Pergamon

0731-7085(93)E0005-8

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 5, pp. 699–703, 1994

0731-7085/94 $7.00 + 0.00

Liquid chromatographic determination of ceftibuten, a new oral cephalosporin, in human plasma and urine

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Abstract: Two liquid chromatographic methods with UV detection were developed for the determination of ceftibuten in human plasma and urine. Diluted plasma samples were directly injected onto a reversed-phase column without prior protein precipitation while diluted urine samples were processed through an automated on-line sample clean-up procedure using column-switching. Both methods were linear over clinically relevant concentration ranges in plasma (from 0.1 to 50 $\mu g\ ml^{-1}$) and urine (from 0.5 to 60 $\mu g\ ml^{-1}$). The methods showed acceptable precision (RSD <20%) and accuracy (bias <15%) at the limit of quantitation (LOQ) for ceftibuten in plasma and urine. These LOQs represented the lowest concentrations of ceftibuten in plasma (0.1 $\mu g\ ml^{-1}$) and urine (0.5 $\mu g\ ml^{-1}$) that could be measured with acceptable precision and accuracy. RSDs for both within-day and between-day analyses were ≤12% for plasma and <7% for urine. These methods have been used successfully for the analysis of ceftibuten in plasma and urine following single oral doses of 200, 400 and 800 mg in man.

Keywords: *Ceftibuten; cephalosporin, ceftibuten*-trans*; liquid chromatography; column switching; plasma pharmacokinetics; urinary excretion.*

Introduction

Ceftibuten; SCH 39720; (+)-(6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)-4-carboxycrotonamidol]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid; (Fig. 1) is a new oral cephalosporin antimicrobial agent which is currently undergoing clinical development. It exhibits antibacterial activity against a wide range of gram-negative and certain gram-positive bacteria. It is highly active against *E.coli*, *Klebsiella*, *Proteus* and *H.influenza*, and moderately active against *Enterobacter*, *Citrobacter*, *Serratia* and *S. pyogenes*. Ceftibuten is stable against most β-lactamase producing organisms [1]. A column-switching LC procedure for the determination of ceftibuten in human plasma was reported by Pan *et al.* [2]. However, the method suffers from major drawbacks which are the lack of an internal standard and inability to determine the ceftibuten-*trans* isomer (a metabolite). A method of measurement of ceftibuten in human urine has not been reported. Therefore, the two LC methods were developed to evaluate the pharmacokinetics and excretion of ceftibuten in human plasma and urine, respectively.

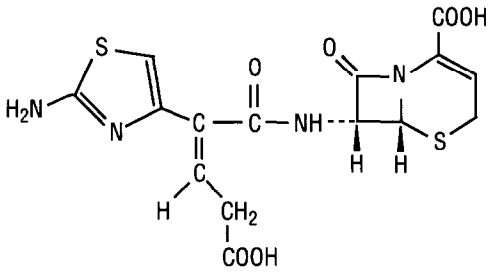

Figure 1
Chemical structure of ceftibuten.

Experimental

Reagents and standards

Sodium salts of ceftibuten and its relatively inactive *trans* isomer, were obtained from Shionogi Research Laboratories, Osaka, Japan. Acyclovir (internal standard) was obtained from Burroughs Wellcome (Research Triangle Park, NC). Other reagents used and their suppliers were as follows: ammonium acetate and ammonium phosphate monobasic (Fisher Scientific, Fairlawn, NJ) and sodium phosphate monobasic and dibasic (Mallinckrodt, Paris, KY).

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A stock solution of ceftibuten was prepared at a concentration of 1 mg ml^{-1} in 0.2 M sodium phosphate buffer (pH 7). Appropriate dilutions were made with the buffer to obtain standard solutions ranging from 1 to 100 μg ml^{-1}. Ceftibuten-*trans* was prepared at a concentration of 100 μg ml^{-1} in buffer. Both ceftibuten and ceftibuten-*trans* were stored at −70°C in 1 ml aliquots. Frozen standards were thawed on the day of the assay, kept cold at ≤4°C and discarded at the end of the day. Acyclovir was prepared in deionized water at a concentration of 50 μg ml^{-1} and stored at 4°C. Spiked plasma and urine samples were prepared by diluting the ceftibuten solution with drug-free human plasma or urine.

Sample preparation

To 100 μl of human plasma was added 10 μl of the internal standard solution and 100 μl of 0.2 M sodium phosphate buffer, pH 7. The resulting solution was transferred into a small conical injection vial and 5 μl of the mixture was injected onto the column. Urine (100 μl) was diluted to 300 μl with 0.2 M sodium phosphate buffer, pH 7, a 15 μl aliquot of which was injected onto the column for analysis.

Plasma chromatographic conditions

The HPLC system consisted of a Waters Intelligent Sample Processor, a model 6000A pump and a model 440 UV detector operating at a wavelength of 254 nm (Waters Associates, Milford, MA). The analytical separation was achieved on a Waters μBondapak C_{18} column (30 cm L × 3.9 mm i.d.). A μBondapak CN guard-PAK was placed just before the inlet of the analytical column. The mobile phase consisted of a mixture of acetonitrile and 0.05 M ammonium acetate (2:98, v/v) at a flow rate of 1 ml min^{-1}.

Urine chromatographic conditions

The HPLC system consisted of a Waters Intelligent Sample Processor, two model 6000A pumps, and a model 440 UV detector with the wavelength set at 254 nm. The column-switching device was a 10-port switching valve connected to a solenoid interface and automated by an Autochrom Chrontrol programmable timer (Autochrom, Milford, MA). Sample was injected onto a Brownlee Spheri-10 amino H2GU MPLC guard cartridge (3 cm L × 4.6 mm i.d.; Rainin Instrument, Emeryville, CA) and eluted with 0.03 M monobasic ammonium phosphate at a flow rate of 0.6 ml min^{-1}. Components of analytical interest were retained while endogenous interfering components were eluted to waste. Four minutes after injection, the valve was automatically rotated and a stronger mobile phase consisting of a mixture of acetonitrile and 0.05 M sodium phosphate buffer pH 7 (2.5:97.5, v/v) at a flow rate of 1 ml min^{-1} eluted the retained components onto a Partisil 10 ODS-3 column (25 cm × 4.6 mm i.d., Whatman, Clifton, NJ).

Results and Discussion

Plasma

Representative chromatograms of a drug-free human plasma sample and a plasma sample spiked with ceftibuten (*cis*-isomer) and ceftibuten-*trans* are shown in Fig. 2. There were no endogenous peaks that co-eluted with ceftibuten (11.0 min) or ceftibuten-*trans* (13.4 min). There was clear resolution between the two compounds as well as the internal stan-

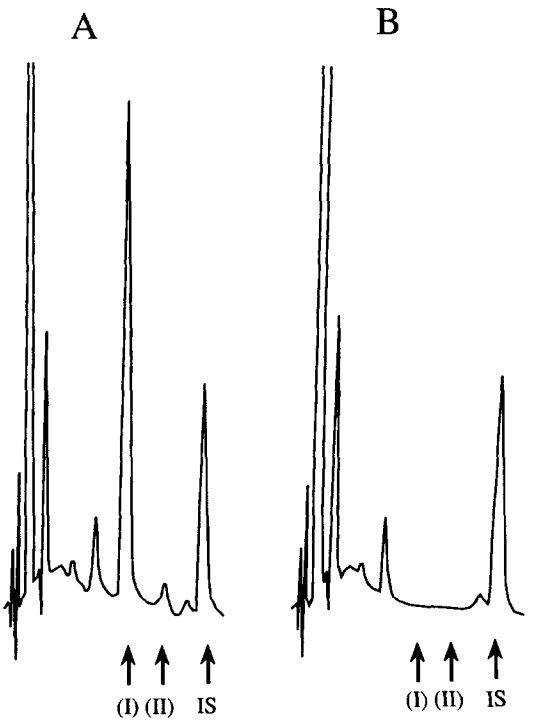

Figure 2
Chromatograms of (A) human plasma containing 20 μg of ceftibuten (I), 1.25 μg of ceftibuten-*trans* (II) and 5 μg of internal standard (IS) per ml and (B) human plasma containing only internal standard.

dard. Furthermore, none of the following drugs interfered with the assay: cefamandole, ceftazidime, cefotiam, ceftriaxone, cefsoludin, cefuroxime, cephaloridine, cephalothin, moxolactam, piperacillin, aztreonam, ampicillin, amoxicillin, gentamicin, nafcillin, vancomycin, ticarcillin, chlorpheniramine, pseudoephedrine, acetaminophen, aspirin, caffeine and theophylline.

The linearity of the assay was demonstrated by multiple analyses ($n = 33$) of plasma samples containing 0.1–50 μg ceftibuten ml^{-1}. Linear regression analysis of the observed concentrations (y) versus added concentrations (x) gave the equation $y = 1.0179x + 0.0009$ with a correlation coefficient (r) of 0.9996. The slope approached unity indicating negligible proportional error in the assay and the intercept was relatively small indicating negligible interference.

The within-day reproducibility and accuracy of the method at five different concentrations of ceftibuten in the plasma was demonstrated by the consistently low relative standard deviations (%RSD, an index of precision) ranging from 0.91 to 2.9% and low percentage bias ranging from −0.70 to 5.0% (Table 1). The between-day reproducibility of the plasma assay was demonstrated by the RSDs of 12, 4.9 and 2.9% at 1.0, 10 and 20 μg ml^{-1}, respectively (Table 2). The limit of quantitation (LOQ) was defined as the lowest concentration on the standard curve which could be measured with acceptable precision (RSD <20%) and accuracy (bias <15%). The method showed acceptable precision (%RSD) and accuracy (%bias) at the LOQ of 0.1 μg ml^{-1}. For ceftibuten at the LOQ, precision and accuracy were 18 and 10%, respectively ($n = 6$).

The absolute recovery of ceftibuten in plasma was determined by comparing the peak height ratio of ceftibuten to internal standard in plasma samples containing 1 and 10 μg ceftibuten ml^{-1} with those in aqueous solutions containing similar concentrations. The average recovery was 104 and 107% for 1 and 10 μg ml^{-1}, respectively.

Table 1
Within-day reproducibility and accuracy of the HPLC assay for ceftibuten in plasma and urine

Assay	Concentration (μg ml^{-1}) Added	Measured*	RSD (%)	Bias† (%)
Plasma	1.0	1.05 ± 0.03	2.9	5.0
	5.0	5.11 ± 0.06	1.2	2.2
	10.0	9.93 ± 0.09	0.91	−0.7
	20.0	20.0 ± 0.28	1.4	0.0
	50.0	51.2 ± 0.62	1.2	2.4
Urine	1.0	1.02 ± 0.07	6.9	2.0
	5.0	4.77 ± 0.03	0.63	−4.6
	10.0	10.2 ± 0.07	0.69	2.0
	20.0	19.2 ± 0.65	3.4	−4.0

*Mean ± standard deviations for five determinations.
† % Bias = $\left(\frac{\text{Measured conc.} - \text{added conc.}}{\text{Added conc.}}\right) \times 100$.

Urine

Chromatograms of human urine containing ceftibuten and ceftibuten-*trans* and that of drug-free human urine are shown in Fig. 3. Baseline separation of ceftibuten and ceftibuten-*trans* from each other and from other endogenous components in the urine allowed simultaneous determinations of both compounds. The retention times were 12.3 and 13.7 min for ceftibuten and ceftibuten-*trans*, respectively. No interference was observed with other tested antimicrobials such as cefmenoxime, cefoxitin, cefotiam, ceftriaxone, cephalexin and aztreonam.

The urine assay was linear for concentrations

Table 2
Day-to-day reproducibility of the HPLC assay for ceftibuten in plasma and urine

Assay	Concentration (μg ml^{-1}) Added	Measured* Day 1	Day 2	Day 3	Between-day RSD (%)
Plasma	1.0	1.02 ± 0.04	1.16 ± 0.01	0.94 ± 0.15	12.0
	10	10.8 ± 0.26	10.3 ± 0.71	10.0 ± 0.03	4.9
	20	19.7 ± 0.53	19.9 ± 0.39	19.9 ± 0.88	2.9
Urine	10	9.53 ± 0.04	9.66 ± 0.28	10.3 ± 0.07	4.0
	20	18.8 ± 0.08	19.4 ± 0.21	19.5 ± 0.63	1.9

*Mean ± standard deviation of three determinations.

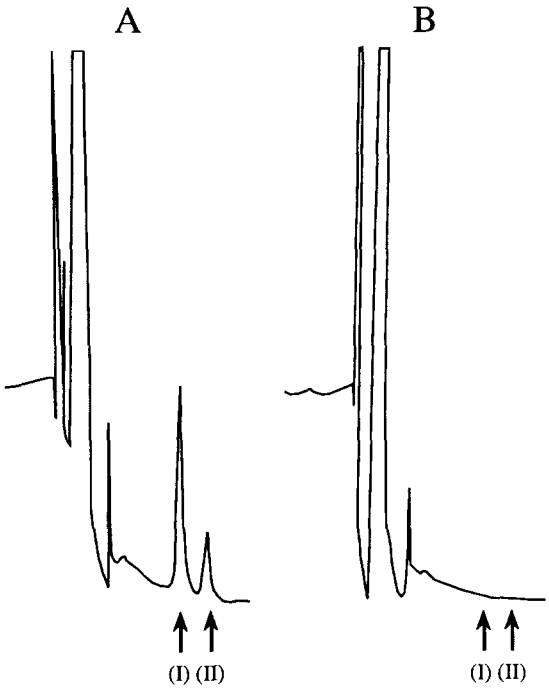

Figure 3
Chromatograms of (A) human urine containing 8.4 μg of ceftibuten (I) and 3.0 μg of ceftibuten-*trans* (II) per ml and (B) blank human urine.

ranging from 0.50 to 60 μg ceftibuten ml^{-1} ($r = 0.9997$). The equation of the regression line was $y = 1.0418x + 0.0149$, where y and x are the observed and nominal concentrations respectively. The slope approached unity, thereby demonstrating the accuracy of the method.

The RSD for the within-day and between-day data ranged from 0.63 to 6.9% and from 1.9 to 4%, respectively (Tables 1 and 2). The LOQ for ceftibuten in the urine was 0.5 μg ml^{-1} with a RSD of 6.1% and an accuracy of 9% ($n = 5$).

Assay feasibility

The methods described above were used to analyse plasma and urine samples from normal volunteers following oral administration of a single dose of 200, 400 and 800 mg of ceftibuten. Mean plasma concentration–time curves for ceftibuten following oral administration are shown in Fig. 4. Pharmacokinetics and urinary excretion data for ceftibuten and ceftibuten-*trans* are summarized in Table 3. The results showed dose proportionality in maximum plasma concentration of ceftibuten (C_{max}) and area under the plasma concentration–time curve (AUC) at the 200 and 400 mg doses, with deviation from dose proportionality at the 800 mg dose. Urinary excretion of ceftibuten accounted for 53–68% of the administered dose. The metabolite, ceftibuten-*trans*, was present in small amounts in plasma and accounted for 7–10% of the dose in urine.

The present study described HPLC methods for the determination of ceftibuten in human

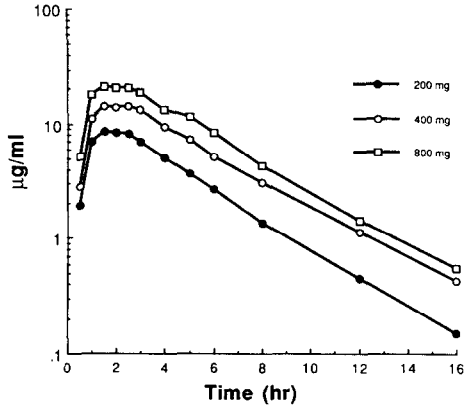

Figure 4
Mean plasma concentration of ceftibuten in normal male volunteers after a single oral dose of 200, 400 and 800 mg of ceftibuten.

Table 3
Plasma pharmacokinetic parameters and urinary excretion data (mean ± standard deviation) for ceftibuten and ceftibuten-*trans* in 12 normal volunteers

Component	Parameter	200 mg	400 mg	800 mg
Ceftibuten	C_{max} (μg ml^{-1})	9.85 ± 1.56	17.0 ± 3.55	23.3 ± 4.19
	T_{max} (h)	1.75 ± 0.54	2.00 ± 0.64	1.96 ± 1.12
	AUC(I) (μg·h ml^{-1})	42.1 ± 5.1	79.2 ± 16.4	118 ± 29.8
	$t_{½}$ (h)	2.01 ± 0.22	2.29 ± 0.56	2.25 ± 0.37
	Urinary excretion (% of dose)	67.9 ± 4.9	62.3 ± 11.0	52.7 ± 13.9
Ceftibuten-*trans*	AUC(0–36 h) (μg·h ml^{-1})	4.7	7.14	10.5
	Urinary excretion (% of dose)	9.7	7.4	8.0

plasma and urine. The methods are sufficiently accurate, reproducible and sensitive for pharmacokinetic evaluation of the drug. As a safety precaution against potential exposure to the AIDS virus when working with biological fluids, we have also investigated the use of NP-40 detergent (Sigma, St Louis, MO) as a disinfectant [3] during sample preparation. In this procedure, 1.5% NP-40 in sodium phosphate buffer (the higher percentage provided an added margin of safety) was used in place of the original phosphate buffer for diluting plasma and urine samples. This treatment did not interfere with the assay and the same criteria for excellent accuracy, specificity and sensitivity were met. This finding was reassuring and particularly important to laboratory personnel who routinely handle clinical specimens. In addition, the column-switching method for urine analysis was also found to be suitable for plasma and was successfully used for the analysis of samples obtained from renally impaired patients [4]. This assay has also been used in numerous other clinical pharmacokinetics studies which have been published previously [5].

Acknowledgements — The authors wish to acknowledge Ms Amelia Lapiguera for her excellent technical assistance, Dr Arthur deSilva for his editorial review of this report and Dr Mitchell N. Cayen for his support of this study.

References

[1] A. Bauernfeind, *Diagn. Microbiol. Infect. Dis.* **14**, 89–92 (1990).
[2] H.T. Pan, P. Kumari, J. Lim and C.C. Lin, *J. Pharm. Sci.* **81**, 663–666 (1992).
[3] L.S. Martin, J.S. McDougal and S.L. Loskoski, *J. Infect. Dis.* **152**, 400–403 (1985).
[4] J.S. Kelloway, W.M. Awni, C.C. Lin, J. Lim, M.B. Affrime, W.F. Keane, G.R. Matzke and C.E. Halstenson, *Antimicrob. Agents Chemother.* **35**, 2267–2274 (1991).
[5] W.M. Barr, C.C. Lin, E. Radwanski, J. Lim, S. Symchowicz and M. Affrime, *Diagnostic Microbiol. Infect. Dis.* **14**, 93–100 (1991).

[Received for review 26 July 1993; revised manuscript received 16 September 1993]