Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 4/5, pp. 375–378, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00
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Determination of ampicillin in human plasma by high-performance liquid chromatography using ultraviolet detection

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Abstract: A high-performance liquid chromatographic method with isocratic elution has been developed for the determination of ampicillin in human plasma. The method comprises injection of the plasma after protein precipitation on to a reversed-phase C_{18} column, using phosphate buffer (pH 3.5)–methanol as the mobile phase. The method involves UV detection at 225 nm. This method provides a simple technique for the rapid analysis of ampicillin within the concentration range 0.2–25 $\mu g\ ml^{-1}$ with a high degree of accuracy and precision.

Keywords: *Ampicillin; reversed-phase HPLC; isocratic élution; UV detection; human plasma.*

Introduction

The traditional method for the analysis of ampicillin in human plasma by high-performance liquid chromatography involves a pre-column [1–2] or a post-column [3–6] derivatization step, because of the low UV-absorbance of the drug. Most antibiotics have a relatively high molar absorptivity within the UV absorption range so that UV detection permits determination with sufficient sensitivity [7]. Several methods for the assay of penicillins in body fluids have been studied. These utilize high-performance liquid chromatographic techniques with UV detection [8–13].

The object of the present work was to develop a simple, accurate and precise HPLC technique with isocratic elution for the monitoring of ampicillin in human plasma within the concentration range 0.2–25 $\mu g\ ml^{-1}$.

Experimental

Materials and reagents

Ampicillin trihydrate was obtained from Astra (Sweden); heptanesulphonic acid sodium salt monohydrate, Cat. No. 51835 Purum, 99%(T) was from Fluka; monobasic potassium phosphate, Cat No. 4871, cryst. extra pure was from Merck; methanol, HPLC grade was from BDH. All chemicals were used without further purification.

Citrate–phosphate buffer (pH 5.5 ± 0.1). Citrate–phosphate buffer (pH 5.5 ± 0.1) was prepared by mixing equal volumes of 0.3 M citric acid monohydrate and 1 M disodium hydrogen phosphate solution. The pH was adjusted if required with 0.3 M citric acid or 1 M disodium hydrogen phosphate.

Buffered TCA solution for protein precipitation. Buffered TCA solution was citrate–phosphate buffer (pH 5.5 ± 0.1)–70% w/v trichloroacetic acid in water (TCA) (1:3, v/v).

Buffered TCA solution was used for protein precipitation, to provide buffer action while 5 M sodium hydroxide was added to the supernatant to maintain the pH at 5 ± 0.1 for the optimal stability of ampicillin.

Chromatography

Equipment. The chromatographic system comprised: a LC-6A pump (Shimadzu, Japan) for delivering the eluent; a SIL-6A injector (Shimadzu, Japan); a SPD-6AV detector (Shimadzu, Japan); a C-R3A integrator (Shimadzu, Japan); and a micro Bonda Pak C_{18} column (300 × 3.9 mm i.d.) protected with an ODS pre-column (Shimadzu, Japan).

Preparation of mobile phase. Transfer 250 ml of 0.2 M KH_2PO_4 into a 1000-ml glass beaker and add 750 ml of distilled water; adjust the pH to 3.5 ± 0.1 with 44% (w/w)

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phosphoric acid. Transfer 900 ml of this solution into a 2000-ml volumetric flask add 3.5 g of heptanesulphonic acid sodium salt monohydrate and dissolve the salt; add 300 ml of methanol of (HPLC grade). Filter the resulting solution using a 0.45 μm HV filter (Millipore, Bedford, MA, USA).

Chromatographic conditions. The flow rate was maintained at 1.5 ml min^{-1}. Detection was performed at 225 nm. The detector output was set at 0.02 aufs. All separations were carried out at ambient temperature.

Sample pre-treatment

The sample was treated in accordance with the following procedure. To 1.0 ml of plasma was added 0.1 ml of buffered TCA solution for protein precipitation and mixed on a whirlimixer for about 30 s. After centrifugation at 4000 rpm for 3 min, 0.8 ml of the supernatant was transferred into the vial for the auto-injector containing 20 μl of 5 M sodium hydroxide. The solution was then sonicated for 10 s using an ultrasonic water bath. A 50 μl volume of this solution was injected on to the chromatographic column.

Sample handling

After the sampling of blood in the heparinized test-tube, the sample was handled with care to avoid haemolysis. Plasma was separated by centrifugation and stored at −60°C or below.

Calibration curves

Calibration standards were prepared by adding microlitre amounts of the 500 μg ml^{-1} stock aqueous solution of ampicillin to 1 ml of drug-free plasma. Two sets of calibration standards were prepared at concentration ranges of 0.2–4 and 5–25 μg ml^{-1}. These standards were treated according to the sample work-up procedure. Three replicates were used for each concentration.

Calibration curves were constructed by plot-

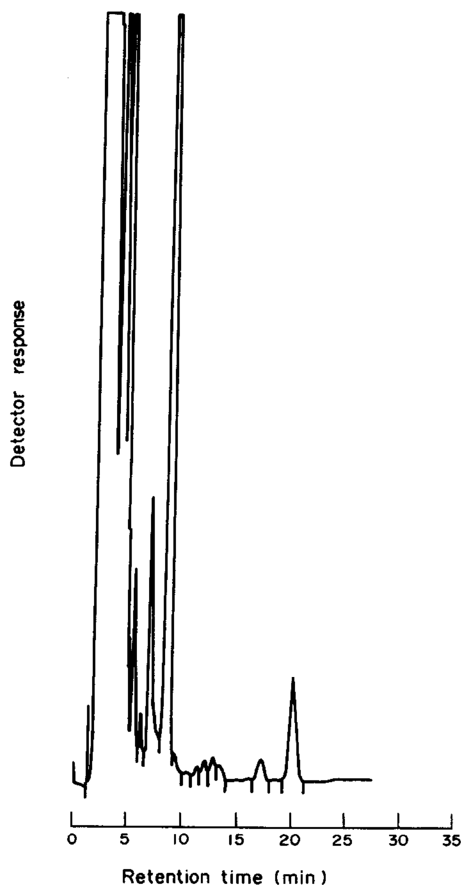

Figure 1
Chromatogram of drug-free human plasma after protein precipitation.

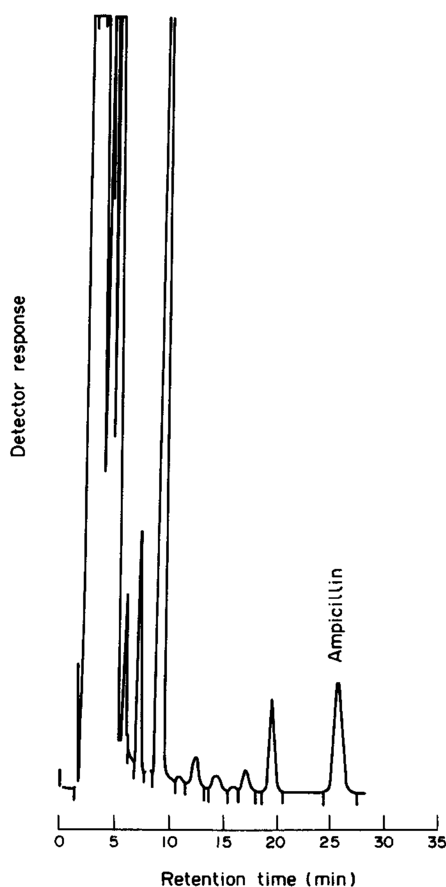

Figure 2
Chromatogram of human plasma spiked with 10 μg of ampicillin and treated to precipitate protein.

Table 1
Typical assay performance data from the mean of three calibration curves for the determination of ampicillin in human plasma

Linear conc. range (μg ml^{-1})	UV (nm)	Slope	Intercept	Correlation coefficient
0.2–4	225	0.385	+0.0096	0.999358
5–25	225	0.239	−0.036	0.997778

Table 2
Precision and accuracy data

Quantity of ampicillin added to 1 ml of drug-free plasma (μg)	Mean determined conc.* (μg ml^{-1})	Recovery (%)	RSD (%)
0.20	0.20	100.64	0.48
0.40	0.40	100.79	1.49
0.60	0.60	100.11	1.16
0.80	0.81	100.59	0.29
1.00	1.00	100.20	1.05
2.01	2.01	100.01	0.92
3.02	3.04	100.66	1.38
4.03	4.06	100.76	1.36
4.98	4.92	98.72	3.57
9.97	9.98	100.06	2.36
14.96	15.07	100.71	2.04
19.95	20.05	100.48	1.90
24.94	23.80	95.41	1.81

*Each concentration represents the mean of three within-day replicates.

ting the peak height of ampicillin against concentration of ampicillin, using linear regression analysis. Unknown concentrations of ampicillin were quantified by relating the respective peak height to the regression line [6].

Results and Discussion

Under the conditions described, the retention time of ampicillin was 23 min. Typical chromatograms obtained from blank and spiked plasma are shown in Figs 1 and 2. No interference by endogenous compounds was observed.

Linearity was observed when a graph of peak height against concentration over a range of 0.2–4 and 5–25 μg ml^{-1} was plotted.

Performance data for the determination of ampicillin in human plasma using the assay procedure are presented in Table 1.

The precision of the method was calculated as the relative standard deviation (RSD) of assays of standards containing ampicillin in concentrations of 0.2–25 μg m^{-1} in plasma. The RSD was 0.29–3.57%. The accuracy of the method was assessed by calculating relative recoveries of ampicillin added to drug-free plasma within the concentration range 0.2–25 μg ml^{-1}. Recoveries were calculated by relating the respective peak height to the regression line of the standard calibration curve. The analytical recoveries were 95.41%–100.71%. The data represent the means of three within-day replicates for each concentration and are shown in Table 2.

The stability of ampicillin is indicated by the constant value of peak heights observed within 24 h of protein precipitation when the drug-free plasma spiked with 10 μg ml^{-1} of ampicillin was analysed. The data tabulated in Table 3 represents three replicates for each time interval. Stability-indicating chromatograms are presented in Fig. 3(a) and (b).

Table 3
Stability indicating data

Time period (h)	Peak height*
Blank	—
0†	2361
16	2326
24	2243

*Each peak height represents the mean of three replicates.

†The sample treated and injected into the chromatograph without delay.

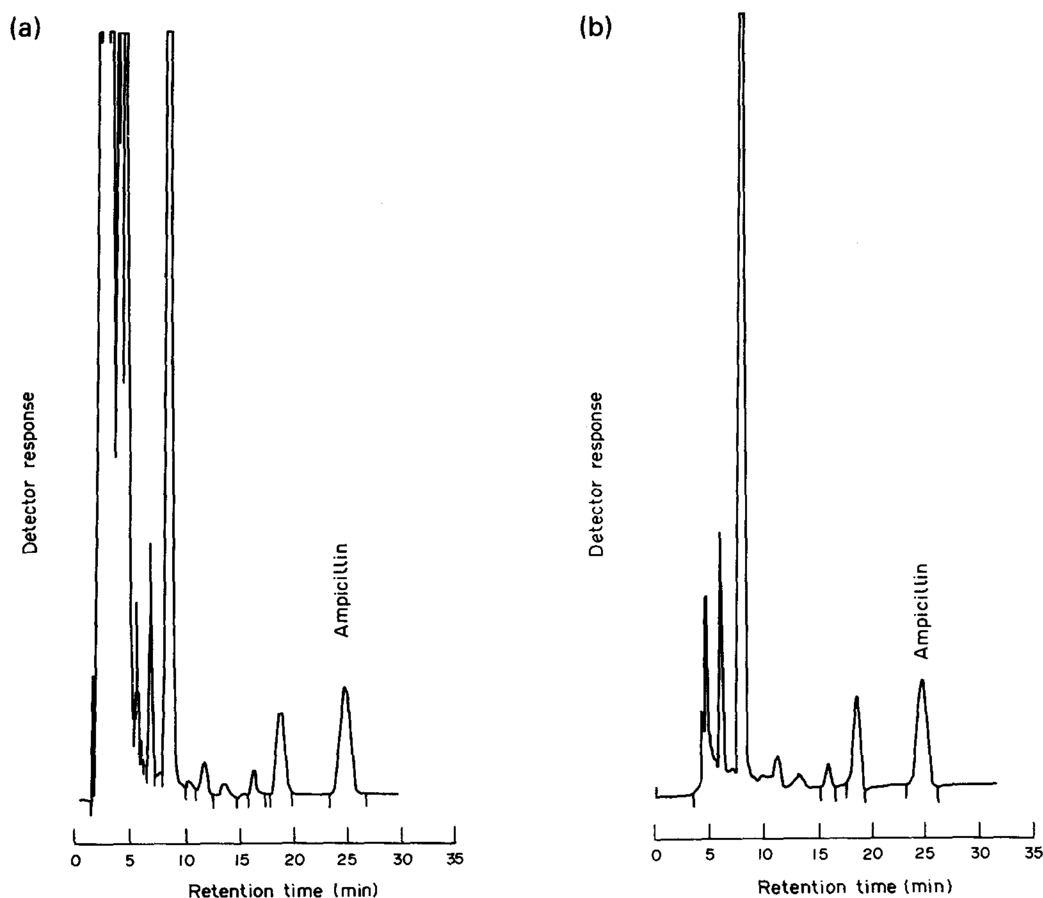

Figure 3
Stability-indicating chromatogram of human plasma (a) 16 and (b) 24 h after treatment to precipitate protein.

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[Received for review 7 May 1992; revised manuscript received 14 October 1992]