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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AREA UNDER CURVE METHOD FOR SIMULTANEOUS ESTIMATION OF EPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN COMBINED CAPSULE DOSAGE FORM

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical area under curve method for the simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in combined capsule dosage form. The proposed area under curve method involves the measurement of area at selected analytical wavelength ranges and performing the analysis using "Cramer's Rule". Two analytical wavelength ranges selected were 251.0-261.0 nm and 276.0-286.0 nm for the estimation of Eperisone Hydrochloride and Diclofenac Sodium, respectively. The linearity of the proposed method was found in the range of 2-20 µg/ml for both Eperisone Hydrochloride and Diclofenac Sodium, respectively. The percentage mean recovery was found to be 101.54±0.25% for Eperisone Hydrochloride and 100.88±0.67% for Diclofenac Sodium. Also the method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % Relative standard deviation value, indicating high grade of precision of the method. The method was successfully applied to pharmaceutical dosage forms because no interference from the capsule excipients was found.

KEY WORDS: Diclofenac Sodium, Eperisone hydrochloride, Area under curve, Validation.

INTRODUCTION

Eperisone Hydrochloride (EPE) is chemically 4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride[1]. It is an antispasmodic agent, a centrally acting muscle relaxant. It is official in Japanese Pharmacopoeia (JP) [2]. JP describe potentiometric method for its estimation. Literature survey reveals liquid chromatography-Electrospray ionization-Mass Spectrometry method & gas chromatography-mass spectrometry method for determination of Eperisone in human plasma.[3,4] HPLC/MS, GC/MS,NMR, UV and IR to identify a degradation product of Eperisone Hydrochloride in the tablet is also available.[5] Diclofenac sodium (DICLO) is chemically 2-[2,6 dichlorophenylamino] benzene acetic acid sodium salt.[6] It is official in IP, BP & USP[7-9]. IP & BP describes liquid chromatography methods & USP describes potentiometric method for its estimation. Literature survey also reveals UV method[10] and HPLC[11-13] for determination of DICLO in single dosage form. Literature survey also reveals UV spectrophotometry[14-19]and HPLC [20-24] method for the determination of DICLO with other drugs in combination. Literature survey reveals spectrophotometric simultaneous equation method for simultaneous estimation of EPE and DICLO in synthetic mixture.[25] However, no references have been found for simultaneous estimation of EPE and DICLO in pharmaceutical formulations by Area Under Curve (AUC) method. A successful attempt has been made to estimate two drugs simultaneously by AUC method. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of EPE and DICLO in their combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise AUC spectrophotometric method for simultaneous estimation of both drugs in capsule dosage forms.

MATERIALS & METHODS

Apparatus

A Shimadzu model UV-1800 series (Japan) double beam UV/Visible spectrophotometer with spectral width of 1 nm and a pair of 1 cm matched quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe version 2.34 system software. An analytical balance (Denver Instruments, Germany), an ultrasonic bath (Fast clean ultrasonic cleaning system, Mumbai) was used in the study.

Reagents and Materials

Eperisone Hydrochloride (EPE) was kindly gifted by Sun Pharmaceuticals ltd., J & K; India & Diclofenac Sodium (DICLO) was kindly gifted by Alembic Ltd., Vadodara, India. The capsule dosage form (EPRY-D sr -containing Eperisone Hydrochloride 150 mg and Diclofenac Sodium 100 mg, Mfd. by : Easai pharmaceutical works) was procured from the local market. Methanol (AR Grade, S.D. Fine Chemicals Ltd., Mumbai, India) were used in the study. All other chemicals and solvents used were of analytical grade.

Preparation of standard stock solutions

An accurately weighed quantity of standard EPE (10 mg) and DICLO (10 mg) were weighed and transferred to separate 100 ml volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solutions containing 100 µg/ml each of EPE and DICLO.

Methodology

For the selection of analytical wavelength, solutions of EPE and DICLO

(10 µg/ml, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the overlain spectra of both drugs (Fig. 3), area under the curve in the range of 251.0-261.0 nm (for EPE) and 276.0-286.0 nm (for DICLO) were selected for the analysis. The calibration curves for EPE and DICLO were prepared in the concentration range of 2-20 µg/ml at 251.0-261.0 nm for

EPE and 276.0-286.0 nm for DICLO. The 'X' values were determined for both the drugs at both selected AUC ranges, i.e. at 251.0-261.0 nm & 276.0-286.0 nm. The 'X' is the ratio of area under the curve at selected wavelength ranges with the concentration of component in µg/ml. These 'X' values were the mean of six independent determinations. A set of two simultaneous equations obtained by using mean 'X' values are given below.

$$A1 = 0.5432\ C_{Epe} + 0.1292\ C_{Diclo} \text{ -- (At } \lambda \text{ 251.0-261.0 nm)} \quad \text{------- (1)}$$
$$A2 = 0.09638\ C_{Epe} + 0.3627\ C_{Diclo} \text{ – (At } \lambda \text{ 276.0-286.0 nm)} \quad \text{------ (2)}$$

Where, A1 and A2 were area under curve of sample at the wavelength range 251.0-261.0 nm and 276.0-286.0 nm, respectively.

0.5432 and 0.09638 were 'X' values of EPE at wavelength range 251.0-261.0 nm and 276.0-286.0 nm, respectively.

0.1292 and 0.3627 were 'X'values of DICLO at the wavelength range 251.0-261.0 nm and 276.0-286.0 nm, respectively.

C_{Epe} and C_{Diclo} were concentration of EPE and DICLO, respectively.

By applying the Cramer's rule to equations (1) and (2), the concentration of EPE and DICLO can be obtained as follows.

$$C_{Epe} = \frac{(A2)0.1292 - (A1)0.3627}{-0.18455}$$

$$C_{Diclo} = \frac{(A1)0.09638 - (A2)0.5432}{-0.18455}$$

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[26]

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 2-20 µg/ml for both EPE and DICLO. Accurately measured standard stock solution of EPE (0.2, 0.4, 0.8, 1.2, 1.6 & 2.0 mL) and standard stock solution of DICLO (0.2, 0.4, 0.8, 1.2, 1.6 & 2.0 mL) were transferred to a separate series of 10 mL of volumetric flasks and diluted to the mark with methanol. The area of solutions of EPE and DICLO were measured between the wavelength ranges of 251.0-261.0 nm and 276.0-286.0 nm against methanol as blank. Calibration curves were constructed at 251.0-261.0 nm for EPE and 276.0-286.0 nm for DICLO by plotting area versus concentrations and the regression equations were calculated. The Beer-Lambert's concentration range was found to be 2-20 µg/ml for both EPE and DICLO.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for EPE and DICLO (8 µg/ml) without changing the parameters of the proposed spectrophotometry method. Percent relative standard deviation (%RSD) was found and was within limit (Not more than 2%).

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 EPE and DICLO (8, 12 and 16 µg/ml for both EPE and DICLO standard solutions.). Percent relative standard deviation (%RSD) was found and was within limit (Not more than 2%).

Accuracy (Recovery study)

The accuracy of the method was determined by calculating the recoveries of EPE and DICLO by the standard addition method. Known amounts of standard solutions of EPE and DICLO were added at 50, 100 and 150 % level to prequantified sample solutions of EPE and DICLO (6 µg/ml for EPE and 4 µg/ml for DICLO). The amounts of EPE and DICLO were estimated by applying obtained values to the respective regression line equations and percent recoveries were found.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs were derived using following equation as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

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

ANALYSIS OF CAPSULE SAMPLE

The powder of 20 capsules was weighed, mixed. The net content of the capsule was found. An accurately weighed quantity of the powder equivalent to about 10 mg of DICLO and 15 mg of EPE was transferred in to 100 mL measuring flasks. The volume was made up to mark. The solution was filtered through Whatman filter paper and diluted in such a way to obtain the final concentration of EPE 15 µg/ml and DICLO 10 µg/ml. The absorbances of the sample solution i.e. A1 and A2, were recorded at 251.0-261.0 nm and 276.0-286.0 nm respectively and relative concentration of two drugs in the sample was calculated using two simultaneous equation (1) and (2) given in methodology section. The analytical procedure was repeated 3 times with capsule formulation.

RESULTS

The standard solutions of EPE and DICLO were scanned separately in the UV range and zero-order spectra for EPE and DICLO were recorded. Maximum absorbance was obtained at 256 nm and 281 nm for EPE and DICLO, respectively. The area for EPE was obtained by taking wavelength range of 251.0-261.0 nm and 276.0-286.0 nm for DICLO. Linear correlation was obtained in the concentration ranges of 2-20 µg/ml for both EPE and DICLO. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. Relative standard deviation for repeatability was found less than 2% which indicates that proposed method is repeatable. The low RSD value of intraday (0.19-0.55 for EPE at 251.0-261.0 nm and 0.09-0.21 for DICLO at 276.0-286.0 nm) and interday (0.66-0.93 for EPE at 251.0-261.0 nm and 0.50-0.72 for DICLO at 276.0-286.0 nm) variation for EPE and DICLO reveal that the proposed method is precise. LOD and LOQ values for EPE were found to be 0.13 and 0.40 µg/ml at 251.0-261.0 nm and for DICLO were found to be 0.16 and 0.48 µg/ml at 276.0-286.0 nm. These data show that method is sensitive for the determination of EPE and DICLO. 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 found 101.54±0.25 and 100.88 ±0.67 for EPE and DICLO respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine EPE and DICLO in their combined capsule dosage form. The

results obtained for EPE and DICLO were comparable with the corresponding labeled amount (Table 3).

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR EPE AND DICLO.

Parameters	EPE	DICLO
Wavelength (nm)	251.0-261.0	276.0-286.0
Beer's law limit (µg/ml)	2-20	2-20
Regression equation (y = mx+c)	y = 0.527x + 0.090	y = 0.338x + 0.156
Slope (m)	0.527	0.338
Intercept(c)	0.090	0.156
Correlation Coefficient (R^2)	0.999	0.999
LOD[a] (µg/ml)	0.13	0.16
LOQ[b] (µg/ml)	0.40	0.48
Repeatability (% RSD[c], n = 6)	0.70	0.29
Precision (% RSD, n=3)		
Interday	0.66-0.93	0.50-0.72
Intraday	0.19-0.55	0.09-0.21
Accuracy±S.D.[d] (% Recovery, n=3)	101.54±0.25	100.88±0.67

[a]LOD= Limit of detection, [b]LOQ= Limit of quantification,
[c]RSD= Relative standard deviation, [d]SD= Standard deviation,

TABLE 2: RECOVERY DATA OF EPE AND DICLO

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D. (n=3)
EPE	6	3	9.06	101.89 ± 0.19
	6	6	12.07	101.17 ± 0.44
	6	9	15.14	101.56 ± 0.11
DICLO	4	2	6.01	100.67 ± 0.76
	4	4	8.05	101.25 ± 0.66
	4	6	10.04	100.72 ± 0.59

SD= Standard deviation, n= Number of determinations.

TABLE 3: ANALYSIS OF EPE AND DICLO IN CAPSULE DOSAGE FORM.

Formulation	EPE			DICLO		
	Amount labeled (mg)	Amount found (mg)	% Amount found ± S.D. (n=3)	Amount labeled (mg)	Amount labeled (mg)	% Amount found ± S.D. (n=3)
EPRY-D sr Capsule	150	151.32	100.88 ± 0.34	100	101.03	101.03 ± 0.33

SD= Standard deviation, n= Number of determinations.

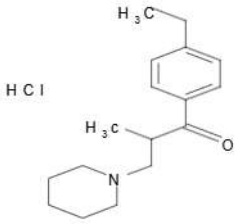

Figure 1: Chemical structure of Eperisone Hydrochloride (EPE)

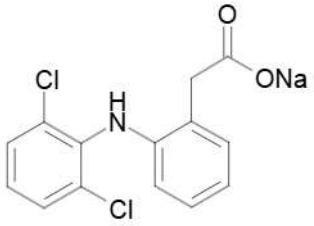

Figure 2: Chemical structure of Diclofenac sodium (DICLO)

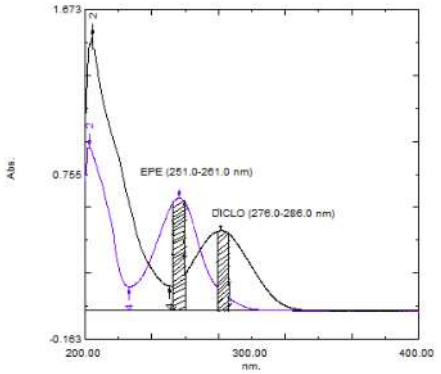

Figure: 3 Overlain absorption spectra of EPE (251.0-261.0 nm) and DICLO (276.0-286.0 nm) in methanol for Area Under Curve method

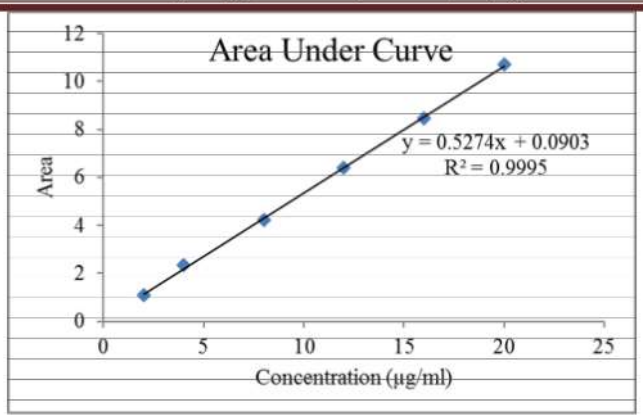

Figure 4: Calibration curve of EPE at 251.0-261.0 nm

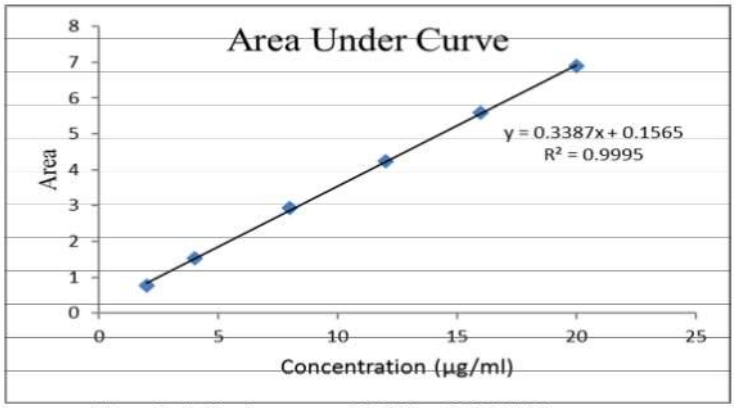

Figure 5: Calibration curve of DICLO at 276.0-286.0 nm.

DISCUSSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of EPE and DICLO in capsule dosage form. The method utilizes easily available and cheap solvent; hence the method was also economic for estimation of EPE and DICLO in capsule dosage form. The common excipients and other additives are usually present in the capsule dosage form do not interfere in the analysis of EPE and DICLO in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in capsule dosage form.

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