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Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 1023–1031

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods

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Received 27 January 2000; received in revised form 14 April 2000; accepted 21 May 2000

Abstract

Three simple, rapid and accurate methods are described for the simultaneous determination of chlorpheniramine maleate and phenylephrine hydrochloride in two component mixtures. The first method comprised of measurement of difference absorptivities derivatized in first order of a nasal drops in 0.1 N NaOH relative to that of an equimolar solution in methanol at wavelengths of 271.6 and 250.2 nm, respectively. The second method, zero-crossing derivative spectrophotometry, is based on recording the first derivative curves and determining each component using the zero-crossing technique. Using first derivative spectrophotometry, the amplitudes in the first derivative spectra at 246.5 and 238.6 nm were selected to simultaneously determine chlorpheniramine maleate and phenylephrine hydrochloride in the mixture. The presence of identical zero-crossing points for pure drugs and nasal drop solutions established the non-interference of the excipients in the absorption at these wavelengths. Absorbance ratio method was also developed for a comparison method. The proposed procedures were successfully applied to the determination of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops, with a high percentage of recovery, good accuracy and precision.

Keywords: Chlorpheniramine maleate; Phenylephrine hydrochloride; Differential-derivative spectrophotometry; Derivative spectrophotometry; Absorbance ratio method; Pharmaceutical formulations

1. Introduction

Chlorpheniramine maleate, is an alkylamine derivative with the actions and uses of the antihistamines [1]. It is one of the most potent antihistamines and causes a moderate degree of sedation.

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PII: S0731-7085(00)00371-X

It is used alone or with phenylephrine hydrochloride and guaiphenesin for the symptomatic treatment of coughs due to acute or chronic bronchitis and bronchial allergic conditions.

Some procedures have been described for the assay of either chlorpheniramine maleate or phenylephrine hydrochloride in pharmaceutical preparations, such as spectrophotometry [2–12] and HPLC [13–26]. The chlorpheniramine maleate–phenylephrine hydrochloride mixture is not yet official in any pharmacopoeia. To my knowledge, no analytical methods could be traced for the analysis of chlorpheniramine maleate–phenylephrine hydrochloride combination in pharmaceutical dosage form. Therefore a simple, rapid and reliable method for simultaneous assay of both drugs in mixture seemed to be necessary.

The aim of this work was to investigate the utility of differential-derivative spectrophotometry, derivative spectrophotometry and absorbance ratio method in the assay of chlorpheniramine maleate and phenylephrine hydrochloride in combination in pharmaceutical preparations without the necessity of sample pre-treatment. Derivative spectrophotometry [27] is a useful means of resolving two overlapping spectra and eliminating matrix interferences in the assay of two-component mixtures using the zero-crossing technique. Difference spectrophotometry has proved to be a powerful technique for determination of drugs [28–30] as well as detection and determination of decomposition products [31]. The derivative-difference spectrophotometry will offer further advantages in cancelling heavy spectral interferences to drug analysis [32,33] when the irrelevant absorption is pH and solvent dependent. The methods had sufficient accuracy and precision and permitted a simple and time-saving assay of chlorpheniramine maleate and phenylephrine hydrochloride in mixtures.

2. Materials and methods

2.1. Apparatus

A Shimadzu 1601 double beam UV-Vis spectrophotometer, in 1 cm quartz cells with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC Software equipped with a Lexmark printer was used for all the absorbance measurements and treatment of data.

2.2. Chemicals

Chlorpheniramine maleate and phenylephrine hydrochloride were kindly donated by the pharmaceutical industry and used without further purification. All solvents and reagents were of analytical reagent grade (Merck Chem. Ind.).

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (RiNOSiL® nasal drop AKDENiZ Pharm Ind.-Turkey, batch no: ER 34) was assayed. Its declared content was as follows: phenylephrine hydrochloride (50.0 mg), chlorpheniramine maleate (80.0) mg per 5 ml drop.

2.4. Calibration graphs

Standard solutions of chlorpheniramine maleate and phenylephrine hydrochloride were prepared by weighing accurately 80.0 mg chlorpheniramine maleate and 50.0 mg phenylephrine hydrochloride and dissolving in 250 ml methanol. Appropriate volume aliquots of the stock solution were transferred to 25 ml calibrated flasks. Accurate volumes were transferred into two sets of 25 ml calibrated flasks. One set was diluted to volume with 0.1 N NaOH and the other set was diluted to volume with methanol. The first series contained a constant concentration of chlorpheniramine maleate (25.0 μg ml^{-1}) and a varying concentration of phenylephrine hydrochloride (4.0–20.0 μg ml^{-1}). The second contained a constant concentration of phenylephrine hydrochloride (8.0 μg ml^{-1}) and a varying concentration of chlorpheniramine maleate (10.0–25.0 μg ml^{-1}).

2.5. Sample preparation

A 5 ml drop (from RiNOSiL® nasal drop), was taken and dissolved in methanol in 100 ml cali-

brated flasks. The solution was diluted 1:50 with 0.1 N NaOH and methanol, separately.

2.6. Spectrophotometric measurements

2.6.1. Differential-derivative spectrophotometry

The difference spectra between the methanolic solution and equimolar 0.1 N NaOH solution of pure drugs and sample were recorded from 240.0 to 330.0 nm by placing the methanolic solution in the reference compartment and the 0.1 N NaOH solution in the sample compartment. A first derivative spectrum of each of the differential curves was subsequently recorded.

2.6.2. First derivative spectrophotometry

The first derivative spectra of the drug solutions in the 0.1 N NaOH were recorded against 0.1 N NaOH as a blank. The absolute values (peak to zeroline) for D_1 were measured at the selected wavelengths.

2.6.3. Absorbance ratio method

Such a method of analysis is based on the linear relationship between the absorbance ratio value of a binary mixture and the relative concentration of such a mixture.The quantification analysis of chlorpheniramine maleate and phenylephrine hydrochloride in binary mixture are performed by using following equations:

$$C_1 = (Q_1 - b_1/a_1)(A_{iso}/a_{iso}) \times 10^3,$$

$$C_2 = (Q_2 - b_2/a_2)(A_{iso}/a_{iso}) \times 10^3$$

where $Q_1 = A_1/A_{iso}$ for chlorpheniramine maleate, $Q_2 = A_2/A_{iso}$ for phenylephrine hydrochloride, C_1 and C_2 = concentrations of the chlorpheniramine maleate and phenylephrine hydrochloride, respectively, A_{iso} = absorbance at isoabsorptive point ($\lambda_{iso} = 275.8$ nm), a_{iso} = absorptivity at isoabsorptive point = $A_{iso}/C_1 + C_2$, a_1 = slope of regression equation (Q_1 vs $C_1/C_1 + C_2$), a_2 = slope of regression equation (Q_2 vs $C_2/C_1 + C_2$), $b_{1,2}$ = intercept values of these regression equations, A_1 and A_2 denotes the absorbances of the mixture solution measured at λ_1 and λ_2 (260.8 and 294.1 nm).

3. Results and discussion

3.1. Differential-derivative spectrophotometry

The difference absorption spectra of chlorpheniramine maleate and phenylephrine hydrochloride is shown in Fig. 1. Fig. 2 shows that the first derivative difference spectrum. The first

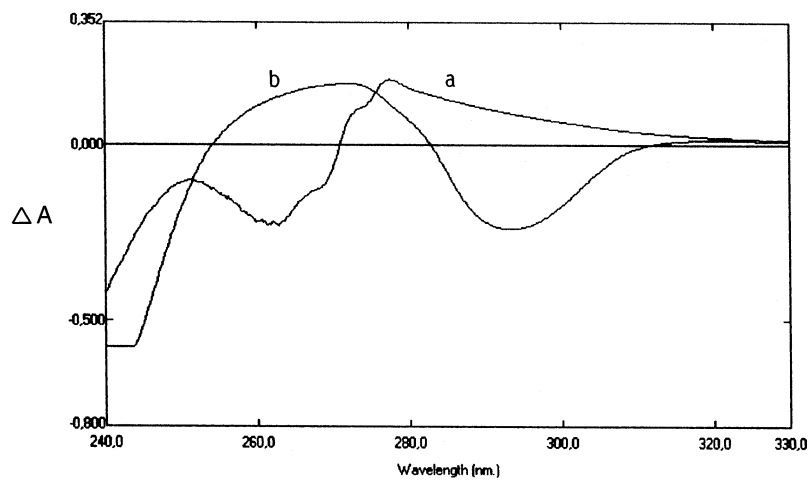

Fig. 1. Differential spectra of (a) 25.0 μg ml^{-1} chlorpheniramine maleate; (b) 8.0 μg ml^{-1} phenylephrine hydrochloride in methanol versus 0.1 N NaOH.

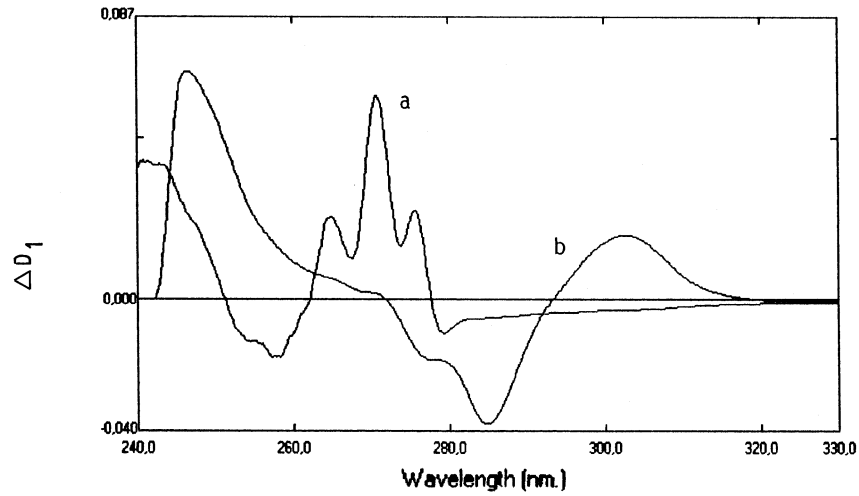

Fig. 2. Differential-derivative spectra of (a) 25.0 μg ml^{-1} chlorpheniramine maleate; (b) 8.0 μg ml^{-1} phenylephrine hydrochloride in methanol versus 0.1 N NaOH.

derivative differential spectra of both drugs offered an advantage for their simultaneous determination by having zero-crossing points (Fig. 2). In particular absorbance at 271.6 nm for chlorpheniramine maleate and at 250.2 nm for phenylephrine hydrochloride were considered as the optimum working wavelengths for their determination. The differential-derivative spectra showed the best linear response to analyte concentrations used at these wavelengths. Under the experimental conditions described, standard calibration curves for chlorpheniramine maleate and phenylephrine hydrochloride were constructed by plotting absorbance versus concentration, respectively. Conformity with Beer's law was evident in the concentration range from 10.0 to 25.0 μg ml^{-1} of chlorpheniramine maleate and from 4.0 to 20.0 μg ml^{-1} of phenylephrine hydrochloride (Table 1).

The regression curve was calculated by the least-squares method. The correlation coefficients were 0.9983 for phenylephrine hydrochloride and 0.9990 for chlorpheniramine maleate, indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations were found to be less than 1.23%, indicating reasonable repeatability of the selected method.

3.2. First derivative spectrophotometry

Fig. 3 shows the absorption (zero-order) spectra of chlorpheniramine maleate and phenylephrine hydrochloride. The large overlap of the spectral bands of the drugs at 230.0–330.0 nm prevents the formation from the total zero-order spectrum of any spectral future that could be used for analytical purposes. The first derivative spectra allowed the simultaneous determination of chlorpheniramine maleate and phenylephrine hydrochloride. Fig. 4 shows the first derivative spectra of chlorpheniramine maleate and phenylephrine hydrochloride. The D_1 spectrum of of chlorpheniramine maleate shows a well-defined minimum at 246.5 nm while phenylephrine hydrochloride has a zero D_1 value at the same wavelength.

Phenylephrine hydrochloride has a maximum D_1 value at 238.6 nm at which chlorpheniramine maleate exhibits no contribution. Therefore, at these selected wavelenghts the two drugs can be quantified in the presence of each other without interference. For quantitative analysis, the analytical data for the calibration graphs are listed in Table 1. The correlation coefficients were 0.9997 and 0.9984 indicating good linearity. Five replicate determinations at different concentration lev-

Table 1
Statistical analysis of calibration graph in the determination of chlorpheniramine maleate and phenylephrine hydrochloride by differential-derivative, first derivative spectrophotometry and absorbance ratio method

Parameters	Chlorpheniramine maleate			Phenylephrine hydrochloride		
	Diff. derivative spectr.	First derivative spectr.	Abs. ratio method	Diff. derivative spectr.	First derivative spectr.	Abs. ratio method
Range (μg ml^{-1})	10.0–25.0	10.0–25.0	8.3–27.8	4.0–20.0	4.0–20.0	3.2–25.0
Detection limits (μg ml^{-1})	0.012	0.10	0.89	0.023	0.038	0.078
Regression equation (Y)[a]						
Slope (b)	4.52×10^{-2}	5.43×10^{-2}	1.98×10^{-2}	6.73×10^{-2}	6.42×10^{-2}	9.91×10^{-4}
Standard deviation on slope (S_b)	1.80×10^{-4}	3.35×10^{-4}	7.89×10^{-4}	8.12×10^{-5}	3.72×10^{-5}	4.25×10^{-4}
Intercept (a)	2.32×10^{-5}	4.51×10^{-5}	6.10×10^{-2}	6.81×10^{-4}	6.37×10^{-4}	3.45×10^{-2}
Standard deviation on intercept (S_a)	7.24×10^{-6}	9.12×10^{-6}	9.81×10^{-4}	8.27×10^{-6}	1.29×10^{-6}	8.79×10^{-4}
Standard error of estimation (S_e)	3.58×10^{-3}	3.19×10^{-3}	4.56×10^{-2}	3.38×10^{-3}	5.17×10^{-3}	7.12×10^{-3}
Correlation coefficient (r)	0.9990	0.9997	0.9999	0.9983	0.9984	0.9990
Relative standard deviation (%)[b]	0.85	0.12	0.67	2.05	3.23	2.58
% Range of error (95% confidence limit)	0.68	0.29	0.93	0.48	0.34	0.65

[a] $Y=a+bC$ where C is concentration in μg ml^{-1} and Y is absorbance units.
[b] Five replicate samples.

els were carried out to test the precision of the methods. The relative standard deviations were found to be less than 0.87%, indicating reasonable repeatability of the proposed method.

3.3. Absorbance ratio method

Derivative spectrophotometry, differential-derivative spectrophotometry tested absorbance ratio method for resolving the binary mixtures. The zero-order (original) spectra of chlorpheniramine maleate and phenylephrine hydrochloride are illustrated in Fig. 3. These spectra indicated that binary mixtures containing chlorpheniramine maleate and phenylephrine hydrochloride could be analyzed by applying the principle absorbance ratio method. By measuring absorbance values at 260.8 nm (λ_{max} for chlorpheniramine maleate), 294.1 nm (λ_{max} for phenylephrine hydrochloride) and 275.8 nm (isosbestic point) in the original spectra of the binary mixture in methanol, the analysis of the binary mixture containing chlorpheniramine maleate and phenylephrine hydrochloride was made by using the formulas

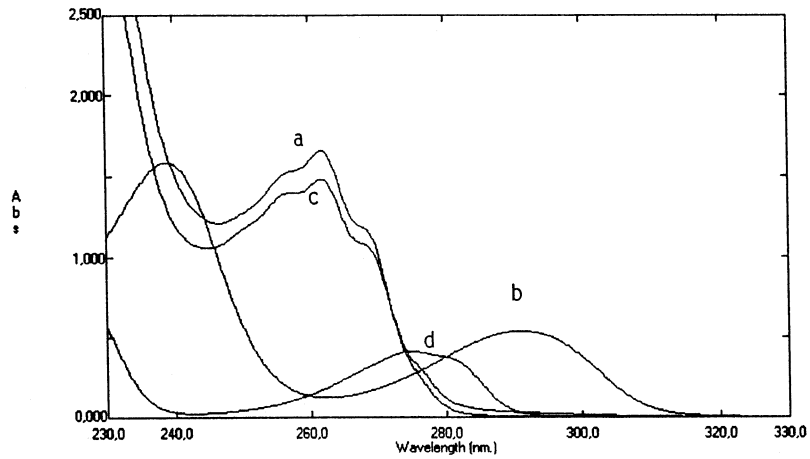

Fig. 3. Zero-order spectra of (a) 25.0 μg ml^{-1} chlorpheniramine maleate; (b) 8.0 μg ml^{-1} phenylephrine hydrochloride in methanol, and (c) 25.0 μg ml^{-1} chlorpheniramine maleate; (d) 8.0 μg ml^{-1} phenylephrine hydrochloride in 0.1 N NaOH.

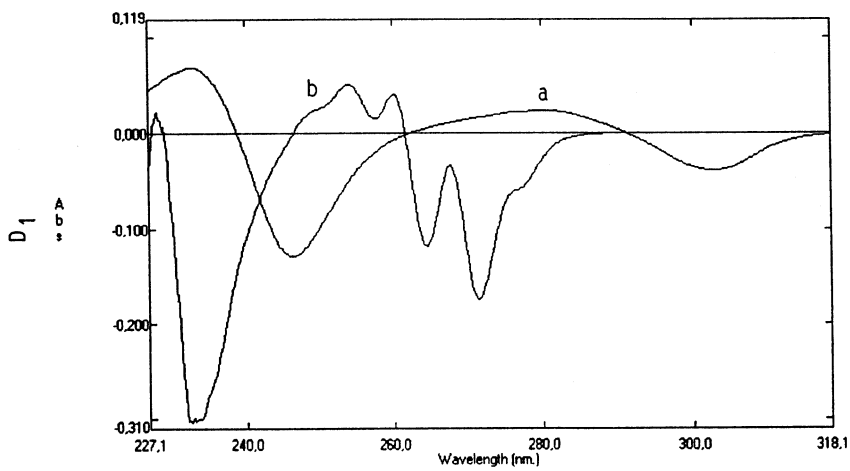

Fig. 4. First derivative spectra of (a) 25.0 μg ml^{-1} chlorpheniramine maleate; (b) 8.0 μg ml^{-1} phenylephrine hydrochloride in 0.1 N NaOH.

Table 2
Assay results for the determination of chlorpheniramine maleate and phenylephrine hydrochloride in laboratory synthetic mixture and commercial tablets

Sample	% Recovery (mean ± SD)[a]					
	Chlorpheniramine maleate			Phenylephrine hydrochloride		
	Differential derivative spectr.	First derivative spectr.	Abs. ratio method	Differential derivative spectr.	First derivative spectr.	Abs. ratio method
Synthetic mixtures	100.7 ± 4.2	99.9 ± 0.9	98.9 ± 0.5	99.8 ± 1.9	99.8 ± 1.5	99.9 ± 1.1
t	0.278[b]	0.719[b]	–	0.529[b]	1.810[b]	–
F	0.627[b]	2.658[b]	–	0.140[b]	1.789[b]	–
Commercial nasal drops[c]	98.1 ± 1.1	100.2 ± 1.6	99.6 ± 1.9	101.0 ± 1.2	101.8 ± 1.9	98.3 ± 0.7
t	0.636	1.000	–	0.355	0.964	–
F	0.516	0.897	–	0.812	1.239	–

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Theoretical values at 95% confidence limits $F = 3.18$; $t = 2.26$.
[c] Commercial nasal drops are the product of Akdeniz Pharm. Ind., Turkey; each 5 ml drop was labeled to contain 80.0 and 50.0 mg of chlorpheniramine maleate and phenylephrine hydrochloride, respectively.

explained in Section 2.6. A critical evaluation of the proposed method was performed by the statistical analysis of the experimental data. The obtained slopes, intercepts and correlation coefficients obtained are summarized in Table 1. In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by analyzing synthetic mixtures of chlorpheniramine maleate and phenylephrine hydrochloride which reproduced different composition ratios. The percentage recoveries of chlorpheniramine maleate and phenylephrine hydrochloride from spiked excipient are summarized in Table 2.

The absorbance ratio method was chosen as the analytical reference method. Differential-derivative spectrophotometry and first derivative spectrophotometry were compared with the absorbance ratio method. The intercept values for differential-derivative spectrophotometry, first derivative spectrophotometry and absorbance ratio method were not statistically ($P < 0.05$) different from zero. The order linearity for the calibration graphs in the ranges stated in Table 1 for the different analytical method was absorbance ratio method > first derivative spectrophotometry = differential-derivative spectrophotometry. The lowest detection limit calculated was obtained for differential-derivative spectrophotometry (0.010–0.023 μg ml^{-1}) indicating the highest sensitivity. The absorbance ratio method was the least sensitivite (0.078–0.89 μg ml^{-1}). Commercially available nasal drops were analyzed using differential-derivative, first derivative spectrophotophotometric and absorbance ratio method. The results obtained are summarized in Table 2. No significant differences were found between the results obtained by the absorbances ratio method and the differential-derivative and first derivative spectrophotometry, for same batch at the 95% confidence level (student's t-test and F-variance ratio test).

4. Conclusions

The proposed methods are simple (as there is no need for solvent extraction), rapid (as it requires measurements of ΔD_1, D_1 and A values at a single wavelength and direct (as it estimates each drug independently of the other). This paper demonstrates the potential of derivative-differential spectroscopy, first derivative spectrophotometry and absorbance ratio method as an analytical technique and its usefulness to accurately, rapidly, simply and simultaneously quantitate active ingredients in multicomponent pharmaceuticals.

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