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Simultaneous determination of ampicillin and metampicillin in biological fluids using high-performance liquid chromatography with column switching

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Abstract

A new high-performance liquid chromatographic method with column switching has been developed for the simultaneous determination of metampicillin and its metabolite ampicillin in biological fluids. The plasma, urine and bile samples were injected onto a precolumn packed with LiChrosorb RP-8 (25–40 μm) after simple dilution with an internal standard solution in 0.05 *M* phosphate buffer (pH 7.0). The polar plasma components were washed out using 0.05 *M* phosphate buffer (pH 7.0). After valve switching, the concentrated drugs were eluted in the back-flush mode and separated by an Ultracarb 5 ODS-30 column with a gradient system of acetonitrile–0.02 *M* phosphate buffer (pH 7.0) as the mobile phase. The method showed excellent precision, accuracy and speed with a detection limit of 0.1 μg/ml. The total analysis time per sample was less than 40 min and the coefficients of variation for intra- and inter-assay were less than 5.1%. This method has been successfully applied to plasma, urine and bile samples from rats after intravenous injection of metampicillin.

1. Introduction

Metampicillin [(6*R*)-6-(D-2-methyleneamino-2-phenylacetamido) penicillanic acid] is a semisynthetic penicillin antibiotic produced by reaction of ampicillin with formaldehyde [1]. Metampicillin has the same broad antimicrobial activity as ampicillin and it is preferred because of the high biliary excretion following parenteral administration [1–6]. Metampicillin is susceptible to hydrolyze to ampicillin, and there is a marked effect of pH on the hydrolysis rate: hydrolysis proceeds more rapidly at acidic pH [1,2,7]. At physiological temperature (37°C) and pH (7.0), the hydrolysis proceeds with a half-life of 41.5 min [7].

High-performance liquid chromatographic (HPLC) methods have been developed previously for the determination of ampicillin in biological fluids [8–10]. These methods require large sample volumes and are time-consuming and tedious because of the liquid–liquid extraction and deproteinization steps needed for sample clean-up. In a recent paper [7], we described an HPLC method for the simultaneous determination of metampicillin and ampicillin in aque-

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ous solution to study the stability kinetics and quality control. However, the instability of metampicillin and difficulties in the simultaneous determination of metampicillin and ampicillin in biological samples have severely hampered pharmacokinetic study and therapeutic drug monitoring.

The purpose of the present study was to develop a rapid, specific and sensitive HPLC method using column-switching technique [11–19] for the simultaneous determination of metampicillin and ampicillin in biological samples. This paper describes an automated column-switching HPLC method involving on-line sample trace enrichment and direct injection of diluted plasma, urine or bile samples.

2. Experimental

2.1. Materials and reagents

Sodium metampicillin and sodium ampicillin were received from Samsung Pharmaceutical Co. (Seoul, Korea). Cephalexin (internal standard) was obtained from Sigma (St. Louis, MO, USA). All other reagents were of HPLC grade.

Stock solutions of metampicillin and ampicillin were prepared from their sodium salt in phosphate buffer (pH 7.0) to yield a final concentration of 1 mg/ml as the free base. The stock solution was aliquoted and stored at −20°C for 1 month. Working standard solutions were prepared on the day of analysis by diluting the stock solution with 0.05 *M* phosphate buffer (pH 7.0) and kept on ice before injection. Internal standard solution was prepared in methanol and diluted with 0.05 *M* phosphate buffer (pH 7.0). This solution could be stored at −20°C for several months.

2.2. Chromatographic system

The HPLC system consisted of two Spectra-Physics Model SP 8800 pumps (Santaclara, CA, USA), a Rheodyne 7125 injector (Cotati, CA, USA), a Rheodyne 7000 switching valve and Spectra-Physics SP 8490 detector. Data handling was performed by a Spectra-Physics 4270 computing integrator. The instrumental set-up for the six-port column-switching system is shown in Fig. 1.

The precolumn (20 × 3.9 mm I.D.) was tap-filled with LiChrosorb RP-8 (25–40 mm, Merck, Darmstadt, Germany) and was changed after injection of 100 samples. A Nova-Pak C_8 guard column (4.0 × 10 mm I.D., Waters Assoc., MA, USA) was used and the analytical column was an Ultracarb 5 ODS-30 packed column (250 × 4.6 mm I.D., 5 μm, Phenomenex, CA, USA).

The washing solvent was 0.05 *M* phosphate buffer (pH 7.0). The mobile phase consisted of two components: (A) acetonitrile in 0.02 *M* phosphate buffer (pH 7.0) (4:96, v/v) and (B) acetonitrile in 0.02 *M* phosphate buffer (pH 7.0) (30:70, v/v). A linear gradient was used: 45% B in A (0–10 min), from 45% B in A to 100% B (10–18 min), 100% B (18–30 min). The flow-rates of washing solvent and mobile phase were 1.0 ml/min, respectively. The column temperature was ambient and the wavelength of detection was 230 nm.

2.3. Analytical procedure

A 50-μl aliquot of the spiked plasma or rat plasma samples and 100 μl of internal standard solution were mixed, and 100 μl of the mixture was injected. Rat bile and urine samples were adequately diluted with 0.05 *M* phosphate buffer (pH 7.0). The prepared samples were kept on ice before injection.

Sample analysis included the following steps and required about 40 min. Step I (0–5 min): The diluted plasma, urine and bile sample was injected onto the precolumn. Polar interfering plasma components were washed out to waste with washing solvent using pump 1. The guard column and analytical column were equilibrated with mobile phase with the composition of the starting eluent in the gradient elution using pump 2. Step II (5–35 min): The washing solvent passed directly to waste. The retained components were eluted in the back-flush mode from the precolumn to the guard column/analytical column by the gradient elution after valve

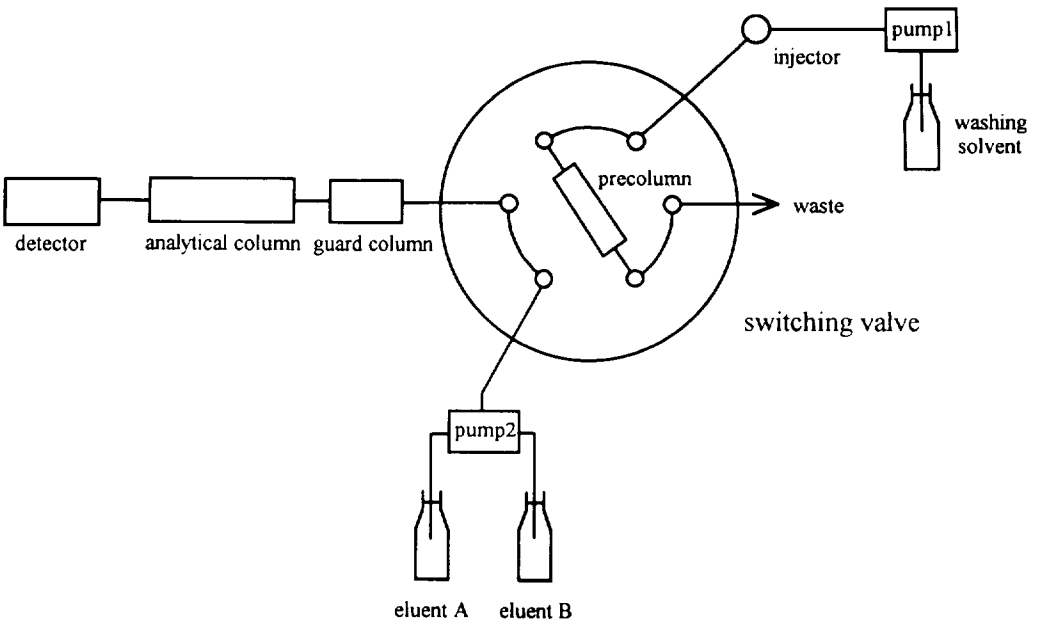

Fig. 1. Schematic diagram of six-port switching system. (——) Load; (- - -) inject.

switching. The eluted drugs were separated in the analytical column with pump 2 starting the gradient elution immediately after valve switching. Step III (35–39 min): The precolumn and analytical column were re-equilibrated with the washing solvent and the initial mobile phase composition, respectively.

3. Results and discussion

3.1. Chromatography and column-switching procedure

The chromatographic conditions for ampicillin on reversed-phase material have been reported previously [7–10]. In a previous paper [7], we described a method for the simultaneous determination of ampicillin and metampicillin in aqueous solution by HPLC for the study of the stability kinetics and quality control and the efficiency of various bonded stationary-phase columns for the separation of ampicillin and metampicillin. For the simultaneous determination of ampicillin and metampicillin from biological samples, an Ultracarb 5 ODS-30 column, which has a very high carbon content (30%) and contains end-capped material, was chosen among the various available columns because interfering peaks are separated from the analyte peaks.

A gradient was necessary for the simultaneous determination of ampicillin and metampicillin in plasma, urine or bile due to the large difference in polarity. Also, the gradient was useful for purging nonpolar interfering components from the analytical column and obtaining a sharp metampicillin peak, which resulted in a low detection limit. Retention times were highly reproducible. Because of the instability of metampicillin at acidic pH, phosphate buffer at pH 7.0 was used as the mobile phase. Typical chromatograms illustrating the separation of metampicillin and ampicillin are shown in Fig. 2. The retention times of cephalexin, ampicillin and metampicillin were 7.4, 9.0 and 20.4 min, respectively. As shown in Fig. 2a, there were practically no interfering peaks at the retention times of metampicillin and ampicillin. The analytical column showed no decrease in efficiency after more

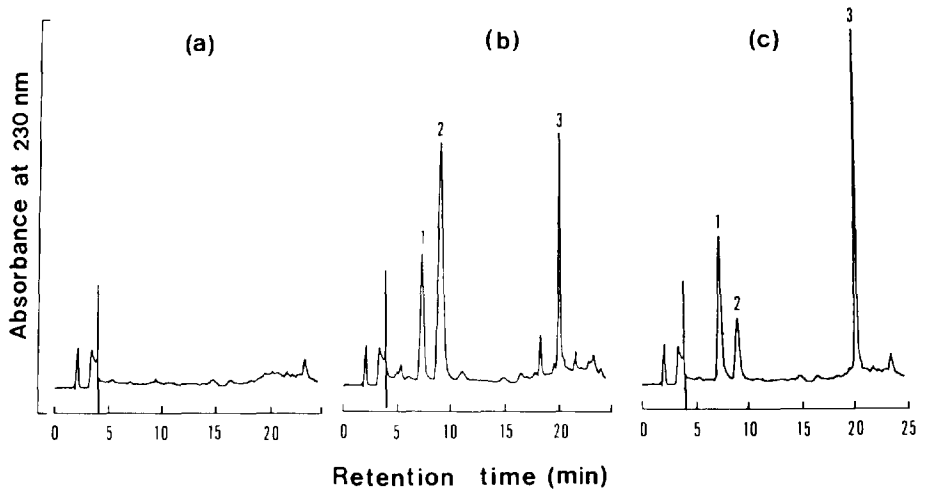

Fig. 2. Chromatograms of (a) blank plasma, (b) blank plasma spiked with metampicillin and ampicillin (each 30 μg/ml) and (c) plasma sample from a rat 10 min after an intravenous injection of 80 mg/kg metampicillin. Peaks: 1 = cephalexin (internal standard); 2 = ampicillin; 3 = metampicillin. For conditions see Experimental.

than 700 injections of biological samples such as diluted plasma, bile and urine.

Possible interferences due to the presence of other drugs such as caffeine, sulbactam, phenobarbital, ibuprofen and acetaminophen were ruled out after analyzing for these compounds at the concentrations normally present in plasma.

In the column-switching technique [11–19], the choice of the precolumn packing material, washing solvent and washing time is crucial in order to obtain complete adsorption of metampicillin and ampicillin from biological samples and to remove unwanted components from the precolumn. LiChrosorb RP-8 (25–40 μm), a nonpolar octylsilane bonded-phase adsorbent, is a suitable precolumn packing material because of its strong adsorptivity for metampicillin and ampicillin at pH 7.0, high stability at pH 1–7 and easy availability.

By using 0.05 *M* phosphate buffer (pH 7.0) as washing solvent, the majority of the plasma components and proteins are relatively unretained while ampicillin and metampicillin exhibit strong retention on the LiChrosorb RP-8 precolumn. To obtain good recovery and clean chromatograms, the clean-up process was completed in less than 5 min by washing the precolumn at a flow-rate of 1.0 ml/min with 0.05 *M* phosphate buffer only.

3.2. Linearity and detectability

The correlation of peak-height ratios with the concentrations of ampicillin and metampicillin in plasma, bile or urine was linear in the range of 0.1–100 μg/ml. The correlation coefficients were better than 0.999. Detection limits were determined as the concentration of compound giving a signal-to-noise ratio greater than 3:1. The limits of detection of ampicillin and metampicillin were respectively 0.1 μg/ml after an injection of 100 μl of diluted plasma (equivalent to 30 μl of plasma).

3.3. Recovery

The recovery of ampicillin and metampicillin from plasma was determined by the analysis of fixed amount of drugs added to plasma, followed by replicate injection of the same amount of a standard in 10 μl of buffer directly onto the analytical column, providing the 100% value. Mean absolute recoveries of ampicillin and

metampicillin in plasma were 93.7 ± 1.1 and 80.6 ± 2.6%, respectively.

3.4. Reproducibility

The precision (defined as the coefficient of variation of replicate analysis) and the accuracy (defined as the deviation between added and found concentration) of the assay for ampicillin and metampicillin were evaluated over the plasma concentration range 0.1–100 μg/ml. The results are shown in Table 1. The coefficient of variation varied from 0.4 to 5.1% of the added amount in the spiked plasma samples.

3.5. Application of the method to biological samples

The present method has been successfully applied to the analysis of more than 400 plasma, bile and urine samples from rats. A plasma chromatogram of a rat after intravenous injection of metampicillin is shown in Fig. 2c. Fig. 3 shows mean plasma concentration–time profiles of ampicillin and metampicillin following an intravenous injection of metampicillin (80 mg/kg) to rats.

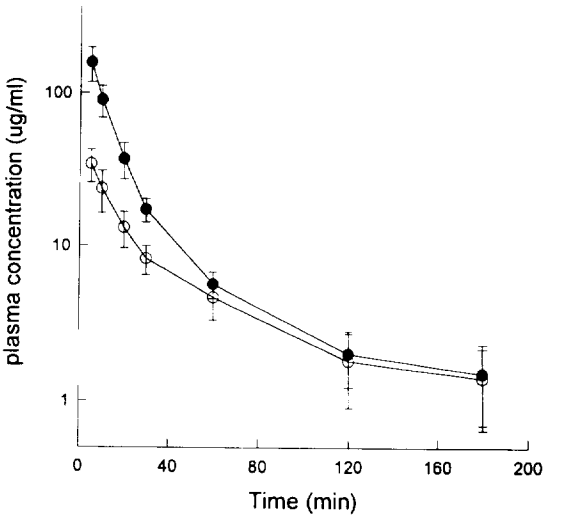

Fig. 3. Mean plasma concentration–time curves of metampicillin and ampicillin in rats after an intravenous injection of 80 mg/kg metampicillin. Each point with vertical bar represents a mean and standard error of 4 rats. (●) Metampicillin; (○) ampicillin.

4. Conclusions

For the simultaneous determination of ampicillin and metampicillin in biological fluids, a

Table 1
Reproducibility of ampicillin and metampicillin assay in plasma samples ($n = 4$)

Concentration (mg/ml)			C.V. (%)	
Added	Found		Ampicillin	Metampicillin
	Ampicillin	Metampicillin		
Within-day				
1.0	0.96	0.97	1.4	1.3
10.0	9.4	9.8	1.3	1.4
20.0	22.8	18.7	0.4	1.3
50.0	53.7	49.8	1.5	4.0
100.0	99.6	96.3	1.6	3.1
Day-to-day				
1.0	1.0	0.96	3.3	3.1
10.0	9.6	9.3	2.4	3.0
50.0	50.4	47.9	4.1	5.1
100.0	101.0	97.9	1.8	3.8

simple column-switching HPLC method with direct injection of a small amount of diluted plasma, urine and bile samples was developed. This method is readily applicable to therapeutic drug monitoring of ampicillin and metampicillin in plasma, urine and bile because of its excellent precision, sensitivity, specificity and speed. An advantage of this automated analytical process is that the hydrolysis of metampicillin to ampicillin can be limited by the direct analysis of the plasma, urine and bile samples and by avoiding time-consuming extraction steps.

References

[1] R. Sutherland, S. Elson and E.A.P. Croydon, *Chemotherapy*, 17 (1972) 145.
[2] J.M. Brogard, M. Pinget, M. Adloff, M. Dorner and J. Lavillaureix, *J. Antimicrob. Chemother.*, 2 (1976) 363.
[3] E. Ferrero and A. Lanfranconi, *Il. Farmaco. Ed. Pr.*, 26 (1970) 230.
[4] M.M.C. Dulong de Rosnay, J. Aubertin, J.Y. Lacut, M. Lesgouarres, B. Leng, J.C. Boget and F. Le Gall, *Bordeaux Medical*, 20 (1972) 2715.
[5] M. Pinget, J.M. Brogard, J. Dauchel and J. Lavillaurex, *J. Antimicrob. Chemother.*, 2 (1976) 195.
[6] R. Labia and F. Le Goffic, *Medecine et Maladies Infectieuses*, 10 (1973) 395.
[7] H. Lee, W.C. Jang and H.S. Lee, *Arch. Pharm. Res.*, 17 (1994) 378.
[8] T. Uno, M. Masada, K. Yamaoka and T. Nakagawa, *Chem. Pharm. Bull.*, 29 (1981) 1957.
[9] R.A. Blum, R.K. Kohli, N.J. Harrison and J.J. Schentag, *Antimicrob. Agents Chemother.*, 33 (1989) 1470.
[10] F. Salto, J.G. Prieto and M.T. Alemany, *J. Pharm. Sci.*, 69 (1980) 501.
[11] R. Wyss and F. Bucheli, *J. Chromatogr.*, 424 (1988) 303.
[12] H.S. Lee, E.J. Kim, O.P. Zee and Y.J. Lee, *Arch. Pharm. Res.*, 12 (1989) 108.
[13] H.S. Lee, E.J. Kim, O.P. Zee and Y.J. Lee, *Arch. Pharm. (Weinheim)*, 322 (1989) 801.
[14] H.S. Lee, O.P. Zee and K.I. Kwon, *J. Chromatogr.*, 528 (1990) 425.
[15] Y.J. Lee and H.S. Lee, *Chromatographia*, 30 (1990) 80.
[16] H.S. Lee, O.P. Zee, B.H. Woo and Y.J. Lee, *J. Environ. Sci. Health*, A26 (1991) 1253.
[17] H.S. Lee, H.C. Shin, S.S. Han and J.K. Rho, *J. Chromatogr.*, 574 (1992) 175.
[18] E.S. Jung, H.S. Lee, J.K. Rho and K.I. Kwon, *Chromatographia*, 37 (1993) 618.
[19] W.C. Jang and H.S. Lee, *J. Liq. Chromatogr.*, 17 (1994) 1375.