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Spectrophotometric Estimation of Tramadol Hydrochloride and Aceclofenac in Combined Dosage form by Second Order Derivative Method

Trupti. P. Patel[1*], Arun. M. Prajapati[1]

1.*Department of Pharmaceutical Analysis, Centre For Health Science Studies, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India.*

ABSTRACT

The present manuscript describes sensitive, rapid, accurate, and precise second order derivative spectrophotometry method for the simultaneous determination of tramadol hydrochloride and aceclofenac in combined dosage form. The absorbance values at 225 nm and 215 nm of second derivative spectrum was used for the estimation of tramadol hydrochloride and aceclofenac in combined dosage form, respectively without mutual interference. This method obeyed beer's law in the concentration range of 5-80µg/ml and 4-28µg/ml for tramadol hydrochloride and aceclofenac respectively. The method was successfully applied to combined dosage form because no interference from the excipients was found. The suitability of this method for the quantitative determination of tramadol hydrochloride and aceclofenac was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of tramadol hydrochloride and aceclofenac in combined dosage form. The results of analysis have been validated by recovery studies.

Keywords tramadol hydrochloride, aceclofenac, second order derivative spectrophotometry method.

*Corresponding Author Email: trups1189@gmail.com

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INTRODUCTION

Tramadol hydrochloride (TRAMA) is a well known anti-inflammatory analgesic drug[1]. Chemically it is (1RS,2RS)-2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride (Figure 1). It is official in Indian Pharmacopoeia (IP)[2] and British pharmacopoeia (BP)[3]. IP & BP describes potentiometric titration and HPLC methods for its estimation. Literature survey reveals UV spectroscopy[4], HPLC[5], chemiluminance[6] methods for determination of tramadol hydrochloride. Literature survey also reveals UV spectroscopy[7-10], HPLC[11-14], GC-MS[14], HPTLC[15] methods for the determination of tramadol hydrochloride with other drugs combination.

Aceclofenac (ACE)[16] is chemically [[[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid (Figure 2). Aceclofenac (ACE) is official in Indian Pharmacopoeia (IP)[17] and British Pharmacopoeia (BP)[18]. IP describes Potentiometric titration and Liquid Chromatography (LC) method for its estimation. BP describes LC method for its estimation. Literature survey reveals UV[19-22], HPLC[23] method for determination of ACE alone. Literature survey also reveals UV[24-30], HPLC[31-35] method for the determination of ACE 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 TRAMA and ACE in their combined dosage forms. Literature survey reveal only first derivative method[36] for TRAMA and ACE in combined dosage form is available. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on second order derivative for simultaneous estimation of both drugs in combined dosage form.

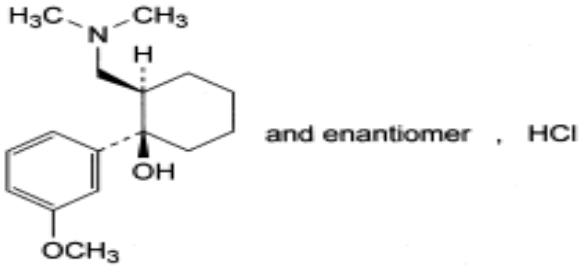

Figure 1 Chemical structure of tramadol hydochloride (TRAMA)

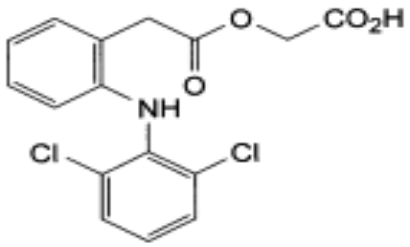

Figure 2 Chemical structure of aceclofenac (ACE)

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

ACE & TRAMA bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. 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 TRAMA and ACE 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 TRAMA and ACE.

Methodology

This method is based on second order derivative spectroscopy to overcome spectral interference from other drug. Zero order spectrums of both the drugs were converted to second order derivative spectra with the help of spectra manager software.

It was observed that TRAMA showed $d^2A/d\lambda^2$ zero at 215 nm in contrast to ACE that has considerable $d^2A/d\lambda^2$ at this wavelength. Further, ACE has zero $d^2A/d\lambda^2$ at 225 nm while at this wavelength TRAMA has significant $d^2A/d\lambda^2$. Therefore wavelengths 225 nm and 215 nm were employed for the determination of TRAMA and ACE respectively without interference of other drug. The calibration curves were plotted at these two wavelengths of concentrations against $d^2A/d\lambda^2$ separately. Working standard solutions having concentration 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml for TRAMA and 4, 8, 12, 16, 20, 24 and 28 µg/ml for ACE were prepared in methanol and the absorbance at 225 nm (zero crossing point for ACE) and 215 nm (zero crossing point for TRAMA) were measured and the calibration curves were plotted at these wavelengths.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[37].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 5-80 µg/ml for TRAMA and 4-28 µg/ml for ACE. Accurately measured standard solutions of TRAMA (0.5, 1, 2, 3, 4, 5, 6, 7, 8 ml) and ACE (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the derivatized spectra were measured at 215 nm and 225 nm for ACE and TRAMA respectively against methanol as blank. Six replicate analysis were carried out. Absorbance Vs concentration were plotted to obtain the calibration graph. Both drugs obey the Beer's law with the above concentration range with R^2 value of 0.998 and 0.999 for TRAMA and ACE respectively.

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for TRAMA and ACE (50 µg/ml and 16 µg/ml for both drugs respectively) 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 TRAMA and ACE (40, 50, 70 µg/ml for TRAMA and 12, 20, 24 µg/ml for ACE). 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 TRAMA and ACE by the standard addition method. Known amounts of standard solutions of TRAMA and ACE were added at 80, 100 and 120 % level to pre-quantified sample solutions of TRAMA and ACE (37.5µg/ml for TRAMA and 20µg/ml ACE). The solutions were measured at 225 nm for TRAMA and 215 nm for ACE and % recovery of the sample were calculated. The experiment was repeated for three times.

Limit of detection and quantification

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From this value the parameters. 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[37]

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

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

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

Analysis of combined dosage form

Accurately weighed the 20 tablets and powdered. The powder equivalent to 228 mg of tablet was transferred to 100 ml volumetric flask which contains 37.5 mg of tramadol hydrochloride and 100 mg of Aceclofenac, then add 20 ml of 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. From this solution, 1 ml was taken into a 10 ml volumetric flask and the volume was adjusted upto mark with methanol to get a final concentration of TRAMA (37.5 µg/ml) and their second derivative spectra was recorded. From the derivative spectra, the absorbance at 225 nm was noted for the estimation of TRAMA and this solution was further 5 times diluted and get a final concentration of ACE (20 µg/ml) and from the second derivative spectra, the absorbance at 215 nm was noted for the estimation of ACE. From these absorbance values, the concentrations of TRAMA and ACE were determined using calibration graph. The analysis procedure was repeated six times with marketed formulation.

RESULTS AND DISCUSSION

Zero-order absorption spectra of TRAMA and ACE showed overlapping peaks that interfere with the simultaneous determination of this formulation (Figure 3). Derivative spectroscopy, based on a mathematical transformation of the spectra zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multicomponent mixture and overcomes the problem of overlapping of a multicomponent system. Derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other component. The spectroscopic parameters including derivative order, wavelength and Δλ values should be optimized to obtain maximum resolution, sensitivity and reproducibility. In this study second derivative technique (D2) traced with Δλ= 8 nm was used to resolve the spectral overlapping. The optimums D2values without interference for TRAMA and ACE were 225 and 215 nm, respectively (Figure 4).

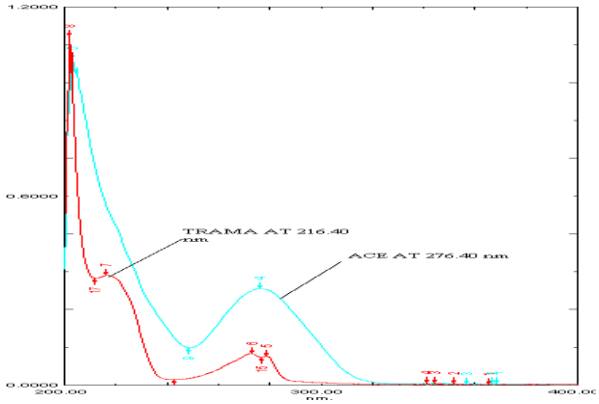

Figure 3 Zero order overlain spectra of TRAMA (10µg/ml) and ACE (10µg/ml)

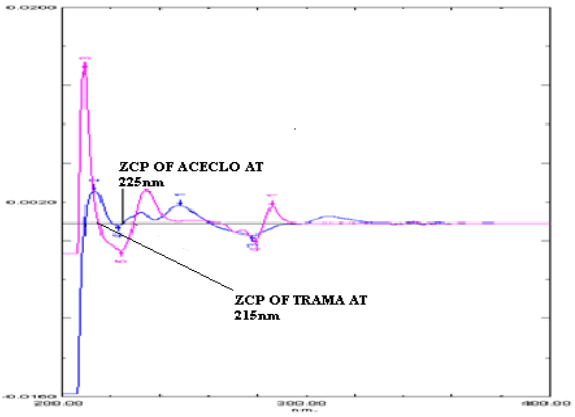

Figure 4 Overlain second order derivative spectra of TRAMA (225 nm) and ACE (215 nm)

The linearity of the method was established from second derivative spectra by measurement of the absorbance of standard solutions. The calibration curves were constructed by plotting the D_2 value against TRAMA and ACE concentration at the zero-crossing wavelength of ACE (215 nm) and TRAMA (225 nm) respectively.

Linear correlation was obtained between absorbances and concentrations of TRAMA and ACE in the concentration ranges of 5-80 µg/ml and 4-28 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The % RSD values of interday (0.3945 – 1.3558 and 0.8408 – 1.4678 for TRAMA at 225 nm and ACE at 215 nm, respectively) and intraday (0.3892 – 1.1364 and 0.6966 – 1.1985 for TRAMA at 225 and ACE at 215 nm, respectively). The % RSD values for TRAMA and ACE, reveal that the proposed method is precise. LOD and LOQ values for TRAMA were found to be 0.7909 and 2.3968 µg/ml at 225 nm. LOD and LOQ values for ACE were found to be 0.2485 and 0.7531 µg/ml at 215 nm. These data show that method is sensitive for the determination of TRAMA and ACE. 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		TRAMA	ACE
Wavelength range (nm)		225	215
Beer's law limit (µg/ml)		10 - 80	4 - 28
Regression equation (y = a + bc)		y = 0.00021x - 0.00037	y = 0.00023x +0.00104
Slope (b)		0.00021	0.00023
Intercept (a)		0.00037	0.00104
Correlation Coefficient (r^2)		0.998	0.999
Accuracy (Recovery) (n = 3)	Level I	101.64±1.83	100.36± 1.57
	Level II	99.51±1.47	100.58 ± 1.26
	Level III	99.86 ± 1.22	100.72 ± 1.81
Method precision (Repeatability) (% RSD, n = 6),		0.4902	1.7614
Interday (n = 3) (% RSD)		0.3945 – 1.3558	0.8408 – 1.4678
Intraday(n = 3) (% RSD)		0.3892 –1.1364	0.6966 – 1.1985
LOD (µg/ml)		0.7909	0.2485
LOQ (µg/ml)		2.3968	0.7531
Assay ± S. D. (n = 3)		100.12 ± 1.36	100.22 ± 1.19

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. S. D. is standard deviation

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.12 ± 1.36 and 100.22 ± 1.19 for TRAMA and ACE, respectively (Table 2).

Table 2 Recovery data of proposed method

Drug	Level	Amount taken(µg/ml)	Amount added(%)	% Mean recovery ± S.D. (n = 3)
TRAMA	I	37.5	80	101.64 ± 1.83
	II	37.5	100	99.51 ± 1.47
	III	37.5	120	99.86 ± 1.22
ACE	I	20	80	100.36± 1.57
	II	20	100	100.58 ± 1.26
	III	20	120	100.72 ± 1.81

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

The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine TRAMA and ACE in their combined dosage form. The results obtained for TRAMA and ACE 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 TRAMA and ACE in combined dosage form.

Table 3 Analysis of TRAMA and ACE in combined dosage form

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D.(n = 6)	
	TRAMA	ACE	TRAMA	ACE	TRAMA	ACE
I	37.5	100	37.54	100.2	100.12 ± 1.36	100.22 ± 1.19

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

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