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Short Communication

Determination of a novel β-lactam antibiotic (E-1100) in rat plasma by high-performance liquid chromatography

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ABSTRACT

A fast, simple and accurate method to determine the concentration of E-1100, a novel β-lactam antibiotic, in rat plasma has been developed. This method involves deproteinization by methanol with 0.2% perchloric acid and reversed-phase high-performance liquid chromatography with ultraviolet detection. Regression analysis showed that the method was linear over the standard-curve range from 0.191 to 191.0 μg/ml. This newly developed method has been applied for the analysis of plasma samples in a preliminary pharmacokinetic study in rats.

INTRODUCTION

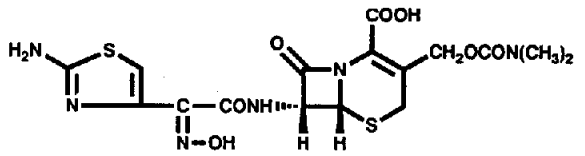
(+)-(6*R*,7*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-N,N-dimethylcarbamoyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (E-1100, Fig. 1) is a novel β-lactam antibiotic which has a wide spectrum of antibacterial activity [1]. However, E-1100 can not be absorbed from the gastrointestinal tract due to its high hydrophilicity according to the pH-partition hypothesis [2]. In order to improve the oral bioavailibility, preparation of a lipophilic pro-drug has been investigated.

This paper describes a simple high-performance liquid chromatographic (HPLC) procedure for the determination of E-1100 in rat plasma and the preliminary pharmacokinetics in rats after intravenous administration of E-1100.

EXPERIMENTAL

Materials

The sodium salts of E-1100 (E-1100-Na) and



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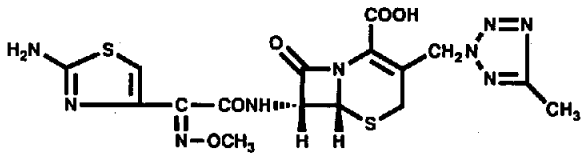

(2)

Fig. 1. Structures of E-1100 (1) and cefteram (2).

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cefteram (Fig. 1) [3,4] were synthesized in our laboratories. E-1100-Na and sodium cefteram were confirmed to be more than 95% pure by HPLC. Other chemicals were of reagent or HPLC grade.

Stock solution

Sodium salt of E-1100 (100 mg) was dissolved in 10 ml of pH 4.0 phosphate buffer (0.2 M, μ = 0.4), yielding a solution of 9.55 mg/ml E-1100, which was separated into 1.0 ml aliquots and stored at −35°C. Sodium cefteram was dissolved in the pH 4.0 buffer solution to make a 10 μg/ml solution. The solution (100 ml) was separated into 5.0 ml aliquots and stored at −35°C. This sodium cefteram solution was used as the internal standard (I.S.) solution.

Apparatus and chromatographic conditions

The HPLC system consisted of a Model LC-6A pump, equipped with a Model SCL-6B system controller, a Model SPD-6A UV spectrophotometric detector, a Model CTO-6A column oven, a Model C-R4AX Chromatopac, and a Model SIL-6B autoinjector, all from Shimadzu (Kyoto, Japan). The mobile phase was acetonitrile–water–perchloric acid (60%)–sodium perchlorate monohydrate (156:844:1:5, v/v/v/w). The chromatographic column was a YMC Pack AM312 ODS (150 mm × 6 mm I.D., particle diameter 5 μm) obtained from Yamamura Chemical Lab. (Kyoto, Japan). The flow-rate, the wavelength for determination, and the temperature of the column were 1 ml/min, 254 nm and 40°C, respectively.

Calibration curve samples

The stock solution of E-1100 was diluted with drug-free rat plasma to obtain plasma standard solutions with a concentration of 0.191, 0.382, 0.955, 1.91, 3.82, 9.55, 19.1, 38.2, 95.5 and 191.0 μg/ml. The final volume of each plasma standard solution was 2 ml. For the daily standard curves, one ml of the plasma standard solution of each concentration was prepared and the standard curves were obtained from analysis of three plasma samples at each concentration.

Assay procedures

Methanol (200 μl) containing 0.2% perchloric acid and 50 μl of I.S. solution was added to 100 μl of plasma cooled in an ice-bath. The mixture was vortex-mixed for 1 min and centrifuged at 3300 g for 10 min at 4°C; 20 μl of the supernatant were injected into the chromatograph.

Recovery

The absolute recovery of E-1100 was calculated by the following method. The plasma standards of E-1100 with concentrations of 0.955, 9.55 and 95.5 μg/ml were treated by the method described in the *Assay procedures*. However, 50 μl of the pH 4.0 buffer solution replaced the I.S. solution. After centrifugation at 3300 g for 10 min the supernatant was removed, and 50 μl I.S. solution was added to the supernatant. The mixture was vortex-mixed for 1 min and 20 μl of the mixture was injected into the chromatograph. The peak-area ratio of E-1100 to I.S. was compared with that of the standard solution prepared with pH 7.4 buffer solution at the equivalent concentration.

The relative recovery of E-1100 was determined by comparing the peak-area ratio obtained from the treatment of the plasma standards with that of the standard solutions prepared with pH 7.4 buffer solution at the equivalent concentration.

Calculations

The peak areas in the HPLC profiles were measured, and the peak-area ratio of E-1100 and I.S. was calculated. A calibration curve was established comparing the peak-area ratio with the concentration of E-1100 in the standards. The slope and intercept of the calibration curve were calculated using weighted ($1/y$) linear regression. The concentrations of E-1100 in the experimental samples were calculated using the equation x (μg/ml) = $(y - b)/a$, where y is the ratio of E-1100 to I.S. in an experimental sample and b (intercept) and a (slope) are constants generated by the linear regression analysis of the calibration curve data.

Animal study

Male Sprague–Dawley rats were used; they were fasted for 12–18 h before drug administration. All rats (272–285 g body weight) were allowed free access to water, but no food was given. The sodium salt of E-1100 was dissolved in saline to a concentration of 19.1 mg/ml of E-1100. An amount of 1.047 ml of this solution per 1 kg body weight was injected into the right jugular vein after the rats were lightly anesthetized with ether. Blood samples (0.3 ml) were withdrawn from the left jugular vein of the lightly anesthetized rat. The samples were centrifuged at 3500 *g* for 15 min to obtain plasma (0.12–0.15 ml), which was subjected to HPLC for E-1100 determination on the same day, according to the method described above.

Data analysis

Pharmacokinetic analysis of the plasma concentration data was performed using model-independent methods. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule and then added to the value of the plasma concentration at the last determined time divided by the terminal elimination rate constant (β), which was calculated by a least squares method on a semi-logarithmic scale. The half-life ($t_{1/2}$) was calculated from the terminal elimination rate constant (β) by the following equation:

$$t_{1/2} = 0.693/\beta$$

Total plasma clearance (Cl) was calculated by the following equation:

$$Cl = \text{dose}/AUC$$

The apparent volume of distribution at steady-state (V_{dss}) and the mean residence time (MRT) were estimated by the following equations:

$$V_{dss} = \text{dose} \times AUMC/AUC^2$$

$$MRT = AUMC/AUC$$

$AUMC$ is the area under curve of the product of time and the plasma drug concentration *versus* time from time zero to infinity,

$$AUC = \int_0^\infty C_t \, dt,$$

$$AUC = \int_0^\infty t \, C_t \, dt,$$

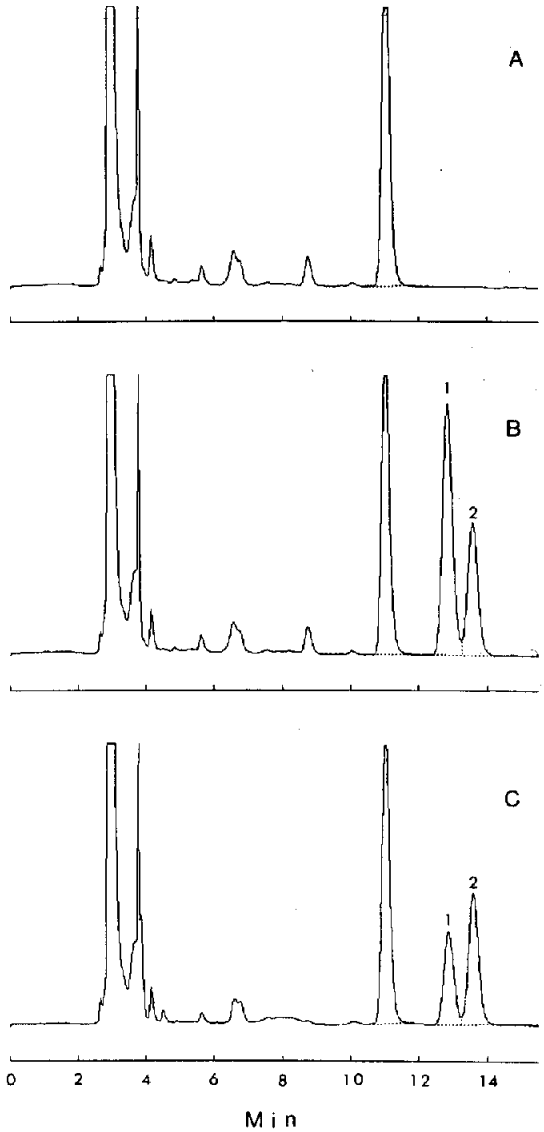

Fig. 2. HPLC of rat plasma containing E-1100 (1) and I.S. (2). (A) Drug-free plasma; (B) plasma spiked with E-1100 at 9.55 μg/ml; (C) plasma of a rat 8 h after administration of 20 mg/kg of E-1100 (concentration of E-1100 is 3.64 μg/ml). Chromatographic conditions as described under Experimental. The sensitivity of the detector was operated at 0.005 AUFS. A 100-μl sample of plasma was used for the assay.

where C_t is plasma concentration at time t. In general, AUC is called the zero(-order) moment of the drug concentration curve and MRT is called the first moment of the curve.

RESULTS AND DISCUSSION

Typical chromatograms of E-1100 obtained under the conditions described above are shown in Fig. 2. The retention times were approximately 12.9 min for E-1100 and 13.6 min for sodium cefteram (I.S.). The retention times of E-1100 and I.S. are fairly close, but this did not interfere with the determination of E-1100 as described below. Cefuroxime [5] and ampicillin with retention times of 20.7 min and 18.1 min, respectively, could have been used as internal standard. However, the run time could be shortened by using sodium cefteram as the internal standaard. The chromatogram of drug-free plasma showed no detectable interference from endogenous substances in the plasma.

Linear regression analysis gave slope, intercept and correlation coefficient values of $y = 0.172221x - 0.00164851$, $r = 0.9994$, weighting factor $= 1/y$, for the plasma calibration curve. The intra-day precision and accuracy were determined by analyzing five replicates at each drug concentration. The precision and accuracy of this method, as shown in Table I, were found to range from 1.6 to 10.0%, and from 95.9 to 102.7%, respectively.

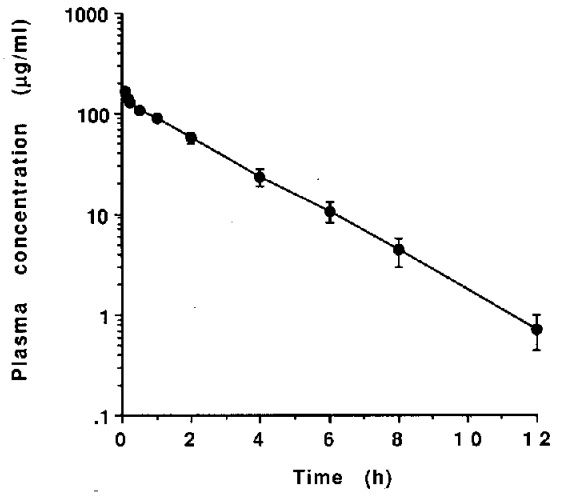

Fig. 3. Plasma levels of E-1100 after intravenous administration of E-1100 at 20 mg/kg to rats. Each point represents the mean ± S.E. of 3 rats.

The inter-day precision and accuracy were determined by analyzing triplicates at each standard concentration over three different days. The result for the calibration curve is also shown in Table I. The precision at concentrations ranging from 0.191 to 191.0 μg/ml was 9.0% or better.

TABLE I

INTRA-DAY AND INTER-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF E-1100 IN RAT PLASMA

Actual concentration (μg/ml)	Intra-day (n = 5)		Inter-day (n = 9)	
	Precision[a] (%)	Accuracy[b] (%)	Precision[a] (%)	Accuracy[b] (%)
0.191	4.5	100.0	9.0	91.3
0.382	10.0	95.9	3.4	97.2
0.955	2.2	99.4	3.1	99.5
1.91	4.6	98.9	4.6	102.2
3.82	1.6	102.3	3.2	105.6
9.55	3.0	102.0	3.5	101.0
19.1	3.1	102.7	3.8	101.7
38.2	4.7	101.7	4.2	103.2
95.5	4.0	98.9	4.0	100.4
191.0	3.0	100.0	3.7	99.1

[a] (S.D. of concentration found/its mean) × 100.

[b] (Concentration found/actual concentration) × 100.

The accuracy ranged from 91.3 to 105.6%. These values are thought to be acceptable.

The absolute recoveries of E-1100 at concentrations of 0.955, 9.55 and 95.5 μg/ml were 76.6 ± 2.3 (mean ± S.D., $n = 4$), 70.2 ± 2.1 and 76.0 ± 2.5%, respectively. The relative recoveries at the same concentrations were 100.9 ± 3.2, 96.2 ± 1.5 and 97.7 ± 2.8%, respectively.

Fig. 3 shows the plasma concentration–time curve of E-1100 after intravenous administration to rats at a dose of 20 mg/kg. All values are the means ± S.E. of three rats. The plasma levels of E-1100 apparently decreased with time following a bi-exponential curve. The value of the AUC was 288.95 ± 9.24 (mean ± S.E.) μg/h/ml. MRT and $t_{1/2}$ were 2.09 ± 0.06 h and 1.55 ± 0.09 h, respectively. The values of Cl and V_{dss} were 69.36 ± 2.27 ml/h/kg and 144.73 ± 2.35 ml/kg, respectively.

In conclusion, the proposed method, requiring only a small volume of plasma (100 μl) and having a rapid sample preparation, is simple, precise and accurate, and can be used in human and animal pharmacokinetic studies.

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