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Note

High-performance liquid chromatographic assay of cephalexin in serum and urine

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There is a current interest in the pharmacokinetics and bioavailability of the orally administered cephalexin, an antibiotic drug used to treat a number of infections. Although methods [1–5] are available for the assay of cephalexin in serum and urine, we have developed a method which is more convenient and efficient for the assay of the large number of samples involved in a pharmacokinetic or bioequivalence study than those previously published. The method, based upon the assay of ceftazidime described by Ayrton [6], was used in a bioequivalence study and found to have adequate reliability, precision, sensitivity and specificity.

EXPERIMENTAL

Standards and reagents

Cephalexin was obtained from Sigma (St. Louis, MO, U.S.A.) (potency 910 mg/g). A stock standard cephalexin solution containing 2.0 mg/ml was prepared in 50.0 ml water, and appropriate volumes were added to pooled Red Cross serum which had been previously screened for lack of interferences to yield standards ranging from 0.5 to 20 μg/ml. An internal standard stock solution containing 2.0 mg/ml ceftazidime was prepared by dissolving an appropriate amount of Tazidime® (Eli Lilly, Indianapolis, IN, U.S.A.) in 50 ml water. A working internal

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standard solution containing 4 μg/ml ceftazidime was prepared by diluting 100 μl of the stock internal standard to 50 ml with 6% perchloric acid. Buffer salts and other compounds were reagent grade. Mobile phase solvents and perchloric acid were HPLC grade. A stock ammonium dihydrogenphosphate stock buffer solution (0.5 *M*) was prepared by dissolving 28.8 g of the salt in 500 ml water after adjustment to pH 3.0 with 10% phosphoric acid. The high-performance liquid chromatography (HPLC) mobile phase was prepared by combining 100 ml acetonitrile and 90 ml stock ammonium phosphate buffer and diluting to 1000 ml with water. The mobile phase was filtered and degassed before use.

Urine standards were prepared by adding appropriate volumes of the cephalexin stock solution to screened urine to yield standards in the range 50–1000 μg/ml. The internal standard used in the assay in urine was 500 μg/ml ceftazidime in water.

Sample preparation

Serum. A 200-μl volume of standard or specimen was pipetted into a 1.5-ml polypropylene microtube, and 250 μl of working internal standard solution were added. Each tube was then mixed thoroughly on a vortex mixer for 15 s and then centrifuged for 15 min at 1500 *g* on an IEC Centra-7 centrifuge (IEC Division, Damon, Needham Heights, MA, U.S.A.). Of each supernatant 25 μl were injected into the HPLC system within 24 h.

Urine. A 250-μl volume of standard or specimen was pipetted directly into a 2-ml borosilicate injection vial, and 1.0 ml working internal standard solution was mixed into it. Of each mixture 10 μl were injected into the HPLC system within 24 h.

Chromatography

Chromatography was performed at ambient temperature using a 15 cm×4.6 mm I.D. Pecosphere 5-μm C_{18} cartridge column (Perkin-Elmer, Norwalk, NJ, U.S.A.) fitted with a direct-connect guard column (3 cm×2 mm I.D.) packed with 30–38 μm CO:PELL ODS (Whatman, Clifton, NJ, U.S.A.). The mobile phase was pumped at 1.7 ml/min with a 6000 solvent delivery system (Waters, Milford, MA, U.S.A.). A Perkin-Elmer ISS-100 autosampler was used to inject the samples. The effluent was monitored using a Waters 440 ultraviolet detector at a wavelength of 254 nm. Peak-height integration was performed on a Waters 740 data module.

Under these conditions the retention times of drug and internal standard were about 8 and 4 min, respectively. Typical chromatograms for urine and serum blanks and subject samples are shown in Fig. 1.

The following antibiotics did not interfere in the assay: imipenem, gentamicin, tobramycin, amikacin, vancomycin, ciprofloxacin, moxalactam, cefoxitin, cefoperazine, cefazolin, carbenicillin, ticarcillin, oxacillin and chloramphenicol. Compounds tested which had retention times similar enough to cephalexin and ceftazidime to cause interference were cefatoxime, cefonicid, methicillin and penicillin G.

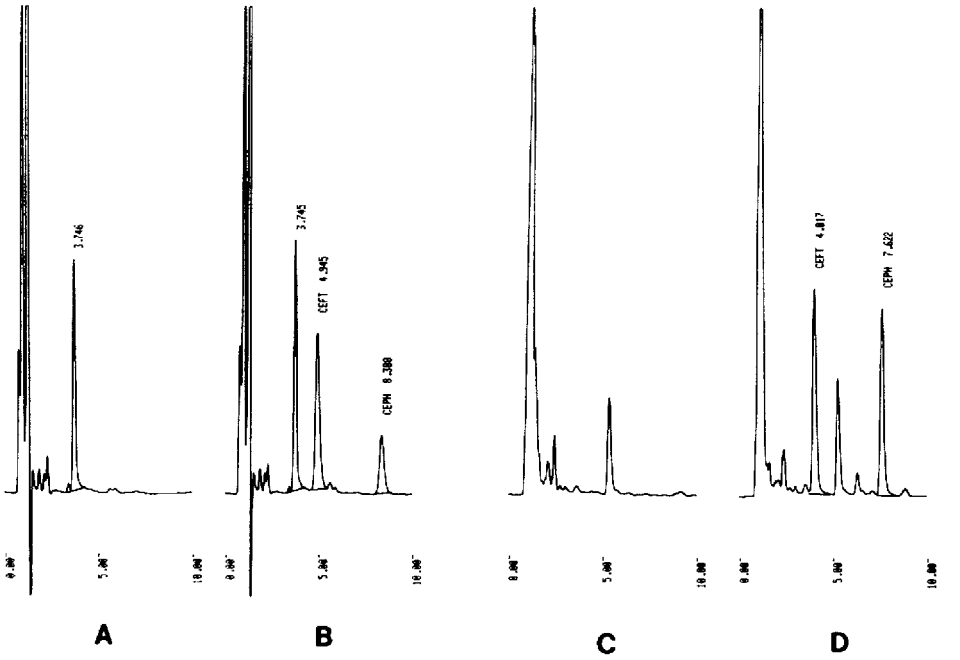

Fig. 1. Representative chromatograms for assay of cephalexin in serum and urine. (A) Blank serum; (B) subject serum, 3 μg/ml cephalexin; (C) blank urine; (D) subject urine, 200 μg/ml cephalexin.

RESULTS AND DISCUSSION

Standard lines consisting of six standards ranging from 20 to 0.50 μg/ml for serum and from 50 to 1000 μg/ml for urine were linear with correlation coefficients typically greater than 0.999 and intercepts not significantly different from 0. Intra-run precision had a relative standard deviation of 1.5% at 20 μg/ml and 1.2% at 0.5 μg/ml for serum and 0.7% at 1000 μg/ml and 2.0% at 50 μg/ml for uring ($n=3$). Inter-run precision was 3.3% at 13.8 μg/ml, 2.3% at 2.8 μg/ml and 10.9% at 0.6 μg/ml for the serum analysis and 2.1% at 760 μg/ml, 1.7% at 260 μg/ml and 2.3% at 40.0 μg/ml for the urine assay ($n=7$). The accuracy was assessed by assaying control samples along with each run of subjects' samples. For serum, the accuracy was > 99.5% at 10 μg/ml and 89% at 1.0 μg/ml and for urine the accuracy was 94% at 750 μg/ml and 97% at 100 μg/ml. The detection limit was 3 ng on-column. When samples were reinjected after standing at room temperature for 24 h the peak-height ratios had changed by less than 5%, attesting to the stability of the drug.

The method was used to analyze the samples from sixteen subjects administered 250 mg cephalexin orally in a two-phase bioequivalence study and found to be consistently reliable. A serum concentration–time profile is illustrated in Fig. 2. No interfering endogenous compounds were found in any of the predose samples.

The method described is a convenient method for the assay of the large number of samples of cephalexin in bioequivalence studies. Sample clean-up for serum is a simple protein precipitation followed by direct injection of the supernatant,

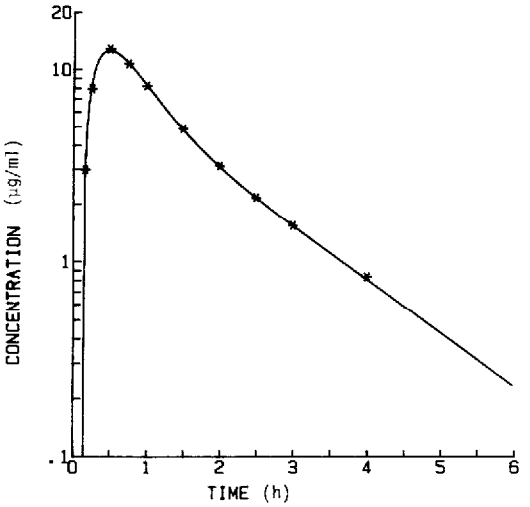

Fig. 2. Pharmacokinetic profile of cephalexin in the serum of a subject after administration of an oral dose of 250 mg of the drug.

whereas for urine the samples are only diluted with internal standard solution prior to injection. This is in contrast to the procedures of Takagishi et al. [1] which involved an extraction of interfering compounds in the serum supernatant with diethyl ether, Welling et al. [2] in which there was a clean-up step of the urine by methylene chloride, Miyazaki et al. [3] who used fluorescent degradation products and Nahata [5] who evaporated the supernatant after protein precipitation. In addition, the use of ceftazidime as internal standard with a retention time of 8 min rather than the use of β-hydroxyethyltheophylline [2] with its retention time of almost 16 min greatly improved the efficiency of the method. Lecaillon et al. [4] have described a method with a simple sample preparation step for the separation of a number of cephalosporins but at the expense of long retention times.

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