Journal of Chromatography, 426 (1988) 406–411
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4086

Note

Sensitive high-performance liquid chromatographic determination of pseudoephedrine in plasma and urine

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(Received November 13th, 1987)

Pseudoephedrine has been clinically used as a nasal and bronchial decongestant for many years. Usual doses are 60–180 mg orally per day. Until 1969 the investigation of the pharmacokinetic behaviour of pseudoephedrine and other "ephedrines" (as ephedrine or norephedrine) was confined to data gained from the urinary excretion of these compounds. This was attributed to the lack of adequate sensitivity of the analytical methods to quantify the concentrations in plasma over a sufficient time period after dosage.

The first analytical method to provide the necessary sensitivity to measure plasma concentrations of pseudoephedrine after therapeutic dosages was reported by Cummins and Fourier [1] in 1969. These authors determined pseudoephedrine by gas chromatography (GC) after derivatization with heptafluorobutyric anhydride. Subsequently several modified GC methods were published [2–7]. These modifications were developed in order to improve the sensitivity or to substitute benzene by a less toxic solvent for the extraction procedure. A different approach was reported by Kuntzman et al. [8], who used a radiolabel technique for the quantification of pseudoephedrine. All these procedures except one [6], however, required a derivatization prior to analysis. Furthermore, the sample preparations were rather time-consuming since several extraction and/or washing steps had to be carried out. Finally, plasma concentrations of pseudoephedrine below 50 ng/ml could not routinely by quantified.

In recent years several high-performance liquid chromatographic (HPLC) methods have been reported for the determination of pseudoephedrine in urine [9] and in pharmaceutical preparations [10–14]. However, to our knowledge no HPLC assay method for plasma has yet been published.

0378-4347/88/$03.50

The literature data (e.g. ref. 7) reveal that an assay sensitivity of at least 20–30 ng/ml is necessary for the measurement of a 24-h pharmacokinetic profile after a single oral dose of pseudoephedrine. We describe here an HPLC procedure that is sensitive enough to quantify 10 ng/ml in a 1-ml plasma sample.

EXPERIMENTAL

Materials

(−)-Pseudoephedrine, free base (No. E-9626) and (±)α-(methylaminomethyl)benzyl alcohol (No. M-7762) were both from Sigma (Deisenhofen, F.R.G.). Hydrochloric acid (analytical grade), ammonia solution 25% (w/v) (Suprapur®) and acetonitrile (LiChrosolv®) were from Merck (Darmstadt, F.R.G.). Methanol (HPLC reagent) was purchased from Baker (Deventer, Netherlands) and heptane-1-sulphonic acid sodium salt (98% purity) from Aldrich (Steinheim, F.R.G.). Bond Elut® C_{18} extraction columns (1 ml, Part No. 607101) were from Analytichem International (Harbor City, CA, U.S.A.). Purified water was obtained by a Milli-Q Type I grade water purification system (Millipore, El Paso, TX, U.S.A.).

Apparatus and chromatographic conditions

The chromatographic system consisted of an M 6000 A pump, a WISP 710 B automatic sample injection system, a μBondapak C_{18} column (300 mm × 4.6 mm I.D.; 10 μm particle size) all from Waters Assoc. (Eschborn, F.R.G.) and a Spectroflow 773 variable-wavelength detector from Kratos (Karlsruhe, F.R.G.) set at 220 nm and 0.002 a.u.f.s. The chromatograms were recorded either with an HP 3390A integrator from Hewlett-Packard (Frankfurt, F.R.G.) or a TRIO chromatography computing integrator from Trivector Systems (Sandy, U.K.).

The mobile phase consisted of 0.03 *M* sodium heptanesulphonate (adjusted to pH 3.0 with 0.1 *M* hydrochloric acid)–acetonitrile (77:23 v/v). The flow-rate was set at 1.5 ml/min.

Preparation of samples

For conditioning the Bond Elut extraction columns, 5 ml of methanol, 5 ml of 0.3 *M* methanolic hydrochloric acid (concentrated hydrochloric acid diluted with methanol) and 10 ml of water were successively passed through within 10 min. A plasma sample (1 ml) was diluted with 1 ml of water in a polypropylene vial and mixed with 50 μl of a 10 μg/ml solution of α-(methylaminomethyl)benzyl alcohol (MAMBA) in dilute hydrochloric acid (1 mg of MAMBA was dissolved in 1 ml of 0.03 *M* hydrochloric acid of which the pH value had been adjusted to 3.0 with sodium hydroxide, and then diluted with water 1:100; this solution is stable for at least five days in a refrigerator). To this solution 30 μl of a 25% ammonia solution were added. The resulting mixture was briefly vortexed and then passed through a pre-conditioned Bond Elut extraction column within 2–3 min. The polypropylene vial was rinsed with 2 ml of a 60:40 (v/v) mixture of 0.03 *M* hydrochloric acid (pH 3.0) and acetonitrile, and the washing solution was also passed through the extraction column which was then dried under suction. The

analytes were then eluted with 300 μl of 0.1 *M* methanolic hydrochloric acid. The eluate was evaporated to dryness under a gentle stream of nitrogen at 40°C. Finally the residue was reconstituted in 100 μl of water, of which 20–40 μl were injected into the HPLC system.

Urine samples were diluted with water either 1:10 (pre-dosing samples) or 1:200 to 1:500, and then processed as described for plasma samples.

Preparation of calibration curves

A stock solution of 1 mg/ml pseudoephedrine in 0.03 *M* hydrochloric acid (pH 3.0) was diluted with water to give working standard solutions with 10, 1 and 0.1 μg/ml pseudoephedrine, respectively. These solutions are stable for at least five days in the refrigerator. Appropriate volumes of working standard solutions were adjusted to 1 ml with pooled human plasma to obtain standards with 10–500 ng/ml pseudoephedrine. For analyses of urine samples the standards were prepared by adjusting appropriate volumes of working standard solutions to 1 ml with water. The standards were worked up according to the procedure described above and then chromatographed. Calibration lines were constructed by linear regression analysis of peak-height ratios of pseudoephedrine to internal standard versus pseudoephedrine concentration units. These calibration lines were prepared daily.

RESULTS AND DISCUSSION

Two aims governed our decision to develop a new pseudoephedrine assay method. Firstly the assay should be sensitive enough to quantify at least 20 ng/ml pseudoephedrine in plasma and, secondly, the work-up procedure should be simpler than existing methods.

Fig. 1 shows chromatograms of a plasma blank and plasma standards containing 10 and 500 ng/ml pseudoephedrine, respectively. The chromatograms demonstrate that pseudoephedrine can be quantified down to 10 ng/ml. The sample preparation includes a single liquid–solid extraction procedure and does not require a derivatization of pseudoephedrine. Calibration lines were linear in the range 10–500 ng/ml with correlation coefficients of $r=0.9989$ to $r=0.9998$ for both plasma and urine. The accuracy and precision of the assay were determined by adding known amounts of pseudoephedrine to pooled human plasma. Samples containing concentrations of 20, 100 and 500 ng/ml were then analysed repeatedly. The coefficients of variation (C.V.) ranged from 1.8 to 8.0% and mean bias ranged from +0.5% to −2.0% (Table I). Mean recoveries were 85–88% for pseudoephedrine and 75% for MAMBA ($n=15$), calculated by the comparison of peak heights of spiked samples (worked up according to the described procedure) with peak heights after injection of standard solutions of equivalent concentrations. The limit of detection was estimated to be less than 5 ng/ml, based on a signal-to-noise ratio of 3:1.

Stability tests were performed with plasma samples spiked with 20, 100 and 500 ng/ml pseudoephedrine which were stored at −20°C. Under these conditions

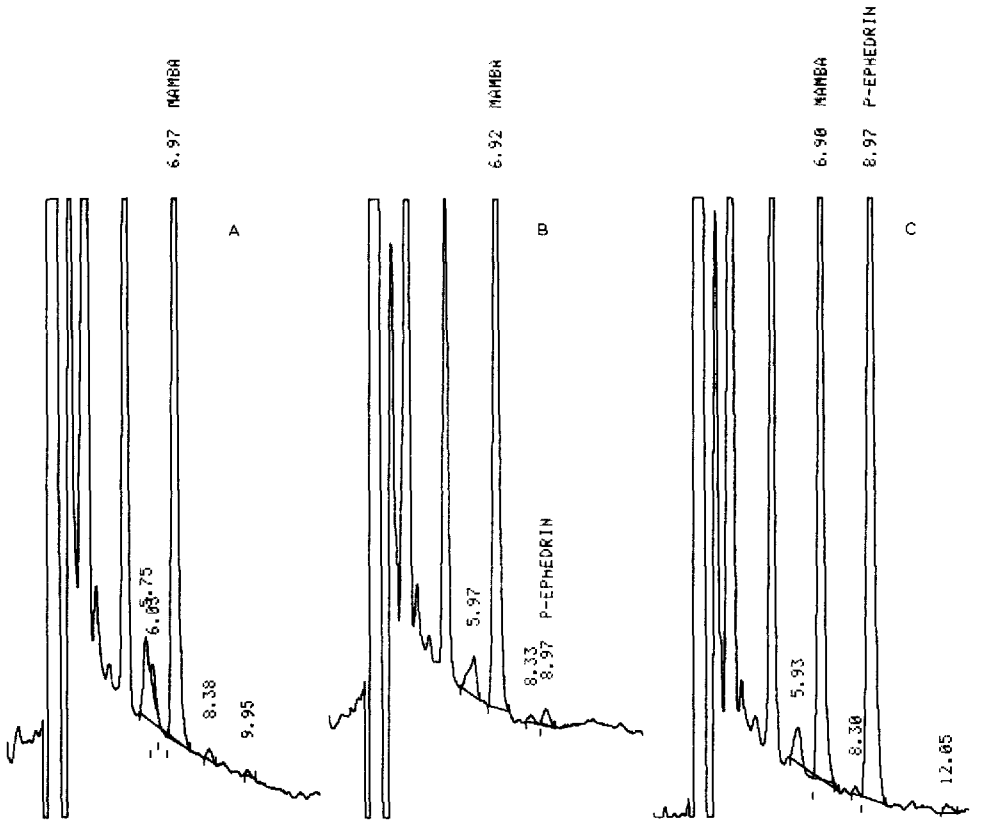

Fig. 1. Chromatograms of a plasma blank (A) and of plasma standards spiked with 10 ng/ml (B) and with 500 ng/ml (C). For chromatographic conditions see text. P-EPHEDRIN = pseudoephedrine.

pseudoephedrine proved to be stable for at least six months. These findings are consistent with those reported earlier [3].

The need to set the detector wavelength at 220 nm in order to achieve a sufficient sensitivity for the assay led to extensive investigations to eliminate interfering peaks. The best results were obtained by optimizing two pivotal steps during

TABLE I

ACCURACY, PRECISION AND MEAN RECOVERY FOR PSEUDOEPHEDRINE IN PLASMA ($n=5$)

Plasma concentration of pseudoephedrine (ng/ml)	Mean concentration found (ng/ml)	Coefficient of variation (%)	Mean bias (%)	Mean recovery (%)
20	20.1	8.0	+0.5	88
100	98.9	7.4	−1.1	85
500	490	1.8	−2.0	85

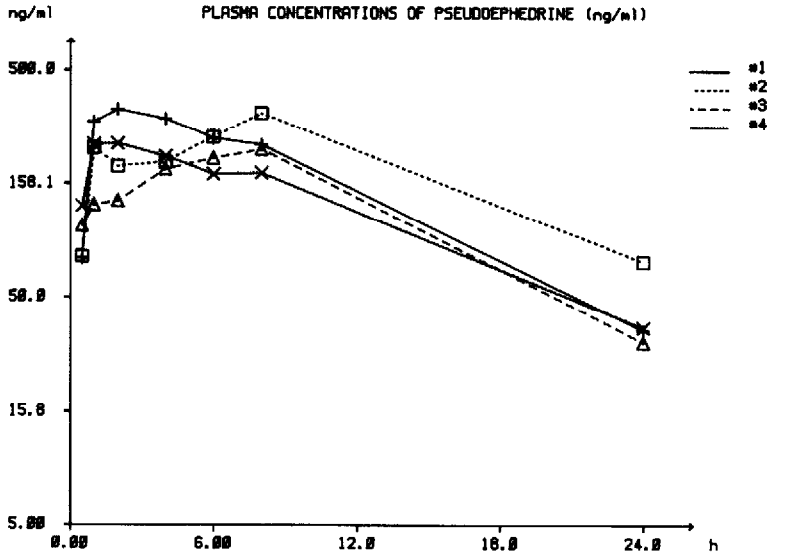

Fig. 2. Plasma concentration-time curves of pseudoephedrine in four healthy subjects after oral administration of a slow-release formulation containing 120 mg of pseudoephedrine.

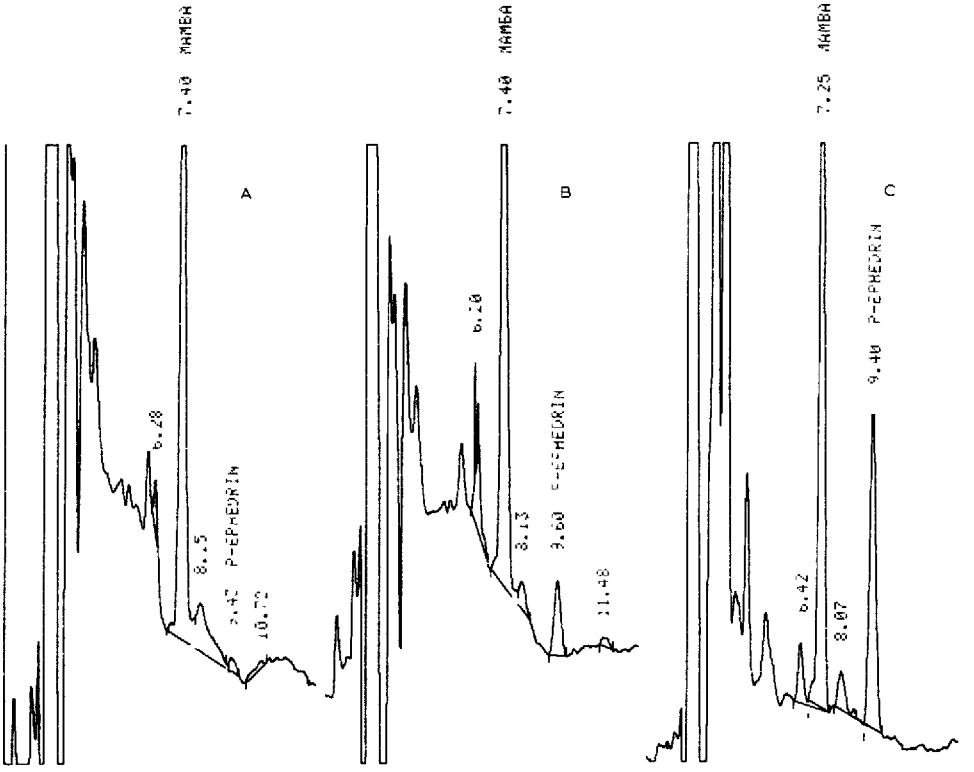

Fig. 3. Chromatograms of plasma and urine samples from subject No. 1. For chromatographic conditions see text. (A) Plasma pre-dose; (B) plasma 24 h after dosing, pseudoephedrine (P-EPHEDRIN) corresponds to 35 ng/ml; (C) urine 0–8 h, diluted 1:200 prior to analysis, pseudoephedrine corresponds to 174 ng/ml in the diluted sample.

the work-up procedure. A large interfering peak with a retention time similar to the MAMBA peak could be eliminated by an additional purging of the Bond Elut extraction columns with 0.3 *M* methanolic hydrochloric acid during the conditioning step. Further impurities were removed by washing the columns with a mixture of 0.03 *M* hydrochloric acid (pH 3.0) and acetonitrile (60:40, v/v) after absorption of the analytes. When the pH value of the hydrochloric acid was lowered to 2.0, distinctly lower recoveries for both pseudoephedrine and MAMBA were observed, whereas a pH value of 4.0 led to incomplete removal of interfering peaks.

This assay was used for a pharmacokinetic study in human volunteers receiving 120 mg of pseudoephedrine in a slow-release formulation. Fig. 2 shows the plasma concentration–time curves of pseudoephedrine in four subjects. The plasma concentrations at 24 h after dosage ranged from 32 to 72 ng/ml. Representative chromatograms from this study are presented in Fig. 3. Some of the chromatograms from pre-dose plasma samples revealed a small interference peak occurring at a slightly shorter retention time than the pseudoephedrine peak (Fig. 3A). Therefore pseudoephedrine plasma concentrations below 10 ng/ml could not routinely be quantified, whereas the absence of pseudoephedrine in pre-dose plasma samples could easily be distinguished. Provided the pharmacokinetics of pseudoephedrine is linear, this assay method is sensitive enough to quantify 24-h plasma concentrations even after single oral doses of 60 mg of pseudoephedrine. The assay is fast and permits the analysis of at least 40 samples plus standards during a normal working day.

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