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DETERMINATION OF CROSS-CONTAMINATION OF THE DIASTEREOMERS EPHEDRINE AND PSEUDOEPHEDRINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, THIN-LAYER CHROMATOGRAPHY AND CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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SUMMARY

A high-performance liquid chromatographic method for separation of the diastereomers ephedrine and pseudoephedrine is described. The method can be used to determine diastereomeric cross-contamination at the 1% level and to detect 0.1% of the contaminant. It employs a phenyl reversed-phase column, mobile phase consisting of 1% acetonitrile in aqueous phosphate buffer and ultraviolet detection at 210 nm.

A thin-layer chromatographic separation, using reversed-phase separation plates and borate buffer, was also developed. Carbon-13 nuclear magnetic resonance spectroscopy was also examined as a method for determining diastereomeric cross-contamination. The three methods are compared for sensitivity and selectivity.

INTRODUCTION

Ephedrine and pseudoephedrine are diastereomers that are resistant to simple chromatographic separation. Because of their therapeutic importance as adrenergic agents, their analysis has received considerable attention. Many of the published chromatographic methods for ephedrine and pseudoephedrine separate these compounds from other drugs[1–5] but not from each other. Although ephedrine and pseudoephedrine have been separated after derivatization[6,7], some of the reported separations[8,9] are not practical as routine analytical techniques. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has been used in this laboratory[10] to determine ephedrine in pseudoephedrine after acetylation, but the method is not especially sensitive. The optical rotatory dispersion (ORD) curve of ephedrine passes through zero, but the ORD curve of pseudoephedrine does not; an assay has been reported[11] that takes advantage of this phenomenon. However, racemic samples cannot be analyzed and any racemic pseudoephedrine present would be quantitated as ephedrine. Because of these analytical difficulties, the United States Pharma-

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copeia[12] does not contain a limits test for either ephedrine in pseudoephedrine or *vice versa*.

Commercial formulations labeled to contain either of the diastereomers have a varying risk of cross-contamination, depending on the actual source of the labeled material. (−)-Ephedrine occurs naturally in certain plants of the *Ma Huang* species[13]; the racemic compound is synthesized and resolved[13]. A synthesis has been reported[14] to yield as much as 20% pseudoephedrine, which is then removed by recrystallization as various salts. (+)-Pseudoephedrine is prepared[13] from (−)-ephedrine by the Welsh rearrangement[15] of N-acetylephedrine. Because of the intimate relationship between the two compounds, there may be ample opportunity for low-level cross-contamination.

We report here a simple, rapid high-performance liquid chromatographic (HPLC) method for the complete separation and determination of ephedrine in pseudoephedrine or *vice versa*. We also report a thin-layer chromatographic (TLC) method for separation of the diastereomers and compare these methods to analysis by ^{13}C NMR.

EXPERIMENTAL

High-performance liquid chromatography

The determinations were performed with a Spectra-Physics Model 8000 high-performance liquid chromatograph equipped with an SP 8000 data system, a Spectra-Physics Model 770 UV-visible detector set at 210 nm and a temperature-controlled column compartment. A stainless-steel μBondapak phenyl (particle size 10 μm) (30 cm × 3.9 mm I.D.) column was used. The injector was equipped with a 10-μl sampling loop. The mobile phase was 1% acetonitrile in 0.05 *M* monobasic sodium phosphate which had been recirculated continuously over the column overnight. A flow-rate of 1 ml/min and a column temperature of 22°C were maintained throughout the analysis.

Ephedrine sulfate and pseudoephedrine sulfate were purchased from the United States Pharmacopeia (USP). All solvents (including water) were of suitable grade for HPLC and all solutions were filtered through micropore filters and then degassed before use.

Standard solutions of ephedrine and pseudoephedrine were prepared in water, either separately or in mixtures, over a concentration range of 0.5–500 μg/ml. The standard solutions (10 μl) were chromatographed and calibration curves were obtained by using the data system's peak area measurements.

Single dosage units (tablets or capsules) were dissolved individually in water in small volumetric flasks with the aid of an ultrasonic bath and diluted to volume with water. Samples were filtered and diluted quantitatively to a final concentration of 0.5 mg/ml. The sample solution (10 μl) was chromatographed and peak areas were used for quantitative analysis.

Thin-layer chromatography

Standards used were USP Reference Standards. Ephedrine hydrochloride powder and pseudoephedrine sulfate powder were used (as secondary standards) to prepare the mixed test solutions. Precoated, prescored reversed-phase separation

(RPS) plates (20 × 20 cm, layer thickness 0.25 mm) were purchased from Analtech (Newark, DE, U.S.A.).

Test solutions were prepared to simulate ephedrine (10 mg/ml) cross-contaminated with 1, 5 and 10% pseudoephedrine by dissolving suitable amounts of ephedrine hydrochloride and pseudoephedrine sulfate in methanol–chloroform (1:1). The test solutions (10 μl) were applied to the plate with disposable micropipets as 1-cm streaks about 2 cm from the bottom of the plate. Standard solutions (1 mg/ml) were prepared in methanol–chloroform (1:1) and were applied (10 μl) as spots. Commercial sample solutions (10 mg/ml) were also prepared in the same solvent. An ultrasonic bath was used to facilitate disintegration of the tablet or capsule material. The solution was filtered and 10 μl were applied to the plate as a 1-cm streak about 2 cm from the bottom of the plate.

The developing solvent consisted of 10% acetonitrile, 2% boric acid and 0.5% acetic acid in methanol. Plates were developed at room temperature in a pre-saturated (0.5 h) chamber lined with filter-paper. The solvent was allowed to develop for 15 cm from the spotting line. The plates were removed, air-dried and sprayed with ninhydrin reagent (0.3% in *n*-butanol with 3 ml acetic acid added). Plates were then heated in a 110°C oven for about 15 min to bring out the color.

Carbon-13 nuclear magnetic resonance

^{13}C NMR analyses were performed with a Varian Associates XL-200 Fourier transform NMR spectrometer equipped with a 10-mm broadband probe and deuterium lock.

About 500 mg of the free base (ephedrine, pseudoephedrine or mixtures) were dissolved in 3 ml deuterochloroform in a 10-mm NMR sample tube. The instrumental conditions were: pulse width 5 μsec (about 30°) acquisition time 0.636 sec and delay 0.864 sec (for a total acquisition cycle of 1.5 sec); spectrum width 12,578 Hz (250 ppm) and 3600 transients. Various standard mixtures of the diastereomers were prepared and ^{13}C NMR spectra were determined.

RESULTS AND DISCUSSION

Baseline separations of ephedrine and pseudoephedrine (down to 5% of the minor component) were obtained with the HPLC system reported here. A representative chromatogram is shown in Fig. 1. Linearity curves (concentrations from 0.5 to 0.0005 mg/ml in water) were prepared for each component (Fig. 2). Standard mixtures of ephedrine and pseudoephedrine were prepared and analyzed; concentrations of one component in the other ranged from 1 to 99% (Fig. 3). Chromatograms of mixtures containing 1% of the contaminant are shown in Fig. 4. Other measurements showed that less than 1% could be detected; however, calculation of diastereomers in amounts less than 0.5% were considered to be estimations only. The limit of detection was 0.1%.

Before the determinations, a preliminary system suitability test should be performed. The retention times should be about 16.4 min for ephedrine sulfate and about 19.8 min for pseudoephedrine sulfate. A complete determination will take about 25 min. The resolution factor[16], *R*, is about 2.6 for equal amounts (25 μg/ml) of a standard mixture of ephedrine and pseudoephedrine. Minor adjustments in the aceto-

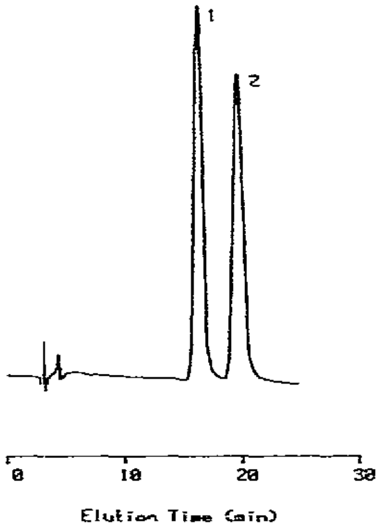

Fig. 1. HPLC separation of ephedrine (1) and pseudoephedrine (2). Column: μBondapak phenyl (10 μm), 30 cm × 3.9 mm I.D. Mobile phase: 1% acetonitrile in 0.05 M monobasic sodium phosphate. Flow-rate: 1 ml/min. Injection volume: 10 μl. Detector wavelength: 210 nm.

nitrile concentration and flow-rate may be necessary to attain the optimum resolution factor and retention times.

Because of the sensitivity of the method, extreme care must be exercised in cleaning the injection loop and port between injections of standards and samples, since cross-contamination can be introduced at this point.

Several dosage forms were assayed by using the HPLC method reported here. Single capsule analysis of pseudoephedrine hydrochloride capsules from two manu-

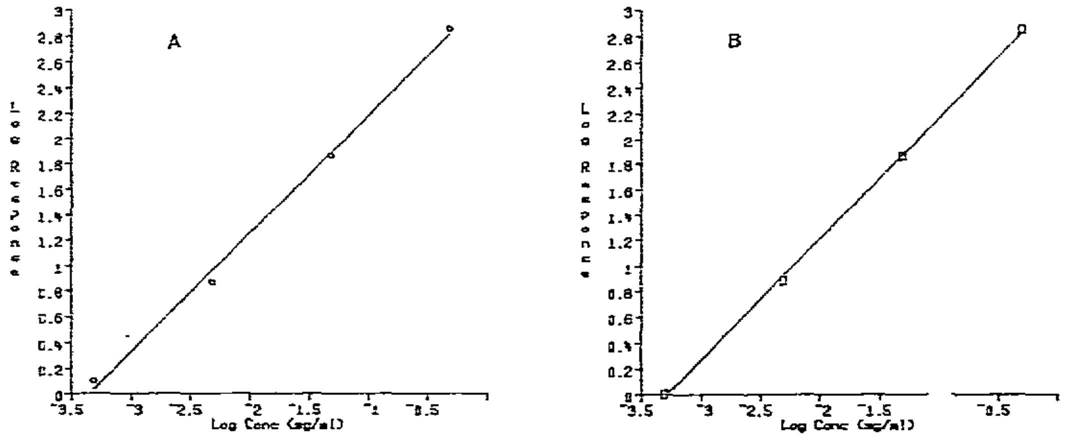

Fig. 2. Linearity curves (log concentration *vs.* log peak response) for ephedrine (A) and pseudoephedrine (B). Concentrations range from 0.5 to 0.0005 mg/ml in water.

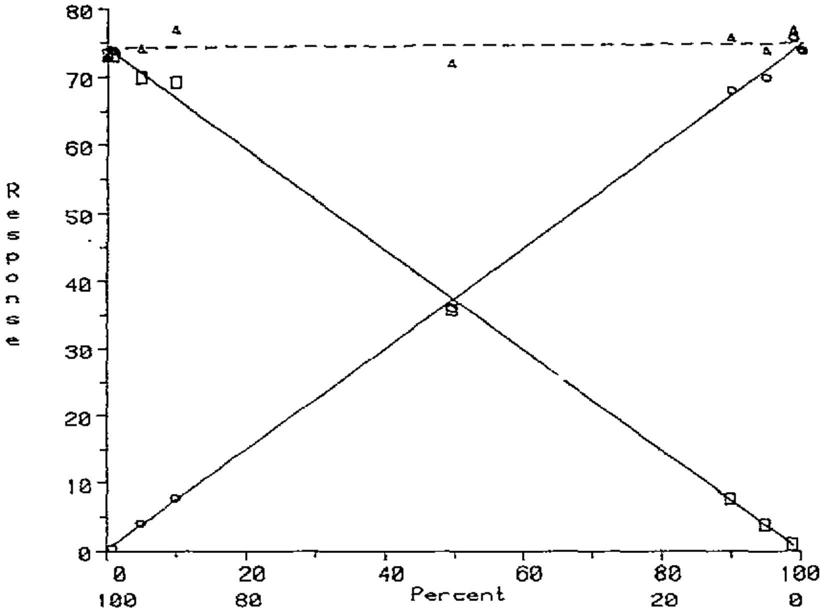

Fig. 3. Plot of peak area response *vs.* composition (in percent) of ephedrine (○), pseudoephedrine (□) and total alkaloid (△).

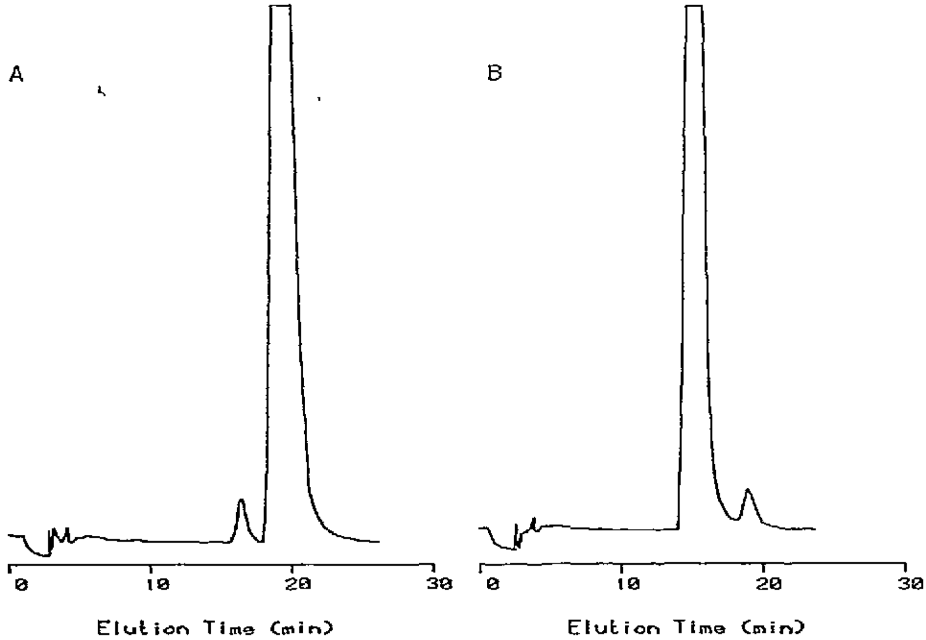

Fig. 4. HPLC chromatograms of 1% cross-contaminated test solutions: A, 1% ephedrine in pseudoephedrine; B, 1% pseudoephedrine in ephedrine.

facturers was performed. One capsule contained 92.7% of label claim and the analysis showed that a small amount of ephedrine was present (about 0.2%). The other capsule contained 102.4% of label claim and the sample had no trace of ephedrine. Ephedrine sulfate syrup was analyzed without any cleanup; the chromatogram is shown in Fig. 5. Despite the potentially interfering presence of sugars, dyes, alcohol, etc., the peak corresponding in retention time to ephedrine sulfate showed that the syrup contained 105.2% of label claim. An ephedrine sulfate capsule contained 92.4% of label claim and no pseudoephedrine was found.

It is important to assay the sample at two different sensitivity levels. The first determination should be performed at the most sensitive attenuation position; this will establish whether or not a small amount of the other diastereomer is present. If there is no indication of the presence of the other diastereomer, the lower (least sensitive) attenuation setting can be used for the remaining sample injections. If the other diastereomer is present, standard mixtures may be prepared containing 1–5% of the contaminant diastereomer in the diastereomer of interest.

The wavelength 210 nm was selected because the extinction coefficients are 40 times greater than the extinction coefficients for the maxima at higher wavelengths, with corresponding increase in sensitivity.

Various HPLC columns and solvent systems were evaluated. A silica column, Zorbax Sil (DuPont), with a normal-phase solvent system successfully separated ephedrine and pseudoephedrine; however, the peak due to pseudoephedrine was unacceptably broad and flat and was therefore unamenable to quantitation. There

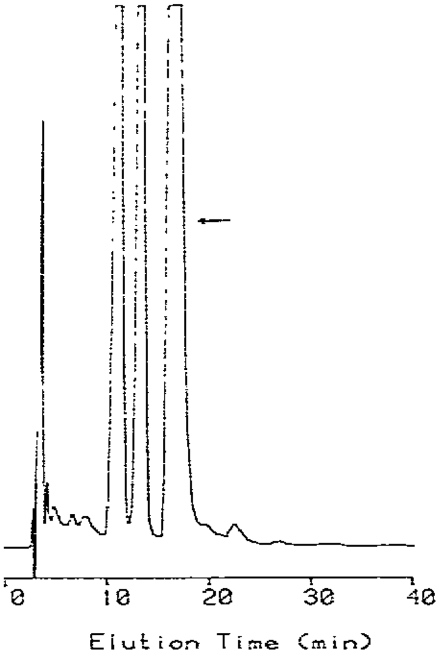

Fig. 5. HPLC chromatogram of an ephedrine sulfate syrup sample diluted with water. The peak corresponding in retention time to ephedrine sulfate is indicated with an arrow.

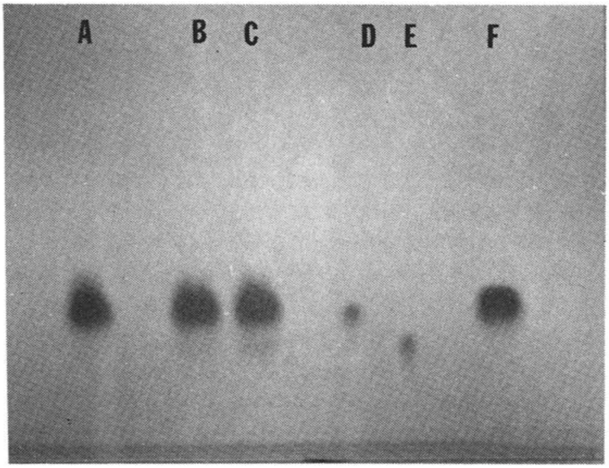

Fig. 6. Photograph of TLC plate: A = 1% pseudoephedrine in ephedrine; B = 5% pseudoephedrine in ephedrine; C = 10% pseudoephedrine in ephedrine; D = ephedrine; E = pseudoephedrine; F = ephedrine sulfate capsule.

was only minimal or no separation of the diastereomers on C_8 columns (E. Merck or DuPont), on Zorbax TMS (DuPont), on μBondapak CN (Waters Assoc.) or on LiChrosorb C_{18} (Altech).

The column of choice was μBondapak phenyl, 10 μm; in fact, separation of ephedrine from pseudoephedrine was observed with three different μBondapak phenyl columns. A 5-μm Spherisorb phenyl column also gave adequate separation of the two diastereomers with 2% acetonitrile. Similar separation was possible if the acetonitrile is replaced by 1% 2-methoxyethanol. In some cases, addition of 2-methoxyethanol sharpened the peaks and gave shorter retention times. A mixture of boric acid, sodium acetate and acetic acid in methanol (pH 4.5) gave the same separation as the phosphate mobile solvent. Other buffers at pH 4.5 gave reasonable separation; we conclude that the identity of the anion is not crucial. The use of phosphate does allow detection at the lower wavelength of 210 nm.

Both silica gel and reversed-phase TLC plates were evaluated for the separation of ephedrine and pseudoephedrine. Good separation was obtained on the reversed-phase plate with the borate system (Fig. 6). The TLC system was initially investigated, but the HPLC assay was found to be more sensitive. Mixtures containing 1, 5 and 10% of pseudoephedrine in ephedrine were prepared and chromatographed by TLC; 5% was the minimum that could be detected with this system.

^{13}C NMR was evaluated because there are usually differences in absorption of the diastereomeric carbons[17]. The proton-decoupled ^{13}C NMR spectrum of ephedrine had absorptions at δ 13.7, 33.7, 60.4, 73.7, 126.2, 126.9, 128.0 and 142.4 ppm; the spectrum of pseudoephedrine had absorptions at δ 15.2, 33.5, 61.1, 77.4, 127.0, 127.4, 128.1 and 142.8 ppm with tetramethylsilane as the reference. A problem encountered with the use of ^{13}C NMR as an analytical method was very low sensitivity, which required either very large samples or very long times, in the latter option, for example, more than 8 h per assay. However, the most serious drawback was that 4% of the minor diastereomer was the minimum that could be detected.

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[*] *Editor's Note:* see also R. Gill, S. P. Alexander and A.C. Moffat, *J. Chromatogr.*, 218 (1981) 639.