



Application of Ratio Derivative Spectrophotometry for Simultaneous Determination of Naphazoline and Antazoline in Eye Drops

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Abstract: Ratio derivative spectrophotometric method has been developed for the simultaneous determination of naphazoline (NAP) and antazoline (ANT) at micromolar levels in Britton Robinson buffer (pH 9) medium. In this method the overlapping spectra of naphazoline and antazoline were well resolved by making use of the first-derivative of the ratios of their direct absorption spectra. The derivative ratio absorbances of naphazoline and antazoline were measured at 227.2 and 235 nm, respectively for their quantification. The method is simple, fast and does not require separation of naphazoline and antazoline. Another salient feature of the method is that simultaneous standard additions of both analytes permitted to resolve matrix effect and quantification at a unique standard addition plot. Naphazoline and antazoline were determined in the concentration range of 10-150 $\mu\text{mol L}^{-1}$ (NAP/ANT ratio varying from about 10 to 150) in the same aliquot with a precision and accuracy of about 1.7% and 1.8%, respectively. The recommended procedure was successfully applied for analysis of naphazoline and antazoline in eye drops.

Keywords: Simultaneous determination, Naphazoline, Antazoline, Spectrophotometry, Ratio derivative spectra.

Introduction

Naphazoline hydrochloride (NAP) [2-(1-naphthylmethyl)-2-imidazoline monohydrochloride] is a sympathomimetic, which belongs to the imidazole group. It is a vasoconstrictor of relatively long-lasting action that acts on the α receptors of the vascular smooth muscle¹. Antazoline (ANT) is another imidazoline ligand which has alpha 2-adrenoceptor antagonistic properties. It is now widely recognized that imidazoline derivatives provided with 2-adrenoceptor antagonist properties not only exhibit a high affinity for 2- adrenoceptors but also bind to non-adrenergic imidazoline sites in various tissues². Several pharmaceuticals containing the two active ingredients are currently commercialized in our country as an eye drops.

Several methods are available for the determination of naphazoline such as heavy-atom-induced room-temperature phosphorescence³, atomic absorption and emission⁴, capillary electrophoresis in the presence of its degradation products⁵ and first-derivative UV spectrophotometry⁶. Some chromatographic methods have been also reported for the separation or quantitative measurement of naphazoline, *e.g.*, GC-MS⁷, sequential injection chromatography⁸ and capillary electrophoresis methods⁹.

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^{10,11}. Gas chromatographic and high performance liquid chromatographic methods were also reported for determination of naphazoline and antazoline in pharmaceutical preparations¹²⁻¹⁴. 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. There is only one report on the application of derivative spectrophotometry for determination of naphazoline and antazoline¹⁵. The main disadvantage of this technique is its dependence on instrumental parameters like speed of scan and the slit width¹⁶. The instrumental conditions of recording parent zero-order spectrum have strong influence on the shape and intensity of its derivative generations.

Direct UV-Visible spectrophotometric method is not suitable for simultaneous determination of naphazoline and antazoline due to the spectral overlapping. 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.

Since UV-Visible spectrophotometry is a rapid, sensitive and inexpensive analytical tool, it is appropriate for dosage control of pharmaceutical preparations. Despite the mentioned advantages, spectroscopy techniques (such as other analytical techniques) suffer from multiplicative (matrix effect) and additive (direct interference) errors. The problem of multiplicative errors can be simply solved by using method of standard addition (MOSA). The applicability of MOSA is limited to the cases where no direct interference (additive error) is present.

Various multivariate methods for handling nonselective signals in spectrophotometric analysis have been proposed. Principal component and partial least squares solutions require no explicit data about the individual interferences to be known in order to model and to eliminate them¹⁷. However, they require a set of calibration samples with known concentrations of the analyte to be determined and the concentration of each interferent in different samples to be varied to allow the calibration algorithm to model its effect. In the most applications of multivariate chemometric methods the matrix effects are not considered.

Following the work of Salinas *et al.*,¹⁸ Ratio Derivative Spectrophotometry based on the use of the first-derivative of the ratios of the spectra was tried and found feasible in resolving the spectra of naphazoline and antazoline.

Experimental

Naphazoline and antazoline were kindly donated by Sina daroo Pharm. Ind., Iran and used without further purification. All the solvents used in spectrophotometric analysis were of analytical reagent grade.

For preparation of 1.0×10^{-3} mol L⁻¹ naphazoline, 246.74 g naphazoline (99%) was weighed and dissolved in distilled water to 100 mL.

Antazoline solution of 1.0×10^{-3} mol L⁻¹ was prepared daily by dissolving 0.0402 g antazoline (99%) in water and made up to 100 mL. Britton–Robinson (B-R) buffer (0.1 mol L⁻¹) at pH 9 was used throughout the experiment.

Apparatus

UV-Vis absorption spectra were measured on an Agilent UV-Vis spectrophotometer, PerkinElmer (Lambda 25), with the use of 1.0 cm quartz cells. A pH meter (Metrohm, Model 691) with a double junction glass electrode was used to check the pH of the solutions.

Recommended procedure

Suitable volumes of naphazoline and antazoline stock solutions (up to 100 μmol L⁻¹) were mixed in a 25 mL calibrated flask and diluted to volume with 0.1 mol L⁻¹ Britton Robinson buffer (pH 9). According to the theory of the ratio spectra derivative method, the absorption spectrum of the mixture was divided, wavelength by wavelength, by a standard spectrum of antazoline (ANT, 4 μmol L⁻¹) for determining NAP and by a standard spectrum of NAP (NAP, 1 μmol L⁻¹) for determining ANT. Then, the first-derivative of the above ratio spectra was recorded and the values of the derivatives were measured at suitably selected wavelengths. In particular, the concentration of NAP was proportional to the value of the first-derivative of the ratio spectra at 227.2 nm (one maxima). The concentration of ANT was proportional to the value of the first-derivative of the ratio spectra at 235 nm (one minima), respectively. The concentration of NAP and ANT in the mixture was computed from the simultaneous standard additions of both analytes for resolving of matrix effect.

Principle of ratio derivative spectrophotometry

Consider a mixture of two compounds A and B. The absorption spectrum of the mixture is given by the equation

$$A_{M,\lambda_1} = E_{A,\lambda_1} C_A + E_{B,\lambda_1} C_B \quad (1)$$

Where A_{M,λ_1} is the absorbance of mixture at wavelength λ_1 , E_{A,λ_1} and E_{B,λ_1} are the molar absorptivity of A and B at λ_1 , C_A and C_B are the concentrations of A and B in the mixture.

Eq. (1) is divided by the absorbance at λ_1 of a standard solution of A whose concentration is C_A^0 i.e. $E_{A,\lambda_1} C_A^0$ then Eq. (1) becomes:

$$\frac{A_{M,\lambda_1}}{E_{A,\lambda_1} C_A^0} = \frac{C_A}{C_A^0} + \frac{E_{B,\lambda_1} C_B}{E_{A,\lambda_1} C_A^0} \quad (2)$$

This can be simplified to:

$$\frac{A_{M,\lambda_1}}{E_{A,\lambda_1}} = C_A + \frac{E_{B,\lambda_1}}{E_{A,\lambda_1}} C_B \quad (3)$$

Differentiating Eq. (3) with respect to λ , gives

$$\frac{d}{d\lambda} \left(\frac{A_{M,\lambda_1}}{E_{A,\lambda_1}} \right) = C_B \frac{d}{d\lambda} \left(\frac{E_{B,\lambda_1}}{E_{A,\lambda_1}} \right) \quad (4)$$

Equation (4) indicates that the derivative ratio spectrum of the mixture is dependent only on the values of C_B , and is independent of the value of C_A in the mixture.

Results and Discussion

The absorption spectra of naphazoline and antazoline under certain experimental conditions are shown in Figure 1. As it is shown, the maximum wavelengths of two compounds are very close to each other and their spectra are highly overlapped. Therefore, direct determination of two pharmaceutical compounds in the presence of each other is impossible by spectrophotometry. Therefore, the combination of ratio derivative spectrophotometry and standard addition methods was used for resolving of additive interference and matrix effect simultaneously.

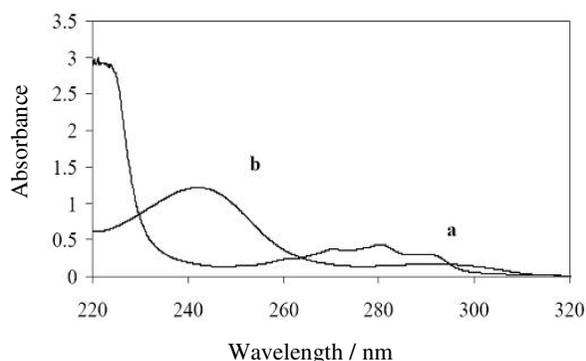


Figure 1. Absorption spectra of (a) naphazoline and (b) antazoline in Britton Robinson buffer at pH 7.0

Effect of operational parameters

In order to optimize the procedure for the simultaneous determination of naphazoline and antazoline, we studied the effect of effective parameters including pH, wavelength selection, $\Delta\lambda$ value and divisor concentrations on the sensitivity and selectivity of the method. As it is shown in Figure 2, there weren't significant changes in the absorption spectra of naphazoline and antazoline at the pH range of 2-10, Therefore pH of 9.0 was selected for obtaining higher selectivity and omitting some ion interferences in alkali media.

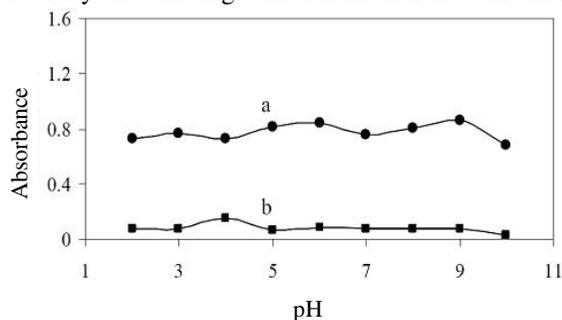


Figure 2. Effect of pH on the maximum absorption spectra of (a) naphazoline and (b) antazoline. Conditions: Britton Robinson buffer (0.01 M), naphazoline $10 \mu\text{mol L}^{-1}$, antazoline $10 \mu\text{mol L}^{-1}$

Wavelength selection

In a preliminary investigation, different concentrations of NAP and ANT as divisors were examined. An accurate choice of both standard divisors and working wavelengths is fundamental

for several reasons¹⁸⁻²⁰. In the wavelength range where the absorbance of the standard spectrum used as divisor approaches zero, the noise of the ratio spectra is greatly enhanced. Consequently, a certain overlap of spectra in the working wavelengths region is actually desirable. Then, by increasing or decreasing the concentration of divisor, the resulting derivative values (hence, the slope of calibration graphs) are proportionately decreased or increased, with potential variation of both sensitivity and linearity range. From several tests, we found the best results in terms of signal-to-noise ratio and sensitivity by using as divisors standard spectra of both 1-400 $\mu\text{ mol L}^{-1}$ NAP and ANT, with minimal differences. Outside the above concentration ranges, the noise was greatly increased; hence it required too high a level of smoothing, with consequent distortion and variation of the shape of curves and location of peaks. For all subsequent measurements, we selected standard divisors of 4 $\mu\text{ mol L}^{-1}$ of NAP and 1 $\mu\text{ mol L}^{-1}$ of ANT.

In Figure 3, there are two series of ratio spectra of NAP (from 10 to 40 $\mu\text{ mol L}^{-1}$, continuous lines) and ANT (from 10 to 40 $\mu\text{ mol L}^{-1}$, dashed lines). In Figure 4, it is shown that the corresponding first-derivative of the ratio spectra of Figure 3. For calibration graphs, we selected the wavelengths which exhibited the best linear response to the analyte concentration, *i.e.*, in the first-derivative mode 227.2 nm and 235 nm to determine NAP and ANT, respectively.

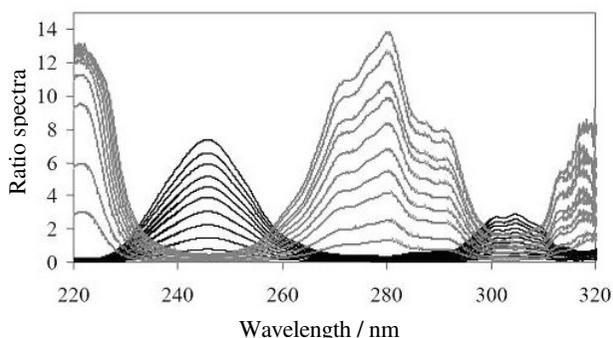


Figure 3. Ratio spectra for different concentrations of naphazoline (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 $\mu\text{ mol L}^{-1}$, continuous lines 1 to 10, divisor ANT, 40 $\mu\text{ mol L}^{-1}$) and of antazoline (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 $\mu\text{ mol L}^{-1}$, dashed lines 1 to 10, divisor NAP, 10 $\mu\text{ mol L}^{-1}$)

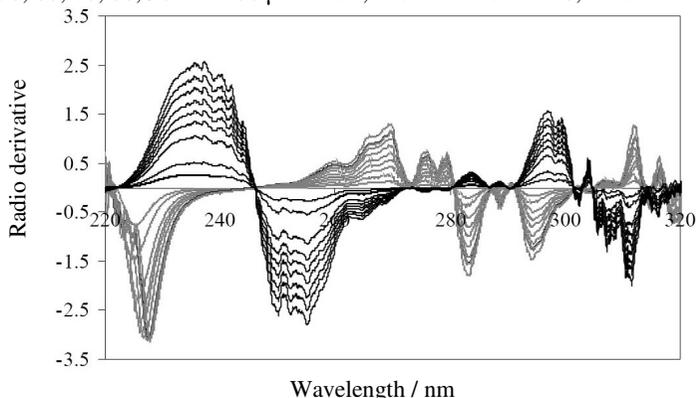


Figure 4. First-derivative spectra of the ratio spectra of naphazoline (continuous lines) in 40 $\mu\text{ mol L}^{-1}$ ANT as divisor and antazoline (dashed lines) shown in 10 $\mu\text{ mol L}^{-1}$ NAP as divisor

The calibration graphs for each drug in both derivative modes were achieved by plotting the values of the first-derivative of the ratio spectra NAP/ANT and ANT/NAP, with variable concentrations of NAP and ANT, at the above working wavelengths against the concentrations of NAP and ANT in the standards.

Divisor concentration

According to the theory¹⁸, the slope of the calibration graphs proportionally increases if the concentration of the divisors decreases. This is shown in Figure 5. These results confirm the reliability of the ratio spectra method in the present instance. According to the above results, divisor concentrations of 4 and 1 $\mu\text{mol L}^{-1}$ were chosen for divisors NAP and ANT respectively.

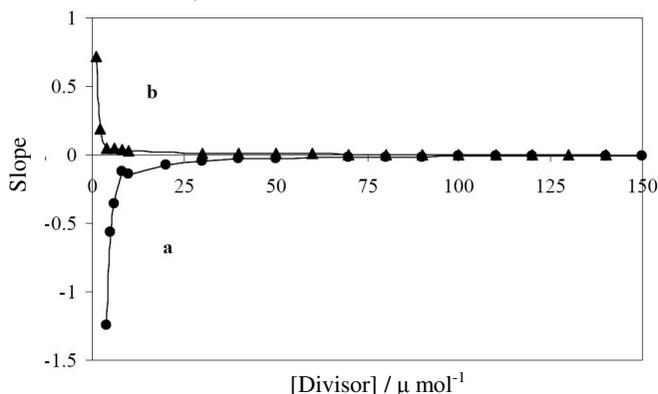
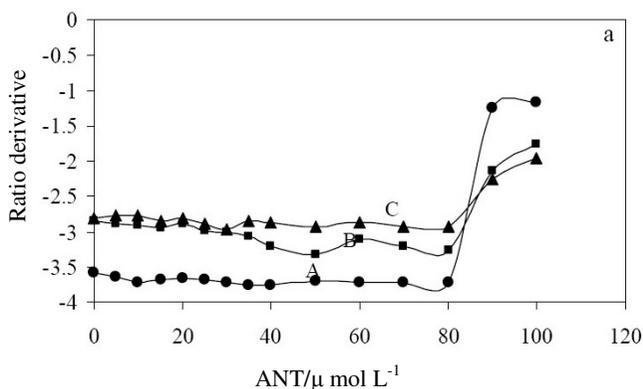


Figure 5. The effect of divisor concentration on the slopes of calibration curves for naphazoline and antazoline. Curve (a) naphazoline, divisor ANT at 227.2 nm and curve (b) antazoline, divisor NAP at 235 nm

$\Delta\lambda$ value

In order to select the $\Delta\lambda$ value for differentiation, a series of first-derivative ratio spectra of a mixture of 10.0 $\mu\text{mol L}^{-1}$ naphazoline with increasing concentration of antazoline ranging from 0.0 to 100.0 $\mu\text{mol L}^{-1}$, were evaluated under the selected conditions, at different $\Delta\lambda$ values. In the selection of $\Delta\lambda$, it is necessary to study the effect of the presence of antazoline on the naphazoline (Figure 6a). Similarly, a series of first-derivative ratio spectra of 30.0 $\mu\text{mol L}^{-1}$ antazoline with increasing concentrations of naphazoline ranging from 0.0 to 100.0 $\mu\text{mol L}^{-1}$ were evaluated under the selected conditions, at different $\Delta\lambda$ values (Figure 6b)



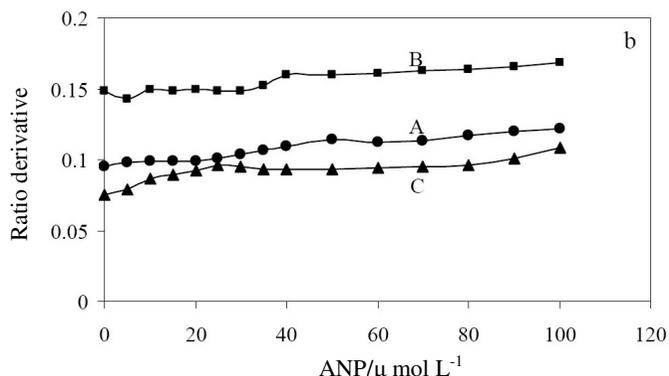


Figure 6. (a) Effect of concentrations of antazoline over the signals to analytical wavelength of $10.0 \mu\text{mol L}^{-1}$ naphazoline at different $\Delta\lambda$ values. (A) $\Delta\lambda = 2.0 \text{ nm}$; (B) $\Delta\lambda = 3.0 \text{ nm}$; (C) $\Delta\lambda = 5.0 \text{ nm}$. (b) Effect of concentrations of naphazoline over the signals to analytical wavelength of $30.0 \mu\text{mol L}^{-1}$ antazoline at different $\Delta\lambda$ values. (A) $\Delta\lambda = 2.0 \text{ nm}$; (B) $\Delta\lambda = 3.0 \text{ nm}$; (C) $\Delta\lambda = 5.0 \text{ nm}$

According to Figure 6(a) & 6(b), when $\Delta\lambda$ is equal to 3 nm, in both cases the signals of the analytes that remain constant are not affected when the concentration of the variable analyte increases up to 80 and $100 \mu\text{mol L}^{-1}$ for naphazoline and antazoline respectively.

Accuracy

Accuracy of the proposed method was tested by determinations of various synthetic mixtures of NAP and ANT using the simultaneous standard additions of both analytes. The results shown in Table 1 indicate that first-derivative ratio spectra method is very effective for the simultaneous determination of NAP and ANT in the presence of each other.

Table 1. Determination of naphazoline and antazoline in some synthetic mixtures

Synthetic samples	$C_{\text{added}}/\mu\text{mol L}^{-1}$		$C_{\text{found}}/\mu\text{mol L}^{-1}$		Recovery, %	
	NAP	ANT	NAP	ANT	NAP	ANT
Synthetic 1	10.0	10.0	11.7	10.7	117.0	107.4
Synthetic 2	30.0	30.0	28.0	30.7	93.6	102.4
Synthetic 3	10.0	30.0	10.1	33.9	101.0	113.0
Synthetic 4	30.0	10.0	31.8	10.1	105.9	101.3
Synthetic 5	10.0	50.0	9.3	50.8	92.6	101.7
Synthetic 6	50.0	10.0	51.3	11.3	102.5	112.7

Precision

To check the reproducibility of the method, six replicate experiments for the analysis of naphazoline and antazoline mixtures were designed (Table 2). As it shown relative standard deviations of calculated concentrations are satisfactory.

Interferents

An attractive feature of an analytical procedure is its relative freedom from interferences. The selectivity of the proposed procedure for the assay of naphazoline and antazoline was identified by studying the effect of excipients that often accompany with NAP and ANT in pharmaceutical formulations. Therefore, samples containing $10 \mu\text{mol L}^{-1}$ NAP and/or ANT in the absence and presence of excipients were analyzed by means of the proposed procedure. For other constituents, Tolerance limit was defined as the concentrations which give an error of $\leq 5\%$ in the determination of each naphazoline and antazoline. The effects of all examined compounds at several molar ratios over NAP and ANT on the measured analytical concentrations

are given in Table 3. The results show no significant interference from excipients. This is due to the fact that in alkali media, pH (>7.0), many cationic ions precipitate in aqueous media.

Table 2. Replicate measurements results of naphazoline and antazoline in some synthetic mixtures

$C_{\text{added}}/\mu\text{ mol L}^{-1}$		$C_{\text{found}}/\mu\text{ mol L}^{-1}$		Average		Standard deviation	
SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP
10.0	10.0	10.74	9.70	11.10	9.29	0.62	0.89
10.0	10.0	10.76	8.28				
10.0	10.0	11.82	9.91				
30.0	30.0	30.71	28.09	30.74	28.67	0.09	0.50
30.0	30.0	30.67	28.91				
30.0	30.0	30.84	29.00				
30.0	10.0	33.90	10.11	10.24	33.39	0.89	0.32
30.0	10.0	33.90	10.00				
30.0	10.0	32.35	10.61				
10.0	30.0	10.13	31.77	33.39	10.24	0.32	0.89
10.0	30.0	10.13	34.40				
10.0	30.0	10.35	34.0				
10.0	50.0	11.46	51.28	11.41	51.26	0.12	0.05
10.0	50.0	11.28	51.20				
10.0	50.0	11.51	51.29				
50.0	10.0	50.85	9.26	50.90	9.37	0.070	0.09
50.0	10.0	50.87	9.42				
50.0	10.0	50.96	9.42				

Table 3. Maximum tolerable concentration of interfering species with naphazoline ($10\ \mu\text{ mol L}^{-1}$) and antazoline ($10\ \mu\text{ mol L}^{-1}$)

Species	Tolerance concentration/ m mol L^{-1}
NO_3^- , I, K^+ , Mg^{2+} , Ca^{2+} , Na^+ , CH_3COO^- , Cu^{2+} , Co^{2+} , Ag^+ , Br^- , Cd^{2+} , Fe^{2+} , Zn^{2+} , Bi^{3+} , Cr^{3+} , Ca^{2+} , Al^{3+} , Fe^{3+} , PO_4^{3-} , SO_4^{2-} , Mn^{2+} , CH_3OH , $\text{C}_2\text{H}_5\text{OH}$	10
Vitamin B ₁ , Vitamin C, Folic acid, Glucose, Sodium benzoate	0.1

Analyses of NAP and ANT in a commercial eye drop

To examine the applicability of the proposed ratio derivative method in the analysis of the real samples, ANT and NAP in a commercial eye drop (Sina Darou Company, Tehran, Iran) were analyzed by the proposed method at the optimum conditions. First, 2.0 mL of the sample was taken and subjected to the successive dilution with Britton Robinson buffer (pH 9.0) to obtain concentrations in the region of calibration curves. For resolving of matrix effect, simultaneous standard additions of both NAP and ANT were designed. The absorbance spectrum of each solution was then recorded and transferred to the ratio first-derivative spectrum to predict the concentrations of the ANT and NAP by standard addition plot. In the other trials, different amounts of ANT or NAP or a mixture of the drugs were spiked into the original eye drop and the procedure for the analysis of ANT and NAP were repeated. Each analysis was repeated three times. The results are listed in Table 4. In this table, the resulting values of ANT and NAP as the mean of three replicates and the mean recoveries are represented. The confidence intervals for ANT and NAP are varied in a small range indicating good reproducibility of the proposed analytical method. Indeed, the recoveries (relative to the declared amounts of drugs in the eye drop and the spiked amounts) varied between 98.5-103.8% and 97.4-105.8% for ANT and NAP, respectively. This confirms the high accuracy of the proposed method for simultaneous analyses of ANT and NAP in a commercial formulation.

Table 4. Determination of naphazoline and antazoline in a commercial eye drop

Sample	$C_{\text{added}}/\mu\text{ mol L}^{-1}$		$C_{\text{found}}/\mu\text{ mol L}^{-1}$		Recovery, %	
	NAP	ANT	NAP	ANT	NAP	ANT
Eye drop*	---	---	10.64 ± 0.47	47.98 ± 3.79	106.4	95.9
	10.0	25.0	21.08 ± 1.20	73.6 ± 2.41	104.1	102.5
	20.0	40.0	31.9 ± 1.53	89.0 ± 3.92	106.3	102.5

* The real concentrations of naphazoline and antazoline are 10.0 and 50.0 $\mu\text{ mol L}^{-1}$ respectively manufactured by Daroo Pakhsh Co. (Tehran, Iran)

Conclusion

First order ratio spectra derivative method enable the quantitation of mixtures of NAP and ANT with good accuracy and precision, either in laboratory samples or in pharmaceutical products. The procedure is fast and specific and works without solving equations or separation steps. As a further advantage of the ratio spectra method proposed over the zero-crossing derivative method, is the possibility of performing measurements in correspondence of peaks, hence a potentially greater sensitivity and accuracy. Disadvantages of the zero-crossing method are the risk of small drifts of the cross over points and the fact that the working wavelengths do not coincide with the peaks. This may be particularly dangerous when the slope of the spectra is very high, with consequent loss of precision and accuracy.

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