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Research Article

SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF CHLORZOXAZONE AND DICLOFENAC SODIUM IN SYNTHETIC MIXTURE

Patel Satish A, Prajapati Kalpesh M*

Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India

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*Email: kelpex.prajapati@gmail.com

ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of diclofenac sodium and chlorzoxazone in bulk and synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using 0.1 N NaOH as solvent. Diclofenac sodium has absorbance maxima at 276 nm and chlorzoxazone has absorbance maxima at 288 nm in 0.1 N NaOH. The linearity was obtained in the concentration range of 2-24 µg/ml and 2-24 µg/ml for diclofenac sodium and chlorzoxazone, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 100.4 ± 1.3 and 100.2 ± 0.56 for diclofenac sodium and chlorzoxazone, respectively. The method was successfully applied to laboratory prepared synthetic mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of diclofenac sodium and chlorzoxazone was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of diclofenac sodium and chlorzoxazone in combination. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS: Diclofenac sodium, Chlorzoxazone, Recovery, Synthetic mixture, Simultaneous equations, 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 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 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 simultaneous equations 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 2.0 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 Whatman 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 of each CLR and DIC.

Methodology

The working standard solutions of CLR and DIC were prepared separately in 0.1 N NaOH having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm against 0.1 N NaOH as blank. Maximum absorbance was obtained at 288 nm and 276 nm for CLR and DIC, respectively. These two wavelengths can be employed for the determination of CLR and DIC without any interference from the other components in their synthetic formulations.

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 2-24 µg/ml for CLR and 2-24 µg/ml for DIC. Accurately measured standard solutions of CLR (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4 ml) and DIC (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.4, 2.0, 2.2, 2.4 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 288 and 276 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 Spectrophotometric 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 2 µg/ml DIC). The amounts of CLR and DIC were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five 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 CLR and DIC from synthetic mixture

Chlorzoxazone (500 mg) and diclofenac (50 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 25 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 responses of the sample solution were measured at 288 nm and 276nm for quantitation of CLR and DIC, respectively. The amounts of the CLR and DIC present in the sample solution were calculated by solving respective simultaneous equations for CLR and DIC as follows.

Cx = (A2 aY1 – A1 aY2) / (aY1 aX2 - aY2 aX1)

Cy = (A1 aX2 - A2 aX1) / (aY1 aX2 - aY2 aX1)

Where,

A_1 and A_2 are absorbances of mixture at 288 nm and 276 nm;

aX_1 and aY_1 are absorptivities of CLR and DIC respectively at 288 nm;

aX_2 and aY_2 are absorptivities of CLR and DIC respectively at 276 nm.

Table 1: Regression analysis data and summary of validation parameters for CLR and DIC

Parameters	CLR		DIC	
Wavelength (nm)	288	276	276	288
Beer's law limit (µg /ml)	2-24	2-24	2-24	2-24
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.041x + 0.008 0.041 0.008	y = 0.022x + 0.006 0.022 0.006	y = 0.067x - 0.127 0.067 -0.127	y = 0.027x + 0.007 0.027 0.007
Correlation coefficient (R^2)	0.9990	0.9990	0.9970	0.9980
LOD[a] (µg/ml)	0.08	0.15	0.04	0.24
LOQ[b] (µg /ml)	0.24	0.45	0.13	0.74
Repeatability (% RSD[c], n = 6)	0.33	0.44	0.37	0.59
Precision (% RSD, n = 3) Interday Intraday	 0.27-0.64% 0.13-0.27%	 0.12-1.02% 0.27-0.35%	 0.24-0.66% 0.16-0.70%	 0.53-1.25% 0.11-0.86%
Accuracy ± S. D[d]. (% Recovery, n = 5)	100.2 ± 0.56		100.4 ± 1.13	

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification [d]S. D. is standard deviation

Table 2: Recovery data of CLR and DIC

Drug	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 5)
CLR	20	50	100.96 ± 0.8
	20	100	99.12 ± 0.5
	20	150	100.5 ± 0.4
DIC	2	50	100.05 ± 1.4
	2	100	100.65 ± 0.8
	2	150	100.45 ± 1.9

S. D. = Standard deviation. n = Number of determinations.

Table 3: Analysis of CLR and DIC in synthetic mixture

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	CLR	DIC	CLR	DIC	CLR	DIC
I	500	50	500.3	50.2	100.0 ± 0.33	100.4 ± 0.37

S. D. = Standard deviation. n = Number of determinations.

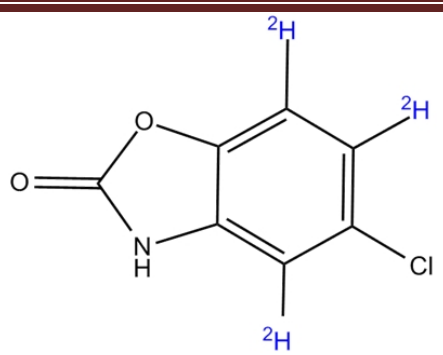

Figure 1: Chemical structure of Chlorzoxazone (CLR)

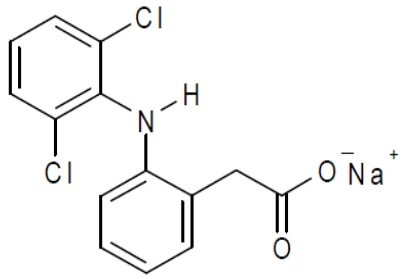

Figure 2: Chemical structure of Diclofenac Sodium (DIC)

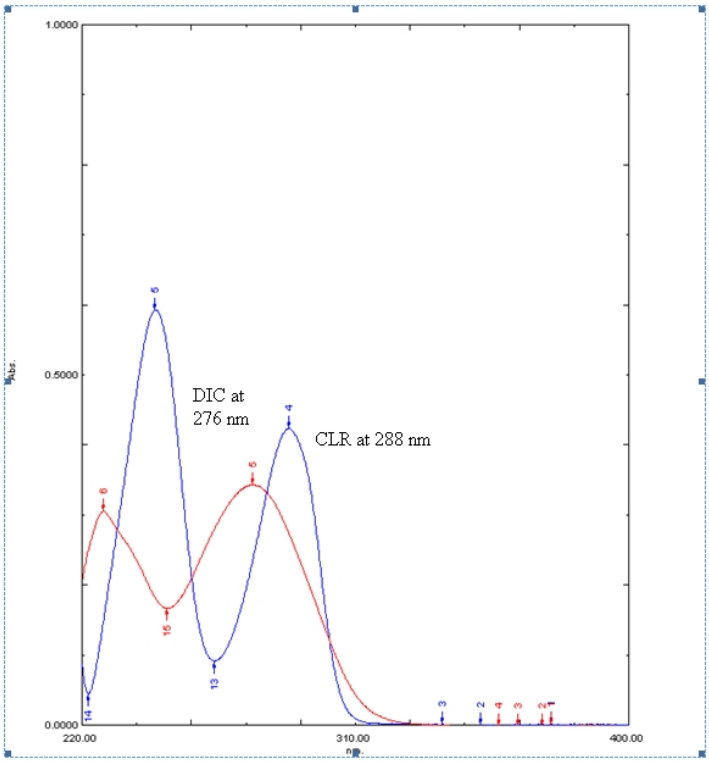

Figure 3: Overlain absorption spectra of CLR (288 nm) and DIC (276 nm) in 0.1 N NaOH

RESULTS

The standard solutions of CLR and DIC were scanned separately in the UV range and zero-order spectra for CLR and DIC were recorded. Maximum absorbance was obtained at 288 nm and 276 nm for CLR and DIC, respectively. These two wavelengths can be employed for the determination of CLR and DIC without any interference from the other drug in their combined synthetic mixture. Overlain zero-order absorption spectrum of CLR and DIC in 0.1 N NaOH is shown in (Figure 3). Linear correlation was obtained between absorbances and concentrations of CLR and DIC in the concentration ranges of 2-24 µg/ml and 2-24 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 0.26 and 0.38 % at 288 and 276 nm, respectively. The RSD value of DIC was found to be 0.64 and 0.18 % at 288 and 276 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.27-0.64% and 0.12-1.02% for CLR at 288 and 276 nm, respectively and 0.53-1.25% and 0.24-0.66% for DIC at 288 and 276 nm, respectively) and intraday (0.13-0.27% and 0.27-0.35% for CLR at 288 and 276 nm, respectively and 0.11-0.86% and 0.16-0.70% for DIC at 288 and 276 nm, respectively) variation for CLR and DIC, reveal that the proposed method is precise. LOD and LOQ values for CLR were found to be 0.08 and 0.24 µg/ml and 0.15 and 0.45 µg/ml at 288 and 276nm, respectively. LOD and LOQ values for DIC were found to be 0.24 and 0.74 µg/ml and 0.04 and 0.13 µg/ml at 288 and 276 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 100.2 ± 0.56 and 100.4 ± 1.3 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 synthetic mixture. 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 synthetic mixture as well as in pharmaceutical dosage forms.

DISCUSSION

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 combined pharmaceutical formulation.

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