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Analytical Method Development and Validation of Cetirizine Hydrochloride and Phenylephrine Hydrochloride in Combined Dosage Form

Mayank K. Patel, K. Jessi Kala Veni

Department of Quality Assurance, Rofel Shri G. M. Bilakhia College of Pharmacy, Vapi,
Gujarat Technological University, Gujarat, India.

ABSTRACT:

A newer, simple, rapid, accurate, precise and sensitive Absorbance ratio and Area under curve methods are developed for the simultaneous estimation of Cetirizine Hydrochloride (CTZ HCl) and Phenylephrine Hydrochloride (PHE HCl) in combined dosage form. The methods employed were I) absorbance ratio and II) area under curve (AUC) method. Beer's law was obeyed in concentration range of 5-25 µg/ml for both drugs and for both proposed methods. The first developed method makes use absorbance ratio method using wavelength at 219 nm (isoabsorptive point) and 230 nm (λ_{max} of CTZ HCl). The second method is area under curve method in which Sampling wavelengths range selected are 225-235 nm and 268-278 nm with linearity for CTZ HCl and PHE HCl respectively. The results of the analysis were analyzed and validated statistically and recovery studies were carried out as per ICH guidelines. It can be used for routine analysis of both drugs in bulk as well as in pharmaceutical formulations.

KEY WORDS: Cetirizine Hydrochloride (CTZ HCl), Phenylephrine Hydrochloride (PHE HCl), Absorbance ratio method (Q-ratio), Area under curve method (AUC)

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For Correspondence:

Mr. Mayank K. Patel

Department of Quality Assurance, Rofel Shri
G. M. Bilakhia College of Pharmacy, Vapi,

Gujarat Technological University, Gujarat,
India.

Email: mayank_patel75@ymail.com

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INTRODUCTION:

Cetirizine Hydrochloride (CTZ HCl), 2-(2-{4-[(4-chlorophenyl) (phenyl) methyl] piperazine-1-yl} ethoxy) acetic acid hydrochloride is selective Histamine H-1 antagonist used primarily as an anti-allergic agent. Phenylephrine Hydrochloride is 3 - [(1R)-1- hydroxyl -2- (methylamino) ethyl] phenol hydrochloride. It is a selective α -1 adrenergic receptor agonist used primarily as a decongestant [1, 2]. Liquid chromatography is the only available official method for the estimation of both drugs in single dosage forms [3]. CTZ HCl and PHE HCl combination is not official in any pharmacopoeia, hence no official method is available for estimation of these two drugs in combined dosage forms.

Literature survey revealed two spectroscopic and one RP-HPLC and one HPTLC methods reported for determination of the drug [4-8]. The aim of the present work was to develop simple, sensitive, accurate, and precise methods for routine analysis. The proposed method was validated according to ICH guidelines [9].

MATERIALS AND METHODS

Apparatus

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched

quartz cells. All weighing was done on Mettler Toledo electronic analytical balance.

Reagents and chemicals

Cetirizine Hydrochloride (CTZ HCl) and Phenylephrine Hydrochloride (PHE HCl) was kindly supplied as a gift samples from Vapi Care Pharma Pvt. Ltd., Vapi, Gujarat (India).

Water used as solvent and all calibrated glass wares were used throughout the work.

Marketed formulation

Combined Tablet formulation Allercet-DC was purchased from the local market.

Preparation of standard solution

The standard stock solution of CTZ HCl and PHE HCl was prepared by dissolving 100 mg of each API in 100 ml of different volumetric flask in water and volume make up with water to produce 1000 µg/ml of each solution. 10ml of aliquot was taken in 100ml volumetric flask and diluted with water to prepare standard stock solution of 100 µg/ml of each.

Selection of analytical wavelength

Standard solutions of CTZ HCl (20 µg/ml) and PHE HCl (20 µg/ml) were scanned in the range of 200 to 400 nm for the determination of wavelength having maximum absorbance. Cetirizine HCl shows 230 nm and Phenylephrine HCl shows 273 nm as the wavelength having maximum absorbance. From the overlain spectra, Isoabsorptive point was found at 219 nm. For the absorbance ratio method 219 nm and 230 nm were selected as analytical wavelengths For Area under curve method wavelengths range selected are 225-235 nm and 268-278 nm with linearity for CTZ HCl and PHE HCl respectively.

Methods:

Method I): Absorbance Ratio Method [10]

From overlain spectra (Fig 1.) 219 nm (Isoabsorptive point) and 230 nm λmax for CTZ HCl were selected for formation of Absorbance ratio equation of two drugs. The absorbance at 219 nm and 230 nm for CTZ HCl and PHE HCl were measured. The absorptivity values of each drug at both wavelengths were determined. The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$C_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A}{A_{X1}} \quad C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A}{A_{Y1}}$$

Q_M, Q_X, and Q_Y were obtained as below:

$$Q_M = A_2/A_1,$$

$$Q_X = a_{x2}/a_{x1},$$

$$Q_Y = a_{y2}/a_{y1}$$

Where, A₁ and A₂ were absorbance of sample at 219 nm and 230 nm respectively,

a_{x1} and a_{x2} are absorptivity of CTZ HCl at 219 nm and 230 nm,

a_{y1} and a_{y2} are absorptivity of PHE HCl at 219 nm and 230 nm.

Validity of above framed equation was checked using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.

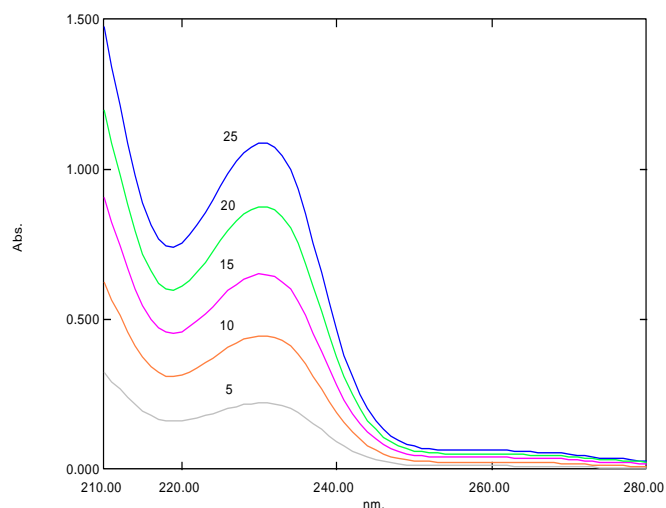


Figure 1: Linearity spectra of CTZ HCl at 230 nm

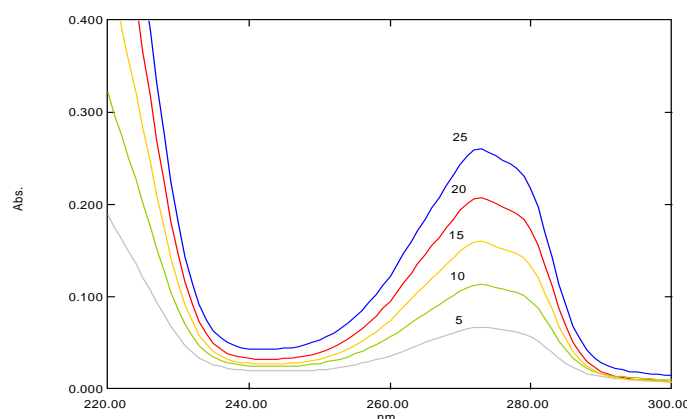


Figure 2: Linearity spectra of PHE HCl at 273 nm

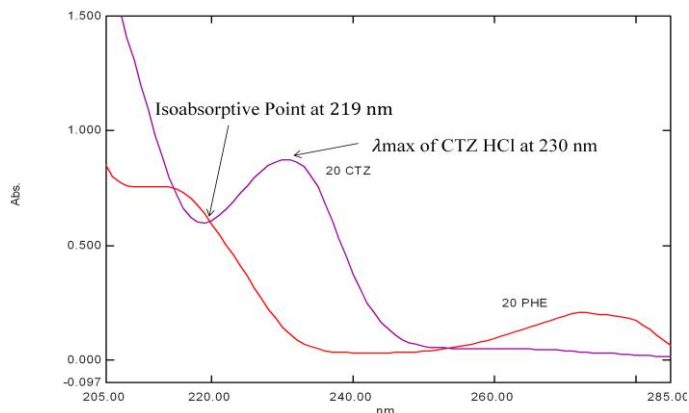


Figure 3: Overlain spectra of CTZ HCl (20 µg/ml) and PHE HCl (20 µg/ml)

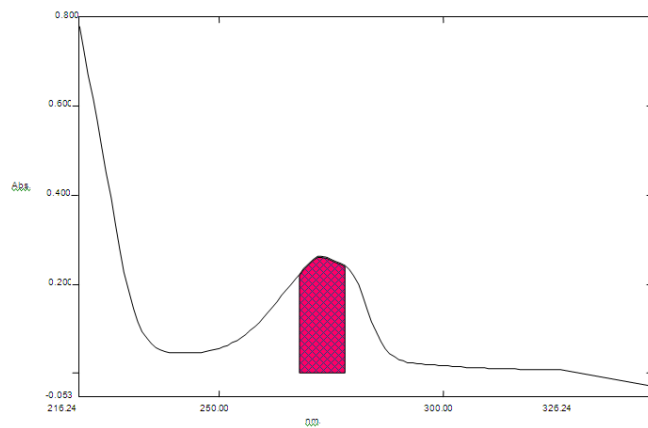


Figure 5: AUC spectra of PHE HCl at 268-278 nm

METHOD II): AREA UNDER CURVE METHOD (AUC) [11]

From overlain spectra (Fig 4-6.) 225-235 nm and 268-278 nm were selected as the two sampling wavelength intervals of CTZ HCl and PHE HCl respectively for area under curve method. The area at 225-235 nm and 268-278 nm for CTZ HCl and PHE HCl were measured. The absorptivity values of each drug at both intervals were determined. The area and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$A1 = ax1 C_{(CTZ)} + ay1 C_{(PHE)} \quad \dots \text{at } 225-235 \text{ nm}$$

$$A2 = ax2 C_{(CTZ)} + ay2 C_{(PHE)} \quad \dots \text{at } 268-278 \text{ nm}$$

Where;

ax1 and ax2 are mean absorptivity value of CTZ HCl at 225-235 nm and 268-278 nm respectively.

ay1 and ay2 are mean absorptivity value of PHE HCl at 225-235 nm and 268-278 nm respectively.

A1 and A2 are the AUC of mixed standard of sample solution at 225-235 nm and 268-278 nm respectively.

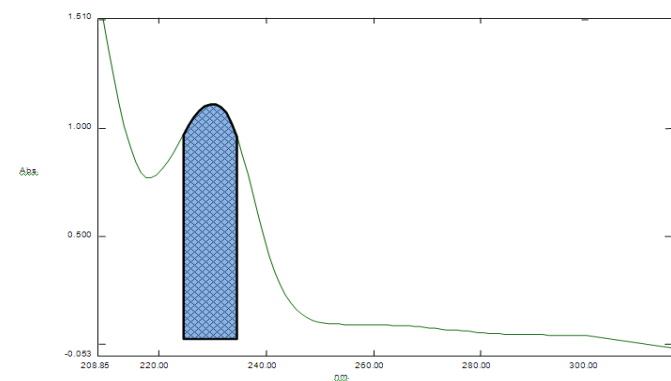


Figure 4: AUC spectra of CTZ HCl at 225-235 nm

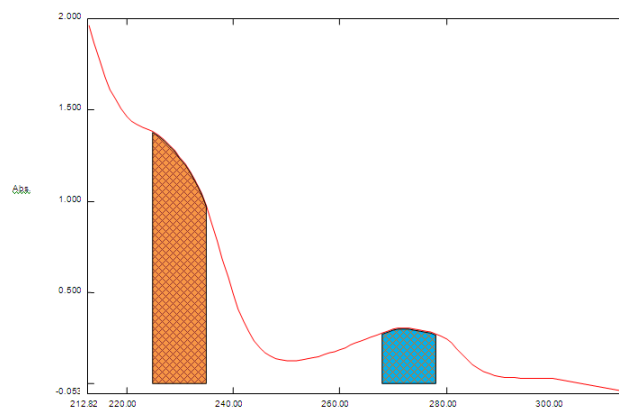


Figure 6: AUC spectra of Mixture at 225-235 nm and 268-278 nm

Validation of the proposed method

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 5-25 µg/ml for CTZ HCl and PHE HCl. Accurately measured standard stock solutions of both the drugs individually (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with water. The absorbance of solution was measured at 219 nm and 230 nm for Absorbance ratio method and area was measured at 225-235 nm and 268-278 nm. The calibration curves were constructed by plotting absorbance versus concentration for absorbance ratio method and area versus concentration for AUC method (Table 1).

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of CTZ HCl (1.5 µg/ml) and PHE HCl (1.5 µg/ml) without changing the parameters of both methods (Table 1).

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of both the drugs (1.0, 1.5 and 2.0 µg/ml). The results were reported in Table 1 in the terms of relative standard deviation (% CV).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of CTZ HCl and PHE HCl by the standard addition method. Known amount of standard solutions of CTZ HCl and PHE HCl were added to prequantified sample solutions of both the drugs (1.5µg/ml). The amounts of CTZ HCl and PHE HCl were obtained by applying regression line equations (Table 2).

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.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of Y-intercept of 6 calibration curves and

S = the mean slope of the 6 calibration curves.

Assay of tablet formulation

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 10 mg of CTZ HCl and 10 mg of PHE HCl was transferred to a 100 ml volumetric flask and make up to volume 100 ml with water. Sonicate it for 10min. The working solution was filtered through whatman filter paper (No. 41) and the volume was made up to the mark with the same solvent. The aliquot portions of above solutions were further diluted with solvent to get final concentration of about 1.5 µg/ml of CTZ HCl and 1.5 µg/ml PHE HCl and absorbance were measured at 219 nm and 230 nm against blank for Absorbance ratio method and the Area were measured at 25-235 nm and 268-278 nm for AUC method. The concentrations of two drugs in sample were determined and results are reported in the Table 3.

RESULT AND DISCUSSION

The proposed both methods were validated as per ICH guideline. Methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of CTZ HCl and PHE HCl. In Absorbance ratio method, wavelengths selected were 219 nm (isoabsorptive point) and 230 nm (λ_{max} of CTZ HCl). The plot of absorbance versus respective concentrations of CTZ HCl and PHE HCl were found to be linear in the concentration range of 5-25 µg/ml for CTZ HCl and PHE HCl with correlation coefficient 0.9999 at 219 nm and 0.9998 at 230 nm for CTZ HCl and 0.9999 at 219 and 0.9999 at 230 for PHE HCl as shown in table 3 and figures 5-8 for absorbance ratio method Correlation coefficient 0.9999 at 225-235 nm and 0.9997 at 268-278 nm for CTZ HCl and 0.9992 at 225-235 nm and 0.9995 at 268-278 nm for PHE HCl for as shown in table 3 and figures 9-12 for AUC method. Precision was calculated in terms of repeatability, intraday and interday variations and % CV (coefficient of variance) was found to be in acceptance range (Table 1). The accuracy of method was determined by standard addition method. The % recovery ranges from 98.74-99.87% for CTZ HCl and 99.00-99.89% for PHE HCl (Table 2).

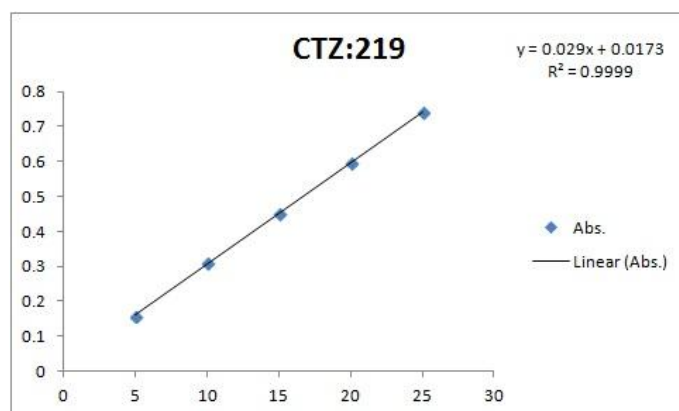


Figure 7: Calibration curve of standard CTZ HCl at 219 nm

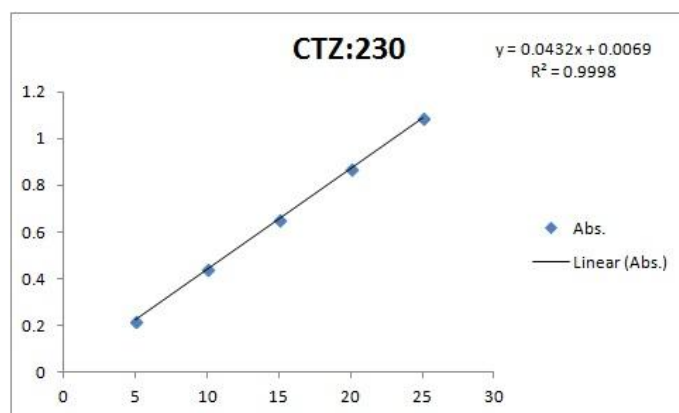


Figure 8: Calibration curve of standard CTZ HCl at 230 nm

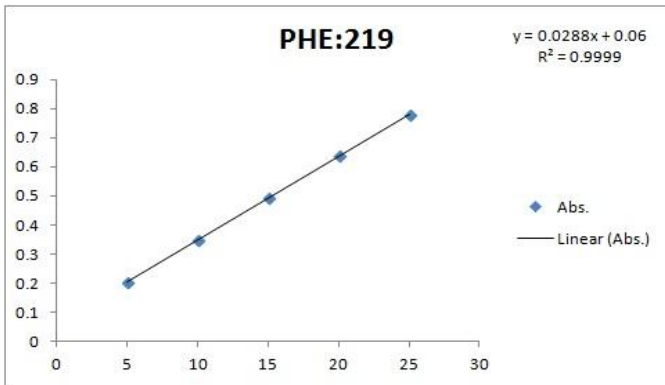


Figure 9: Calibration curve of standard PHE HCl at 219 nm

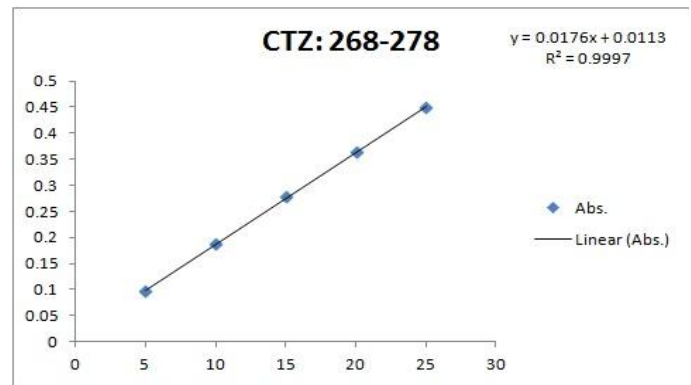


Figure 12: Calibration curve of standard CTZ HCl at 268-278 nm

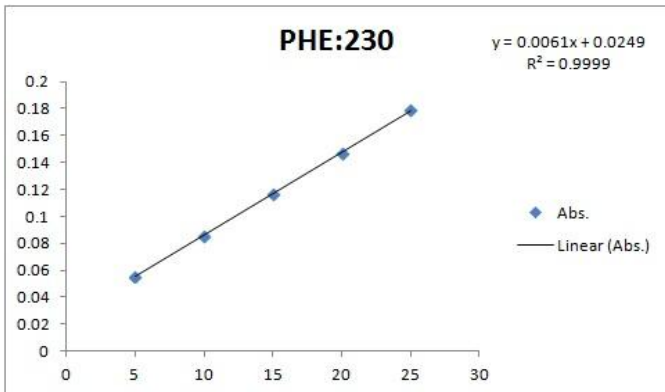


Figure 10: Calibration curve of standard PHE HCl at 230 nm

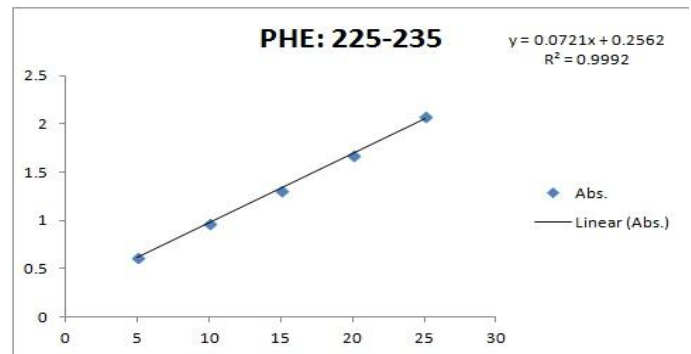


Figure 13: Calibration curve of standard PHE HCl at 225-235 nm

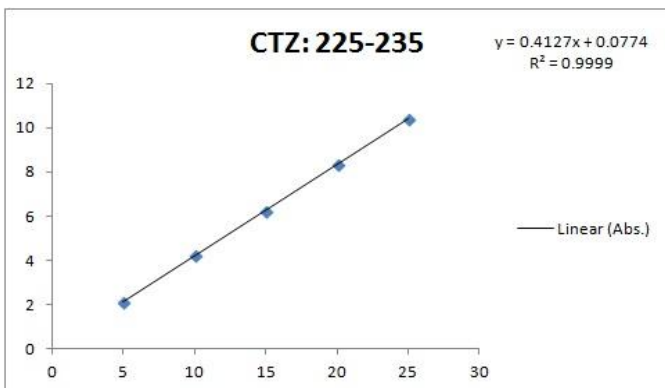


Figure 11: Calibration curve of standard CTZ HCl at 225-235 nm

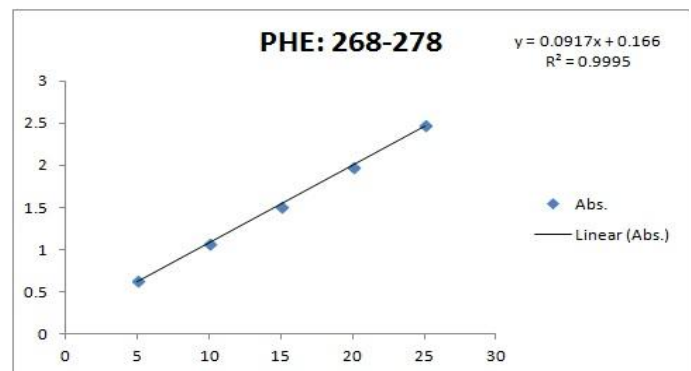


Figure 14: Calibration curve of standard PHE HCl at 268-278 nm

Table 1: Validation parameters

Parameters (Absorbance ratio)	CTZ HCl		PHE HCl	
	219nm	230nm	219nm	230nm
Conc. Range (µg/ml)	5-25	5-25	5-25	5-25
Regression Equation	0.029x + 0.0173	0.0432x + 0.0069	0.0288x + 0.06	0.0061x + 0.0249
Slope (m)	0.029033	0.0432	0.02875	0.006083
Intercept (C)	0.000804	0.000463	0.000843	0.001017
Regression coefficient (r ²)	0.9999	0.9998	0.9999	0.9999
Intraday Precision (n = 3) % R.S.D.	0.127263 – 0.192557	0.066033 – 0.15361	0.166704 – 0.180799	0.49206 – 1.332347
Interday Precision (n = 3) % R.S.D.	0.167224 – 0.221239	0.066159 – 0.154083	0.090874 – 0.203252	0.704225 – 1.847813
Repeatability (n = 6) % R.S.D.	0.113828	0.137393	0.16506	0.440112
LOD (µg/ml)	0.091386	0.035368	0.096762	0.551718
LOQ (µg/ml)	0.276926	0.107176	0.293217	1.671872

Parameters (AUC)	CTZ HCl		PHE HCl	
	225 – 235 nm	268 – 278 nm	225 – 235 nm	268 – 278 nm
Conc. Range ($\mu\text{g/ml}$)	5-25	5-25	5-25	5-25
Regression Equation	$0.4127x + 0.0774$	$0.0176x - 0.0113$	$0.0721x + 0.2562$	$0.0917x + 0.166$
Slope (m)	0.412717	0.017633	0.072133	0.0917
Intercept (C)	0.000716	0.00055	0.001097	0.000779
Regression coefficient (r^2)	0.9999	0.9997	0.9992	0.9995
Intraday Precision (n = 3) % R.S.D.	0.006906 – 0.027123	0.206689 – 0.306558	0.034278 – 0.131017	0.029032 – 0.065703
Interday Precision (n = 3) % R.S.D.	0.006909 – 0.016069	0.316645 – 0.427081	0.0344 – 0.075873	0.038017 – 0.142316
Repeatability (n = 6) % R.S.D.	0.008291	0.195965	0.088508	0.033921
LOD ($\mu\text{g/ml}$)	0.005725	0.102932	0.050186	0.028034
LOQ ($\mu\text{g/ml}$)	0.017348	0.311915	0.15208	0.084951

Table2: Recovery study

Concentration ($\mu\text{g/ml}$)		Spiked level ($\mu\text{g/ml}$)		Percent recovery % \pm SD (n=3)	
CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
15	15	0	0	-	-
15	15	12	12	98.74 % \pm 0.0100	99.00 % \pm 0.0116
15	15	15	15	99.36 % \pm 0.0152	99.50 % \pm 0.0252
15	15	18	18	99.87 % \pm 0.0057	99.89 % \pm 0.0057

Table 3: Results of simultaneous estimation of CTZ HCl and PHE HCl in marketed formulation

Marketed Formulation (Tablet)	Labeled claim		Amount Obtained		% Assay	
	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl	CTZ HCl	PHE HCl
Allercet-DC	10	10	9.83	9.85	98.30%	98.50%

This method can be successfully used for simultaneous estimation of CTZ HCl and PHE HCl in their combined tablet dosage form. Marketed tablets were analysed and results obtained were within the range of 98-102% (Table 3).

CONCLUSION

The low value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage forms without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the simultaneous estimation of these two drugs in combined dosage form.

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