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ANALYTICAL STUDIES ON β-LACTAM ANTIBIOTICS

III AUTOMATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THE ORALLY ACTIVE ANTIBIOTIC CEFTIBUTEN IN HUMAN PLASMA AND URINE

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SUMMARY

A fully automated high-performance liquid chromatographic method was established for the determination of the oral cephalosporin antibiotic ceftibuten The procedure for plasma assay involves on-line sample clean-up with a precolumn of BSA-ODS (ODS coated with bovine serum albumin) and subsequent determination of the drug with a reversed-phase C_{18} column using a column-switching technique The precolumn effectively removed protein components and hydrophilic substances from plasma, with ceftibuten and its metabolite, the *trans*-isomer of ceftibuten, being retained using an ion-pairing reagent, tetra-*n*-butylammonium bromide, in the mobile phase In urine assay, an ODS precolumn was used in place of the BSA-ODS column The urine sample, after 10-fold dilution, was analysed in a similar manner to that used in the plasma assay A large proportion of hydrophilic substances was eliminated by the on-line clean-up and the residual interfering substances introduced into the analytical column were separated from ceftibuten and its metabolite using the ion-pairing reagent This method permits the determination of 0 1–20 μg/ml of ceftibuten and its metabolite in human plasma and 1–200 μg/ml of both compounds in urine The advantages of the method are easy performance without manual sample preparation, saving of plasma (50 μl) and high sensitivity The method was applied to pharmacokinetic studies of ceftibuten after oral administration to healthy subjects

INTRODUCTION

Ceftibuten, (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-4-carboxy-2-butenoylamino]-8-oxo-5-thia-1-azabicyclo[4 2 0]oct-2-ene-2-carboxylic acid (I, Fig. 1), is an oral cephem antibiotic developed by Shionogi (Osaka, Japan) It ex-

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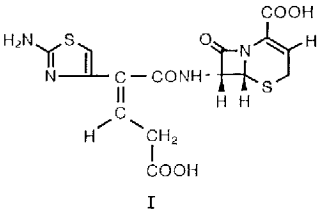

I

Fig 1 Structure of ceftibuten (I)

hibits antibacterial activity against a wide range of Gram-negative and certain Gram-positive bacteria [1,2] Previously, we reported a high-performance liquid chromatographic (HPLC) method for the determination of the plasma and urine levels of compound I after its administration [3] Further studies of HPLC analysis were essential for extending the pharmacokinetic and pre- and clinical investigations.

Automated HPLC methods for the assay of drugs using two-column systems with a column-switching device are now widely used Such a method allows direct injection of the plasma sample with on-line sample clean-up and/or concentration of analytes by the pretreatment column(s), followed by analysis with the analytical column [4–10] In order to automate sample preparation in the plasma assay, we selected a BSA–ODS (ODS coated with bovine serum albumin) column (Tosoh, Tokyo, Japan) for the pretreatment column A large proportion of the protein fragments and hydrophilic components in plasma could be eluted from it because of the low interaction with BSA, whereas hydrophobic substances with a small molecular size interacted with the ODS phase and could be retained on the column [9,11,12] In this study, we established an automated HPLC method for the assay of compound I and its metabolite, the *trans*-isomer of ceftibuten (II), in plasma, which permitted on-line clean-up and drug analysis using two columns, BSA–ODS and reversed-phase C_{18}, which were switched during the procedure We also automated the urine assay using two reversed-phase C_{18} columns. Compounds I and II retained on the precolumn were transported to the analytical column A fraction of the analytes was cut off by column switching and the urinary components were effectively eliminated The method allowed direct injection of plasma and diluted urine The efficiency of the analysis was improved by eliminating the manual sample preparation steps in the previous method

EXPERIMENTAL

Materials and reagents

Compounds I and II were supplied by our laboratories Methanol, acetonitrile and distilled water (HPLC grade) were purchased from Wako (Osaka, Japan) All other reagents were of JIS special grade

Apparatus

Flow diagrams of the fully automated system for the determination of compounds I and II in plasma and urine samples are shown in Fig 2. The chromatographic system consisted of a Shimadzu LC-6A instrument (Shimadzu, Kyoto, Japan) connected with a WISP 710B automatic injector (Waters Assoc , Milford, MA, U S A) with a cooling unit (4°C)

Solvent delivery unit and valve One of the two LC-6A pumps carries S_1 or S_2 to the precolumn (column 1) through the low-pressure channel selection valve (FCV-3AL) Another pump carries S_3 to the analytical column (column 2) through the high-pressure channel selection valve (FCV-2AH)

System controller The SCL-6A system controller module controls the solvent delivery units (flow-rate) and the column-switching times It is activated automatically according to the conditions programmed before the analysis

Data integration system A Shimadzu Chromatopac C-R3A data processor was used

Detector A Shimadzu SPD-6AV variable-wavelength UV detector was used with the wavelength set at 256 nm

Column In both plasma and urine assays, a Nucleosil $5C_{18}$ (5 μm) column (15 cm×4 6 mm I.D , Chemco Scientific, Osaka, Japan) was used as an analytical column A TSK BSA–ODS (20 μm) column (3 5 cm×4 6 mm I.D., Tosoh) was used as a precolumn in the plasma assay A Cosmosil $5C_{18}$ (5 μm) column (5 cm×4 6 mm I D , Nacalai Tesque, Kyoto, Japan) was used as a precolumn in the urine assay The column temperature was ambient

Mobile phase for plasma assay

The pretreatment solvent (S_1) was [5 m*M* tetra-*n*-butylammonium bromide (TBAB)–2 m*M* monobasic ammonium phosphate (pH 5 0)]–methanol (50 1, v/v) The washing solvent (S_2) for column 1 was [5 m*M* TBAB–10 m*M* monobasic ammonium phosphate (pH 7 0)]–acetonitrile (10 3, v/v) The mobile phase for analysis (S_3) consisted of [10 m*M* TBAB–2 m*M* monobasic ammonium phosphate (pH 5.0)]–acetonitrile–methanol (25 6 3, v/v)

TBAB and monobasic ammonium phosphate were dissolved in water, the pH was adjusted to 5.0 or 7.0 with 0 1 *M* sodium hydroxide and then water was added to the prescribed volume.

Mobile phase for urine assay

The common solvent for pretreatment and washing (S_1) was 10 m*M* monobasic ammonium phosphate (pH 5)–methanol (10 1, v/v) The mobile phase for analysis (S_3) consisted of an aqueous mixture of three salts [5 m*M* TBAB, 5 m*M* tetra-*n*-amylammonium bromide (TAAB) and 8 m*M* monobasic ammonium phosphate]–acetonitrile–methanol (65 25 10, v/v) TBAB, TAAB and monobasic ammonium phosphate were dissolved in water, the pH was

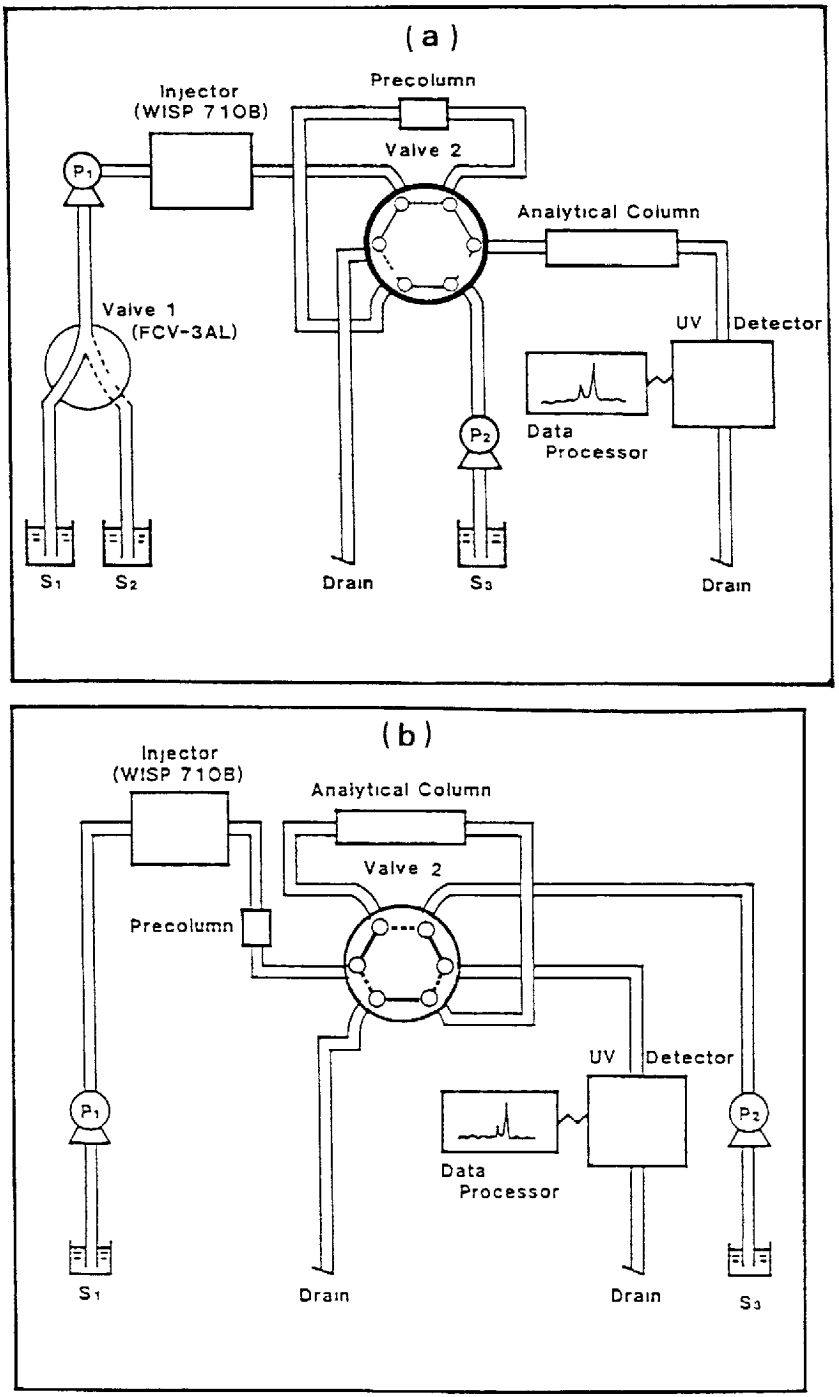

Fig 2 Flow diagram of the automated HPLC system Valve 2 is positioned at "2", after treatment of plasma, sample is transported in the back-flush mode (a) and after treatment of urine, sample is transported in the forward-flush mode (b) P_1 and P_2 = pumps, valve 1 = low-pressure channel selection valve, valve 2 = high-pressure channel selection valve, S_1 = pretreatment solvent, S_2 = washing solvent, S_3 = mobile phase for separation

adjusted to 5 0 with 0 1 *M* sodium hydroxide and then water was added to the prescribed volume

Column-switching procedure for plasma assay

The switching times for the two valves and the flow-rates of the three solvents in the chromatographic run (time programme) were programmed and stored in the system controller (Fig 2a)

Conditions of the two columns The two valves were positioned at "1" S_1 and S_3 flowed through the precolumn (column 1) and the analytical column (column 2), respectively, along the dotted lines in valve 2, at flow-rates of 1 2 and 1 0 ml/min, respectively Both solvents were finally flushed into the drain

Injection of the sample The sample was injected from the autoinjector (0 min) S_1 flowed for 6 min at a rate of 1 2 ml/min Compounds I and II were retained near the head of column 1

Transfer of the analytes from the precolumn to the analytical column At 6 min after sample injection, valve 2 was positioned at "2" The solvent delivery into column 1 was changed from S_1 to S_3 The analytes in column 1 were back-flushed (flow-rate, 1 0 ml/min) along the solid lines in valve 2 to column 2 S_1 was flushed into the drain

Separation of the analytes and washing of precolumn At 9 min after sample injection, valves 1 and 2 were switched to positions "2" and "1", respectively Through the continuous flow of S_3 at the same rate, further separation of the analytes and other substances from plasma took place Compounds I and II peaked at 23 and 21 min, respectively Column 1 was then eluted with S_2 (flow-rate, 1 5 ml/min) to wash out the remaining substances

Conditioning of the pretreatment column At 14 min after sample injection, valve 1 was turned back to the original position ("1") Column 1 was re-equilibrated by elution with S_1 at a flow-rate of 1.5 ml/min until the next sample injection The injection interval for this procedure was 25 min

Column-switching procedure for urine assay (Fig 2b)

Conditioning of the two columns The valve was positioned at "1" S_1 and S_3 flowed through the precolumn and the analytical column, respectively, along the dotted lines in the valve at flow-rates of 1 0 ml/min Both solvents were finally flushed into the drain

Injection of the sample The sample was injected from the autoinjector (0 min) S_1 flowed for 1 5 min at a rate of 1 0 ml/min Compounds I and II were retained near the end of the precolumn

Transfer of the analytes from the precolumn to the analytical column At 1 5 min after sample injection, the valve was positioned at "2" The analytes in the precolumn were transferred to the analytical column with S_1 in the forward-flush mode (flow-rate, 1 0 ml/min) along the solid lines in the valve to the analytical column S_3 was flushed into the drain

Separation of the analytes and washing of the precolumn At 3.5 min after sample injection, the valve was switched to position "1" The continuous flow of S_3 at the same rate led to further separation of the analytes and other substances from urine in the analytical column Compounds I and II peaked at 20 0 and 18 6 min, respectively The precolumn was then eluted with S_1 (flow-rate, 1 5 ml/min) for at least 10 min to wash out the remaining substances until the next sample injection The injection interval for this procedure was 22 min

Collection of blood and urine samples

Blood was first collected before administration of the drug (0 h), and then at 0 5, 1, 1 5, 2, 2 5, 3, 4, 5, 6, 8, 12, 16 and 24 h after administration All the samples were placed in heparinized polyethylene tubes and centrifuged at 2000 *g* for 15 min at 4°C The separated plasma portions were transferred to polyethylene tubes The plasma samples, after being immediately frozen at −70°C in a dry ice–methanol bath, were stored in a freezer at −70°C until the assay

Urine was first collected before drug administration (0 h), and then at 0–2, 2–4, 4–6, 6–8, 8–10, 10–12 and 12–24 h after the administration The urine volumes were measured and aliquots of the sample were placed in a glass tube The urine samples, after being immediately frozen in a dry ice–methanol bath, were stored in a freezer at −70°C until the assay

Sample preparation

About 200 μl of a plasma sample was filtered with an Ekicrodisc 13 membrane (0 45 μm) (Gelman Sciences, Tokyo, Japan) and the filtrate was introduced into a tube (Limited Volume Inserts, 300 μl, Waters Assoc) which was then put in the tray of the autoinjector A 50-μl portion of the filtrate was injected into the column from an autoinjector

A 1-ml portion of the urine was pipetted into a 10-ml volumetric flask and diluted to volume with phosphate buffer (pH 7 0) About 2 ml of diluted urine was filtered with an Ekicrodisc 13 membrane (0 45 μm) and the filtrate was introduced into a vial (Limited Volume Inserts, 4 ml) which was then put in the tray of the autoinjector A 20-μl portion of the solution was injected into the column from the autoinjector The areas of the peaks of componds I and II were measured The concentrations of compounds I and II were calculated using a calibration graph

Standard solution

About 11 6 mg of reference standard I, equivalent to 10 mg potency, was accurately weighed in a 20-ml volumetric flask It was dissolved and diluted to volume with phosphate buffer (pH 7 0) (a 0 1 *M* solution was prepared with monobasic and dibasic sodium phosphate) A 1-ml portion of the solution was pipetted into a 25-ml volumetric flask and diluted to volume with phosphate

buffer (pH 7 0) Next, 0 5, 1, 2, 3, 4 and 5 ml of the solution were pipetted into 10-ml volumetric flasks and diluted to volume with phosphate buffer (pH 7 0) [equivalent to 1, 2, 4, 6, 8 and 10 μg (potency)/ml] These solutions were prepared just before the analysis

Calibration graph

A portion (50 μl for plasma assay and 20 μl for urine assay) of each of the six standard solutions of compound I was injected from the autoinjector into the chromatographic system The peak area was plotted against the concentration of compound I to give a calibration graph This calibration graph was also used for the assay of compound II

Stability of compound I in plasma and urine

Two spiked solutions were prepared by mixing 0 1 volume of I standard solution with 9 9 volumes of human plasma or urine The concentrations were 5 and 100 μg/ml, respectively A 1-ml volume of each of the samples was transferred into a glass centrifuge tube. The samples were allowed to stand for the specified times and then assayed The amounts of residual compounds I and II produced were measured

RESULTS AND DISCUSSION

Chromatographic conditions

As compounds I and II are very polar, acidic compounds, they were only weakly retained on the reversed-phase ODS column (Nucleosil $5C_{18}$) To enhance their retention, an ion-pairing reagent, TBAB, was used. The optimum chromatographic conditions were established after determining the effect of the TBAB and phosphate buffer concentrations and the pH on the retention time The data shown below were obtained using the analytical column alone

Effect of TBAB concentration on retention time Both compounds I and II were retained at pH 5.0 by the ion-pair interaction with good resolution as the TBAB concentration increased A good resolution was obtained at 10 m*M*

Effect of pH on retention time Phosphate buffers (2 m*M*) of various pH containing TBAB (10 m*M*) were tested and, as shown in Fig 3, the maximum retention times were obtained at pH 5 0 for both compounds Strong retention was useful for avoiding interfering peaks from the plasma components. A pH of 5 0 was used as the optimum pH of the mobile phase

Effect of phosphate buffer concentration on retention time The concentration of pH 5 0 monobasic ammonium phosphate also affected the retention time in the presence of TBAB (10 m*M*) As shown in Fig 4, the lower the concentration, the more strongly both compounds were retained and the better was their separation

Effect of solvent composition on retention time A ternary mixture of solvents

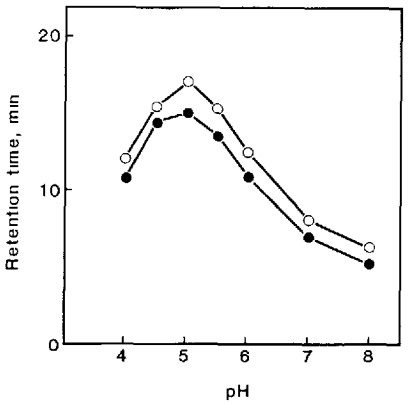

Fig 3 Effect of pH of the mobile phase on retention time for ceftibuten (○) and its metabolite (●)

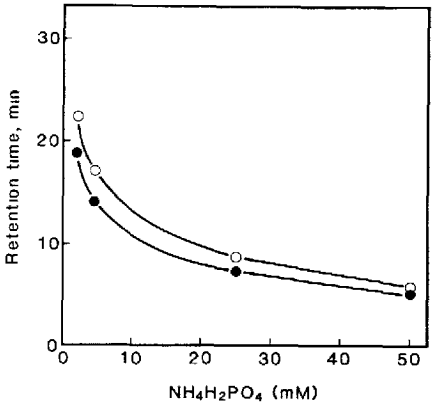

Fig 4 Effect of $NH_4H_2PO_4$ concentration in the mobile phase at pH 5 0 on retention time for ceftibuten (○) and its metabolite (●)

(phosphate buffer–acetonitrile–methanol) was finally used In the phosphate buffer (pH 5 0)–acetonitrile system there was a large difference in the retention times of compounds I and II Addition of methanol led to closer retention times and also caused faster elution of the interfering substances The optimum proportions of phosphate buffer (pH 5 0)–acetonitrile–methanol were set at 25 6 3 (v/v)

Conditions of on-line sample clean-up for plasma assay

A large proportion of proteins and highly polar water-soluble substances were not retained on the BSA–ODS precolumn [11,12] with the buffer solutions (pH 5 0–7 0), whereas compounds I and II were trapped near the head of the column, showing the possibility of sample cleaning Testing showed the

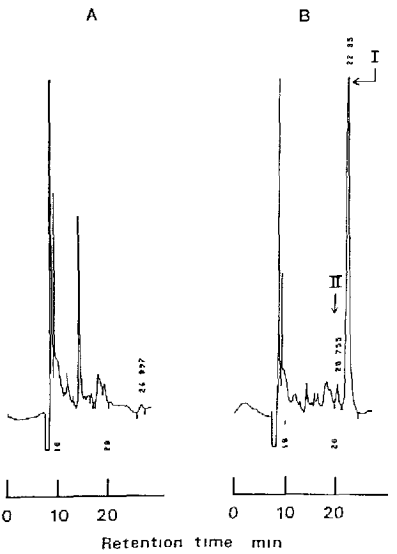

Fig 5 Chromatograms of (A) human plasma from a healthy subject and (B) human plasma from the same subject collected 1 h after oral administration of 200 mg of compound I Retention times I=22 9 min (7 54 μg/ml), II=20 8 min (0 22 μg/ml)

time required for the clean-up to be 4–10 min Interfering substances from the plasma could be almost eliminated over 4 min, giving the same chromatogram (blank) after subsequent elution on to the analytical column After the clean-up, compounds I and II were eluted with the mobile phase for analysis in the back-flush mode The time required for complete recovery from the precolumn was 1–4 min Compounds I and II were completely recovered through the analytical column in 4 min, appearing as the sharpest peaks after further elution with the same mobile phase When both compounds were eluted in the forward-flush mode, peak broadening took place The conditions selected were 6 min for the clean-up (flow-rate, 1.2 ml/min) and 3 min (flow-rate, 1.0 ml/min) for elution in the precolumn

A human plasma sample was treated and assayed under the above conditions Fig 5 shows a typical chromatogram for a plasma sample from a healthy subject after an oral dose of 200 mg of compound I. Compounds I and II were detected at 22.9 and 20 8 min, respectively The background peaks (control plasma before the administration) from human plasma did not interfere with the analyte peaks.

Conditions of on-line sample clean-up for urine assay

The HPLC system for plasma assay could not be applied to urine samples. Much larger amounts of interfering substances were retained on the BSA–ODS column with compounds I and II when urine samples were treated than

with plasma. Compounds I and II, after being transported to the analytical column, could not be detected because of the elution of urinary substances

Clean-up of the urine sample was performed using a precolumn (Cosmosil $5C_{18}$) connected on-line in the system To examine the effect of the precolumn, the elution pattern of urine was tested using the precolumn alone without an analytical column Urine was injected into the precolumn and the effluent was sent directly to the detector Urinary components were found at retention times between 0 1 and 22 min Compounds I and II, after being injected separately, were eluted at 1 6 min and 2 1 min, respectively To eliminate as much of the urinary components as possible and then transport the analytes to the analytical column, urinary components eluted with buffer–methanol (S_1) for 1 5 min were flushed into the drain and then eluted fragments were carried to the analytical column by adjusting the column-switching valve. All the analytes were removed with the buffer in the forward-flush mode Less polar materials from the urine retained on the column were cut off Thus, a large proportion of the urinary components were eliminated At 3 5 min after the sample injection, the switching valve was returned to its original position and the mobile phase (S_3) for analysis was eluted into the analytical column (Nucleosil $5C_{18}$) Although compounds I and II were weakly retained on the reversed-phase column, the retention time could be enhanced by using a mobile phase containing ion-pairing reagents, which led to good separation from urinary components A satisfactory chromatogram without interfering peaks was obtained Fig 6 shows

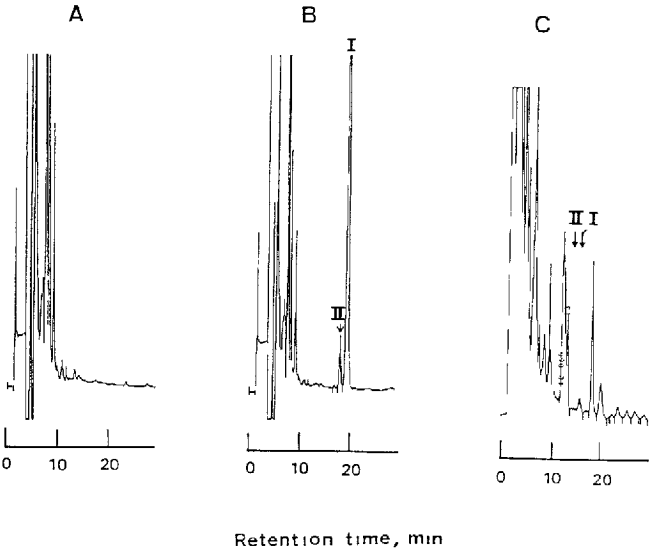

Fig 6 Chromatograms of (A) human urine from a healthy subject (control), (B) human urine from the same subject collected 2–4 h after oral administration of 200 mg of compound I (I, 94 6 μg/ml, II, 5 5 μg/ml) and (C) control urine not subjected to pretreatment

the chromatograms for a control urine sample and a sample after oral administration of compound I to a healthy subject Both compounds I and II were detected, at 20 0 and 18 6 min, respectively When a urine sample was introduced directly into the analytical column without use of the precolumn, interfering peaks appeared around the retention times of both compounds A trace amount of the peak around the retention time of compound I eluted from urine was effectively removed by using the two ion-pairing reagents (TBAB and TAAB)

Recovery test

Plasma Standard of compound I was spiked with human serum and seven samples containing 0 5–10 μg/ml were injected into the system and assayed A calibration graph was prepared by the same method using a standard solution Regression data are shown in Table I. A linear relationship was obtained between the amounts of compound I found by this analytical procedure and the added amounts, the line passed through the origin ($y=1\ 011x+0\ 024$, $s=0\ 066$, $r=0.9998$). Without any influence from the plasma components, 100% recovery was obtained The recovery of compound II, also determined by the same method, was 100% The recovery rate was reproducible between days The data confirmed that the absorption strengths of compounds I and II were the same at 256 nm; the amount of compound II on the chromatogram was calculated using a common calibration graph constructed for compound II Although compound I was bound to human serum albumin (64% at 5 μg/ml concentration by an ultrafiltration method [13]), its weaker binding to the albumin compared with the ODS phase in the presence of TBAB led to retention on the precolumn

A recovery test was also conducted using various amounts (μl) of a spiked sample containing 2 5 μg/ml compound I The assayed values for 10–100 μl samples were proportional, showing complete trapping of the analytes and no interference by plasma components in this range

Urine Standard of compound I was spiked with human urine and seven samples containing 1–200 μg/ml compound I were injected into the system and assayed A calibration graph was prepared using six standard solutions A lin-

TABLE I

TYPICAL REGRESSION DATA FOR CALIBRATION GRAPHS CALCULATED FROM THE ASSAY METHODS

Sample	Concentration range (μg/ml)	Regression equation[a]	Correlation coefficient	n	s^b
Plasma	1- 10	$y=59644x-4363$	0 99999	6	1138
Urine	10-100	$y=27641x-857$	0 99996	6	1067

[a] x = concentration, y = peak area

[b] Estimate of residual standard deviation

ear relationship was obtained between the amounts of compound I found by this analytical procedure and the added amounts; the line passed through the origin ($y=1\ 002x-0\ 205$, $s=0\ 761$, $r=0\ 99998$) The recovery was 100% without any influence from the urine components The recovery of compound II was also determined by the same method and found to be 100%. The recovery rate was reproducible between days

Accuracy, precision and limit of determination

Two spiked plasma samples with different concentrations were assayed using this system and the results showed good accuracy and precision. The coefficients of variation were 1 5% ($n=7$) for a 1 μg/ml sample and 0 5% ($n=7$) for a 5 μg/ml sample Inter-day assays also maintained both accuracy and the same level of precision The limit of determination was 0 1 μg/ml for compounds I and II

Five spiked urine samples with different concentrations were assayed using this system with sufficient accuracy and precision The coefficients of variation were below 1% for the samples of 10–100 μg/ml The precision decreased as the sample concentration was lowered Inter-day assays showed the same levels of accuracy and precision The limit of determination was 1 0 μg/ml for both compounds I and II

Plasma levels and urinary excretion of compounds I and II in humans

Fig 7 shows typical I and II plasma level–time profiles after an oral dose of 200 mg to three subjects in the phase I test The concentrations reached the maximum levels ($C_{max}=9\ 9$–$11\ 9$ μg/ml for compound I and 0 4–0 6 μg/ml for

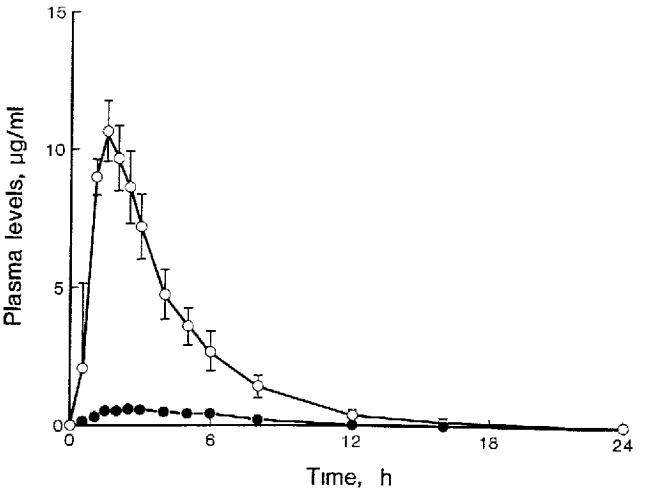

Fig 7 Plasma levels of ceftibuten (○) and its metabolite (●) after oral administration of a 200-mg capsule of ceftibuten to three healthy subjects

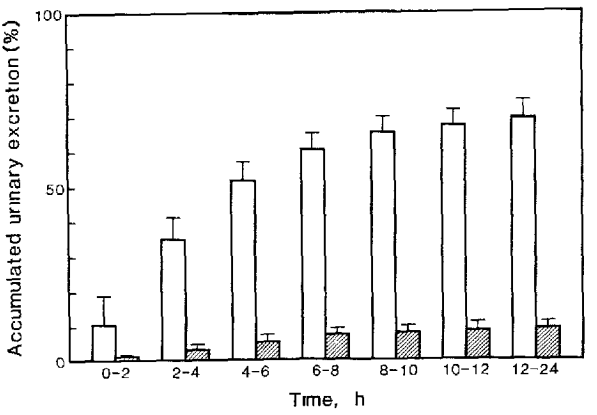

Fig 8 Urinary excretion of ceftibuten (open boxes) and its metabolite (hatched boxes) for 24 h after an oral dose of 200 mg of ceftibuten to a subject in a fasting state (mean ± S D , $n=6$)

compound II) 1 5–2 h after the dosing and thereafter compound I decreased slowly to 0 3–0 5 μg/ml at 12 h At the maximum concentration of compound I, the proportion of the metabolite II present was about 5%

The urinary excretion of compound I from six human subjects after an oral dose of 200 mg of compound I was studied (phase I test) The accumulated urinary rates of compound I are shown in Fig. 8 The mean urinary recovery in 24 h was 69 5 ± 5 3% of the dosed amount, and that of the metabolite II was 9 2 ± 2 3% of the dosed amount The same samples were assayed with our previous HPLC method [3] and good agreement was found between the results

Stability of compound I in plasma and urine

Compound I in plasma and urine was stable below 4 °C, but it was converted into its isomer II over 5 h. The conversion rate was higher at room temperature These samples were preserved in a freezer for prolonged storage

A great difference in stability at −20 and −70 °C was observed Table II shows that at −70 °C compound I was stable for 60 days, but that at −20 °C compound II was gradually formed and reached a level of 25–45% A similar phenomenon was observed for an aqueous solution of latamoxef (LMOX) between −4 and −17 °C [14]. Conversion of the *R*- to the *S*-epimer occurred below −4 °C with its rate decelerating towards the lower temperature and stopping at −20 °C This solution below the freezing point was apparently in the solid state, but an unfrozen liquid region was found in the solid ice The solute was highly concentrated in this region because of solidification of water alone, and epimerization had occurred. It seems that compound I stored in urine at −20 °C was concentrated in such an unfrozen liquid region and was rapidly converted into its isomer by the same type of epimerization Such compounds, which are labile in the unfrozen liquid, become stable below the temperature

TABLE II

STABILITY OF COMPOUND I IN HUMAN PLASMA AND URINE AT −20 AND −70°C

Specimen	Compound I (μg/ml)	Temperature (°C)	Compound	Residual content (%) after n days					
				$n=0$	$n=1$	$n=3$	$n=7$	$n=30$	$n=60$
Plasma	5	−20	I	100	100	101	96	81	73
			II	0	0	0	4	20	25
		−70	I	100	100	101	101	104	108
			II	0	0	0	0	0	0
Urine	100	−20	I	100	91	84	72	51	51
			II	0	4	13	26	40	45
		−70	I	100	101	99	98	99	100
			II	0	0	1	0	1	1

(the eutectic point or the collapse point) at which such liquid regions disappear completely. LMOX in water below −20°C and compound I in urine at −70°C were stable

CONCLUSION

An automated HPLC method has been developed for the determination of compound I in human plasma and urine Two versions were employed for plasma and urine samples after oral administration of compound I to healthy subjects, and the assay data were used for pharmacokinetic studies The simplicity of these methods enhanced the efficiency of analysis They are suitable for the treatment of many samples, the lifetime of the two precolumns is long enough for use with more than 100 samples This method can also be used for the direct analysis of other cephem antibiotics, e g , cefpiramide and cefoperazone, in plasma

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