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Simultaneous Analysis of Phenylpropanolamine, Chlorpheniramine and Bromhexine in Syrups by Derivative Spectrophotometry

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The absorbance maxima of phenylpropanolamine and chlorpheniramine maleate in 0.1 N hydrochloric acid are 257, 265 nm, respectively, whereas, bromhexine hydrochloride shows absorbance maxima at 245 and 311 nm. This paper presents two methods based on first derivative spectrophotometry for simultaneous estimations of these three drugs in combination in pharmaceutical formulations. The first derivative amplitude at 264, 280.6 and 327 nm is utilized for simultaneous estimation. Linearity was validated by Least Squares Method. The results of analysis have been validated statistically. The proposed methods are simple, economical, accurate, reproducible and rapid.

Phenylpropanolamine hydrochloride (PPA) is a sympathomimetic agent primarily used in symptomatic relief of nasal congestion[1]. Bromhexine hydrochloride (BH) is a mucolytic drug used as an expectorant and chlorpheniramine maleate (CPM) is an antihistaminic drug used in allergic and vasomotor rhinitis[2]. Fixed combination of PPA (25 mg), BH (8 mg) and CPM (4 mg) per 10 ml are marketed as syrup formulation for symptomatic relief of coughs and upper respiratory symptoms such as irritation of throat, running nose, nasal congestion and watery eyes associated with allergy or common cold.

Official methods for quantitative estimation of PPA includes a potentiometric (BP)[3], nonaqueous titration method (USP)[4] and HPLC methods[4] in various formulations. Nonaqueous method with potentiometric end point determination is official for CPM in IP[5], BP[6], USP[7] where, UV spectrophotometry is specified in BP[8] and USP[7] for estimation in tablet and injection and a GC method for syrups as per BP[8]. BH is specified to be estimated by potentiometric method in IP[9] and BP[10] and by spectrophotometry in tablets in IP[9]. UV spectrophotometric[11-13], GLC[14,15] and HPLC[16-21] methods are reported for estimation of CPM along with PPA or BH in other multicomponent formulations and biological fluids. Formulation containing PPA, BH and CPM is an unofficial combination for which only one simultaneous spectrophotometric method based on multicomponent analysis[22] is available. However, this method requires a number of mixed standards prior to analysis and sophisticated instrument having such multicomponent analysis mode in which it processes the signals received to generate matrix equations for further analysis of samples.

The paper presents two simple, accurate, reproducible and economical methods based on derivative spectroscopy for determination of PPA, CPM and BH in multicomponent formulations.

MATERIALS AND METHODS

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. PPA (BP), CPM (IP), BH (IP), hydrochloric acid (Ranbaxy, A.R. grade, S.A.S. Nagar), sodium hydroxide (Qualigens, ExcelaR, Mumbai),

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anhydrous sodium sulfate and double distilled water were used in the present investigation.

Stock solutions of PPA (1000 μg/ml), CPM (200 μg/ml) and BH (200 μg/ml) were prepared separately in 0.1 N HCl. Each stock solution was suitably diluted to different concentrations and the linearity was studied at respective absorbance maxima namely 257, 265 and 311 nm, respectively.

Method I:

The overlain zero order spectra of PPA, CPM and BH (fig. 1) show that the absorption maxima of PPA and CPM lie in close proximity and at absorption maxima of one other exhibits substantial absorbance. Again BH shows considerable absorbance at these two wavelengths. This clearly indicates the existence of spectral interference in estimation of PPA and CPM. To overcome this, spectra of all the three drugs were derivatised to first order between 220 nm and 400 nm with δλ of 24 nm using a scan speed of 1500 nm/min. The overlain first derivative spectra of PPA, CPM and BH (fig. 2) revels that BH has maximum amplitude (DA=dA/dλ) at 327.0 nm (z) where, both PPA and CPM show no amplitude. Hence at this wavelength BH is estimated with no interference from PPA and CPM.

Here, $DA_z^{BH}=DA_z$ ---- (1) where, DA_z^{BH} is first derivative amplitude contributed by BH to total amplitude (DA_z) of samples containing PPA, CPM and BH at 327 nm.

CPM has substantial amplitude in the wavelength vicinity of 279 to 281 nm. At this wavelength region, PPA shows positive amplitude while BH shows negative amplitude which indicates considerable interference by both analytes in estimation of CPM. However, this was nullified by selecting a wavelength where PPA and BH were having amplitude of same magnitude but with opposite exponential, so that the net contribution by both interferrents to the overall amplitude at this wavelength tends to zero. This was successfully found to be at 280.6 nm(y). At this wavelength, $DA_y^{CPM}=DA_y$ - ---- (2). Hence CPM is estimated at 280.6 nm.

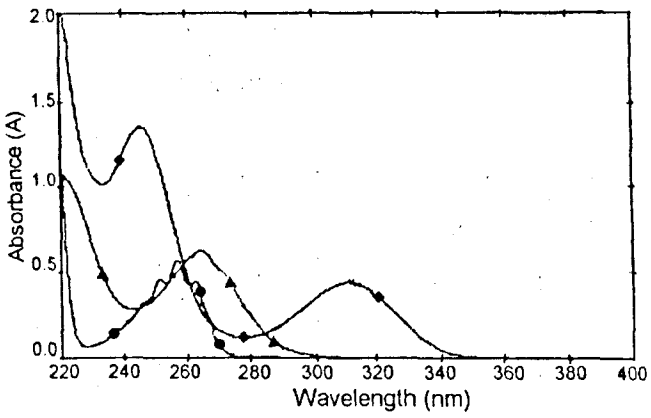


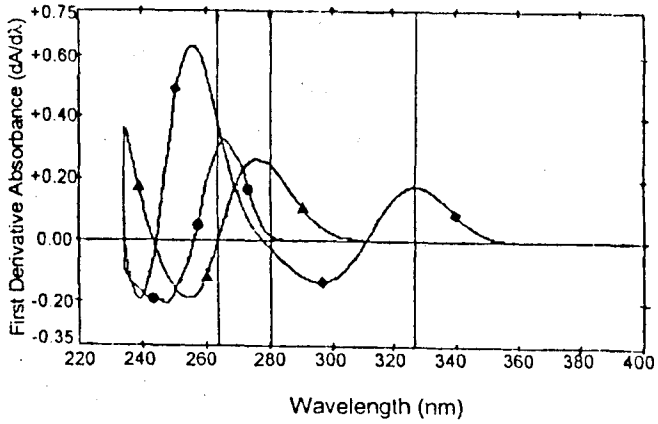
Fig. 1: Overlain spectra of PPA, CPM and BH.

Absorbance spectra obtained at different wavelengths in the range of 400-220 nm of PPA (●), CPM (▲) and BH (◆) in 0.1 N HCl overlain over each other.

For estimation of PPA, the wavelength 264 nm (x) is selected which is the zero crossing point of CPM i.e. dA/dλ of CPM is zero. The only interferrent is BH. However, the contribution of BH to first derivative amplitude at 264 nm (DA_x^{BH}) can be assessed from that at 327 nm (DA_z^{BH}) through a linear regression equation (3). This equation is framed through simultaneous estimation of first derivative amplitude of samples containing different concentrations of BH in the range 0-64 μg/ml by least squares method (n=9, r=0.99996).

$DA_x^{BH}=2.1868xDA_z+0.0004$ ---- (3). The first derivative amplitude due to PPA at 264.0 nm (DA_x^{PPA}) is given by: $DA_x^{PPA}=DA_x-DA_x^{BH}$. Substituting the value of DA_x^{BH} from equation (3), we get: $DA_x^{PPA}=DA_x-(2.1868xDA_z+0.0004)$ ---- (4) where, DA_x is the first derivative amplitude of samples containing PPA, CPM and BH at 264.0 nm (x). Hence PPA is estimated from first derivative amplitude at 264 nm utilizing equation (4).

The linearity between first derivative amplitude and concentration of PPA, CPM and BH were examined at se-



* Markers Showing the selected Wavelengths

Fig. 2: Overlain first derivative spectra of PPA, CPM and BH.

First derivative absorbance spectra obtained at different wavelengths in the range of 400-220 nm of PPA (●), CPM (▲) and BH (◆) in 0.1 N HCl overlain over each other.

lected wavelengths i.e. 264 nm (x), 280.6 nm (y) and 327 nm (z) respectively. Beer's law is followed in the concentration range of 0-800 μg/ml for PPA, 0-40 μg/ml for CPM and 0-70 μg/ml for BH.

Seven mixed standards containing PPA, CPM and BH in the concentrations of 75xn μg/ml, 4xn μg/ml and 8xn μg/ml (where n = 1,2,---,7), 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 nm, 280.6 nm and 327 nm were recorded. By employing equations (1), (2) and (4) the amplitudes contributed by PPA, CPM and BH 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 PPA, CPM and DXB in samples.

$$C_{PPA}=1975.54\ DA_x^{PPA}+2.571\ (r=0.9999) \quad \text{---------- (5)}$$

$$C_{CPM}=127.593\ DA_y+0.1243\ (r=0.9990) \quad \text{---------- (6)}$$

$$CBH=342.814\ DA_z+0.1035\ (r=0.9997) \quad \text{---------- (7)}$$

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

Method II:

This method is also based on first derivative spectrophotometry. The wavelengths selected for estimations of PPA, CPM and BH are same as described in method I, i.e. 264, 280.6 and 327 nm. However, in contrast to method-I, this method utilizes simultaneous equations (Vierdot's method) on derivative spectra to overcome spectral interference at selected wavelengths.

First derivative absorptivity coefficients of individual drugs were determined at 264, 280.6 and 327 nm. A set of three equations framed using these coefficient values is given below.

$$DA_x=0.506\ C_{PPA}+6.44\ C_{BH} \quad \text{---------- (8)}$$

$$DA_y=8.084\ C_{CPM} \quad \text{---------- (9)}$$

$$DA_z=2.935\ C_{BH} \quad \text{---------- (10)}$$

The numericals in above equations denote first derivative absorption coefficients of corresponding drugs at selected wavelengths and are mean of six independent determinations in the concentration range that obey Beer's law.

By solving above three equations we get

$$C_{PPA}=1.9763\ DA_x-4.3364\ DA_z \quad \text{---------- (11)}$$

$$C_{CPM}=0.1237\ DA_y \quad \text{---------- (12)}$$

$$C_{BH}=0.34072\ DA_z \quad \text{---------- (13)}$$

Before analyzing the commercial formulations, the methods were validated by analyzing standard samples containing PPA, CPM and BH in ratio 75:4:8 μg/ml and random samples prepared in laboratory. The results of replicate determinations (n=5) by both proposed methods were validated statistically and are shown in Table 1(A). Precise and more accurate results are obtained with samples containing minimum 200 μg/ml of PPA.

TABLE 1: ANALYSIS OF AUTHENTIC SAMPLES (A) AND RECOVERY EXPERIMENTS (B).

		Method - I			Method - II		
	Analyte	C.I.	SD	% SE	C.I.	SD	%SE
	PPA	98.62±0.825	0.665	0.297	97.88±1.144	0.922	0.412
A	CPM	99.78±1.320	0.967	0.432	98.83±1.549	1.248	0.554
(n=5)	BH	98.69±1.557	1.254	0.561	98.46±1.539	1.239	0.554
	PPA	100.44±2.221	1.396	0.698	100.01±2.026	1.274	0.637
B	CPM	99.47±1.653	1.039	0.519	100.03±1.42	0.893	0.446
(n=4)	BH	99.32±2.001	1.258	0.629	99.12±1.751	1.101	0.550

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, t: theoretical 't' values at 95% confidence level for n-1 degrees of freedom are t (0.05,4)=2.776, t (0.05,3)=3.182.

Commercial syrup formulations procured from local market were used for analysis by methods developed in this investigation. A selective extraction procedure was adopted to avoid interference due to formulation adjuvants. Ten milliliters of each syrup was taken and made alkaline with 10 ml of 1 N NaOH. The resulting alkaline syrup solution was extracted successfully 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 80 μg/ml of PPA, CPM and BH respectively. Different dilutions were prepared from above solution and the amplitude at 264, 280.6 and 327 nm were recorded from the first derivative spectra. The concentration of each analyte was determined using the equations generated in both methods. 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 PPA, CPM and BH, preanalyzed 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 complex spectra and makes it possible to analyse drugs in multicomponent pharmaceutical formulations in presence of various interferences. The technique resolves the overlapped interference by smoothing peaks and loss of background signals and so increases sensitivity of detection[23]. Proper selection of wavelengths with utilization of correlative regression equation and simultaneous equation to avoid complex interference raised in estimation of one drug by others in derivative spectra leads to successful development of methods for simultaneous estimation. Optimum resolution of complex interferences is achieved through first order derivatisation with $\delta\lambda$ of 24 nm of normal spectra.

The proposed methods were found to be accurate, simple and convenient for simultaneous analysis of PPA, CPM and BH in pharmaceutical formulations. The modalities adopted in experimentation were 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 subsequently validated by analysing simulated syrup prepared in laboratory. The results of analysis of authentic samples and the average recoveries obtained in each instance were compared with theoretical value of 100 per cent by means of student's 't' test at a 95 per cent confidence level. The recoveries obtained as indicated from Table 1(B) for each drug do not differ significantly from 100 per cent and there was no interference from common adjuvants used in the formulation indicating accuracy and reliability of both methods.

To overcome spectral interferences, method-I utilizes correlative linear regression equations where as method-II utilizes application of simultaneous equations to first derivative spectra similar to application in normal (zero order) spectra (Vierdot's method). The proper selection of wavelengths with compensation of net spectral interference leads to an advantage that three component system is considered as

TABLE 2: RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS.

	PPA		CPM		BH	
Method	**C.I.**	**SD**	**C.I.**	**SD**	**C.I.**	**SD**
M - I	99.15±0.169	0.136	102.6±1.926	1.552	100.28±1.684	1.357
M - II	97.668±0.991	0.798	98.135±0.868	0.669	99.09±1.814	1.461
M - R*	98.76±1.448	1.167	101.36±0.876	0.706	98.54±2.231	1.878

M-R* implies result obtained by reported methods[22], * each data represents results of five determinations in the analytical range (n=5)

single or two component system, thus avoiding complex situation in solving the equations framed.

The results of analysis of commercial syrup are found to be satisfactory with standard deviation values within acceptable limits. Again both the methods are in well agreement with reported method[22], but with comparatively higher degree of precision as indicated from lower standard deviation values.

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