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Spectrophotometric Estimation of Tolperisone Hydrochloride and Diclofenac Sodium In Synthetic Mixture by Q-Absorbance Ratio Method

Satish A. Patel[1], Kaushik P. Hariyani[*1]

1.S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India..

ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of diclofenac sodium and tolperisone hydrochloride 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. Tolperisone hydrochloride and diclofenac sodium show an isoabsorptive point at 267 nm in methanol. The second wavelength used is 255 nm, which is the λ-max of tolperisone hydrochloride in methanol. The linearity was obtained in the concentration range of 2-20 µg/ml for both tolperisone hydrochloride and diclofenac sodium. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of tolperisone hydrochloride. 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 tolperisone hydrochloride and diclofenac sodium was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of tolperisone hydrochloride and diclofenac sodium in synthetic mixture or pharmaceutical dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Diclofenac sodium, Tolperisone hydrochloride, Recovery, Absorbance ratio method, Isoabsorptive point, Validation

*Corresponding Author Email: hariyanikaushik@gmail.com

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INTRODUCTION

Tolperisone (TOL) is chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one (Figure 1) is a well known antispasmodic drug[1]. It is official in Japanese Pharmacopoeia (JP). JP[2] describe potentiometry method for its estimation. Literature survey reveals HPLC[3] and UV[4] method for estimation of TOL alone. Literature survey also reveals HPLC[5] and UV spectrophotometry[6] methods for determination of TOL with other drugs in combination. Diclofenac sodium (DIC) is chemically 2-[2,6dichlorophenylamino] benzene acetic acid sodium salt[7] (Figure 2). Diclofenac sodium (DIC) is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP[8] and BP[9] describes liquid chromatography method for its estimation. Literature survey reveals HPLC[10, 11] and UV[12] method for determination of DIC alone. Literature survey also reveals HPLC[13, 14, 15], UV spectrophotometry[16] and HPTLC[17] method for the determination of DIC 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 TOL and DIC in their combined synthetic mixture or dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of TOL and DIC 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.

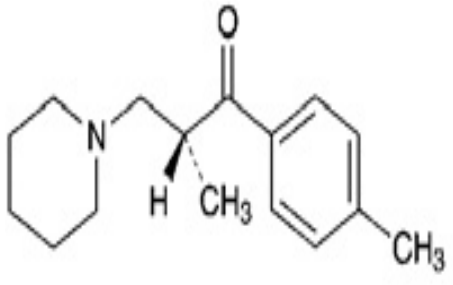

Figure 1: Chemical structure of Tolperisone (TOL

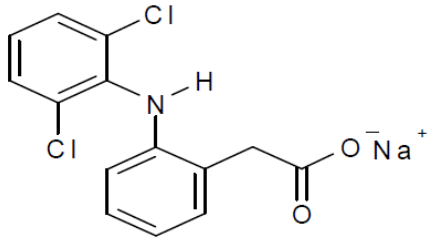

Figure 2: Chemical structure of Diclofenac Sodium (DIC)

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

TOL and DIC bulk powder was kindly gifted by Torrent Research Centre, Gandhinagar, India and Acme Pharmaceuticals Ltd. Ahmedabad, Mehsana, Gujarat, India, respectively. 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 TOL and DIC 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 TOL 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 λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that TOL and DIC show an isoabsorptive point at 267 nm. The second wavelength used is 255 nm, which is the λ-max of TOL. Eight working standard solutions having concentration 2, 4, 6, 8, 10, 12, 16 and 20 µg/ml for TOL and 2, 4, 6, 8, 10, 12, 16 and 20 µg/ml for DIC were prepared in methanol and the absorbances at 267 nm (isoabsorptive point) and 255 nm (λ-max of TOL) 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 267 nm and 255 nm; aX_1 and aY_1 are absorptivities of TOL and DIC at 267 nm; aX_2 and aY_2 are absorptivities of TOL and DIC respectively at 255 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[18].

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 2-20 µg/ml for TOL and 2-20 µg/ml for DIC. Accurately measured standard solutions of TOL (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 ml) and DIC (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 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 267 and 255 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 TOL and DIC (6 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 TOL and DIC (4, 6, 8 µg/ml for TOL and 4, 6, 8 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 TOL and DIC by the standard addition method. Known amounts of standard solutions of TOL and DIC were added at 50, 100 and 150 % level to prequantified sample solutions of TOL and DIC (7.5 µg/ml for TOL and 2.5 µg/ml for DIC). The amounts of TOL 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

Tolperisone (75 mg) and diclofenac (25 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 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 (0.2 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 TOL (15 µg/ml) and DIC (5 µg/ml). The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 267 nm (isoabsorptive point) and 255 nm (λ-max of TOL) 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 267 nm (isoabsorptive point) and 255 nm (λ-max of TOL) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of TOL (255 nm) and DIC (281 nm) showing isoabsorptive point (267 nm) in methanol is shown in Figure 3.

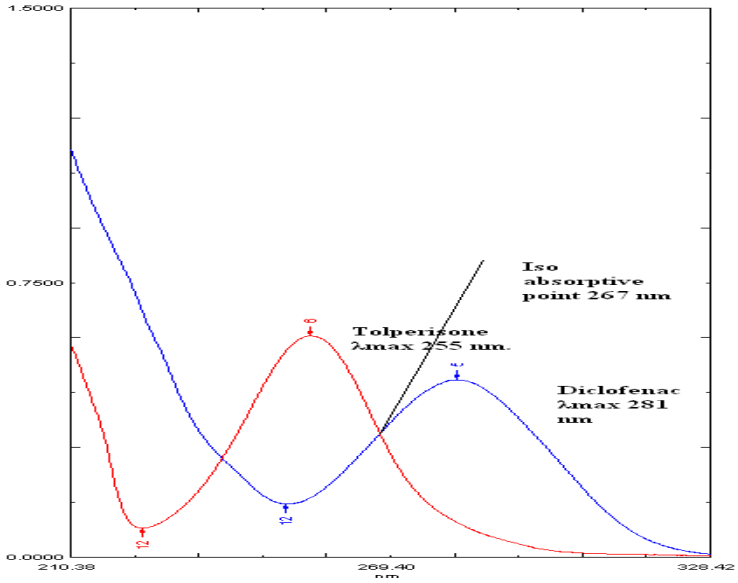

Figure 3: Overlain absorption spectra of TOL (255 nm) and DIC (281 nm) showing isoabsorptive point (267nm) in methanol

Linear correlation was obtained between absorbances and concentrations of TOL and DIC in the concentration ranges of 2-20 µg/ml and 2-20 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of TOL were found to be 0.64 and 0.79 % at 267 and 255 nm, respectively. The RSD value of DIC was found to be 0.64 and 0.74 % at 267 and 255 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.54-0.81% and 0.31-0.89% for TOL at 267 and 255 nm, respectively and 0.54-0.81% and 0.30-0.53% for DIC at 267 and 255 nm, respectively) and intraday (0.55 - 0.87% and 0.29 – 0.82% for TOL at 267 and 255 nm, respectively and 0.55 - 0.87 % and 0.44 – 0.98% for DIC at 267 and 255 nm, respectively) variation for TOL and DIC, reveal that the proposed method is precise. LOD and LOQ values for TOL were found to be 0.11 and 0.26 µg/ml and 0.33 and 0.79 µg/ml at 267 and 255nm, respectively. LOD and LOQ values for DIC were found to be 0.11 and 0.22 µg/ml and 0.33 and 0.66 µg/ml at 267 and 255 nm, respectively. These data show that method is sensitive for the determination of TOL and DIC. 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		**TOL**	**DIC**	**TOL & DIC**
Wavelength range (nm)		255	255	267
Beer's law limit (µg/ml)		2 - 20	2 - 20	2 - 20
Regression equation (y = a + bc)		y = 0.063x - 0.021	y = 0.015x - 0.001	y = 0.030x + 0.000
Slope (b)		0.063	0.015	0.030
Intercept (a)		-0.021	-0.001	0.00
Correlation Coefficient (r^2)		0.998	0.998	0.997
Molar extinction co-efficient ($l\ mol^{-1}\ cm^{-1}$)		14708.5	4451.57	8587.84 (TOL) 10365.19 (DIC)
Accuracy (Recovery)	Level I	99.92±0.53	100.35± 0.21	-
(n = 3)	Level II	99.80±0.84	99.30 ± 0.42	-
	Level III	99.19±0.13	100.4 ± 0.14	-
Method precision (Repeatability) (% RSD, n = 6),		0.49	0.98	0.93
Interday (n = 3) (% RSD[a])		0.31 - 0.89	0.30 - 0.53	0.54 - 0.81
Intraday(n = 3) (% RSD)		0.29 – 0.82	0.44 – 0.98	0.55 - 0.87
LOD[b] (µg/ml)		0.26	0.22	0.11
LOQ[c] (µg/ml)		0.79	0.66	0.33
Assay ± S. D[d]. (n = 3)		99.64 ± 0.5	100.0 ± 0.25	-

[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 99.64 ± 0.5 and 100.0 ± 0.25 for TOL 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 TOL and DIC in their combined dosage form. The results obtained for TOL 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 TOL and DIC in pharmaceutical dosage forms.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
	I	7.5	50	99.92 ± 0.53
TOL	II	7.5	100	99.80 ± 0.84
	III	7.5	150	99.19 ± 0.13
	I	2.5	50	100.35 ± 0.21
DIC	II	2.5	100	99.30 ± 0.42
	III	2.5	150	100.4 ± 0.14

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

Table 3: Analysis of TOL and DIC in synthetic mixture

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D (n = 3)	
	TOL	DIC	TOL	DIC	TOL	DIC
I	150	50	150.04	50.15	100.02 ± 0.78	100.3 ± 0.98

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 TOL and DIC in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of TOL and DIC hence the method was also economic for estimation of TOL and DIC from synthetic mixture. The common excipients and additives are usually present in the synthetic mixture do not interfere in the analysis of TOL 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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