# Rapid determination of pseudoephedrine in human plasma by high-performance liquid chromatography

J. Macek*, P. Ptáček, J. Klíma

*Pharmakl spol. s.r.o., Laboratory and Clinical Facility, U Vojenské Nemocnice 1200, CZ-16200 Prague 6, Czech Republic*

## Abstract

A rapid high-performance liquid chromatographic method for the quantitation of pseudoephedrine in human plasma is presented. The sample preparation involved liquid–liquid extraction of pseudoephedrine from alkalised plasma with hexane–isoamylalcohol (9:1, v/v) and back-extraction of the drug to 0.02 $M$ hydrochloric acid. Liquid chromatography was performed on an octadecylsilica column (50×4 mm, 5 μm particles); the mobile phase consisted of acetonitrile–phosphate buffer containing 0.1% of triethylamine, pH 2.4 (5:95, v/v). The run time was 4 min. The spectrophotometric detector was operated at 195 nm. Codeine was used as the internal standard. The limit of quantitation was 5.8 ng/ml using 0.5 ml of plasma. Within-day and between-day precision expressed by relative standard deviation was less than 7% and inaccuracy did not exceed 8%. The assay was applied to the analysis of samples from a pharmacokinetic study. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Pseudoephedrine is an effective sympathomimetic agent for use as a nasal decongestant with peripheral effects similar to epinephrine and central effects similar to, but less intense than, amphetamines. At the recommended oral dosage, it has little or no pressor effect in normotensive adults. The drug produces fewer adverse effects than ephedrine, specifically, tachycardia, hypertension, and central nervous system stimulation [1].

Several gas chromatographic [2,3] and high-performance liquid chromatographic (HPLC) [4–7] methods have been described for determination of pseudoephedrine in plasma. The limit of quantitation (LOQ) of HPLC assays ranges between 10 and 50 ng/ml. Sample preparation is performed by liquid–liquid extraction [4], solid-phase extraction [5] or column switching [6,7]. Pseudoephedrine is detected by spectrophotometric detection at low wavelengths (205–220 nm).

The aim of this study was to develop a rapid HPLC method for pseudoephedrine determination in plasma with LOQ 5 ng/ml to enable pharmacokinetic studies after lower doses of pseudoephedrine.

*Corresponding author. Tel.: 420-2-2020-3673; Fax: +420-2-2431-9969.

*E-mail address:* pharmakl@mbox.vol.cz (J. Macek).

## 2. Experimental

### 2.1. Chemicals

Pseudoephedrine hydrochloride was obtained from Léčiva, Czech Republic. Acetonitrile (for liquid chromatography) was a Riedel–de Haën (Seelze, Germany) product. Potassium dihydrogenphosphate (analytical grade) and hexane (for spectroscopy, Uvasol) were manufactured by Merck (Darmstadt, Germany). All other reagents and chemicals were of analytical grade. Isoamylalcohol and triethylamine were Fluka products (Buchs, Switzerland). Sodium hydroxide, hydrochloric and *o*-phosphoric acids were purchased from Lachema (Brno, Czech Republic). Codeine dihydrogenphosphate sesquihydrate (internal standard) was obtained in the local pharmacy in the form of tablets (Codein Slovakofarma 15 mg).

### 2.2. Apparatus

All HPLC instruments were obtained from Thermo Separation Products (Riviera Beach, FL, USA). The system consisted of a membrane degasser, pump ConstaMetric 4100, automatic sample injector AS 3000, spectrophotometric detector UV2000 and datastation with PC1000 software, version 2.5. The separation was performed on a 50×4 mm I.D. column (Watrex, Prague, Czech Republic) filled with Nucleosil 120-5 $C_{18}$ stationary phase, particle size 5 μm.

The mobile phase consisted of acetonitrile–phosphate buffer containing 0.1% of triethylamine, pH of the buffer was adjusted to 2.4 with *o*-phosphoric acid (5:95, v/v). The flow-rate was 2 ml/min at 35°C. The spectrophotometric detector was operated at 195 nm and the time constant was set to 1 s.

### 2.3. Standards

Stock solutions of pseudoephedrine hydrochloride were made by dissolving approximately 15 mg in 25 ml of methanol (conversion factor to the free base 0.81907). Separate solutions were prepared for the calibration standards and quality control samples. Further solutions were obtained by serial dilutions of stock solutions with methanol. These solutions were added to drug-free plasma in volumes not exceeding 2% of the plasma volume.

One tablet of Codein Slovakofarma (containing 15 mg of codeine dihydrogenphosphate sesquihydrate) was dissolved in 5 ml of methanol and 335 μl of the supernatant was diluted with methanol to the final volume of 10 ml. This solution containing 100.5 ng/μl was used as the internal standard. All samples and standards were analyzed with the same solution of internal standard.

All solutions were stored at −18°C and protected from light.

### 2.4. Preparation of the sample

The samples were stored in the freezer at −18°C and allowed to thaw at room temperature before processing. Ten μl of the internal standard solution (1005 ng of codeine dihydrogenphosphate sesquihydrate) were added to 0.5 ml of plasma and the tube was briefly shaken. Fifty μl of 1 *M* NaOH was added and the tube was shaken again. Then the mixture was vortex-mixed with 4 ml of hexane–isoamylalcohol (9:1, v:v) for 2 min at 2000 rpm. The tube was centrifuged 3 min at 2600 *g* and the upper organic phase was transferred to another tube. One hundred μl of 0.02 *M* HCl was added and the tube was vortex-mixed again (2 min, 2000 rpm). The phases were separated by centrifugation for 3 min at 2600 *g* and approximately 80 μl of the lower aqueous phase was transferred to the polypropylene autosampler vial. Thirty μl were injected into the chromatographic system.

### 2.5. Calibration curves

The calibration curve was constructed in the range 5.86–509 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor $1/y^2$): the ratio of pseudoephedrine peak height to codeine peak height was plotted vs. the ratio of pseudoephedrine concentration to that of internal standard. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

## 3. Results and discussion

### 3.1. Chromatography

We aimed at optimizing sensitivity and speed of the chromatography and therefore column dimensions 50×4 mm were selected. The benefit of this column is not only the increased sensitivity due to small peak volume, but also the possibility to obtain relatively large values of the capacity factor (5–8) in a short time. The limited efficiency of the column was compensated by optimization of the mobile phase composition to obtain the desired selectivity. No ion-pair reagent was required to separate pseudoephedrine from endogeneous interferences and the separation system was thus simpler than in some previous methods [4,5]. At flow-rate 2 ml/min the retention time of pseudoephedrine and codeine (internal standard) was 1.3 and 2.0 min, respectively (Fig. 1) and the analysis was completed within 4 min. The typical column efficiency expressed as the number of theoretical plates was 1500 for both compounds.

In order to maximize the detector signal the detection wavelength was lowered to 195 nm. At this wavelength the response is doubled when compared with 205–210 nm. The content of acetonitrile in the mobile phase is only 5% and thus the absorbance of the mobile phase and baseline noise are low even at 195 nm.

The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks. The typical chromatogram of blank plasma is shown in Fig. 2 and the chromatogram of a plasma sample from a subject 24 h after administration of 60 mg of pseudoephedrine is shown in Fig. 3. The concentration of pseudoephedrine was 8.34 ng/ml.
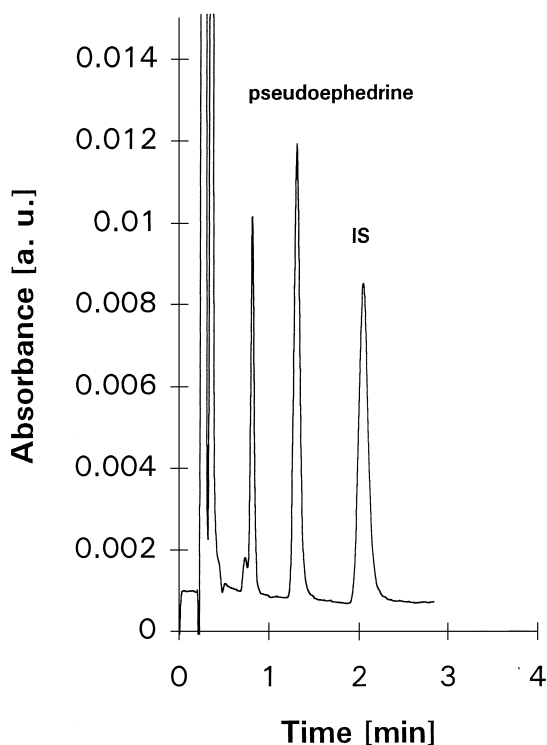


Fig. 1. Chromatogram of a mixture of pseudoephedrine and codeine (internal standard).
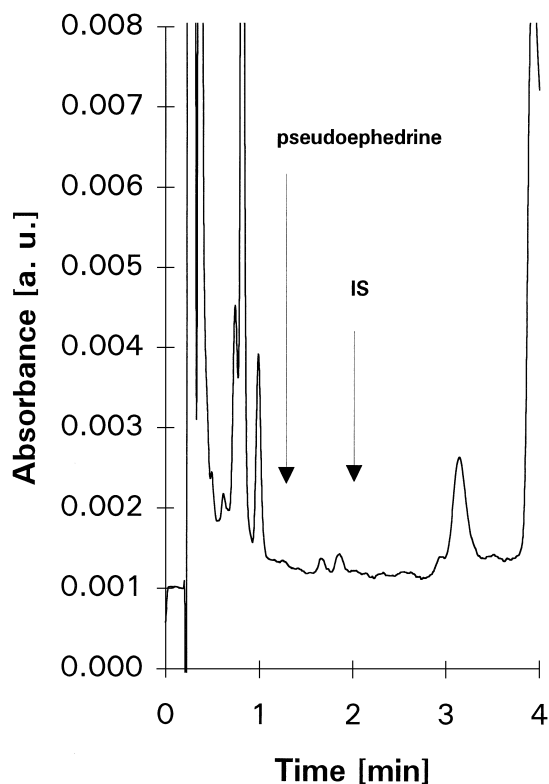


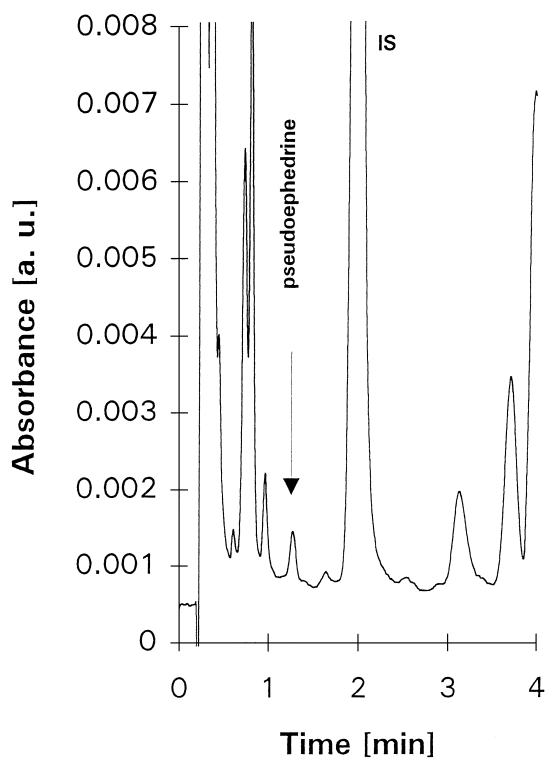Fig. 2. Typical chromatogram of drug-free human plasma.

Fig. 3. Chromatogram of a plasma sample from a volunteer 24 h after administration of 60 mg of pseudoephedrine. The measured concentration of pseudoephedrine was 8.34 ng/ml.

Table 1
Intra-assay precision and accuracy

| $n$ | Concentration (ng/ml) | | Bias (%) | RSD (%) |
|---|---|---|---|---|
| | Added | Measured | | |
| 6 | 11.66 | 12.28 | 5.0 | 6.2 |
| 6 | 52.58 | 52.59 | 0.0 | 1.3 |
| 6 | 421.5 | 414.9 | −1.6 | 1.4 |

the limit of quantitation could be further lowered, if desired.

### 3.2.1. Intra-assay precision

Intra-assay precision of the method is illustrated in Table 1. It was estimated by assaying the quality control samples (low, medium and high concen-

### 3.2. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y = bx + c$, where $y$ represents the pseudoephedrine to codeine peak height ratio and $x$ represents the ratio of pseudoephedrine concentration to that of internal standard. The mean equation (curve coefficients±standard deviation) of the calibration curve ($n=5$) obtained from six points was $y = 4.34(\pm 0.16)x + 0.00056(\pm 0.00147)$ (correlation coefficient $r = 0.9999$).

The limit of quantitation was 5.86 ng/ml ($n=6$). This level was selected with respect to expected concentrations of the samples from the pharmacokinetic study. The precision, characterised by the relative standard deviation, was 2.7% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was 0.8% at this concentration ($n=6$). These values suggest that
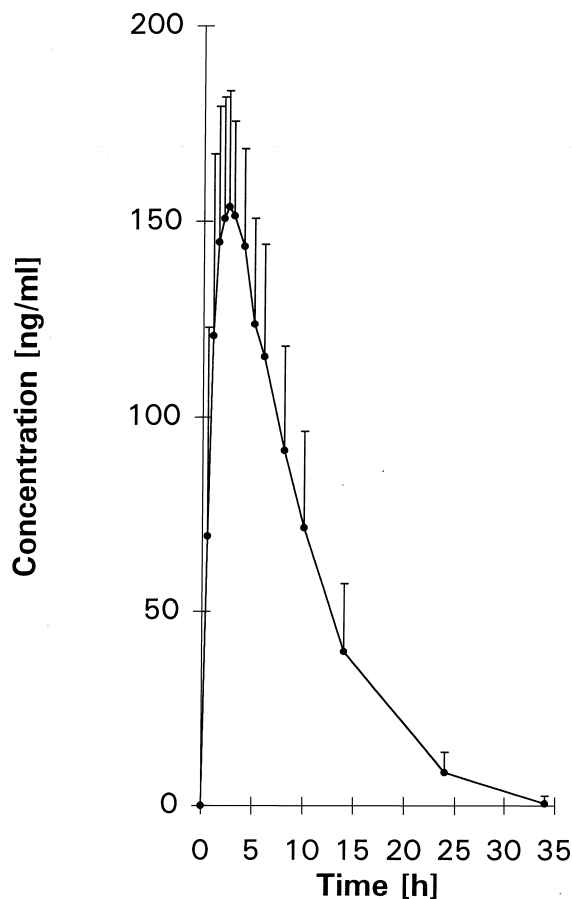


Fig. 4. Mean plasma concentrations (±SD) of pseudoephedrine after administration of 60 mg single oral dose (26 healthy volunteers).

Table 2
Inter-day precision and accuracy

| n | Concentration (ng/ml) | | Bias (%) | RSD (%) |
|---|---|---|---|---|
| | Added | Measured | | |
| 6 | 11.66 | 12.54 | 7.5 | 5.9 |
| 6 | 52.58 | 52.59 | −0.2 | 1.9 |
| 6 | 421.5 | 405.2 | −3.9 | 1.7 |

tration) six times in the same analytical run. The precision was better than 7% and the bias did not exceed 5% at all levels.

### 3.2.2. Inter-assay precision and accuracy

Inter-assay precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analysed twice, results averaged for statistical evaluation) on six separate runs. The samples were prepared in advance and stored at −18°C. The respective data are given in Table 2. The precision was at most 6% and the inaccuracy was better than 8% at all levels.

### 3.2.3. Stability study

#### 3.2.3.1. Freeze and thaw stability.
Stock solutions of a low and high concentration sample were prepared. The solutions were stored at −18°C and subjected

for three thaw–freeze cycles. During each cycle triplicate 0.5-ml aliquots were processed, analysed and the results averaged. The results are shown in Table 3. The concentrations found are well within the allowed limit ±15% of nominal concentration, indicating no significant substance loss during repeated thawing and freezing.

#### 3.2.3.2. Processed sample stability.
Two sets of samples with a low and a high concentration of pseudoephedrine were analysed and left in the autosampler at ambient temperature. The samples were analysed using a freshly prepared calibration samples 3 days later. The results are presented in Table 3. The processed samples are stable at room temperature for 3 days.

#### 3.2.3.3. Long term stability.
Two sets of samples (low and high concentration of pseudoephedrine) were stored in the freezer at −18°C for 5 weeks. The samples were then analysed using freshly prepared calibration samples. The results are within the acceptable ±15% limit of the nominal concentration (see Table 3). The samples are stable at −18°C for the studied period.

### 3.3. Application to biological samples

The proposed method was applied to the de-

Table 3
Stability of the samples

| Sample conc. [ng/ml] | | n | Freeze and thaw stability | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cycle 1 | | Cycle 2 | | Cycle 3 | |
| | | | Measured | Bias (%) | Measured | Bias (%) | Measured | Bias (%) |
| 19.75 | | 3 | 20.03 | 1.4 | 20.15 | 2.0 | 20.52 | 3.9 |
| 421.5 | | 3 | 386.1 | −8.4 | 376.6 | −10.7 | 407.2 | −3.4 |
| | | | Conc found [ng/ml] | RSD (%) | Bias (%) | Difference (%) | | |
| *Processed sample stability* | | | | | | | | |
| 11.66 | New | 6 | 12.28 | 6.2 | – | – | | |
| | 3 days old | 6 | 11.43 | 5.4 | – | −6.9 | | |
| 421.5 | New | 6 | 414.9 | 1.4 | – | – | | |
| | 3 days old | 6 | 432.0 | 3.4 | – | 4.1 | | |
| *Long-term stability* | | | | | | | | |
| 19.75 | | 6 | 18.66 | – | −5.5 | – | | |
| 421.5 | | 6 | 398.0 | – | −5.6 | – | | |

termination of pseudoephedrine in plasma samples from a bioequivalence study. The plasma samples were periodically collected up to 34 h after oral administration of a 60-mg single dose to 26 healthy male volunteers. Fig. 4 shows the mean plasma concentrations of pseudoephedrine. The plasma level of pseudoephedrine reached its maximum 2.5 h after the administration and thereafter the plasma level declined with an elimination half-time of ca. 5 h. These values agree with previously published reports [1]. The extrapolated fraction of the AUC from 0 to infinity accounted only for 5% which indicates a suitability of the analytical method for pharmacokinetic studies.

## 4. Conclusions

The validated method allows determination of pseudoephedrine in the 5.9–510 ng/ml range. The assay is rapid, the analysis time is only 4 min. About 200 samples can be prepared and analysed in one working day. The precision and accuracy of the method are well within the limits required for bioequivalence study methods. The limit of quantification 5.9 ng/ml permits the use of the method for pharmacokinetic studies.

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