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**Note****High-performance liquid chromatographic assay for cefotiam and d3-cefotiam in human serum**

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Cefotiam is a second-generation cephalosporin for parenteral use, and the active moiety of a recently developed ester of cefotiam for oral administration, cefotiam hexetil. In vitro, cefotiam hexetil is partially hydrolysed to the inactive d3-isomer of cefotiam (Fig. 1) [1], and to a minor extent d3-cefotiam has also been detected in serum [2,3]. This paper describes a high-performance liquid chromatographic (HPLC) assay for the simultaneous determination of cefotiam and d3-cefotiam in biological fluids. HPLC methods for the determination of the parent drug have already been published [4–7]. The method

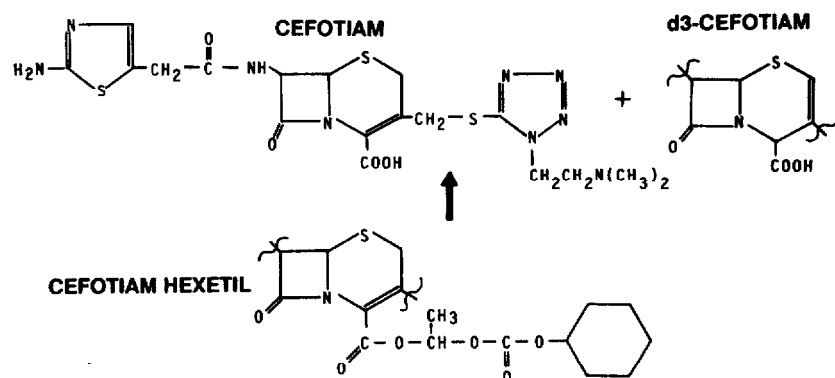


Fig. 1. Structures of cefotiam, its d3-isomer and cefotiam hexetil.

was used for analysing serum and blister fluid of healthy volunteers following intravenous injection of 200 mg of cefotiam or oral administration of 400 mg of cefotiam as cefotiam hexetil.

## EXPERIMENTAL

### *Chemicals and reagents*

Cefotiam dihydrochloride (Lot. No. 8 B 2907, activity 835.1  $\mu\text{g/g}$ ) and d3-cefotiam (Lot. No. M378-R0103, purity 99.0%) were gifts from Takeda Pharma (Stolberg, F.R.G.). Acetonitrile, HPLC-reagent grade, was obtained from Baker Chemicals (Gross-Gerau, F.R.G.); all other chemicals (analytical grade) were from E. Merck (Darmstadt, F.R.G.). Water was purified through a Milli-Q water purification system purchased from Millipore (Eschborn, F.R.G.).

Stock solutions (100  $\mu\text{g/ml}$ ) were prepared by weighing 11.97 mg of cefotiam dihydrochloride in a 100-ml volumetric flask and dissolving in water, and 10.10 mg of d3-cefotiam in 25 mM sodium phosphate (pH 7.0), respectively. Aliquots of 0.4 ml were stored at  $-20^\circ\text{C}$ , used once and discarded. Further dilution for standard and control samples was done in serum or 25 mM sodium phosphate (pH 7.0).

### *Apparatus*

The chromatographic system consisted of a Model M 6000A pump, a WISP 710B autosampler, a TCM column oven (set to  $30^\circ\text{C}$ ), a Model M 720 system controller (all from Millipore Waters Chromatography, Eschborn, F.R.G.), a 2141 variable-wavelength monitor (set to 254 nm, Pharmacia, Freiburg, F.R.G.), and a C-R4A integrator (Shimadzu, Duisburg, F.R.G.). Initially, a LiChrocart<sup>®</sup> column (125 mm  $\times$  4 mm I.D.) filled with LiChrospher<sup>®</sup> RP-18, 5  $\mu\text{m}$  particle size (E. Merck) was used for separation, but higher selectivity and performance were achieved with a Hyperchrome<sup>®</sup> column (125 mm  $\times$  4.6 mm I.D.) filled with Spherisorb ODS-2, 5  $\mu\text{m}$  particle size (Bischoff, Leonberg, F.R.G.). The mobile phase was prepared by combining 1000 ml of water, 80 ml of acetonitrile (100 ml when LiChrospher was used) and 2 ml of acetic acid, and adjusted to pH 5.1 with 10 M sodium hydroxide. The eluent was degassed and filtered through a Millipore filter (Type FH 0.45  $\mu\text{m}$ ). At a flow-rate of 1.0 ml/min (back-pressure 5–6 MPa) the retention times for cefotiam and the d3-isomer were 4.8–5.4 and 4.3–4.7 min, respectively (Fig. 2).

### *Treatment of serum and blister fluid samples*

Sample preparation was performed according to an already published procedure [8]. Because of the lability of  $\beta$ -lactam antibiotics in serum [9], all frozen specimens were thawed in iced water. In brief, 200  $\mu\text{l}$  of serum or blister fluid were buffered with 200  $\mu\text{l}$  of 50 mM sodium phosphate (pH 6.2) and deproteinized with 400  $\mu\text{l}$  of acetonitrile. The acetonitrile and the lipids were

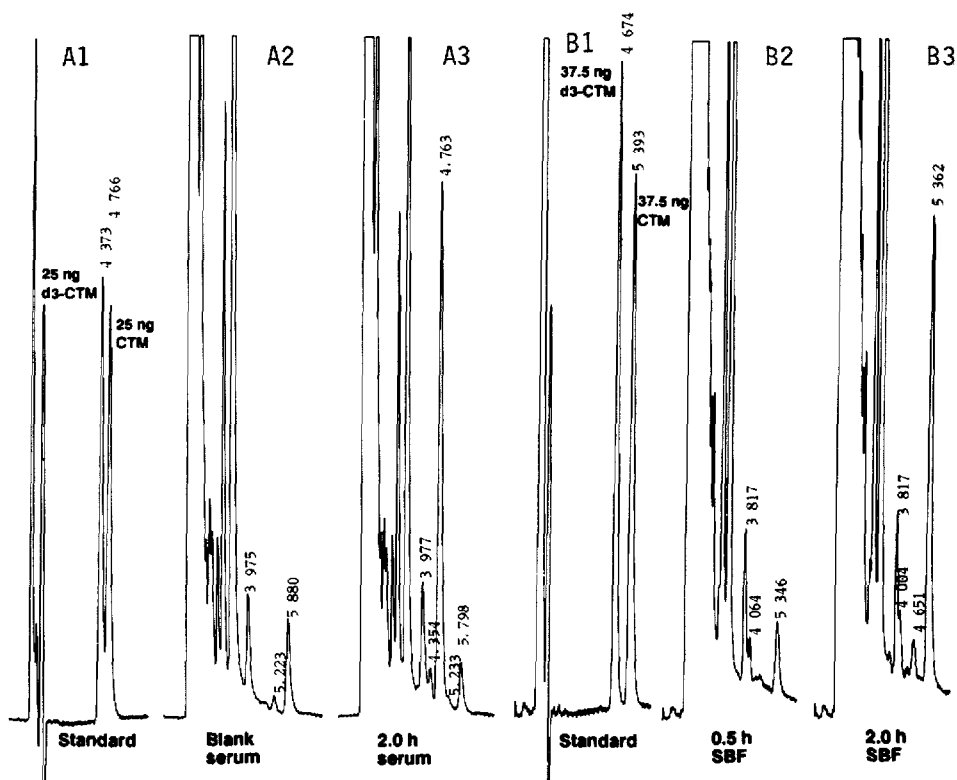


Fig. 2. Chromatograms of serum and suction blister fluid (SBF) of a healthy volunteer before and after oral administration of 400 mg of cefotiam as cefotiam hexetil. Chromatographic conditions as described in Experimental. Stationary phase: (A) LiChrospher, eluent containing 10% acetonitrile; (B) Spherisorb, eluent containing 8% acetonitrile. Samples: A1, aqueous solution of 25 ng of cefotiam (CTM, 4.766 min) and 25 ng of d3-cefotiam (d3-CTM, 4.373 min); A2, blank serum; A3, serum of the subject 2 h after dosing (concentration 0.062  $\mu\text{g/ml}$  d3-CTM, 2.35  $\mu\text{g/ml}$  CTM); B1, aqueous solution of 37.5 ng of CTM (5.393 min) and 37.5 ng of d3-CTM (4.674 min); B2 and B3, suction blister fluids of the same subject 0.5 h (B2, 0.202  $\mu\text{g/ml}$  CTM) and 2 h (B3, 1.32  $\mu\text{g/ml}$  CTM, 0.099  $\mu\text{g/ml}$  d3-CTM) after dosing.

then removed by extraction with 2 ml dichloromethane. After centrifugation, up to 50  $\mu\text{l}$  of the aqueous supernate were injected onto the HPLC column. Samples were kept in a refrigerator prior to analysis, because the analytes are unstable at room temperature (10% decay within 6 h in the autosampler at 25–27°C).

#### *Calibration and quality control*

For calibration, three serum samples spiked with 5 or 2  $\mu\text{g/ml}$  cefotiam were assayed with each run. For assessing assay variability and accuracy, control samples spiked with 10, 5, 1 and 0.2  $\mu\text{g/ml}$  cefotiam were prepared and ana-

lysed with each run. For the blister fluid assay the calibration and control samples were prepared in 25 mM sodium phosphate (pH 7.0, concentrations 2  $\mu\text{g}/\text{ml}$  for standards and 0.5 and 0.1  $\mu\text{g}/\text{ml}$  for controls). As the recovery, stability and detector response of d3-cefotiam were equal to those for cefotiam, the same analytical characteristics were assumed for both compounds.

## RESULTS AND DISCUSSION

As with other  $\beta$ -lactam antibiotics [9], the recovery at concentrations of 5 and 2  $\mu\text{g}/\text{ml}$  was quantitative for cefotiam (serum:  $104.9 \pm 3.1\%$ ,  $n=7$ ; buffer:  $98.8 \pm 3.0\%$ ,  $n=5$ ) and also for d3-cefotiam (serum: 103–107%,  $n=3$ ; buffer: 99.2–102%,  $n=3$ ). Linearity was checked by analysing three standard sets of 10, 5, 2, 1, 0.5, 0.2 and 0.1  $\mu\text{g}/\text{ml}$  cefotiam in serum and water, using a linear regression weighing factor of  $1/x$ . The coefficients of correlation were better than 0.99990. The results obtained from spiked control samples were used for assessing precision and accuracy, and are summarized in Table I. The assay variability was less than 7% in the concentration range 10–0.2  $\mu\text{g}/\text{ml}$ . The limit of quantitation (determined by replicate analysis of serum dilutions of 100, 50 and 20 ng/ml) was 50 ng/ml (found,  $56.4 \pm 1.6$  ng/ml,  $n=4$ ). The limit of detection (defined as the smallest peak height that was three times the baseline noise level) was 10 ng/ml in buffer and blister fluid and 20 ng/ml in serum. Chromatograms of serum and blister fluid of a subject receiving 400 mg cefotiam orally as hexetil are shown in Fig. 2.

The described reversed-phase HPLC assay for cefotiam and its d3-isomer

TABLE I

INTER-ASSAY VARIATION AND ACCURACY FOR CEFOTIAM DETERMINATION IN SERUM AND BUFFER OBTAINED FROM SPIKED CONTROL SAMPLES ASSAYED ON SUCCESSIVE DAYS

Number of assays	Concentration added ( $\mu\text{g}/\text{ml}$ )	Concentration found (mean $\pm$ S.D.) ( $\mu\text{g}/\text{ml}$ )	Coefficient of variation (%)	Accuracy (mean $\pm$ S.D.) (%)
<i>Serum</i>				
4	10.0	$10.038 \pm 0.313$	3.1	$2.5 \pm 1.3$
4	5.0	$4.851 \pm 0.284$	5.9	$3.4 \pm 5.4$
4	1.0	$0.988 \pm 0.010$	1.0	$1.2 \pm 1.0$
6	0.2	$0.200 \pm 0.012$	6.1	$4.9 \pm 2.9$
<i>Buffer<sup>a</sup></i>				
5	0.5	$0.498 \pm 0.019$	3.7	$2.8 \pm 2.1$
5	0.1	$0.097 \pm 0.006$	6.5	$6.1 \pm 2.7$

<sup>a</sup>25 mM phosphate buffer (pH 7.0).

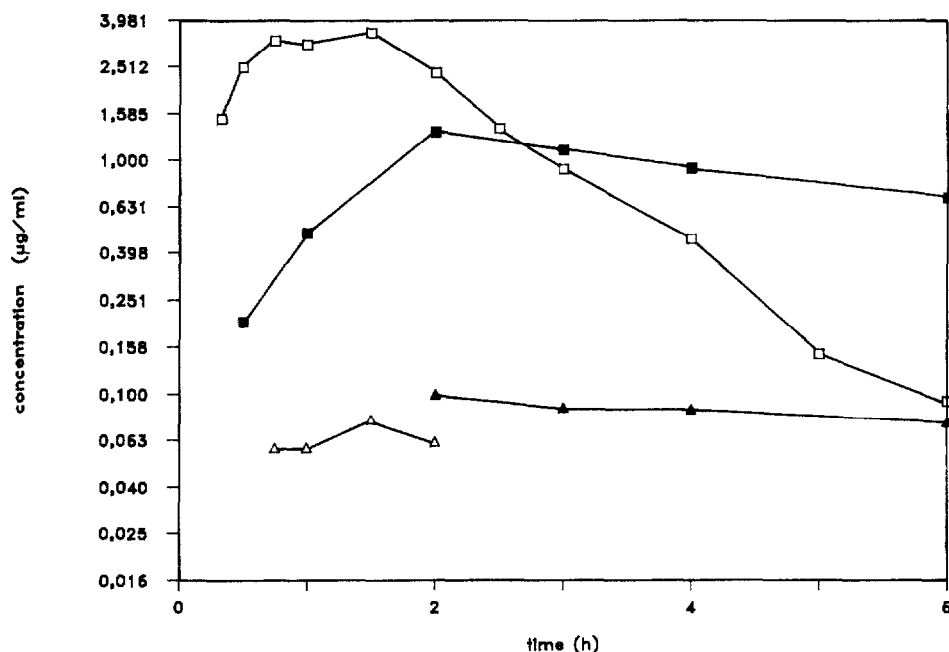


Fig. 3. Concentration-time course of cefotiam in serum (□), d3-cefotiam in serum (△), cefotiam in suction blister fluid (■) and d3-cefotiam in suction blister fluid (▲) of a healthy volunteer following oral administration of 400 mg of cefotiam as cefotiam hexetil.

provides sensitive and reliable determinations in biological fluids. It is based on a published sample treatment procedure (refs. 8–10 and references cited herein) which is well studied for polar, hydrophilic substances. The pH of mobile phase had strong influence on the separation of cefotiam and d3-cefotiam from endogenous substances; therefore the pH had to be adjusted accurately for each run and varied between 5.1 and 5.2. Unfortunately, because of late-eluting peaks, the run time had to be set to 15 min although the retention time of cefotiam was 5–6 min. This could be prevented by using an ion-pair chromatographic method with tetrabutylammonium salt on a LiChrocart column (125 mm × 4 mm I.D.) filled with LiChrospher RP-18 (5 µm particle size) and an eluent of 1000 ml of water, 80 ml of acetonitrile, 200 mg of tetrabutylammonium hydrogensulphate and 600 µl of acetic acid, pH adjusted to 4.75 with 10 M sodium hydroxide. In this case, however, separation of cefotiam from its d3-isomer was not achieved because of identical retention times (5.4 min at a flow-rate of 1.0 ml/min).

The method was used for the determination of cefotiam and d3-cefotiam in serum and blister fluid of six volunteers after intravenous injection of 200 mg of cefotiam and oral administration of 400 mg of cefotiam as hexetil [11]. Only

in some of the specimens were low concentrations (up to 0.1  $\mu\text{g/ml}$ ) of d3-cefotiam detected. In one subject, the determination of the d3-isomer was not possible because of an interfering peak, whereas no interferences were found in the case of cefotiam itself. The concentration-time course of cefotiam and of d3-cefotiam in serum and suction blister fluid of a volunteer following oral administration of 400 mg cefotiam as hexetil is shown in Fig. 3. After intravenous injection of 200 mg of cefotiam, the concentrations of d3-cefotiam were at the detection limit or not found at all. Therefore, isomerization of cefotiam hexetil may occur at the absorption site [1,12].

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