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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 for isocratic mode of elution has been developed for the determination of ampicillin in human plasma. The method comprises injection of the plasma after protein precipitation, separation by mined-phase Cu column, using Implant sulphonic add, sodium salt monohydrate as an ion-pairing agent in the mobile phase which consists of pH 33 phosphate buffer-methanol. The method involves ITV-detection at 225 nm. This method provides a simple tool for the rapid analysis of ampicillin with high degree of accuracy and precision, where the drug level is in microgram range.

Introduction

The traditional trend for the analysis of ampicillin in human plasma by high performance liquid chromatographic (HPLC) method involves pre-column (Miyazaki *et al,* 1983; Haginaka and Wakai, 1985) or a post column (Westerlund *et al,* 1979; Haginaka and Wakai, 1987; Haginaka *et al,* 1987; Rasseel *et al,* 1989) derivatization step due to the low UV absorbance of the drug. Most antibiotics have relatively high molar absorptivity within the UV absorption range, so that UV detection permits determinations with sufficient sensitivity (Ronan, 1985). Several methods for the assay of antibiotics in body fluids have been investigated. These utilise HPLC technique with UV detection (Nakagawa *et al,* 1978a, 19786; Miyazaki *et al,* 1983).

In the present work an isocratic HPLC method with UV detection has been developed for the determination of ampicillin in human plasma. The minimum detectable concentration is 1 µg/ml. To and above this concentration, the method provides a high degree of accuracy and precision. The object of this study is to develop a simple chromatographic method for monitoring ampicillin in human plasma within the concentration range 1-25µg/ml.

Materials and Methods

Materials

Ampicillin dihydrate was obtained from Astra (Sweden). Heptane sulphonic add, sodium salt monohydrate and monobasic potassium phosphate were obtained from Fluka and Merck respectively. Methanol obtained from BDH was HPLC-grade. All chemicals were used without further purification. Citrate/phosphate buffer pH 55 ± 0.1 was prepared by dissolving 2.0 g of disodium hydrogen phosphate and 0.84 g citric acid monohydrate in 25 ml deionized water.

The solution for protein precipitation consisted of 75 ml trichloroacetic add (70% w/v) and 25 ml citrate/phosphate buffer pH 5.5 ± 0.1.

Chromatographic procedure

The Chromatographic set up (Shimadzu) used in this study included a LC-6A pump for delivering the eluent, SIL-6A, Injector, SPD-6AV Detector; C-R3A Integrator; and micro Bonda Pak Cu Column, 250 mm x 3.9 mm, protected with pre-column ODS (Shimadzu). The mobile phase used was as follows: A 250 ml quantity of 0.2 M KH_2PO_4 solution was transferred into a 1000 ml pyrex beaker and 750 ml of distilled water was added. The pH was adjusted to 35±0.1 with 44% phosphoric add. A 900 ml quantity of this solution was transferred into a 2000 ml volumetric flask, 3.5 g of heptane sulphonic add sodium salt monohydrate was added and the salt was dissolved. To this solution 300 ml of methanol (HPLC grade) was added and the resulting solution was filtered with 0.45 pm HV type filter (Millipore Corporation, USA). The flow rate was maintained at 1.5 ml/min. and the detection was performed at 225 um. All separations were carried out at ambient temperature.

Sample handling

After the sampling of blood in the haparinized test tube, the sample was handled with care to avoid hemolysis. The plasma was separated by centrifugation and stored at -60C or below.

Sample pre-treatment

The sample was treated according to the following procedure.

1.0 ml of plasma was taken and the proteins were precipitated by the addition of 0.1 ml of solution for protein precipitation and mixing on a whirlmixer for about 30 seconds. After centrifugation at 4000 rpm for 3 minutes, 0.8 ml of the supernatant was transferred into a vial for auto-injector containing 20 pl of 5 M sodium hydroxide and sonicated for 10 seconds on a ultrasonic water bath. 50µl of

this solution was injected on to the chromatographic column.

Calibration curves

Calibration standards for the assay of plasma were prepared by adding known quantities of the 500 pg/ml stock aqueous solution of ampicillin to 1 ml of drug-free plasma. Calibration standards were prepared at concentrations of 5, 10, 15, 20 and 25 pg/ml. These standards were treated according to the sample work up procedure.

Calibration curves were constructed by plotting peak height against known concentration of ampicillin. Using linear regression analysis unknown concentrations of ampicillin were quantified by relating the respective peak height to the regression line R2 used *et al*., 1989).

Results and Discussion

Chromatography

Under the conditions described for HPLC, the retention time of the ampicillin sample is 23 minutes. Typical chromatograms obtained from the blank and spiked plasma are shown in Fig. 1. In the chromatograms no interference from endogenous compounds was observed.

A linear behaviour has been observed when a graph is plotted between peak height and concentrations of ampicillin over the range 5-25 µg/ml (Fig. 2). Typical standard curves give a regression of Y=239.631 x -36.12 with correlation coefficient (R) = 0.997778. The performance characteristics for the determination of ampicillin in human plasma using the present assay procedure are given in Table 1.

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

Concentration range (µg/ml)	Slope	Intercept	Correlation coefficient
5-25	0.239	-0.036	0.997778

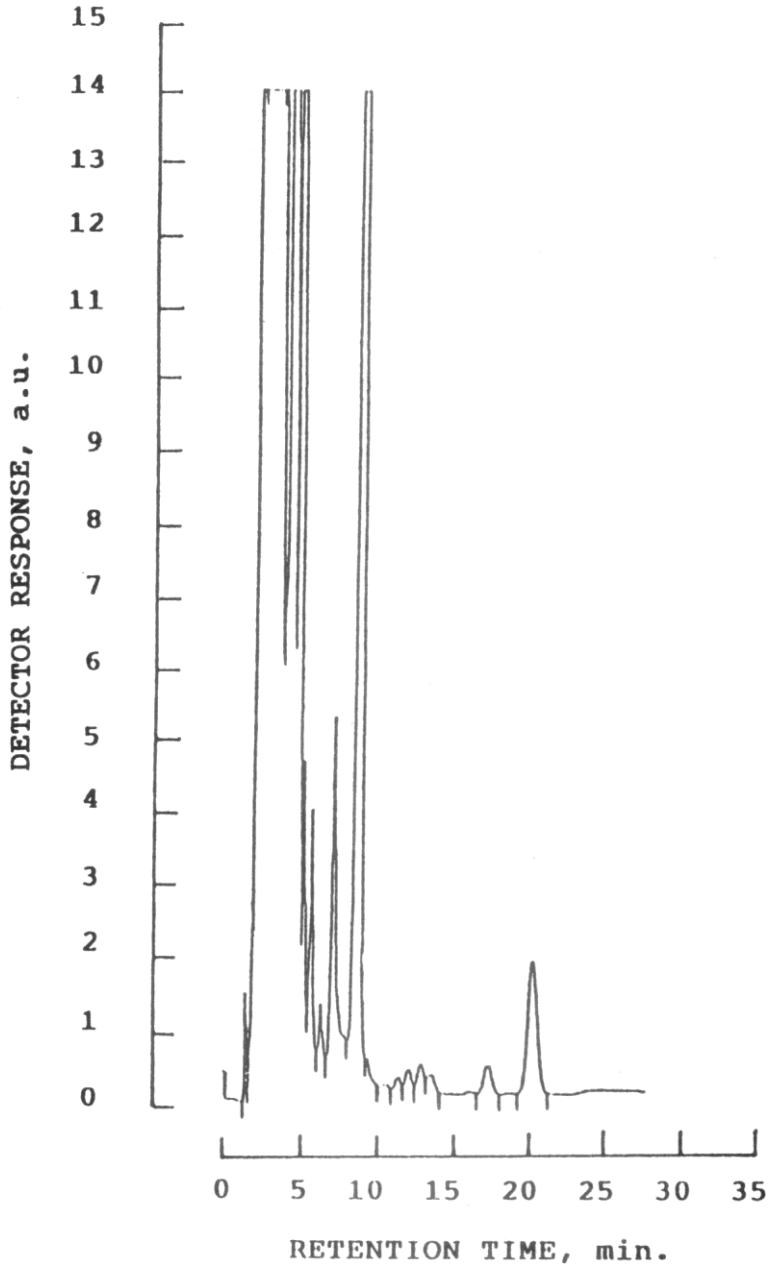

Fig. 1. Chromatogram of drug-free human plasma after protein precipitation.

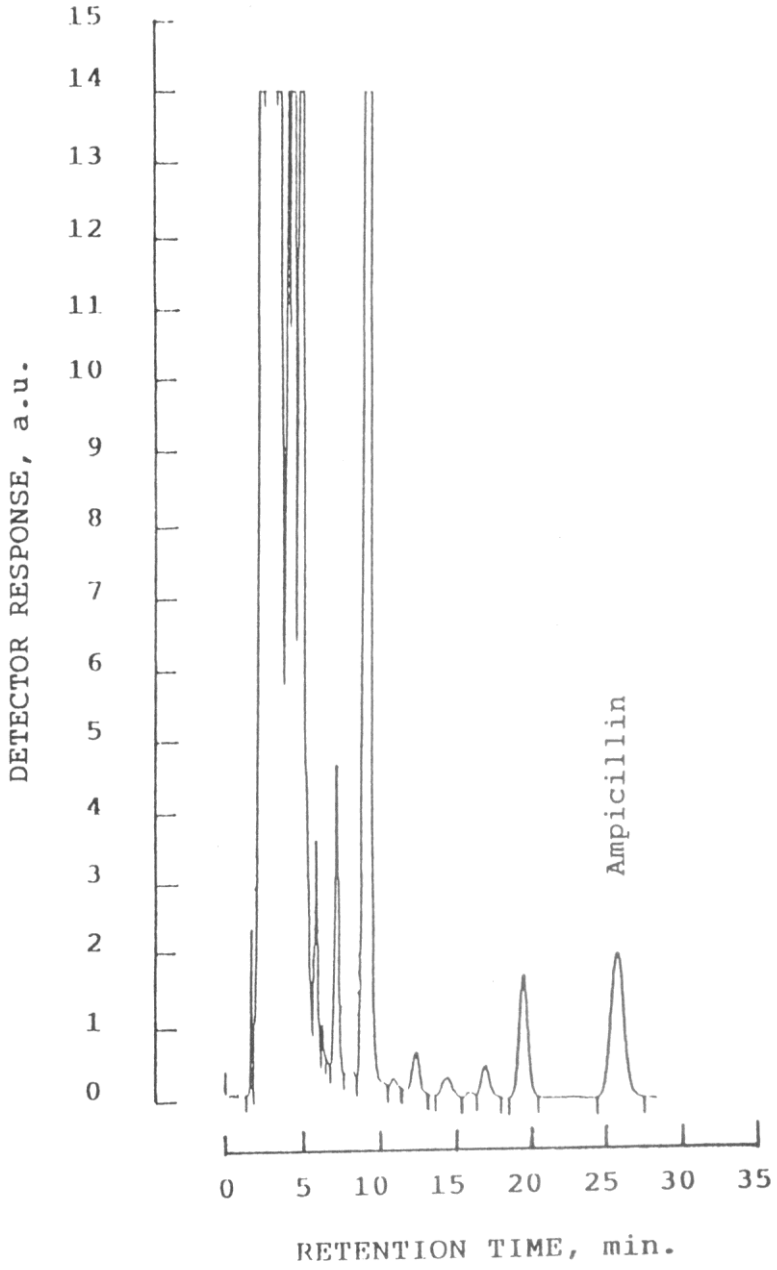

Fig. 2. Chromatogram of human plasma spiked with 10 µg ampicillin and treated for protein precipitation.

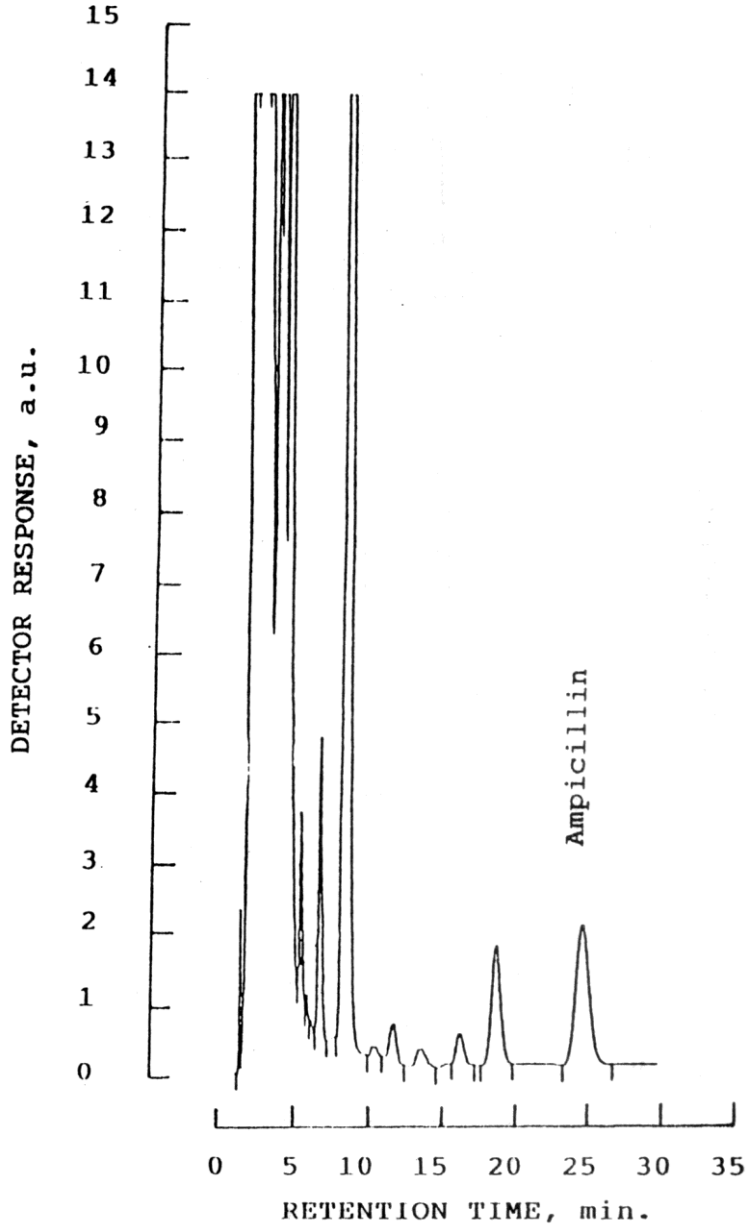

Fig. 3a. Stability-indicating chromatogram of human plasma after 16 hours of treatment for protein precipitation.

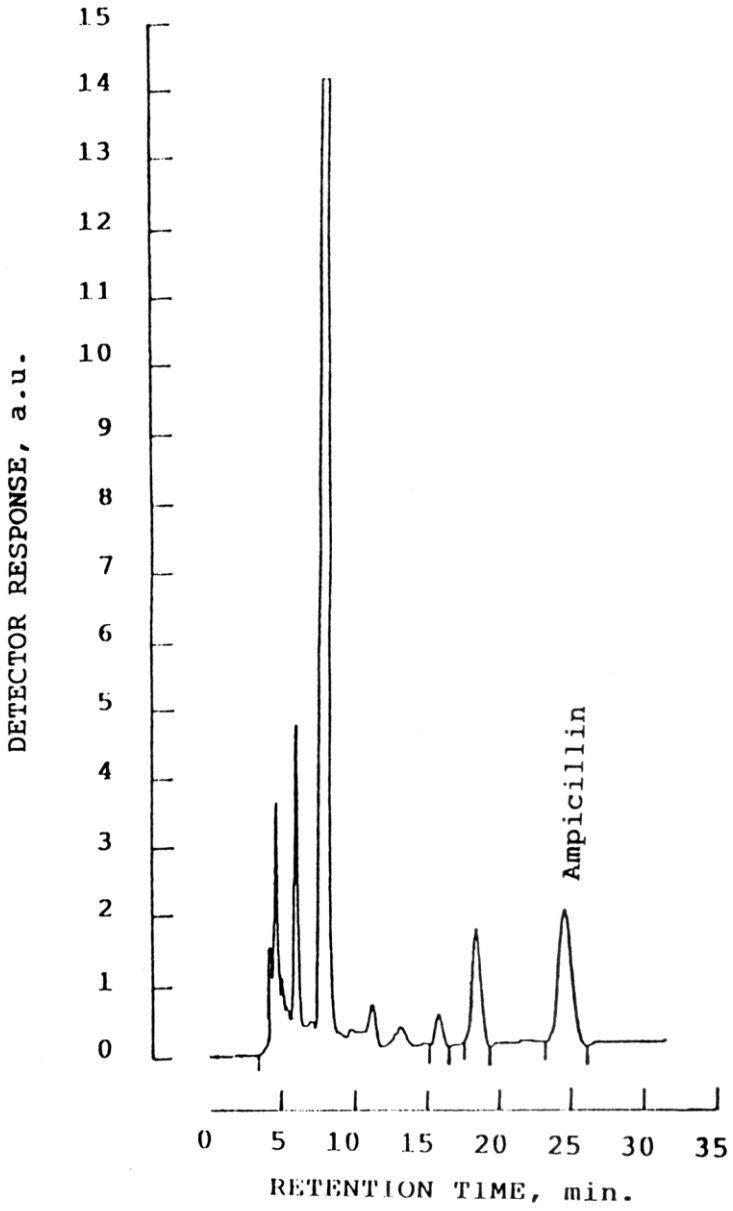

Fig. 3b. Stability-indicating chromatogram of human plasma after 24 hours of treatment for protein precipitation.

Accuracy and precision

The accuracy of the method was assessed by calculating recoveries of the drug of known amount added to one ml of drug-free plasma. The analytical recoveries ranged from 95.41% to 100.71% (Table 2).

The precision of the method was calculated as the coefficient of variation of assays of standards containing ampicillin at concentrations of 5-15 pg/ml for the plasma. The precision was between 128% and 233% C.V.

Table 2.

Recovery data for ampicillin added to human plasma

Quantity of Ampicillin added to 1 ml plasma* μg	Mean ±SD μg/ml	Recovery %	C.V. %
4.9885	4.925±0.176	98.72	2.53
9.9770	9.983±0.236	100.06	1.67
14.9655	15.072±0.308	100.71	1.44
19.9540	20.05±0.381	100.48	1.34
24.9425	23.80±0.432	95.41	1.28

* drug-free plasma.

Study of ampicillin in treated plasma

Penicillins are rather unstable in aqueous solution, the degradation being catalyzed by both acids and bases. The optimum stability for ampicillin is at pH 4.9 (Westerland *et al,* 1979). The stability of ampicillin within 24 hours of treatment according to the sample pre-treatment procedure can be judged from the peak heights given in Table 3. The chromatograms are presented in Fig. 3. The peak heights indicate that there is no degradation of the drug within 24 hours. Thus the method is quite safe for the determination of ampicillin in plasma.

Table 3

Stability data for ampicillin in treated plasma.

Time (hrs.)	Peak height (a.u.)
Blank	-
*0	2361
16	2326
24	2243

*** The sample just treated and injected to L.C. without delay.**

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