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Note

Determination of cefadroxil in serum by high-performance liquid chromatography with cephradine as internal standard

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Cefadroxil (Fig. 1) is a semi-synthetic cephalosporin active against many Gram-positive and Gram-negative bacteria. The use of high-performance liquid chromatography (HPLC) for the determination of cefadroxil in serum has been previously reported [1]. No internal standard is used and, being one of the most polar cephalosporins, cefadroxil is less retained on a reversed-phase column, so late-eluting peaks lengthen the analysis time. In this paper an HPLC method is presented for the determination of cefadroxil in 100 μl of serum, using a strong cation-exchange column and cephradine (Fig. 1) as internal standard. Quantitation is based on the relative response of cefadroxil to the internal standard. The method has been used in a pharmacokinetic study of the drug.

EXPERIMENTAL

Chromatographic system

HPLC analyses were performed using a Perkin-Elmer (Västra Frölunda, Sweden) chromatograph consisting of a Series 2 pump, an ISS-100 automatic injector

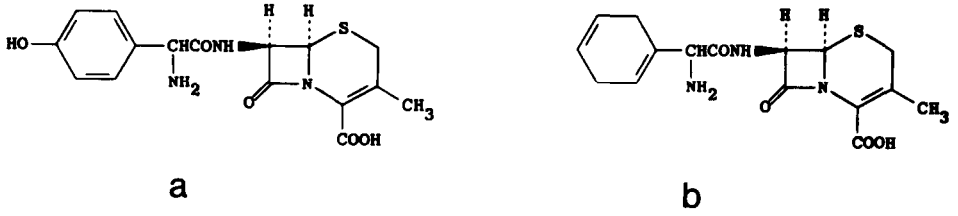

Fig. 1. Structures of (a) cefadroxil and (b) the internal standard cephradine.

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and an LC-55 UV–visible detector equipped with a 10-μl cell. Peak areas were measured with a Perkin-Elmer M2 Minigrator with attenuation set to obtain approximately 0.04 a.u.f.s. deflection. The cefadroxil analysis was performed on a 200×4.6 mm I.D. column packed with Nucleosil SA, particle size 5 μm, batch No. 4101 (Macherey, Nagel, Düren, F.R.G.). A Guard-Pak pre-column module (Millipore-Waters, Västra Frölunda, Sweden) supplied with an exchangeable insert containing C_{18} bonded-phase silica was used in conjunction with the analytical column.

Reagents

All chemicals used were of analytical-reagent grade from Merck (Darmstadt, F.R.G.) unless stated otherwise.

The mobile phase consisted of ammonium dihydrogen phosphate to a final concentration of 20 m*M* in a mixture of water–methanol–acetonitrile (30:35:35, v/v/v). The pH was adjusted to 3.0 with concentrated phosphoric acid. The mobile phase was filtered through a 0.45-μm Millipore FHUP filter and degassed by ultrasonic treatment before use.

A 1 mg/ml stock solution of cefadroxil (Bristol-Myers, Solna, Sweden) was prepared in water and stored at −20°C. Working standards of 2.5, 5.0, 10.0, 20.0 and 40.0 μg/ml were prepared fresh in a serum pool each day. A 200 μg/ml internal standard solution of cephradine (Squibb, Lidingö, Sweden) was prepared in water and stored at −20°C.

Procedure

To 100 μl of standard or sample solution were added 10 μl of cephradine internal standard solution (200 μg/ml). Proteins were precipitated with 100 μl of 6% (w/v) trichloroacetic acid, vortexed and centrifuged at 9000 *g* for 10 min. Part of the supernatant (100 μl) was transferred into a sample cup and 25 μl were injected on to the HPLC column. The detector was set to 240 nm and the mobile phase flow-rate at 1.5 ml/min. The mobile phase was used at ambient temperature.

A calibration graph was constructed by plotting the standard concentration versus the cephalosporin-to-internal standard (cefadroxil to cephradine) peak-area ratio.

RESULTS

Fig. 2A shows a typical chromatogram from a patient receiving cefadroxil per os. The serum concentration was 13.8 μg/ml. The retention times of cefadroxil and cephradine were 7.7 and 10.5 min, respectively, when the flow-rate was set at 1.5 ml/min. Cephradine was added as an internal standard. No interference was encountered when serum samples from healthy volunteers known not to take the drug were analysed (Fig. 2B).

The peak-area ratio of cefadroxil to the internal standard was linearly related to concentrations up to 40 μg/ml. The regression line for cefadroxil concentration (x) versus the cefadroxil-to-internal standard peak-area ratio (y) was $y=0.038+0.138x$ ($r=0.999$).

The precision was determined by analysing, in duplicate, aliquots from a serum

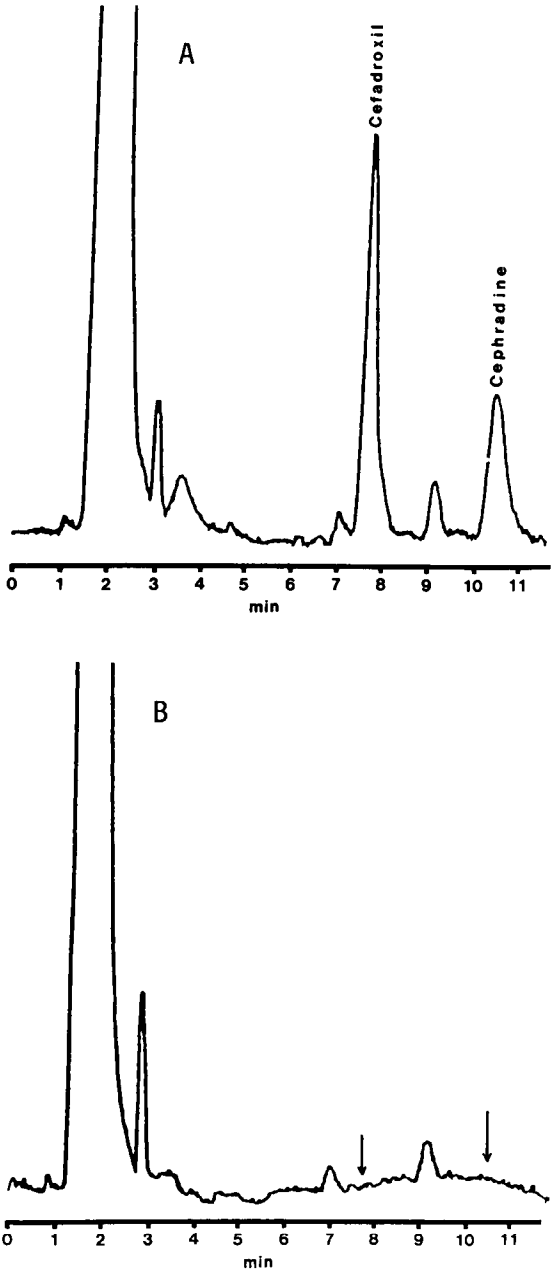

Fig. 2. Typical chromatograms of cefadroxil and cephradine in serum from a patient receiving cefadroxil (A) and a blank serum (B). The serum concentration was 13.8 μg/ml.

pool on seven different occasions. The mean concentration was 9.9 μg/ml and the within-run and between-run coefficients of variation were 5.3 and 5.5%, respectively. The limit of quantitation was approximately 1 μg/ml.

DISCUSSION

The polar and amphoteric character of cefadroxil makes it difficult to extract from serum with organic solvents, in contrast to the acidic cephalosporins [2,3]. Thus, in this method the serum proteins are precipitated with trichloroacetic acid and the supernatant is injected on to the column. The strong cation exchanger with the mobile phase used is fully compatible with the injected trichloroacetic acid. The use of an internal standard makes the analysis less influenced by errors in transferred or injected volumes.

The retention of cefadroxil on a reversed-phase column is low and the selectivity is difficult to predict, as has been thoroughly investigated [4,5]. Hence, there are difficulties in separating cefadroxil from polar serum components on a reversed-phase column. In this method the strong cation exchanger separates cefadroxil and the internal standard from naturally occurring substances in serum at a low pH.

The method described has been used to determine cefadroxil in serum specimens from elderly hospitalized patients participating in a pharmacokinetic study of the drug [6].

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