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# DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN COMBINED DOSAGE FORM

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## ABSTRACT

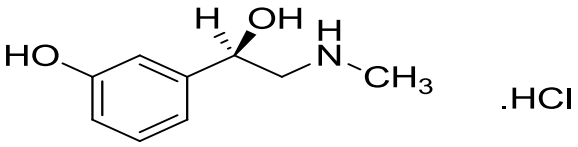
Three simple, accurate, rapid and precise uv spectrophotometric methods have been developed for simultaneous estimation of phenylephrine hydrochloride and cetirizine hydrochloride in combined tablet formulation. First Method is dual wavelength method, in which two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Cetirizine hydrochloride has equal absorbance at 222.6 nm and 236.4nm. Where the differences in absorbance were measured for the determination of phenylephrine hydrochloride; similarly differences in absorbance at 231.8nm and 260.8nm were measured for the determination of Cetirizine hydrochloride. Second method is mean centering of ratio spectra (MCR) in which absorption spectra of each drug were recorded, divided by suitable divisor and the obtained ratio spectra were mean centered. Third method based upon absorption ratio method which involves measurement of absorbance at 246.0nm (iso-absorptive point) and 230.2nm ($\lambda$max of cetirizine hydrochloride). These methods were validated as per ICH norms.

**KEYWORDS:** *Dual wavelength method, Mean centering of ratio spectra, Absorbance ratio method, Validation.*

## 1. INTRODUCTION

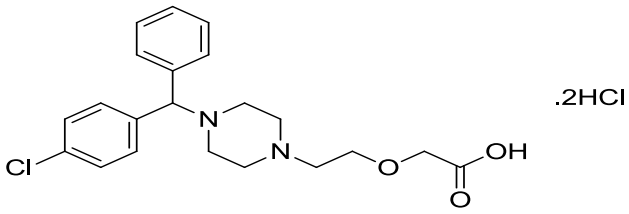
Phenylephrine hydrochloride (PHE), chemically (R)-l-(3-hydroxyphenyl)-2methylaminoethanol hydrochloride (Fig.1)[1] is a direct sympathomimetic agent, a selective $\alpha$ lagonis, and causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations.

Cetirizine hydrochloride (CTZ), chemically [2-[4-[(4-chlorophenyl)phenymethyl]-1 piperazinyl]ethoxy] acetic acid (Fig.2)[2], belongs to the group of second generations antagonists of H1-receptors, inhibits the allergic reaction mediated by histamine. It is a non-sedative antihistamine, used in the treatment of seasonal rhinitis, hay fever, running nose, control sneezing of allergic origin.




**Phenylephrine hydrochloride**

**Fig.1 Chemical structure of Phenylephrine hydrochloride.**




**cetirizine hydrochloride**

**Fig. 2: Chemical structure of Cetirizine hydrochloride.**

Literature survey revealed that a number of methods have been reported for estimation of PHE[3] and CTZ[4-6] [individually. These drugs available in combination with other drugs have also been simultaneously estimated by UV. HPLC. There is only one reference available for simultaneous determination of doses forms by UV spectroscopy using simultaneous equation and first order derivative methods. In combination with other drugs.[3-7] However, there is no Dual wavelength, Mean centering of ratio spectra and Absorbance ratio reported for the simultaneous estimation of PHE and CTZ in a combined dosage formulation. Although methods like Dual wavelength method, Absorption ratio method and mean centering of ratio offer simple, sensitive and accurate methods for simultaneous estimation of drugs, simultaneous determination using above methods have not been reported yet. This

prompted the authors to carry out the work in these lines. The successful results are obtained and communicated in this paper.

## 2. MATERIAL AND METHOD

**Instrumentation**: A double-beam Elico UV-Visible spectrophotometer model SL 210 with a pair of 1-cm matched quartz cells and UV-PC personal software version 4.01.01 was used.

**Pure samples:** Phenylephrine hydrochloride and Cetirizine hydrochloride were obtained as a gift sample from Hetero drugs Pvt. Ltd. Distilled water was used to prepare all solutions.

### Preparation of standard stock solution

Accurately weighed 40 mg of PHE and CTZ was transferred to 100.0 ml volumetric flask and 50.0 ml of distilled water was added to dissolve the drug and diluted up to the mark with distilled water, to get 400 $\mu$g mL$^{-1}$ of PHE and 400 $\mu$g mL$^{-1}$ of CTZ in separate volumetric flask. The standard stock solutions (400 $\mu$g mL$^{-1}$) were further diluted separately to obtain working standard of concentration 28 $\mu$g mL$^{-1}$ of CTZ and 200 $\mu$g mL$^{-1}$of PHE.

### 3.1 Spectral characteristics and wavelengths selection

The absorption spectra of 100 $\mu$g mL$^{-1}$of PHE and 14 $\mu$g mL$^{-1}$ were recorded over the range of 200-300 nm using water as a blank. From the overlay spectra (Figure.3) suitable wavelengths for Dual wavelength method, mean centering of ratio spectra and Absorption ratio methods have been selected. The $\lambda_{max}$ value of Phenylephrine hydrochloride is 273.2 nm and $\lambda_{max}$ of Cetirizine hydrochloride is 230.2 nm.

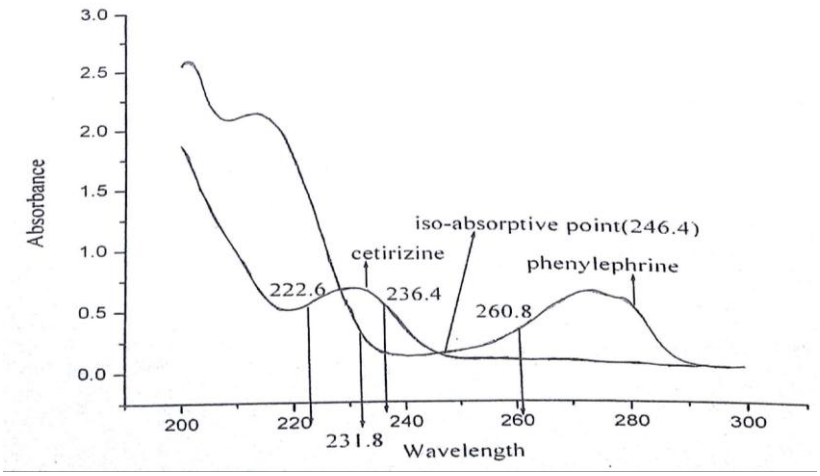



**Figure 3: Overlay spectra of Phenylephrine hydrochloride and Cetirizine hydrochloride.**

### 3.2 LINEARITY

#### 3.2.1 Method 1: Dual wavelength method

Different aliquots equivalent to 20-140 and 2.8.-19.6$\mu$g mL$^{-1}$of PHE and CTZ were separately transferred from their respective standard working solutions into two separate series of 10 mL volumetric flasks and then the volume was completed using distilled water. They were scanned in the wavelength range of 200-300 nm. From the overlain spectra (Figure.3), four wavelengths 222.6 nm, 231.8nm, 236.4nm and 260.8nm were selected for quantization of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of CTZ is carried out by measuring the absorbance difference value at between 231.8nm and 260.8nm where PHE has same absorbance at both the wavelength. The difference between 231.8nm and 260.8nm is directly proportional to concentration of CTZ in the mixture. The quantitative determination of PHE is carried out by measuring the absorbance difference value 222.6nm and 236.4nm where CTZ has same absorbance at both the wavelength. The difference between 222.6nm and 236.4nm is directly proportional of PHE in the mixture.

#### 3.2.2. Method II: Mean centering of ratio spectra (MCR)

Aliquots of PHE equivalent to 20-140 $\mu$g mL$^{-1}$were accurately transferred from its standard working solution into a set of 10mL measuring flasks and the volume was adjusted using water. The absorption spectra of the prepared solutions were recorded in the range of 200-300nm, were divided by the standard spectrum of 16.8 $\mu$g mL$^{-1}$ of CTZ and then the obtained ratio spectra mean centered. By the same way the spectra of different concentrations of standard solutions of CTZ in the range of 2.8-19 $\mu$g mL$^{-1}$ o were recorded. The stored spectra were divided by the standard spectrum of 120 $\mu$g mL$^{-1}$ of PHE to obtain the ratio spectra which were the mean centered. Calibration curves for both PHE and CTZ were constructed by plotting the amplitude values of their respective mean centered ratio spectra (peak to peak) against their corresponding concentrations.

#### 3.2.3. Method III: Absorption ratio method

It uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the $\lambda_{max}$ of one of the two components. From the overlay spectra of two drugs (Figure.3), it is evident that PHE and CTZ show an isoabsorptive point at 246.4nm. The second wavelength used is 230.2nm, which is the $\lambda_{max}$ of CTZ. Seven working standard

solutions having concentration 20,40,60,80, 100, 120 $\mu$g/ml for PHE and 2.8, 5.6,8.4, 11.2, 14, 16.8, 19.6 $\mu$g /ml for CTZ were prepared in distilled water and the absorbances at 246.4 nm (isoabsorptive point) and 230.2nm ($\lambda_{max}$ of CTZ) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance / Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

$C_p = [(Q_M - Q_c)/ (Q_p - Q_c)] \times A_1/aX_1$…… (1)

$C_C = (A_1/ aX_1) - C_p$……(2)

Where, $A_1$ and $A_2$ are absorbances of mixture at 246.4 nm and 230.2 nm;

$aX_1$ and $aY_1$ and absorptivities of PHE and CTZ at 246.4 nm;

$aX_2$ and $aY_2$ and absorptivities of PHE and CTZ respectively at 230.2 nm;

$Q_M = A_2/ A_1$

$Q_p = aX_2/ aX_1$ and $Q_c = aY_2/ aY_1$

### 3.3. Analysis of laboratory prepared mixtures

Zero order absorption spectra of different laboratory prepared mixtures containing different ratios of PHE and CTZ were recorded using distilled water as blank and the procedure under linearity for each method was then followed. Concentrations of PHE and CTZ in the prepared samples were calculated from the computed regression equations.

### 3.4. Analysis of the pharmaceutical dosage form

An accurately weighted quantity (40 mg) of PHE and CTZ was transferred to a 100-mL volumetric flask and 75 mL of distilled water was added and ultrasonicated for 20 min, volume was then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatmann filter paper no.42. Appropriate dilutions of the prepared solution were made to prepare its working solution and the procedures under linearity were followed.

#### 3.4.1. Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by application of the standard addition technique (Table.2). Known amounts of the studied drugs were separately added to a definite amount of the powdered tablet; the prepared samples were then analyzed as under linearity and the percentage recoveries were then calculated.

## 4. RESULTS AND DISCUSSION

PHE and CTZ drugs act as anti Allergic and anti Cold. Hence it is very important to develop analytical methods which are not only accurate, precise, and rapid but also simple and economic for determination of the studied drugs in their pharmaceutical dosage form and this is the main task of the developed spectrophotometric methods. Since UV-spectrophotometric methods have the advantages of saving time and cost when compared to the HPLC technique, this work concerns with the development and validation of three spectrophotometric methods, dual wavelength, mean centering of ratio spectra and absorption ratio methods for determination of the suggested drugs. Moreover, the suggested methods provide a simple, rapid, sensitive and accurate way for simultaneous analysis of PHE and CTZ in their combined dosage without derivatization step.

### 4.1 Dual wavelength method

The developed dual wavelength method provides a simple method for selective determination of both PHE and CTZ using their zero order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest independent to the interfering component. The pre-requisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value while the component of interest shows significant difference in absorbance with concentration. Using the absorbance values at 222.6 and 236.4nm (where CTZ has the same absorbance) gave the best selectivity when used for determination of PHE. On the other hand absorbance values at 231.8 and 260.8nm were chosen for determination of CTZ where the best results were obtained. Calibration curves for PHE and CTZ were constructed by plotting the difference in absorbance values at the selected wavelengths for each drug against their corresponding concentrations, PHE and CTZ obeyed Beer Lambert's law in the concentration ranges of 20-140 and 2.8-19.6$\mu$g $mL^{-1}$ for PHE and CTZ respectively with good correlation coefficients. Regression equation parameters are given in Table1.

### 4.2. Mean centering of ratio spectra

The developed MCR method is based on the mean centering of ratio spectra; the mathematical explanation of the developed method was illustrated by *Afkhami and Bahram.*[8] This method was applied for resolving binary and ternary mixtures in the complex samples with unknown matrices.[5] To optimize the developed MCR method, different parameters were tested. Since the wavelength range taken has a great effect on the obtained mean

centered ratio spectra, different wavelength ranges were tested and the best results were obtained when using the wavelength range from 200 to 300 nm for PHE and 200-280 nm for CTZ. The effect of divisor concentration on the selectivity was checked by testing several concentrations each of PHE (normalized spectrum, 20, 60, 80 and 120$\mu$gML$^{-1}$) and CTZ(normalized spectrum 5.6, 11.2, 14,16.8, 19.6 $\mu$gML$^{-1}$). The best results regarding sensitivity and selectivity were obtained using 120 and 16.8 $\mu$gML$^{-1}$ each of PHE and CTZ, respectively as divisors. To construct the calibration curves of the proposed method, the absorption spectra of the standard solutions of PHE with different concentrations were recorded in the wavelength range of 200-300 nm and divided by the standard spectrum of CTZ (16.8 $\mu$gML$^{-1}$). Then mean centering of the resulted ratio spectra (figure.4) has been obtained and the concentrations of PHE were determined by measuring the amplitude values of the mean centered ratio spectra (peak to peak) as shown in (figure.6). By the same way different standard solutions of CTZ with different concentrations were recorded and divided by the standard spectrum of PHE (120 $\mu$gML$^{-1}$) and the ratio spectra (figure.5) were obtained which were mean centered. The amplitude values (peak to peak) in the obtained mean centered ratio spectra were used for CTZ as shown in (figure.7). The computed regression equation parameters for each of the studied drugs are given in Table1.

The developed spectrophotometric methods were also applied for determination of PHE and CTZ in Citrizet-D tablet without interferences from the excipients and satisfactory results were obtained. Standard addition technique was performed in order to asses the validity and accuracy of the methods where good percentage recoveries were obtained indicating no interference from excipients Table (2). The results obtained by applying the proposed methods were statistically compared with those obtained by applying the reported method[3] for determination of the proposed drugs in their pure forms and no significance differences were obtained between them. (Table.3).

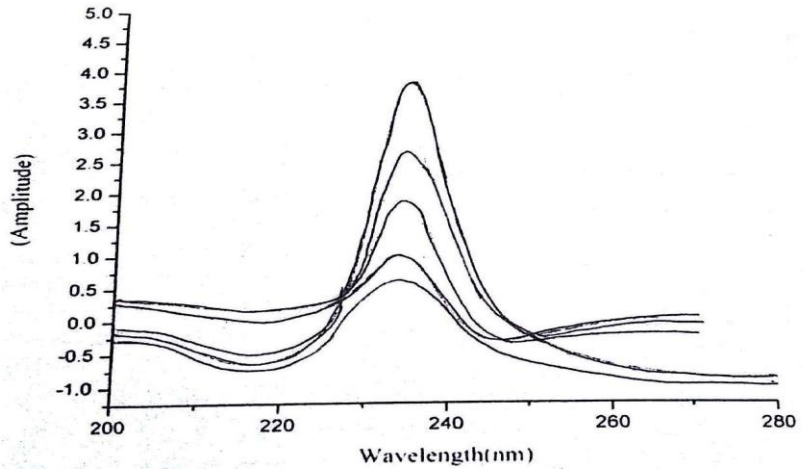



**Figure 4: Ratio spectra of PHE (20-140 $\mu$gML$^{-1}$) using 16.8 $\mu$g/ml of CTZ as a divisor and distilled water as blank.**

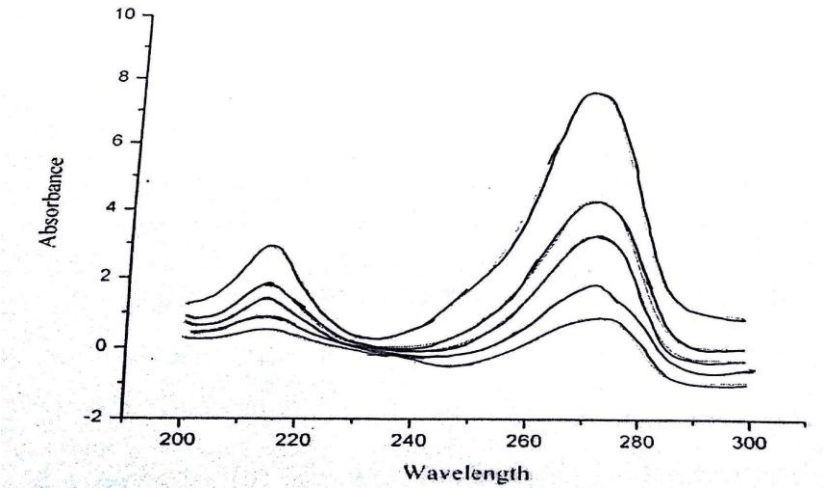



**Figure.5 Ratio spectra of CTZ (2.8-19.6 $\mu$gML$^{-1}$) using 120 $\mu$g/ml of PHE as a divisor and distilled water as blank.**

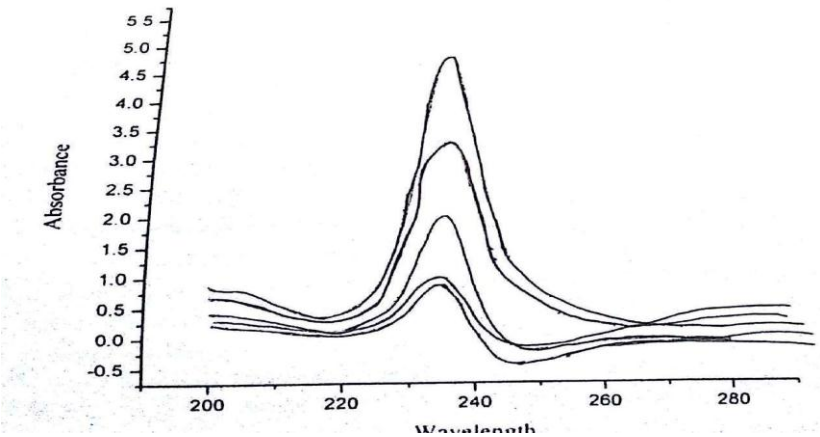



**Figure 6: Mean centered ratio spectra of PHE (20-140 $\mu$gML$^{-1}$) using 16.8 $\mu$g/ml of CTZ as a divisor and distilled water as blank.**

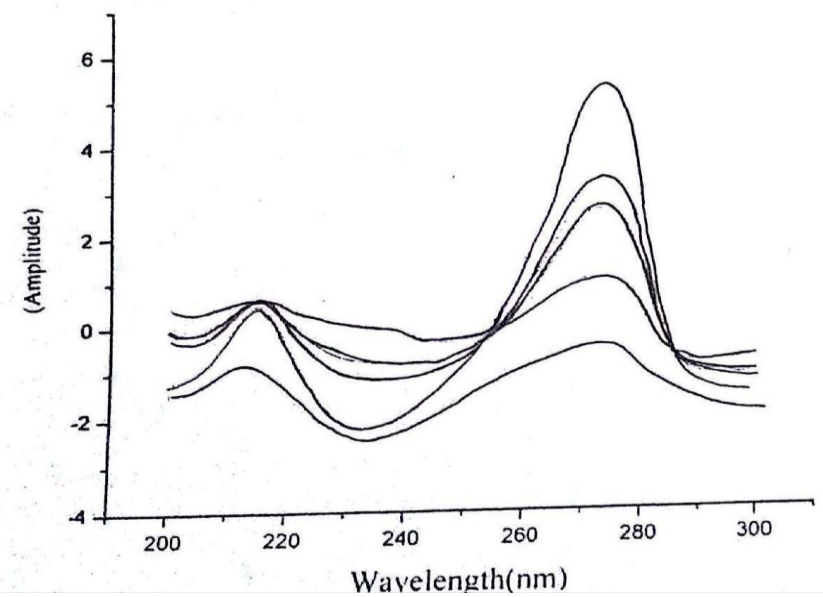



**Figure 7: Mean centered ratio spectra of CTZ (2.8-19.6 $\mu$gML$^{-1}$) using 120 $\mu$g/ml of PHE as a divisor and distilled water as blank.**

**Table 1: Regression and analytical parameters of the proposed dual wavelength method, MCR and Absorption ratio methods for determination of Phenylephrine hydrochloride and Cetirizine hydrochloride.**

| Parameters | Dual wavelength | | Mean centering of ratio spectra | | Absorbtion ratio method | |
|---|---|---|---|---|---|---|
| | PHE | CTZ | PHE | CTZ | PHE | CTZ |
| Range | 20-140 | 2.8-19.6 | 20-140 | 2.8-19.6 | 20-140 | 2.8-19.6 |
| Slope | 0.010 | 0.040 | 0.061 | 0.214 | 0.071 | 0.057 |
| Intercept | 0.007 | -0.006 | -0.550 | 0.034 | -0.0245 | -0.041 |
| Correlation Coefficient | 0.9985 | 0.9984 | 0.9964 | 0.9989 | 0.9979 | 0.9924 |
| Sandell sensitivity ($\mu$g cm$^{-2}$) | 0.100 | 0.025 | 0.016 | 0.0046 | 0.014 | 0.0175 |
| Accuracy (mean ±SD) | 99.97±1.658 | 100.1±.546 | 98.12±1.478 | 99.96±1.894 | 100.35±1.24 | 99.55±1.1256 |
| Precision<br>Repeatability | 99.97±0.987 | 99.85±0.981 | 100.0±0.984 | 99.58±1.25 | 99.87±1.254 | 100.07±0.874 |
| Intermediate precision | 100.88±0.84 | 100.15±0.97 | 99.44±0.877 | 100.45±1.04 | 100.76±0.97 | 100.44±0.657 |

**Table 2: Quantitative determination of PHE and CTZ in Citrizet-D tablets by dual wavelength method, MCR and Absorption ratio methods and application of standard addition technique.**

| Parameters | Dual wavelength | | Mean centering of ratio spectra | | Absorption ratio Method | |
|---|---|---|---|---|---|---|
| | PHE` | CTZ | PHE | CTZ | PHE | CTZ |
| Takenμg $mL^{-1}$ | 30 | 5 | 30 | 5 | 30 | 5 |
| Mean % | 100.05 | 99.58 | 99.87 | 100.96 | 99.87 | 99.95 |
| Added $\mu g\ mL^{-1}$ | 5<br>10<br>15 | 3<br>6<br>9 | 5<br>10<br>15 | 3<br>6<br>9 | 5<br>10<br>15 | 3<br>6<br>9 |
| % recovery[b] | 99.88<br>99.76<br>100.10 | 99.78<br>100.87<br>100.01 | 99.87<br>99.86<br>100.09 | 101.25<br>100.89<br>101.41 | 98.96<br>100.24<br>98.45 | 100.98<br>101.25<br>98.24 |
| Mean ≠SD | 99.91±0.1724 | 100.22±0.580 | 99.94±.130 | 101.41±0.266 | 99.21±0.922 | 100.15±1.665 |

Note: a-average of 6 determinations.

b- Average of 3 determinations.

**Table 3: Statiscal analysis of the proposed dual wavelength method, MCR and Absorption methods and reported one for determination of PHE and CTZ in their pure forms.**

| Parameters | Dual wavelength method | | Mean centering of ratio spectra | | Absorption ratio Method | | Reported method[3] | |
|---|---|---|---|---|---|---|---|---|
| | PHE | CTZ | PHE | CTZ | PHE | CTZ | PHE | CTZ |
| **Mean %** | 100.25 | 99.82 | 100.87 | 101.58 | 99.58 | 99.25 | 99.95±0.983 | 100.54±0.615 |
| **SD** | 1.025 | 0.987 | 0.974 | 0.784 | 0.754 | 0.843 | 0.983 | 0.615 |
| **N** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Student t-test (2.22)*** | 1.95 (2.477) | 0.784 (2.477) | 0.015 (2.477) | 0.416 (2.477) | 0.4534 (2.477) | 0.535 (2.477) | | |
| **F- Value** | 1.087 (4.28) | 2.575 (4.28) | 0.981 (4.28) | 1.625 (4.28) | 0.588 (4.28) | 1.878 (4.28) | | |

### 5. Method validation

Validation of the methods was carried out according to ICH recommendation.[9]

### 5.1. Linearity and range

The calibration range for PHE and CTZ was established through considerations of the practical range necessary according to adherence to Beer-lambert's law to give accurate, precise and linear results. Linearity ranges of PHE and CTZ are shown in (Table 1).

### 5.2 Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations and the results are shown in Table (1). Accuracy was further assessed by applying the standard addition technique to Citrizet-D tablets, where good recoveries were obtained revealing no interference from excipients Table (2).

### 5.3 Precision

#### 5.3.1. Repeatability

Three concentrations (20, 60 and 80 $\mu$g mL$^{-1}$ of PHE and 2.8, 8.4 and 11.2 $\mu$g mL$^{-1}$ of CTZ) were analyzed three times intra-daily using the proposed methods. Good results and acceptable relative standard deviations (RSDs) were obtained. Table (1).

#### 5.3.2. Intermediate precision

The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable RSDs values were obtained. Table (1).

## 6. CONCLUSION

These validated methods are new, rapid, accurate, precise, sensitive and reproducible and can be employed for routine analysis for simultaneous estimation Phenylephrine hydrochloride and Cetirizine hydrochloride in combined form.

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