

# SIMULTANEOUS DETERMINATION OF DICLOFENAC SODIUM AND THIOCOLCHICOSIDE IN FIXED DOSE COMBINATION BY SPECTROPHOTOMETRY

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**Abstract:** Three accurate, precise, sensitive and economical procedures for simultaneous determination of diclofenac sodium and thiolcolchicoside in tablet dosage form have been developed. The methods employed were absorbance correction method (I), first order derivative spectroscopic method (II) and dual wavelength method (III). In the first method diclofenac sodium concentration was determined directly from calibration plot by measuring absorbance at 276.6 nm and thiolcolchicoside was determined after correction for absorbance of diclofenac sodium at 372.8 nm. The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug. Wavelengths 278.6 nm and 243.2 nm were selected for the determination of the diclofenac sodium and thiolcolchicoside, respectively. In the third method, diclofenac sodium was determined by plotting the difference in absorbance at 244 and 269 nm (difference is zero for thiolcolchicoside) against the concentration of diclofenac sodium. Similarly for the determination of thiolcolchicoside, the difference in absorbance at 266.8 and 290 nm (difference is zero for diclofenac sodium) was plotted against the concentration of diclofenac sodium. The Beer's law obeyed in the concentration range 5-30 µg/ml for diclofenac sodium and 10-60 µg/ml for thiolcolchicoside. The results of analysis have been validated statistically and by recovery studies.

**Keywords:** Diclofenac sodium, Thiolcolchicoside, Absorbance correction method, Dual wavelength method.

## INTRODUCTION

Diclofenac sodium (DIC) [Sodium (o- {2, 6-dichlorophenyl} amino) phenyl] acetate] is a synthetic non steroidal anti-inflammatory drug (NSAID), has been proved to be safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders [1]. Thiolcolchicoside (THIO) chemically, (s)-N-[3-(B-D-glucopyranoxyl)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo (a) heptalen-7yl] acetamide, is a muscle relaxant which has been claimed to possess GABA mimetic and glycinergic actions. It is used in the symptomatic treatment of painful muscle spasm [2].

Literature survey reveals spectrophotometric [3, 4] and HPTLC [5, 6] determination of DIC in combination with other drugs. HPLC [7] and bioanalytical chromatographic methods [8] for quantification of DIC are also reported. For simultaneous determination of THIO with other drugs spectrophotometric [9], HPTLC [10] and HPLC methods [11, 12] are reported. No reports were found for determination of DIC and THIO by HPTLC method in fixed dose combination. Aim of present work was to develop simple, economical, rapid, accurate and precise spectrophotometric methods for determination of these drugs in fixed dose combination. The proposed methods were optimized and validated as per the International Conference on Harmonization (ICH) guidelines [13].

## MATERIALS AND METHODS

### Instrumentation

The instrument used in the present study was JASCO double beam UV/ Visible spectrophotometer (Model UV-550) with slit width fixed at 2 nm, equipped with spectra manager software (Version 1.54A). All weighing were done on electronic balance (Shimadzu, Model AY-120).

### Reagents and chemicals

Analytically pure samples of DIC and THIO were kindly supplied by Cipla Pvt. Ltd., Mumbai, India and Aventis Pharma Pvt. Ltd., Goa, India respectively and were used as such without further purification. The pharmaceutical dosage form used in this study was THIOACT-D4 capsules (Sun Pharmaceuticals Industries Ltd, Mumbai, India) labeled to contain 4 mg of thiolcolchicoside and 50 mg of diclofenac sodium as enteric coated tablet, per capsule.

### Methods

#### Absorbance correction method (Method I)

This method involves absorbance correction for DIC determination by subtracting absorbance of THIO from total absorbance of sample at 276.6 nm ( $\lambda_{max}$  of DIC). THIO concentration was determined directly from calibration plot by measuring absorbance at 372.8 nm

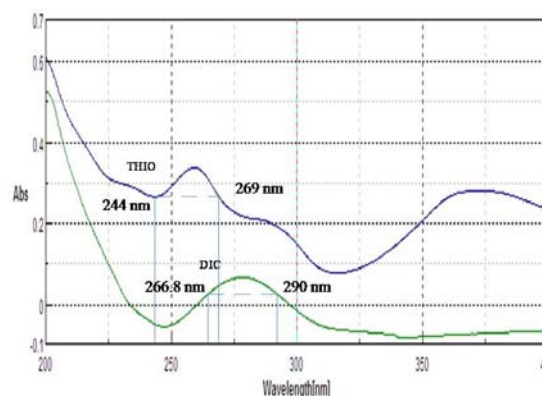
where DIC shows zero absorbance. The equations obtained for the determination of concentration are

$$C_{THIO} = A_{372.8}/\alpha_{\lambda_1} \dots\dots\dots (1)$$

$$C_{DIC} = A_{276.6} - (23.335 \times C_{THIO})/10.2346 \dots (2)$$

#### First order derivative spectroscopic method (Method II)

This method is based on first order derivative spectroscopy to overcome spectral interference from other drug. Zero order spectra of both the drugs were converted to first order derivative spectra with the help of spectra manager software (Figure 1 and Figure 2).



**Figure 1.** Zero order overlain spectra of DIC (10 µg/ml) and THIO (10 µg/ml)

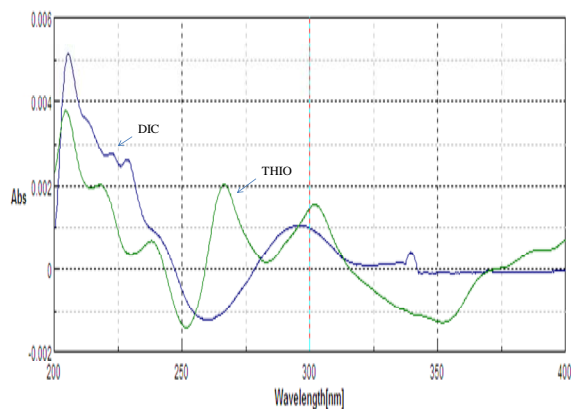
It was observed that DIC showed  $dA/d\lambda$  zero at 278.6 nm in contrast to THIO that has considerable  $dA/d\lambda$  at this wavelength. Further, THIO has zero  $dA/d\lambda$  at 243.2 nm while at this wavelength DIC has significant  $dA/d\lambda$ . Therefore wavelengths 278.6 nm and 243.2 nm were employed for the determination of THIO and DIC respectively without interference of other drug. The calibration curves were plotted at these two wavelengths of concentrations against  $dA/d\lambda$  separately. The equations of line obtained to determine concentrations of DIC and THIO were as follows

$$C_{DIC} = (dA/d\lambda_{243.2} + 0.00003)/0.0009 \dots (3)$$

$$C_{THIO} = (dA/d\lambda_{278.6} + 0.00005)/-0.0006 \dots (4)$$

#### Dual wavelength method (Method III)

In this method difference in absorbance at two selected wavelengths



**Figure 2.** First order derivative overlain spectra of DIC (10 µg/ml) and THIO (10 µg/ml)

is calculated. The difference in absorbance at 266.8 and 290 nm was found to be zero for DIC. Hence these two wavelengths were selected for the determination of THIO. Similarly, 244 and 269 nm were selected for the determination of DIC, where the difference in absorbance was found to be zero for THIO. Zero order spectra were recorded for solutions of different concentration of DIC and THIO between 200-400 nm. The difference in absorbance at 244 and 269 nm were plotted against the concentration of DIC and that 266.8 and 290 nm were plotted against the concentration of THIO to construct two separate calibration curves for DIC and THIO. The equations of line obtained to determine concentrations of DIC and THIO are as follows

$$C_{\text{THIO}} = A_{266.8-290} - 0.0863/0.0206 \quad \dots\dots (5)$$

$$C_{\text{DIC}} = A_{244-269} - 0.0036/0.0067 \quad \dots\dots\dots (6)$$

#### Preparation of standard stock solutions

Standard stock solution of DIC was prepared by dissolving 5 mg of pure DIC in 10 ml methanol to get concentration of 0.5 mg/ml. Standard stock solution of THIO was prepared by dissolving 10 mg of pure THIO in 10 ml of methanol to get concentration of 1 mg/ml. Aliquots of the stock

solution were further diluted to 10 ml with distilled water and scanned in the wavelength range of 200-400 nm to determine linearity.

#### Analysis of capsule formulation

Contents of twenty capsules were weighed accurately and powdered. Powder equivalent to 10 mg of DIC was weighed and dissolved in 5 ml of methanol with the aid of sonication for 5 min in 10 ml volumetric flask. Volume was made up to the mark with methanol to get sample stock solution. Solution was filtered through whatman filter paper no. 41 and first few ml were rejected. From the filtrate 0.1 ml was taken and diluted to 10 ml with distilled water to get final concentration of 10 µg/ml of DIC. Similarly powder equivalent to 2 mg of THIO was weighed and dissolved in 5 ml of methanol with the aid of sonication for 5 min in 10 ml volumetric flask. Volume was made up to the mark with methanol to get sample stock solution. Solution was filtered through whatman filter paper no.41. From the filtrate 0.5 ml was taken and diluted to 10 ml with distilled water to get final concentration of 10 µg/ml of THIO. Absorbance of these solutions was recorded in the wavelength range of 200-400 nm. Spectrums were processed separately as mentioned in theory section to determine the concentration of each drug by mentioned methods. Analysis was repeated six times to study the precision of the method.

#### Recovery studies

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels within the range of linearity for both the drugs.

#### RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets, precision and recovery studies were performed. The drugs obey beer's law in the concentration range 5-30 µg/ml for DIC and 10-60 µg/ml for THIO for all the methods with good correlation coefficient > 0.998. The results of commercial formulation analysis are presented in Table 1. Results of recovery studies are shown in Table 2. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed methods are simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of DIC and THIO in tablet formulation. Of the three methods developed dual wavelength method found to be more accurate and precise as standard deviation is less.

**Table 1. Results of commercial formulation analysis**

Method	Label claim (mg/tab)	% Label claim estimated * (Mean ± SD)	% RSD
Absorbance Correction Method	DIC-50	101.09 ± 0.858	0.849
	THIO-4	100.10 ± 0.535	0.534
First Derivative Method	DIC-50	101.80 ± 0.589	0.578
	THIO-4	101.91 ± 0.468	0.459
Dual Wavelength Method	DIC-50	100.56 ± 0.346	0.344
	THIO-4	101.13 ± 0.345	0.340

\* Mean of six determinations, RSD is relative standard deviation

**Table 2. Recovery studies of DIC and THIO**

Drug	Conc. of drug added		% Recovery * (Mean ± S.D)		
	µg/ml	% Level	Method I	Method II	Method III
DIC	5	50	100.78 ± 0.507	100.50 ± 0.806	100.87 ± 0.507
	10	100	101.35 ± 0.679	102.17 ± 0.603	100.54 ± 0.378
	15	150	100.12 ± 0.688	100.88 ± 0.329	102.08 ± 0.290
THIO	10	50	101.96 ± 0.827	101.03 ± 0.706	100.31 ± 0.437
	20	100	100.59 ± 0.427	100.49 ± 0.382	100.74 ± 0.417
	30	150	102.38 ± 0.446	100.94 ± 0.514	101.66 ± 0.292

\*Average of three determinations

#### CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine simultaneous determination of DIC and THIO in tablet dosage form.

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