

Research Article


SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF DICLOFENAC POTASSIUM B.P. AND THIOCOLCHICOSIDE IN COMBINED TABLET DOSAGE FORM BY ABSORPTION CORRECTED METHOD AND AREA UNDER CURVE METHOD

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ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Diclofenac potassium (DICLO K) and Thiocholchiside (THIO) in combined tablet dosage form has been developed. The first method is based on Absorption corrected and second method is Area Under Curve (AUC) Spectrophotometry. The first method was based on the absorption corrected method in which DICLO K and THIO exhibit λ_{max} at 264.99 nm and 373.84 nm, respectively in methanol. Quantitative estimation of THIO was carried out by subtracting the absorption due to DICLO K at 333.74 nm using experimentally calculated absorption factor and wavelength ranges of 252.56-260.59 nm and 278.51-285.53 nm were selected to determine DICLO K and THIO by AUC method respectively in combined formulations. Beer's law is obeyed in the concentration range of 25-75 $\mu\text{g mL}^{-1}$ for DICLO K and 4-12 $\mu\text{g mL}^{-1}$ for THIO for both the methods. The % assay for commercial formulation was found to be in the range 99.01 – 100.89 % for DICLO K and 98.91 – 101.72 % for THIO by the proposed methods. These methods were validated with respect to linearity, precision and accuracy as per ICH analytical method development guidelines. Recovery was found in the range of 98.16 – 100.02 % for DICLO K and 98.56 - 99.81 % for THIO by absorption corrected method and 98.56-99.87% for DICLO K and 99.68-100.21% for THIO by AUC method respectively.

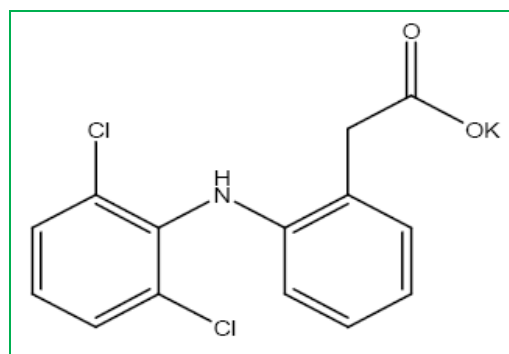
Keywords: Diclofenac potassium (DICLO K), Thiocholchiside (THIO), Absorption corrected Spectrophotometry, Area Under Curve, combined dosage form.

INTRODUCTION

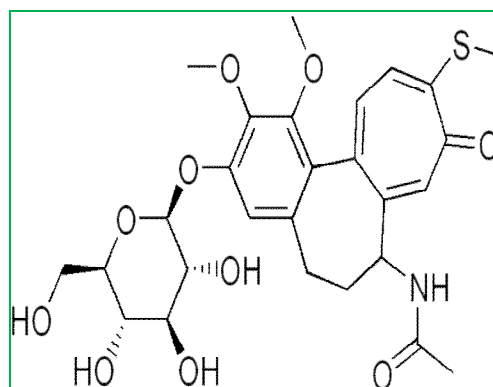
Diclofenac potassium, potassium [O-(2, 6-dichloroanilino) phenyl] acetate, is non-steroidal anti-inflammatory agent. ^[1] Diclofenac is officially in B.P. where as its potassium salt is official in European pharmacopeia. Other method reported for its estimation are by laser desorption ionisation mass spectra, ² thermal and fractional analysis, UV Spectrophotometry method, ^{4,10} Direct rapid colorimetric detection, ⁸ sensitive HPLC and HPTLC ⁹ and gas chromatography ¹⁰.

Thiocholchiside is (s)-N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio) -9-oxobenzo[a]heptalen-7yl] acetamide. Thiocholchiside is a synthetic sulphur derivative of colchicoside. Thiocholchiside has a selective affinity for γ -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 ¹³. 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 ¹¹ 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



THIOCOLCHICOSIDE



MATERIALS AND METHODS

Instrumentation

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

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. Spectroscopic grade methanol purchased from Merck, Mumbai was used throughout the study.

Theoretical aspects

Method A: Absorption Corrected Method

λ_{max} of DICLO K and THIO was determined by scanning the drug solution in methanol was found to be at 264.99 nm and 373.84 nm respectively. DICLO K also showed absorbance at 373.84 nm, while THIO did not show any interference at 264.99 nm. To construct Beer's plot for DICLO K and THIO dilutions were made in the solvent using stock solution of 1000 $\mu\text{g/ml}$. Also Beer's plot was constructed for DICLO K and THIO in solution mixture at different concentration (25:4, 37.5:6, 50:8, 62.5:10, 75:12 $\mu\text{g/ml}$) levels. Both the drugs followed linearity individually and in mixture within the concentration range 25-75 $\mu\text{g/ml}$ and 4-12 $\mu\text{g/ml}$ for DICLO K and THIO respectively (Fig.1).

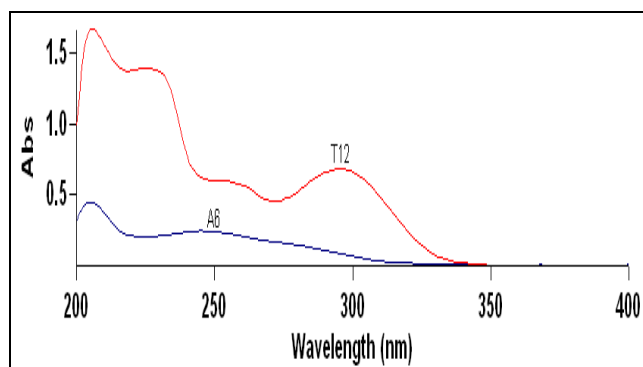


Figure 1: Overlain zero order absorbance spectra of DICLO K (6 $\mu\text{g/mL}$) and THIO (12 $\mu\text{g/mL}$)

Determination of Absorption Factor at Selected Wavelengths

DICLO K and THIO solution in methanol of known concentrations were scanned against blank on spectrophotometer. The value of absorption factor was found to be 1.152. Quantitative estimation of DICLO K and THIO was carried out using following equation:

Corrected Absorbance of THIO at 373.84nm = $abs_{333.74}(\text{DICLO K} + \text{THIO}) - [(abs_{373.84}(\text{DICLO K}) / abs_{264.99}(\text{DICLO K})) \times abs_{264.99}(\text{DICLO K})]$ or Corrected Absorbance of THIO at 373.84nm = $abs_{373.84}(\text{DICLO K} + \text{THIO}) - 1.152 \times abs_{264.99}(\text{DICLO K})$

Where; abs: Absorption value at given wavelengths.

Method B: Area under Curve

For the simultaneous determination using the area under the curve method, suitable dilutions of the standard stock solutions (1000 $\mu\text{g/ml}$) of DICLO K and THIO were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-450 nm. For Area Under Curve method, the sampling wavelength ranges selected for estimation of DICLO K and THIO are 252.56-260.59 nm (λ_1 - λ_2) and 278.51-285.53 nm (λ_3 - λ_4). Mixed standard were prepared and their Area under the Curve were measured at the selected wavelength ranges. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and (2).

$$A1 = 2253.33 C_{\text{ATO}} + 3031.25 C_{\text{THIO}} \dots \dots \dots (1) \text{ at } 243\text{-}248\text{nm.}$$

$$A2 = 1502.33 C_{\text{ATO}} + 5566.58 C_{\text{THIO}} \dots \dots \dots (2) \text{ at } 291.08\text{-}299.86 \text{ nm.}$$

Where,

2253.33 and 1502.33 are absorptivities of DICLO K at (λ_1 - λ_2) and (λ_3 - λ_4) respectively.

3031.25 and 5566.58 are absorptivities of THIO at (λ_1 - λ_2) and (λ_3 - λ_4) respectively.

A1 and A2 are absorbances of mixed standard at (λ_1 - λ_2) and (λ_3 - λ_4) respectively. C_{DICLOK} and C_{THIO} are the concentrations in $\text{g}/100\text{mL}$.

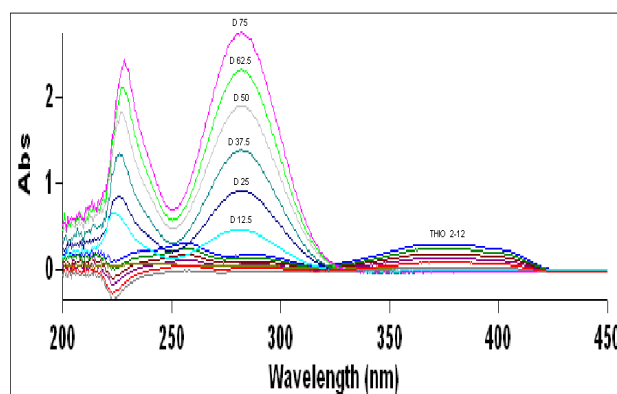


Figure 2: Overlay spectrum of THIO and DICLO K

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solutions of pure drug containing 1000 $\mu\text{g mL}^{-1}$ of DICLO K and THIO were prepared separately in methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in methanol. Derivative amplitudes of spectrum, by using the above mentioned procedure, were

used to prepare calibration curves for both the drugs. Beer's law obeyed in the concentration range of 25-75 $\mu\text{g mL}^{-1}$ for DICLO K and 4-12 $\mu\text{g mL}^{-1}$ for THIO for ratio derivative and AUC spectroscopic methods respectively.

Preparation of Sample Stock Solution and 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 the 40 mL of methanol with the aid of ultrasonication for 15 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 $\mu\text{g mL}^{-1}$ of DICLO K and 6 $\mu\text{g mL}^{-1}$ of THIO

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

The proposed methods for simultaneous estimation of DICLO K and THIO in combined dosage form were found to be accurate, simple and rapid. Since none of the method is reported for simultaneous analysis of the two drugs earlier, the developed methods can be used for routine analysis of two drugs in combined dosage forms.

Method A Practically no interference from tablet excipients was observed in these methods. As their λ_{max} differ more than 20 nm, absorption corrected method was tried for their simultaneous estimation in formulation. Quantitative estimation of THIO was carried out by subtracting interference of DICLO K using experimentally calculated absorption factor.

Method B involves formation and solving of simultaneous equation. Once the equations are formed, then only measurement of the area of sample solution at two wavelength ranges and simple calculations are required.

Table 1: Optical characteristics of the proposed methods

Parameter		Diclofenac potassium		Thiocolchicoside			
		Method A	Method B	Method A	Method B		
λ (nm)		264.99	252.56-260.59	373.84	278.51- 285.53		
Beer's law limit ($\mu\text{g mL}^{-1}$)		25 – 75	4 - 12	25-75	4 – 12		
Regression Equation ($y = mx + c$)	Slope (m)	0.02204	-	0.02646	-		
	Intercept (c)	-0.03232	-	-0.0267	-		
Correlation coefficient		0.9993	-	0.9998	-		
Precision (%R.S.D.)	Repeatability (n=5)	0.68	0.76	0.59	0.82		
	Intra-day (3x3)	1.10	0.78	0.67	1.40		
	Inter-day(3x3)	1.24	0.95	0.87	0.83		
Formulation Analysis (% Assay, %RSD) n=6		Formulation - I	99.01, 0.89	98.91, 1.1	100.89, 0.56	101.72, 1.04	
		Formulation - II	100.11, 0.63	99.24, 0.93	99.84, 0.75	101.5, 0.62	
Result of recovery study	Level	Recovery of		Amount ($\mu\text{g/mL}$)		% Mean Recovery (n=3)	% R.S.D.
				Spiked	Recovered		
	80%	DICLO K	Formulation I	2.4	2.400	100.02	0.52
			Formulation II	2.4	2.379	99.12	1.2
		THIO	Formulation I	4.8	4.731	98.56	0.05
			Formulation II	4.8	4.765	99.27	0.95
	100%	DICLO K	Formulation I	3	2.945	98.16	1.92
			Formulation II	3	2.972	99.06	1.5
		THIO	Formulation I	6	5.968	99.46	0.79
			Formulation II	6	6.012	100.2	0.97
	120%	DICLO K	Formulation I	3.6	3.581	99.47	1.03
			Formulation II	3.6	3.579	99.41	1.57
THIO		Formulation I	7.2	7.187	99.81	0.19	
		Formulation II	7.2	7.166	99.52	1.34	

R.S.D. is relative standard deviation



A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table 1. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision. Beer's law obeyed in the concentration range 25-75 $\mu\text{g mL}^{-1}$ for DICLO K and 4-12 $\mu\text{g mL}^{-1}$ for THIO with correlation coefficient of > 0.999 for both the drugs. For DICLO K, the recovery study results ranged from 98.16 – 100.02 % and 98.56 - 99.81 for THIO by Absorption corrected method and 98.56-99.87% for DICLO K and 99.68-100.21% for THIO by AUC method respectively. Results of recovery studies are also shown in Table 1. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low.

CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. Thus, it can be used as IPQC test and for routine simultaneous determination of DICLO K and THIO in tablet dosage form.

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