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Spectrophotometric Estimation of Tolperisone Hydrochloride and Diclofenac Sodium in Synthetic Mixture By Derivative Spectroscopic Method

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical derivative spectroscopic method for the simultaneous determination of tolperisone hydrochloride (TOL) and diclofenac sodium (DIC) in bulk and synthetic mixture. Derivative spectroscopy offers a useful approach for the analysis of drugs in mixtures. In this study a first-derivative spectroscopic method was used for simultaneous determination of tolperisone hydrochloride and diclofenac sodium using the zero-crossing technique. The measurements were carried out at wavelengths of 250.60 and 311.20nm for tolperisone hydrochloride and diclofenac sodium respectively. The method was found to be linear (r2>0.9970) in the range of 5- 40 µg/ml for tolperisone hydrochloride at 250.60 nm. The linear correlation was obtained (r2>0.9990) in the range of 5-50 µg/ml for diclofenac sodium at 311.20 nm. The limit of determination was 0.65 and 0.55 µg/ml for tolperisone hydrochloride and diclofenac sodium respectively. The limit of quantification was 2.01 and 1.67 µg/ml. The method was successfully applied for simultaneous determination of tolperisone hydrochloride and diclofenac sodium in binary mixture.

Keywords: Diclofenac sodium, Tolperisone hydrochloride, Derivative spectroscopic method, Zero-crossing point.

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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-[2,6diclohlorophenylamino] 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] describe 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 derivative spectroscopic method for simultaneous estimation of both drugs in bulk and combined synthetic mixture.

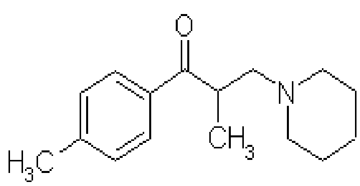

Figure 1: Chemical structure of Tolperisone (TOL)

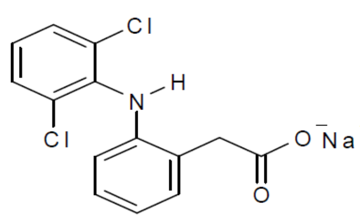

Figure 2: Chemical structure of DICfenac Sodium (DIC)

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. Ahmadabad, 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.

Determination of the zero crossing points

The standard solutions of TOL (10 µg/ml) and DIC (10 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 TOL showed zero crossing at 225.60nm and 311.20 nm while DIC showed zero crossing at 250.60nm and 281.60nm. At 311.20nm TOL showed zero absorbance and DIC showed reasonable absorbance, while at 250.60nm DIC showed zero absorbance and TOL 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[18].

Linearity (calibration curve)

To check linearity of the method, working standard solution having concentration in range of 5-40 µg/ml for TOL and 5-50 µg/ml for DIC were prepared from the standard stock solutions of both drugs. Aliquots of standard solution of TOL (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 ml) and DIC (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 ml) of standard stock solutions of both drug were transferred separately to a series of 10 ml volumetric flasks and diluted to mark with methanol, and first-derivative absorbance (D1) were measured at 250.60 nm for TOL and 311.20 nm for DIC. The calibration curves were constructed by plotting absorbance vs. concentration.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solution of (n = 6) TOL and DIC (10 µg/ml) without changing the parameters of First Derivative 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 (10, 20, 30 µg/ml for TOL and 15, 25, 35 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 (10 µg/ml for TOL and 10 µg/ml for DIC). The amounts of TOL and DIC were estimated by applying obtained values to the regression equation of the calibration curve.

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 DICfenac (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). and the stock solution (0.4 ml) was taken in to 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of TOL (30 µg/ml) and DIC (10 µg/ml). The absorbance of the sample solution was measured at 250.60 nm and 311.20 nm for quantitation of TOL and DIC, respectively. The amounts of the TOL and DIC present in the sample solution were calculated by fitting the responses into the regression equation for TOL and DIC in the First Derivative Method.

RESULTS AND DISCUSSION

In derivative spectroscopic method, 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 250.60 nm (ZCP of DIC) and 311.20 nm (ZCP of TOL) at which the calibration curves were prepared for both the drugs. The first derivative overlain UV absorption spectrum of TOL (at 250.60 nm) and DIC (at 311.20 nm) is shown in Figure 3.

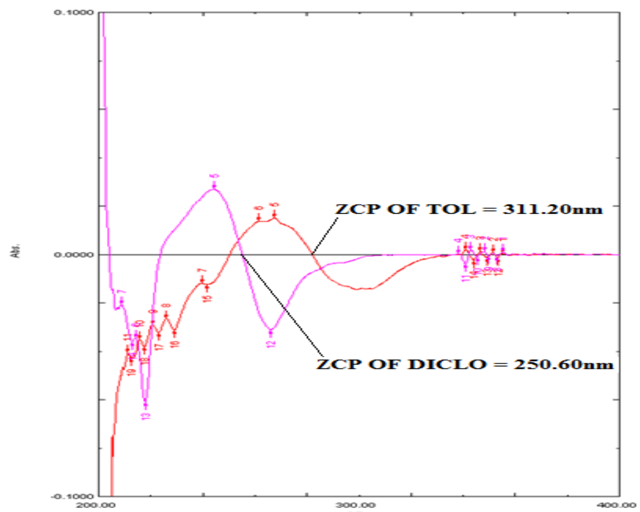

Figure 3: Overlain first derivative absorption spectra of standard solution of TOL and DIC in methanol

Linear correlation was obtained between absorbance and concentrations of TOL and DIC in the concentration ranges of 5-40 µg/ml and 5-50 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 1.51% at 250.60 nm, respectively. The RSD value of DIC was found to be 1.11% at 311.20 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.80-1.99% and for TOL at 250.60 nm, respectively and 0.39-1.44% for DIC at 311.20 nm, respectively) and intraday (1.14 – 1.48% for TOL 250.60 nm, respectively and 0.60 – 1.01% for DIC at 311.20 nm, respectively) variation for TOL and DIC, reveal that the proposed method is precise. LOD and LOQ values for TOL were found to be 0.66 and 2.01 µg/ml and at 250.60 nm, respectively. LOD and LOQ values for DIC were found to be 0.55 and 1.67 µg/ml and 0.33 and 0.66 µg/ml at 311.20 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
Wavelength range (nm)		250.60	311.20
Beer's law limit (µg/ml)		5-40	5-50
Regression equation (y = a + bc)		y = 0.002x - 0.004	y = 0.001x - 0.000
Slope (b)		0.002	0.001
Intercept (a)		-0.004	-0.000
Correlation Coefficient (r^2)		0.997	0.999
Accuracy (Recovery) (n = 3)	Level I	100.44±0.50	98.99±0.88
	Level II	102.75±0.25	99.00±0.50
	Level III	100.46±0.30	100.00±1.05
Method precision (Repeatability) (% RSD, n = 6),		1.15	1.11
Interday (n = 3) (% RSD[a])		0.80-1.99	0.39-1.44
Intraday(n = 3) (% RSD)		1.14 – 1.48	0.60 – 1.01
LOD[b] (µg/ml)		0.66	0.55
LOQ[c] (µg/ml)		2.01	1.67
Assay ± S. D[d]. (n = 3)		100.40 ± 0.67	100.35 ± 1.26

[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 proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
TOL	I	10	50	100.44±0.50
	II	10	100	102.75±0.25
	III	10	150	100.46±0.30
DIC	I	10	50	98.99±0.88
	II	10	100	99.00±0.50
	III	10	150	100.00±1.05

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
1	150	50	151.50	50.05	101.15 ± 0.50	101.00 ± 1.00
2	300	100	298.90	99.70	99.65 ± 0.75	99.70 ± 1.53

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

The recovery experiment was performed by the standard addition method. The mean recoveries were 101.22 ± 0.35 and 99.33 ± 0.81 for TOL 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 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.

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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