Hydrolysis kinetics of Metampicillin by High Performance Liquid Chromatography

Heeyong Lee, Won Cheoul Jang and Hye Suk Lee

Screening and Toxicology Center, Korea Research Institute of Chemical Technology, Yoosung, P.O. Box 107, Taejeon 305-606, Korea

(Received September 5, 1994)

The hydrolysis of metampicillin to ampicillin was investigated using high performance liquid chromatography. We developed the simultaneous determination of metampicillin and ampicillin using a Zorbax CN column and 5% acetonitrile and 8% methanol in 0.02 M phosphate buffer (pH 7.0) as mobile phase. Metampicillin was hydrolyzed to ampicillin with half life of 41.5 min at physiological pH and temperature. In acidic pH, metampicillin was rapidly hydrolyzed to ampicillin within a chromatographic separation.

Key words: Metampicillin, Ampicillin, High performance liquid chromatography, Kinetics of hydrolysis

INTRODUCTION

Metampicillin [(16R)-6-(D-2-methyleneamino-2-phenylacetamide) penicillanic acid](MAP) is a semisynthetic penicillin antibiotic produced by combination of ampicillin (AP) with formaldehyde (Sutherland *et al.*, 1972). MAP has the same broad antibacterial activity as AP and it is preferred because of high biliary excretion following parenteral administration (Berte *et al.*, 1971; Brogard *et al.*, 1976, 1985; Ferrero and Lanfranconi, 1971; Pinget *et al.*, 1976; Sutherland *et al.*, 1972). According to the electrophoretic bioassay, MAP is hydrolyzed to AP and there is a marked effect of pH on the hydrolysis rate (Sutherland *et al.*, 1972). However, this method is not appropriate to study the kinetics of hydrolysis of MAP because of the time-consuming procedure during which MAP is hydrolyzed to AP. There has been no method for the simultaneous determination of MAP and AP using high performance liquid chromatography (HPLC).

In this paper, we developed the HPLC method for the simultaneous determination of MAP and AP and evaluated the kinetics of hydrolysis of MAP at various pH and temperature. This method can be further applied to the quality control of MAP.

MATERIALS AND METHODS

Correspondence to: Hye Suk Lee, Screening & Toxicology Center, Korea Research Institute of Chemical Technology, Yoosung, P.O. Box 107, Taejeon 305-606, Korea

Materials

The sodium salts of metampicillin (MAP) and ampicillin (AP) were obtained from Samsung Pharmaceutical Co. (Seoul, Korea). HPLC grade acetonitrile and methanol were from Burdick & Jackson. All other reagents were analytical grade.

HPLC System

HPLC system consisted of a Spectra-Physics Model SP 8800 pump (SantaClara, CA, USA), a Spectra-Physics 8875 autosampler and a 1000S diode array detector (Applied Biosystems). The chromatographic data were analyzed using a Spectra-Physics 4270 integrator. The detection wavelength of the effluent was 220 nm. Nova-Pak C8 guard column (4.0×10 mm i.d., Waters Assoc., MA, USA), a Zorbax CN column (Dupont, USA), a Nucleosil phenyl column (Machery-Nagel, Duren, Germany), and an Inertsil ODS-2 column (GL Sciences, Tokyo, Japan) were used. The size of the analytical columns used was 250×4.6 mm i.d..

Chromatographic Separation of MAP and AP

To evaluate the effect of the polarity of stationary phase on the capacity factors (k') of MAP and AP, a Zorbax CN, a Nucleosil phenyl and an Inertsil ODS-2 columns were used. The mobile phase was optimized by changing the ionic strength of buffer and the percentage of organic modifier on a Zorbax CN column.

Table I. The effects of stationary phase on the capacity factor of ampicillin and metampicillin

Stationary phase	Antibiotic	capacity factor (k')	
		13%[a]	18%[a]
Zorbax CN	Ampicillin	0.3	0.0
	Metampicillin	0.7	0.0
Nucleosil phenyl	Ampicillin	0.6	0.2
	Metampicillin	8.1	1.3
Inertsil ODS-2	Ampicillin	6.5	0.8
	Metampicillin	b	10.3

mobile phase: [a]percent of acetonitrile in 0.02 M disodium phosphate buffer (pH 7.0).
detection wavelength: 220 nm; flow rate: 1 ml/min.
b: k'>30.

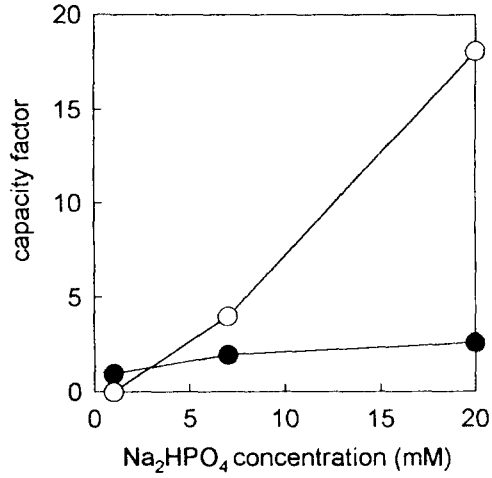

Fig. 1. Effect of disodium phosphate concentration on the capacity factor of metampicillin and ampicillin. (○) metampicillin; (●) ampicillin. The chromatographic separation was performed using a Zorbax CN column and 5% acetonitrile and 8% methanol in given concentration of disodium phosphate (pH 7.0) as mobile phase. The flow rate was 1 ml/min and detection wavelength was 220 nm.

Kinetic Stability Studies of MAP

Buffer mixtures of Clark and Lubs were used for the studies of the effect of pH on the hydrolysis of MAP to AP; pH 1.2, pH 4.0 and pH 7.0. The simultaneous determination of MAP and AP was performed using a Zorbax CN column and 5% acetonitrile and 8% methanol in 0.02 M disodium phosphate buffer (pH 7.0). The flow rate was 1 ml/min.

RESULTS AND DISCUSSION

Table I shows the effect of the polarity of the stationary phase on the k' of MAP and AP. Decreasing the polarity of the stationary phase from CN to C-18 caused the k' of MAP and AP to increase. As the bonded phase become more nonpolar, the narrow

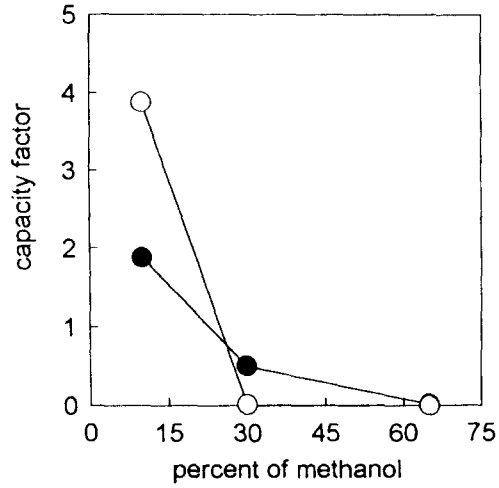

Fig. 2. Effect of methanol content on the capacity factor of metampicillin and ampicillin. (○) metampicillin; (●) ampicillin. The chromatographic separation was performed using a Zorbax CN column and given percent of methanol in 0.02 M disodium phosphate buffer (pH 7.0) as mobile phase. The flow rate was 1 ml/min and detection wavelength was 220 nm.

decrease of acetonitrile concentration caused excessive retention of MAP and the great differences in the k' values of MAP and AP. These results could be explained by the increase of the hydrophobicity of MAP due to the additon of a methylene group to amino group of AP.

The simultaneous determination of MAP and AP was obtained with an isocratic separation on a Zorbax CN column. The pH of mobile phase was selected as 7.0 considering the stability of MAP because it has been reported that MAP is liable to hydrolyze to AP and its hydrolysis rate exeeds up at acidic condition (Sutherland *et al.*, 1972). The effect of the ionic strength of mobile phase on the k' of MAP and AP is shown in Figure 1. As the ionic strength of mobile phase increased, the k' of MAP steeply increased but the k' of AP was not significantly affected.

Figure 2 is the plot for the k' of MAP and AP versus various percent of methanol in mobile phase. At more than 30% of methanol, AP and MAP were not separated. Reduction of methanol concentration led to the increase of the resolution between MAP and AP. The k' of MAP steeply changed in the various concentration range of methanol compared to the k' of AP. The use of ternary mixture of acetonitrile, methanol and buffer as mobile phase caused the optimum k' values of MAP and AP in the isocratic separation and symmetrical peaks.

Figure 3 showed the HPLC chromatograms of MAP kept in different pH solutions for a specific time. At acidic and physiological pH, the degradation product was AP. After hydrolysis of MAP to AP, further degra-

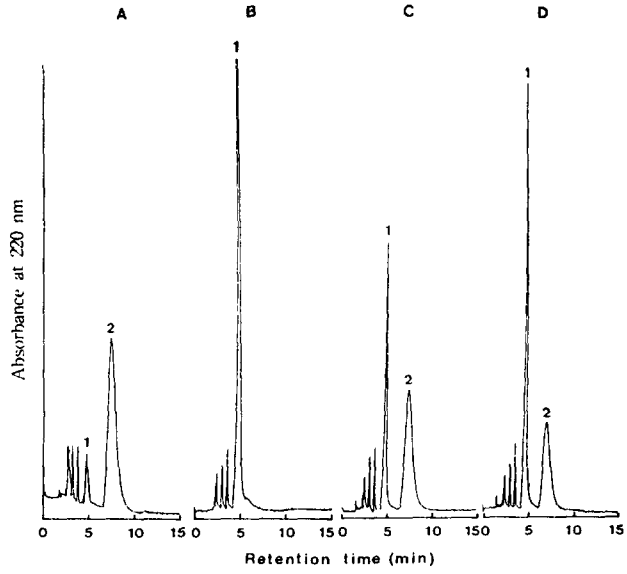

Fig. 3. HPLC chromatograms of metampicillin (50 μg/ml) incubated in different pH solutions for a given time. (A) pH 7.0, 0 min at 20℃; (B) pH 4.0, 0 min at 20℃; (C) pH 7.0, 100 min at 20℃; (D) pH 7.0, 40 min at 37℃. peaks: 1, ampicillin; 2, metampicillin.

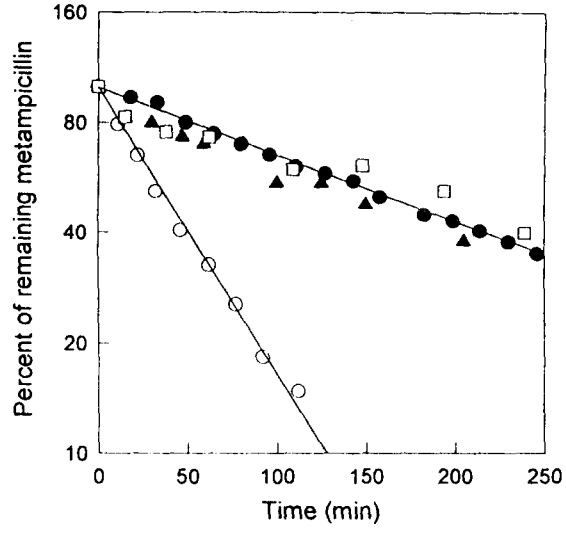

Fig. 4. Semilogarithmic plots for the hydrolysis of metampicillin to ampicillin in various physiological solutions. (○) pH 7.0 buffer at 37℃; (●) pH 7.0 buffer at 20℃; (▲) deionized water at 20℃; (□) saline solution at 20℃.

dation was not observed. These results were consistent with the fact that AP was not hydrolyzed to other compounds in acidic or physiological pH for several days. However, some degradation products of AP as well as MAP were observed at basic pH and seemed to be formed by beta-lactam ring opening.

The hydrolysis kinetics of MAP to AP in various pH solution, deionized water and saline solution were studied. It was found that the pH critically affected the hydrolysis rate. At pH 1.2 or 4.0, MAP was rapidly hydrolyzed to AP within a chromatographic separation. Figure 4 shows a semilogarithmic plot of the residual percentage of MAP amounts versus time in various solution. The hydrolysis reaction of MAP to AP approximately followed first-order kinetics. By statistical regression analysis of the semilog plots of concentration versus time, the half life of the hydrolysis reaction was obtained. The calculated half life of MAP in pH 7.0 buffer at room temperature (20℃) was 167 min. The hydrolysis rate increased as a function of the temperature. At 4℃ and pH 7.0, less than 10% of MAP was hydrolyzed to AP for 1 day. At physiological temperature (37℃) and pH (7.0), the half-life was 41.5 min.

In conclusion, the present study described the HPLC method for the simultaneous determination of MAP and AP and the kinetics of hydrolysis of MAP to AP as a function of pH and temperature. At physiological temperature and pH, the hydrolysis proceeds with a half-life of 41.5 min.

REFERENCES CITED

Berte, F., Manzo, L., de Bernardi, M., and Benzi, G., Distribution of ampicillin administered orally in three different forms in rabbit. *J. Pharm. Sci.*, 60, 805-806 (1971).

Brogard, J. M., Pinget, M., Adloff, M., Domer, M., and Lavillaureix, J., Experimental and clinical pharmacology of metampicillin in the biliary tract. *J. Antimicrob. Chemother.*, 2, 363-371 (1976).

Brogard, J. M., Pinget, M., Domer, M., and Blickle, J. F., Biliary elimination of beta-lactam antibiotics by the isolated perfused rabbit liver. *Liver*, 5, 147-155 (1985).

Ferrero, E. and Lanfranconi, A., Tropismo Biliare Della Metampicillina e Dell'ampicillina. *Il Farmaco. Ed. Pr.*, 26, 230-236 (1971).

Pinget, M., Brogard, J. M., Dauchel, J., and Lavillaureix, J., Biliary excretion of ampicillin, metampicillin and carbenicillin. *J. Antimicrob. Chemother.*, 2, 195-201 (1976).

Sutherland, R., Elson, S., and Croydon, E. A. P., Metampicillin: antibacterial activity and absorption and excretion in man. *Chemotherapy*, 17, 145-160 (1972).