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Spectrophotometric Estimation of Ibuprofen and Chlorzoxazone 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 ibuprofen and chlorzoxazone 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 λ-max of one of the two components. Ibuprofen and Chlorzoxazone show an isoabsorptive point at 227 nm in methanol. The second wavelength used is 221 nm, which is the λ-max of Ibuprofen in methanol. The linearity was obtained in the concentration range of 2-20 μg/ml for both Ibuprofen and Chlorzoxazone. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of Ibuprofen. The method was successfully applied for the determination of these two drugs in synthetic mixture. No interference was observed from excipients present in the synthetic mixture. The suitability of this method for the quantitative determination of Ibuprofen and Chlorzoxazone was proved by validation. The proposed method was found to be simple and sensitive for the routine analysis of these two drugs in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

Keywords: Ibuprofen, Chlorzoxazone, Recovery, Absorbance ratio method, Isoabsorptive point, Validation.

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INTRODUCTION

Ibuprofen (IBU) is chemically (*RS*)-2-(4-(2-methylpropyl) phenyl) propanic acid (Figure 1) is a well known Nonsteroidal anti-inflammatory drug[1]. It is official in Indian Pharmacopoeia (IP)[2], British Pharmacopoeia (BP)[3], United states Pharmacopeia (USP)[4], Japanese Pharmacopoeia (JP)[5]. IP and JP describe aqueous titration method for its estimation, while BP, IP, USP describes liquid chromatography method for its estimation. Literature survey reveals UV[6-7], HPLC[8-10], HPTLC[11] methods for estimation of IBU alone. Literature survey also reveals UV[12-15], HPLC[16-18] HPTLC[19] spectrophotometry methods for determination of IBU with other drugs in combination. Chlorzoxazone (CHL) is chemically 5-chloro-3H-benzooxazol-2-one (Figure 2). Chlorzoxazone (CHL) is official in United states Pharmacopeia (USP)[20]. USP describes UV and liquid chromatography method for its estimation. Literature survey reveals Flourimetry[21], HPLC[22-25] methods for determination of CHL alone. Literature survey also reveals UV[26-28] , HPLC[29-31], HPTLC[32] methods for the determination of CHL with other drugs combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of IBU and CHL in their combined synthetic mixture or dosage forms. Literature survey reveal only simultaneous method[33] for IBU and CHL in synthetic mixture or combined 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.

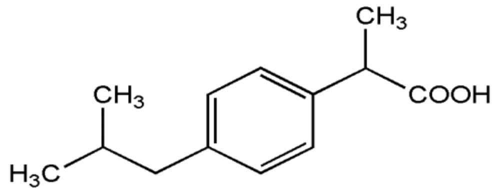

Figure 1: Chemical structure of Ibuprofen (IBU)

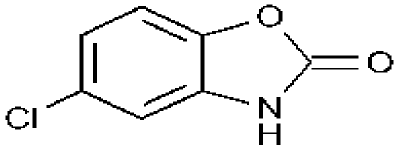

Figure 2: Chemical structure of Chlorzoxazone (CHL)

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

IBU and CHL bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. Ahmedabad, Mehsana, Gujarat, India. Methanol (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 standard IBU and CHL powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml of each IBU and CHL.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that IBU and CHL show an isoabsorptive point at 227 nm. The second wavelength used is 221 nm, which is the λ-max of IBU. Nine working standard solutions having concentration 2, 4, 6, 8, 10, 12, 16,18 and 20 µg/ml for IBU and 2, 4, 6, 8, 10, 12, 16,18 and 20 µg/ml for CHL were prepared in methanol and the absorbances at 227 nm (isoabsorptive point) and 221 nm (λ-max of IBU) were measured and absorptivity coefficients were calculated using calibration curve.

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

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \qquad (1)$$

$$C_Y = (A_1/aX_1) - C_X \qquad (2)$$

Where, A_1 and A_2 are absorbances of mixture at 227 nm and 221 nm; aX_1 and aY_1 are absorptivities of IBU and CHL at 227 nm; aX_2 and aY_2 are absorptivities of IBU and CHL respectively at 221 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[34].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-20 µg/ml for IBU and 2-20 µg/ml for CHL. Accurately measured standard solutions of IBU (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6,1.8, 2.0 ml) and CHL (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6,1.8, 2.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 227 and 221 nm against methanol 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 IBU and CHL (10 µg/ml for both drugs) without changing the parameter of the proposed 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 IBU and CHL (8, 12, 16 µg/ml for IBU and 8, 12, 16 µg/ml for CHL). 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 IBU and CHL by the standard addition method. Known amounts of standard solutions of IBU and CHL were added at 50, 100 and 150 % level to pre-quantified sample solutions of IBU and CHL (4µg/ml for IBU and 5 µg/ml for CHL). The amounts of IBU and CHL 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

Ibuprofen (40 mg) and Chlorzoxazone (50 mg) standard drug powder were accurately weighed and then mixed with commonly used excipients like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol 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 methanol. This solution (0.1 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of IBU (4 µg/ml) and CHL (5 µg/ml). The absorbances of the sample solution i.e.

A_1 and A_2 were recorded at 227 nm (isoabsorptive point) and 221 nm (λ-max of IBU) 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 227 nm (isoabsorptive point) and 221 nm (λ-max of IBU) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of IBU (221 nm) and CHL (281 nm) showing isoabsorptive point (227 nm) in methanol is shown in Figure 3.

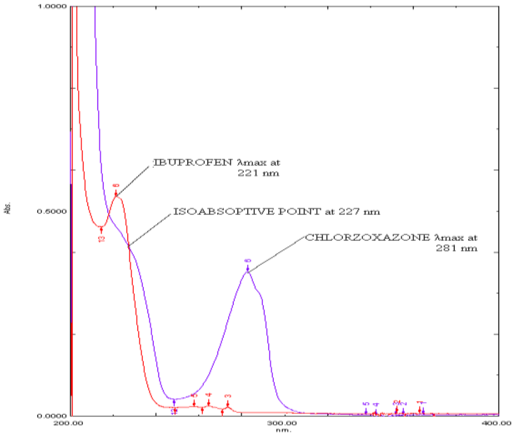

Figure 3: Overlain absorption spectra of IBU (221 nm) and CHL (281 nm) showing isoabsorptive point (227nm) in methanol

Linear correlation was observed in the concentration range of 2-20 µg/ml and 2-20 µg/ml for IBU and CHL respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient. The % RSD values of IBU were found to be 0.8217 and 0.6344 at 227 and 221 nm respectively. The % RSD value of CHL was found to be 0.8217 and 0.5927 at 227 and 221 nm respectively. Low % RSD indicates the repeatability of the proposed method. The low RSD values for interday and intraday reveals that the proposed method is precise. LOD and LOQ values for IBU were found to be 0.1276 and 0.3868 µg/ml and 0.1366 and 0.4141 µg/ml at 227 and 221nm, respectively. LOD and LOQ values for CHL were found to be 0.1276 and 0.3868 µg/ml and 0.0737 and 0.2236 µg/ml at 227 and 221 nm, respectively. These data show that method is sensitive for the determination of IBU and CHL. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

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

Parameters		IBU	CHL	IBU & CHL
Wavelength range (nm)		221	221	227
Beer's law limit (µg/ml)		2 - 20	2 - 20	2 - 20
Regression equation (y = a + bc)		y = 0.045x + 0.013	y = 0.040x + 0.013	y = 0.033x + 0.018
Slope (b)		0.045	0.040	0.033
Intercept (a)		0.013	0.013	0.018
Correlation Coefficient (r^2)		0.997	0.997	0.999
Molar extinction co-efficient (l mol^{-1} cm^{-1})		475.77	425.74	358.3
Accuracy (Recovery) (n = 3)	Level I	100.33±0.76	100.4± 0.4	-
	Level II	99.66±1.01	100.96 ± 0.76	-
	Level III	99.99±0.16	99.49 ± 0.89	-
Method precision (Repeatability) (% RSD, n = 6),		0.6510	0.9734	0.7672
Interday (n = 3) (% RSD[a])		0.8753 – 1.6435	0.1077 – 1.4331	0.6669 – 1.3165
Intraday(n = 3) (% RSD)		0.1312 –0.3364	0.2767 – 0.6591	0.3012 - 0.6210
LOD[b] (µg/ml)		0.1366	0.07379	0.1276
LOQ[c] (µg/ml)		0.4141	0.2236	0.3868
Assay ± S. D[d]. (n = 3)		98.87 ± 1.022	99.83 ± 1.022	-

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

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.32 ± 0.87 and 100.28 ± 0.74 for IBU and CHL, 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 IBU and CHL in their combined dosage form. The results obtained for IBU and CHL were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of analyte of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of IBU and CHL in mixture.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
IBU	I	4	50	100.33 ± 0.76
	II	4	100	99.66 ± 1.01
	III	4	150	99.99 ± 0.16
CHL	I	5	50	100.4 ± 0.4
	II	5	100	100.96 ± 0.76
	III	5	150	99.49 ± 0.89

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

Table 3: Analysis of IBU and CHL in synthetic mixture

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	IBU	CHL	IBU	CHL	IBU	CHL
I	400	500	395	499	98.87 ± 1.022	99.83 ± 1.022

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 IBU and CHL in mixture. The method utilizes easily available and cheap solvent for analysis of IBU and CHL hence the method was also economic for estimation of IBU and CHL from mixture. The common excipients and additives are usually present in the synthetic mixture do not interfere in the analysis of IBU and CHL in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture.

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