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Research Article

Simultaneous estimation of Thiocolchicoside and Hydrochlorothiazide by Q-absorption ratio method

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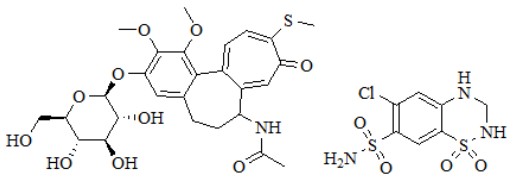
ABSTRACT

A simple, precise and accurate UV spectrophotometric method has been developed and validated for the Simultaneous estimation of Thiocolchicoside(TCS) and Hydrochlorothiazide(HCTZ) by UV spectrophotometric method by Q-Absorption ratio method.For development of Q-Absorption ratio method, uses the ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. Thiocolchicoside and Hydrochlorothiazide have shown an iso-absorptive point at 282.60 nm in methanol. The second wavelength used is 271 nm, which is λ-max of Hydrochlorthiazide in methanol.Calibration curves were linear in range of 5 - 25µg/mL (r2=0.999) and 5-25 µg/mL (r2=0.999).

Key words: Thiocolchicoside, Hydrochlorothiazide , Q-Absorption ratio method, Methanol

INTRODUCTION

Thiocolchicoside (TCS) chemically, N-[3-(β-D-glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2-imethoxy-10-(methylthio)-9-oxobenzo [a] heptalen-7yl]acetamide. It has selective affinity for Y-amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA- inhibitory pathways thereby acting as a potent muscle relaxant. Thiocolchicoside is official in IP[2],USP[4]. Hydrochlorothiazide(HCTZ) is diuretic and antihypertensive, which inhibits the reabsorption of sodium and calcium at the beginning of distal convoluted. It is the 3,4dihydroderivetive of chlorothiazide. It is chemically 6-chloro-3,4-dihydro-2H-1, 2, 4-benzothiadi- azine-7-sulphonamide-1,1-dioxide. Hydrochlorothiazide is official in IP[2], BP[3], USP[4].Thiocolchicoside is Centrally Acting Skeletal Muscle Relaxant having Analgesic And Anti-Inflammatory Effect and Hydrochlorothiazide is Thiazide class of Diuretics Used in treatment of Hypertension and Chronic Heart Failure[1]. Longer use of Hydrochlorothiazide may Cause Joint Pain, Back Pain, Muscle Pain, to overcome these Hydrochlorothiazide is given with combination of Thiocolchicoside. On detailed literature survey, it was found that Thiocolchicoside can be estimated by spectrophotometry[5-11], HPLC[12,14], HPTLC[13] methods individually or in combination with other drugs. The aim of the present work is to develop and validate new spectrophotometric methods for the estimation of Thiocolchicoside and Hydrochlorothiazide. The proposed method was developed and validated according to guidelines.



MATERIALS AND METHODS

Instrumentation

A double beam UV visible spectrophotometer (UV – 1800, Shimadzu) connected to Computer loaded with spectra manager software UV probe was employed with spectral band width 1 nm and wavelength accuracy of 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance.

Chemicals and Reagents

The bulk drug Hydrochlorothiazide was obtained from (Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat) and Thiocolchicoside was obtained from (West Coast,Gota, Ahmedabad, Gujarat).Methanol was used as a solvent throughout the experimentation.

Preparation of Standard Solutions and Synthetic Mixture

(A) Preparation of Standard stock solution:

A 10 mg of standard HCTZ and TCS were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of HCTZ and TCS. This stock solution was used to prepare further dilutions of standard solutions.

(B) Preparation of Synthetic Mixture of Hydrochlorothiazide and Thiocolchicoside:

Acenac MR is a medicine that contain two active substances, Thiocolchicoside and Hydrochlorothiazide.The synthetic mixture of Hydrochlorothiazide and Thiocolchicoside was prepared in the ratio of 1:25. Accurately weighed Hydrochlorothiazide (1 mg) and (25mg)Thiocolchicoside were transferred into 100 ml volumetric flask and dissolved in Methanol.Excipients such as such as magnesium stearate, Carmellose Sodium, Polysorbate 80were added in this mixture and sonicated for 20 minutes. This solution was filtered through Whatman filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 10 µg/ml of Hydrochlorothiazide and 250 µg/ml of Thiocolchicoside. From this solution 1 ml transferred into a 10 ml volumetric flask and the volume was adjusted up to the mark with methanol to make final concentration of Hydrochlorothiazide 1 µg/ml and Thiocolchicoside 25 µg/ml.

(C) Selection of Wavelength for Estimation of Hydrochlorothiazide and Thiocolchicoside:

The standard stock solution of Hydrochlorothiazide and Thiocolchicoside were scanned in the range of 200 nm to 400 nm against methanol as a blank. Maximum absorbance was obtained at 271 nm and 257 nm for Hydrochlorothiazide and Thiocolchicoside. Isoabsorptive point was found at 282.60 nm.

Q- Absorption ratio method

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptivepoint and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that HCTZ and TCS show an isoabsorptive point at 282.60 nm. The second wavelength used is 271 nm, which is the λ-max of HCTZ. Working standard solutions having concentration range 5-25 µg/ml for HCTZ and TCS were prepared in methanol and the absorbance at 271 nm and 282.60 nm were measured and absorptivity coefficients were calculated using calibration curve.The concentration of two drugs in the mixture can be calculated using following equations.

CX = [(QM-QY) / (QX-QY)] ×A1/ax1

CY = [(QM-QX) / (QY-QX)] ×A2/ay1

Where,

Cx and Cy= Concentration of Hydrochlorothiazide and Thiocolchicoside

Qx= The ratio of absorptivity of Thiocolchicoside at 271 and 282.60 nm

Qy= the ratio of absorptivity of Hydrochlorothiazide at 271 and 282.60 nm

QM = A2/A1, QX = ax2 / ax1 and QY = ay2 / ay1

A1 and A2= Absorbance of mixture at 271 nm and 282.60nm

ax1 and ay1 = Absorptivities of HCTZ and TCS at 271 nm

ax2 and ay2 = Absorptivities of HCTZ and TCS at 282.60 nm

Method Validation

UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The Analytical method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection(LOD) and limit of quantitiation (LOQ).

Calibration Curve for Hydrochlorothiazide and Thiocolchicoside

For Hydrochlorothiazide: An aliquots of stock solution of Hydrochlorothiazide (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were pipette out in five different 10 ml volumetric flasks and further diluted to attain concentration of about 5, 10, 15, 20 and 25 µg/ml respectively. Graph of Absorbance Vs. Concentration was plotted.

For Thiocolchicoside: An aliquots of stock solution of Thiocolchicoside (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were pipette out in five different 10 ml volumetric flasks and further diluted to attain concentration of about 5, 10, 15, 20 and 25 µg/ml respectively. Graph of Absorbance Vs. Concentration was plotted.

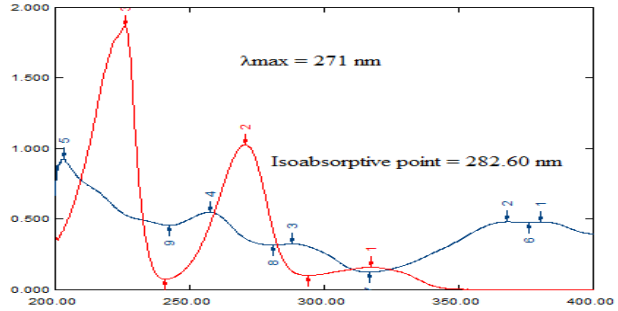


Figure.1: Overlain UV Spectrum of Thiocolchicoside and Hydrochlorothiazide (each 15µg/mL)

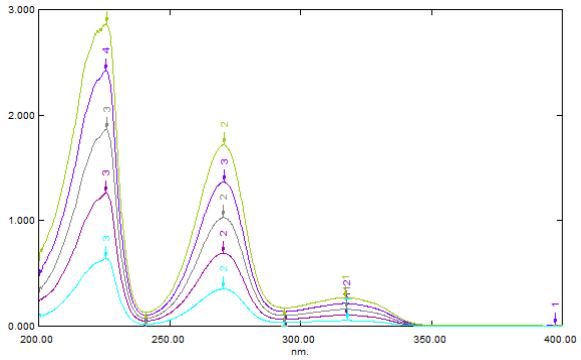


Figure. 2: "Overlain spectra of Hydrochlorothiazide (5-25 µg/ ml)"

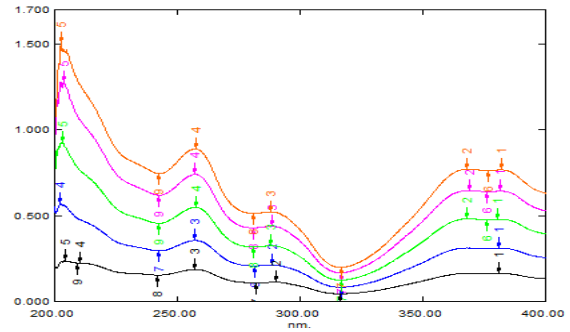


Figure.3:"Overlain spectra of Thiocolchicoside (5 - 25 µg/ml)"

Table 1: "Calibration data at Isoabsorptive Point (282.60 nm)"

Hydrochlorothiazide (282.60 nm)			Thiocolchicoside (282.60 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**% RSD**
5	0.105 ± 0.0010	0.9523	**5**	0.105 ± 0.0010	0.9433
10	0.207 ± 0.0015	0.7367	**10**	0.207 ± 0.0015	0.7211
15	0.315 ± 0.0020	0.6594	**15**	0.314 ± 0.0025	0.7911
20	0.422 ± 0.0020	0.4925	**20**	0.421 ± 0.0026	0.6132
25	0.526 ± 0.0015	0.2902	**25**	0.528 ± 0.0035	0.6591

Table 2: "Calibration data at λmax (271 nm)"

Hydrochlorothiazide (271 nm)			Thiocolchicoside (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**% RSD**
5	0.352 ± 0.0020	0.5649	**5**	0.124 ± 0.0015	1.2096
10	0.691 ± 0.0040	0.5755	**10**	0.235 ± 0.0020	0.8438
15	1.028 ± 0.0055	0.5324	**15**	0.359 ± 0.0026	0.7202
20	1.365 ± 0.0070	0.5109	**20**	0.485 ± 0.0030	0.6160
25	1.719 ± 0.0085	0.4918	**25**	0.582 ± 0.0035	0.5982

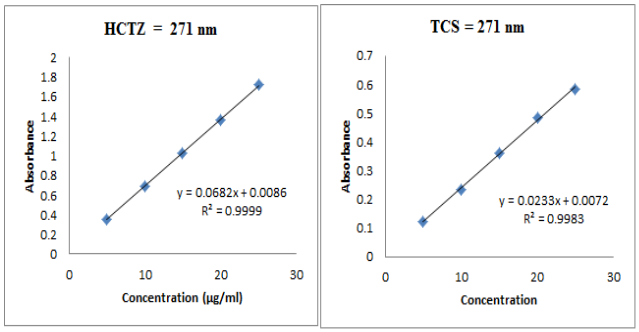


Figure.4: "Calibration Curve of Hydrochlorothiazide and Thiocolchicoside (5 – 25 µg/ml) at 271 nm"

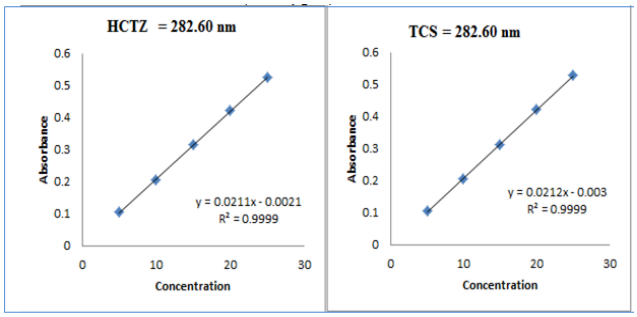


Figure. 5: "Calibration Curve of Hydrochlorothiazide and Thiocolchicoside (5 – 25 µg/ml) at 282.60 nm"

ACCURACY

To study the accuracy of the proposed methods, recovery studies were carried out by standard edition method at three different levels (80%, 100%, 120% of the test concentrations as per ICH guidelines). A known amount of drug was added and percentage recoveries were calculated. The results of recovery studies were satisfactory.

Table 3: "Accuracy data"

Drug	Amount Taken (µg/ml)	Amount Added (µg/ml)	Total amount of Drug (µg/ml)	Amount of Drug Recovered (µg ± SD)	% Recovery ± SD
Thiocolchicoside	10	8	18	17.87 ± 0.0035	99.27 ± 1.017
	10	10	20	19.98 ± 0.0040	99.9 ± 0.678
	10	12	22	22.41 ± 0.0055	101.86 ± 1.340
Hydrochlorothiazide	10	8	18	17.96 ± 0.0045	99.77 ± 0.750
	10	10	20	20.13 ± 0.0052	100.65 ± 0.900
	10	12	22	21.89 ± 0.0040	99.5 ±0.8407

PRECISION

Precision of the method was measured in terms of the interday and intraday precision and repeatability. The experiment was repeated for three times in a day using three different concentrations (3×3) for intraday and three different concentrations on three consecutive days for interday precision. The experiment was repeated for six times for repeatability.

Table 4: "Intraday Precision Data (n=3) for Hydrochlorothiaizide"

Hydrochlorothiazide (282.60 nm)			Hydrochlorothiazide (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
10	0.209 ± 0.0025	1.2022	**10**	0.698 ± 0.0035	0.5026
15	0.317 ± 0.0030	0.9463	**15**	1.034 ± 0.0058	0.5609
20	0.423 ± 0.0025	0.5940	**20**	1.374 ± 0.0090	0.6550

Table 5: "Interday Precision Data (n=3) for Hydrochlorothiaizide"

Hydrochlorothiazide (282.60 nm)			Hydrochlorothiazide (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
10	0.209 ± 0.0030	1.4570	**10**	0.696 ± 0.0043	0.6178
15	0.317 ± 0.0035	1.1055	**15**	1.035 ± 0.0066	0.6376
20	0.424 ± 0.0035	0.8276	**20**	1.375 ± 0.0100	0.7272

Table 6: "Repeatability Data (n=6) for Hydrochlorothiaizide"

Hydrochlorothiazide (282.60 nm)			Hydrochlorothiazide (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
15	0.318 ± 0.0019	0.6200	**15**	1.034 ±0.0046	0.4452

Table 7: "Intraday Precision Data (n=3) for Thiocolchicoside

Thiocolchicoside (282.60 nm)			Thiocolchicoside (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
10	0.209 ± 0.0025	1.1961	**10**	0.237 ± 0.0025	1.0548
15	0.317 ±0.0026	0.8201	**15**	0.363 ± 0.0045	1.2396
20	0.423 ±0.0030	0.7092	**20**	0.489 ± 0.0040	0.8179

Table 8: "Interday Precision Data (n=3) for Thiocolchicoside"

Thiocolchicoside (282.60 nm)			Thiocolchicoside (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
10	0.210 ± 0.0032	1.5238	**10**	0.239 ± 0.0041	1.7154
15	0.318 ± 0.0040	1.2578	**15**	0.364 ± 0.0055	1.5109
20	0.424 ± 0.0035	0.8254	**20**	0.491 ± 0.0065	1.3238

Table 9: "Repeatability Data (n=6) for Thiocolchicoside"

Thiocolchicoside (282.60 nm)			Thiocolchicoside (271 nm)		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**%RSD**	**Conc. (µg/ml)**	**Mean Absorbance ± SD**	**%RSD**
15	0.318 ±0.0033	1.037	**15**	0.365 ±0.0052	1.4246

Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 × (σ / S)

Where, σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve

Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × (σ / S)

Where, σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve

Table 10: "LOD and LOQ data"

Drug Name	LOD(µg/ml)	LOQ(µg/ml)
Thiocolchicoside	0.210	0.630
Hydrochlorothiazide	0.248	0.744

Table 11: "Analysis of synthetic mixture by Q – absorption ratio method"

Drug Name	Label Claim (mg)	Amt. in Synthetic Mix. (mg)	Total amt. obtained ± SD	% Recovery
Hydrochlorothiazide	100	25	24.87± 0.003	99.48 %
Thiocolchicoside	4	1	1.012± 0.0020	101.2 %

RESULTS AND DISCUSSION

The proposed method was based on spectrophotometric simultaneous estimation ofThiocolchicoside and Hydrochlorothiazide in UV region using Methanol as solvent. The proposed methods were found to be accurate, simple, rapid and reproducible.Thus the Proposed method can beapplied in the routine analysis of the Thiocolchicoside and Hydrochlorothiazide.

Table 13: "Regression analysis data and summary of validation parameters"

Parameters	HCTZ		TCS	
Wavelength (nm)	271 nm	282.60 nm	271 nm	282.60 nm
Beer's Law Limit (µg/ml)	5 – 25	5 – 25	5 – 25	5 – 25
Regression equation (y = mx + c)	y = 0.0682x+ 0.0086	y = 0.0211x - 0.0021	y = 0.0233x + 0.0072	y = 0.0212x - 0.003
Correlation coefficient (r^2)	0.999	0.999	0.998	0.999
Repeatability (%RSD, n = 6)	0.445	0.620	0.670	0.682
Intraday (n = 3) (%RSD)(µg/ml)	0.50 – 0.65	0.54 -1.20	0.81 – 1.23	0.70 – 1.19
Interday (n = 3) (%RSD)(µg/ml)	0.61- 0.72	0.82 – 1.45	1.32 – 1.71	0.82 – 1.52
LOD (µg/ml)	0.248		0.210	
LOQ (µg/ml)	0.744		0.630	
Accuracy (%)	99.27 – 101.86		99.5 – 100.65	

CONCLUTION

Simple, accurate, rapid and precise Q-Absorption ratio method was developed and validated for simultaneous estimation of both these drugs. The method utilizes easily available solvent for analysis of both drugs hence the method was also economic for estimation of HCTZ and TCS from synthetic mixture. The plot of absorbance versus respective concentration was found to be linear in the concentration range of 5-25 µg/ml for both Hydrochlorothiazide and Thiocolchicoside respectively. Accuracy of method was found between 98-102%.The precision(intra-day, inter-day and repeatability) of method was found within limits. This method can be successfully applied for the simultaneous estimation of Hydrochlorothiazide and Thiocolchicoside in synthetic mixture.

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