Spectrophotometric Estimation Of Chlorzoxazone And Diclofenac Sodium In Synthetic Mixture By Q-Absorbance Ratio Method

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Abstract: The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Chlorzoxazone and diclofenac sodium in bulk and synthetic mixture. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the -max of one of the two components. Chlorzoxazone and diclofenac sodium show an isoabsorptive point at 281 nm in 0.1 N NaOH. The second wavelength used is 244 nm, which is the -max of Chlorzoxazone in 0.1 N NaOH. The linearity was obtained in the concentration range of 4-22 µg/ml for both Chlorzoxazone and diclofenac sodium. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the -max of chlorzoxazone. The method was successfully applied to pharmaceutical dosage form because no interference from the synthetic 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 synthetic mixture or pharmaceutical dosage form. The results of analysis have been validated statistically and by recovery studies.

KeyWords: Chlorzoxazone, Diclofenac sodium, Recovery, Q-Absorbance ratio method, Isoabsorptive point, Validation.

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 UV[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 IP and BP. IP[14] and BP[15] describes 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 simultaneous estimation of CLR and DIC in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for

simultaneous 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 absorbance ratio method (Q-analysis) for simultaneous estimation of both drugs in bulk and combined synthetic mixture.
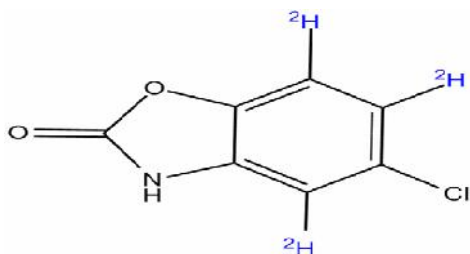
Figure 1: Chemical structure of Chlorzoxazone (CLR)
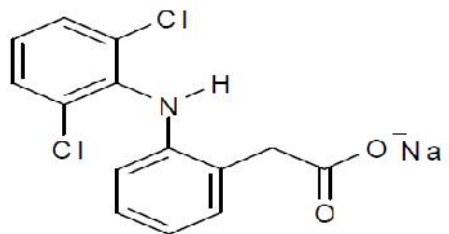
Figure 2: Chemical structure of Diclofenac Sodium (DIC)

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

DIC and CLR bulk powder was kindly gifted by Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India, 0.1 N NaOH (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatmann filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of standard CLR (10 mg) and DIC (10 mg) powder 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 each of CLR and DIC.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the -max of one of the two components. From the overlay spectra of two drugs, it is evident that CLR and DIC show an isoabsorptive point at 281 nm. The second wavelength used is 244 nm, which is the -max of CLR. Ten working standard solutions having concentration 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 μg/ml for CLR and 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 μg/ml for DIC were prepared in 0.1 N NaOH and the absorbances at 281 nm (isoabsorptive point) and 244 nm (-max of CLR) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{..} (1)$$

$$C_Y = (A_1/aX_1) - C_X \quad \text{.....................................} (2)$$

Where, A_1 and A_2 are absorbances of mixture at 281 nm and 244 nm; aX_1 and aY_1 are absorptivities of CLR and DIC at 281 nm; aX_2 and aY_2 are absorptivities of CLR and DIC respectively at 244 nm; $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$

Validation of the proposed method

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

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 4-22 µg/ml for CLR and 4-22 µg/ml for DIC. Accurately measured standard solutions of CLR (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 ml) and DIC (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with 0.1 N NaOH. The absorbances of the solutions were measured at 281 and 244 nm against 0.1 N NaOH as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

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 (10 µg/ml for both drugs) without changing the parameter of the proposed Spectrophotometry method.

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 (8, 10, 12 µg/ml for CLR and 8, 10, 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 and DIC (20µg/ml CLR and 2µg/ml DIC). The amounts of CLR and DIC were estimated by applying putting value in equation no.1 and 2. The experiment was repeated for three times.

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[18].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

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

Analysis of synthetic mixture

Chlorzoxazone (50 mg) and diclofenac (5 mg) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like starch, lactose, magnesium stearate and talc. 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). The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 281 nm (isoabsorptive point) and 244 nm (λ-max of CLR) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1. Relative concentration of two drugs in the sample was calculated using above equation (1) and (2). The analysis procedure was repeated six times with synthetic mixture.

RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 281 nm (isoabsorptive point) and 244 nm (-max of CLR) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CLR (244 nm) and DIC (276 nm) showing isoabsorptive point (281 nm) in 0.1 N NaOH is shown in **Figure 3.**
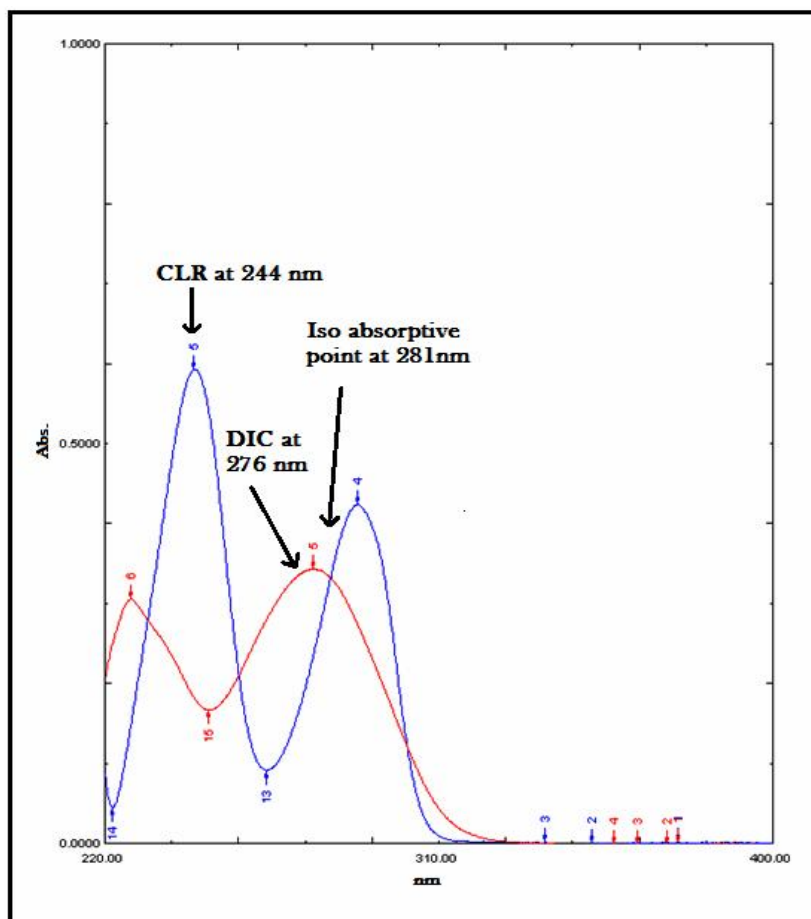
Figure 3: Overlain absorption spectra of CLR (244 nm) and DIC (276 nm) showing isoabsorptive point (281nm) in 0.1 N NaOH

Linear correlation was obtained between absorbances and concentrations of CLR and DIC in the concentration ranges of 4-22 µg/ml and 4-22 µg/ml, respectively. 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 0.38 and 0.26 % at 281 and 244 nm, respectively. The RSD value of DIC was found to be 0.64 and 0.18 % at 281 and 244 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of intraday (0.54-0.81% and 0.06-0.17% for CLR at 281 and 244 nm, respectively and 0.54-0.81% and 0.16-0.45% for DIC at 281 and 244 nm, respectively) and interday (0.55 - 0.87 and 0.45-1.42% for CLR at 281 and 244 nm, respectively and 0.55 - 0.87 % and 0.52-1.60% for DIC at 281 and 244 nm, respectively) variation for CLR and DIC, reveal that the proposed method is precise. LOD and LOQ values for CLR were found to be 0.09 and 0.29 µg/ml and 0.08 and 0.25 µg/ml at 281 and 244nm, respectively. LOD and LOQ values for DIC were found to be 0.08 and 0.25 µg/ml and 0.45 and 1.37 µg/ml at 281 and 244 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 100.17 ± 0.44 and 100.24 ± 0.19 for CLR and DIC, respectively (Table 2). The results of recovery studies indicate 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.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS		CLR	DIC	CLR & DIC
Wavelength range (nm)		244	244	281
Beer's law limit (µg/ml)		4 - 24	4 - 24	4 - 24
Regression equation (y = a + bc) Slope (b) Intercept (a)		y = 0.058x + 0.004 0.058 0.004	y = 0.017x + 0.020 0.017 0.020	y = 0.065x + 0.070 0.065 0.070
Correlation Coefficient (r^2)		0.9990	0.9980	0.9990
Accuracy (Recovery) (n = 3)	Level I	100.1 ± 0.21	99.94 ± 0.20	-
	Level II	100.8 ± 1.01	100.5 ± 0.31	-
	Level III	99.63 ± 0.12	100.3 ± 0.08	-
Method precision (Repeatability) (% RSD, n = 6),		0.2375	0.3318	0.4037
Intraday (n = 3) (% RSD)		0.06-0.17%	0.16-0.45%	0.54 - 0.81
Interday(n = 3) (% RSD)		0.45-1.42%	0.52-1.60%	0.55 - 0.87
LOD (µg/ml)		0.08	0.45	0.09
LOQ (µg/ml)		0.25	1.37	0.29

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

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added(%)	% Mean recovery ± S.D. (n = 3)
CLR	I	20	50	100.1 ± 0.21
	II	20	100	100.8 ± 1.01
	III	20	150	99.63 ± 0.12
DIC	I	2	50	99.94 ± 0.20
	II	2	100	100.5 ± 0.31
	III	2	150	100.3 ± 0.08

S. D. is Standard deviation and n is number of replicate

Table 3: Analysis of CLR and DIC in synthetic mixture

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 5)	
	CLR	DIC	CLR	DIC	CLR	DIC
I	500	50	500	50.19	100.00 ± 0.12	100.64 ± 0.79

S. D. is standard deviation and n is number of replicate

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.

ACKNOWLEDGEMENT

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