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Method Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Diclofenac Sodium and Thiocolchicoside from its Pharmaceutical Formulation

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ABSTRACT

The objective of this study was to evaluate the utility of derivative spectrophotometric method for analysis of Diclofenac sodium (DIC) and Thiocolchicoside (THIO) in combination. Derivative spectrophotometry has allowed specific determination of Diclofenac sodium at 249 nm with negligible contribution by Thiocolchicoside. Similarly, Thiocolchicoside was determined at 246nm with negligible interference by Diclofenac sodium. The described method obeyed Beer's law in the range of and 4-36µg/ml for DIC 2-10µg/ml for THIO. Validation parameters such as linearity, accuracy, precision, specificity, and LOD and LOQ values were performed. The percentage recovery was 99.52% for DIC and 99.32% for THIO. Intra and Interday precision %RSD values were <2. Thus from the results obtained it can be concluded that proposed method is simple, rapid and specific.

INTRODUCTION

Diclofenac sodium (DIC) [Sodium (o-{(2, 6-dichloro phenyl) 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 (Bucci R *et al*, 1998).

Thiocolchicoside (THIO) chemically, (s) -N- [3-(B-D glucopyranoxyloxy) -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 (Wikipedia.org, DOA 16/05/2013). Literature survey reveals spectrophotometric (Ciapina *et al*, 2005).

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and PO Fernandez de Cordova ML *et al*, 1998) and HPTLC (Dighe VV *et al*, 2006 and Shah HJ, 2003) determination of DIC in combination with other drugs. HPLC (Vora A *et al*, 2007) and bioanalytical chromatographic methods (Kulhmann O *et al*, 1998) for quantification of DIC are also reported.

For simultaneous determination of THIO with other drugs spectrophotometric (Lu Q *et al*, 2006), HPTLC (El-Ragehy NA *et al*, 2003) and HPLC methods (Rosso A *et al*, 1998 and Forni G *et al*, 1977) are reported. No reports were found for determination of DIC and THIO by second order derivative spectrophotometric 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.

MATERIALS AND METHODS

Instrument

Shimadzu UV 1800 spectrophotometer, Kyoto, Japan with cuvette of 1 cm path length. Weighing balance, Denver SI234, Germany was used throughout the study.

Reagents and chemicals

DIC and THIO bulk powder was purchased from Lincoln pharmaceutical Ltd, Ahmedabad Gujarat, India. The commercial fixed dose combination product **DYNAPAR MR 8**(DIC – 50 mg, THIO – 8 mg) was procured from the local market which is manufactured by Troikaa Pharmaceuticals Ltd. All chemicals and solvents were of analytical grade and were purchased from Thermo fisher scientific Pvt. Ltd, Mumbai- India.

Preparation of standard stock solution

Standard stock solutions of pure drug containing 100µg/mL of DIC and THIO were prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analyte in the concentration range of 4-36µg/ml for DIC 2-10µg/ml for THIO, respectively and scanned in the range of 200-400nm.

Calibration curve and wavelength selection

Diclofenac sodium and Thiocolchicoside mixtures were initially scanned to determine the zero-crossing absorption bands. Calibration graphs were constructed in the concentration range of 4-36µg/ml for Diclofenac sodium and 2-10µg/ml of Thiocolchicoside against methanol as blank. The values of the second derivative amplitudes at 249nm (zero-crossing of THIO) were measured for the determination of DIC in presence of THIO and the second derivative amplitude values, at 226nm (zero-crossing for THIO) were used for the determination of DIC in presence of THIO.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50mg of DIC and 8mg of THIO was weighed and dissolved in methanol with the aid of ultra-sonication for 10min and solution was filtered through Whatman paper No. 41 into a 100mL volumetric flask. Filter paper was washed with solvent, adding washings to the volumetric flask volume were made up to the mark with methanol. The solution was suitably diluted with methanol to get 50µg/mL of DIC and 8µg/mL of THIO.

The concentrations of both DIC and THIO were determined by measuring the absorbance of derivative spectra at 249nm and 226nm for second order derivative spectroscopic method.

Method Validation

Methods were validated according to ICH guidelines.

Linearity

The linearity of method was evaluated by analysing different concentrations of the standard solutions of DIC and THIO. The Beer-Lambert's concentration range was found to be 4-36µg/ml for DIC and 2-10µg/ml for THIO for Derivative Spectrophotometric method. (FIGURE-1, FIGURE-2)

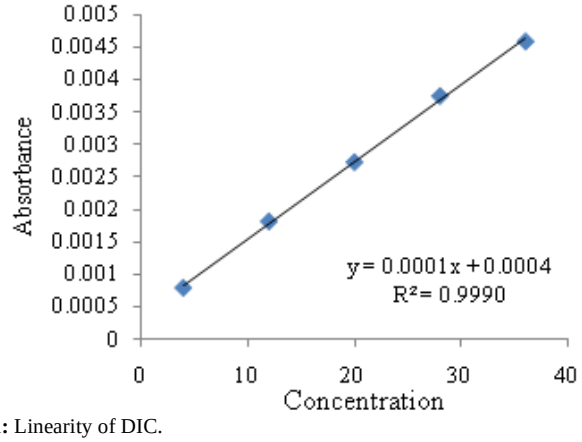

Fig. 1: Linearity of DIC.

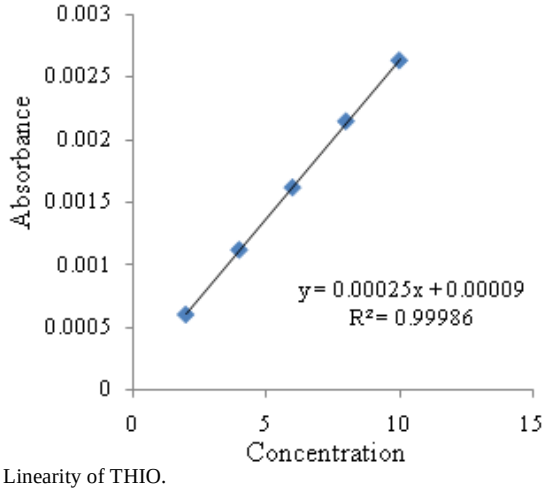

Fig. 2: Linearity of THIO.

Accuracy

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 100% & 150%). Percentage recovery for DIC and THIO was found in the range of 99.02 -99.94 % to 98.46 -99.86% respectively.(TABLE-1)

Precision

The reproducibility of the proposed method was determined by performing tablet assay at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision) and was expressed in % RSD. % RSD for Inter-day precision was found to be 1.04 –1.81% (for DIC) and 1.77 - 1.88% (for THIO).

Intraday precision was found to be 0.76-1.53% (for DIC) and 1.01-1.39% (for THIO) in second order derivative method. (TABLE-2)

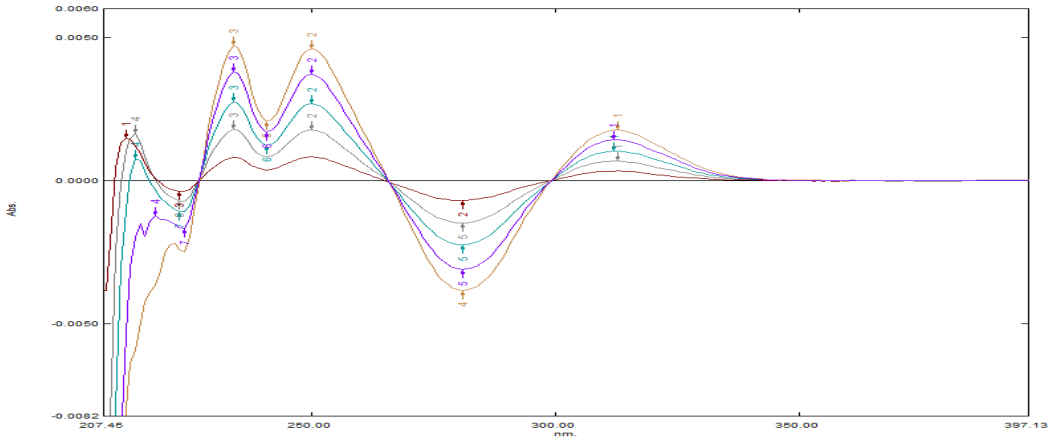

Fig. 3: second order derivative spectra of DIC.

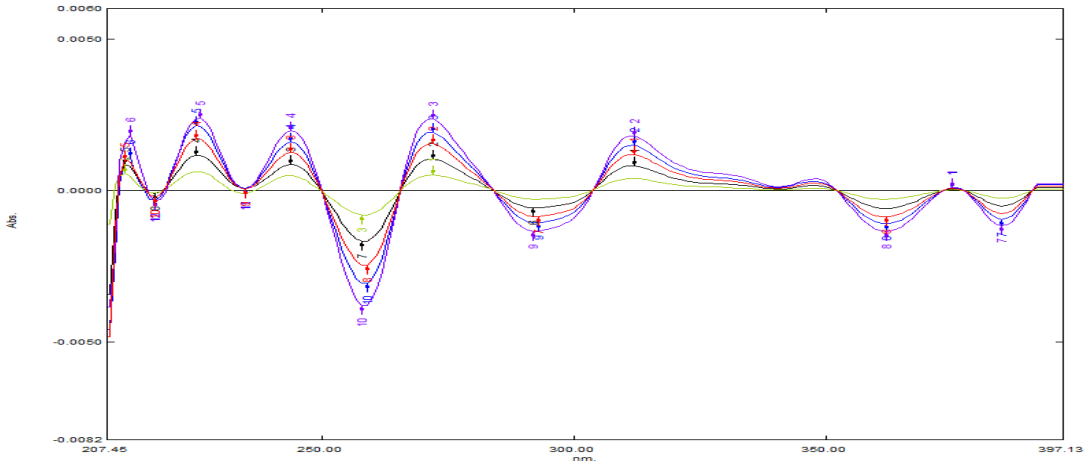

Fig. 4: Second order derivative spectra of THIO.

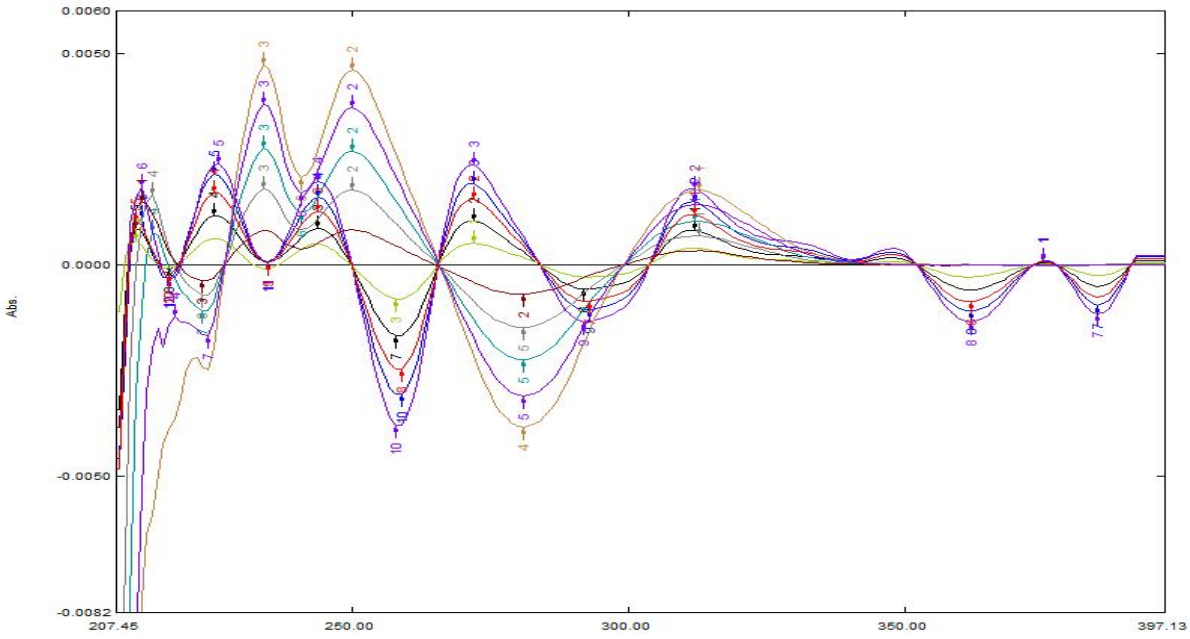

Fig. 5: Zero cross point spectra of DIC and THIO.

Table. 1: Recovery study for DIC and THIO n=3.

Name of Drug	Level	Amount of drug in powder (mg)	Amount of drug in powder (µg/ml)	Amount of Standard Spiked (µg/ml)	Actual amount (µg/ml)	Average of Amount Recoverd (Mean) (µg/ml)	Recovery (%) (mean ± S.D)	% RSD
	50	50	12.5	25	6.25	18.56	99.02±1.11	1.121
DIC	100	50	12.5	50	12.5	24.9	99.60±1.05	1.062
	150	50	12.5	75	18.7	31.23	99.94±1.57	1.579
	50	8	2	4	1	2.95	98.46±1.60	1.632
THIO	100	8	2	8	2	3.98	99.66±1.52	1.532
	150	8	2	12	3	4.99	99.86±1.28	1.287

Table. 2: Precision of DIC and THIO n=3.

Precision	SD		%RSD	
	DIC	THIO	DIC	THIO
Inter-day	0.0003	0.0003	1.45	1.84
Intraday	0.0003	0.0002	1.25	1.18

Table. 3: Validation Parameter.

Summary of Validation Parameters		
Parameters	DIC	THIO
Recovery (%)	99.52	99.32
Repeatability (%RSD)	1.25	1.48
	Precision (%RSD)	
Intra-day (n=3)	1.25	1.18
Inter-day (n=3)	1.45	1.84
LOD	0.90	1.48
LOQ	0.30	0.92

RESULTS AND DISCUSSION

The methods were successfully validated with respect to linearity, accuracy, intra and inter day precision etc. In Derivative spectroscopic method, linearity for detector response was observed in the concentration range of 4-36µg/ml for DIC and 2-10µg/ml for THIO. (FIGURE-1, FIGURE-2)

As shown in Table-2 %RSD values during both intra and inter day precision studies were <2.0% demonstrating repeatability and intermediate precision. Apparently the mean percentage recovery values for DIC and THIO by derivative spectroscopic method were 99.52% and 99.32% respectively. The proposed spectroscopic methods allow for accurate, precise and reliable measurement of DIC and THIO simultaneously in combined dosage form. The developed methods were found to be simple, rapid, linear, accurate and precise for the concurrent estimation of drugs in respective two-component oral dosage forms of DIC and THIO.

The methods were evaluated for validation parameters, linear relation including accuracy, reproducibility and precision. The RSD for all parameters and assay results obtained by this method are in fair agreement. The developed methods can be used for routine quantitative simultaneous estimation of DIC and THIO in pharmaceutical preparations.

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