Journal of Chromatography, 578 (1992) 71–76
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6373

Determination of cefcanel in plasma and urine by high-performance liquid chromatography using coupled columns, after administration of the new cephalosporin prodrug cefcanel daloxate hydrochloride

K. Lanbeck-Vallén and J. Carlqvist

Department of Bioanalysis, Astra Arcus AB, S-151 85 Södertälje (Sweden)

(First received November 29th, 1991; revised manuscript received February 18th, 1992)

ABSTRACT

A rapid and sensitive high-performance liquid chromatographic method has been developed for the determination in plasma and urine of the new cephalosporin cefcanel. The method involves a simple deproteinizing step followed by separation on a coupled-column chromatographic system with ultraviolet detection. Limits of quantification were 0.2 μM for plasma samples and 2 μM for urine samples. The method has been used for the determination of cefcanel in various clinical studies.

INTRODUCTION

Cefcanel daloxate hydrochloride [1] (Fig. 1a) is a new cephalosporin prodrug for oral use. Cefcanel (Fig. 1b) is the active principle released in the body. In animal studies [2] only cefcanel could be detected in the portal blood after administration of cefcanel daloxate hydrochloride, indicating that the release of the active drug from the prodrug is completed during the absorption process in the gastrointestinal tract. The aim of this work was to develop a method suitable for routine determination of cefcanel in plasma and urine samples from clinical trials.

Analysis of cephalosporins in body fluids is often performed using high-performance liquid chromatography (HPLC) [3,4]. Reversed-phase chromatography with UV detection is the most frequently used technique. Sample preparation is often based on a simple deproteinization step, sometimes followed by pH adjustment for optimal stability of the samples.

This paper describes the use of a column-switching system to achieve selectivity from endogenous compounds. A similar chromatograph-

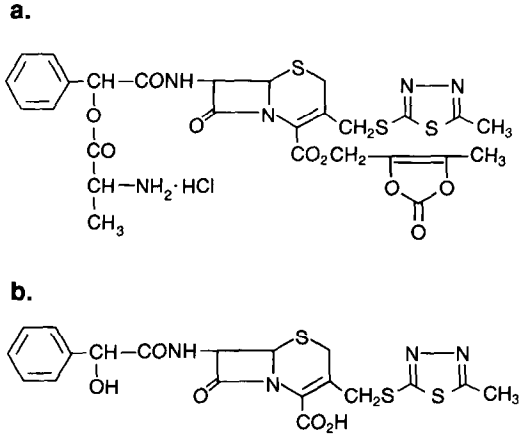

Fig. 1. Structures of cefcanel daloxate hydrochloride (a) and cefcanel (b).

Correspondence to: Dr. K. Lanbeck-Vallén, Department of Bioanalysis, Astra Arcus AB, S-151 85, Södertälje, Sweden.

0378-4347/92/$05.00

ic system has previously been used to determine ampicillin in plasma [5].

EXPERIMENTAL

Chemicals

Cefcanel was supplied from Kyoto Pharmaceutical Industries (Kyoto, Japan). All other chemicals were of HPLC or analytical grade.

Apparatus

The HPLC system consisted of two pumps, an LDC ConstaMetric III (Laboratory Data Control, Riviera Beach, FL, USA) and a Waters M510 (Waters Assoc., Milford, MA, USA), an autosampler (Waters WISP 710B) and a UV detector (LDC SpectroMonitor 3100). Three Waters automated switching valves, controlled by an SP4100 (Spectra Physics, Santa Clara, CA, USA), were used for the switching procedures. An Access*Chrom GC/LC data system (PE Nelson Systems, Cupertino, CA, USA) or a Spectra Physics SP4270 was used for computation of the results. A Microlab M (Hamilton, Bonaduz, Switzerland) was used for dilution of the samples.

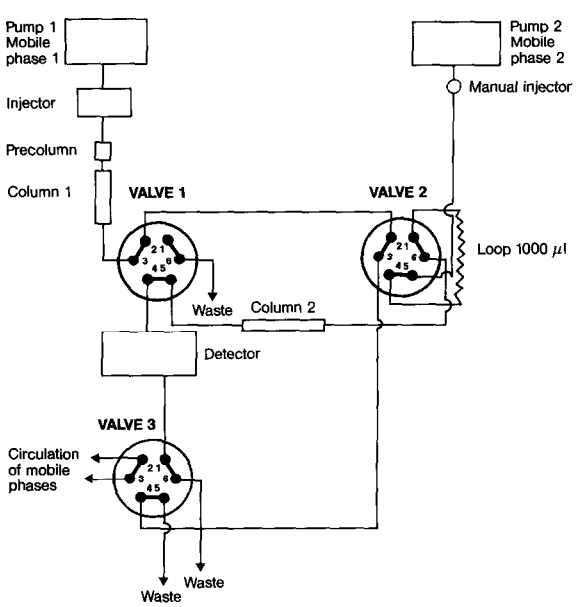

Fig. 2. Chromatographic system.

Chromatography

The chromatographic system (Fig. 2) contained a New Guard RP2 precolumn (15 mm × 3.2 mm I.D., 7 µm particle size; Brownlee Labs., Santa Clara, CA, USA), a Supelcosil LC8 column (33 mm × 4.6 mm I.D., 3 µm particle size; Supelco, Bellefonte, PA, USA) as column 1, and an Ultrasphere ODS column (150 mm × 4.6 mm I.D., 5 µm particle size; Beckman Instruments, San Ramon, CA, USA) as column 2.

The mobile phases for plasma samples were acetonitrile–0.02 *M* sodium dihydrogenphosphate buffer (pH 4.7) (13:87, v/v) for column 1 and acetonitrile–0.1 *M* phosphate buffer (pH 2) (30:70, v/v) for column 2. The mobile phases for urine samples were acetonitrile–0.02 *M* phosphate buffer (pH 6) (14:86, v/v) for column 1 and acetonitrile–tetrahydrofuran–0.1 *M* phosphate buffer (pH 2) (28:2:70, v/v) with addition of 5 m*M* tetrabutylammonium hydrogensulphate for column 2.

Flow-rates were 1.0 ml/min. The loop in valve 2 had a volume of 1 ml and an I.D. of 0.5 mm. The UV detector was set at 272 nm.

Valve 1, which was placed between column 1 and column 2, made it possible to check the performance and the retention time of cefcanel on column 1. This was done every day, and the switching times for valve 2 were adjusted if necessary. The switching procedures are further described in Table I. The manual injector placed after pump 2 allowed the performance of column 2 to be checked.

Sample handling

After a blood sample was taken, the plasma was separated and frozen as quickly as possible. Urine samples were also frozen immediately after sampling. The samples, which were stable for at least one year, were stored at −70°C until analysis.

Sample preparation for plasma

Plasma proteins were precipitated by adding 500 µl of acetonitrile to 400 µl of plasma in a 4-ml glass tube. After mixing for *ca.* 2 min in a Buchler vortex evaporator and centrifuging at 2400 *g* for

TABLE I

SCHEME OF COLUMN-SWITCHING PROCEDURES

Time after injection (min)	Switch of valve No.	Event
0		Sample injected on column 1
2.5	2	Eluate from column 1 trapped in loop 1
3.5	2 reset	Trapping completed; fraction from column 1, containing cefcanel, injected on column 2 for enrichment and separation
11		Separation completed
12		Next sample injected and sequence restarted
20	3	Injection of all samples completed; circulation of mobile phases starts

10 min, 80 μl of the supernatant were diluted directly in the Waters sample vial with 720 μl of 0.1 *M* phosphate buffer (pH 6) in order to optimize the stability of cefcanel. A sample of 200 μl was injected.

Sample preparation for urine

Urine (80 μl) was diluted directly in the Waters sample vial with 720 μl of 6% (v/v) acetonitrile in 0.1 *M* phosphate buffer (pH 6). Samples containing more than 400 μmol of cefcanel per litre of urine were diluted further with the same solvent to a suitable concentration. After mixing, a sample of 20 μl was injected.

Preparation of standard and control samples in plasma

A stock solution of cefcanel was made from 5 10 mg of cefcanel in 0.1 *M* phosphate buffer (pH 7) in a 50.00-ml volumetric flask. The stock solution was made fresh immediately before use. To prepare standards a small volume (less than 8%, v/v) of the stock solution was diluted with plasma in a 25.00-ml volumetric flask. The concentration of cefcanel in the standards was normally between 5 and 10 μ*M*.

Control samples at three concentrations were prepared in the same way as the standards from different weighings. The concentrations of the control samples were usually between 0.5 and 20 μ*M*.

Standard and control samples in plasma were stored at −70°C in polypropylene tubes (Nunc cryo tubes) in 1-ml portions until required for analysis.

Preparation of standard and control samples in urine

Standard samples of cefcanel in urine were made by dissolving 4–6 mg of cefcanel in *ca.* 5 ml of 0.1 *M* phosphate buffer (pH 7) and making it up to volume with urine in a 50.00-ml volumetric flask.

"Low" control samples of cefcanel in urine were prepared by making a stock solution of 4–6 mg of cefcanel in 0.1 *M* phosphate buffer (pH 7) in a 25.00-ml volumetric flask. A ten-fold dilution of the stock solution with buffer (pH 7) was made in a 50.00-ml volumetric flask. From the diluted stock solution, 5 ml were placed in a 50.00-ml volumetric flask and made up to volume with urine.

"Middle" control samples of cefcanel in urine were prepared by making another stock solution of 4–6 mg of cefcanel in 0.1 *M* phosphate buffer (pH 7) in a 25.00-ml volumetric flask. From the stock solution, 5.00 ml were placed in a 50.00-ml volumetric flask and made up to volume with urine.

"High" control samples of cefcanel in urine were prepared by dissolving 4–6 mg of cefcanel in 5 ml of 0.1 *M* phosphate buffer (pH 7) in a 25.00-ml volumetric flask and making it up to volume with urine. Standard and control samples in urine were stored at −70°C in Nunc tubes in 1-ml portions until analysis.

Quantitation

Five to eight standard samples of the same concentration (treated as described in *Sample preparation*) were used to construct a two-point calibration curve through the origin. The origin was confirmed every day by analysing blank plasma. The standards were spread evenly among the unknowns so that every tenth sample was a stan-

dard. Peak-height measurements were used for the calculations. To check the performance of the analytical system, three to six control samples were analysed together with the unknowns.

RESULTS AND DISCUSSION

Stability of samples after work-up

After precipitation with acetonitrile, plasma samples were diluted with pH 6 buffer. Urine samples were diluted with 6% (v/v) acetonitrile in pH 6 buffer. Both a pH of 6 and the use of acetonitrile improved the stability of the samples, which was necessary because they were usually put in an autosampler and analysed overnight. Using this procedure the processed samples were stable for at least 20 h at ambient temperature. The sensitivity obtained was sufficient, in spite of the dilution of the samples. Furthermore, for plasma samples the dilution of acetonitrile makes it possible to inject a large volume without loss of efficiency on column 1.

Evaluation of analytical procedure

During the development of the method, standard curves were constructed from spiked samples to confirm the linearity of the method. For plasma, ten samples were made with concentrations ranging from 0.5 to 40 μM. They were analysed together with blank plasma samples according to the described method. For urine, ten samples were made in the concentration range 2–400 μM. They were analysed together with blank urine samples according to the described method. Standard curves were made by plotting the peak heights against the concentration of cefcanel in plasma or urine, respectively. The correlation coefficients were >0.999.

The limit of quantification, defined as the concentration giving a peak height corresponding to five times the noise level of a processed blank sample, was 0.2 μM for plasma and 2.0 μM for urine.

The accuracy and intra-assay precision were determined from spiked samples, which were made as described in *Preparation of standard and control samples*. For these determinations, five standards at one concentration and ten control samples at three concentrations were made. The samples were processed according to the described method. The accuracy and the relative standard deviation (R.S.D.) were calculated, and the results are given in Table II.

TABLE II

INTRA-ASSAY PRECISION (n = 10)

Concentration added (μM)	Found (%)	R.S.D. (%)
Plasma[a]		
0.58	100.2	2.75
5.76	101.0	1.13
17.29	102.0	1.53
Urine[b]		
3.80	101.3	6.0
38.2	98.9	1.0
381.4	100.1	0.8

[a] Standards at 5.86 μM were used for plasma determinations.
[b] Standards at 156.9 μM were used for urine determinations.

The inter-assay precision was determined from spiked samples, made as described above, which were analysed over a fourteen-day period (thirteen days for urine). On each day the control samples were analysed in duplicate, and the average was calculated from the found concentrations. From these results the R.S.D. was calculated to give the inter-assay precision. These results are given in Table III.

TABLE III

INTER-ASSAY PRECISION

Concentration added (μM)	Found (%)	R.S.D. (%)	n (days)
Plasma			
0.64	104.7	5.87	14
7.30	100.4	0.71	14
17.40	100.8	1.02	14
Urine			
3.85	101.3	1.9	13
47.2	99.4	0.5	13
390.7	98.7	0.7	13

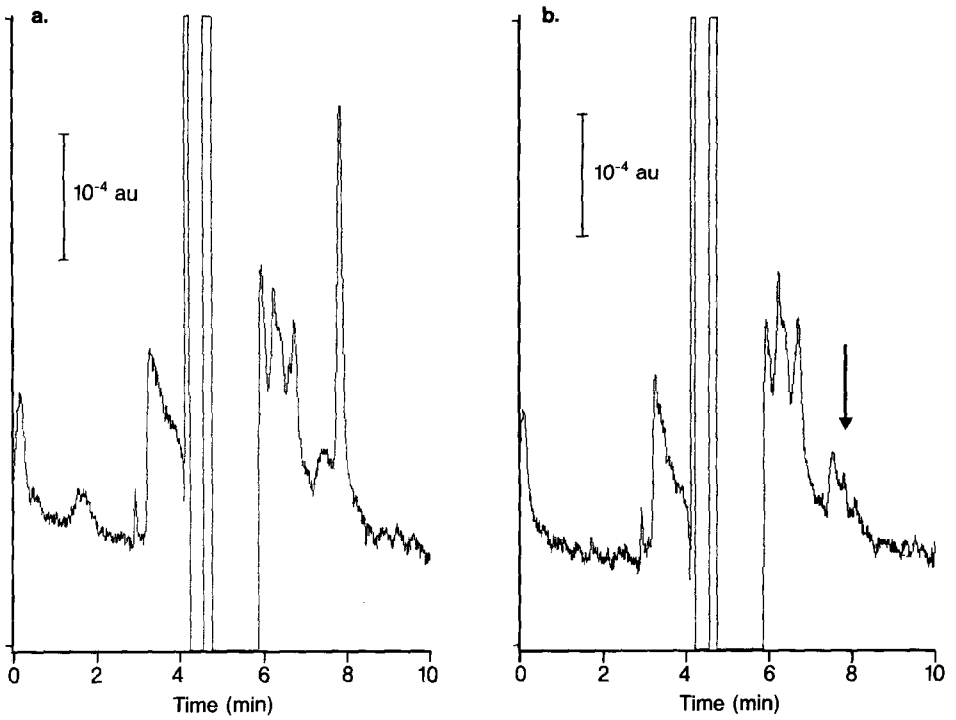

Fig. 3. Chromatograms of (a) spiked plasma sample containing 0.45 μM cefcanel and (b) blank plasma sample. The position of cefcanel is indicated with an arrow.

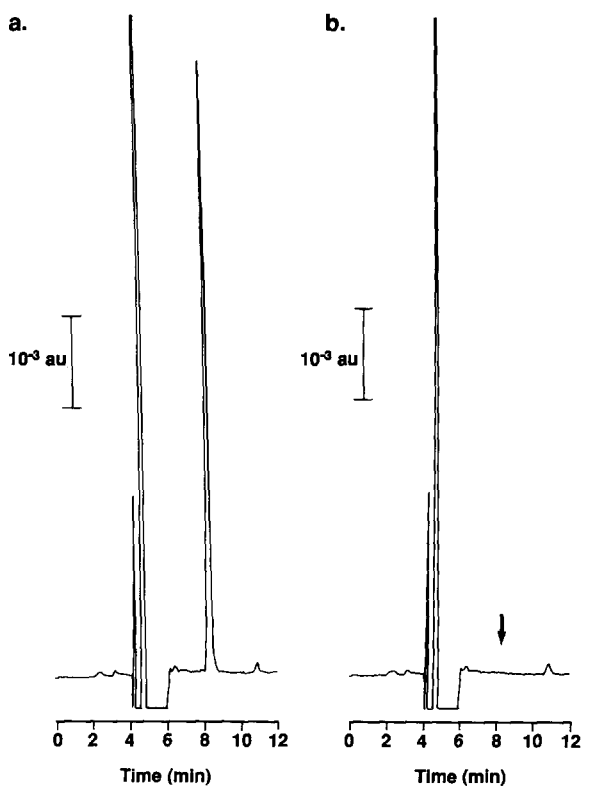

Fig. 4. Chromatograms of (a) plasma sample taken 0.75 h after the administration of a 300-mg tablet of cefcanel daloxate hydrochloride (the sample contains 10.98 μM cefcanel) and (b) blank plasma sample taken before dosage. The position of cefcanel is indicated with an arrow.

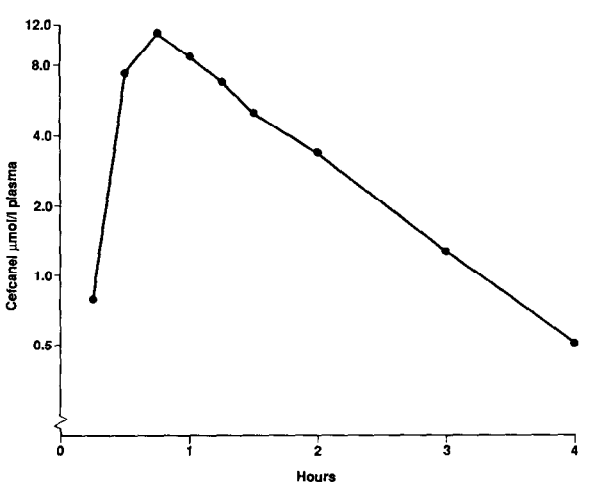

Fig. 5. Semi-logarithmic plot of the plasma concentration of cefcanel *versus* time after a 300-mg tablet of cefcanel daloxate hydrochloride.

Based on the results in Tables II and III, it was concluded that a two-point standard curve, together with control samples, as described in *Quantitation* could be used for the routine determinations. Typical chromatograms from spiked samples are shown in Fig. 3.

CONCLUSION

The described method comprises a simple sample work-up followed by reversed-phase liquid chromatography with coupled columns and UV detection. This method is simple and robust and has been used for more than three years. During this time *ca.* 6500 clinical samples have been analysed. Typical chromatograms from clinical samples are given in Fig. 4, and the plasma concentration *versus* time curve from the same person and dosage is shown in Fig. 5.

REFERENCES

1 N. Kakeya, S. Nishizawa, K.-I. Nishimura, A. Yoshimi, S. Tamaki, T. Mori and K. Kitao, *J. Antibiot.*, 38 (1985) 380.
2 K. Nishimura, S. Nishizava, A. Yoshimi, S. Nakamura, M. Nishimura and N. Kakeya, *Chem. Pharm. Bull.*, 36 (1988) 2128.
3 M. C. Rouan, *J. Chromatogr.*, 340 (1985) 361.
4 F. Jehl, C. Gallion and H. Monteil, *J. Chromatogr.*, 531 (1990) 509.
5 K. Lanbeck-Vallén, J. Carlqvist and T. Nordgren, *J. Chromatogr.*, 567 (1991) 121.