Derivative Spectrophotometric Methods for Simultaneous Estimations of Phenylpropanolamine, Chlorpheniramine and Dextromethorphan in Syrups

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The absorbance maxima of phenylpropanolamine hydrochloride, chlorpheniramine maleate and dextromethorphan hydrobromide in 0.1 N hydrochloric acid are 257, 265 and 278 nm, respectively. This paper presents two methods based on first derivative spectrophotometry for simultaneous estimations of these three drugs in combinations in pharmaceutical formulations. The first derivative amplitudes at 264.4, 278.5 and 288.9 nm are utilized for simultaneous estimations. The first method utilizes correlational regression equations and the second method utilizes simultaneous equations to avoid complex interferences at selected wavelengths in derivative spectra. All three drugs obey Beer’s law in the concentration range employed for analysis. Linearity was validated by Least Squares Method. The results of analysis have been validated statistically and by recovery studies. Both methods are simple, economical, accurate, reproducible and rapid.

Cough syrups containing phenylpropanolamine hydrochloride (PPM), chlorpheniramine maleate (CPM) and dextromethorphan hydrobromide (DXB) are significant marketed formulation often prescribed for symptomatic relief of nonproductive dry and irritating coughs and upper respiratory symptoms such as irritation of throat, running nose, nasal congestion and watery eyes associated with allergy or common cold. Fixed combinations of PPM (25 mg), CPM (4 mg) and DXB (20 mg) per each 10 ml of syrup are marketed by various manufacturers including Ranbaxy, E.Merck, Indoco, Blue Cross and many others.

The official assay methods available for analysis of individual drugs and their formulations are described in IP1,2, BP3,4 and USP5,6. UV Spectrophotometric7–9, GLC10,11 and HPLC12–15 methods are reported for estimation of CPM along with PPM or DXB in multicomponent formulations. The combined dosage form of PPM, CPM and DXB is nonofficial and none of official compendia specify simultaneous analysis of said analyte in multicomponent formulations. The traditional methods of separation of individual components followed by estimation by various official/reported methods are tedious and moreover time consuming. Reported HPLC methods16–19 for simultaneous estimation are comparatively expensive and time consuming. The paper presents two simple, accurate, reproducible and economical spectrophotometric methods for determination of PPM, CPM and DXB in multicomponent formulations.

EXPERIMENTAL

A Shimadzu UV/Vis recording spectrophotometer (Model 160A) with spectral band width of 3 nm and wavelength accuracy of ± 0.5 nm (with automatic wavelength correction) was employed for all spectroscopic measurements using a pair of 10 mm matched quartz cells. PPM (BP), CPM (IP), DXB (IP), hydrochloric acid (Ranbaxy A.R. Grade), chloroform (Ranbaxy, A.R. Grade), sodium hydroxide (Qualigens ExcelAR), anhydrous sodium sulfate and double distilled water were used in the present investigation.

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drugs lie in close proximity and at absorption maximum of each component the other two components exhibit substantial absorbencies. This clearly indicates the existence of considerable spectral interferences in estimation of one analyte by other. To overcome this, spectra of all three drugs were derivatised to first order between 220.0 nm and 320.0 nm with $\Delta \lambda$ of 16.0 nm using a scan speed of 1500 nm/min. The overlaid first derivative spectra of PPM, CPM and DXB (Fig. 2) reveals that CPM has considerable amplitude ($DA_{CPM} = DA_y$) at 278.5 nm ($y$), where both PPM and DXB have zero amplitude. Hence CPM is estimated at this wavelength with no interference from PPM and DXB.

Here, $DA_y^{CPM} = DA_y^{CPM}$ ______ (1)

where, $DA_y^{CPM}$ is first derivative amplitude contributed by CPM to total amplitude $DA_y$ of samples containing PPM, CPM and DXB at 278.5 nm.

DXB has maximum first derivative amplitude at 288.9 nm ($z$). At this wavelength PPM has no contribution and the only other interfering ingredient during the estimation of DXB is CPM. However, the contribution of CPM to first derivative amplitude at 288.9 nm ($DA_y^{CPM}$) can be accessed from that at 278.5 nm ($DA_y^{CPM}$) through a linear regression equation (2). This equation is framed through simultaneous estimation of first derivative amplitude of sample containing different concentrations of CPM in the range 0-40 $\mu$g/ml by Least Squares method ($n=9$, $r = 0.9998$).

$DA_y^{CPM} = DA_y^{CPM} \times 0.4385 + 0.0002$ ______ (2) is given as $DA_y^{DXB} = DA_y - DA_y^{CPM}$

Here the first derivative amplitude due to DXB at 288.9nm is $DA_y^{DXB}$

Substituting the value of $DA_y^{CPM}$ from equation (2), we get

$DA_y^{DXB} = DA_y - (DA_y^{CPM} \times 0.4385 + 0.0002)$ ______ (3)

Where $DA_y$ is the first derivative amplitude of samples containing PPM, CPM and DXB at 288.9 nm ($z$). Hence DXB is estimated from first derivative amplitude at 288.9 nm utilizing equation (3).

For estimation of PPM, the wavelength 264.4 nm ($x$) is selected which is the zero crossing point of CPM i.e. $DA_y^{C}$ of CPM is zero. The only other interfering substance is DXB. However, through a similar framing of linear regression equation as in estimation of DXB described above, the contribution to amplitude at 264.4 nm

Fig. 1: Overlaid Spectra of PPM, CPM And DXB

Absorbance spectra obtained at different wavelengths in the range of 220-320 nm of PPM ($\Delta$) (250 $\mu$g/ml), CPM (□) (32 $\mu$g/ml) and DXB (O) (160 $\mu$g/ml) in 0.1 N HCl overlaid over each other.

Fig. 2: Overlaid First derivative spectra of PPM, CPM and DXB

First derivative Absorbance spectra obtained at different wavelengths in the range of 220-320 nm of PPM ($\Delta$) (600 $\mu$g/ml), CPM (□) (32 $\mu$g/ml) and DXB (O) (160 $\mu$g/ml) in 0.1 N HCl overlaid over each other.

Stock solutions of PPM (1000 $\mu$g/ml), CPM (200 $\mu$g/ml) and DXB (500 mg/ml) were prepared separately in 0.1 N HCl. Each stock solution was suitably diluted to different concentrations and the linearity was studied. Linear relationships were observed in the range 0-800 mg/ml for PPM, 0-50 mg/ml for CPM and 0-200 mg/ml for DXB.

Method-I:

The overlaid zero order spectra of PPM, CPM and DXB (Fig.1) show that the absorption maxima of all three
by DXB (DA_x^{DXB}) can be accessed from that at 288.9 nm (DA_y^{DXB}) by least squares method, Equation No-4 (r=0.9999)

\[ DA_x^{DXB} = DA_z^{DXB} \times 0.3445 + 0.0004 \quad (4) \]

The first derivative amplitude contributed by PPM at 264.4 nm (DA_x^{PPM}) is given by:  
\[ DA_x^{PPM} = DA_y - DA_x^{DXB} \]

Substituting the value of DA_x^{DXB} from equation (4)

\[ DA_x^{PPM} = DA_x - (DA_z^{DXB} \times 0.3445 + 0.0004) \quad (5) \]

Where, DA_x is first derivative amplitude of sample containing PPM, CPM, and DXB at 264.4 nm.

The linearity between first derivative amplitude and concentration of PPM, CPM and DXB were examined at selected wavelengths 264.4 nm (x), 278.5 nm (y) and 288.9 nm (z), respectively. Beer's law is followed in the concentration range 0-800 μg/ml for PPM, 0-40 μg/ml for CPM and 0-180 μg/ml for DXB. The coefficient of correlation (r) as evaluated by Least Squares method in each case is 0.9999.

Eight mixed standards containing PPM, CPM and DXB in the concentrations of 75 x n μg/ml, 4 x n μg/ml and 20 x n μg/ml (where n=1, 2, 3 - - - - 8), respectively were prepared in 0.1N HCl. From the first derivative spectra of all mixed standards the amplitudes at selected wavelengths i.e. 264.4 nm, 278.5 nm and 288.9 nm were recorded. By employing equations (1), (3) and (5) the amplitudes contributed by PPM, CPM and DXB to total amplitude at selected wavelengths were worked out. Linearity of resulting data against concentration of corresponding components in the mixed standards were checked by Least Squares method. The following linear regression equations were obtained and utilized for direct estimation of PPM, CPM and DXB in samples.

\[ C_{PPM} = 4310.6 DA_x^{PPM} + 2.0004 \quad (r = 0.9999) \quad (6) \]
\[ C_{CPM} = 329.22 DA_y - 0.2941 \quad (r = 0.9999) \quad (7) \]
\[ C_{DXB} = 505.55 DA_z^{DXB} - 0.6586 \quad (r = 0.9999) \quad (8) \]

Where C_{PPM}, C_{CPM} and C_{DXB} are concentrations of PPM, CPM and DXB, respectively in samples or mixed standards.

**Method – II:**

This method is also based on First derivative spectrophotometry. The wavelengths selected for estimations of PPM, CPM and DXB are as described in method-I, i.e. 264.4, 278.5 and 288.9 nm. However, in contrast to method-I, this method utilizes simultaneous equations (Vierdot's method) on derivative spectra to overcome spectral interferences at selected wavelengths.

First derivative absorptivity coefficients of individual drugs were determined at 264.4, 278.5 and 288.9 nm. A set of three equations framed using these coefficient values are given below.

\[ DA_x = 0.23 C_{PPM} - 0.6853 C_{DXB} \quad (9) \]
\[ DA_y = 3.116 C_{CPM} \quad (10) \]
\[ DA_z = 1.3775 C_{CPM} + 2.0109 C_{DXB} \quad (11) \]

Where DA_x, DA_y and DA_z are the first derivative amplitude (dA/dλ) of sample containing PPM, CPM and DXB at 264.4 nm (x), 278.5 nm (y), and 288.9 nm (z), respectively. The numericals in equations denotes first derivative absorption coefficients of corresponding drugs at selected wavelengths and are mean of seven independent determinations in the concentration range that obey Beer's law.

From equation (10) we get, \[ C_{CPM} = 0.320773 DA_y \quad (12) \]

By substituting this value of \[ C_{CPM} \] in equation (11), the value of \[ C_{DXB} \] is obtained as:

\[ C_{DXB} = 0.497289 DA_x - 0.219803 DA_y \quad (13) \]

Now substituting this value of \[ C_{DXB} \] in equation (10) the value of \[ C_{PPM} \] is found as

\[ C_{PPM} = 4.347826 DA_x + 1.48185 DA_y - 0.654966 DA_y \quad (14) \]

Before analyzing the commercial formulations, the methods were validated by analyzing standard samples containing PPM, CPM and DXB in ratio of 75:4:20 μg/ml and random samples prepared in laboratory. The results of replicate determinations (n=7) by both proposed methods were validated statistically and are shown in Table-1(A). Precise and accurate results are obtained with samples containing PPM in the range of 150 to 600 μg/ml.
### TABLE 1: ANALYSIS OF AUTHENTIC SAMPLES (A) AND RECOVERY EXPERIMENTS (B)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method-I</th>
<th>Method-II</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C.I.</td>
<td>SD</td>
</tr>
<tr>
<td>A</td>
<td>PPM</td>
<td>99.7±0.64</td>
</tr>
<tr>
<td>(n=7)</td>
<td>CPM</td>
<td>99.3±0.89</td>
</tr>
<tr>
<td></td>
<td>DXB</td>
<td>100.1±0.52</td>
</tr>
<tr>
<td>B</td>
<td>PPM</td>
<td>100.4±2.16</td>
</tr>
<tr>
<td>(n=4)</td>
<td>CPM</td>
<td>99.5±1.56</td>
</tr>
<tr>
<td></td>
<td>DXB</td>
<td>99.7±2.20</td>
</tr>
</tbody>
</table>

SD: Standard deviation, %SE: Per cent standard error, C.I. (Confidence Interval within which true value may be found at 95% confidence level) = R ± tσ/√n, R= mean per cent result of analysis of authentic samples or recovery. Theoretical 't' values at 95% confidence level for n-1 degrees of freedom are t(0.05,6) = 2.447, t(0.05.3) = 3.182. PPM stands for phenylpropanolamine, CPM for chlorpheniramine maleate and DXB for dextromethorphan.

Three batches of syrup formulations, procured from local market were used for analysis by the method developed in this investigation. A selective extraction procedure was adopted to avoid interferences due to adjuvant in formulations. Ten millilitres of each syrup was taken and made alkaline with 10 ml of 1 N NaOH. The resulting alkaline syrup solution was extracted successively five times with each 10 ml of chloroform and the extracts were collected. The solvent was driven off completely under reduced pressure at 45 ± 2°C. The residue was dissolved in 0.1 N HCl and the volume was made up to 100 ml in a volumetric flask containing 50 mg of pure drug of PPM. The resulting solution was treated as stock sample solution to contain 750 μg/ml, 40 μg/ml and 200 μg/ml of PPM, CPM and DXB, respectively. Different dilutions were prepared from above solutions and the amplitude at 264.4 nm, 278.5 nm and 288.9 nm were recorded from the first derivative spectra. The concentration of each analyte was determined using the equations generated in both the methods. However, one of commercial formulation containing menthol as adjuvant was found to interfere seriously in analysis, showing spectral interference in the wavelength regime selected. Hence, a slight modification for this formulation was effected to exclude adjuvant menthol. The residue after complete withdrawal of chloroform from the extract was further dried under reduced pressure at 45° ± 2°C for another 2 h and subsequent process was followed as in other formulations. The statistical data of results obtained after replicate determinations (n=4) are shown in Table 2.

The solvent extraction process adopted was quantitatively assured through analysis of a simulated syrup containing the three analytes without any formulation adjuvants, prepared in laboratory, by these proposed methods.

To study the recovery of PPM, CPM and DXB, preanalysed samples were taken to which different quantities of pure drugs (reference standards) were added at a level of 25 to 200 per cent but within the analytical concentration range limitations in proposed methods. The added quantities of individual drugs were estimated by both methods and the statistical data are given in Table 1 (B) (n=4).

### RESULTS AND DISCUSSION

Derivative spectrophotometry provides a versatile technique for resolving complexes spectra and makes it possible to analyse drugs in multicomponent pharmaceutical formulations in presence of various interferences. The technique resolves the overlapped interference by smoothening peaks at the cost of loss of background signals. This in turn increases sensitivity of detection. Optimum resolution of complex interferences is achieved through first order derivatisation with Δλ of 16.0 nm of normal spectra.

The proposed methods were found to be accurate, simple and convenient for simultaneous determinations of PPM, CPM, and DXB in pharmaceutical formulations. The modalities adopted in the experimentation were
<table>
<thead>
<tr>
<th>Formul</th>
<th>PPM</th>
<th>C.I.</th>
<th>SD</th>
<th>C.PM</th>
<th>SD</th>
<th>C.I.</th>
<th>SD</th>
<th>DXB</th>
<th>SD</th>
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<tbody>
<tr>
<td>M-I</td>
<td>100.2±0.71</td>
<td>0.449</td>
<td>99.2±0.62</td>
<td>0.391</td>
<td>100.6±1.35</td>
<td>0.851</td>
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<tr>
<td>(n=4)  B</td>
<td>100.4±0.43</td>
<td>0.269</td>
<td>102.5±1.13</td>
<td>0.715</td>
<td>100.4±0.98</td>
<td>0.620</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>100.1±1.0</td>
<td>0.631</td>
<td>100.1±1.05</td>
<td>0.663</td>
<td>100.2±1.0</td>
<td>0.629</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-II</td>
<td>100.0±0.6</td>
<td>0.379</td>
<td>98.6±1.59</td>
<td>1.003</td>
<td>99.9±1.73</td>
<td>1.092</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=4)  B</td>
<td>100.2±1.09</td>
<td>0.690</td>
<td>99.4±1.38</td>
<td>0.868</td>
<td>99.4±0.73</td>
<td>0.460</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>102.0±0.71</td>
<td>0.451</td>
<td>99.19±1.15</td>
<td>0.723</td>
<td>99.6±0.85</td>
<td>0.534</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* n = 4, t (0.05,3) = 3.182. PPM stands for phenylpropanolamine, CPM for chlorpheniramine maleate and DXB for dextromethorphan.

The mean percentage of recoveries at 95 per cent confidence limit were calculated for three degrees of freedom (n=4) and were found to be 100.4 ± 2.16, 99.5 ± 1.57, 99.7 ± 2.20 in method -I and 99.8 ± 1.45, 100.4 ± 0.92, 100.5 ± 1.43 in method-II for PPM, CPM and DXB, respectively. This show the recoveries obtained do not differ significantly from 100 per cent and there was no interference from common adjuncts used in the formulation, indicating the accuracy and reliability of both methods.

Method-I utilizes correlative linear regression equations to overcome spectral interferences. The estimation of all three drugs requires a number of steps involving mathematical calculations. However, method-II utilizes application of simultaneous equations to first derivative spectra similar to the application in normal (zero order) spectra (Vierdot's Method). The proper selection of wavelengths leads to an advantage that three-component system is considered as single or two component system, thus avoiding complex situation in solution of equations framed. Once equations were framed, the method becomes very easy and only requires measurement of first derivative amplitudes at selected wavelengths. Three commercial syrups were analyzed by these proposed methods. The results are found to be satisfactory with standard deviation values below unity (Table 2).

Standard addition of PPM up to 200 per cent was effected considering its lower absorptivity values as compared to CPM and DXB. This amount of standard addition was quantitatively established after a series of preliminary trials.

Both methods are suitable for routine analysis in laboratory. However, a new innovative method based on developed simultaneous equations simplifies the quantification of drugs in combination and shows adherence to sensitivity, accuracy and precision. The method developed for simultaneous estimation through mathematical manipulation is a new technique in Derivative Spectrophotometry.

REFERENCES