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Short communication

Simple high-performance liquid chromatography determination of ampicillin in human serum using solid-phase extraction disk cartridges

Mitsuhiro Ishida*, Kayoko Kobayashi, Norio Awata, Fumio Sakamoto

Department of Pharmacokinetics, Pharmaceutical Research Laboratories Pharmaceuticals R & D Center, Kanebo LTD., 5-90 Tomobuchi-cho 1-chome Miyakojima-ku, Osaka 534-8666, Japan

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Abstract

A simple and reproducible method for the analysis of ampicillin in human serum was developed. Serum samples were extracted using solid-phase extraction disk cartridges containing a sorbent of styrene divinyl/benzene. Extracts were separated by reversed-phase C_{18} high-performance liquid chromatography with UV detection at 220 nm. The mobile phase consisted of acetonitrile–10 m*M* NaH_2PO_4 (6.5:93.5, v/v). Using this extraction procedure, recovery from serum was 98.4±5.6%. The quantitation limit was 0.19 μg/ml using 0.5 ml of serum. The calibration curves from 0.19 to 9.41 μg/ml were linear with correlation coefficients of 0.999. This method is suitable for therapeutic drug monitoring of ampicillin (ABPC) after oral administration of lenampicillin hydrochloride.

Keywords: Ampicillin; Lenampicillin

1. Introduction

Ampicillin (ABPC), D(−)-α-aminobenzylpenicillin is widely used in clinical chemotherapy, and lenampicillin (LAPC) hydrochloride, ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl) methyl ester (Fig. 1), is an ester prodrug of ABPC [1]. LAPC showed good oral absorption and has been used clinically for urinary and respiratory tract infection [2]. The absorbed LAPC is readily hydrolyzed in the intestinal mucosa. The hydrolyzed products of LAPC are ABPC and acetoin. LAPC is not detected in the systemic circulation [3]. A method for the determination of ABPC in human serum after oral administration of LAPC hydrochloride has been reported [4]. However, this method was insufficiently sensitive for LAPC pharmacokinetics studies at doses equal to or less than 250 mg ABPC equivalent.

In the present paper, we describe a simple, sensitive and reproducible assay for determination of ABPC in human serum using solid-phase extraction disk cartridges containing a sorbent of styrene divinyl/benzene (SDB) and reversed-phase C_{18} high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Materials

ABPC sodium, acetoin, 2,3-butandiol, HPLC

*Corresponding author.

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grade acetonitrile and methanol were obtained from Wako Pure Chemical industries (Osaka, Japan). The HPLC internal standard, N^2, N^4, N^6-trimethylmelamine (Fig. 1) [5], was supplied by Drug Discovery Laboratory of Kanebo. Water used was purified by Milli-Q Labo (Nihon Millipore, Tokyo, Japan). Other Chemicals used were analytical grade. Empore™ disk cartridges SDB-XC (10 mm/6 ml, sorbent weight 24 mg, 3M, MN, USA) were used for solid-phase extraction.

Stock solutions of ABPC and the internal standard were prepared weekly in water at concentrations of 0.5 and 0.2 mg/ml, respectively. These solutions were stored at 4°C.

Blank human blood was collected from five healthy and drug free volunteers. Serum was prepared by centrifugation at 1636×*g* for 5 min and stored at −20°C until used.

2.2. Sample preparation

For each sample or standard, 0.5-ml volume of serum was pipetted into a 10-ml glass test tube, and then 2 μl of internal standard solution and 0.5 ml water were added, followed by vortex mixing. Solid-phase extraction disks were attached to a vacuum manifold (GL Science, Tokyo, Japan) and conditioned with 0.5 ml of methanol followed by 0.5 ml of water twice (Note: did not allow sorbent to dry). Samples were loaded onto the cartridges and allowed to filter at approximately 3.5 inch Hg vacuum. The disk cartridges were washed with 1 ml of water and eluted with 400 μl of acetonitrile. The eluted solution (10 μl) was directly injected into the HPLC system.

2.3. HPLC conditions

The HPLC system consisted of a Waters 510 pump, a 710 autoinjector, a column oven and a 481 UV detector (Waters, Milford, MA, USA). The column was Inertsil ODS-3 (250×4.6 mm I.D., GL Science). The mobile phase consisted of acetonitrile–10 m*M* NaH_2PO_4 (6.5:93.5, v/v). The column temperature was maintained at 30°C. The UV detector was set at 220 nm and the flow-rate was 1.0 ml/min throughout the analysis.

2.4. Calibration curves

Appropriate dilutions of the stock solution were made with water. Standard solutions of ABPC in serum were prepared by spiking 10 μl of diluted stock solution to 0.5 ml of serum to give a final concentrations of 0.19, 0.47, 0.94, 1.88, 3.76 and 9.41 μg/ml. The sample preparation and HPLC analysis were carried out as described in Section 2.2 and 2.3. Calibration curves for the determination of ABPC were produced by the plotting the ABPC/internal standard peak height ratios against ABPC concentration.

3. Results

3.1. Recovery for solid-phase extraction from serum

We tested the correlation between elution volume of acetonitrile and the recovery of serum ABPC from the solid-phase extraction cartridges. Recovery was assessed at 10 μg/ml by comparing to the peak-height ratios obtained from unextracted solutions. The recovery of ABPC from serum was increased with increasing volume of acetonitrile from 100 to 500 μl. A 400 μl volume was sufficient to obtain a high recovery of ABPC from serum and was chosen for this procedure. The recovery of ABPC from human serum was 98.4±5.6% (n=7). Under the same conditions, the recovery of internal standard was 86.4±4.9%.

3.2. Specificity

Chromatograms of a blank serum extract (a), an extract of serum spiked with ABPC and internal standard (b), and an extract of serum sample taken 3 h after a single oral dosage of LAPC (250 mg ABPC equivalent) into a healthy volunteer (c) are shown in Fig. 2. ABPC and internal standard were eluted at 20.7 min and 15.4 min, respectively. No interference peaks of the retention times of ABPC and internal standard were observed in the blank serum. ABPC was free of interference from LAPC and the metabolites of LAPC, acetoin and 2,3-butandiol.

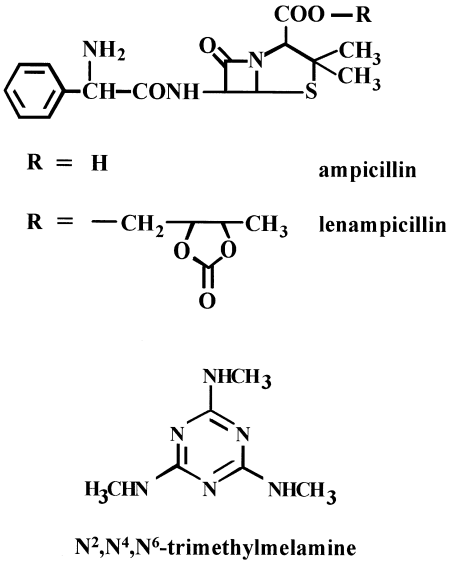

Fig. 1. Chemical structures of ampicillin and lenampicillin and N^2,N^4,N^6-trimethylmelamine used as an internal standard.

3.3. Calibration curves

The daily slopes and correlation coefficients of the calibration curves are shown in Table 1. The slopes were between 0.0775–0.0796 for each of five days. The calibration curves were linear in the concentration range of 0.19–9.41 μg/ml of ABPC with correlation coefficients of 0.999.

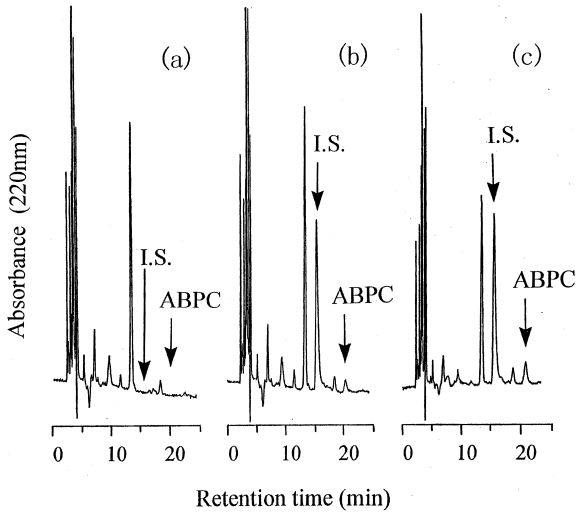

Fig. 2. Chromatograms obtained from (a) blank human serum; (b) serum spiked with ampicillin (ABPC, 0.47 μg/ml) and N^2,N^4,N^6-trimethylmelamine as an internal standard (I.S.); (c) healthy volunteer serum sample (1.27 μg/ml) 3 h after an oral administration of lenampicillin hydrochloride (250 mg ABPC equivalent). It is the same scale on (a), (b) and (c).

Table 1
Daily slopes and correlation coefficients of the calibration curves obtained from inter-day assay

Day	Slope	r[a]
1	0.0786	0.9996
2	0.0793	0.9998
3	0.0796	0.9994
4	0.0793	0.9996
5	0.0775	0.9992

[a] r=Correlation coefficients.

3.4. Precision and accuracy

The precision and accuracy data in the intra- and inter- day assay of quality control sample are shown in Table 2. The intra- and inter-day precision of the assay was within 13.9% and 14.1%, respectively, at ABPC concentrations of 0.19–9.41 μg/ml. The intra- and inter-day accuracy of assay was in the range of 90.4–103.1% and 90.4–102.1%, respectively.

Table 2
The precision and accuracy of the proposed method for the determination of ampicillin in human serum

Ampicillin (μg/ml)[a]		Accuracy[b] (%)	Coefficients of variation[c] (%)
Added	Found		
Intra-day assay			
0.19	0.17	90.4	13.9
0.47	0.48	102.1	11.5
1.88	1.81	95.6	2.6
9.41	9.74	103.1	5.8
Inter-day assay			
0.19	0.17	90.4	9.9
0.47	0.48	102.1	9.9
0.94	0.96	102.0	14.1
1.88	1.81	95.6	6.3
3.76	3.64	96.7	3.9
9.41	9.10	96.7	6.3

[a] All values based on n=5.

[b] Accuracy=[(concentration found−concentration spiked)/concentration spiked]×100%.

[c] Coefficients of variation=(S.D./mean×100%).

4. Discussion

The results of our extraction experiments showed high recovery of ABPC from serum using the Empore™ Disk Cartridges SDB-XC. SDB, a copolymer of styrene and divinyl/benzene, has both reversed-phase and ion-exchange characteristics [6]. Using these cartridges, the elution volume is important to the assay sensitivity since the eluant is directly injected onto the HPLC system. High recovery (98.4%) of ABPC was obtained using a 400 μl volume of acetonitrile.

The intra- and inter-day precision and accuracy of the method of 0.19 μg/ml ABPC concentrations were within 15% of nominal. This is the lower limit of quantitation of ABPC in serum for this method, which was better than that obtained by previous methods [4,7]. The serum ABPC concentration was 5 μg/ml at 1 h and 0.2 μg/ml at 6 h after an oral administration of LAPC hydrochloride (250 mg ABPC equivalent) [2]. Therefore, calibration curves between 0.19 and 9.41 μg/ml are required for pharmacokinetic investigations. A method using fluorescent derivatives has high sensitivity (0.5 ng/ml) [8], but this method required several pretreatment steps. The microbiological plate assay for ABPC in human serum is complex and could be affected by the other antibiotics [9].

In conclusion, we developed a simple, sensitive and reproducible assay for determination of ABPC in human serum by means of solid-phase extraction disk cartridges and HPLC. It seems that this method is suitable for therapeutic drug monitoring of ABPC after an oral administration of LAPC hydrochloride.

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