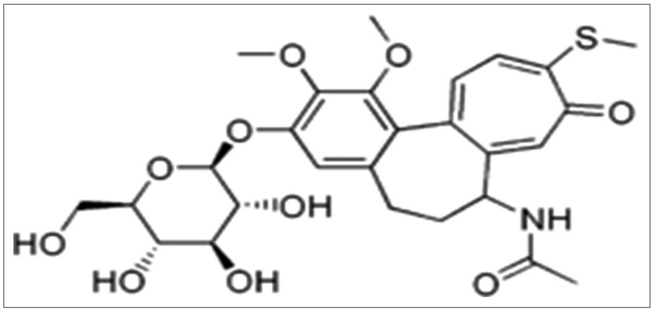

Figure 1: Chemical structure of thiocolchicoside

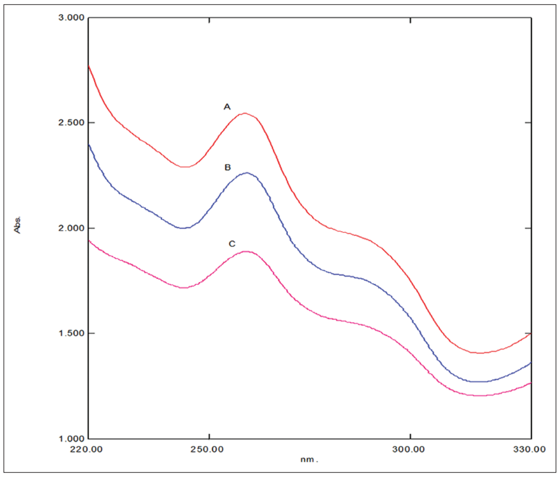

Figure 2: Absorption spectrum of thiocolchicoside (D_0) (a) hydrochloric acid 0.1 N (40 µg/ml) (b) phosphate buffer pH 7.0 (4 µg/ml) (c) sodium acetate buffer (10 µg/ml)

Method Validation

Linearity

Zero-order spectroscopy (D_0)

A series of thiocolchicoside solutions 1–70, 0.1–2, and 0.1–6 µg/ml were prepared from the stock solution on dilution with hydrochloric acid (0.1 N) (Method I), sodium acetate buffer pH 4.0 (Method II), and phosphate buffer pH 7.0 (Method III) and scanned (200–400 nm) against their reagent blanks. The zero-order spectrum so obtained has shown maximum absorbance (λ_{max}) at 259 nm in all the three methods. The absorbance of all the solutions was noted at λ_{max}, and a calibration curve was drawn by taking the concentration on the x-axis and the corresponding absorbance on the y-axis for Method I, II, and III, respectively.

First-order derivative spectroscopy (D_1)

The individual zero-order absorption spectra of thiocolchicoside so obtained in Method I, II, and III were converted into their first-order derivative spectra with the help of inbuilt software of the instrument in all the three reagents. The resultant derivative spectra have shown both minima and maxima in all the three buffers, and therefore, the amplitude was selected for the construction of calibration curves for Method I, II, and III.

Precision and Accuracy Studies

Precision studies were performed by calculating the % relative standard deviation (RSD) values of nine independent assays at three different concentration levels, whereas the accuracy studies were carried out by standard addition method.

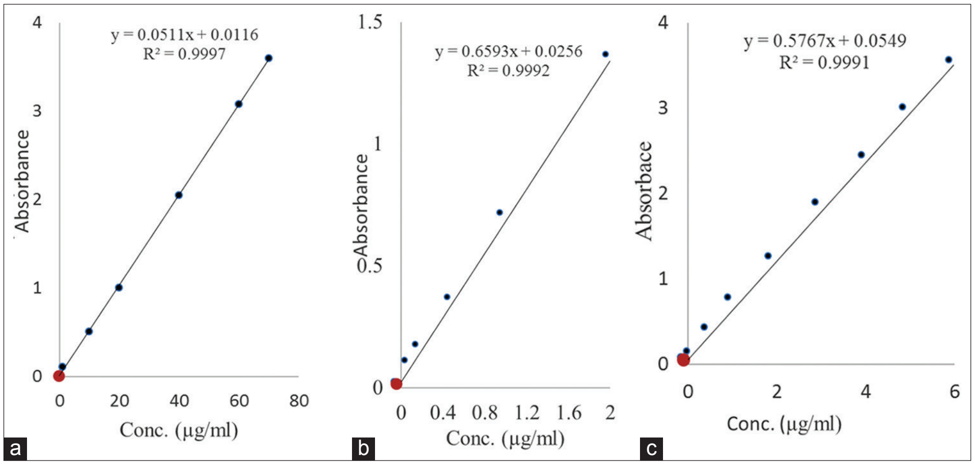

Figure 3: Calibration curves of thiocolchicoside (D_0) in (a) hydrochloric acid (b) sodium acetate buffer (c) phosphate buffer

Assay of Thiocolchicoside

A total of 20 thiocolchicoside capsules were procured and the contents inside were weighed. The powder content equivalent to 25 mg of thiocolchicoside was extracted with methanol in a 25 ml volumetric flask, and dilutions were made with hydrochloric acid, sodium acetate buffer, and phosphate buffers for Method I, II, and III, respectively, and the assay was carried out as per the procedure explained.

RESULTS AND DISCUSSION

Two different techniques – zero-order (D_0) and first-order derivative spectroscopy (D_1) have been developed for the

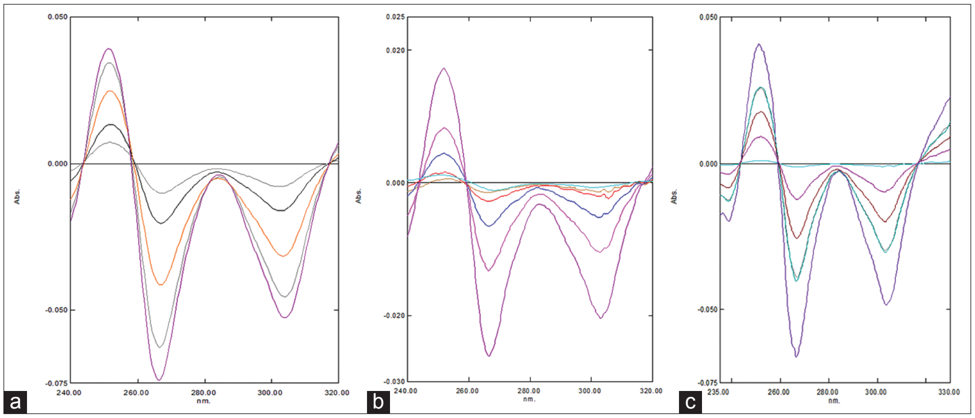

Figure 4: Overlay first derivative spectrum (D_1) of thiocolchicoside in (a) hydrochloric acid, (b) sodium acetate buffer, (c) phosphate buffer

Table 1: Comparison of present spectrophotometric method with the reported methods

Reagents	λ_{max} (nm)	Linearity(µg/mL)	Reference
Methanol	251.8	1–14	[4]
Distilled water	259.8	5–50	[5]
Sodium hydroxide	259	2.5–50	[6]
PDAB and HCl HCl and water MBTH and $FeCl_3$	400 400 410	10–50	[7]
Water(D_0) Water(AUC)	259.8 269.8–249.8	5–50 5–50	[8]
Hydrochloric acid Sodium acetate buffer pH 4.0 Phosphate buffer pH 7.0	259 259 259	1.0–70 (Method I) 0.1–2 (Method II) 0.1–6 (Method III)	Present method

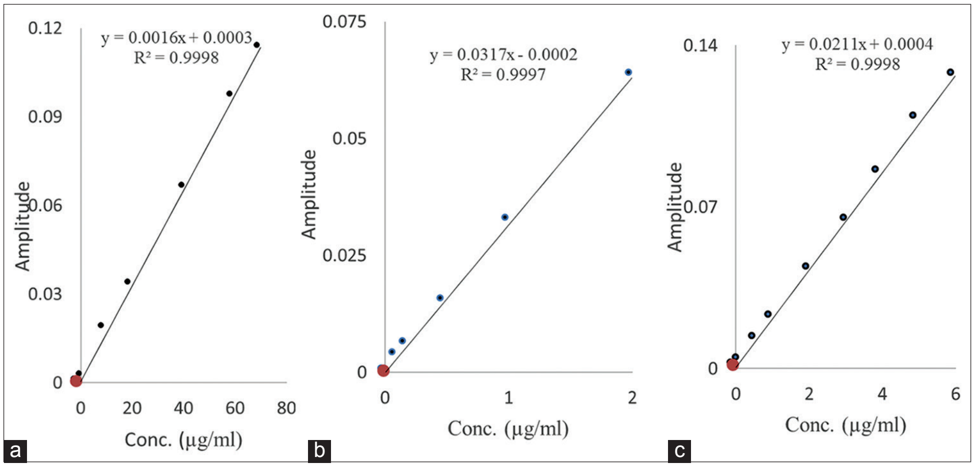

Figure 5: Calibration curves of thiocolchicoside (D_1) in (a) hydrochloric acid, (b) sodium acetate buffer, (c) phosphate buffer

determination of thiocolchicoside capsules in three different reagents such as hydrochloric acid, sodium acetate buffer, and phosphate buffers. The present proposed method was compared with the previously reported methods in Table 1.

Zero-order Spectroscopy (D_0)

The overlay absorption spectrum obtained in zero-order spectrophotometric technique (D_0) in hydrochloric acid, sodium acetate buffer, and phosphate buffers for Method I, II, and III was shown in Figure 2. Thiocolchicoside has shown absorption maxima at 259 nm and obeys Beer–Lambert's law over the concentration range of 1.0–70, 0.1–2, and 0.1–6 µg/ml in hydrochloric acid, sodium acetate buffer, and phosphate buffer, respectively [Table 2]. The linear regression equations were found to be $y = 0.0511x + 0.0116$ ($R^2 = 0.999$), $y = 0.6593x + 0.0256$ ($R^2 = 0.999$), and $y = 0.5767x + 0.0549$ ($R^2 = 0.999$) for Method I, II, and III [Figure 3], respectively. The optical characteristics were given in Table 2. The percentage RSD in precision and accuracy studies was found to be <2 in all the three methods indicating that the methods are precise and accurate.

First-order Derivative Spectroscopy (D_1)

The overlay first-order derivative spectra of thiocolchicoside in hydrochloric acid, sodium acetate buffer, and phosphate

Table 2: Optical characteristics of thiocolchicoside - zero-order spectroscopy

Parameters	Method		
	I	II	III
Linearity range (µg/ml)	1–70	0.1-2.0	0.1–6
λ_{max} (nm)	259	252	259
Molar extinction coefficient (Liter/mole/cm)	2.8800×10^4	37.118×10^4	32.4682×10^4
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.212	0.0373	0.0934
Slope	0.0511	0.6593	0.5767
Intercept	0.0116	0.0256	0.0549
Correlation coefficient	0.999	0.999	0.999
Precision (% RSD)	0.56–0.74	0.21–0.35	0.24–0.39
Accuracy (% RSD)	0.79–0.86	0.52–0.37	0.14–0.45
Assay(%)	99.78	99.82	99.49

RSD: Relative standard deviation

Table 3: Linearity of thiocolchicoside - first derivative spectroscopy

Conc. (µg/ml)	Method I Hydrochloric acid			Method II Sodium acetate buffer			Method III Phosphate buffer		
	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude
0.1	-	-	-	0.000	0.102	0.102	0.001	0.001	0.002
0.2	-	-	-	0.085	0.079	0.165	0.002	0.003	0.005
0.5	-	-	-	0.163	0.195	0.359	0.005	0.007	0.012
1	0	0.001	0.001	0.359	0.338	0.697	0.009	0.012	0.021
2	0.001	0.002	0.003	0.753	0.583	1.336	0.017	0.026	0.043
3	0.002	0.003	0.005	-	-	-	0.025	0.039	0.064
4	0.003	0.004	0.007	-	-	-	0.035	0.050	0.085
5	0.003	0.005	0.008	-	-	-	0.041	0.066	0.107
6	0.004	0.006	0.010	-	-	-	0.044	0.082	0.126
10	0.007	0.010	0.017	-	-	-	-	-	-
20	0.013	0.020	0.033	-	-	-	-	-	-
40	0.025	0.041	0.066	-	-	-	-	-	-
60	0.036	0.063	0.097	-	-	-	-	-	-
70	0.046	0.072	0.118	-	-	-	-	-	-

buffers for Method I, II, and III, respectively, were shown in Figure 4, and the spectral characteristics observed were shown in Table 3. Thiocolchicoside obeys Beer–Lambert's law over the concentration range of 1.0–70, 0.1–2, and 0.1–6 μg/ml in hydrochloric acid, sodium acetate buffer, and phosphate buffer, respectively, and the linear regression equations were found to be $y = 0.0016x + 0.0003$ ($R^2 = 0.9998$), $y = 0.0317x - 0.0002$ ($R^2 = 0.9997$), and $y = 0.0211x + 0.0004$ ($R^2 = 0.9998$) for Method I, II, and III [Figure 5], respectively, and the %RSD values in precision and accuracy studies were found to be <2 indicating that all the methods are precise and accurate.

CONCLUSION

The two spectrophotometric techniques were validated and found to be simple, precise, accurate, and economical and can be used for the routine analysis of thiocolchicoside capsules.

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