

*ijcrr*

Vol 02 issue 12

Category: Research

Received on: 28/10/10

Revised on: 13/11/10

Accepted on: 20/11/10

## SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF THIOCOLCHICOSIDE AND DICLOFENAC POTASSIUM B.P. IN COMBINED DOSAGE FORM BY RATIO DERIVATIVE AND DUAL WAVELENGTH METHOD

Choudhari V.P.<sup>1</sup>, Chabukswar A.R.<sup>1</sup>, Savakhande S.N.<sup>1</sup>,  
Tryambake M.U.<sup>1</sup>, Suryawanshi V.M.<sup>1</sup>, Sayal P.K.<sup>1</sup>

<sup>1</sup>Pharmaceutical Analysis and Quality Assurance Department, MAEER's  
Maharashtra Institute of Pharmacy, M.S., India

E-mail of Corresponding Author: mangesh.tryambake@gmail.com

### ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Diclofenac potassium (DICLO K) and Thiocholchiside (THIO) in combined dosage form has been developed. The first method is based on Ratio Derivative (Method A) and second method is based on Dual Wavelength (Method B). The amplitudes 268.78 nm and 355.62 nm wavelength were selected to determine THIO and DICLO K respectively, by method A. In method B, two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. At wavelengths 252.47 nm and 260.74 nm Thiocholchiside have equal absorbance therefore these two wavelengths were used to determine DICLO K and on similar basis 263.22 nm and 301.65 nm were selected to determine THIO in combined formulation. The drugs obey Beer's law in the concentration range of 12.5-75  $\mu\text{g mL}^{-1}$  for DICLO K and 2-12  $\mu\text{g mL}^{-1}$  for THIO by the method A and 25-75  $\mu\text{g mL}^{-1}$  for DICLO K and 4-12  $\mu\text{g mL}^{-1}$  for THIO by the method B. The % assay of DICLO K and THIO was found to be in the range 99.95 – 100.29 % by the proposed methods. Recovery was found in the range of 98.6 - 101.0 % for both analytes by both methods. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out by following ICH guidelines.

**Keywords:** Diclofenac potassium (DICLO K) Thiocholchiside. (THIO), Ratio Spectra Derivative Spectrophotometry, Dual wavelength Spectrophotometry.

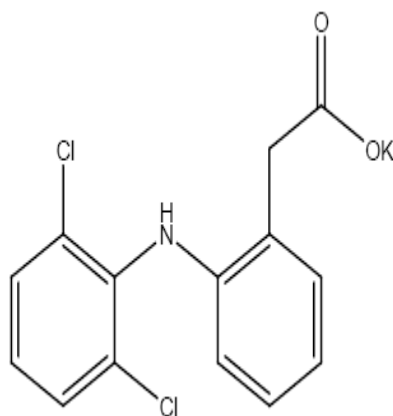
### INTRODUCTION

Diclofenac potassium, potassium [0-(2,6-dichloroanilino) phenyl] acetate, is non-steroidal anti-inflammatory agent.

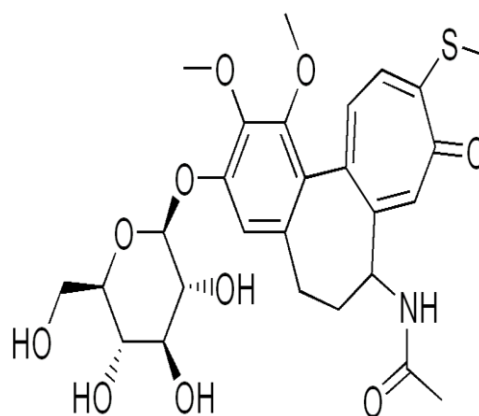
Diclofenac is officially in B.P. where as its potassium salt is official in European pharmacopeia. Other method reported for its estimation are by laser desorption ionisation mass spectra, thermal and fractional analysis, UV Spectrophotometry method, Direct rapid and sensitive HPLC and HPTLC.

Thiocolchicoside is (s)-N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1,2-dimethoxy-10-(methylthio) -9-oxobenzo[a]heptalen-7yl] acetamide (Fig.1). Thiocolchicoside is a synthetic sulphur derivative of colchicoside. Thiocolchicoside has a selective affinity for  $\gamma$ -amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA-nergic inhibitory pathways thereby acting as a potent muscle relaxant<sup>4</sup>. For THIO various analytical methods have been reported or its individual estimation and in combined dosage form which includes HPLC, electrophoresis, LC-MS, extractive

Spectrophotometry, visible Spectrophotometry, HPLC with electrochemical detection. Two methods have been reported for simultaneous analysis of DICLO K and THIO in its combination which includes TLC- Densitometry and second order derivative Spectrophotometry. Here an attempt has been made to develop simple, rapid and accurate Dual Wavelength and Ratio Derivative spectroscopic methods for simultaneous estimation of DICLO K and THIO from its formulation. The proposed methods are optimized and validated as per International Conference on Harmonization (ICH) guidelines.



**DICLOFENAC POTASSIUM**



**THI COLCHICOSIDE**

## MATERIALS AND METHODS

### Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used for Spectrophotometric method. All weighing were done on electronic balance (Model Shimadzu AUW-220D). Spectroscopic grade methanol was used

throughout the study. Ultrasonicator (Model 5.5 150H) was used for sample solution preparation.

### Reagents and chemicals

Pure drug sample of DICLO K and THIO were kindly supplied as a gift sample by Zest Pharma, Indore and Glenmark Pharmaceuticals, Sinner,

Nasik, respectively. These samples were used without further purification. Tablet formulation manufactured by Sun Pharmaceutical Industries (Lotensyl, Batch No. AD 92286) was purchased from local market containing DICLO K (50 mg) and THIO (10 mg) per tablet. Spectroscopy grade methanol purchased from Merck, Mumbai was used throughout the study.

### **Preparation of Standard Stock Solutions and calibration Curve**

Standard stock solutions each containing  $1000 \mu\text{g mL}^{-1}$  of DICLO K and THIO were prepared separately in the methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in methanol. For verification of Beer's law a series of dilutions in the concentration range of  $12.5\text{-}62.5 \mu\text{g mL}^{-1}$  for DICLO K and  $2\text{-}10 \mu\text{g mL}^{-1}$  for THIO were prepared in mixture for method A. Solutions in the concentration range of  $25\text{-}75 \mu\text{g mL}^{-1}$  for DICLO K (**series A**) and  $4\text{-}12 \mu\text{g mL}^{-1}$  for THIO (**series B**) were prepared and mixture of both the drugs (**series C**) in same concentration range was prepared for method B.

### **Preparation of Sample Stock Solution**

For formulation analysis, twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of DICLO K (8 mg of THIO) was weighed and dissolved in 40 mL of methanol with the aid of ultrasonicator for 10 min and solution was filtered through Whatman paper No. 41 into a 50 ml volumetric flask. Filter paper was

washed with methanol, adding washings to the volumetric flask and volume was made up to mark. The solution was suitably diluted with methanol to get of  $37.5 \mu\text{g/ml}$  of DICLO K and  $6 \mu\text{g/ml}$  of THIO. The proposed methods were followed for analysis

### **Procedure**

#### **Method A: RATIO DERIVATIVE METHOD**

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different DICLO K standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of THIO ( $6 \mu\text{g mL}^{-1}$ ) and the first derivative of these spectra traced, illustrated in Fig 1. Wavelength  $268.78 \text{ nm}$  was selected for the quantification of DICLO K in DICLO K + THIO mixture. The ratio and ratio derivative spectra of the solutions of THIO at different concentrations were obtained by dividing each with the stored standard spectrum of the DICLO K ( $37.5 \mu\text{g mL}^{-1}$ ) (Fig. 2). Wavelength  $355.62 \text{ nm}$  was selected for the quantification of THIO in THIO + DICLO K mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the

selected wavelength and concentration of the standard solutions. The amount of DICLO K ( $C_{\text{DICLO K}}$ ) and THIO ( $C_{\text{THIO}}$ ) in tablets and capsules was calculated by using equations 1 and 2, respectively.

$$C_{\text{DICLO K}} = [\text{Derivative amplitude at } 268.78 - (0.0094)] / (1.4434) \dots (1)$$

$$C_{\text{THIO}} = [\text{Derivative amplitude at } 355.62 - (-0.0137)] / (0.0792) \dots (2)$$

### Method B: DUAL WAVELENGTH METHOD

The spectrum of DICLO K shows identical absorbance at 263.22 nm ( $\lambda_3$ ) and 301.65 nm ( $\lambda_4$ ) therefore these two wavelengths were selected for the analysis of THIO. All the solutions of **series A** were scanned to ensure that the difference between  $\lambda_3$  and  $\lambda_4$  is zero. Similarly, the THIO solutions were scanned to determine the two wavelengths, where absorbance is same. These two wavelengths were found to be 252.47 nm ( $\lambda_1$ ) and 260.74 nm ( $\lambda_2$ ). All the solutions of **series B** were scanned to ensure that difference between ( $\lambda_1$ ) and ( $\lambda_2$ ) is zero. All the solutions of **series c** were scanned to verify the results.

### Recovery studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 37.5  $\mu\text{g/ml}$  of

DICLO K and 6  $\mu\text{g/ml}$  of THIO for both the methods.

### Precision of the Method

Reparability of the methods was studied by repeating the methods six times. To study intra-day precision, method was repeated 3 times in a day. Similarly the method was repeated on five different days to determine inter-day precision.

## RESULTS AND DISCUSSION

DICLO K and THIO the proposed methods for simultaneous estimation of in combined dosage form were found to be accurate, simple and rapid. The developed methods can be used for routine analysis of two drugs in combined dosage forms. Practically no interference from tablet excipients was observed in these methods. Both the methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical.

These methods were validated as per ICH guidelines. The values of % RSD and correlation of coefficient were satisfactory and result of the recovery study indicates that there is no interference due to excipients present in the formulation. Result of formulation analysis and precision study are summarized in table 1 indicated that method is precise. % Recovery (%RSD) for DICLO K by Method A and Method B was found to be in the range of 99.9-101.0(0.38-1.27) and 99.50-100.6 (0.25-1.42) respectively and that of THIO by Method A and Method B was found to be in the range of 98.6-100.2(0.82-1.56) and 99.5-100.1(1.10-1.50) (Table 2).

## CONCLUSION

The proposed methods are simple, precise, accurate and rapid for the determination of DICLO K and THIO in combined tablet dosage forms. Analysis of authentic samples containing DICLO K and THIO showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations. Thus these

methods can be easily and conveniently adopted for routine quality control analysis.

## ACKNOWLEDGEMENT

The authors are thankful to Zest Pharma, Indore and Glenmark Pharmaceuticals for providing gift samples. The authors are thankful to Management of MAEER's Maharashtra Institute of Pharmacy, Pune for providing necessary facility for the work.

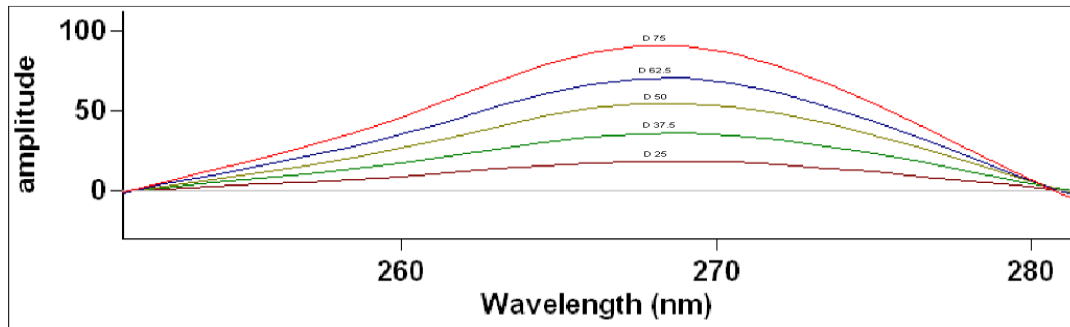


Fig 1. First derivative of the ratio spectra of DICLO K solution ( $25-75 \mu\text{g mL}^{-1}$ ) when  $8 \mu\text{g mL}^{-1}$  solution of THIO is used as divisor

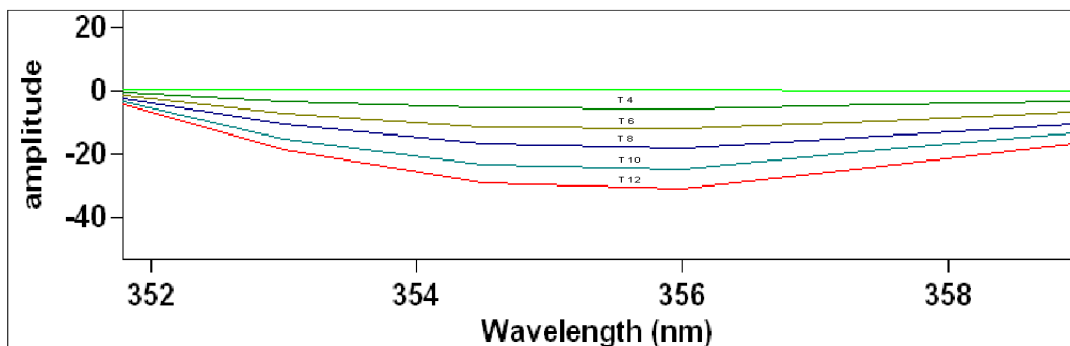


Fig 2. First derivative of the ratio spectra of THIO solution ( $4 - 12 \mu\text{g mL}^{-1}$ ) when  $50 \mu\text{g mL}^{-1}$  solution of DICLO K is used as divisor

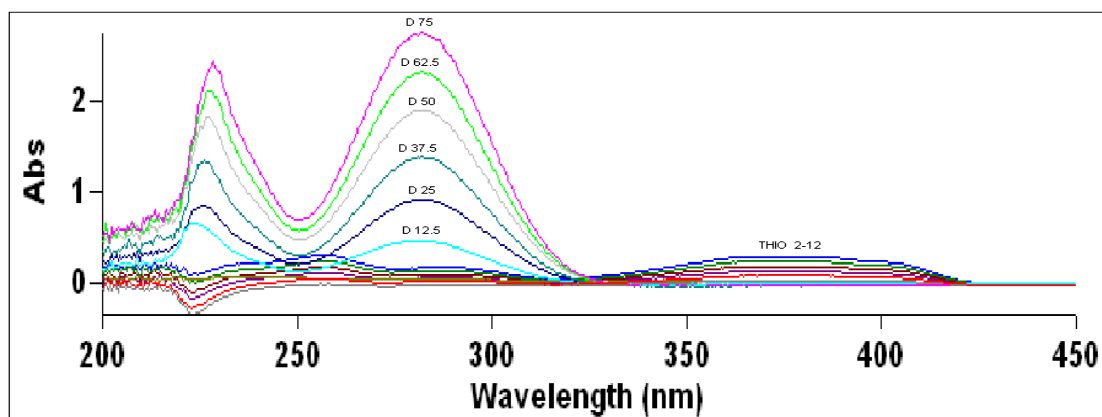


Fig 3. Overlain spectra of DICLO K and THIO in methanol

Table 1: Optical characteristics of the proposed methods and results of formulation analysis and precision study

Parameter		Diclofenac potassium		Thiocolchicoside	
		Method A	Method B	Method A	Method B
$\lambda$ (nm)		268.78	Difference in absorbance between 252.47 nm	355.62	Difference in absorbance between 263.22 nm
Beer's law limit ( $\mu\text{g mL}^{-1}$ )		12.5-62.5	25-75	2-10	4-12
Regression Equation	Slope (m)	1.4434	0.0065	0.07925	0.0147
	Intercept (c)	0.0094	0.0049	-0.0137	-0.365
Correlation coefficient		0.9999	0.99964	0.99964	0.99965
Precision (%R.S.D.)	Repeatability (n=5)	0.59	0.71	0.78	0.63
	Intra-day	0.58	0.69	0.56	0.94
	Inter-day	0.69	0.77	0.55	0.73
	Analyst	0.55	0.63	0.73	0.72
Formulation Analysis (% Assay, %RSD) n=6		99.98, 0.84	99.95, 1.01	100.29, 1.1	100.15, 1.24

RSD: Relative Standard Deviation

**Table 2: Result of Recovery studies**

Formulation	Recovery Level (%)	Amount spiked ( $\mu\text{g mL}^{-1}$ )		Recovery (%), % RSD n=3			
				Method A		Method B	
		DICLOK	THIO	DICLO K	THIO	DICLO K	THIO
Tablet	50	18.75	3	99.9, 0.58	98.6, 1.10	100.2, 0.68	99.5, 1.31
	100	37.5	6	100.0, 1.27	99.7, 1.56	100.6, 1.42	99.30, 1.50
	150	65.25	9	101.0, 0.38	100.2, 0.82	99.50, 0.25	100.1, 1.10

**REFERENCES**

1. A.H. Beckett, J.B. Stenlake, Practical pharmaceutical chemistry, the Athlone press of university of London, 3<sup>rd</sup> ed., part two, 1976, 249.
2. M. Carta \*, L. Murru, P. Botta, G. Talani, G. Pietro Sechi, P. De Riu, E. Sanna, G. Biggio, The muscle relaxant thicolchicoside is an antagonist of GABAA receptor function in the central nervous system, *Neuropharmacology* 51 (2006) 805-815.
3. W. Schneider, P.H. Degen, Simultaneous determination of diclofenac sodium and its hydroxy metabolites by capillary column gas chromatography with electron-capture detection, *Journal of Chromatography A*, Volume 217, 6 November 1981, Pages 263-271.
4. Gao, Songmei, Analysis of diclofenac potassium by laser desorption ionization mass spectrum.
5. The Merck index, an encyclopedia of chemicals, drugs & Biologicals, Merck research laboratories, 13<sup>th</sup> ed., 1997, 3108, 9397.
6. A. fini. et al, Thermal & fractional analysis of sodium & potassium salts of Diclofenac.
7. R. K. Prasad\* and R. Sharma, Simultaneous estimation and validation of Rabepazole Sodium and Diclofenac Sodium in capsule dosage form, *J. Chem. Pharm. Res.*, 2010, 2(2): 186-196.
8. Y.K. Agrawal\* and K. Shivramchandras, Spectrophotometric determination of diclofenac sodium in tablets, *Journal of Pharmaceutical & Biomedical Analysis*, Vol. 9, No. 2, 1991, pp. 97-100.
9. N.A. El-Ragehy \*, M.M. Ellaihy, M.A. El-Ghobashy, Determination of thicolchicoside in its binary mixtures (Thicolchicosideglafenine and Thicolchicoside-floctafenine) by

- TLC-densitometry, 58 (2003) 463-468.
10. Ming-Thau Sheu, Huei-Lan Chou, Ching-Cheng Kao, Cheng-Hsiung Liu, Theodore D. Sokoloski, Dissolution of diclofenac sodium from matrix tablets, *International Journal of Pharmaceutics, Volume 85, Issues 1-3, 20 September 1992, Pages 57-63*
  11. British Pharmacopoeia, London: The Majesty stationary office, 2004; I: 626.
  12. M.S. Bhatia, S.R. Dhaneshwar, Simple colorimetric detection of diclofenac sodium from tablets.
  13. M.Riegel\*, P.P. Ellis, High-performance liquid chromatographic assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *Journal of Chromatography B*, 654 (1994) 140-145.
  14. Julio c. Botello and guadalupe pi~rez-caballero\*, spectrophotometric determination of Diclofenac sodium with methylene blue, *Talanta*, Vol. 42, No. I, pp. 105-108, 1995.