Journal of Chromatography, 391 (1987) 303–308
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 320

Note

Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography

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(Received December 2nd, 1986)

The stability testing of drugs in dosage forms usually involved a non-specific determination (*e.g.*, by titrimetry or spectrophotometry) coupled with a thin-layer chromatographic method for the determination of degradation products and for the identification of the drug. In recent years, high-performance liquid chromatography (HPLC) has become increasingly important in the quality control of drugs in pharmaceuticals, as it is both selective and quantitative.

Although many over-the-counter decongestant products containing 2-imidazolidine-derived drugs are available, few papers have dealt with the determination of such drugs in pharmaceutical preparations using chromatographic techniques[1,2]. The use of acidic aqueous methanolic eluents containing both amines and alkylsulphonates has proved to be very succesful for the reversed-phase ion-pair liquid chromatographic separation of several 2-imidazoline drugs[3].

To investigate the suitability of these eluent systems in the quality control of such drugs in terms of reproducibility of the assay and specificity against accompanying ingredients, a specific stability-indicating determination for tetryzoline hydrochloride, 2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazoline (I), in a commercially available nasal solution, was studied.

EXPERIMENTAL

Chemicals and solvents

Tetryzoline hydrochloride reference compound was a kind gift from Pfizer (Brussels, Belgium). Tolazoline hydrochloride was purchased by Janssen (Beerse, Belgium).

Tetryzoline hydrochloride was analysed in Neocor-Tyzine (Roerig) nasal solution stated to contain 1 mg/ml. N,N-Dimethyloctylamine (DMOA) was obtained from Aldrich (Milwaukee, WI, U.S.A.) and was used as received; anhydrous sodium 1-octanesulphonate (SOS) was obtained from Janssen, 85% orthophosphoric acid from Merck (F.R.G.) and analytical-reagent grade methanol from UCB (Belgium).

Water was purified by ion-exchange chromatography and subsequent distillation.

0021-9673/87/$03.50

HPLC equipment

Chromatography was performed on an SP 8000 liquid chromatograph (Spectra Physics, Darmstadt, F.R.G.) equipped with a Model 770 variable-wavelength detector (Spectra Physics) and a BD 8 single-channel recorder (Kipp & Zonen, Delft, The Netherlands).

Chromatographic conditions

A 5-μm particle size RSIL C_{18} column (150 × 4.1 mm I.D.) (RSL, Eke, Belgium) was used throughout. The mobile phase was pumped at 1.0 ml/min and the column effluent was monitored at 220 nm. All separations were conducted at 25°C (heated air oven). Injections of 10 μl were made with a Valco six-port injection valve.

The mobile phase was prepared by dissolving 4.325 g of SOS (20 m*M*) and 2.0 ml of DMOA (10 m*M*) in *ca.* 990 ml of methanol–water (40:60), adjusting the pH to 3.0 with orthophosphoric acid and diluting to 1000 ml with the same mixture. Before chromatography, the mobile phase was filtered through a 5-μm filter and degassed with helium.

Calibration standards

Stock solutions containing *ca.* 62.5 mg of I and *ca.* 50 mg of tolazoline hydrochloride (internal standard) in 100.0 ml were prepared in methanol–water (40:60). Working standard solutions were prepared by pipetting 6.0, 7.0, 8.0, 9.0 and 10.0 ml of the stock solution of I and 10.0 ml of the stock solution of the internal standard into a 50-ml volumetric flask and diluting to volume with methanol–water (40:60).

Determination of the tetryzoline hydrochloride content of the nasal solution

A 5.0-ml volume of Neocor-Tyzine nasal solution and 10.0 ml of the internal standard stock solution were pipetted into a 50 ml volumetric flask and diluted to volume with methanol–water (40:60). Aliquots of this solution were injected into the liquid chromatograph.

Precision

The precision was tested by subjecting portions of the appropriate sample preparation to the entire assay procedure and calculating the coefficient of variation (C.V.) of the results.

Accuracy

The accuracy was tested using synthetic samples. A placebo mixture was prepared by combining all ingredients except I. Portions of the placebo were taken and an accurately weighed amount of I, approximately equivalent to the label claim was added. The samples were analysed and the recoveries of I were calculated.

RESULTS AND DISCUSSION

The development of a stability-indicating determination for I in Neocor-Tyzine nasal solution was complicated as this dosage form also contains prednisolone, preservatives (propyl- and methylparaben) and neomycin sulphate. The last compound did not interfere as it cannot be detected spectrophotometrically at 220 nm; it can be determined using a microbiological technique.

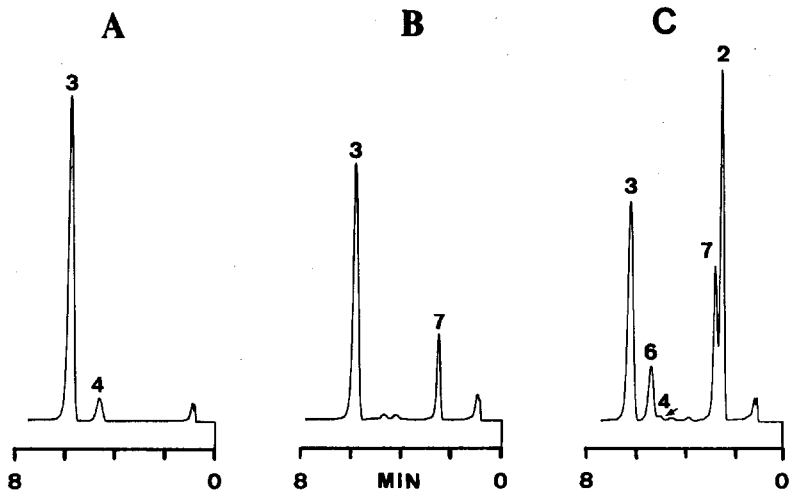

Fig. 1. Separation of tetryzoline hydrochloride and accompanying compounds after partial decomposition with 0.1 *M* sodium hydroxide solution. (A) Blank nasal solution; (B) blank nasal solution partially decomposed; (C) commercial nasal solution, partially decomposed. Mobile phase, stationary phase and other chromatographic conditions as in text. Peaks: 1 = tolazoline hydrochloride (internal standard); 2 = tetryzoline hydrochloride (I); 3 = methyl *p*-hydroxybenzoate; 4 = prednisolone; 5 = propyl *p*-hydroxybenzoate; 6 = degradation product of tetryzoline hydrochloride (II); 7 = 4-hydroxybenzoic acid.

Initial work was carried out with a methanol–water (40:60) mobile phase containing 20 m*M* SOS and 20 m*M* DMOA, adjusted to pH 3.0. The chromatogram obtained after appropriate sample preparation showed satisfactory resolution of all (active) ingredients. However, stability experiments revealed interference of I by 4-

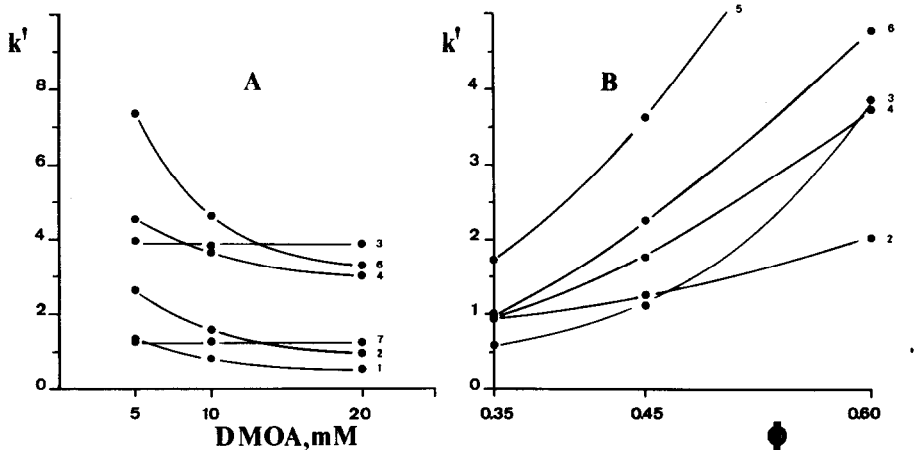

Fig. 2. Change in retention of tetryzoline hydrochloride and related compounds as a function of (A) the DMOA concentration and (B) the volume fraction of water (Φ) in the eluent. Mobile phase: (A) methanol–water (40:60), containing 20 m*M* SOS (pH 3.0); (B) methanol–water containing 20 m*M* SOS and 10 m*M* DMOA (pH 3.0). Stationary phase and other chromatographic conditions as in text. Peaks as in Fig. 1.

hydroxybenzoic acid, the hydrolysis product of the preservatives (Fig. 1). The degradation product of I, N-(2-aminoethyl)-1,2,3,4-tetrahydro-1-naphthylamide (II), as identified previously[4], was completely resolved from the parent compound.

The selectivity of the separation is strongly affected by the DMOA content of the eluent through an increase in the capacity of the basic compounds (I, II and the internal standard) and of prednisolone with decreasing DMOA concentration (Fig. 2A). A baseline separation of I and the internal standard from all possible interferents could be obtained using a mobile phase containing 20 m*M* SOS and 10 m*M* DMOA in methanol–water (40:60) (pH 3.0). This eluent composition was used for the quantitative analysis (Fig. 3).

The eluent system can be adapted for the simultaneous stability-indicating determination of I, prednisolone and methylparaben by further decreasing the DMOA content of the eluent (Fig. 2A). However, this possibility was not studied further as the internal standard coelutes with 4-hydroxybenzoic acid. Attempts were also made to reduce the retention of propylparaben by decreasing the water content of the eluent. From Fig. 2B, it can be seen that the use of such eluents results in either coelution of I and its degradation product II, or in unsatisfactory separations between I and methylparaben.

The SOS concentration of the eluent was not altered throughout the experiments. SOS is present in the eluent in order to provide sufficient capacity and resolution of the basic compounds through ion-pair formation at relatively high DMOA concentrations. The latter ensures elution of such compounds as sharp, symmetrical

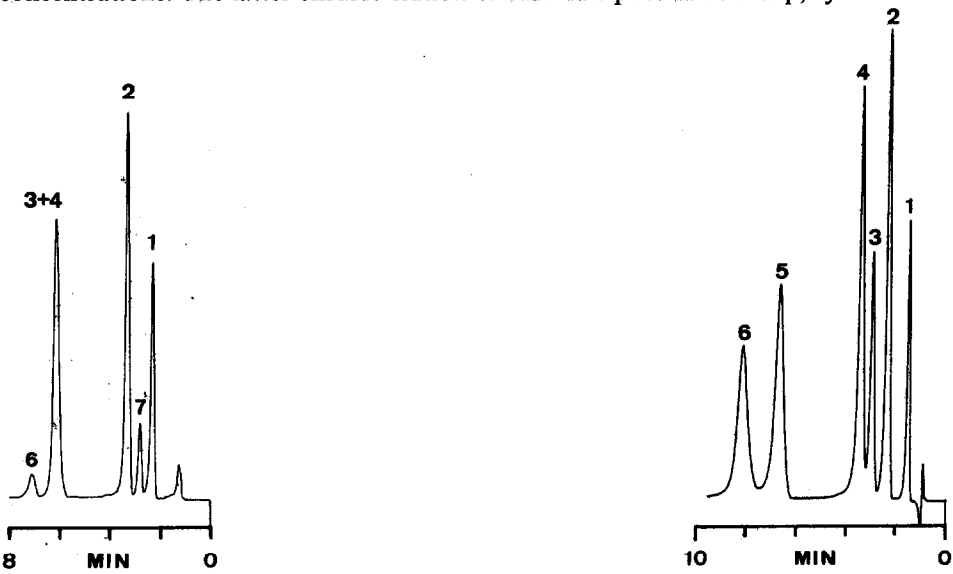

Fig. 3. Representative chromatogram for the HPLC analysis after accelerated (incomplete) degradation and appropriate sample preparation of the nasal solution. Mobile phase, stationary phase and chromatographic conditions as in text. Peaks as in Fig. 1.

Fig. 4. Separation of a standard mixture of 2-imidazolines, sulphonamides and local anaesthetics. Mobile phase: methanol–water (20:80) containing 20 m*M* SOS and 10 m*M* DMOA (pH 3.0). Stationary phase and chromatographic conditions as in text. Peaks: 1 = sulphanilamide; 2 = sulphadimidine; 3 = sulphathiazole; 4 = tolazoline hydrochloride; 5 = tetryzoline hydrochloride; 6 = lidocaine hydrochloride.

peaks but also reduces the resolution owing to a strong decrease in capacity. Neither eluent parameter altered the elution of neutral and acidic solutes. Hence selectivity changes can easily be obtained. Additional small changes in the water content can be made to improve further the resolution between the samples of interest.

Careful optimization of these three eluent parameters, *i.e.*, DMOA concentration, SOS concentration and water content, permits the separation of numerous classes of compounds, such as quaternary ammonium drugs[5], benzodiazepines, butyrophenones, corticosteroids, sulphonamides and local anaesthetics.

2-Imidazoline drugs are frequently administered in combination with local anaesthetics and sulphonamides. The simultaneous determination of the former compounds and 2-imidazolines is possible using similar appropriate eluent conditions to those described earlier in this paper. However, using all of the eluent (and chromatographic) conditions described so far, the sulphonamides are not retained on the column ($k' < 1$). To move these compounds away from the dead volume, the mobile

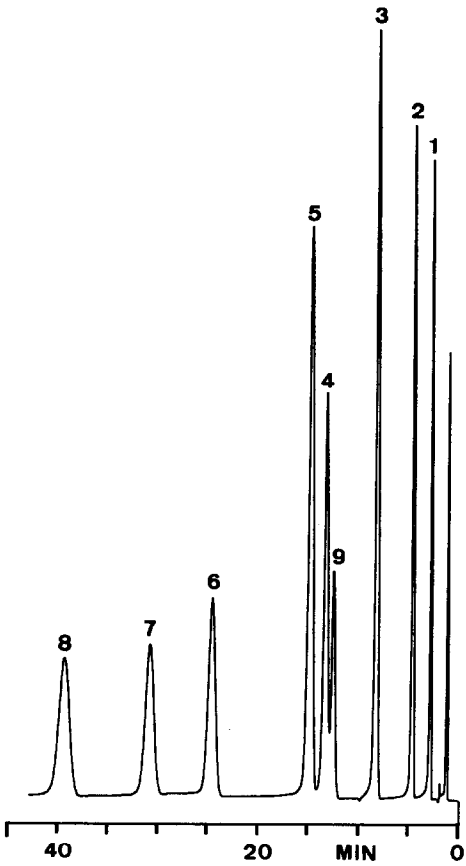

Fig. 5. Reversed-phase HPLC separation of some commercially available 2-imidazoline drugs as their octanesulphonic acid ion pairs. Mobile phase, stationary phase and chromatographic conditions as in text. Peaks: 1 = tolazoline; 2 = tetryzoline; 3 = naphazoline; 4 = tramazoline; 5 = coumazoline; 6 = antazoline; 7 = oxymetazoline; 8 = xylometazoline; 9 = tenaphtoxaline.

phase should contain not less than 80% of water. Under such conditions, the simultaneous determination of 2-imidazoline drugs, local anaesthetics and sulphonamides becomes possible. An example of such a separation is given in Fig. 4.

The main aim of this paper was to provide useful methodology for checking the content and purity of tetryzoline hydrochloride-containing dosage forms. It is convenient, however, if the chromatographic analysis simultaneously allows the specific identification of I. The selectivity towards related commercially available 2-imidazoline drugs is illustrated in Fig. 5. This chromatogram clearly demonstrates the separating power of the proposed reversed-phase ion-pair chromatographic approach, allowing the specific identification of any of the 2-imidazolines without the need for an additional thin-layer chromatographic system.

Quantitative analysis

Calibration graphs are constructed by plotting the peak-area ratios of I to the internal standard *versus* the weights of I in the standard solutions and analysed by least-squares regression.

A linear relationship with a correlation coefficient of $r = 0.9996$ was obtained over the concentration range studied (75–125% of the label claim). Standard addition–recovery experiments performed on placebo mixtures showed a mean recovery of 103.3% ($n = 3$, C.V. = 0.84%). Seven replicate determinations of I in the nasal solution, with a declared potency of 1 mg/ml, gave a mean recovery of 101.1% with a relative standard deviation of 1.64%. Fairly good reproducibility and sufficient precision were obtained.

In order to avoid system peaks, causing the peak-area integration to be irreproducible, it should be stressed that all injections must be made in solvent of the same strength (methanol:water ratio) as that of the mobile phase. Under such conditions, the mobile phase, despite its complexity, is well suited for quantitative work, as can be seen from the results presented here.

CONCLUSION

This work demonstrates the utility of the reversed-phase ion-pair liquid chromatographic separation technique, based on the combined effects of amines and alkylsulphonates, as a versatile analytical tool for practical applications. It appears that the eluent used here yields superior resolution and peak shape to those in previous reports in which 2-imidazoline drugs have been separated and quantitated.

The assay method for tetryzoline hydrochloride described here is specific, selective, simple and reliable and shows good reproducibility (C.V. 1–2%). Adaptation of the method to the determination of numerous commercially available 2-imidazoline drugs should be possible.

REFERENCES

1 J. Bauer and S. Krogh, *J. Pharm. Sci.*, 72 (1983) 1347.
2 G. Andermann and A. Richard, *J. Chromatogr.*, 298 (1984) 189.
3 J. A. De Schutter, W. Van den Bossche and P. De Moerloose, *J. Pharm. Biomed. Anal.*, submitted for publication.
4 G. Andermann, A. Richard and A. Tritz, *J. Pharm. Belg.*, 37 (1982) 309.
5 J. A. De Schutter, W. Van den Bossche and P. De Moerloose, *J. Chromatogr.*, 366 (1986) 321.