Comparative evaluation of four spectrophotometric methods for the simultaneous determination of paracetamol and phenylephrine hydrochloride in fixed dose pharmaceutical formulations

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ABSTRACT

Four simple, rapid, accurate and precise spectrophotometric methods have been developed for the simultaneous estimation of paracetamol and phenylephrine hydrochloride in pharmaceutical preparations. Determinations were carried out using the graphical method, Vierordt’s simultaneous equation method, first order derivative and absorbance-ratio methods. Linearity was established in the concentration range of 1.0-12 µg/mL for paracetamol and 5.0-25 µg/mL for phenylephrine hydrochloride by all four methods. The limit of detection (LOD) for all the methods varied from 0.05 to 0.33 for paracetamol and 0.75 to 1.67 for phenylephrine hydrochloride. The methods were validated for their linearity, accuracy and precision, recovery and ruggedness according to the ICH guidelines. The performance of the methods was evaluated by analyzing synthetic mixture of the drugs and the results obtained were statistically compared by Student t-test and by the variance ratio F-test. Further, all four methods were applied to commercial pharmaceutical preparations without any interference of commonly used excipients and additives.

INTRODUCTION

Paracetamol (PAR, N-(4-hydroxyphenyl) acetamide) is an analgesic drug, which is extensively used to alleviate headaches, body-ache and postoperative pain. Paracetamol was firstly introduced as a medicine by Von Mering in 1893 and has since been used as an analgesic for more than three decades. It is accepted as a very effective treatment for the relief of pain and fever in adults and children (Bosch et al., 2006). Phenylephrine hydrochloride (PHE) chemically is (1R)-1-(3-hydroxy-phenyl)-2-(methylamino) ethanol hydrochloride and is a selective α1-adrenergic receptor agonist (Martindale: The Extra Pharmacopoeia, 1996). PHE is widely used as a decongestant, as a mydriatic agent to dilate the pupil and as a vasopressor to increase blood pressure. PHE as a decongestant is sold as an oral medicine (tablets, capsules or sachets) and as a nasal spray or drops. These substances are frequently present in pharmaceutical formulations and are used against nasal congestion and for symptoms of sinus.

Several methods are reported for the determination of PHE and PAR either alone or in combination, including spectrophotometry (Ahmed and Amin, 2007; Beyene and Van Staden, 2004; Bosch et al., 2006; Knochen and Giglio, 2007).
Fani et al., 2004; Sirajuddin et al., 2007) electrochemical (Fan et al., 2011; Ghadimi et al., 2013; Özcan and Sahin, 2011; Khaskheli et al., 2013) and chromatographic techniques (McEvoy et al., 2007; Mostafa, 2010; Németh, 2008). The rationale for the present work was to develop and validate four accurate, precise, simple and economical spectrophotometric methods for the simultaneous determination of PAR and PHE in their combined dosage forms.

The methods have been developed and validated as per the ICH guidelines (ICH Guidelines, 2005) for simultaneous estimation of PAR and PHE in synthetic mixtures as well as in pharmaceutical preparations. In addition, all the four methods showed absence of interferences due to presence of commonly used excipients and additives.

MATERIALS AND METHODS

Instrumentation and analysis conditions

A Shimadzu UV-1700 double beam spectrophotometer (Kyoto, Japan) with matched 10 mm quartz cells was used for spectral measurements. The wavelength accuracy was within ± 0.5 nm and a bandwidth of 1 nm was kept for all the methods. Data processing was done with Shimadzu UV PC software version 2.0. The spectra were recorded at a scan speed of 400 nm min⁻¹. Weighing of samples was done on a Sartorius GD503 (Bradford, MA, USA) analytical balance having a readability of 0.0001 g. For studying the ruggedness of the methods, HITACHI 3210 UV-Visible Spectrophotometer (Tokyo, Japan) was also used.

Chemicals and materials

Reference standards of paracetamol (99.38%) and phenylephrine hydrochloride (99.13%) were obtained from Clearsynth Labs (P) Ltd. (Mumbai, India). Spectroscopic grade methanol was procured from E. Merck (Mumbai, India). Five pharmaceutical formulations of PAR and PHE, Contac C® (500 mg PAR + 10 mg PHE tablet, GlaxoSmithKline, PA, USA), Robitussin® nasal relief® (325 mg PAR + 5 mg PHE tablet, Pfizer, Madison, NJ, USA), Excedrin® Sinus Headache (325 mg PAR + 5 mg PHE tablet, Novartis Farmaceutica, S.A. DE C.V., México), Feniapal® (100 mg PAR + 5 mg PHE capsule, Novartis Farmaceutica, S.A. DE C.V., México) and Vicks® DayQuil Sinex® (325 mg PAR + 5 mg PHE capsule, Procter & Gamble Hygiene & Healthcare Ltd., LA, USA) were purchased from a local pharmacy.

Standard stock and calibrations standards

Separate stock solutions of PAR and PHE equivalent to 200 μg mL⁻¹ were prepared by dissolving 20 mg of each reference standard in 100 mL methanol. Calibration standards for PAR and PHE were prepared in the concentration range of 1.0-12 μg mL⁻¹ for PAR and 5.0-25 μg mL⁻¹ for PHE from their respective stock solutions in methanol.

Graphical method

This method is generally useful when the individual spectra are not well resolved (Blanco et al., 1989). It requires absorbance measurement on multiple wavelengths for standard solutions of each component (A_MSG and A_PHE) and the synthetic sample mixture (A_MIX). The mathematical expression for the method is given the equation 1.

\[
A_{\text{MIX}} = \frac{C_{\text{PHE}}}{C_{\text{PHE}_0}} + \frac{C_{\text{PAR}}}{C_{\text{PAR}_0}} \times \frac{A_{\text{PAR}}}{A_{\text{PHE}}}
\]

(1)

In the present study, the measurements were performed by analyzing standard solutions of the drugs, C_MSG, and C_PHE (10 μg mL⁻¹ each). A linear graph of A_MSG/A_PAR versus A_PHE/A_PAR was plotted and the concentration of PHE and PAR were calculated from the values of slope and intercept respectively.

Simultaneous equation method (Vierordt’s method)

This method is based on the selection of wavelengths for each drug, at which another component has minimal interference. It requires formation and solution of simultaneous equations to determine the concentration of both the drugs in a given mixture (Davidson et al., 2001). For this purpose, the absorption spectra were recorded from 200-400 nm for the drugs and two wavelengths, 248.0 nm (λ₁) and 217.0 nm (λ₂) corresponding to absorption maxima of PAR and PHE respectively were selected for measurement. Molar absorptivity were calculated at these wavelengths for both the drugs and quantification analysis was performed using the following equations 2 and 3.

\[
A_1 = \epsilon_{\text{PAR}} \times C_{\text{PAR}} + \epsilon_{\text{PHE}} \times C_{\text{PHE}}
\]

(2)

\[
A_2 = \epsilon_{\text{PAR}} \times C_{\text{PAR}} + \epsilon_{\text{PHE}} \times C_{\text{PHE}}
\]

(3)

where, A₁ and A₂ are the absorbance values for the sample mixture at 248.0 nm and 217.0 nm respectively; \(\epsilon_{\text{PAR}}\) and \(\epsilon_{\text{PHE}}\) represent the molar absorptivities of PAR and PHE respectively.

These equations were solved and directly utilized for the simultaneous estimation of PAR and PHE in standard laboratory mixture as well as in the marketed formulation.

Derivative spectrophotometric method

This method is based on the use of derivative spectra of the absorption spectra of analytes and their combinations, and thus clearly distinguishes between moderate to highly overlaying spectra. This method is most useful while considering analytes which are difficult to analyze directly but have different spectrophotometric profiles (El-Sayed and El-Salem, 2005). It requires selection of wavelengths in derivative spectra of both the analytes, so that estimation of one analyte shows negligible interference due to another component in the mixture. In our present work, the zero-order (D⁰) absorption spectra were recorded for 10.0 μg mL⁻¹ for PAR and PHE respectively and a binary mixture of PAR and PHE with same concentration using methanol as a blank over 200–400 nm wavelength range. These zero order spectra of PAR and PHE were treated to obtain corresponding first derivative spectra with an inter-point distance of 5 nm and scaling factor of 10. Using memory channels, the zero and first order derivative spectra of both
the drugs were overlapped for wavelength selection and data treatment.

**Absorbance ratio method**

This method depends on the linear relationship between the ratio of absorbance values of a binary mixture and the relative amount of components in such mixture for quantitative measurement (Erk, 2000). It is based on the selection of optimum wavelengths ($\lambda_1$, $\lambda_2$ and $\lambda_{iso}$) and the mathematical expression of the method is as shown below in equations 4 and 5,

$$C_1 = \frac{Q_1-a_1}{a_2} \times \frac{A_{iso}}{a_{iso}} \times 10^3$$  \hspace{1cm} (4)

$$C_2 = \frac{Q_2-b_2}{a_2} \times \frac{A_{iso}}{a_{iso}} \times 10^3$$  \hspace{1cm} (5)

where, $Q_1$ is the absorbance ratio ($A_1/A_{iso}$) for the first component (PAR); $Q_2$ is the absorbance ratio ($A_2/A_{iso}$) for the second component (PHE); $C_1$ and $C_2$ are concentrations of PAR and PHE respectively; $A_{iso}$ is the absorbance at isoabsorptive point; $a_{iso}$ is the absorptivity at isoabsorptive point which equals to ($A_{iso}/(C_1+C_2)$); $a_1$ is the slope of regression equation ($Q_1$ vs. $C_1/(C_1+C_2)$); $a_2$ is the slope of regression equation ($Q_2$ vs. $C_2/(C_1+C_2)$); $b_1$ and $b_2$ are intercept values of these regression equations and $A_1$ and $A_2$ denotes the absorbance values of the mixture measured at $\lambda_1$ and $\lambda_2$ respectively.

(a) Series 1: (for PAR)

Five aliquots equivalent to 20, 40, 60, 80 and 100 µg of PAR were transferred to a series of 10-mL measuring flasks, containing an aliquot equivalent to 100 µg of PHE. Each flask was diluted to volume with methanol. The absorbance of each solution was measured at 248.0 nm ($\lambda_{max}$ of PAR) and 222.0 nm (isoabsorptive point). The relative absorbance ($Q_{PAR}=A_{PAR}/A_{iso}$) and the corresponding relative concentration ($C_{PAR}/(C_{PAR}+C_{PHE})$) was plotted and the regression equation for PAR was computed.

(b) Series 2: (for PHE)

Five aliquots equivalent to 75, 100, 125, 150 and 200 µg of PHE were transferred to a series of 10-mL measuring flasks, containing an aliquot equivalent to 50 µg of PAR. Each flask was diluted to volume with methanol. The absorbance of each solution was measured at 217.0 nm ($\lambda_{max}$ of PHE) and 222.0 nm (isoabsorptive point). The relative absorbance ($Q_{PHE}=A_{PHE}/A_{iso}$) and the corresponding relative concentration ($C_{PHE}/(C_{PAR}+C_{PHE})$) was plotted and

Fig. 1: Zero order spectra of (A) phenylephrine hydrochloride (10 µg/mL), (B) paracetamol (10 µg/mL) and (C) synthetic mixture of paracetamol and phenylephrine hydrochloride (10 µg/mL each).

Fig. 2: Plot of $A_{MIX}/A_{PAR}$ versus $A_{PHE}/A_{PAR}$ obtained using paracetamol (10 µg/mL), phenylephrine hydrochloride (10 µg/mL) and synthetic mixture of paracetamol and phenylephrine hydrochloride (10 µg/mL each).

Fig. 3: Univariate calibration curves for (A) paracetamol and (B) phenylephrine hydrochloride at 248.0 and 217.0 nm respectively.
the regression equation for PHE was computed.

**Validation of the proposed methods**

All the four spectrophotometric method were validated as per ICH guidelines for parameters accuracy, precision, specificity, recovery and reproducibility.

**Linearity**

Linearity of each method was proved by analyzing five calibration standards for absorbance-ratio method and seven calibration standards for other methods, within the range of 1.0-12 μg mL\(^{-1}\) for PAR and 5.0-25 μg mL\(^{-1}\) for PHE. The analytical response of the method was plotted against the concentration of each drug and slope, intercept and correlation coefficient were calculated. For a method to be acceptable the value of \(r^2\) should be \(\geq 0.99\).

**Accuracy**

The accuracy of the methods was established by standard addition method. Standard mixtures were prepared and analyzed after addition of known amounts of each drug. The study was carried out by adding known amounts of PAR and PHE standards to a pre-quantified sample solution of known concentration. The amounts recovered were

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**Table 1**: Experimental parameters and calibration data for graphical method

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>(A_{\text{PAR}}^a)</th>
<th>(A_{\text{PHE}}^b)</th>
<th>(A_{\text{MIX}}^c)</th>
<th>(A_{\text{MIX}}/A_{\text{PAR}})</th>
<th>(A_{\text{PHE}}/A_{\text{PAR}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>0.6382</td>
<td>0.3092</td>
<td>0.9545</td>
<td>1.4956</td>
<td>0.4845</td>
</tr>
<tr>
<td>220</td>
<td>0.2739</td>
<td>0.3338</td>
<td>0.6130</td>
<td>2.2382</td>
<td>1.2188</td>
</tr>
<tr>
<td>230</td>
<td>0.5264</td>
<td>0.1394</td>
<td>0.6694</td>
<td>1.2717</td>
<td>0.2649</td>
</tr>
<tr>
<td>240</td>
<td>0.8854</td>
<td>0.0141</td>
<td>0.8988</td>
<td>1.0151</td>
<td>0.0160</td>
</tr>
<tr>
<td>250</td>
<td>1.0333</td>
<td>0.0188</td>
<td>1.0422</td>
<td>1.0087</td>
<td>0.0182</td>
</tr>
<tr>
<td>260</td>
<td>0.7220</td>
<td>0.0481</td>
<td>0.7727</td>
<td>1.0701</td>
<td>0.0666</td>
</tr>
<tr>
<td>270</td>
<td>0.3127</td>
<td>0.1013</td>
<td>0.4189</td>
<td>1.3400</td>
<td>0.3239</td>
</tr>
<tr>
<td>280</td>
<td>0.1786</td>
<td>0.1122</td>
<td>0.2927</td>
<td>1.0701</td>
<td>0.0731</td>
</tr>
<tr>
<td>290</td>
<td>0.1486</td>
<td>0.0173</td>
<td>0.1674</td>
<td>1.1259</td>
<td>0.1164</td>
</tr>
<tr>
<td>300</td>
<td>0.0827</td>
<td>0.0060</td>
<td>0.0855</td>
<td>1.0703</td>
<td>0.0731</td>
</tr>
</tbody>
</table>

\(a\) PAR: paracetamol (10 μg mL\(^{-1}\)); \(b\) PHE: phenylephrine hydrochloride (10 μg mL\(^{-1}\)); \(c\) MIX: mixture

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**Table 2**: Recovery results of paracetamol and phenylephrine hydrochloride in laboratory prepared mixtures by proposed methods using standard addition technique (n = 5)

<table>
<thead>
<tr>
<th>Amount added (μg mL(^{-1}))</th>
<th>Method 1(^a)</th>
<th>Method 2(^b)</th>
<th>Method 3(^c)</th>
<th>Method 4(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found (μg mL(^{-1}) ± SD)</td>
<td>Recovery (%)</td>
<td>Amount found (μg mL(^{-1}) ± SD)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>Paracetamol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.00</td>
<td>7.94±0.09</td>
<td>99.27</td>
<td>7.95 ± 0.09</td>
<td>99.43</td>
</tr>
<tr>
<td>10.00</td>
<td>10.08±0.11</td>
<td>100.75</td>
<td>9.96 ± 0.16</td>
<td>99.85</td>
</tr>
<tr>
<td>12.00</td>
<td>12.10±0.10</td>
<td>100.83</td>
<td>12.03 ± 0.15</td>
<td>100.26</td>
</tr>
<tr>
<td>Phenylephrine hydrochloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.00</td>
<td>8.02±0.10</td>
<td>100.28</td>
<td>7.98±0.10</td>
<td>99.83</td>
</tr>
<tr>
<td>10.00</td>
<td>9.92±0.09</td>
<td>99.24</td>
<td>9.97±0.12</td>
<td>99.77</td>
</tr>
<tr>
<td>12.00</td>
<td>12.07±0.10</td>
<td>100.61</td>
<td>11.96±0.10</td>
<td>99.66</td>
</tr>
</tbody>
</table>

\(a\) Graphical method; \(b\) Simultaneous equation method; \(c\) First derivative method; \(d\) Absorbance ratio method; SD: standard deviation
calculated and expressed in terms of mean recovery based on ICH guidelines (ICH Guidelines, 2005).

**Precision**

Intra and inter-batch precision were evaluated by carrying out measurements of five replicate samples on the same day and on five consecutive days respectively. The concentration values for known standards were calculated and the precision of the method was reported in terms of percent relative standard deviation (% RSD). The method was considered well reproducible if the results were within the recommended range as suggested by ICH guidelines (ICH Guidelines, 2005).

**Specificity**

The interference due to commonly used excipients was also evaluated. Known amount of commonly used excipients present in the selected formulations were spiked into a pre-analyzed standard sample solution for both the drugs. The % recovery was considered for the assessment of the specificity (ICH Guidelines, 2005).

**Ruggedness**

The robustness of the method was evaluated by analyzing standard samples of both the drugs and their combinations on two different spectrophotometers and also by using methanol obtained from different sources. The results were obtained and % RSD was calculated. The method was considered robust enough for the proposed application if the % RSD were within the acceptable range (ICH Guidelines, 2005).

**Analysis of marketed formulations**

Ten marketed tablets/capsules each of Contac C®, Robitussin® nasal relief and Excedrin® Sinus Headache, Feniapal® and Vicks® DayQuil Sinex® were separately weighed and ground to a fine powder. From the resultant powder, an accurately weighed amount equivalent to 50 mg PAR was taken in a calibrated volumetric flask containing methanol. The solution was filtered through Whatman filter paper 41 and made up to a final volume (100 mL) with methanol. A 5 mL aliquot of this solution was further diluted to 25 mL with the same diluent to get 100 μg mL⁻¹ of PAR and corresponding concentration of PHE. From the
above solution, 1.0 mL was transferred to a 10 mL volumetric flask and 0.4 mL of PHE stock solution was added and diluted to volume with methanol. The purpose of this addition was to bring the concentration of PHE within the linear range. This solution was used for the estimation of both the drugs.

RESULTS AND DISCUSSION

Molecular absorption spectroscopy is a simple, rapid and accurate analytical technique. It is applicable in almost all laboratories for pharmaceutical analysis as most of the drugs absorb in UV region. Using UV spectrophotometric technique, it is much simpler to simultaneously determine multiple components in a given mixture without prior separation. In addition to conventional graphical method, various types of spectrophotometric methods have been developed with improved accuracy and precision, some of which are Vierordt’s simultaneous equation method (Davidson et al., 2001), Absorbance ratio method (Erk, 2000), H-point standard addition method (Reig and Falco, 1988), Bivariate method (López-de-Alba et al., 1996) and a number of Derivative methods (El-Sayed and El-Salem, 2005). In the present work, we have developed and validated four different spectrophotometric methods for comparative evaluation.

Graphical method

The overlain zero order absorption spectra of PAR (10 μg mL⁻¹), PHE (10 μg mL⁻¹) and their combined mixture (10 μg mL⁻¹ each) are shown in Fig. 1. The absorbance values for the standard samples of both the drugs and synthetic mixtures were measured in the range of 200-300 nm with inter-point distance of 10 nm (Table 1). The calibration curve of A_mix/A_PAR versus A_PHE/A_PAR showed good linearity (Fig. 2). The linear regression was obtained using equation 6.

\[
\frac{A_{\text{mix}}}{A_{\text{PAR}}} = 1.018 \times \frac{A_{\text{mix}}}{A_{\text{PAR}}} + 1.001 \quad (r^2 = 0.9992)
\]  (6)

From the above equation, slope and intercept values were used for the quantification of PHE and PAR respectively. Table 2 summarizes mean recovery and standard deviation results calculated by analyzing standard known mixtures of PAR and PHE.

Simultaneous equation method

In the zero-order spectra, PAR showed maximum absorbance at 248.0 nm and 204.0 nm, while for PHE they were 217.0 and 275.0 nm respectively. However, wavelength 248.0 nm and 217.0 nm were selected for the analysis of PAR and PHE as they provided consistent and better results with minimum interference. The absorbance values for the standards and mixture were measured at these wavelengths which showed good linearity for the standard drug solutions (Fig. 3). The mean absorptivity at these wavelengths was calculated for both the components and substituted in equations 2 and 3 as below.

\[
A_1 = 0.10827 C_{\text{PAR}} + 0.00157 C_{\text{PHE}}
\]  (7)

\[
A_2 = 0.22407 C_{\text{PAR}} + 0.01207 C_{\text{PHE}}
\]  (8)

These equations 7 and 8 were directly utilized for the determination of PAR and PHE in standard laboratory mixtures. Summary of the mean recovery and standard deviation is presented in Table 2.

First derivative spectrophotometry

Adequate separation of overlapped peaks can be achieved by correct selection of derivative order. Optimal derivative order is a function of signal height and the distance between maxima in basic spectrum. Several manipulations were made to enable mixture resolution using first, second and third derivative of the absorption spectra with an inter-point distance of 5 nm and scaling factor of 10. Using memory channels, the zero, first, second and third order derivative spectra of both the drugs were overlapped for wavelength selection and data treatment. First order derivative spectra (D₁) afforded highest accuracy and detection limits compared to higher order spectra and hence was selected for the analysis. In contrast to zero-order spectra, first derivative spectra showed enhanced resolution in terms of zero crossing point. The first derivative spectra (Fig. 4) were obtained using Δλ=5 nm and scaling factor of 10 for the calibration standards of both the drugs. PAR was determined at 204.0 nm without significant interference of PHE. Since PAR shows negligible contribution at 209.0 nm, PHE was accurately determined at this wavelength. Two calibration curves were established for both the drugs using the analytical response at respective zero crossing points and a straight line curve was observed in the concentration range of 1.0-12 μg mL⁻¹ for PAR and 5.0-25 μg mL⁻¹ for PHE (Fig. 5). The regression equations and correlation coefficients for both the drugs were calculated using the following equations 9 and 10.

\[
\frac{dA}{dx} = 0.0114 C_{\text{PAR}} + 0.0004 \quad (r^2 = 0.9992) \quad \text{at 209 nm}
\]  (9)

\[
\frac{dA}{dx} = 0.0059 C_{\text{PHE}} - 0.0013 \quad (r^2 = 0.9992) \quad \text{at 204 nm}
\]  (10)

The mean recovery and relative standard deviation for both the drugs, obtained from derivative spectrophotometry are presented in Table 2.

Absorbance ratio method

The zero order absorption spectra (Fig. 1) of PAR (10 μg mL⁻¹) and PHE (10 μg mL⁻¹) showed an isosorptive point at 222.0 nm. The synthetic mixtures of the components were prepared as described in section 2.4.4 and the absorbance values were measured at 248.0 nm (λ_max of PAR), 217.0 nm (λ_max of PHE), and 222.0 nm (λ_min). A linear relation between the absorbance ratio value of the binary mixture and the relative concentration of such a mixture was established (Fig. 6). It shows good linearity between the two variables as evident from the correlation coefficients values in the following equations 11 and 12.

\[
Q_1 = 3.294 \left( \frac{C_{\text{PAR}}}{C_{\text{PAR}}+C_{\text{PHE}}} \right) + 0.073 \quad (r^2 = 0.9988)
\]  (11)

\[
Q_2 = 0.266 \left( \frac{C_{\text{PHE}}}{C_{\text{PAR}}+C_{\text{PHE}}} \right) + 0.857 \quad (r^2 = 0.9993)
\]  (12)
The linearity of the method was determined by analysis of five linearity curves containing five/seven non-zero concentrations. The calibration curves were linear in the range from 1.0-12 μg mL$^{-1}$ and 5.0-25 μg mL$^{-1}$ for PAR and PHE respectively for all the developed methods. The detailed results of linearity along with regression equation, correlation coefficient, LOD and LOQ are presented in Table 3.

### Validation of the proposed methods

#### Linearity

The linearity of the method was determined by analysis of five linearity curves containing five/seven non-zero concentrations. The calibration curves were linear in the range from 1.0-12 μg mL$^{-1}$ and 5.0-25 μg mL$^{-1}$ for PAR and PHE respectively for all the developed methods. The detailed results of linearity along with regression equation, correlation coefficient, LOD and LOQ are presented in Table 3.

### Accuracy

For the assessment of the accuracy, to a pre-analyzed known sample solution, standard drug solutions were added and then percentage drug content was calculated. Accuracy of

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**Table 3**: Results of validation parameters obtained for developed spectrophotometric methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method 1$^a$</th>
<th>Method 2$^b$</th>
<th>Method 3$^c$</th>
<th>Method 4$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAR</td>
<td>PHE</td>
<td>PAR</td>
<td>PHE</td>
</tr>
<tr>
<td>Range (μg mL$^{-1}$)</td>
<td>1.0-12</td>
<td>5.0-25</td>
<td>1.0-12</td>
<td>5.0-25</td>
</tr>
<tr>
<td>Slope</td>
<td>--</td>
<td>--</td>
<td>0.0152</td>
<td>0.0361</td>
</tr>
<tr>
<td>Intercept</td>
<td>--</td>
<td>--</td>
<td>0.0035</td>
<td>-0.0082</td>
</tr>
<tr>
<td>Correlation-coefficient ($r^2$)</td>
<td>--</td>
<td>--</td>
<td>0.9992</td>
<td>0.9989</td>
</tr>
<tr>
<td>Intra batch accuracy (%)</td>
<td>100.28</td>
<td>99.13</td>
<td>100.09</td>
<td>100.43</td>
</tr>
<tr>
<td>Inter batch precision (%)</td>
<td>1.81</td>
<td>1.01</td>
<td>1.37</td>
<td>1.04</td>
</tr>
<tr>
<td>Reproducibility (%)</td>
<td>0.91</td>
<td>1.18</td>
<td>0.94</td>
<td>1.08</td>
</tr>
</tbody>
</table>

$^a$Graphical method; $^b$Simultaneous equation method; $^c$First derivative method; $^d$Absorbance ratio method; PAR: paracetamol; PHE: phenylephrine hydrochloride; LOD: limit of detection; LOQ: limit of quantitation; RSD: related standard deviation.

**Table 4**: Assay performance of the developed methods for the determination of paracetamol and phenylephrine hydrochloride in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug component</th>
<th>Claimed value (mg)</th>
<th>Method 1$^a$</th>
<th>Method 2$^b$</th>
<th>Method 3$^c$</th>
<th>Method 4$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAR</td>
<td>PHE</td>
<td>PAR</td>
<td>PHE</td>
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<tr>
<td>Feniapal®</td>
<td>PAR</td>
<td>100</td>
<td>99.98 ± 0.88</td>
<td>100.44 ± 0.72</td>
<td>100.82 ± 0.75</td>
<td>100.39 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>PHE</td>
<td>5</td>
<td>5.01 ± 0.07</td>
<td>4.97 ± 0.06</td>
<td>5.05 ± 0.05</td>
<td>5.06 ± 0.05</td>
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<tr>
<td>Contac C®</td>
<td>PAR</td>
<td>500</td>
<td>500.44 ± 5.94</td>
<td>502.34 ± 6.49</td>
<td>494.68 ± 6.03</td>
<td>503.72 ± 5.19</td>
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<tr>
<td></td>
<td>PHE</td>
<td>10</td>
<td>10.09 ± 0.09</td>
<td>9.99 ± 0.10</td>
<td>9.96 ± 0.08</td>
<td>9.97 ± 0.07</td>
</tr>
<tr>
<td>Robitussin® nasal</td>
<td>PAR</td>
<td>325</td>
<td>323.74 ± 3.91</td>
<td>323.57 ± 4.34</td>
<td>323.39 ± 3.73</td>
<td>326.74 ± 2.74</td>
</tr>
<tr>
<td></td>
<td>PHE</td>
<td>5</td>
<td>5.03 ± 0.05</td>
<td>5.04 ± 0.05</td>
<td>5.04 ± 0.06</td>
<td>4.99 ± 0.06</td>
</tr>
<tr>
<td>Vicks® DayQuil</td>
<td>PAR</td>
<td>325</td>
<td>325.69 ± 3.07</td>
<td>325.17 ± 4.04</td>
<td>322.22 ± 4.20</td>
<td>323.67 ± 2.89</td>
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<tr>
<td></td>
<td>PHE</td>
<td>5</td>
<td>5.02 ± 0.05</td>
<td>5.03 ± 0.06</td>
<td>4.98 ± 0.06</td>
<td>5.02 ± 0.07</td>
</tr>
<tr>
<td>Sinex®</td>
<td>PAR</td>
<td>325</td>
<td>323.03 ± 5.20</td>
<td>323.15 ± 2.30</td>
<td>326.24 ± 2.66</td>
<td>325.99 ± 3.72</td>
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<tr>
<td>Headache</td>
<td>PHE</td>
<td>5</td>
<td>5.04 ± 0.06</td>
<td>5.04 ± 0.06</td>
<td>5.03 ± 0.05</td>
<td>5.04 ± 0.06</td>
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</tbody>
</table>

$^a$Graphical method; $^b$Simultaneous equation method; $^c$First derivative method; $^d$Absorbance ratio method; PAR: paracetamol; PHE: phenylephrine hydrochloride; SD: standard deviation. From the calculated regression equations, values of slopes and intercepts were obtained and used to analyze PAR and PHE using equations 4 and 5. The mean recovery and relative standard deviation were also calculated for both the drugs as presented in Table 2.
mean recoveries were considered which were within the range of 98-102 % for both PAR and PHE and the detailed results of accuracy study are provided in Table 3.

**Precision**

Intra and inter-batch precision were calculated by analyzing replicate samples as described previously. The detailed summary of the precision values are given in Table 3, which indicates that the % RSD were within the acceptable range.

**Specificity**

The specificity studies revealed that the excipients normally present in proposed formulations like starch, stearic acid, gelatin, glycerin, polyethylene glycol, povidone, propylene glycol, sorbitol, cellulose do not interfere even in excess than the anticipated amount. The four spectrophotometric methods were found to be specific for the proposed application using methanol as the diluent.

**Ruggedness**

In the ruggedness study with different instrumentation and solvent source, no significant changes in the response of the methods were observed for both drugs. The % RSD of the four methods was within 0.474-0.853 % for both the drugs (Table 3).

**Analysis of marketed formulations**

The proposed methods were successfully applied for the determination of PAR and PHE in different marketed formulations. The mean recoveries of each method were in good agreement with the claimed values of the formulations. The comparative study for the marketed formulation is presented in Table 4.

**Statistical evaluation of the developed methods**

The developed methods were statistically compared by analyzing known mixture of PAR and PHE using standard addition method. The results obtained were verified by Student t-test and by the variance F-test. According to the t-test and F-test, all the calculated t-values and F-values were less than the theoretical values at the 95 % confidence level, indicating no significant differences among the results obtained these methods. The detailed summary of the results is shown in Table 5.

**CONCLUSIONS**

In the present study, four different spectrophotometric methods have been proposed for the simultaneous estimation of paracetamol and phenylephrine hydrochloride in their combined dosage forms without prior separation. The methods were found to be simple, rapid, accurate, precise and economical. The results indicate their adequacy for routine analysis of paracetamol and phenylephrine hydrochloride in tablets as good agreement was seen in the mean recoveries of each method.
Table 5: Statistical comparison of the results obtained by the developed methods (n = 7)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 3</th>
<th>Method 4</th>
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<tbody>
<tr>
<td>Paracetamole</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>rcalculated</td>
<td>0.486</td>
<td>0.482</td>
<td>0.490</td>
<td>0.500</td>
</tr>
<tr>
<td>rtheoretical</td>
<td>1.782</td>
<td>1.782</td>
<td>1.782</td>
<td>1.782</td>
</tr>
<tr>
<td>Fcalculated</td>
<td>0.491</td>
<td>0.493</td>
<td>0.499</td>
<td>0.489</td>
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<tr>
<td>Ftheoretical</td>
<td>4.284</td>
<td>4.284</td>
<td>4.284</td>
<td>4.284</td>
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<tr>
<td>Phenylephrine HCle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rcalculated</td>
<td>0.493</td>
<td>0.479</td>
<td>0.484</td>
<td>0.488</td>
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<tr>
<td>rtheoretical</td>
<td>1.782</td>
<td>1.782</td>
<td>1.782</td>
<td>1.782</td>
</tr>
<tr>
<td>Fcalculated</td>
<td>0.499</td>
<td>0.466</td>
<td>0.475</td>
<td>0.478</td>
</tr>
<tr>
<td>Ftheoretical</td>
<td>4.284</td>
<td>4.284</td>
<td>4.284</td>
<td>4.284</td>
</tr>
</tbody>
</table>

*Graphical method;  Simultaneous equation method;  First derivative method;  Absorbance ratio method

assay results of marketed formulations. Finally, the developed methods were statistically valid as evident from Student t-test and F-test results and can be readily applied in quality control labs where sophisticated instruments like HPLC are not available.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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