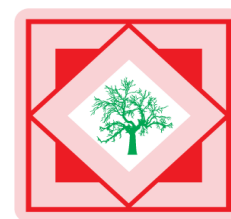




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# Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in Pharmaceutical formulation

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## ABSTRACT

*A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Thiocolchicoside and Diclofenac in pharmaceutical formulation by UV-Spectrophotometric method which includes Simultaneous Equation method (Method I), Absorbance Correction method (Method II). For development of Method I, wavelengths were selected 260.0 nm  $\lambda_{max}$  for thiocholchicoside and 276.5 nm  $\lambda_{max}$  for diclofenac sodium, while for Method II, 276.5 nm  $\lambda_{max}$  for diclofenac sodium and 373.0 nm is isoabsorptive point of thiocholchicoside and diclofenac sodium. The two drugs follow Beer-Lambert's law over the concentration range of 1-10  $\mu\text{g/mL}$  for thiocholchicoside and 6.25-62.5  $\mu\text{g/mL}$  for diclofenac sodium in both the methods. The percent recoveries of the drugs were found to be nearly 100 % representing the accuracy of both the methods. Validation of the proposed methods was carried out for its accuracy, precision, and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of thiocholchicoside and diclofenac sodium in combined dosage form. Also the method II can be applied for estimation of thiocholchicoside even in presence of the diclofenac sodium in the formulation matrices without interference.*

**Keywords:** Thiocolchicoside, Diclofenac sodium, Absorbance Correction method, Simultaneous Equation method and validation

## INTRODUCTION

Thiocolchicoside (TCD) is (s)-N-[3-(B-D-glucopyranoxyloxy)- 5,6,7,9- tetrahydro - 1,2 dimethoxy- 10- (methylthio)-9-oxobenzo [a]heptalen-7yl] acetamide. It is an anti-inflammatory analgesic agent with muscle relaxant action<sup>1</sup>. Literature survey reveals the Ultraviolet absorbance

detection of colchicine and related alkaloids on a capillary electrophoresis microchip<sup>2</sup>, the determination of thiocolchicoside in its binary mixtures (thiocolchicoside/glafenine and thiocolchicoside / floctafenine by TLC/densitometry<sup>3</sup>, The validation of analytical procedure for the determination of thiocolchicoside drug by HPLC<sup>4</sup>, HPLC/UV method for quantitative analysis of Thiocolchicoside and its active metabolites in human<sup>5</sup>, UV-Spectrophotometric determination of Thiocolchicoside in Capsule<sup>6</sup>. Diclofenac Sodium (DICLO) is Sodium [o-(2,6dichloroanilino) phenyl] acetate<sup>7</sup>. It is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and an analgesic, reducing pain in conditions such as arthritis or acute injury. Literature survey reveals Spectrophotometric determination of Diclofenac in the presence of cyclodextrin<sup>8</sup>, High Performance Thin Layer Chromatographic Method for the Determination of Diclofenac Sodium in Pharmaceutical Formulation<sup>9</sup>, The High-performance liquid chromatographic determination of diclofenac in human plasma after solid-phase extraction<sup>10</sup>, The Stability indicating HPLC method for simultaneous determination of mephenesin and diclofenac diethylamine<sup>11</sup>.

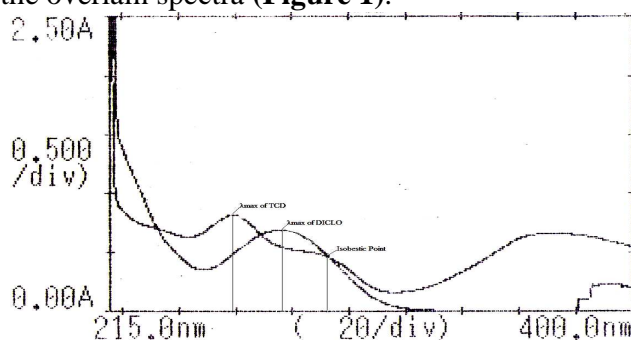
To the best of our knowledge, there is no published spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of Thiocolchicoside and Diclofenac in combined capsule dosage form by two simple UV Spectrophotometric methods (absorbance correction method, Simultaneous equation method).

## MATERIALS AND METHODS

SHIMADZU double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Shimadzu AUX220 balance was used for weighing the samples. 0.1N NaOH was used as solvent throughout the experimental work. Multicomponent tablet Thioact D 8mg (TCD 8.0 mg and DICLO 50.0 mg) was procured from local market.

### UV Spectrophotometry

Standard stock solutions of TCD and DICLO were prepared in 0.1N NaOH having concentration of 100 $\mu$ g/mL and 100 $\mu$ g/mL respectively. Aliquot portions of stock solutions were diluted with 0.1N NaOH to get final concentration of 10 $\mu$ g/mL each. The final diluted solution was scanned in the range of 400-200 nm in 1.0 cm cell against blank separately to get the absorbance graph for both the drugs and the overlain spectra (**Figure 1**).



**Figure 1: Overlain spectra of TCD and DICLO**

**Simultaneous Equation Method (Method I)**

Simultaneous equation method<sup>12</sup> the wavelength selected was  $\lambda_{\max}$  of both the drugs. At the  $\lambda_{\max}$  of the DICLO, TCD shows the considerable absorbance and at the  $\lambda_{\max}$  of TCD, DICLO shows considerable absorbance. The wavelengths 260.0 nm ( $\lambda_{\max}$  of TCD) and 276.5 nm ( $\lambda_{\max}$  of DICLO) were selected.

**Absorbance correction method (Method II)**

Absorbance correction method<sup>[12]</sup> uses the absorbances at two selected wavelengths, one at  $\lambda_{\max}$  of one drug where other drug also shows considerable absorbance and other being the wavelength at which the first drug has practically nil absorbance. The wavelengths 276.5 nm ( $\lambda_{\max}$  of DICLO) and 373.0 nm (where DICLO does not interfere in absorbance of TCD) were selected for analysis by proposed method.

**Study of Beer-Lambert's law**

Aliquots of working stock solution of TCD and DICLO were diluted with 0.1N NaOH to get a concentration in 1-10 $\mu$ g/mL for TCD and 6.25-62.5 $\mu$ g/mL for DICLO individually and in mixture. Absorbances of each of the resulting solution were measured at 260.0, 276.5 and 373.0 nm in 1.0 cm cell using solvent blank. The graphs were plotted as concentration vs. absorbance and correlation coefficients were found to be less than 1.

**Absorptivity Value for TCD and DICLO**

Working standard solutions of TCD and DICLO were used for the present study. Absorbances of each of the final dilutions were measured in duplicate against solvent blank at 260.0, 276.5 and 373.0 nm and the absorptivities values of each drug at selected wavelengths was determined which the mean of five independent readings.

**Estimation in Standard laboratory mixture**

An accurately weighed quantities of TCD and DICLO in the ratio of 1:6.25 were transferred to different 25.0 mL volumetric flask separately, diluted with 0.1 N NaOH to get the mix standard solutions. 5.0 mL portions of above solution were further diluted up to 50.0 mL in volumetric flask. Aliquots of the above solutions were further diluted to 25.0 mL with 0.1N NaOH to get the final concentration about 6.4 $\mu$ g/mL and 40 $\mu$ g/mL of TCD and DICLO respectively. The absorbencies of the above solution were measured at 260.0, 276.5 and 373.0 nm against blank.

**METHOD I**

The absorbance and the absorptivity values at these particular wavelengths calculated and substituted in the following equation, to obtain respective concentration.

$$C_{\text{TCD}} = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad (1)$$

$$C_{\text{DICLO}} = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad (2)$$

$A_1$  and  $A_2$  are absorbance of diluted laboratory mixture at 260.0 and 276.5 nm respectively.  $C_x$  and  $C_y$  are concentrations of TCD and DICLO respectively (g/100mL).  $a_{x_1}$  and  $a_{x_2}$  are

absorptivities of TCD and DICLO at 260.0 nm while  $a_{y1}$  and  $a_{y2}$  are absorptivities of TCD and DICLO at 276.5 nm respectively.

## METHOD II

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{\text{TCD}} = A_2 / a_{y1} \quad (3)$$

$$A \text{ (Conc. of TCD at 276.5 nm)} = a_{x1} \times C_{\text{TCD}}$$

$$\text{Corrected absorbance (Cabs)} = A_1 - A$$

$$C_{\text{DICLO}} = \text{Cabs} / a_{x2} \quad (4)$$

Where,  $A_1$  and  $A_2$  are the absorbances of mixture at 276.5 nm and 373.0 nm,  $a_{x1}$  and  $a_{x2}$  are absorptivities of two drugs at 276.5 nm.  $a_{y1}$  absorptivity of TCD at 373.0 nm. Results are mentioned in **Table 1**.

**Table 1: Results of estimation in Laboratory mixture**

Sample		Percent Drug Estimated*			
		Method I		Method II	
		TCD	DICLO	TCD	DICLO
Std. lab Mixture	Mean	99.81	100.23	101.42	100.00
	±S.D.	0.8946	0.4961	0.5935	0.5327
	% R.S.D.	0.8966	0.4950	0.5851	0.5327

\*Percent label claim is mean of five observations, Method I is simultaneous equation method, Method II is Absorption correction method, S.D. is standard deviation and R.S.D. is relative standard deviation

## Analysis in Pharmaceutical Formulation

For the simultaneous estimation of commercial formulation twenty capsule of Thiact D 8mg (Label claim: Thiocholchicoside 8 mg and Diclofenac Sodium 50 mg) was taken. The average weight of capsule was determined. The capsule contents were emptied. Average weight of empty shells was again taken for and subtracted from the respective capsule weight to find out average weight of capsule. Contents were finely powdered by mortar and pestle.

For methods 1 and 2, powder equivalent to about 8.0 mg of Thiocolchicoside and 50.0 mg of Diclofenac was transferred to 50.0 mL volumetric flask, dissolved in 0.1N NaOH and solicited for 20 min. The volume was then made up to the mark using 0.1N NaOH as solvent. The resulting solution was filtered first through Whatmann filter paper no. 41, first few drops were rejected and if particles found in filtrate then filtered through membrane filter paper. Filtrate was appropriately diluted to get concentration of 6.4µg/mL of TCD and 40µg/mL of DICLO (On label claim basis). Absorbencies of sample solutions were recorded at 260.0 and 276.5 nm and

the concentration of two drugs in the sample were determined by using eqns. 1 and 2 (Method I). Absorbencies of sample solutions were also recorded at selected wavelengths 276.5 nm, 373.0 nm and the concentration of two drugs in the sample were determined by using eqns. 3 and 4 (Method II). The results of analysis are mentioned in **Table 2**.

### Recovery Studies

To check the accuracy of the developed methods and to study the interference from formulation additives, analytical recovery experiment was performed by standard addition method. To the pre-analyzed powder, known quantities of TCD and DICLO were added at four different levels. The contents were dissolved in 0.1N NaOH, filtered and the absorbance of each solution was measured at 260.0, 276.5, and 373.0 nm. Percent recovery was calculated by using the formula

$$\% \text{ Recovery} = \frac{\text{Total drug Estimated} - \text{Amount Contributed} \times 100}{\text{Amount of Pure Drug added}}$$

The Total drug estimation was calculated using eq 1 and 2 (Method I) and eq 3 and 4 (Method II) and results are shown in **Table 2**

**Table 2: Results of Estimation in marketed formulation and recovery**

Sample		Percent label Claim estimated*				Percent Recovery**			
		Method I		Method II		Method I		Method II	
Marketed Formulation	Drug Mean ±S.D. %R.S.D	TCD	DICLO	TCD	DICLO	TCD	DICLO	TCD	DICLO
				99.81	100.23	101.42	100.00	98.87	100.14
		0.8946	0.4961	0.5935	0.5327	0.41	2.02	0.58	1.96
		0.8966	0.4950	0.5851	0.5327	0.42	2.02	0.57	1.96
	95% CI of mean	100.6%	100.4%	100.5%	103.3% - 100.6%	-	-	-	-
		99.51%	100.32%	98.96%					

\* Percent label claim is mean of five observations, \*\* Recovery is mean of four observations, CI- confidence interval  
Method I is simultaneous equation method, Method II is Absorption correction method  
S.D. is standard deviation, R.S.D. is relative standard deviation

### Validation [13,14]

#### Accuracy

Accuracy was ascertained on the basis of recovery studies by standard addition method. Results were recorded in **Table 2**.

#### Precision

Precision of analytical method is expressed in terms of SD, %RSD of series of measurements. Study was carried out by replicate analysis of homogeneous samples of capsule powder. Results are recorded in **Table 1**.

**Intraday Precision and Inter-day precision**

An accurately weighed quantity of capsule powder equivalent to about 8 mg of TCD was diluted to get the final concentration (6.4µg/mL TCD and 40µg/mL DICLO) on label claim basis. The absorbance of the solution was taken at an interval of 1h for a period of 3h at selected wavelength for intraday study. Similarly the same solution was measured on 1st, 3rd, and 5th day and % label claim was calculated and recorded in **Table 3**.

**Ruggedness**

Different Analyst: The capsule samples were analyzed by proposed method by three different analysts and results were recorded in **Table 3**.

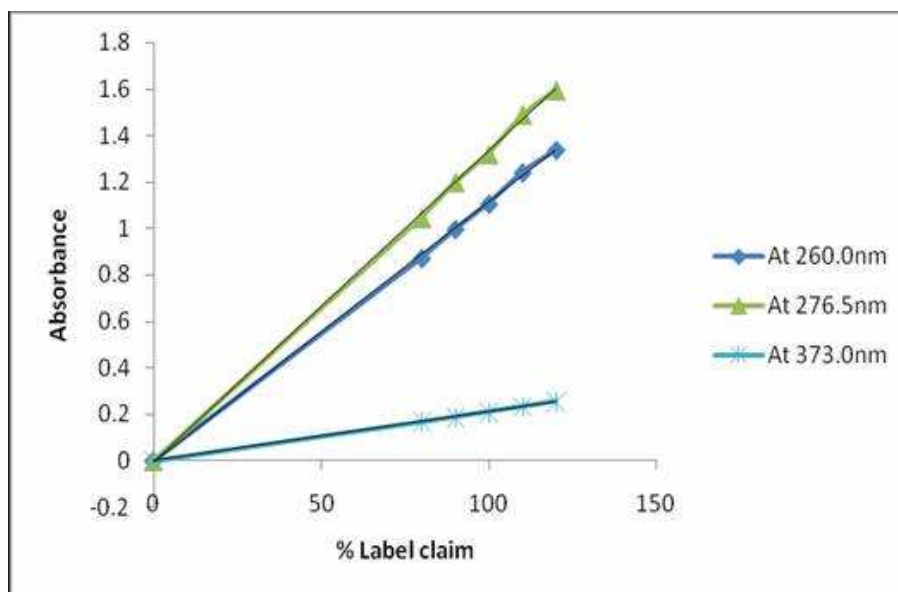
**Linearity and Range**

Accurately weighed quantities of capsule powder equivalent to 80, 90, 100, 110 and 120% of label claim of TCD were taken and dilutions were made as described under marketed formulation. The absorbencies of the resulting solution were measured at 260.0, 276.5 and 373.0 nm against blank. The graphs of concentration vs. absorbance were plotted and found to be linear (**Figure 2**).

**Table 3: Summary of Validation Parameters**

Parameters	Percent Label claim			
	Method I		Method II	
	TCD	DICLO	TCD	DICLO
Intraday Precision				
% label claim (0h)	100.27	100.31	99.65	100.40
% label claim (3h)	119.96	100.01	96.75	102.91
Interday Precision (n=3)				
Mean % label Claim	118.60	103.42	72.72	109.98
% R.S.D.	20.12	5.35	42.31	8.85
% Bias	18.6	3.42	-27.28	9.98
Ruggedness (% R.S.D.)				
Analyst to Analyst n=3				
% Bias	0.49	1.04	0.99	1.11
	0.82	0.65	-1.33	0.95

% Bias = 100 (Found amt – label claim)/ Label claim.



**Figure 2: Plot of linearity and range at selected wavelengths**

## RESULTS AND DISCUSSION

Both the UV Spectrophotometric methods were found to be simple, accurate, economic, and rapid for routine simultaneous estimation of TCD and DICLO in capsule dosage form. For two methods linearity was observed in the concentration range of 1-10 $\mu$ g/ml for TCD and 6.25-62.5 $\mu$ g/ml for DICLO. Marketed brand of capsule was analyzed and amount of drug determined by proposed methods ranges from 99 to 102%. The proposed methods were validated as per ICH guideline. The % recovery ranges from 98.0 to 101.0 for both the methods. Intermediate precision was calculated as was inter-day and intraday variations for both drugs. The results of both parameters indicate that the drug solution was found to be stable for a period of 1h after the preparation of solution and their after the percent label claim of TCD changes, indicates the drug undergoes some sort of degradation. Further DICLO was found to be stable for period of 3day and thereafter the percent label claim differs from normal. Hence it could be suggested that the stability of drug in solution remains for 1h. The results obtained by method A and B for the estimation of Thiocolchicoside and Diclofenac sodium were studied statistically by unpaired t-test with Welch correction. In the result of analysis of marketed formulations by proposed methods, t-calculated values ( $p < 0.05$ ) was found to be 1.524 for TCD and 0.2615 for DICLO. F-test was used to compare variance of the two methods and the f-calculated values was found to be 4.437 for TCD and 1.045 for DICLO, 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.

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