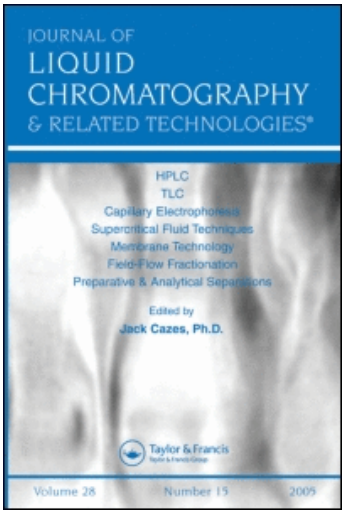

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SIMULTANEOUS DETERMINATION OF EPHEDRINE AND 2-IMIDAZOLINES IN PHARMACEUTICAL FORMULATIONS BY REVERSED-PHASE HPLC

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ABSTRACT

A simple,rapid, and accurate method for the determination of naphazoline, oxymetazoline, xylometazoline, and ephedrine in rinological solutions using reversed-phase HPLC was developed. It involves the use of alumina coated with polybutadiene as the stationary phase, acetonitrile-aqueous 10^{-3} M NaOH (10:90) as the initial mobile phase with a linear gradient from 10 to 80% acetonitrile in 25 min, and detection at 224 nm. The only sample preparation is its dilution with water. Linearity and precision of the method have been assessed. The assay results obtained for various formulations were in agreement with the declared amounts.

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INTRODUCTION

Some 2-imidazoline derived drugs have a potent vasoconstrictor action. Among them naphazoline (I), oxymetazoline (II) and xylometazoline (III) display a rapid and prolonged effect in reducing swelling and congestion when applied to mucous membranes. They represent the active principle of many commercially available nasal solutions.

Also ephedrine (IV) salts have been used alone or in combination with other agents, especially 2-imidazolines, for the symptomatic relief of nasal congestion associated with the common cold, hay-fever, or rhinitis or sinusitis.

The determination of the above compounds has been essentially performed by gas liquid chromatography (1,2) or reversed-phase high performance liquid chromatography (RP-HPLC) (1, 3-5). The major problem associated with the analysis of basic drugs by RP-HPLC is peak tailing caused by ionic interactions between the analytes and free silanol groups of the packing material. Mobile phase additives (6) represent the first form of in situ column modifications to reduce peak asymmetry and to effect selectivity changes.

Alkylamines and alkylsulfonated ion-pairing reagents are the most common ones. Alkylamines act as silanol-masking agents, primarily by hydrogen bonds, thereby reducing ion-exchange and/or adsorption effects (7). The addition of counter ions and/or basic modifiers to the eluent may cause deterioration of the column packing and usually shortens column life. Therefore there is a growing interest in developing reversed phase sorbents from alkaline-stable support materials such as alumina.

A different approach to the analysis of basic drugs has been the use of base-deactivated columns (8) in which

free silanol groups are masked through various procedures. Kountourellis and Raptouli proposed the use of a basic alumina column and non-aqueous mobile phase containing very small amounts of ammonia for determining some 2-imidazolines in pharmaceutical preparations.

We describe here the simultaneous analysis of compounds I-IV by RP-HPLC performing the separation with an alkaline-stable stationary phase, namely Aluspher^{R} RP-select B. Validation data are presented for the determination of the drugs in commercial samples of rinological formulations.

EXPERIMENTAL

Chemicals

All reagents used were of analytical-reagent grade (Merck, Darmstadt, Germany) and were used as obtained. The pure pharmacologically active ingredients were naphazoline nitrate (I), ephedrine hydrochloride (IV) (Lepetit SpA, Milan, Italy), oxymetazoline hydrochloride (II) (Procter&Gamble, Milan, Italy), and xylometazoline hydrochloride (III) (Zyma SpA, Varese, Italy). Acetonitrile was of HPLC grade (J.T.Baker, Deventer, Holland). Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a nylon Milllipore filter (pore size 5 μm).

HPLC Equipment

Chromatography was performed on a Varian 9000 liquid chromatograph equipped with a 10 μL sample loop, a Varian Polychrom 9065 photodiode-array detector, serially connected with a 2550 Varian variable-wavelength detector and a personal computer IBM PS/2. The analytical column was a LiChroCART (125 mm x 4.0 mm I.D.) packed with 5 μm Aluspher^{R} RP-select B (Merck,Darmstadt, Germany).

HPLC Conditions

The operating conditions were as follows: initial mobile phase, acetonitrile-water containing 10^{-3} M sodium hydroxide (10:90, v/v), then a linear gradient up to 80% acetonitrile in 25 min;flow-rate, 1.2 mL min^{-1}; injection volume, 10 μL; column temperature 25 °C detection wavelength, 224 nm.

Calibration Standards

Stock solutions were prepared by dissolving 100 mg of compounds II,III, and IV and 50 mg of compound I in 100 mL of water. They were kept refrigerated at 4 °C. Working standard solutions were prepared by diluting aliquots of the stock solutions to give concentrations ranging from 10 to 800 μg mL^{-1} for (II), (III), and (IV), and from 2 to 160 μg mL^{-1} for (I). The calibration graphs were constructed by plotting the peak areas obtained at the wavelength of 224 nm versus the amounts (μg) injected.

Sample Preparation

An accurately measured volume (1-2 mL) of each commercial nasal solutions was transferred into a volumetric flask and diluted to volume with water. Usually the dilution factor was 10. After filtration through a nylon filter (pore size, 0.45 µm) the solution was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

As mentioned in the introduction, the separation of organic bases on silica-based reversed phase materials is usually very problematic. This is due to the presence of active sites which complicate the retention mechanism and lead to peak tailing with polar solutes. Aluspher^{R} RP-

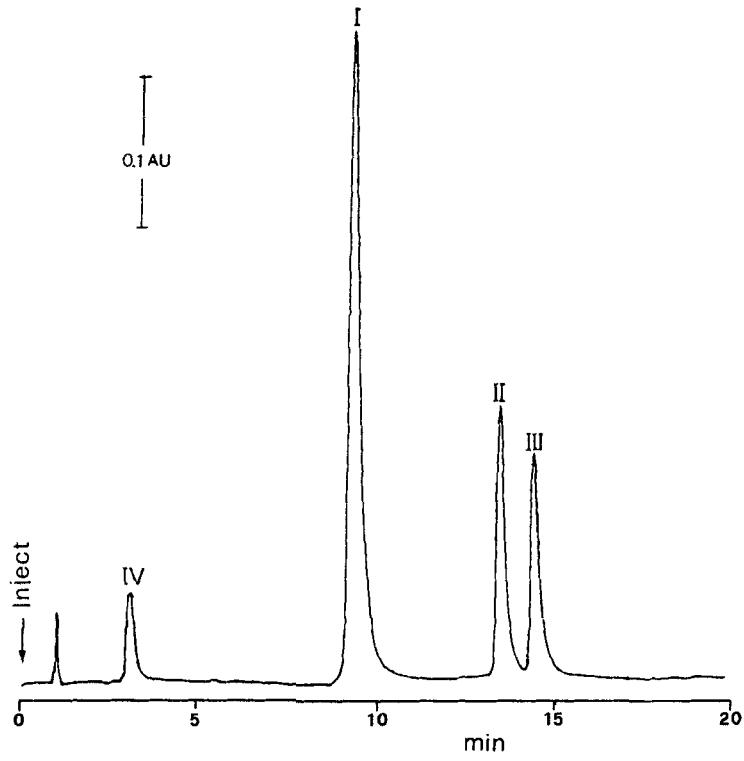

Figure 1 Typical chromatogram obtained at 224 nm for a standard solution containing 0.25 mg mL^{-1} of (II), (III), and (IV), and 0.125 mg mL^{-1} of (I).

select B is a new alkaline-stable stationary phase based on porous aluminium oxide coated with polybutadiene, which displays characteristic hydrophobic properties which are comparable to those shown by silica-based RP-phases (10).

Figure 1 shows the chromatogram of a standard solution of compounds I-IV, obtained with this stationary phase. As can be seen, a very good separation without peak tailing was achieved.

The detection wavelength of 224 nm was chosen since this value allowed the simultaneous analysis of the compounds investigated with a good sensitivity. In case the drugs are to be analyzed alone the following wavelengths can be selected to obtain the maximum sensitivity: 259 nm for ephedrine, 283 nm for naphazoline and oxymetazoline, and 224 nm for xylometazoline.

The photodiode-array detector allowed the estimation of the purity parameter format values (11). These values represent an absorbance-weighted mean wevelength of a spectrum and are very useful in the analysis of a pharmaceutical preparation both in confirming peak purity and peak identification, since these values provide an absorbance-weighed mean wavelength of a spectrum. The purity parameters were calculated for the compounds of interest over the range 220-367 nm and are reported on Table 1.

The capacity factors (reported in Table 1) were reproducible under the experimental conditions used, the coefficient of variation (CV) ranging from 1.0 to 1.5 for within-day and from 1.8 to 2.7% for between-day studies. The average analysis time was about 20 min.

The linearity was evaluated over the range of concentrations reported in the experimental section. The equations obtained by the least square regression fit are reported in Table 2.

The limits of detection, defined as the lowest concentration of each compound resulting in a signal-to-noise ratio of 3:1, are reported in Table 1.

The procedure was applied to the determination of active ingredients in six commercially available formulations. The results obtained are shown in Table 3. The quantities found were in conformity with the values claimed by the manufacturers.

TABLE 1
Analytical Parameters for Compounds I-IV[a]

Compound	Capacity factor	Purity parameter mean(nm) ± SD	Ideal detection wavelength (nm)	Detection limit at 224nm (ng injected)
I	8.3	223.82 ± 0.09	283	0.5
II	12.3	226.23 ± 0.11	283	1.0
III	13.3	223.58 ± 0.09	224	1.0
IV	2.0	221.59 ± 0.10	259	5.0

[a] Each value is the mean of five determinations

TABLE 2
Calibration Curves for Compounds I-IV: Linear Regression of the amount injected (x) versus the peak area (y); mean value ± standard deviation at 95% confidence interval (t=3.18; n=5)

Compound	Intercept	Slope	R^2
I	(-30.0 ± 0.6)E3	(95.4 ± 0.9)E4	0.999
II	(-5.2 ± 0.3)E3	(14.4 ± 0.2)E4	0.999
III	(-3.5 ± 0.1)E3	(12.4 ± 0.1)E4	0.999
IV	(-2.3 ± 0.2)E3	(2.11 ± 0.04)E4	0.998

TABLE 3
Analysis of Pharmaceutical Formulations*

Commercial preparation	Naphazoline Recovery(%)	CV	Oxymetazoline Recovery(%)	CV	Xylometazoline Recovery(%)	CV	Ephedrine Recovery(%)	CV
A					98.7	0.2		
B					92.2	0.3		
C					98.9	0.2		
D	99.1	0.2					96.7	0.1
E			99.8	0.3				
F	98.7	0.3						

*mean of five determinations.

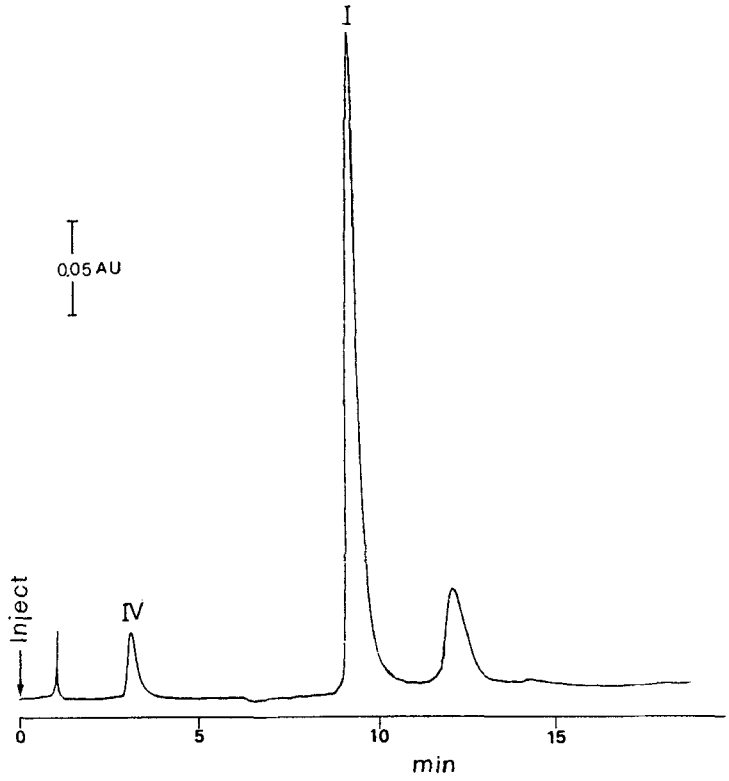

Figure 2 Chromatogram obtained after injecting the pharmaceutical formulation D, containing naphazoline nitrate and ephedrine hydrochloride as active ingredients.

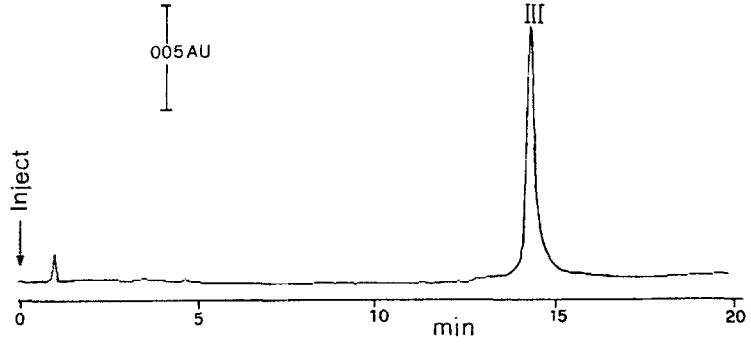

Figure 3 Chromatogram obtained after injecting the pharmaceutical formulation A, containing xylometazoline hydrochloride as active ingredient.

Figures 2 and 3 show the chromatograms obtained from the analysis of the commercial preparations D and A, respectively. No interference was observed from the excipients. In the case of sample D where both active compounds naphazoline and ephedrine are present, no problem was encountered with the possible interfering paraben, added as preservative to the preparation, since its peak eluted as the last one.

The analytical results obtained lead to the conclusion that the developed method is simple, rapid, and precise and therefore it can be successfully adopted for the routine analysis of I-IV in liquid pharmaceutical formulations.

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