Ceftibuten, (+)-(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. 1a) is a third generation cephalosporin antibiotic, given orally once daily, that has a potent and broad spectrum of activity against a wide variety of gram-negative and some gram positive pathogens. It is highly active against *E. coli*, *Klebsiella*, *Proteus*, and *H. influenza*, and moderately active against *Enterobacter*, *Citrobacter*, *Serratia*, and *S. pyrogenes*. Ceftibuten is stable against most β-lactamase producing organisms [1, 2].

Ceftibuten has been shown to be well absorbed orally in rat, mouse and dog. Metabolism of the drug was negligible in all species. In man, ceftibuten was shown to have a terminal-phase half-life (T_{1/2}) of 2–3 h and did not accumulate when given in multiple doses [3]. The pharmacokinetic studies of ceftibuten in human have been well established by a number of research laboratories [4–8].

In pharmacokinetic studies, HPLC methods have been used for determination of ceftibuten in biological fluids such as plasma and urine [5, 7–9]. The research groups have developed the HPLC methods for the determination of ceftibuten in plasma with column-switching [5, 9], dilution [8] or protein precipitation [7]. The analytical HPLC method developed by Shionogi Research Laboratories [9] needs some sample pretreatment. Although an automated system is

![Chemical structures of (a) ceftibuten and (b) sodium ceftizoxime.](image)

**Fig. 1.** Chemical structures of (a) ceftibuten and (b) sodium ceftizoxime.

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1 The article is published in the original.
available to circumvent the labor-intensive liquid-liquid or solid-phase extraction in steps for pretreating sample, problems including sample decomposition at room temperature and cross-contamination are still remaining. Moreover, developments in column-switching techniques have made possible the direct injection [8] or simple sample preparation [7].

Recently, various brands of ceftibuten have been launched and prescribed to the patients for the treatment of community-pneumonia, acute exacerbation of chronic bronchitis, symptomatic pharyngitis, and otitis media. Thus, it is essential to monitor the pharmacokinetics in clinical uses by routinely available and validated HPLC methods. The previously reported HPLC methods with column-switching for the determination of ceftibuten have some advantages including low limit of quantitation and easy sample preparation. However, the column-switching system is too expensive to apply and hard to handle. Moreover, no validation data conforming to the ICH guidelines are available [10].

The aim of the present study was to validate a reliable and accurate HPLC method with UV detection for the determination of ceftibuten in human plasma. This assay method was applied to determine the plasma levels after oral administration of ceftibuten to humans.

EXPERIMENTAL

Materials. Ceftibuten, ceftizoxime disodium (internal standard, IS, Fig. 1b), ammonium acetate and triethylamine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and dichloromethane were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were analytical grade and used without further purification.

Preparation of standards. Stock solutions of ceftibuten (10 mg/mL) were made by dissolving in 0.2 M sodium phosphate buffer and diluted to concentration of 50, 100, 500, 1000, 2000, and 3000 μg/mL. Standard solutions of ceftibuten in human plasma were prepared by spiking the appropriate volume (less than 10 μL per mL) of various diluted stock solutions giving trial concentrations of 0.5, 1, 5, 10, 20, and 30 μg/mL. Internal standard, sodium ceftizoxime, structurally similar to ceftibuten, as shown in Fig. 1, was dissolved in distilled water to make a stock solution at a final concentration of 2000 μg/mL.

Preparation of samples. The samples were stored in the freezer at −70°C and allowed to thaw at room temperature before processing. Each 0.5 mL of plasma, 5 μL of internal standard (2000 μg/mL of sodium ceftizoxime) and 0.6 mL of acetonitrile were added to a glass tube. After brief vortex-mixing for 10 s, 6 mL of dichloromethane were added and the mixture was vortexed for 1 min. Each sample was centrifuged at 2500 rpm for 10 min, and a 30 μL aliquot of the supernatant was injected into the HPLC system.

Apparatus and chromatographic conditions. The determination of ceftibuten was carried out using HPLC system consisting of a Waters Alliance 2690 system (Waters, Milford, MA, USA), equipped with Waters 996 Photodiode Array detector and a Millennium 32 software. The separation was performed on a Capcell Pak C18 UG120 column (4.6 mm × 250 mm i.d., 5 μm, Shiseido, Tokyo, Japan) with a mobile phase of acetonitrile/50 mM ammonium acetate containing 0.15% of triethylamine (5 : 95, v/v) with subsequent UV detection at a wavelength of 262 nm. The flow rate was 1.0 mL/min at 30°C.

Validation of the method. Evaluation of the reversed-phase HPLC method was based on linearity, precision and accuracy assay.

Specificity: Drug-free blank human plasma was tested for interference using the proposed HPLC method, and the result was compared with those obtained from ceftibuten and the internal standard.

Linearity: The calibration curve consisted of the six concentrations: 0.5, 1, 5, 10, 20, and 30 μg/mL of ceftibuten. The calibration curves were obtained by linear regression; the ratio of ceftibuten peak area to internal standard peak area was plotted vs. ceftibuten concentration in μg/mL.

Precision and accuracy: The intra- and inter-day precision (coefficients of variation, CV %) and inter-day accuracy (bias %) of the assay procedure were determined by the analysis of five samples at each concentrations in the same day and one sample at each concentrations in 5 different days, respectively.

Sensitivity: The limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by CV % was lower than 20%, the accuracy expressed by bias % was within 80–120% and ratio of signal to noise was better than 10.

Matrix effect: To show signal suppression caused by matrix components, matrix effects were determined by comparing the peak area of the analytes at the concentrations of 1 and 20 μg/mL in the samples after extraction to that of each analyte obtained in neat solution.

Stability. Freeze and thaw stability: Human plasma samples containing 1 and 20 μg/mL of ceftibuten were prepared. The samples were stored at −20°C for 24 h, subjected to three thaw and freeze cycles and analyzed by HPLC.

Short term stability: Human plasma samples containing 1 and 20 μg/mL of ceftibuten were exposed to room temperature for 4 h and analyzed by HPLC.

Long term stability: Human plasma samples containing 1 and 20 μg/mL of ceftibuten were stored in the deep freezer at −70°C for 4 weeks and analyzed by HPLC.

Standard solution stability: Stock solution of ceftibuten (1 mg/mL) was left at room temperature for 6 h and analyzed by HPLC.