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“Spectrophotometric Estimation of Chlorzoxazone and Diclofenac Sodium in Synthetic Mixture by First Order Derivative Spectrophotometry”.

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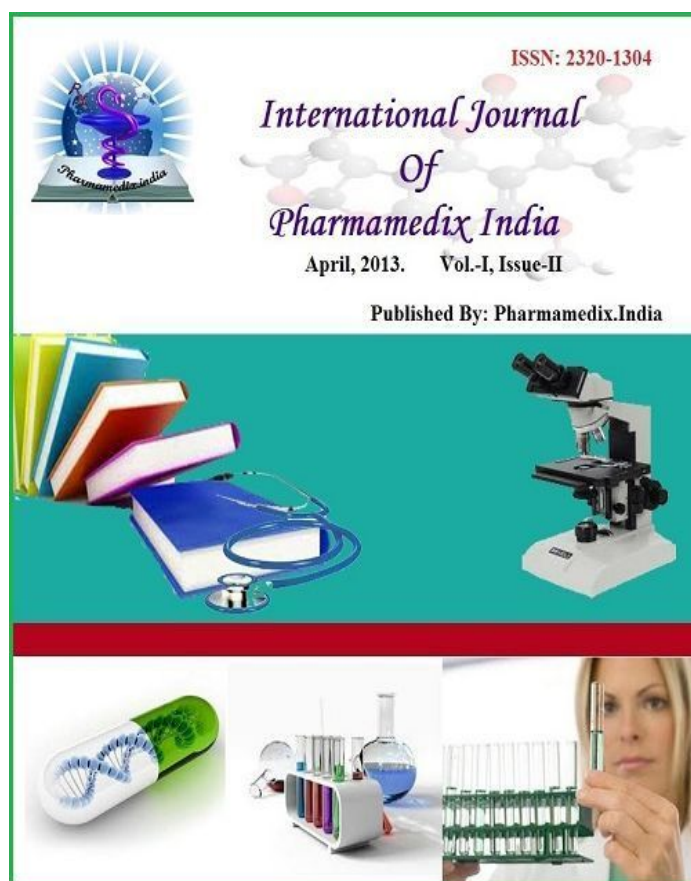
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Abstract:

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical first order derivative spectrophotometry method for the simultaneous determination of Chlorzoxazone and Diclofenac Sodium in synthetic mixture. The absorbance values at 276 nm and 288 nm of first derivative spectrum was used for the estimation of Chlorzoxazone and Diclofenac Sodium, respectively without mutual interference. This method obeyed beer's law in the concentration range of 2-24 µg/ml for both Chlorzoxazone and Diclofenac Sodium, respectively. The method was successfully applied to laboratory prepared mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of Chlorzoxazone and Diclofenac Sodium was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Chlorzoxazone and Diclofenac Sodium in mixture. The results of analysis have been validated statistically and by recovery studies.

Keywords: Chlorzoxazone, Diclofenac sodium, First order Derivative spectrophotometry, Zero-crossing point.

Introduction:

Chlorzoxazone (CLR) is chemically 5-chloro-2, 3-dihydro-1, 3 -benzoxazol-2-one (Figure 1) is a well known muscle relaxant drug ^[1]. It is official in United States Pharmacopoeia (USP). USP^[2] describe spectrophotometric method for its estimation. Literature survey reveals HPLC^[3] and UV^[4] method for estimation of Chlorzoxazone alone. Literature survey also reveals HPLC ^[5-8], HPTLC ^[9] and Spectrophotometric^[10-12] method for estimation of chlorzoxazone with other drug combination. Diclofenac sodium (DIC) is

chemically 2-[2,6dichlorophenylamino] benzene acetic acid sodium salt^[13] (Figure 2). Diclofenac sodium (DIC) is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP^[14] and BP^[15] describe liquid chromatography method for its estimation. Literature survey reveals HPLC ^[16] and UV ^[17] methods for determination of DIC in single dosage form. Literature survey also reveals HPLC ^[18-19] and HPTLC^[20] method for the determination of DIC with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is

available for the first order derivative estimation of CLR and DIC in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for first order derivative estimation of CLR and DIC in synthetic mixture or dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on first order derivative estimation for simultaneous estimation of both drugs in their combined synthetic mixture.

MATERIALS AND METHODS:

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and materials

Chlorzoxazone (CLR) and Diclofenac sodium (DIC) bulk powder was kindly gifted by Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. AR Grade NaOH (S.D. Fine Chemical Ltd., Mumbai, India.). Whatman filter paper no. 41 (Whatman International Ltd., England).

Preparation of standard stock solutions

An accurately weighed standard CLR and DIC powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in 0.1 N NaOH. The flasks were shaken and volumes were made up to mark with 0.1 N NaOH to give a solution containing 100 µg/ml of each CLR and DIC.

Determination of the zero crossing points

The standard solutions of CLR (12 µg/ml) and DIC (12 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. It appeared that CLR showed zero crossing at 288 nm and 244.2 nm while DIC showed zero crossing at 276 nm and 248.2 nm. At 288 nm CLR showed zero absorbance and DIC showed reasonable absorbance, while at 276 nm DIC showed zero absorbance and CLR showed reasonable absorbance so these two wavelengths were selected for further measurement.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines^[21].

Linearity (calibration curve)

Aliquots of Standard solution of CLR (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml) and DIC (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml) were transferred in a series of 10 ml volumetric flask. The

volume was adjusted to the mark with 0.1 N NaOH and mixed. The absorbances of derivatised spectra were measured at 276 nm (zero crossing point for DIC) and 288 nm (zero crossing point for CLR) against 0.1 N NaOH as blank. The linearity was observed in the concentration range of 2-24 µg/ml and 2-24 µg/ml respectively for CLR and DIC.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ($n = 6$) for CLR and DIC (16 µg/ml for both drugs) without changing the parameter of the proposed first order derivative method. The results are reported in terms of relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CLR and DIC (4, 8, 12 µg/ml for CLR and 4, 8, 12 µg/ml for DIC). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of CLR and DIC by the standard addition method. Known amounts of standard solutions of CLR and

DIC were added at 50, 100 and 150 % level to prequantified sample solutions of CLR (20µg/ml) and DIC (2 µg/ml). The solutions were measured at 276 nm for CLR and 288 nm for DIC and % recovery of the sample were calculated.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines ^[21].

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of synthetic mixture

Synthetic mixture of CLR and DIC were prepared in laboratory. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml 0.1 N NaOH and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with 0.1 N NaOH. This solution (0.4 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with 0.1 N

NaOH to get a final concentration of CLR (20 µg/ml) and DIC (2 µg/ml) and their first derivative spectra were recorded. From the derivative spectra, the absorbance at 276 nm and 288 nm were noted against 0.1 N NaOH as blank for the estimation of CLR and DIC, respectively. From these absorbance values, the concentrations of CLR and DIC were determined using calibration graph.

RESULTS AND DISCUSSION:

The working standard solution of CLR (12 µg/ml) and DIC (12 µg/ml) were prepared separately in 0.1 N NaOH. They were scanned in the wavelength range of 200-400 nm. The overlain spectra of CLR and DIC were shown in the figure 3. From the overlay derivatised spectra of two drugs, it is evident that CLR and DIC show a zero crossing point at 288 nm and 276 nm. These two wavelengths were employed for the determination of CLR and DIC. Overlain derivatised spectra of both the drugs are shown in Figure 4. Linear correlation was obtained between absorbance and concentrations of CLR and DIC in the concentration ranges of 2-24 µg/ml for both the drugs. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of CLR were found to be 2.0 % at 276 nm, respectively. The RSD value of DIC was found to be 1.99 % at 288 nm, respectively.

Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low % RSD values of interday (0.39 - 2.05 and 0.76 - 1.99 for CLR at 276 and 288 nm and intraday (0.26 - 0.81 and 0.77 - 1.43 for CLR at 276 and 288 nm. Low % RSD values for CLR and DIC, reveal that the proposed method is precise. LOD and LOQ values for CLR were found to be 0.48 and 1.31 µg/ml and at 276 nm, respectively. LOD and LOQ values for DIC were found to be 0.54 and 1.63 µg/ml at 288 nm, respectively. These data show that method is sensitive for the determination of CLR and DIC. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 101.7 ± 1.17 and 100.8 ± 1.14 for CLR and DIC, respectively (Table 2). The results of recovery studies in DIC state that the proposed method is highly accurate. The proposed validated method was successfully applied to determine CLR and DIC in their combined dosage form. The results obtained for CLR and DIC were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of CLR and DIC in pharmaceutical dosage forms.

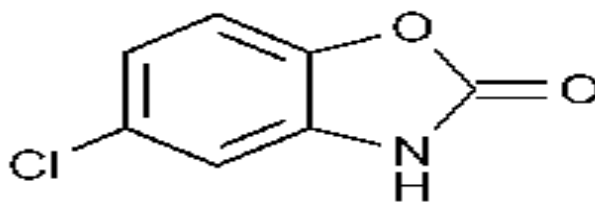


Figure 1: Chemical structure of Chlorzoxazone (CLR)

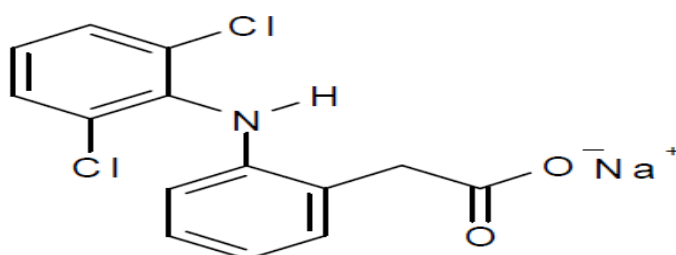


Figure 2: Chemical structure of diclofenac Sodium (DIC)

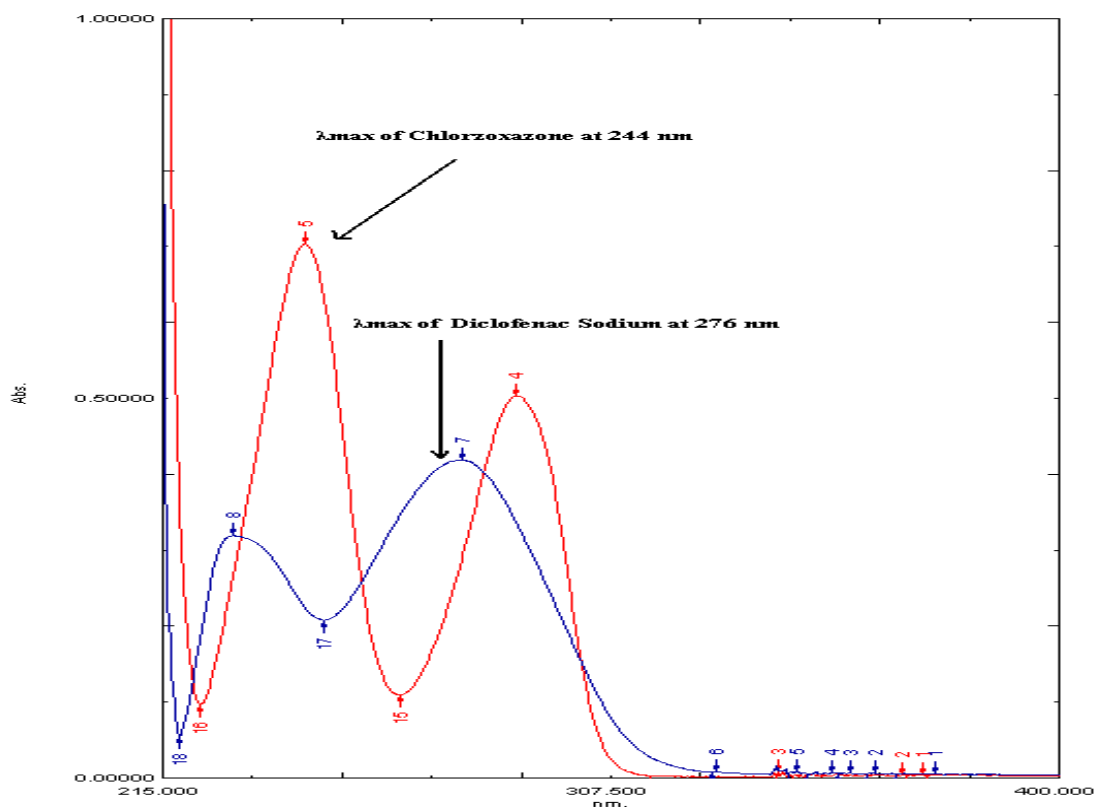


Figure 3: Overlain zero order absorption spectra of CLR and DIC in 0.1 N NaOH.

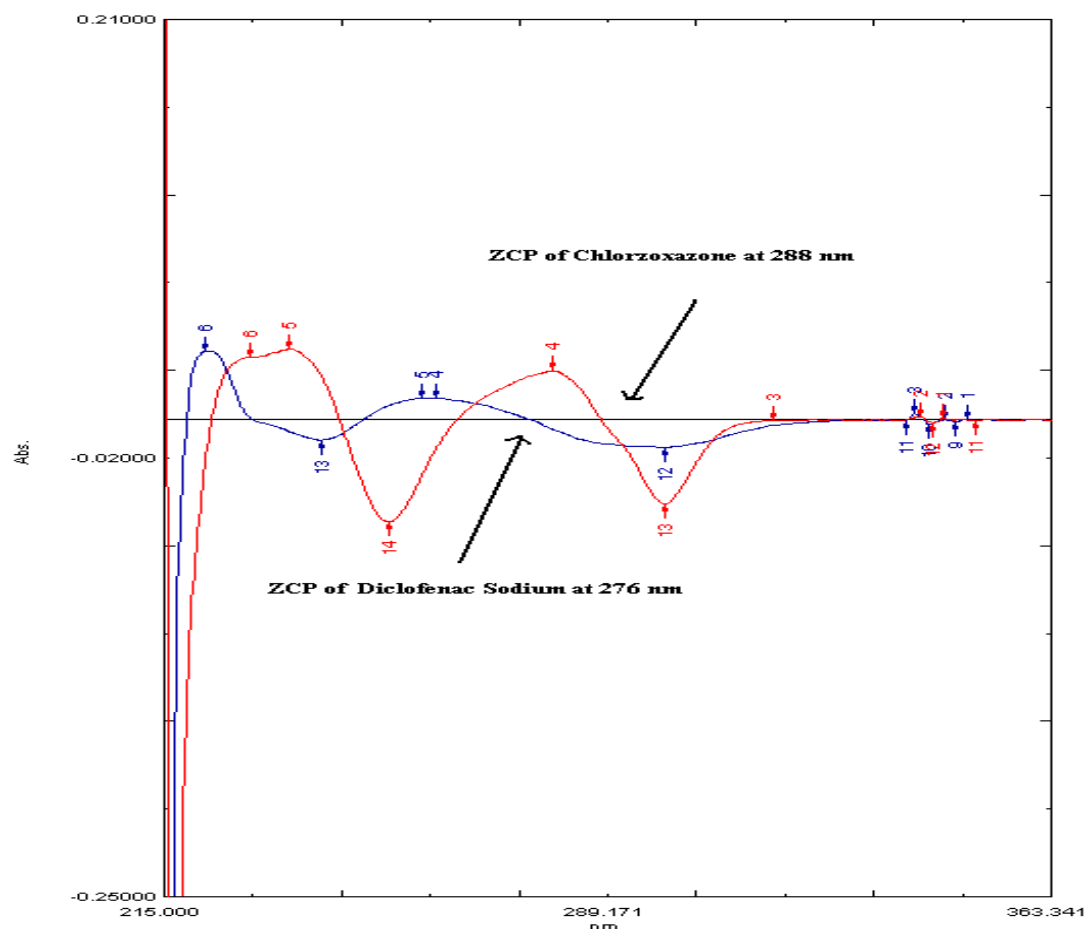


Fig. 4: Overlain first derivative absorption spectra of CLR and DIC in 0.1 N NaOH.

Table 1: Regression analysis data and summary of validation parameters for the first Derivative Spectrophotometric method.

PARAMETERS	CLR	DIC
Wavelength range (nm)	276	288
Beer's law limit ($\mu\text{g/ml}$)	2 - 24	2 - 24
Regression equation ($y = a + bc$)	$y = 0.0018x + 0.0004$	$y = 0.0009x + 0.0006$
Slope (b)	0.0018	0.0009

Intercept (a)		0.0004	0.0006
Correlation Coefficient (r^2)		0.9998	0.9992
Accuracy (Recovery) (n = 3)	Level I	101.9 $\pm$ 1.69	101.1 $\pm$ 1.11
	Level II	101.5 $\pm$ 0.69	100.7 $\pm$ 1.39
	Level III	101.7 $\pm$ 1.15	100.6 $\pm$ 0.93
Method precision (Repeatability) (% RSD, n = 6),		0.18	1.25
Interday (n = 3) (% RSD ^a)		0.39 - 2.05	0.76 - 1.99
Intraday(n = 3) (% RSD)		0.26 - 0.81	0.77 - 1.43
LOD ($\mu\text{g/ml}$)		0.48	0.54
LOQ ($\mu\text{g/ml}$)		1.31	1.63
Assay $\pm$ S. D. (n = 3)		100.2 $\pm$ 1.12	100.8 $\pm$ 1.69

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. S.D. is standard deviation.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken ($\mu\text{g/ml}$)	Amount added (%)	% Mean recovery $\pm$ S.D. (n = 3)
CLR	I	20	50	101.9 $\pm$ 1.69
	II	20	100	101.5 $\pm$ 0.69
	III	20	150	101.7 $\pm$ 1.15
DIC	I	2	50	101.1 $\pm$ 1.11
	II	2	100	100.7 $\pm$ 1.39
	III	2	150	100.6 $\pm$ 0.93

Table 3: Analysis of CLR and DIC in Synthetic mixture by Derivative Spectrophotometric method (n=6).

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim (mg) (n = 6)	
	CLR	DIC	CLR	DIC	CLR	DIC
1	50	5	50.02	4.94	100.0	98.88
2	50	5	49.30	5.02	98.61	100.5
3	50	5	50.13	5.13	100.3	102.7
4	50	5	50.40	4.97	100.8	99.44
5	50	5	50.82	5.11	101.6	102.2
6	50	5	49.85	5.02	99.72	100.5
MEAN			50.07	5.027	100.2	100.8
SD					1.128	1.694

CONCLUSION:

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of CLR and DIC in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of CLR and DIC hence the method was also economic for estimation of CLR and DIC from synthetic mixture. The common excipients and additives are usually present in the synthetic mixture do not interfere in the analysis of CLR and DIC in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture or combined pharmaceutical formulation.

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