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# ANALYSIS OF NASAL SOLUTIONS CONTAINING PHENYLEPHRINE HYDROCHLORIDE AND PHENIRAMINE MALEATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ON A CYCLODEXTRIN BONDED STATIONARY PHASE AND DIODE ARRAY SPECTROPHOTOMETRY

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## ABSTRACT

Methods based on high performance liquid chromatography (HPLC) and diode array spectroscopy for the analysis of phenylephrine hydrochloride and pheniramine maleate in nasal spray solutions have been developed. No interferences from excipients in the formulation occur with either method of analysis which should be applicable to uniformity of content testing. There was no statistical difference ($P<0.01$) in the analyses of the two drugs by either method. The novelty of the HPLC method lies in the use of a cyclodextrin bonded stationary phase which allows simultaneous analysis of phenylephrine and pheniramine. These two drugs could not be analysed by reversed phase HPLC since pheniramine did not elute from the column under conditions which produced adequate retention of phenylephrine. The advantage of diode array spectroscopy lies in its speed of analysis. For analysis by HPLC, the accuracies were 100.3% for phenylephrine and 100.8% for pheniramine, the system precision (% RSDs) was 0.48% for phenylephrine and 0.23% for pheniramine and the method precision was 1.82% for phenylephrine and 1.63% for pheniramine. Various methods of manipulating the raw spectroscopic data were investigated. For analysis at two





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wavelengths, determinations at 262 nm and 273 nm gave the best results. For analysis over a range of wavelengths, 255 to 266 nm was preferred for determination using zero or first order derivatives. A range of 273 to 290 nm gave better results for determinations using second order derivatives. The accuracies and precisions varied with the method of data manipulation and the same method did not give optimal values of these parameters for both drugs. The best compromise for the analysis of pheniramine and phenylephrine was obtained with zero order spectra obtained over the range 255 to 262 nm. Under these conditions, the accuracies were 102.9% for phenylephrine and 99.7% for pheniramine, the system precisions were 0.12% for phenylephrine and 0.31% for pheniramine and the method precisions were 1.44% for phenylephrine and 1.45% for pheniramine.

## INTRODUCTION

It is common to have certain combinations of sympathomimetics and antihistamines in pharmaceutical dosage forms, and fast and sensitive quantitative methods are needed for the analysis of these products. One existing combination is an aqueous solution of phenylephrine hydrochloride and pheniramine maleate, which is indicated for temporary relief of nasal congestion.

Both the spectrophotometric and chromatographic systems described here for the quantitative analysis of combinations of phenylephrine hydrochloride and pheniramine maleate make use of recent advances in their respective fields. The HPLC system has the novelty of utilizing a beta-cyclodextrin column to separate the two active components. Cyclodextrins can be effective in chemical separations because of their ability to form selective inclusion complexes by either a hydrophobic effect, hydrogen bonding, the release of solvent during complex formation or a combination of these factors[1]. The spectrophotometric system is composed of a linear photodiode array spectrophotometer and a microcomputer which are capable of obtaining absorbance spectra at a maximum rate of 10 $s^{-1}$ and then performing either single or multicomponent analysis utilizing zeroth to seventh derivative order spectra. Linear photodiode array spectrophotometers have been described as having a great potential for enhanced specificity in bioanalytical investigations[2] and microcomputer UV/VIS

spectrophotometers have been described as beneficial due to faster set-up procedure and more accurate results[3].

Although multicomponent systems have been analyzed spectrophotometrically for many decades it is only recently that this type of analysis regained some of its popularity due to the proliferation of spectrophotometers with interfaced or built-in computers[4,5]. During the last few years several papers have described spectrophotometric methods which used derivative spectra to quantify two[6-8] or three components [9] in various pharmaceutical preparations. All of these studies compared the results from derivative spectra to the results of absorbance spectra but did not compare their results to any chromatographic technique; the overall conclusion was that the derivative spectra provided better analytical results than those obtained using absorbance spectra.

This paper describes the quantitative analysis of pharmaceutical preparation containing pheniramine maleate and phenylephrine hydrochloride. Techniques involving zero to fifth derivative spectra are compared with an HPLC method.

## EXPERIMENTAL

Reagents-Phenylephrine hydrochloride and pheniramine maleate were obtained from Sigma Chemical Co., St. Louis, Missouri. Dristan nasal spray (Whitehall Laboratories, New York, New York) was purchased from a local retail pharmacy. This spray is formulated with 0.5% phenylephrine hydrochloride, 0.2% pheniramine maleate, 0.2% benzalkonium chloride, 0.4% alcohol, 0.002% thimerosal and a few other exciptients at very low concentrations in a buffered isotonic aqueous solution. All reagents were of analytical grade. Deionized water was used throughout for both the HPLC and the UV analysis.

Standards Preparation- Standard solutions containing phenylephrine hydrochloride and pheniramine maleate were prepared separately by diluting stock solutions of the drugs dissolved in 0.01 N HCl. The pheniramine hydrochloride standards contained 10,20,30,40,and 50 µg/ml in 0.01 N HCl, while the phenylephrine hydrochloride standards contained 25,50,75,100, and 125 µg/ml in 0.01 N HCL.

Sample Preparation- Five laboratory mixtures were made from the stock solutions to contain 100 and 40 µg/ml of phenylephrine hydrochloride and pheniramine maleate respectively, using 0.01 N HCl as the solvent. Five dilutions of the the nasal solution were prepared by diluting 2 ml of the pharmaceutical preparation to 100 ml with 0.01 N HCl, thereby giving the same approximate concentrations as the laboratory mixtures of the two drugs.

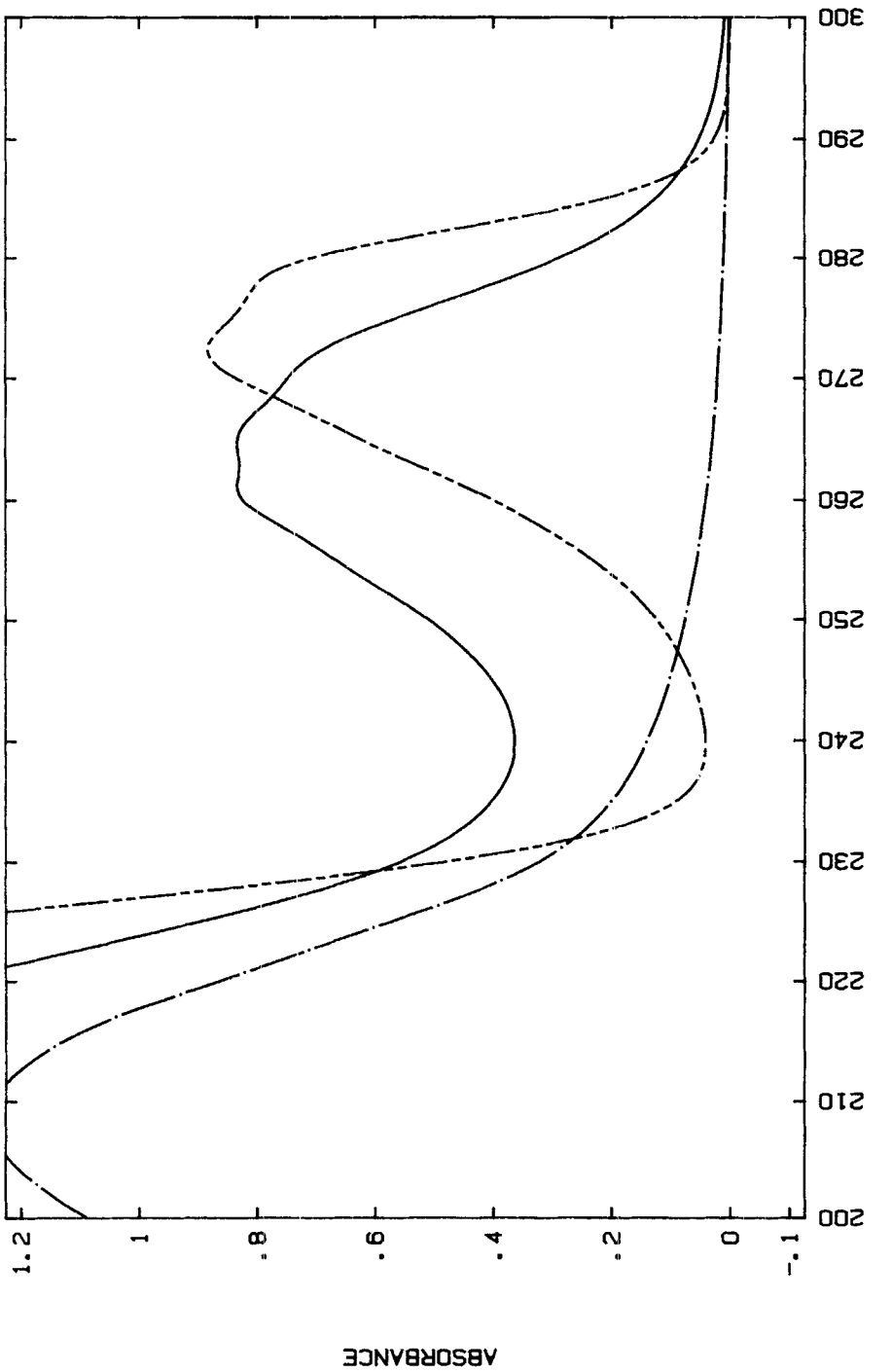
Chromatography- The liquid chromatographic system used in this study consisted of an LC high pressure pump model 110B (Beckman, Palo Alto, CA), a model 7125 sample injector fitted with a 20 µl loop (Rheodyne, Cotati, California), a multiwavelength UV detector (LDC/Milton Roy, Riviera Beach, Florida) set at 262 nm. The analytical column was a Cyclobond I (ASTEC, Whippany, New Jersey). A guard column filled with Permaphase ODS (Dupont, Wilmington, Delaware) was placed between the pump and the injector.

The mobile phase was 60:40 (v/v) methanol/0.05 M phosphate buffer, pH 7.0; flow rate was 2.0 ml/min. The temperature was ambient.

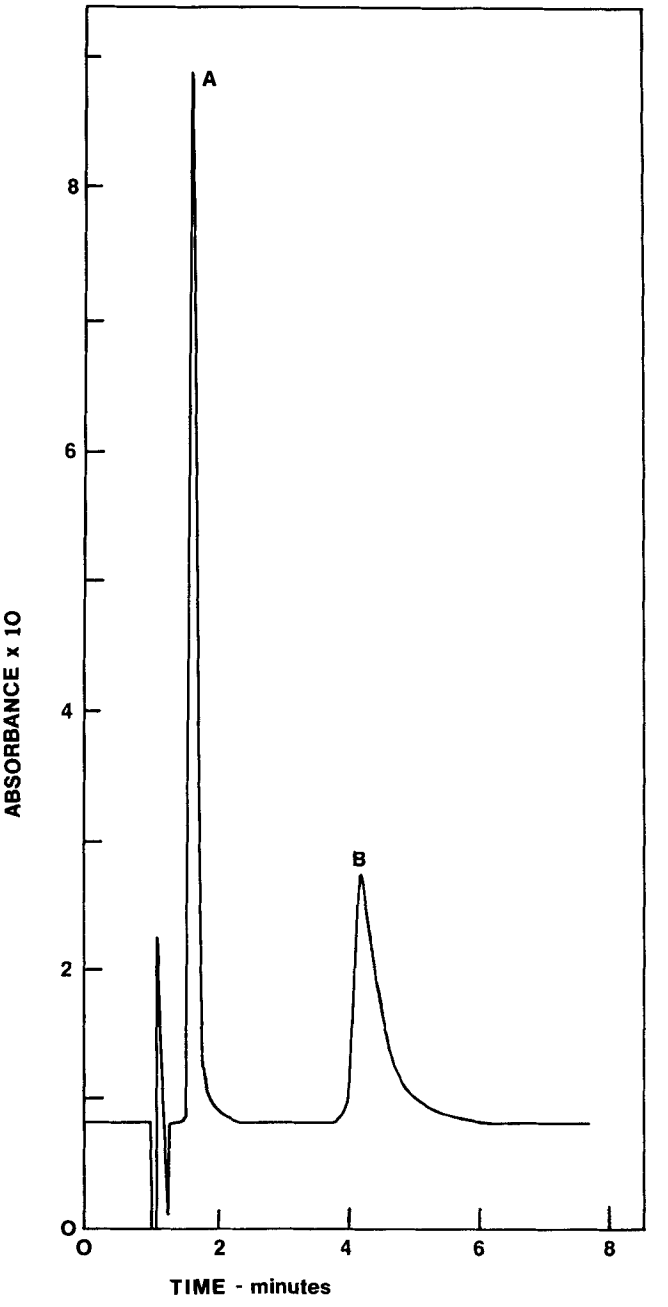
Spectrophotometry- Ultraviolet spectra were obtained using an HP 8451A UV/VIS spectrophotometer (Hewlett-Packard Co.,Palo Alto, California) and recorded on an HP 7470A plotter. Mathematical manipulations for single and multicomponent analysis and derivative spectra were performed using the integrated HP 85 microcomputer and associated software. All derivative spectra were produced post-run by using three smoothing points of the digitized spectra based on Savitzky-Golay functions[10]. The spectral data were stored on 3½" discs using an HP 9151 dual disc drive. The same 1 cm quartz cell was used throughout.

## RESULTS AND DISCUSSION

Figure 1 shows the ultraviolet absorption spectra of phenylephrine hydrochloride, maleic acid and pheniramine maleate in 0.01 N HCl. Both drugs, phenylephrine hydrochloride and pheniramine maleate, exhibit two absorption bands over the range 200 to 290 nm, with the higher amplitude bands being at the lower wavelengths. The two compounds exhibit significantly different spectral features over the range 245 to 290 nm. The spectrum of pheniramine maleate is the result




1. UV Spectra of phenylephrine HCl 100 µg/ml (_____), pheniramine maleate 40 µg/ml (_ _ _ _ _) and maleic acid 13 µg/mL (_ . _ . _).




2. Separation of phenylephrine (A) and pheniramine (B) on a cyclodextrin bonded stationary phase.
Mobile phase: methanol - 0.05 M phosphate buffer, pH 7.0 (60:40)
Flow rate: 2.0 ml/min, Detection: 262 nm.

of the absorption from both the maleate and the pheniramine. Maleic acid has a band with a peak at 209 nm and at the concentrations studied it makes negligible contributions to the overall absorbances above 255 nm. Absorption maxima of 263 and 272 nm are observed for pheniramine maleate and phenylephrine hydrochloride respectively. Phenylephrine hydrochloride also has a shoulder around 277 nm.

A HPLC method was developed to analyze mixtures of phenylephrine hydrochloride and pheniramine maleate. Two hundred and sixty two nanometers was chosen as the analytical wavelength since this gave similar responses (peak heights) for the two drugs in the diluted formulations. Attempts using an ODS Hypersyl column and gradient elution with methanol and phosphate buffer (pH 7.0) failed. The Hypersyl column adequately separated maleic acid from phenylephrine but the pheniramine molecule was always strongly retained on the column. The beta-cyclodextrin column was able to separate the two drugs using a mobile phase of phosphate buffer (pH 7.0) in methanol (40:60). Preliminary studies indicate that the phenylephrine hydrochloride has minimal interaction with the stationary phase while pheniramine seems to form a stronger inclusion complex with the beta-cyclodextrin. This results in a baseline resolution of phenylephrine (retention time 1.6 min) and pheniramine (5.3 min) and an overall analysis time of 6.5 minutes (Figure 2). The maleic acid eluted at the solvent front. Despite the slightly asymmetrical peak for pheniramine, linear relationships were obtained between concentration and peak height for the range of 10 to 50 and 25 to 125µg/ml for pheniramine maleate and phenylephrine hydrochloride, respectively.

To determine the precision and accuracy of the HPLC procedure, five laboratory mixtures prepared to contain 100 and 40 µg/ml of phenylephrine hydrochloride and pheniramine maleate, respectively, were analyzed by HPLC. The results of the analysis of these mixtures are included in Table 1. The concentrations were determined for both drugs based on the best linear fit of a calibration curve of peak heights vs concentration for standard solutions. Very good results were obtained with mean sample concentrations of 100.3±0.26 and 40.3± 1.2 µg/ml for phenylephrine hydrochloride and pheniramine maleate respectively. After it was known that good analytical results could be obtained for simple

Table 1 - HPLC Results for the Analysis of Laboratory Mixtures and a Pharmaceutical Preparation Containing Phenylephrine HCl and Pheniramine Maleate

A. Laboratory Mixtures

| Mixture# | Phenylephrine HCl (µg/ml) | Pheniramine Maleate (µg/ml) |
|---|---|---|
| 1 | 100.7 | 40.0 |
| 2 | 102.1 | 40.1 |
| 3 | 99.0 | 40.4 |
| 4 | 99.4 | 40.2 |
| 5 | 100.2 | 40.6 |
| Mean | 100.3 | 40.3 |
| Std. Dev. | 1.2 | 0.24 |

B. Dilutions of the Pharmaceutical Preparation

| Dilution | Phenylephrine HCl (µg/ml) | Pheniramine Maleate (µg/ml) |
|---|---|---|
| 1 | 102.3 | 40.8 |
| 2 | 107.3 | 42.5 |
| 3 | 103.4 | 41.1 |
| 4 | 103.6 | 41.8 |
| 5 | 105.3 | 42.0 |
| Mean | 104.3 | 41.6 |
| Std. Dev. | 1.9 | 0.68 |

C. Repetitive Determinations for a Dilution of the Pharmaceutical Preparation

| Trial# | Phenylephrine HCl (µg/ml) | Pheniramine Maleate (µg/ml) |
|---|---|---|
| 1 | 106.2 | 41.9 |
| 2 | 105.6 | 41.8 |
| 3 | 106.0 | 41.8 |
| 4 | 106.1 | 42.0 |
| 5 | 104.9 | 41.8 |
| 6 | 105.3 | 42.0 |
| Mean | 105.68 | 41.88 |
| Std. Dev. | 0.51 | 0.098 |

mixtures of the two drugs using the developed HPLC method, five dilutions of the nasal solution containing phenylephrine hydrochloride and pheniramine maleate were assayed using the same method. Table 1 shows the results of the analysis of the dilutions of the pharmaceutical preparation. Mean sample concentrations of 104.3±1.9 and 41.6±0.68 μg/ml were obtained for phenylephrine hydrochloride and pheniramine maleate respectively. Also, one dilution was assayed six times by means of six injections and these results are also included in Table 1.

The UV spectra of the standard solutions of both drugs were fitted separately by the single component software of the HP 8451A to a calibration curve model of a linear relationship between concentration (c) and absorbance (A) with an intercept of zero:

$$c = kA \qquad (1)$$

where k is a proportionality constant. The software also calculated how closely each of the standards fell to the calibration curve and reported the slope. As expected, the correlation was not good above 290 for both drugs, due to the very low absorption in the region of 290 to 300 nm. Also, phenylephrine showed poor correlation between 200 and 205 nm, probably due to the very high absorbance values obtained for the standards of higher concentration or to solvent effects.

The same five laboratory mixtures and five dilutions of the pharmaceutical preparation were used to assess the potential of the spectrophotometric method for the assay of of pharmaceutical products containing pheniramine maleate and phenylephrine hydrochloride. Based on the results of single component analyses, an appropriate wavelength range for the assay of mixtures of phenylephrine hydrochloride and pheniramine maleate by multicomponent analysis was 205 and 290 nm (Figure 1). However, the characteristic differences in spectral features of the two compounds of interest (Figure 1) allowed us to narrow the range further.

UV spectra were obtained for all the standards and samples and the concentrations were determined based on multicomponent analysis. The analysis of the mixtures was performed assuming a two component mixture. The absorbance, A, of a two-component mixture at a particular wavelength is linearly related to the concentration of the absorbing species, 1 and 2, according to Beer-Lambert's Law.

$$A = k_1 c_1 L + k_2 c_2 L \qquad (2)$$

where k is the absorptivity and L = 1 cm. Similarly, the concentration of the

Table 2 - Results for the Multicomponent Analysis for the Laboratory Mixtures Containing Phenylephrine HCl and Pheniramine Maleate at Different Conditions

Method A

| Sample# | Phenylephrine HCl (μg/ml) | RSD[a] ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E.[b] | Ind. Stds.[c] |
|---|---|---|---|---|---|---|
| 1 | 102.2 | 2.54 | 41.56 | 2.17 | ND | 7.36 |
| 2 | 100.9 | 2.87 | 41.64 | 2.40 | ND | 7.00 |
| 3 | 100.9 | 2.67 | 41.76 | 2.32 | ND | 6.80 |
| 4 | 101.1 | 3.27 | 41.73 | 2.64 | ND | 6.84 |
| 5 | 103.5 | 2.32 | 41.58 | 2.07 | ND | 6.91 |
| Mean | 101.7 | | 41.65 | | - | 6.98 |
| Std. Dev. | 1.13 | | 0.089 | | - | 0.22 |

Method B

| Sample# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.2 | 1.84 | 39.80 | 1.03 | 0.306 | 20.8 |
| 2 | 101.9 | 1.86 | 39.87 | 1.03 | 0.396 | 21.3 |
| 3 | 102.1 | 1.96 | 39.99 | 1.03 | 0.457 | 23.9 |
| 4 | 102.5 | 1.95 | 39.88 | 1.05 | 0.222 | 22.6 |
| 5 | 104.8 | 1.91 | 39.76 | 1.06 | 0.195 | 23.5 |
| Mean | 102.9 | | 39.86 | | 0.315 | 22.42 |
| Std. Dev. | 1.17 | | 0.088 | | 0.112 | 1.35 |

Method C

| Sample# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.5 | 5.60 | 39.50 | 9.11 | 0.174 | 2.30 |
| 2 | 102.2 | 5.58 | 39.60 | 9.09 | 0.456 | 2.28 |
| 3 | 102.5 | 5.66 | 39.61 | 9.29 | 0.162 | 2.46 |
| 4 | 102.7 | 6.33 | 39.71 | 9.32 | 0.273 | 2.57 |
| 5 | 105.0 | 5.81 | 39.60 | 9.09 | 0.137 | 2.44 |
| Mean | 103.2 | | 39.60 | | 0.240 | 2.41 |
| Std. Dev. | 1.13 | | 0.074 | | 0.131 | 0.120 |

Method D

| Sample# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 96.4 | 84.0 | 38.30 | 26.1 | 0.116 | 5.49 |
| 2 | 93.5 | 86.6 | 38.03 | 26.3 | 0.485 | 5.52 |
| 3 | 94.2 | 92.5 | 38.35 | 26.1 | 0.056 | 6.35 |
| 4 | 98.4 | 82.4 | 39.07 | 25.6 | 0.494 | 5.45 |
| 5 | 100.1 | 82.9 | 38.69 | 25.8 | 0.098 | 5.78 |
| Mean | 96.5 | | 38.49 | | 0.245 | 5.72 |
| Std. Dev. | 2.78 | | 0.401 | | 0.220 | 0.376 |

(continued)

Table 2 (Continuation)

Method E

| Sample# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 102.5 | 2.93 | 39.52 | 13.2 | 0.425 | 6.94 |
| 2 | 101.3 | 2.86 | 39.65 | 12.6 | 1.073 | 6.46 |
| 3 | 101.2 | 2.76 | 39.86 | 11.8 | 0.730 | 6.47 |
| 4 | 101.6 | 2.76 | 39.94 | 12.0 | 1.233 | 6.62 |
| 5 | 104.0 | 2.98 | 39.46 | 13.9 | 1.683 | 7.95 |
| Mean | 102.1 | | 39.69 | | 1.029 | 6.89 |
| Std. Dev. | 1.17 | | 0.209 | | 0.481 | 0.625 |

[a]RSD Relative standard deviation; RSD = standard deviation (SD)/concentration; SD is determined by the built-in multicomponent analysis software of the HP 8451A.

[b]R.F.E. Relative fit error and is determined by the built-in multicomponent analysis software of the HP 8451A.

[c]Ind. Stds. Independence of the standards and is determined by the built-in multicomponent analysis software of the HP 8451A.

[d]ND Not determined; because the software is not able to determine R.F.E. when only two measurements are used.

Methods
A - = 262, 273 nm
B - = 255 to 266 nm; zeroth derivative
C - = 255 to 266 nm; first derivative
D - = 255 to 266 nm; second derivative
E - = 273 to 290 nm; second derivative

two major components can be determined using derivative spectra according to the following relationship (10)

$$\frac{d^n A}{d\lambda^n} = \frac{c_1 d^n k_1}{d\lambda^n} + \frac{c_2 d^n k_2}{d\lambda^n} \qquad (3)$$

where n is the derivative number, which ranged in this studies from 0 to 5.

Although linear calibration curves for the single components were obtained over the range 205 and 290 nm, a smaller range of 245 to 290 nm, provided the most significant differences in spectral features and was chosen for further analysis of two component mixtures.

Analysis of the laboratory mixtures was performed for many different wavelength ranges within 245 to 290 nm for derivative order from 0 to 2. Data obtained with higher order derivatives gave unacceptably poor precision. Results for the spectrophotometric multicomponent analysis of the laboratory mixtures by a few methods are presented on Table 2. The simplest case is when the concentrations are determined based on absorbance measurements at only two wavelengths. The concentrations were determined at 262 and 273 nm, which correspond to the absorption maximum of the two drugs within the 245 to 290 nm range. For two component analysis using a range of wavelengths, the highest reproducibility was obtained over 255-266 nm and this range was chosen for further study. Table 2 shows the results of spectroscopic analysis of 5 solutions containing known concentrations of phenylephrine HCl (100 µg/mL) and pheniramine maleate (40 µg/ml), using zero, first and second derivative analysis. None of the values obtained by spectroscopy were significantly different. ($P < 0.01$, students t-test) from those obtained by HPLC, for both drugs. Although in the case of the second derivative, this was due to the unacceptably high standard deviations obtained by this method. Interesting good agreement, with high precision, was obtained between the data obtained by HPLC and that obtained by second derivative spectroscopy, over the range 273 - 290 nm.

The accuracy of the HPLC as determined by $(C_{found}/C_{theoretical}) \times 100\%$ was 100.3±1.2% for phenylephrine HCL and 100.8±0.6% for pheniramine maleate (Table

Table 3 - Results for Multicomponent Analysis of Dilutions of the Pharmaceutical Preparations Containing Phenylephrine HCl and Pheniramine Maleate at Different Conditions

Method A

| Dilution# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 99.68 | 3.10 | 41.26 | 2.33 | ND | 7.87 |
| 2 | 103.2 | 3.01 | 42.73 | 2.31 | ND | 7.62 |
| 3 | 100.7 | 3.07 | 41.55 | 2.64 | ND | 6.79 |
| 4 | 102.1 | 2.94 | 42.14 | 2.37 | ND | 6.99 |
| 5 | 102.3 | 2.93 | 42.53 | 2.30 | ND | 7.21 |
| Mean | 101.6 | | 42.04 | | - | 7.30 |
| Std. Dev. | 1.40 | | 0.627 | | - | 0.44 |

Method B

| Dilution# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 101.8 | 2.06 | 40.72 | 1.08 | 1.14 | 24.6 |
| 2 | 105.5 | 2.27 | 42.16 | 1.09 | 1.77 | 25.6 |
| 3 | 103.0 | 2.14 | 40.99 | 1.07 | 1.42 | 23.7 |
| 4 | 104.5 | 2.20 | 41.57 | 1.10 | 1.90 | 23.0 |
| 5 | 104.8 | 2.19 | 41.89 | 1.10 | 1.03 | 24.0 |
| Mean | 103.9 | | 41.46 | | 1.45 | 24.2 |
| Std. Dev. | 1.50 | | 0.60 | | 0.380 | 0.980 |

Method C

| Sample# | Phenylephrine HCl ($\mu$g/ml) | RSD ($10^3$) | Pheniramine Maleate ($\mu$g/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 102.0 | 6.08 | 40.56 | 9.13 | 1.68 | 2.40 |
| 2 | 106.1 | 6.97 | 41.66 | 9.84 | 2.32 | 2.69 |
| 3 | 103.4 | 7.15 | 40.63 | 9.84 | 1.56 | 2.78 |
| 4 | 105.0 | 6.66 | 41.03 | 9.99 | 2.51 | 2.49 |
| 5 | 105.2 | 6.46 | 41.41 | 9.90 | 1.44 | 2.36 |
| Mean | 104.3 | | 41.06 | | 1.90 | 2.54 |
| Std. Dev. | 1.63 | | 0.479 | | 0.481 | 0.183 |

Method D

| Dilution# | Phenylephrine HCl ($\mu$g/ml) | RSD ($10^3$) | Pheniramine Maleate ($\mu$g/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 83.21 | 102.0 | 37.04 | 29.7 | 1.51 | 6.02 |
| 2 | 88.02 | 96.6 | 38.97 | 28.2 | 3.15 | 4.96 |
| 3 | 83.03 | 96.4 | 37.39 | 29.4 | 1.10 | 4.61 |
| 4 | 88.11 | 98.7 | 38.42 | 31.2 | 2.92 | 4.93 |
| 5 | 93.73 | 100.0 | 39.33 | 33.2 | 1.91 | 5.51 |
| Mean | 87.22 | | 38.23 | | 2.12 | 5.21 |
| Std. Dev. | 4.40 | | 0.989 | | 0.888 | 0.558 |

(continued)

Table 3 (continuation)

Method E

| Dilution# | Phenylephrine HCl (µg/ml) | RSD ($10^3$) | Pheniramine Maleate (µg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 100.5 | 2.69 | 39.44 | 12.2 | 2.12 | 7.82 |
| 2 | 104.3 | 2.68 | 41.28 | 11.6 | 1.10 | 7.62 |
| 3 | 101.7 | 2.66 | 40.28 | 11.7 | 0.57 | 7.39 |
| 4 | 103.2 | 2.62 | 40.95 | 11.7 | 1.28 | 7.73 |
| 5 | 103.6 | 2.61 | 41.02 | 11.5 | 1.04 | 7.56 |
| Mean | 102.7 | | 40.59 | | 1.22 | 7.62 |
| Std. Dev. | 1.54 | | 0.743 | | 0.567 | 0.165 |

See Table 2 for explanation of methods and abbreviations.

Table 4 - Results for Repetitive Determinations of the Same Dilution of the Pharmaceutical Preparation Containing Phenylephrine HCl and Pheniramine Maleate by Two Different Multicomponent Analysis Methods

Method A

| Trial# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.0 | 2.14 | 40.99 | 1.07 | 1.42 | 23.7 |
| 2 | 102.7 | 2.14 | 41.11 | 1.07 | 1.16 | 24.2 |
| 3 | 102.7 | 2.14 | 41.16 | 1.07 | 1.31 | 26.4 |
| 4 | 102.7 | 2.04 | 41.18 | 1.04 | 1.87 | 24.1 |
| 5 | 102.7 | 2.14 | 41.18 | 1.07 | 1.63 | 23.8 |
| 6 | 102.8 | 2.04 | 41.09 | 1.07 | 0.758 | 23.4 |
| Mean | 102.8 | | 41.12 | | 1.36 | 24.3 |
| Std. Dev. | 0.121 | | 0.073 | | 0.385 | 1.08 |

Method B

| Trial# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.4 | 7.15 | 40.63 | 9.84 | 1.56 | 2.78 |
| 2 | 103.4 | 6.48 | 40.49 | 9.88 | 1.23 | 2.51 |
| 3 | 103.4 | 6.19 | 40.49 | 9.14 | 1.48 | 2.58 |
| 4 | 103.4 | 6.09 | 40.42 | 9.15 | 1.63 | 2.46 |
| 5 | 103.4 | 6.77 | 40.41 | 9.90 | 1.35 | 2.57 |
| 6 | 103.1 | 6.21 | 40.73 | 9.33 | 1.10 | 2.41 |
| Mean | 103.35 | | 40.53 | | 1.39 | 2.55 |
| Std. Dev. | 0.122 | | 0.126 | | 0.203 | 0.129 |

See Table 2 for explanation of methods and abbreviations.

Table 5 - Summary of Accuracy, System Precision and Method Precision for Analysis of Solutions Containing Phenylephrine Hydrochloride and Pheniramine Maleate

| Method | Accuracy (%) | System Precision (% RSD) | Method Precision (%RSD) |
|---|---|---|---|
| HPLC[a] | | | |
| Phenylephrine HCl | 100.3 | 0.48 | 1.82 |
| Pheniramine maleate | 100.8 | 0.23 | 1.63 |
| Spectroscopy (Method B)[b] | | | |
| Phenylephrine HCl | 102.9[c] | 0.12[d] | 1.44[e] |
| Pheneramine maleate | 99.7[c] | 0.31[d] | 1.45[e] |

a. Table 1
b. See Table 2 for explanation of Method
c. Table 2
d. Table 4
e. Table 3

Table 6 - Results for Two Methods of Multicomponent Analysis for the Laboratory Mixtures Containing Phenylephrine HCl and Pheniramine Maleate Measured 14 hr After Preparation Using the Original Standard Measurements and Standards Measured 14 hr After Preparation

Method B

1. Concentration determined from standards measured 14 hr after preparation

| Mixture# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.6 | 1.83 | 39.48 | 1.01 | 0.399 | 23.4 |
| 2 | 102.3 | 1.96 | 39.65 | 1.03 | 0.492 | 23.6 |
| 3 | 102.4 | 2.05 | 39.79 | 1.06 | 0.458 | 24.4 |
| 4 | 102.8 | 1.94 | 39.66 | 1.06 | 0.365 | 24.0 |
| 5 | 105.1 | 1.90 | 39.56 | 1.03 | 0.394 | 23.2 |
| Mean | 103.2 | | 39.63 | | 0.422 | 23.7 |
| Std. Dev. | 1.16 | | 0.116 | | 0.052 | 0.482 |

(continued)

Table 6 (continuation)

2. Concentrations determined from original standard measurements

| Mixture# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 102.8 | 1.85 | 40.32 | 1.02 | 1.52 | 23.2 |
| 2 | 101.4 | 1.97 | 40.50 | 1.04 | 1.84 | 23.2 |
| 3 | 101.5 | 2.07 | 40.65 | 1.06 | 1.54 | 24.1 |
| 4 | 102.0 | 1.96 | 40.51 | 1.02 | 1.78 | 23.6 |
| 5 | 104.2 | 1.92 | 40.42 | 1.04 | 1.35 | 22.8 |
| Mean | 102.4 | | 40.48 | | 1.61 | 23.4 |
| Std. Dev. | 1.14 | | 0.122 | | 0.201 | 0.492 |

Method C

1. Concentrations determined from standards measured 14 hr after preparation

| Mixture# | Phenylephrine HCl (μg/ml) | RSD ($10^3$) | Pheniramine Maleate (μg/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.6 | 5.41 | 39.58 | 8.33 | 0.286 | 2.44 |
| 2 | 102.3 | 5.77 | 39.66 | 8.57 | 0.708 | 2.46 |
| 3 | 102.4 | 6.74 | 39.84 | 9.29 | 0.437 | 2.69 |
| 4 | 102.9 | 6.22 | 39.60 | 9.09 | 0.483 | 2.56 |
| 5 | 105.0 | 5.71 | 39.67 | 8.57 | 0.455 | 2.38 |
| Mean | 103.2 | | 39.67 | | 0.474 | 2.51 |
| Std. Dev. | 1.11 | | 0.102 | | 0.152 | 0.122 |

2. Concentrations determined from original standard measurements

| Mixture# | Phenylephrine HCl ($\mu$g/ml) | RSD ($10^3$) | Pheniramine Maleate ($\mu$g/ml) | RSD ($10^3$) | R.F.E. | Ind. Stds. |
|---|---|---|---|---|---|---|
| 1 | 103.7 | 5.40 | 39.51 | 8.35 | 0.327 | 2.41 |
| 2 | 102.4 | 5.76 | 39.60 | 8.59 | 0.653 | 2.44 |
| 3 | 102.5 | 6.73 | 39.76 | 9.31 | 0.416 | 2.66 |
| 4 | 103.1 | 6.21 | 39.52 | 9.36 | 0.527 | 2.52 |
| 5 | 105.2 | 5.61 | 39.58 | 9.09 | 0.512 | 2.35 |
| Mean | 103.4 | | 39.59 | | 0.487 | 2.48 |
| Std. Dev. | 1.14 | | 0.100 | | 0.123 | 0.120 |

See Table 2 for explanation of methods and abbreviations.

1). These were not statistically different from 100% ($P < 0.01$) indicating that there is no bias in the HPLC method. The accuracy and precision of the diode spectroscopy methods depended on the derivative order used to manipulate the raw data. The accuracy values ranged between 101.7±1.3% for phenylephrine HCl (analysis at two wavelengths) and 96.2 ±1.06% for pheniramine maleate (255 - 266 nm, second derivative). A good compromise between precision and accuracy for the analysis of both drugs is Method B (Table 2) (255 - 266 nm, zero derivative), in which the accuracy was 102.9±1.9% for phenylephrine HCl and 99.7±0.2% for pheniramine maleate. The relative standard deviations (method and system) increased markedly with higher derivative number (> 2) over the range 255-266 nm. However, it is of interest to note that good results (Table 2E) were obtained using second order derivative spectra over the range 273-290 nm.

The applicability of the methods was determined by analysing a single nasal spray solution five times by HPLC (Table 1B) and spectroscopy (Table 3). Again, there was no statistical difference between the results obtained by the two analytical procedures ($P < 0.01$).

The reproducibilities of the two systems were determined by analysing the same diluted pharmaceutical preparation six times by both methods (Tables 1C and 4). The accuracy, the system reproducibilities and method reproducibilities are summarized in Table 5 and were comparable for the two methods.

The stability of the laboratory mixtures and the integrity of the analytical standards were assessed by measuring the absorbance of the solutions about 14 hours after the original measurements. The concentrations for the two drugs were determined using the original standard measurements and the standards measured 14 hr after preparation. These results are shown on Table 6. No statistically significant differences ($P < 0.01$) were seen when using the earlier or the older standards, and when compared to the results from the fresh solutions.

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