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Short communication

Simultaneous high performance liquid chromatographic and derivative ratio spectra spectrophotometry determination of chlorpheniramine maleate and phenylephrine hydrochloride

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Abstract

Rapid, precise, accurate and specific high performance liquid chromatographic and derivative ratio spectra spectrophotometry procedures are described for the simultaneous analysis of chlorpheniramine maleate and phenylephrine hydrochloride in combined pharmaceutical dosage forms. The chromatographic methods were standardised using a LiChrosorb RP- C_{18} column (5 μm , 20 cm × 4.6 mm), UV detection at 269.0 nm and mobile phases consisting of methanol/phosphate buffer (50 ml 0.2 M monobasic potassium phosphate (KH_2PO_4) + 34.7 ml 0.2 M NaOH; 70:30, apparent pH 7.2). Using derivative ratio spectra spectrophotometry, the amplitudes in the first derivative of the ratio spectra at 238.9 and 280.0 nm were selected to determine chlorpheniramine maleate and phenylephrine hydrochloride in the mixture. Commercial nasal drops and laboratory-prepared mixtures containing both drugs in different proportions were assayed using the methods developed. Both methods showed good linearity, precision and reproducibility.

Keywords: Chlorpheniramine maleate; Phenylephrine hydrochloride; High performance liquid chromatography; Derivative ratio spectra spectrophotometry; Pharmaceutical formulations

1. Introduction

Combinations of decongestant and antihistamine pharmaceutical preparations are widely used for cough and cold treatments. Generally, such preparations contain one decongestant and one antihistamine, but several contain more than one decongestant. These combination preparations are made in various forms, e.g., syrup, tablet, and nasal drops.

Several methods have been described for the quantitative determination of chlorpheniramine maleate and phenylephrine hydrochloride in combination with other drugs, including spectrophotometry [1–8] and HPLC [9–20] in pharmaceutical preparations either separately or in combination with other drugs.

Recently, Salinas et al. [21] and Berzas Nevado et al. [22] developed a new spectrophotometric method for resolving binary mixtures. This method is based on use of the first derivative of the ratio of the spectra. The absorption spectrum of the mixture is obtained and divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph. This method can be applied for resolving binary mixtures of chlorpheniramine maleate and phenylephrine hydrochloride.

This paper reports on high performance liquid chromatography (HPLC) and derivative ratio spectra spectrophotometry assays of chlorpheniramine maleate and phenylephrine hydrochloride in binary mixtures without a previous separation step. The utility of the developed methods to determine the contents of nasal drops is demonstrated.

2. Experimental

2.1. Instruments

The chromatographic system consisted of a Jasco model PU-980 pump with a 7725 Rheodyne value injector 20 μl fixed loop, equipped with a Jasco UV-975 UV/VIS detector. The detector was set at 269.0 nm (0.02 a.u.f.s) and peak areas were integrated automatically by computer using the Borwin software program.

Spectrophotometric analysis was carried out on a Shimadzu1601 double beam spectrophotometer with a

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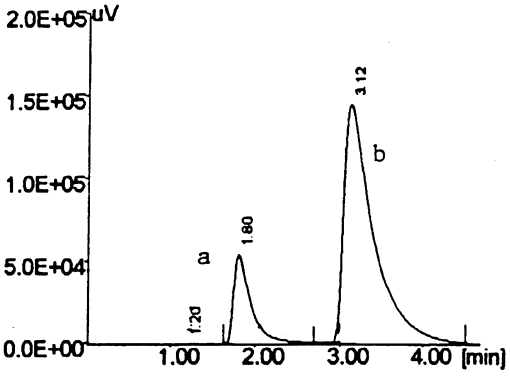

Fig. 1. HPLC trace of a 20 μl injection containing (a) 20.0 μg ml^{-1} of chlorpheniramine maleate (1.80 min); and (b) 12.0 μg ml^{-1} of phenylephrine hydrochloride (3.12 min).

fixed slit width (2 nm) connected to an IBM-PC computer with a Lexmark printer; this was used for all the absorbance signals and treatment of data. Other apparatus used included a Radiometer NEL pH 890 digital pH meter equipped with a combined glass-calamol electrode and ultrasound generator.

2.2. Materials

Pharmaceutical grades of chlorpheniramine maleate (Akdeniz Pharm. Ind., Turkey) and phenylephrine hydrochloride (Akdeniz Pharm. Ind., Turkey) were used. Chromatographic grade, double distilled water, and analytical reagent grade methanol (Merck), NaOH (Merck), monobasic potassium phosphate (Merck), and HCl (Merck) were used.

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, 50.00 mg; chlorpheniramine maleate, 80.00 mg/5 ml drop.

2.4. Procedures

2.4.1. Chromatographic conditions

Solutions and mobile phases were prepared at the moment of use. The mobile phases used were methanol/phosphate buffer (50 ml 0.2 M monobasic potassium phosphate (KH_2PO_4) + 34.7 ml 0.2 M NaOH), 70:30, adjusted to pH 7.2 with phosphoric acid). The analytical column was a RP-LiChrosorb C_{18} (5 μm, 20 cm × 4.6 mm) column. All analyses were done under isocratic conditions at a flow rate of 1.2 ml min^{-1} and at room temperature. All solvents were filtered through a 0.45 μm millipore filter before use and degassed in an ultrasonic bath.

2.4.2. Calibration

An external standard method was used for quantitative determinations. Calibration graphs were prepared from authentic samples of chlorpheniramine maleate and phenyl-ephrine hydrochloride in the mobile phase. Triplicate 20 μl injections were made for each solution. The final concentrations of chlorpheniramine maleate and phenylephrine hydrochloride in the samples were calculated by comparison of the sample and standard peak area obtained with the average of three injections of standard solutions.

2.4.3. Analysis of nasal drops

A 5 ml drop (from RiNOSiL® nasal drop), was taken and dissolved in mobile phase in 100 ml calibrated flasks. The solution was diluted 1:50 with the mobile phase. A 20.0 μl volume of the final solution was injected into the chromatograph.

2.5. Derivative ratio spectra spectrophotometry

2.5.1. Calibration

Samples were prepared in 50 ml calibrated flasks containing 400–1600 μg ml^{-1} of chlorpheniramine maleate and 200–1000 μg ml^{-1} of phenylephrine hydrochloride in methanol/0.1 M HCl (1:1). The absorption spectra were recorded with 1 nm resolution against methanol/0.1 M HCl (1:1) and stored in the IBM-PC. The stored spectra of the binary mixtures, chlorpheniramine maleate and phenyl-ephrine hydrochloride, were divided by a standard spectrum of

Table 1
Analytical data for the calibration graphs ($n = 5$) for the determination of chlorpheniramine maleate and phenylephrine hydrochloride by derivative ratio spectra spectrophotometry and HPLC

Drug	Method	Regression equation				
		Range (μg ml^{-1})	Slope	Intercept	Correlation coefficient (r)	RSD (%)
Chlorpheniramine maleate	Derivative ratio spectrophotometry	8.0–32.0	0.582	0.098	0.9991	0.908
	HPLC	1.0–38.0	0.066	− 0.012	0.9996	0.568
Phenylephrine hydrochloride	Derivative ratio spectrophotometry	4.0–20.0	0.491	0.198	0.9997	1.000
	HPLC	1.5–40.0	0.089	0.489	0.9992	0.088

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

Sample	Recovery (mean ± rsd) %[a]			
	Chlorpheniramine maleate		Phenylephrine hydrochloride	
	HPLC	Derivative ratio spectra	HPLC	Derivative ratio spectra
Synthetic mixtures	99.42 ± 0.67	99.17 ± 0.63	99.47 ± 0.60	99.84 ± 0.29
Commercial nasal drops[b]	99.75 ± 0.96	98.62 ± 1.05	100.2 ± 0.25	99.60 ± 0.15

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Rinosil® nasal drops are the product of Akdenúz Pharm. Ind., Turkey; each 5 ml drop was labeled to contain 80.0 and 50.0 mg of chlorpheniramine maleate and phenylephrine hydrochloride, respectively.

phenylephrine hydrochloride (12.0 μg ml^{-1}). The ratio spectrum were smoothed through the use of 15 experimental points and the first derivates calculated with $\Delta\lambda = 8$ nm. In the binary mixtures, chlorpheniramine maleate can be determined by measuring the amplitudes at 238.9 nm corresponding to a maximum. On the other hand, stored spectra of binary mixtures were divided by a standard spectrum of chlorpheniramine maleate of 20.0 μg m^{-1} concentration. In the same way as we have previously described, we obtained the first derivates from smoothed ratio spectra. Now, phenylephrine hydrochloride can be determined by measuring the signals at 280.0 nm corresponding to a maximum wavelength.

Chlorpheniramine maleate and phenylephrine hydrochloride are soluble in methanol/0.1 M HCl (1:1), respectively, and their solutions were found to be stable for 5 days at least.

2.5.2. Analysis of nasal drop

A 5 ml drop (from RiNOSiL® nasal drop), was taken and dissolved in methanol/0.1 M HCl (1:1) in 100 ml calibrated flasks. The solution was diluted 1:50 with the same solvent. The method described above was applied to the prepared solutions.

3. Results and discussion

3.1. Chromatography

Fig. 1 displays a HPLC chromatogram obtained, under the conditions indicated above, from a standard solution containing chlorpheniramine maleate and phenylephrine hydrochloride. The retention times for the investigated drugs were 1.08 min (chlorpheniramine maleate) and 3.02 min (phenylephrine hydrochloride). To find the appropriate HPLC conditions for separation of the examined drugs, various reversed phase columns, isocratic and gradient mobile phase systems were tried. Successful attempts were performed using a reversed phase RP-LiChrosorb C_{18} (5 μm, 20 cm × 4.6 mm) with (70:30) mobile phase of methanol and

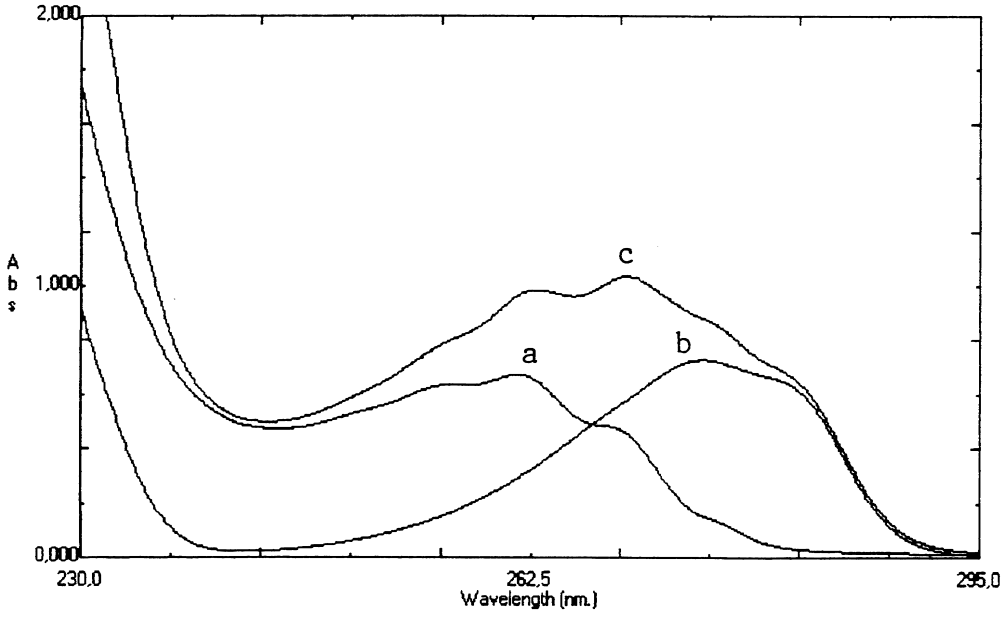

Fig. 2. Absorption spectra of (a) chlorpheniramine maleate (20.0 μg m^{-1}), (b) phenylephrine hydrochloride (12.0 μg m^{-1}) and (c) their mixture in methanol/0.1 N HCl (1:1).

phosphate buffer (50 ml 0.2 M monobasic potassium phosphate (KH_2PO_4) + 34.7 ml of 0.2 M NaOH).

Under the HPLC parameters described, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times. For quantitative applications, linear calibration graphs were obtained with correlation coefficients better than 0.9995 (Table 1). The good precision of the HPLC procedure was indicated by the relative standard deviation (0.60–0.67%). Results of HPLC analysis of laboratory-prepared mixtures with different proportions of nasal drops are given in Table 2.

3.2. Derivative ratio spectra spectrophotometry

The absorption spectra of chlorpheniramine maleate and phenylephrine hydrochloride and their mixture in the 230.0–295.0 nm wavelength region are shown in Fig. 2. As can be seen, the spectra of the compounds overlap sufficiently and a mathematical treatment of the data is recommended for resolving the mixture.

Fig. 3(A) shows the ratio spectra of different chlorpheniramine maleate standards (spectra divided by the spectrum of a 12.0 μg ml^{-1} phenylephrine hydrochloride solution) and their first derivatives were calculated. The first

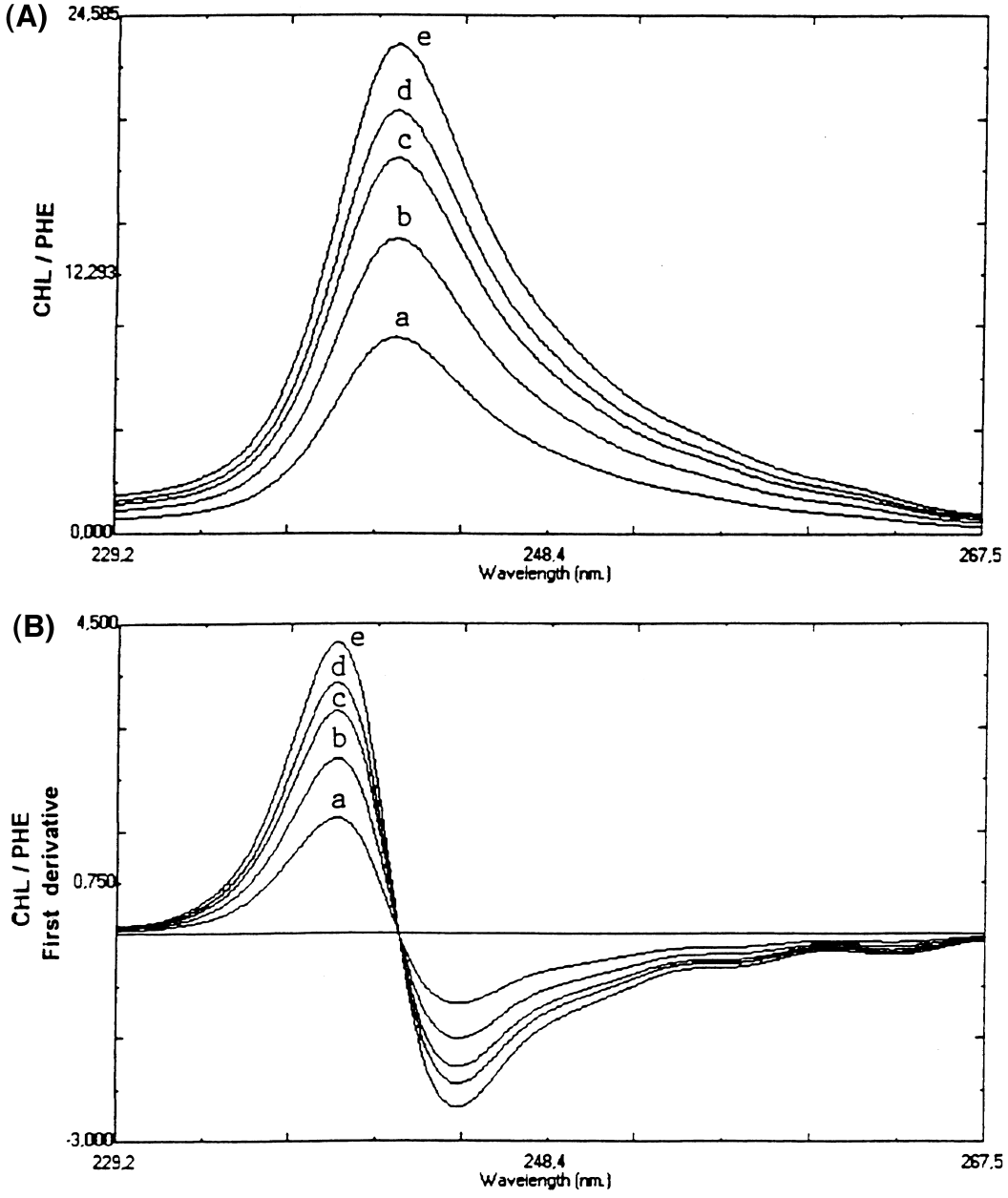

Fig. 3. Ratio spectra (A) and first derivative of the ratio spectra (B) of chlorpheniramine maleate of (a) 8.0 μg m^{-1}, (b) 14.0 μg m^{-1}, (c) 20.0 μg m^{-1}, (d) 26.0, (e) 32.0 μg m^{-1}, when 12.0 μg m^{-1} phenylephrine hydrochloride was used as divisor in methanol/0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

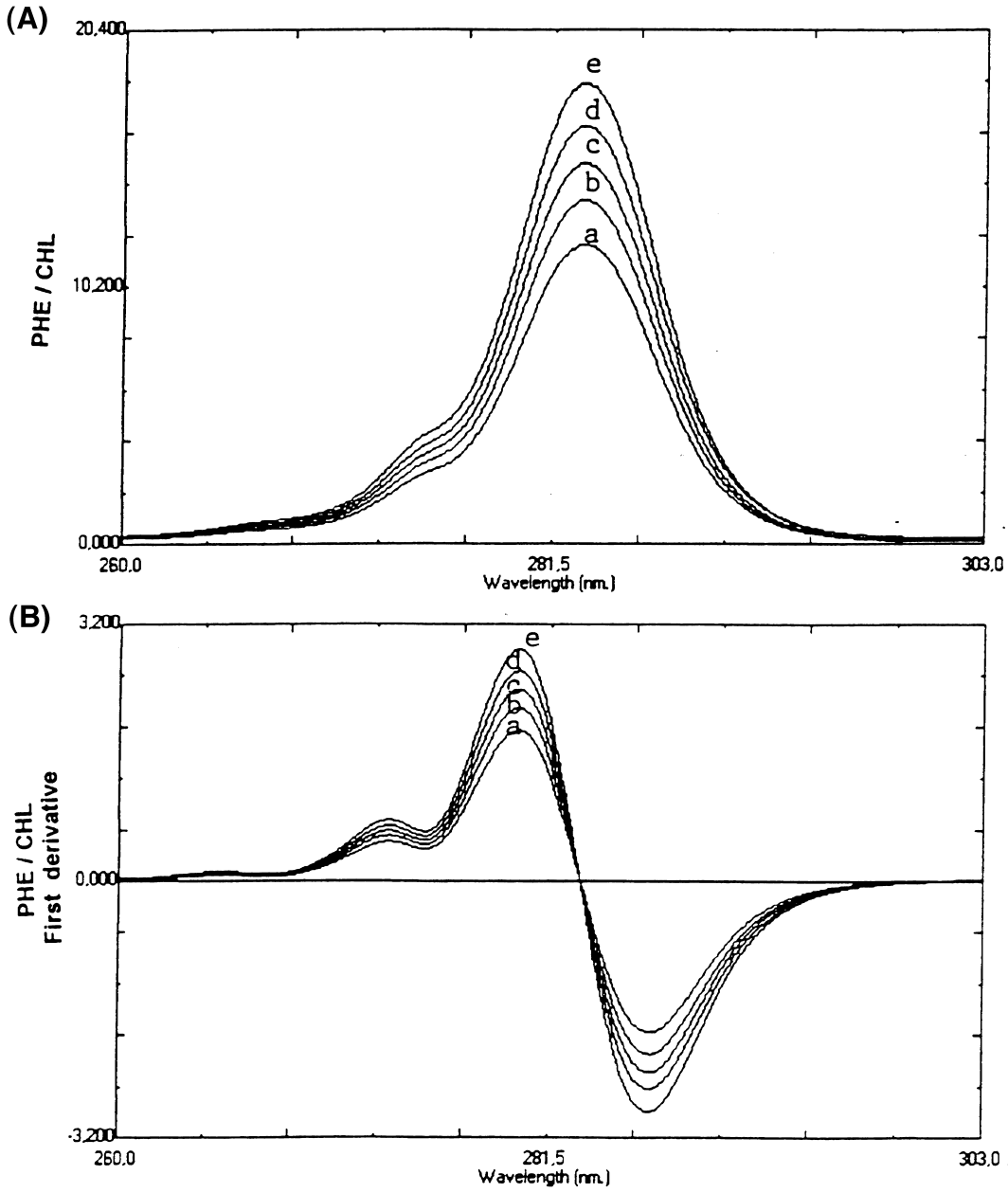

Fig. 4. Ratio spectra (A) and first derivative of the ratio spectra (B) of phenylephrine hydrochloride of (a) 4.0 μg m^{-1}, (b) 8.0 μg m^{-1}, (c) 12.0 μg m^{-1}, (d) 16.0 μg m^{-1} and (e) 20.0 μg m^{-1}, when 20.0 μg m^{-1} chlorpheniramine maleate was used as divisor in methanol/0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

derivative signals at a given wavelength are proportional to the chlorpheniramine maleate concentration (Fig. 3(B)).

Due to the extent of the noise levels on the ratio spectra, a smoothing function was used on the basis of the Steinier et al. method [23] and 15 experimental points were considered as optimum.

The influence of $\Delta\lambda$ for obtaining the first derivative was tested; $\Delta\lambda = 8$ nm was considered suitable. The concentration of divisor (phenylephrine hydrochloride in this case) can be modified, and different calibration graphs are then obtained and the concentration of phenylephrine hydrochloride selected (12.0 μg ml^{-1}). The calibration graph was established by measuring at 238.9 nm corresponding to a maximum. For determining the other component (phenylephrine hydrochloride), Fig. 4(A) shows the ratio spectra of different phenylephrine hydrochloride standards (spectra divided by the spectrum of a 20.0 μg ml^{-1} chlorpheniramine maleate solution) and their first derivative were calculated. The first derivative signals at a given wavelength are proportional to the phenylephrine hydrochloride concentration (Fig. 4(B)). The ratio spectra were smoothed with experimental points and the first derivatives were calculated with $\Delta\lambda = 8$ nm. The concentration of the divisor (chlorpheniramine maleate) was 20.0 μg ml^{-1}.

Several mixtures of chlorpheniramine maleate and phenylephrine hydrochloride were prepared and resolved

by the proposed method. The results are summarised in Table 2. Table 1 shows the regression coefficients and the linear ranges of the calibration graphs for active ingredients at suitable wavelengths for the determinations of chlorpheniramine maleate and phenylephrine hydrochloride.

Good agreement was observed for the assay results of the pharmaceutical formulation by application of the two methods in this paper (Table 2).

4. Conclusions

Chlorpheniramine maleate and phenylephrine hydrochloride were simultaneously determined in nasal drops using two different analytical techniques. The methods developed are simple, accurate, and specific. HPLC and derivative ratio spectra spectrophotometry may be recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations.

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