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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ANTAZOLINE PHOSPHATE AND TETRAHYDROZOLINE HYDROCHLORIDE IN OPHTHALMIC SOLUTION

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SUMMARY

A method for the determination of 2-[(N-phenyl)benzylaminomethyl]-2-imidazoline · H_3PO_4 (antazoline phosphate) and 2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazoline · HCl (tetrahydrozoline hydrochloride) in ophthalmic solution is described. The pharmaceutical preparation is analysed directly by reversed-phase ion-pair high-performance liquid chromatography and the method is very rapid, selective and simple.

INTRODUCTION

Antazoline {2-[(N-phenyl)benzylaminomethyl]-2-imidazoline} (I) is an H_1 receptor antagonist of histamine that has been used as a topical agent in the treatment of ocular disorders[1,2]. This drug is much less irritating to ocular tissues than are other members of the histamine antagonizing group and it is used in the treatment of ocular allergy, usually in combination with decongestants.

Among them, tetrahydrozoline [2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazoline] (II), a sympathomimetic agent with marked α-adrenergic activity[3], has recently been introduced in some ophthalmic solutions to replace the more widely used naphazoline.

Although several analytical procedures have been developed for the determination of either I or II in pharmaceutical preparations, no method has been reported to achieve the simultaneous quantification of the two compounds.

Colorimetric or spectrophotometric methods previously reported for the quantification of imidazolines[4–8] can be tedious and most are not applicable to formulations containing mixtures of these compounds. A gas chromatographic approach[9] for the determination of a number of imidazolines has been also described but it does

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not seem to be reliable as it requires a difficult preliminary extraction of these strong bases from an aqueous phase into organic solvents and yield peaks with heavy tailing; in addition, II was not taken into account in that work.

In recent years, high-performance liquid chromatography (HPLC) has appeared to be the method of choice in developing rapid and specific analyses for imidazoline derivatives. A number of imidazolines have been analysed by HPLC using a strong cation-exchange column[10], but no separation has been achieved for I and II, whose peaks totally overlapped. An ion-pair HPLC assay of decongestants and antihistamines[11] has also been described; the chromatographic behaviour of I and naphazoline was reported, but not that of II. Finally, the only published procedure for determining tetrahydrozoline alone in pharmaceutical preparations involved reversed-phase HPLC on a C_{18} column[12].

This paper describes an HPLC method that can be routinely used to assay I and II simultaneously in ophthalmic solution; the method is simple and combines specificity and sensitivity that are not attainable by previously described methods.

EXPERIMENTAL

Materials

Disodium phosphate, hydrochloric acid and 4-dimethylaminobenzaldehyde were purchased from Carlo Erba (Italy). 1-Octanesulphonic acid sodium salt was a product of Fluka (Switzerland). Antazoline phosphate and tetrahydrozoline hydrochloride were obtained from Sigma (U.S.A.). These chemicals were all of analytical-reagent grade and were used as received. Water, acetonitrile and methanol were of HPLC grade (Waters Assoc., U.S.A.).

Ophthalmic solutions

Ophthalmic solutions A, B and C were prepared containing different amounts of each drug as reported in Table II. Solution A contained sodium ethylmercurithiosalicylate as a preservative and benzalkonium chloride was present in B and C.

Mobile phase

The solvent systems used were (A) acetonitrile–methanol (1:1) and (B) 0.005 *M* octanesulphonic acid sodium salt in aqueous 0.005 *M* disodium phosphate (adjusted to pH 7 with hydrochloric acid). The mobile phase contained 10% of B in A and was filtered through 0.45-μm microporous PTFE membrane filters (Millipore, U.S.A.) before use. It was pumped through the column at a flow-rate of 1.5 ml/min.

Instrumentation

A Spectra-Physics Model SP 8700-8750 high-performance liquid chromatograph equipped with a 10-μl loop injector and fitted with a variable-wavelength UV detector (Polychrom 9060) set at 222 nm was used with a Hypersil C_8 column (150 mm × 4.5 mm I.D.) packed with a spherical silica particulate (dimethyloctylsilane).

Spectroscopic analysis

UV spectra of aqueous solutions of antazoline phosphate (0.01 mg/ml) and tetrahydrozoline hydrochloride (0.01 mg/ml) were recorded on a Perkin-Elmer Model 330 double-beam spectrophotometer fitted with a UV data station.

Standards

Separate stock solutions of I and II were prepared in the mobile phase and contained 2 mg/ml. These solutions could be stored in a refrigerator for at least 2 weeks without deterioration. Working standards were prepared daily using the mobile phase to make the desired dilutions.

The internal standard solution was prepared by dissolving dimethylaminobenzaldehyde (0.1 mg/ml) in the mobile phase. Accurately measured volumes of this solution were added to standard or sample solutions to obtain a final concentration of 0.05 mg/ml.

Preparation of ophthalmic aqueous solutions

Each opthalmic solution (1 ml) was diluted with the mobile phase to a final concentration of near 0.1 mg/ml for I and 0.05 mg/ml for II. A portion of this solution was filtered and diluted 1:1 with internal standard solution before injection into the chromatograph.

Assay procedure and quantitation

Using the chromatographic conditions described, injections of the sample and standard solutions were made. Results were calculated from the linear regression of five standards of I and five standards of II relating peak-area ratio (standard-to-internal standard) and concentration.

Degraded solutions of I and II

According to Andermann and Richard[12], tetrahydrozoline hydrochloride solutions were treated with 10 *M* sodium hydroxide solution at 50°C for 24 h. The pH was then adjusted to 7 with concentrated hydrochloric acid and the solution was injected directly into the chromatograph. Accelerated degradation of antazoline phosphate solutions was achieved with the same procedure.

RESULTS AND DISCUSSION

Preliminary studies involved trying several C_8 and C_{18} reversed-phase columns from various manufacturers and testing several mobile phase compositions for the effective separation of the sample compounds and for minimizing peak tailing. A C_8 column (5 μm spherical silica particles; 25 mm × 4.6 mm I.D.) eluted with a mobile phase containing sodium octylsulphonate in aqueous phosphate mixed with acetonitrile and methanol, as described under Experimental, afforded the best separation of I, II and the internal standard and provided better separations of the active compounds from excipients such as benzalkonium chloride and sodium ethylmercurithiosalicylate, used as preservatives in the ophthalmic solutions tested.

UV spectra of aqueous solutions containing I and II at the same concentration were also preliminarly studied (Fig. 1a and b) in order to select the wavelength at which the UV detector had to be operated.

These spectra showed the same absorption value at 222 nm and this wavelength was chosen for the UV detection. A representative chromatogram of a standard mixture of I, II and the internal standard is given in Fig. 2; the overall chromatographic time of 10 min provides efficient assay capability.

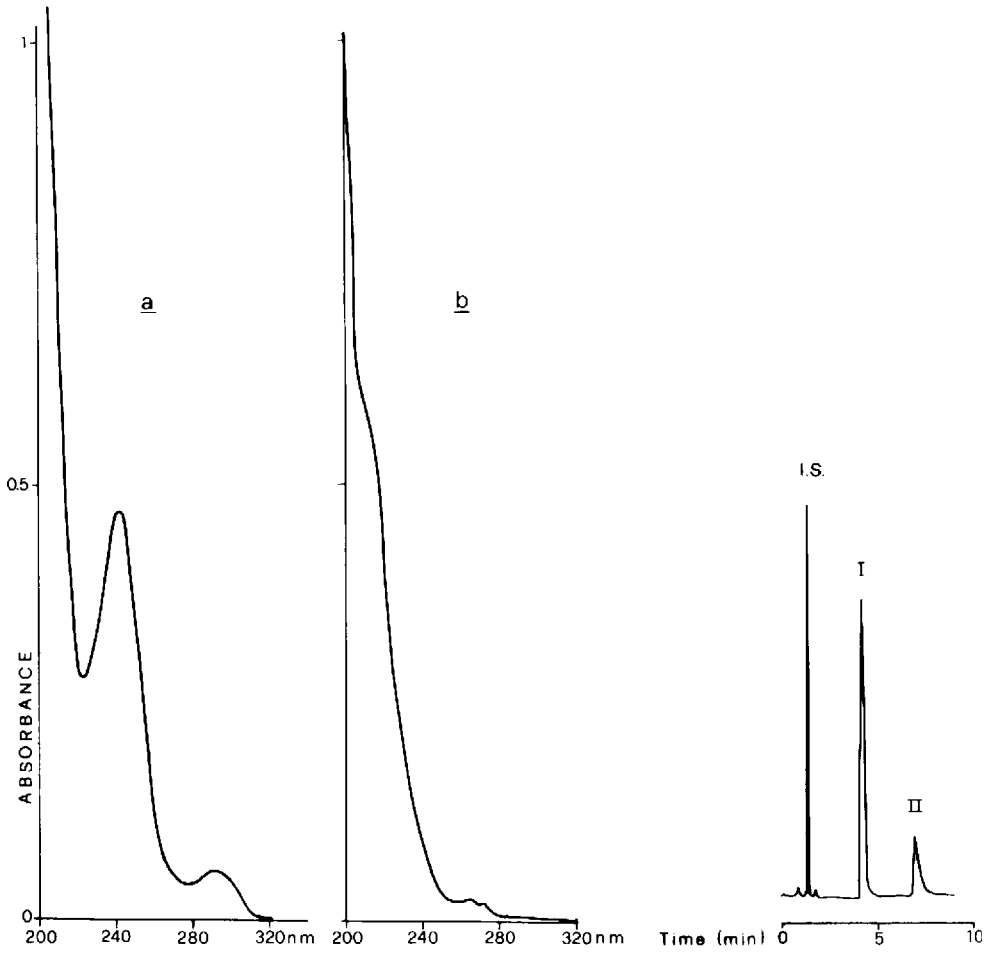

Fig. 1 UV spectra of 0.01 mg/ml solutions of (a) antazoline phosphate and (b) tetrahydrozoline hydrochloride.

Fig. 2. Typical chromatographic separation of I, II and the internal standard (I.S.).

The precision of the assay was determined by independent analyses of six aliquots of the same sample, using solution C; the method was shown to be reproducible with relative standard deviations (R.S.D.) of 0.36 and 0.42% for I and II, respectively (Table I).

The precision was evaluated by making several injections of the same standard solution containing I, II and the internal standard. The R.S.D. values of the response ratios for I and II were 0.35 and 0.36%, respectively.

The linearity of the method was also studied; five standards containing concentrations of I and II spanning 50–200% of the expected working range were analysed by the method. The calibration graph thus obtained for antazoline showed a correlation coefficient of 0.9996 in the range 0.4–10 μg injected. The equation of the linear regression line was $y = 27.096x - 0.082$ where y is the peak-area ratio (an-

TABLE I

PRECISION OF THE ASSAY OF I AND II IN OPHTHALMIC SOLUTION

*Sample No.**	*Assay value (mg/ml)*	
	Compound I	*Compound II*
1	1.038	0.5029
2	1.032	0.5021
3	1.031	0.4989
4	1.039	0.5030
5	1.038	0.4985
6	1.032	0.4994
Mean	1.035	0.5008
R.S.D.	0.36	0.42

* Aliquots of ophthalmic solution C.

tazoline to internal standard) at 222 nm and x the concentration (mg/ml) of antazoline. The equation of the linear regression line for tetrahydrozoline was $y = 29.324x - 0.232$ with a correlation coefficient of 0.9992 in the range 0.25–1 μg injected. These statistics indicate that HPLC analysis gives a linear response, and a single point standard may be used.

Three different ophthalmic solutions (see under Experimental) were analysed for I and II content by the method and the results are summarized in Table II. All the chromatograms obtained were essentially devoid of any interfering peaks due to excipients.

The chromatographic behaviour of potential degradation products from I and II was also studied. Solutions of tetrahydrozoline hydrochloride subjected to accelerated degradation by alkali[12] were chromatographed and a typical chromatogram is given in Fig. 3. The purity of the peak of the undegraded tetrahydrozoline II which is present in this chromatogram was confirmed by acquiring several UV spectra during its elution. Solutions of antazoline phosphate, treated in the same way with alkali, gave a typical chromatogram as shown in Fig. 4. These results show that the method is capable of the separation of the potential degradation products.

The analysis of an aged (21 months) sample of ophthalmic solution C gave the

TABLE II

RESULTS OF HPLC ASSAY OF I AND II IN OPHTHALMIC SAMPLES

Ophthalmic solution	*Formulated (mg/ml)*		*Determined by HPLC (mg/ml)*	
	I	*II*	*I**	*II**
A	1.553	0.502	1.572 (0.46)**	0.506 (0.50)**
B	5.176	0.412	5.179 (0.62)	0.419 (0.46)
C	1.037	0.502	1.039 (0.45)	0.499 (0.51)

* Each value is the mean of three determinations.

** R.S.D. (%) in parentheses.

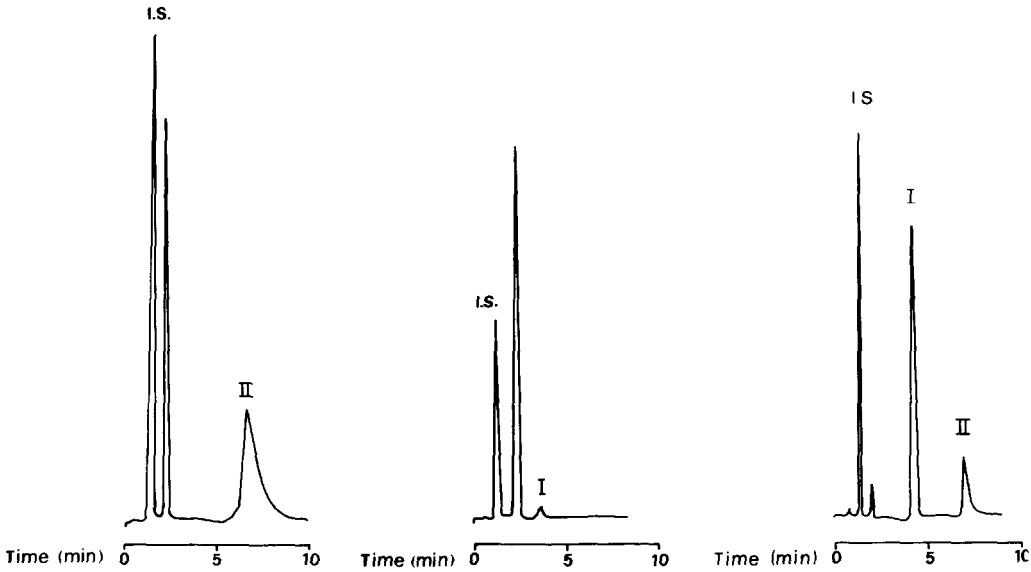

Fig. 3. Typical chromatogram of a degraded tetrahydrozoline hydrochloride solution.

Fig. 4. Typical chromatogram of a degraded antazoline phosphate solution.

Fig. 5. Typical chromatogram the ophthalmic solution C stored at room temperature for 21 months.

chromatogram in Fig. 5, which shows that little decomposition of the two active compounds had occurred.

The HPLC assay method for I and II described here is simple, rapid and precise and provides a sufficiently sensitive method for the determination of these two drugs in ophthalmic solution.

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