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Note

Determination of cefaclor and cephradine in serum by ion-pair reversed-phase chromatography

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Cefaclor and cephradine (Fig. 1) are semi-synthetic cephalosporins active against many Gram-positive and Gram-negative bacteria. Several workers have reported high-performance liquid chromatographic (HPLC) methods for the determination of cefaclor or cephradine in serum [1–5], although the compounds are not separated in the same chromatogram. Some workers used an internal standard, but measured peak height rather than peak area [4,5]. The compounds are usually separated on a reversed-phase column at either pH 5, close to the isoelectric point, or at pH 3. Mixed separation mechanisms under these chromatographic conditions make the selectivity difficult to predict.

In this paper an HPLC method is described for the determination of cefaclor and cephradine in 100 μl of serum using isocratic reversed-phase ion-pair chromatography at pH 7.0 on a bonded C_{18} column. Quantitation is based on the relative response of cefaclor or cephradine to the internal standard. The cephalosporins are analysed separately, with the cephalosporin not found in the patient's serum as an internal standard. The methods have been used in a pharmacokinetic study.

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 and an LC-55 UV–visible detector equipped with a 10-μl cell. Peak areas were measured with a Perkin-Elmer M2 Minigrator with the attenuation set to obtain

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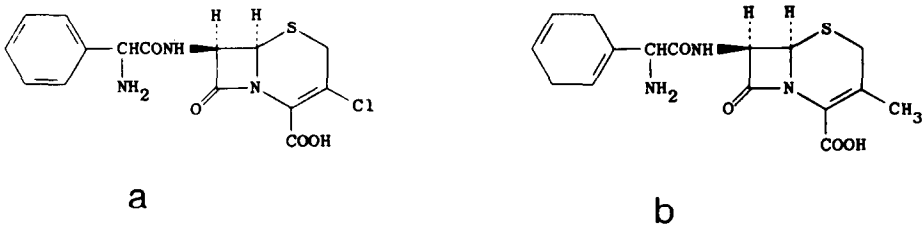

Fig. 1. Structures of (a) cefaclor and (b) cephradine.

approximately 0.04 a.u.f.s. deflection. The cefaclor and cephradine analyses were performed on a 200×4.6 mm I.D. column packed with Nucleosil C_{18} (5 μm particle size), batch No. 5012 (Macherey, Nagel, Düren, F.R.G.). A Guard-Pak precolumn module (Millipore-Waters, Västra Frölunda, Sweden) supplied with an exchangeable insert containing C_{18} bonded-phase silica was used in conjuction with the analytical column.

The mobile phase consisted of 30% (v/v) of acetonitrile in water, with sodium phosphate (pH 7.0) and tetrabutylammonium hydrogen sulphate (TBA) added to a final concentration of 20 m*M* and 5 m*M*, respectively. The pH was adjusted to 7.0 with sodium hydroxide. The mobile phase was filtered through a 0.45-μm Millipore FHUP filter and degassed by ultrasonic treatment before use. All chemicals were of analytical-reagent grade from Merck (Darmstadt, F.R.G.), unless stated otherwise.

Reagents

Stock solutions of 1 mg/ml cefaclor (Eli Lilly, Stockholm, Sweden) and cephradine (Squibb, Lidingö, Sweden) were prepared in water and stored at −20°C. Working standard solutions of 2.5, 5.0, 10.0, 20.0 and 40.0 μg/ml were prepared in a serum pool each day. An internal standard solution consisting of 100 μg/ml cefaclor and cephradine was prepared in water and stored at −20°C.

Procedure

To 100 μl of standard or sample solution were added 10 μl of internal standard solution (100 μg/ml cephradine or cefaclor). Proteins were precipitated with 100 μl of acetonitrile, vortexed and centrifuged at 9000 *g* for 10 min. Part of the supernatant (100 μl) was transferred into a sample cup and evaporated to dryness at room temperature under reduced pressure. The residue was dissolved in 100 μl of 20 m*M* sodium dihydrogen phosphate solution adjusted to pH 3.5 with phosphoric acid. Undissolved material was centrifuged at 9000 *g* for 5 min and 20 μl of the supernatant were injected on to the HPLC column. The detector was set at 265 nm and the mobile phase flow-rate at 1.0 ml/min. The mobile phase was used at ambient temperature.

Calibration graphs were constructed by plotting the standard concentration versus the peak-area ratio of standard to internal standard, i.e., cefaclor to cephradine or cephradine to cefaclor.

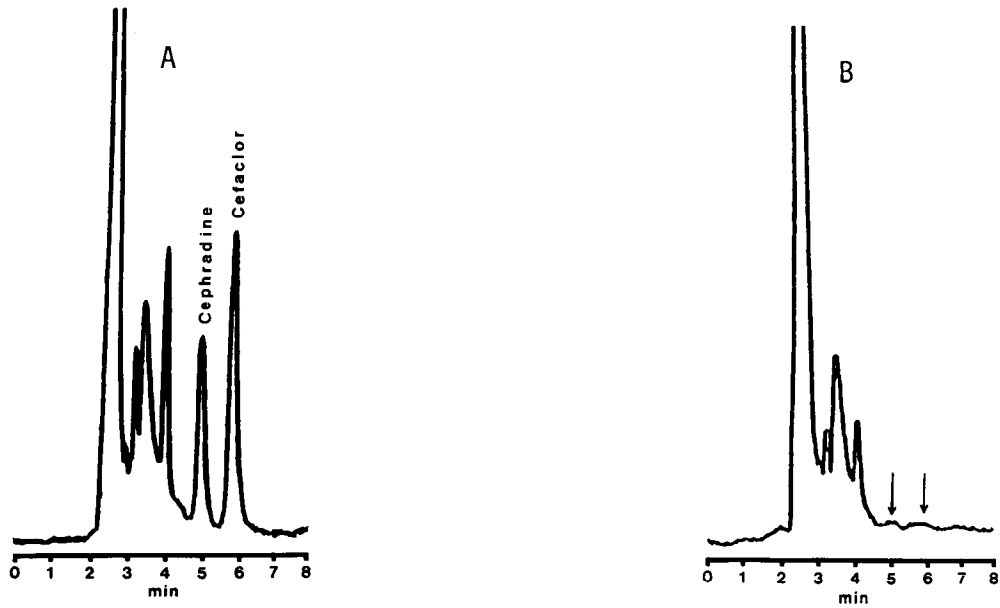

Fig. 2. Typical chromatograms of cephradine and cefaclor in serum from a patient receiving cephradine (A) and a blank serum (B). The serum concentration was 6.7 μg/ml.

RESULTS

Fig. 2A shows a typical chromatogram from a patient receiving cephradine per os. The serum concentration was 6.7 μg/ml. The retention times of cephradine and cefaclor were 5.0 and 5.8 min, respectively, when the flow-rate was set at 1.0 ml/min. Cefaclor was added as an internal standard. No interference was encountered when serum samples from healthy volunteers known to be not taking the drugs were analysed (Fig. 2B).

The peak-area ratio of the analyte to the internal standard was linearly related to concentrations up to 40 μg/ml. The regression line for cephradine concentration (x) versus the cephradine-to-internal standard peak-area ratio (y) was $y = -0.070 + 0.098x$ ($r = 0.999$). The corresponding calibration graph for cefaclor had a typical regression line of $y = -0.071 + 0.104x$ ($r = 1.000$).

The within-run and between-run precisions were determined by analysing a serum pool containing cephradine or cefaclor. Detailed precision data are given in Table I. The described method makes it possible to determine down to approximately 1.0 μg/ml of either antibiotic.

DISCUSSION

The acidic cephalosporins can be extracted from serum as previously described [6,7]. The polar and amphoteric character of cefaclor and cephradine makes them difficult to extract from serum with organic solvents. Thus, in this method the serum proteins are precipitated and the supernatant is used for analysis. The

TABLE I

PRECISION DATA ($n=12$)

Compound	Mean concentration (μg/ml)	Coefficient of variation (%)	
		Within-run	Between-run
Cefaclor	7.9	6.1	7.2
Cephradine	9.0	3.3	2.3

acetonitrile used in the precipitation step has to be removed because of peak distortion when acetonitrile is injected on to the column. The addition of an internal standard makes the analysis less influenced by errors in transferred or injected volumes.

The retention of cephalosporins on different brands or batches of reversed-phase packing materials is difficult to predict because of considerable variations in selectivity [8,9]. The use of an ion-pairing agent (TBA) in the proposed method obviates the risk of mixed separation mechanisms.

Cephradine and cefaclor have a tendency to tail on reversed-phase columns because of the ionized state of the amphoteric molecules at any pH. TBA and sodium pentanesulphonate have been used as ion-pairing agents when analysing cephalexin, another amphoteric cephalosporin [10]. TBA is preferred over a sulphonate in this method because of the better separation achieved from naturally occurring compounds in serum.

The method described has been used to determine cefaclor and cephradine in serum specimens from hospitalized elderly patients participating in a pharmacokinetic study of the drugs [11].

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