A Rapid Derivative Spectrophotometric Method for Simultaneous Determination of Naphazoline and Antazoline in Eye Drops

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A zero-crossing first-derivative spectrophotometric method is applied for the simultaneous determination of naphazoline hydrochloride and antazoline phosphate in eye drops. The measurements were carried out at wavelengths of 225 and 252 nm for naphazoline hydrochloride and antazoline phosphate, respectively. The method was found to be linear (r^2>0.999) in the range of 0.2—1 μg/ml for naphazoline hydrochloride in the presence of 5 μg/ml antazoline phosphate at 225 nm. The same linear correlation (r^2>0.999) was obtained in the range of 1—10 μg/ml of antazoline phosphate in the presence of 0.5 μg/ml of naphazoline hydrochloride at 252 nm. The limit of determination was 0.2 μg/ml and 1 μg/ml for naphazoline hydrochloride and antazoline phosphate, respectively. The method was successfully used for simultaneous analysis of naphazoline hydrochloride and antazoline phosphate in eye drops without any interference from excipients and prior separation before analysis.

Key words naphazoline hydrochloride; antazoline phosphate; derivative spectrophotometry; eye drops

Naphazoline hydrochloride (0.05%), a vasoconstrictor and decongestant, has been marketed as combination drug with antazoline phosphate (0.5%), an antihistamine, in eye drops. The combination is used to relieve redness, puffiness, itchy and watering eyes associated with colds, allergics or eye irritations.

Literature survey showed few old reports on the simultaneous spectrophotometric analysis of naphazoline and antazoline in drops based on two-component spectrophotometric methods using orthogonal functions or least squares.[1,2] Gas chromatographic and high performance liquid chromatographic methods were also reported for determination of naphazoline and antazoline in pharmaceutical preparations.[3—5] These methods are complicated and need the expensive instruments. No official procedure is given in well-known pharmacopeias for simultaneous determination of naphazoline and antazoline. Direct UV-visible spectrophotometric method is not suitable for simultaneous determination of naphazoline and antazoline due to spectral overlapping. Derivative spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures, especially in the field of pharmaceutical analysis to overcome some problems by interferences, due to substances other than analytes.[6—21]

This report describes a simple, sensitive and rapid procedure for simultaneous determination of naphazoline hydrochloride and antazoline phosphate in eye drops based on derivative spectrophotometry.

Experimental

Chemicals Naphazoline hydrochloride form LOBA Feinchemie AG (Austria) and antazoline phosphate from SIMS S. P. A. (Milano, Italy) were donated by Sina-Darou Pharmaceutical Company (Tehran, Iran). Methanol was of analytical grade and obtained from Merck (Darmstadt, Germany).

Apparatus Absorption and derivative spectra were recorded in 1 cm quartz cells using a Shimadzu UV-160 double beam UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) with a fixed bandwidth (2 nm) and data processing capacity. The zero-order absorption spectra were recorded over the wavelength range 200—400 nm against a solvent blank. The derivative spectra were obtained over the same range at different slit width ($\Delta\lambda$). The ordinate, maximum and minimum, was adjusted to the magnitude of derivative values.

Standard and Calibration Solutions Standard stock solutions of naphazoline hydrochloride and antazoline phosphate were prepared by dissolving 10 mg and 100 mg of each drug in 100 ml methanol respectively. Both stock solutions were subsequently diluted to achieve a final solution of 10 μg/ml for naphazoline hydrochloride and 100 μg/ml for antazoline phosphate respectively.

Accurate volumes of final solutions were transferred into two sets of 100 ml calibrated flasks. The first series contained a constant quantity of naphazoline hydrochloride (0.5 μg/ml) and varying concentrations of antazoline phosphate (1—10 μg/ml). The second series contained a constant amount of antazoline phosphate (5 μg/ml) and varying concentrations of naphazoline hydrochloride (0.2—1 μg/ml).

Pharmaceutical Preparation A commercial pharmaceutical preparation, Naphazoline+ Antazoline eye drop (Sina-Darou Pharm. Co., Tehran, Iran, Lot No: 411499) containing 0.05% naphazoline hydrochloride, 0.5% antazoline phosphate, hydroxypropyl methylcellulose (HPMC), benzalkanium chloride, borate buffer and sodium chloride was assayed.

Spectrophotometric Measurements Zero-order spectra of standard solutions of naphazoline hydrochloride (0.5 μg/ml) and antazoline phosphate (5 μg/ml) *versus* their solvent blank were recorded in the range of 200—400 nm. The first-order derivative spectra of standard solutions of each drug were obtained in the same range of wavelength against their blanks. The values of D_1 amplitudes for naphazoline hydrochloride in the presence of antazoline phosphate and *vice versa* were measured at 225 nm (zero-crossing of antazoline phosphate) and 252 nm (zero-crossing of naphazoline hydrochloride), respectively.

The calibration curves for derivative spectrophotometry were constructed by plotting the drug concentration *versus* the absorbance values of the first-derivative spectrum (D_1), at 225 nm for naphazoline hydrochloride and at 252 nm for anthazoline phosphate.

Linearity Calibration curves were constructed using eight series of naphazoline hydrochloride solutions between 0.2—1 μg/ml in the presence of 5 μg/ml antazoline phosphate. The same procedure was used for solutions contained antazoline phosphate (1—10 μg/ml) in the presence of 0.5 μg/ml of naphazoline hydrochloride. The calibration curves were constructed and statistical analysis was performed.

Precision To establish the reliability of the proposed method two series of solutions containing 0.2, 0.4 and 1 μg/ml of naphazoline hydrochloride plus 5 μg/ml of anthazoline phosphate and 1, 4 and 10 μg/ml of antazoline phosphate plus 0.5 μg/ml naphazoline hydrochloride were prepared respectively and analyzed as discussed above. To evaluate the repeatability of the method three series of these synthetic mixtures were assessed in one day using their corresponding calibration curves. The reproducibility of the procedure was determined at similar concentrations on three consecutive days.

Accuracy For accuracy assays the same synthetic mixtures mentioned above were analyzed by the proposed method and the percentage of deviation between added and measured concentrations was calculated.

Analysis of Eye Drop One milliliter of each commercial eye drops was

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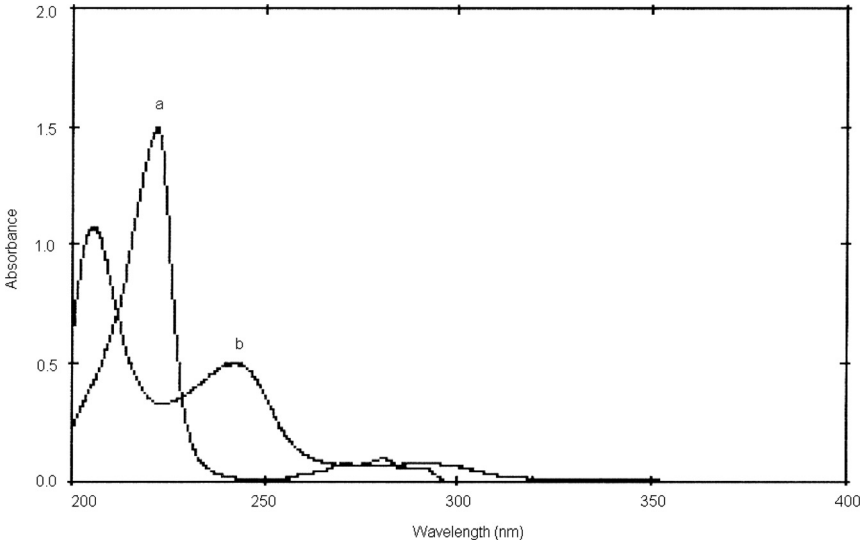

Fig. 1. Zero-Order Spectra of (a) Naphazoline Hydrochloride (5 μg/ml) and (b) Antazoline Phosphate (5 μg/ml)

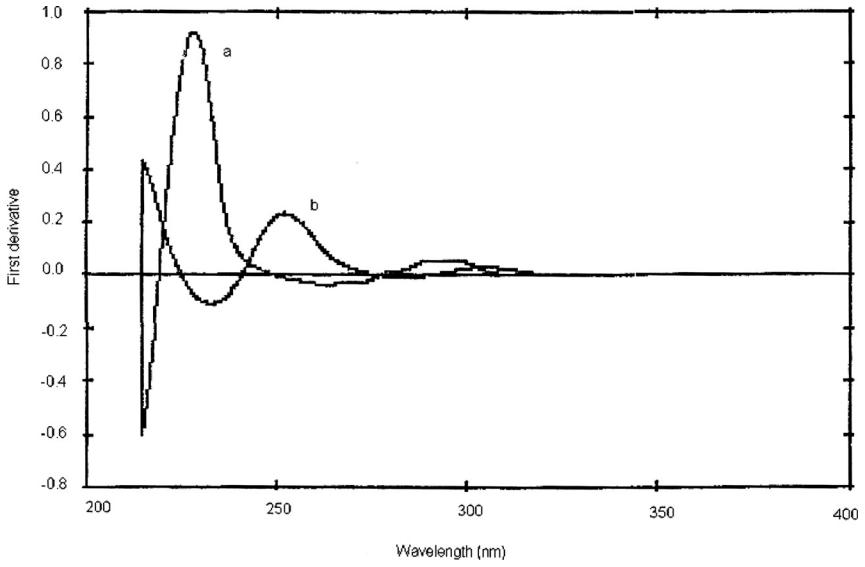

Fig. 2. First-Derivative Spectra of (a) Naphazoline Hydrochloride (5 μg/ml) and (b) Antazoline Phosphate (5 μg/ml)

transferred into a 100 ml volumetric flask and diluted with methanol. Further dilution was done with methanol to reach a concentration of 0.5 μg/ml of naphazoline hydrochloride and 5 μg/ml of antazoline phosphate. The general procedure was followed and the concentrations of napahzoline hydrochloride and antazoline phosphate were calculated.

Results and Discussion

Derivative Spectrophotometric Method Figure 1 shows the zero-order absorption spectra of naphazoline hydrochloride and antazoline phosphate. Since spectral overlap is quite clear in this figure, simultaneous determination of these components can not be performed. Derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem.[6,7]

In this investigation the spectrophotometic parameters were optimized through derivative spectra of naphazoline hydrochloride and antazoline phosphate at different orders and $\Delta\lambda$ values. The first-order derivative spectra traced with $\Delta\lambda$=4.2 (n=6) shows zero-crossing points with evidently useful characteristics from the analytical view point (Fig. 2).

The selection of the optimum wavelength for each component was based on the fact that the absolute value of the total derivative spectrum at these wavelengths has the best linear response to analyte concentration with an intercept very close to zero and least interference of other component. Therefore zero-crossing points of antazoline phosphate (225 nm) and naphazoline hydrochloride (252 nm) were used for simultaneous determination of these compounds.

Calibration Curves and Statistical Analysis Under the optimized conditions, the absorbance of the standard solutions of naphazoline and antazoline were measured at the specified wavelengths. The calibration curves were constructed by plotting the D_1 values against naphazoline hydrochloride or antazoline phosphate over the concentration range mentioned in Table 1. Separate determinations (eight repetitions) at same concentration levels were performed. The statistical data are summarized in Table 1. The linearity of the calibration curves and conformity of the proposed

Table 1. Statistical Data of Calibration Curves of Naphazoline Hydrochloride and Antazoline Phosphate in Mixtures with Different Concentrations Using First-Derivative Spectra

Parameters	Naphazoline hydrochloride	Antazoline phosphate
Linearity	0.20—1.00 (μg/ml)	1.00—10.00 (μg/ml)
Regression equation	$y=1.67\times10^{-1}x-1.85\times10^{-3}$ a)	$y=3.3\times10^{-2}x-1.83\times10^{-3}$ a)
SD of slope	1.60×10^{-3}	1.06×10^{-4}
RSD of slope (%)	0.96	0.82
SD of intercept	8.32×10^{-4}	1.13×10^{-3}
Correlation coefficient	0.9998	0.9999

a) $y=bx+a$, where x is the concentration of naphazoline hydrochloride or antazoline phosphate in μg/ml and y is the amplitude at the specified wavelength.

Table 2. Accuracy and Precision Data of Determination of Naphazoline Hydrochloride (0.2—1.0 μg/ml) in the Presence of Antazoline Phosphate (5.0 μg/100 ml) by First-Derivative Spectrophotometry

Added naphazoline hydrochloride (μg/ml)	Within-day ($n=3$)			Between-day ($n=9$)		
	Found (μg/ml)	CV (%)	Error (%)	Found (μg/ml)	CV (%)	Error (%)
0.200	0.204±0.001	0.49	2.00	0.204±0.005	2.45	2.00
0.400	0.394±0.016	1.52	−1.50	0.397±0.007	1.76	−0.75
1.000	0.995±0.015	1.51	−0.50	0.998±0.014	1.40	−0.20

Table 3. Accuracy and Precision Data of Determination of Antazoline Phosphate (1—10 μg/ml) in the Presence of Naphazoline Hydrochloride (0.5 μg/ml) by First-Derivative Spectrophometry

Aded antazoline phosphate (μg/ml)	Within-day ($n=3$)			Between-day ($n=9$)		
	Found (μg/ml)	CV (%)	Error (%)	Found (μg/ml)	CV (%)	Error (%)
1.00	0.98±0.01	0.61	−2.00	0.98±0.02	1.64	−2.00
4.00	3.98±0.03	0.76	−0.50	3.95±0.04	0.91	−1.25
10.00	9.96±0.05	0.46	−0.40	9.96±0.04	0.43	−0.40

method to Beer's law are validated by the high values of correlation coefficients ($r^2>0.999$) of the regression equations and value of intercept on ordinate which is close to zero (Table 1).

Limit of Quantification The limit of quantification with CV<2.45% was found to be 0.2 μg/ml and 1 μg/ml for naphazoline hydrochloride and antazoline phosphate respectively.

The lowest detection limit that can be reliably detected with a S/N ration of 3 was found to be 0.1 μg/ml and 0.3 μg/ml for naphazoline hydrochloride and antazoline phosphate, respectively.

Accuracy and Precision In order to determine the accuracy and precision of the method, synthetic mixtures of naphazoline hydrochloride and antazoline phosphate were prepared and analyzed in three replicates in three days. The mean recoveries and CV_s are illustrated in Tables 2 and 3. The within-day and between-day coefficient of variation (CV) and the relative error (%) values are considered very satisfactory for both compounds in all three selected concentrations. The data indicate that the proposed derivative spectrophotometric method is highly reproducible during one run and between different runs.

Specificity No interference was observed from the presence of HPMC, benzalkanium chloride, borate buffer and soldium chloride in the amounts commonly present in eye drops.

Stability Study of stability of naphazoline hydrochloride and antazoline phosphate in solutions during the analytical method showed that the analytes were stable for at least 24 h in solutions when protected from light.

Application The proposed method was successfully applied to the analysis of a commercial formulation. No interference from the sample matrix was observed. The results were in good agreement with the labeled content and the error of the determination was 0.60% and 0.64% for naphazoline hydrochloride and antazoline phosphate respectively.

Conclusion

From the results of this study it can be concluded that the proposed first-derivative spectrophotometric method can be used for simultaneous determination of naphazoline hydrochloride and antazoline phosphate. This method is simple, rapid, practical, reliable and inexpensive and can be used for routine analysis of simultaneous determination of these compounds without any prior separation in quality control laboratories.

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