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Simultaneous Derivative Spectrophotometric Analysis of Pseudoephedrine, Chlorpheniramine and Bromhexine in Combined Dosage Forms

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The absorbance maxima of pseudoephedrine and chlorpheniramine maleate in methanolic hydrochloric acid are 257 and 265 nm, respectively whereas; bromhexine hydrochloride shows two 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 amplitudes at 264, 278.2 and 327 nm were utilized for simultaneous estimations. 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 lead to successful development of methods for simultaneous estimations. Linearity was validated by Least Squares Method. The results of analysis were validated statistically and by recovery studies. Both methods are simple, economical, accurate, reproducible and rapid.

Pseudoephedrine hydrochloride (PSD) is a sympathomimetic agent, primarily used as nasal decongestant in patients with allergic or vasomotor rhinitis with upper respiratory tract infections[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 PSD (30 mg), BH (4 mg) and CPM (2 mg) are marketed as dispersible tablets and syrup (per 5 ml) for symptomatic relief of coughs and upper respiratory symptoms, such as irritation of throat, running nose and nasal congestion associated with allergy or common cold.

Official methods for quantitative estimation of PSD include nonaqueous titration (IP, BP)[3,4] and HPLC (USP)[5] methods in various formulations. Non aqueous method with potentiometric end point determination is official for CPM in IP[6], BP[7], USP[5], while UV spectrophotometry is specified in BP[7] and USP[5] for estimation in tablet and injection and a GC method for syrups in BP[7]. BH is recommended to be estimated by potentiometric method and by spectrophotometry in tablets in IP[6]. In other multicomponent formulations or biological samples CPM and PSD are reported to be estimated by UV spectrophotometric[8,9] and HPLC[10-13] methods. UV spectrophotometric[14,15] and HPLC[16] methods are reported for simultaneous analysis of CPM and with BH in other multicomponent formulations. The combined dosage forms of PSD, CPM and BH are not official and none of official compendia and reported methods specify simultaneous analysis of said analytes in multicomponent formulations. The paper presents two simple, accurate, reproducible and economical methods based on derivative spectroscopy for estimation of PSD, CPM and BH in multicomponent formulations.

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MATERIALS AND METHODS

PSD (IP), CPM (IP), BH (IP), hydrochloric acid AR (S. D. Fine Chem., Mumbai), methanol AR (Sara Fine Chem., Vadodara), chloroform AR (CDH, New Delhi), sodium hydroxide AR (CDH, New Delhi), anhydrous sodium sulfate

and double distilled water were used in the present investigation.

Stock solutions of PSD (6 mg/ml), CPM (400 mg/ml) and BH (400 mg/ml) were prepared separately in 5% methanolic HCl (0.1 N). Each stock solution was suitably diluted to different concentrations and the linearity was studied at respective absorbance maxima i.e. 257, 265 and 311 nm, respectively. A Thermo Spectronic Genesys 2 Spilt beam dual detector UV/Vis recording spectrophotometer with spectral band width of 2 nm was employed for all spectroscopic measurements using a pair of 10 mm matched quartz cells.

Method I:

The overlain zero order spectra of PSD, CPM and BH (fig. 1) show that the absorption maxima of PSD 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 PSD and CPM. To overcome this, spectra of all the three drugs were derivatised to first order between 220 nm and 360 nm with 1 of 3 nm using a scan speed of 1800 nm/min. The overlain first derivative spectra of PSD, CPM and BH (fig. 2) reveals that BH has maximum amplitude (DA=dA/1) at 327 nm (z) where, both PSD and CPM show no amplitude. Hence at this wavelength BH can be estimated without any interference from PSD 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 PSD, CPM and BH at 327 nm.

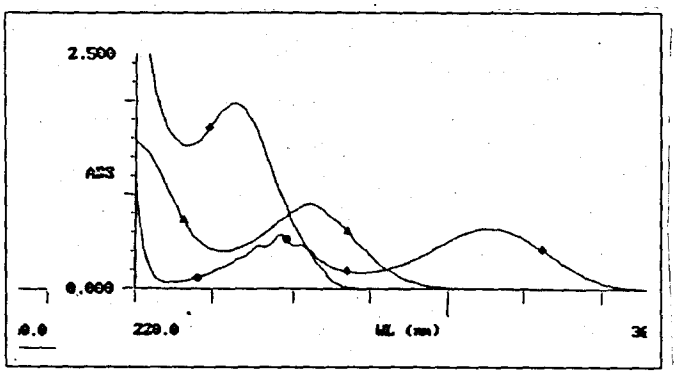

Fig. 1: Overlain Spectra of PSD, CPM and BH
Absorbance Spectra obtained at different wavelengths in the range of 360-220 nm of PSD (●), CPM (◆) and BH (◆) in 0.1 N HCl overlain over each other.

CPM is estimated at 279 nm (y) because here PSD and BH shows amplitude of same magnitude with opposite exponential resulting net contribution by both interferrent tends to zero to overall amplitude. At this wavelength, $DA_y^{CPM} = DA_y$ (2)

For estimation of PSD, the wavelength of 264 nm (x) is selected which is the zero crossing point of CPM, i.e. dA/1 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 Eqn (3). This equation is framed through simultaneous estimation of first derivative amplitude of samples containing different concentrations of BH in the range 0-200 mg/ml by least squares method (n=6, r = 0.9993).

$DA_x^{BH} = 1.6984 \cdot DA_z + 0.003$ (3). The first derivative amplitude due to PSD at 264.0 nm (DA_x^{PSD}) is: $DA_x^{PSD} = DA_x - DA_x^{BH}$. Substituting the value of DA_x^{BH} from equation (3), we get: $DA_x^{PSD} = DA_x - (1.6984 \cdot DA_z + 0.003)$ (4), where DA_x is the first derivative amplitude of samples containing PSD, CPM and BH at 264 nm (x). Hence PSD is estimated from first derivative amplitude at 264 nm utilizing Eqn (4).

The linearity between first derivative amplitude and concentration of PSD, CPM and BH were examined at selected wavelengths, i.e., 264 nm (x), 280 nm (y) and 327 nm (z), respectively. Beer's law was followed in the concentration range of 0-2 mg/ml for PSD, 0-100 mg/ml for CPM and 0-240 mg/ml for BH. The coefficient of correlation (r) as evaluated by least squares method in each case was >0.99.

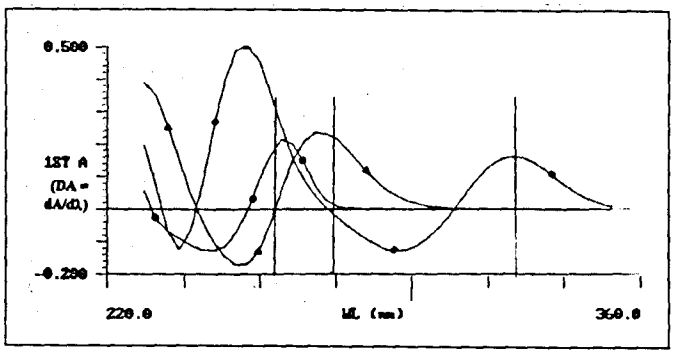

Fig. 2: Overlain First Derivative Spectra of PSD, CPM and BH
First Derivative Absorbance Spectra obtained at different wavelengths in the range of 360-220 nm of PSD (●), CPM (◆) and BH (◆) in 0.1 N HCl overlain over each other. *Markers showing the selected wavelengths

Five mixed standards containing PSD, CPM and BH in the concentrations of 300'n mg/ml, 20'n mg/ml and 40'n mg/ml (where n=1,2,.. 5), respectively, were prepared and the first derivative amplitudes at selected wavelengths, i.e., 264 nm, 278.2 nm and 327 nm were recorded. By employing Eqns. (1), (2) and (4), the amplitudes contributed by PSD, 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. Three linear regression equations obtained and utilized for direct estimation of PSD, CPM and BH in the samples were: C_{PSD} = 5923 DA_x^{PSD} - 4.296 (r = 0.996) (5), C_{CPM} =353.35 DA_y - 0.8235 (r = 0.999) (6), C_{BH} = 982.5 DA_z -0.5649 (r = 0.9997) (7), where, C_{PSD}, C_{CPM} and C_{BH} are concentrations of PSD, CPM and BH, respectively in samples or mixed standards.

Method II:

This method is also based on first derivative spectrophotometry and the wavelengths selected for estimations of PSD, CPM and BH are same as described in method I. However, in contrast to method-I, this method utilized simultaneous equations (Vierdot's method)[17] on derivative spectra to overcome spectral interference at selected wavelengths.

First derivative absorptivity coefficients of individual drugs were determined at 264, 279 and 327 nm. A set of three equations framed using these coefficient values are: DA_x = 0.166 C_{PSD} + 1.79 C_{BH} (8), DA_y = 3.04 C_{CPM} (9), DA_z = 1.026 C_{BH} - (10). The numericals in above equations denote first derivative absorptivity coefficients of corresponding drugs at selected wavelengths and are mean of five independent determinations in the concentration range that were found to obey the Beer's law. By solving above three equations, one got: C_{PSD} = 0.55866 DA_x - 0.09274 DA_z (11), C_{CPM} = 0.329 DA_y -(12), C_{BH} = 0.97466 DA_z (13).

Before analyzing the commercial formulations, the methods were validated by analyzing standard samples containing PSD, CPM and BH in ratio 300:20:40 mg/ml and random samples prepared in the laboratory. The results of replicate determinations (n=4) by both proposed methods were validated statistically and are shown in Table 1 Precise and accurate results were obtained with samples containing PSD in the range of 600-1500 mg/ml.

Preparation of tablet sample solutions:

Twenty tablets were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 120 mg of PSD was transferred to a 100 ml volumetric flask. The powder was dissolved in 5 ml of methanol and the volume was made up to mark with 0.1 N HCl. The solution was then filtered through Whatman Filter paper No 41.

Preparation of syrup sample solutions:

A selective extraction procedure was adopted to avoid interference due to formulation adjuvants in commercial formulations. Ten milliliters of each syrup was taken and made alkaline with NaOH. The resulting alkaline syrup solution was extracted successfully five times with 10 ml portions of chloroform and the extracts were collected. The solvent was driven off completely under reduced pressure at 45±2°. The residue was dissolved in 5% methanolic 0.1

TABLE 1: ANALYSIS OF AUTHENTIC SAMPLES (AS) AND RECOVERY EXPERIMENTS (RS)

	Analyte	Method – I			Method – II		
		C.I.	SD	%SE	C.I.	SD	%SE
AS	PSD	98.6±0.83	0.665	0.297	97.9±1.14	0.922	0.412
(n=5)	CPM	99.8±1.32	0.967	0.432	98.8±1.55	1.248	0.554
	BH	98.7±1.56	1.254	0.561	98.5±1.54	1.239	0.554
RS	PSD	100.4±2.22	1.396	0.698	100±2.026	1.274	0.637
(n=4)	CPM	99.5±1.65	1.039	0.519	100±1.42	0.893	0.446
	BH	99.3±2.00	1.258	0.629	99.1±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 ± ts/Ön, where R is 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, that are t(0.05,4)=2.776, t(0.05,3)=3.182

TABLE 2: RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS (N=3)

FormIn	Method	PSD		CPM		BH	
		C.I.	SD	C.I.	SD	C.I.	SD
Tablet	M - I	99.15±0.169	0.136	102.6±1.926	1.552	100.28±1.684	1.357
	M - II	97.67±0.991	0.798	98.14±0.868	0.669	99.09±1.814	1.461
Syrup	M - I	98.76±1.448	1.167	101.36±0.876	0.706	98.54±2.231	1.878
	M - II	99.59±1.268	0.797	100.32±0.853	0.536	99.23±1.742	1.905

N HCl and the volume was made up to 50 ml in a volumetric flask.

The above resulting solution from tablet and syrup was treated as stock sample solution, labeled to contain 1200 mg/ml, 80 mg/ml and 160 mg/ml of PSD, CPM and BH, respectively. Different dilutions (equivalent to PSD more than 600 mg/ml) were prepared from these solution and the amplitudes at 264 nm, 279 nm and 327 nm were recorded from the first derivative spectra. The concentration of each analyte was determined using the equations generated in both methods. One of commercial syrup 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 was further dried under reduced pressure at 45±2° for another 2 h. The statistical data of results obtained after replicate determinations (n=4) are shown in Table 2.

To study the recovery of PSD, 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 the proposed methods. The added quantities of individual drugs were estimated by both methods and the statistical data are given in Table 1 (n=4).

RESULTS AND DISCUSSION

Derivative spectrophotometry provides a versitile 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[18]. Optimum resolution of complex interferences is achieved through first order derivatisation of normal spectra with dl of 3 nm and smoothing of 6 nm.

The proposed methods were developed using this derivative spectrophotometric technique and found to be accurate, simple and convenient for simultaneous analysis of PSD, 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. The solvent extraction process adopted for syrup formulation was quantitatively assured through analysis of a simulated syrup containing the three analytes without any formulation adjutants, prepared in laboratory, by these proposed methods.

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 percent confidence level. The recoveries obtained as indicated from Table 1for 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 utilized correlative linear regression equations where as method-II utilized 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 lead to an advantage that three component system was considered as single or two component systems, thus avoiding complex situation in solving the equations framed.

The results of analysis of commercial formulations were

found to be satisfactory with standard deviation values within acceptable limits. Again, both the methods were in well agreement with each other. Both methods were suitable for routine analysis in laboratory. However, a comparative new innovative method based on developed simultaneous equations simplifies the quantification of drugs in combination and showed enough sensitivity, accuracy and precision.

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