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Note

Determination of the third generation oral cephalosporin cefpodoxime in biological fluids by high-speed high-performance liquid chromatography

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Cefpodoxime proxetil (RU 51807) is a new third generation oral cephalosporin currently under clinical investigation. It is the prodrug of the active form, cefpodoxime (RU 51763). As a parent compound, cefpodoxime exhibits a broad spectrum of activity against both Gram-positive and Gram-negative bacteria [1–3], but is poorly absorbed from the gastrointestinal tract. Oral absorption is dramatically enhanced on esterification of the molecule leading to cefpodoxime proxetil. After oral administration of the latter, and its enteral uptake within the brush border of the small intestine, the prodrug is rapidly hydrolysed, yielding active cefpodoxime, which finally enters the blood circulation. As further pharmacokinetic investigations are needed, we developed a rapid, sensitive and accurate high-performance liquid chromatographic (HPLC) method for the measurement of cefpodoxime in biological fluids and renal tissue. This method has yet proved to be very suitable for the monitoring of cephalosporin [4–8].

EXPERIMENTAL

Reagents and chemicals

Cefpodoxime (RU 51763) was obtained from Roussel Uclaf (Paris, France). Stock solutions of 10 mg/ml were prepared in water and stored at −80°C. Acetonitrile, dichloromethane, ammonium acetate and acetic acid were all of analytical-reagent grade (E. Merck, Darmstadt, F.R.G.). Water was obtained daily from a Milli-Ro–Milli-Q System (Millipore, Velizy, France).

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Chromatographic conditions

The liquid chromatograph was constituted from the following units: a Model 112 solvent-delivery module (Beckman, Fullerton, CA, U.S.A.), a Model 210 sample-injection valve with a 20-μl loop (Beckman), a Model 160 variable-wavelength UV absorption detector (Beckman) and a Model 740 recording data processor (Millipore, Waters Division, Milford, MA, U.S.A.).

Chromatography was performed on a high-speed analytical column (70 mm × 4.6 mm I.D.) packed with 3-μm diameter particles (Ultrasphere XL-ODS; Beckman). The mobile phase was ammonium acetate (21.5 mmol/l)–acetonitrile (93:7, v/v), adjusted to pH 5 with glacial acetic acid. The flow-rate was set at 2 ml/min and the eluent was monitored at 254 nm. The range setting of the spectrophotometer depended on the concentration of cephalosporin measured.

Sample pretreatment

Serum. As described previously [4–6], an aliquot of serum (0.5 ml) was mixed with an equal volume of acetonitrile in a 5-ml screw-capped glass tube on a vortex mixer (Vortex Manufacturer, Cleveland, OH, U.S.A.). The tube was gently shaken for 10 min by rotation (20 rpm) and then centrifuged for 10 min at 1000 *g*. The supernatant was transferred to another screw-capped glass tube and 3.2 ml of dichloromethane were added. After shaking by rotation (20 rpm) for 10 min and centrifugation at 1000 *g* for 10 min, a 20-μl aliquot of the upper aqueous layer was injected into the column.

Urine. Urine samples were diluted (1:10) with water and then injected directly into the HPLC system.

Renal tissue. Cefpodoxime was measured in per-operatively collected samples of renal tissue in patients undergoing surgical intervention for renal disease and treated with cefpodoxime (200 mg, orally). Immediately after collection, the tissue sample was rapidly washed in phosphate buffer (pH 7) to discard blood contamination, dried and weighed exactly. It was then frozen at −80°C until analysis. For analysis, the tissue sample was ground frozen under liquid nitrogen in an impact grinder 6700 Freeze/Mill (Spex, Edison, NJ, U.S.A.). Cefpodoxime was then extracted from the ground material with 3 ml of phosphate buffer (pH 7) for 12 h at 4°C. Experimental controls showed that no degradation of the drug occurs under these conditions. After centrifugation, the drug was measured in the clear supernatant by direct injection into the chromatograph. Calibration was performed by injection of a standard prepared in phosphate buffer and the results were expressed as micrograms of drug per gram of tissue.

Linearity and limit of determination

A calibration graph for cefpodoxime was prepared for every biological fluid by supplementing cefpodoxime-free samples (from normal subjects) with increasing amounts of this antibiotic, leading to concentrations of 0.02, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5 and 10 μg/ml. Quantification was based on peak areas, as measured by the integrator. Each concentration was measured three times.

The limit of determination was defined as the lowest concentration resulting in a signal-to-noise ratio of 4.

Reproducibility

Both between- and within-day reproducibilities were assessed for serum and urine sample at different concentrations as indicated in Table I. Ten aliquots of each sample were tested on the same day and the resulting relative standard deviations (R.S.D.s) indicated the within-day reproducibility. Aliquots of the same sample were tested once a day for 10 days and the resulting R.S.D. indicated the between-day reproducibility.

Selectivity

Specificity was assessed in the presence of most β-lactam antibiotics (*e.g.*, cefotaxime, ceftazidime, cefadroxil, cefpiramide, amoxicillin, aztreonam, cefixime, ampicillin) as possible interfering compounds with this assay. Other antibiotics and other classes of drugs were included in this study (aminoglycosides, quinolones, salicylate, phenobarbital, carbamazepine, theophylline, digitoxin, furosemide, quinidine, lidocaine).

RESULTS AND DISCUSSION

Under the high-speed HPLC conditions described above, the retention time of cefpodoxime was 2.6 min. No interference from endogenous substances or assessed drugs could be detected in either matrix even at the lowest range setting of the photometer. The good reproducibilities of the assays (Table I) in conjunction with good linearity indicate that the use of an internal standard to overcome sample-to-sample variation is not necessary. The 15-fold higher determination limit for urine is due to the 1:10 dilution used as pretreatment.

TABLE I

CHARACTERISTICS OF CEFPODOXIME ASSAY IN SERUM AND URINE

Sample	Detection limit (μg/ml)	Concentration (μg/ml)	Reproducibility (R.S.D.) (%)	
			Within-day	Between-day
Serum	0.02	1	5.5	5.9
		5	4	4.3
Urine	0.3	2	5.4	6.1
		10	5.1	5.7
		100	2.3	3.3

Determination of cefpodoxime in human serum

Representative chromatograms of drug-free serum and serum spiked with CPD are shows in Fig. 1.

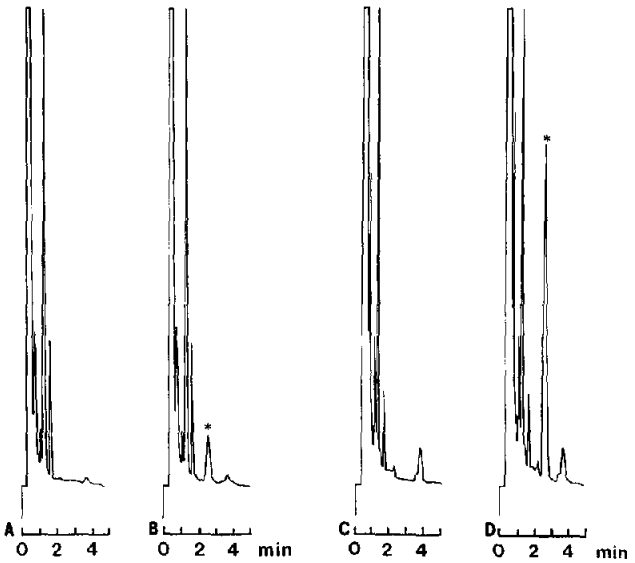

Fig. 1. Chromatograms of (A) cefpodoxime-free normal serum; (B) serum spiked with 0.5 μg/ml cefpodoxime; (C) cefpodoxime-free normal urine; (D) urine spiked with 50 μg/ml cefpodoxime. Detector wavelength, 254 nm; range, 0.01 a.u.f.s.

The linearity was checked from 0.02 to 10 μg/ml. The regression analysis between peak area and serum concentration revealed that the method is linear ($r = 0.999$). The equation of the regression line is $y = 28.202x - 19$, where y = concentration and x = peak area.

Table I shows the principal characteristics of the developed methods. The degree to which a column keeps the peak zones narrow is termed the efficiency. One way to enhance the narrowness of peaks is to increase the rate of transfer of the drug molecules between the mobile phase and stationary phase. This can be achieved by using a packing with very small particles [9]. Thus, the use of short columns filled with 3-μm diameter particles of silica leads to an increased efficiency, and thus to an improved resolution, in comparison with chromatography on 150 or 250 mm columns filled with 5-μm particles. This results in a lowering of the limit of determination and in a shortening of retention times. Therefore, we propose the present high-speed HPLC procedure as a very suitable method for the determination of cefpodoxime in human serum.

Determination of cefpodoxime in human urine

Typical chromatograms resulting from the analysis of drug-free urine and urine spiked with cefpodoxime by high-speed HPLC are shown in Fig. 1. The cefpodoxime peak is well resolved from the endogenous urine peaks flanking it.

A good linear relationship was obtained between peak area and cefpodoxime concentration in the range 2–200 μg/ml ($y = 2.580x + 1.370$; $r = 0.999$). The detection limit and the R.S.D.s obtained for different concentrations are summarized in Table I.

Clinical and pharmacokinetic applications

The method proposed here has proved to be suitable for clinical use as a routine method or for pharmacokinetic purposes. Analysis of serum sampled before and after drug administration in one patient included in a renal tissue diffusion study is depicted in Fig. 2. Fig. 2 also shows chromatograms obtained with tissue samples. If one considers the first pharmacokinetic data (particularly serum peak and trough levels) obtained after single or multiple doses (every 12 h) of both 200 or 400 mg of this drug [10,11] it can be assumed that the limits of determination of our method (0.02 μg/ml in serum; 0.3 μg/ml in urine) are compatible with the serum and urine trough concentrations (*e.g.*, 0.15–0.30 μg/ml in

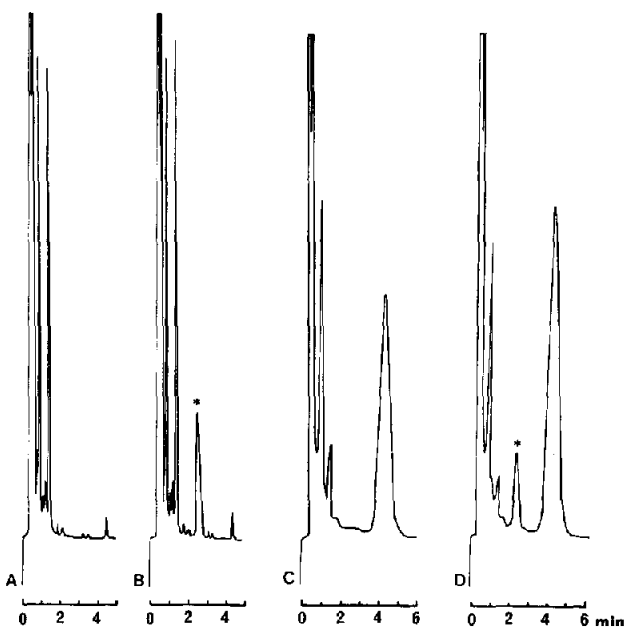

Fig. 2. Chromatograms of (A) patient serum sampled before treatment; (B) patient serum sampled 6 h after oral administration of 200 mg of cefpodoxime (concentration 1.2 μg/ml); (C) cefpodoxime-free renal tissue; (D) renal tissue sampled 6 h after oral administration of 200 mg of cefpodoxime (concentration 5.44 μg/g).

serum [10,11]). In the same studies, serum peak levels never exceeded 5 μg/ml, even during a multiple 400-mg dose study [11], indicating that the linearity range of the methodology reported here is suitable for further pharmacokinetic investigations.

CONCLUSION

The analytical characteristics of the proposed method and the minimum sample handling required are satisfactory for routine clinical applications. Indeed, as no late peak elutes, the maximum time required for serum and urine analysis is 5 min and for tissue samples 6 min. Including preteatment, about 2 h are necessary for the measurement of ten serum samples.

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REFERENCES

1 N. X. Chin and H. C. Neu, *Antimicrob. Agents Chemother.*, 32 (1988) 671.
2 R. Jones and A. L. Barry, *Antimicrob. Agents Chemother.*, 32 (1988) 443.
3 Y. Utsui, M. Inoue and S. S. Mitsuhashi, *Antimicrob. Agents Chemother.*, 31 (1987) 1085.
4 F. Jehl, P. Birckel and H. Monteil, *J. Chromatogr.*, 413 (1987) 109.
5 F. Jehl and H. Monteil, *Rev. Fr. Lab.*, 187 (1989) 47.
6 F. Jehl, C. Gallion and H. Monteil, *J. Chromatogr.*, 531 (1990) 509.
7 A. F. Falkowski, Z. M. Look, H. Noguchi and B. M. Silber, *J. Chromatogr.*, 422 (1987) 145.
8 T. Marunaka, E. Matsushima and M. Maniwa, *J. Chromatogr.*, 420 (1987) 329.
9 S. R. Bakalyar, in P. M. Kabra and L. M. Marton (Editors), *Liquid Chromatography in Clinical Analysis*, Humana Press, Clifton, NJ, 1981, p. 3.
10 M. T. Borin, G. S. Hughes, C. R. Spillers and R. K. Patel, *Antimicrob. Agents Chemother.*, 34 (1990) 1094.
11 P. O'Neill, K. Nye, G. Douce, J. Andrews and R. Wise, *Antimicrob. Agents Chemother.*, 34 (1990) 232.