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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 correlative 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 IP[1,2], BP[3,4] and USP[5,6]. UV Spectrophotometric[7-9] GLC[10,11] and HPLC[12-15] 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 methods[16-19] 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.

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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.

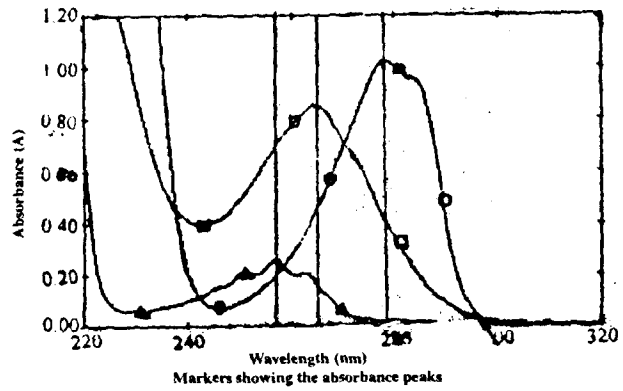

Fig. 1: Overlain Spectra of PPM, CPM And DXB

Absorbance spectra obtained at different wavelengths in the range of 220-320 nm of PPM (Δ) (250 µg/ml), CPM (□) (32 µg/ml) and DXB (O) (160 µg/ml) in 0.1 N HCl overlain over each other

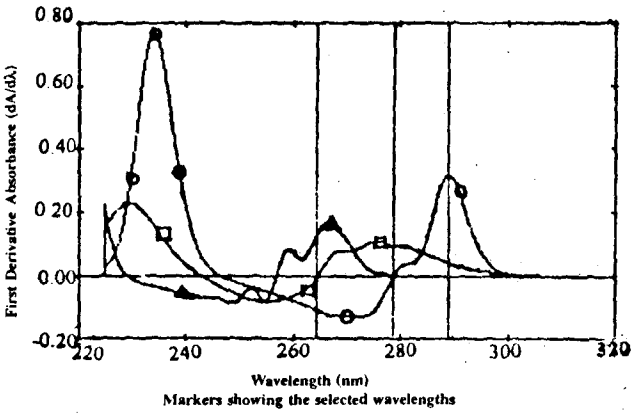

Fig. 2: Overlain First derivative spectra of PPM, CPM and DXB

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

Stock solutions of PPM (1000 µg/ml), CPM (200 µg/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 overlain zero order spectra of PPM, CPM and DXB (Fig.1) show that the absorption maxima of all three drugs lie in close proximity and at absorption maximum of each component the other two components exhibit substantial absorbences. 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 6λ of 16.0 nm using a scan speed of 1500 nm/min. The overlain first derivative spectra of PPM, CPM and DXB (Fig. 2) reveals that CPM has considerable amplitude (DA=dA/6λ) 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.

$$\text{Here, } DA_y^{CPM} = DA_y \text{ ———————— (1)}$$

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_z^{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 µg/ml by Least Squares method (n=9, r = 0.9998).

$DA_z^{CPM} = DA_y^{CPM} \times 0.4385 + 0.0002$ ———(2) is given as

$DA_z^{DXB} = DA_z - DA_z^{CPM}$

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

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

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

Where DA_z 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/dλ 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

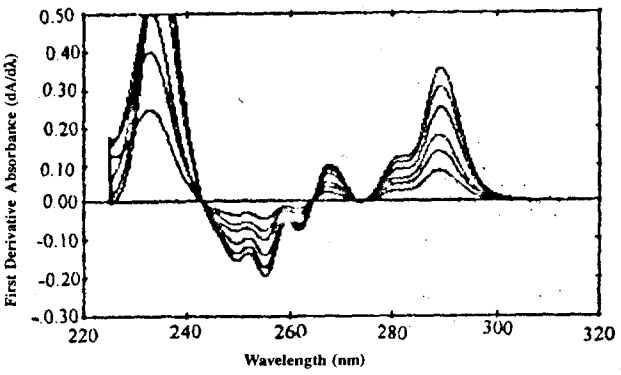

Fig. 3: Overlain first derivative absorbance spectra of mixed standards containing PPM, CPM and DXB

by DXB (DA_x^{DXB}) can be accessed from that at 288.9 nm (DA_z^{DXB}) by least Squares method, Equation No-4 (n=9, r=0.9999)

$$DA_x^{DXB} = DA_z^{DXB} \times -0.3445 + 0.0004 \text{ - - - - (4)}$$

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

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

$$DA_x^{PPM} = DA_x - (DA_z^{DXB} \times -0.3445 + 0.0004) \text{ - - - (5)}$$

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 0.1 N 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.

$$C_{PPM} = 4310.6\ DA_x^{PPM} + 2.0004 \quad (r = 0.9999) \quad \text{- - (6)}$$

$$C_{CPM} = 329.22\ DA_y - 0.2941 \quad (r = 0.9999) \quad \text{- - (7)}$$

$$C_{DXB} = 505.55\ DA_z^{DXB} - 0.6586 \quad (r = 0.9999) \quad \text{- (8)}$$

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.

$$DA_x = 0.23C_{PPM} - 0.68535\ C_{DXB} \text{ ———— (9)}$$

$$DA_y = 3.1165C_{CPM} \text{ ———— (10)}$$

$$DA_z = 1.3775C_{CPM} + 2.0109\ C_{DXB} \text{ ——— (11)}$$

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, $C_{CPM} = 0.320773\ DA_y$—(12)

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

$C_{DXB} = 0.497289\ DA_z - 0.219803\ DA_y$——(13)

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

$C_{PPM} = 4.347826\ DA_x + 1.48185\ DA_z - 0.654966\ DA_y$ (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)

Analyte		Method-I C.I.	SD	%SE	't'	Method-II C.I.	SD	%SE	't'
A	PPM	99.7±0.64	0.696	0.263	1.220	100.0±0.47	0.513	0.194	0.272
(n=7)	CPM	99.3±0.89	0.963	0.364	1.862	99.5±1.08	1.168	0.442	1.04
	DXB	100.1±0.52	0.570	0.215	0.298	99.6±0.43	0.467	0.176	2.334
B	PPM	100.4±2.16	1.358	0.682	0.703	99.8±1.45	0.915	0.457	0.475
(n=4)	CPM	99.5±1.56	0.985	0.493	0.955	100.4±0.92	0.579	0.289	1.595
	DXB	99.7±2.20	1.387	0.694	0.435	100.5±1.43	0.903	0.451	1.229

SD: Standard deviation, %SE : Per cent standard error, C.I. (Confidence Interval within which true value may be found at 95% confidence level) = $R \pm ts/\sqrt{n}$, R= mean per cent result of analysis of authentic samples or recovery. Theoritical 't' values at 95% confidence level for n-1 degrees of freeedom 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°. 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 labeled 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° 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[20]. Optimum resolution of complex interferences is achieved through first order derivatisation with $\delta\lambda$ 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 2 : RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS

Form[n]		PPM C.I.	PPM SD	CPM C.I.	CPM SD	DXB C.I.	DXB SD
M-I	A	100.2±0.71	0.449	99.2±0.62	0.391	100.6±1.35	0.851
(n=4)	B	100.4±0.43	0.269	102.5±1.13	0.715	100.4±0.98	0.620
	C	100.4±1.0	0.631	100.1±1.05	0.663	100.2±1.0	0.629
M-II	A	100.0±0.6	0.379	98.6±1.59	1.003	99.9±1.73	1.092
(n=4)	B	100.2±1.09	0.690	99.44±1.38	0.868	99.4±0.73	0.460
	C	102.0±0.71	0.451	99.19±1.15	0.723	99.6±0.85	0.534

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

successfully validated as per standard analytical procedures. Both methods were validated by preliminary analysis of authentic laboratory samples and by recovery studies. To exclude the interference by other excipients, a solvent extraction procedure was used, which was validated by analyzing simulated syrup prepared in the laboratory. The results of analysis of authentic samples and the average recoveries obtained in each instance were compared with the theoretical value of 100 per cent by means of Student's 't' test. As the calculated 't' values are less than theoretical 't' values (Table 1), it is concluded that the results of analysis and recoveries obtained were 100 per cent in agreement for each analyte.

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.

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