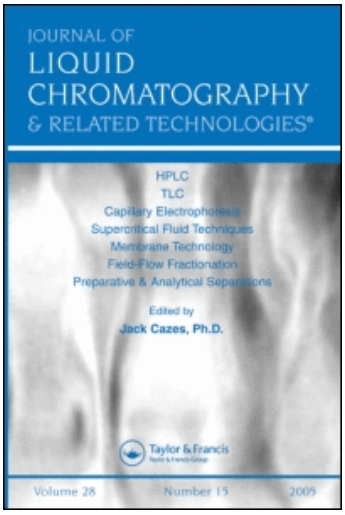

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Cefodizime in Human Plasma by High-Performance Liquid Chromatography with Column-Switching

S. Bompadre[a]; L. Ferrante[a]; L. Leone[a]; M. De Martinis[b]; L. Ginaldi[b]; D. Quaglino[b]
[a] Institute of Biomedical Sciences, University of Ancona, Ancona, Italy [b] Department of Internal Medicine, University of L'Aquila, L'Aquila, Italy

To cite this Article Bompadre, S. , Ferrante, L. , Leone, L. , De Martinis, M. , Ginaldi, L. and Quaglino, D.(1995) 'Determination of Cefodizime in Human Plasma by High-Performance Liquid Chromatography with Column-Switching', Journal of Liquid Chromatography & Related Technologies, 18: 14, 2895 — 2909
To link to this Article: DOI: 10.1080/10826079508009333
URL: http://dx.doi.org/10.1080/10826079508009333

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(14), 2895-2909 (1995)

DETERMINATION OF CEFODIZIME IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN-SWITCHING

S. BOMPADRE[1], L. FERRANTE[1], L. LEONE[1]*, M. DE MARTINIS[2], L. GINALDI[2], AND D. QUAGLINO[2]

[1]*Institute of Biomedical Sciences*
University of Ancona
60131 Ancona, Italy
[2]*Department of Internal Medicine*
University of L'Aquila
67100 L'Aquila, Italy

ABSTRACT

A column-switching high-performance liquid chromatographic assay with ultraviolet detection, at 263 nm, was developed to determine cefodizime, a new parenteral cephalosporin, in human plasma. The method is based on pre-column extraction in a closed system allowing direct injection of plasma samples without any sample pretreatment. The method allows direct, rapid, precise, and simple determination of cefodizime in plasma over the range of 1-400 μg/ml using a 100 μl loop and 25 μl of plasma. The detection limit of the assay was 0.1 μg/ml. Recovery for spiked plasma

* To whom the correspondence should be addressed

samples was near 100% and relative retention time showed good repeteability. An application of the method to study the pharmacokinetics of cefodizime in immunodepressed patients is given.

INTRODUCTION

Cefodizime is a new aminothiazolyl third generation cephalosporin for parenteral use, with a broad antibacterial spectrum and possessing immunomodulating properties (1).

Microbiological and high-performance liquid chromatographic (HPLC) assays have been used to determine cefodizime in biological fluids (2-5). Microbiological methods are slow and give poor precision and specificity (since other antibiotics interfere). Conventional HPLC methods used so far for the determination of drugs are based on time-consuming extraction procedures which increase the potential of introducing a bias in the results. These problems have increased the interest in methods for biological samples not requiring sample preparation. Newer methods used to analyse drugs in biological fluids include direct injection of biological samples by column switching. The use of a column-switching system allows to achieve selectivity from endogenous compounds.

We have developed a pre-column extraction HPLC method allowing direct, rapid and sensitive analysis of cefodizime in plasma. It has been applied to study the kinetics of this drug after intravenous administration to immunodepressed patients.

EXPERIMENTAL

Reagents and chemicals

All chemicals were reagent or analytical grade. Acetonitrile was obtained from E. Merck (Darmstadt, Germany). Acetic acid was purchased from Carlo Erba Farmitalia (Milano, Italy). Sodium 1-heptanesulfonate was supplied by Sigma Chemical Company. Cefodizime was from Hoechst. Analytical grade, filtered water was obtained daily from an Elgastat UHQ PS apparatus (ELGA, High Wycombe, Bucks, England).

Apparatus

The HPLC system consisted of a VARIAN (Walnut Creek, CA, USA) model Vista 5500 HPLC pump, a Spectra Physics model SP-8000B solvent-delivery system, and a Model 166 Programmable ultraviolet detector from

Beckman. The chromatograms were integrated with a System Gold laboratory data system (Beckman, Berkeley, CA, U.S.A.). The injector was a Rheodyne model 7725i manual injection valve equipped with a 100 µl sample loop. The coupled-column system was operated by a pneumatic, six-port, automated switching valve (VALCO Schenkon, Switzerland), controlled by the HPLC system.

Chromatographic conditions

The analytical column was a HP ODS analytical column (20 cm x 0.4 cm I.D., particle size 5 µm) from Hewlett-Packard (Palo Alto, CA, U.S.A.). The extraction column was a 5 cm x 0.4 cm I.D., dry-filled with CN 40 µm silica. The mobile phase 1 consisted of a mixture of 2% acetic acid and 10% acetonitrile in 5 mM sodium 1-heptanesulfonate (pH 3); mobile phase 2 consisted of 2% acetic acid and 27% acetonitrile in 5 mM of sodium 1-heptanesulfonate. Sample aliquots of 100 µl (plasma + mobile phase 1, 1:3, v/v) were injected into the chromatograph. The flow-rate for both columns was set at 1.0 ml/min. The effluent from the analytical column was monitored by UV at a wavelength of 263 nm. The retention time of cefodizime was 7.6 minutes and cycle time of one analysis was 10 minutes. Chromatographic analyses were performed at ambient temperature.

Column switching procedure

The scheme for the switching procedure is shown in Figure 1.

Plasma samples were diluted 1:3, v/v, with mobile phase 1 and a total of 100 µl of the diluted sample was injected, without any sample preparation, directly onto the extraction column. Mobile phase 1, which passed through the column and was directed to waste, was delivered by the pump 1, while the pump 2 delivered the same mobile phase 1 to the analytical column (switching valve at the initial position: Figure 1A). After a flushing period of 1 min to remove matrix components (while cefodizime was retained on the stationary phase) the first column was connected with the analytical column via the switching valve for 2,5 minutes. Cefodizime retained in the precolumn was eluted into the second (analytical) column for quantitation with the mobile phase 1 from the pump 1. At this stage the mobile phase in pump 2 changed from mobile phase 1 to mobile phase 2 (Figure 1B). After 3,5 minutes mobile phase 1 was switched back to pass through the extraction column to prepare it for next sample, while pump 2 maintained the flow of mobile phase 2 through the analytical column where cefodizime was separated and detected by UV (Figure 1C).

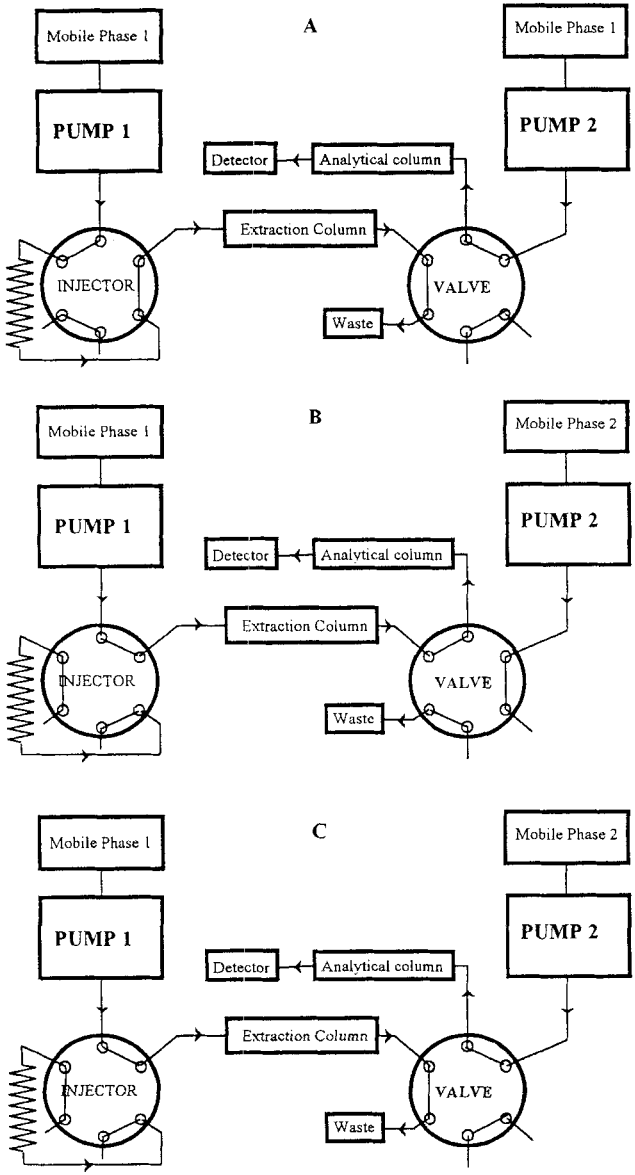

FIGURE 1. Scheme of column switching. A: extraction of injected sample (mobile phase 1 from pump 1 to extract cefodizime and from pump 2 to prepare analytical column). B: elution of the drug from the pre-column into the analytical column with mobile phase 1 from the pump 1. C: separation of the analyte with the mobile phase 2 from pump 2 and conditioning of pre-column with mobile phase 1 from pump 1.

Sample processing

Blood samples were drawn from patients treated with cefodizime by i.v. injection and collected into heparinized glass tubes. Plasma was quickly separated by centrifugation and stored at - 20°C until analysis.

Quantification

Standards for the calibration curve were made by spiking control plasma at the following concentrations: 0, 1, 5, 10, 25, 50, 100, 250, and 400 µg/ml. Each spiked plasma standard was injected eight times. The calibration data of peak-area against the concentration of the drug were fitted to a linear, unweighted, model. The resultant linear regression curves were used to calculate the drug concentrations in the samples.

Recovery

Analytical recoveries were calculated by comparing the peak areas of standards or spiked samples passed through both the extraction and analytical columns with the peak areas of standard of the same concentration injected directly onto the analytical column.

RESULTS AND DISCUSSION

Problems such as time-consuming extraction steps and low recoveries have increased the interest in methods for biological samples not requiring sample preparation. The proposed method is extremely simple since it requires no extraction or clean-up steps and no internal standard. Cefodizime was determined by direct injection of plasma onto a precolumn with sample clean-up in combination with a column-switching method. The cleanup column was connected before the switching valve, and the desired portion was eluted into the second column.

In order to optimize the extraction process 5 cm x 0,4 cm I.D. columns dry packed with different phases were tested. Three kinds of packings (particle size 40 μm) were tentatively used: C2 (ethyl), C8 (octyl), and CN (cyanopropyl) silica phases. Since the selected analytical eluent consisted of a mixture of 2% acetic acid and 27% acetonitrile in 5 mM of sodium heptanesulfonate, experiments were carried out with the same mobile phase containing various acetonitrile concentrations, from 10 to 25%. By using different stationary phases the lower concentration of the organic modifier that ensure a suitable sample clean-up, a good separation of the analyte, and a retention

time as short as possible was investigated. After several experiments the best results were found to be provided by CN packing material and 10% acetonitrile. C8 and C2 required higher concentrations of organic modifier (22% and 18% respectively) in the mobile phase and gave larger peaks. The lenght of the extraction step needed to retain the drug in the column was optimized by studying recoveries after different extraction times.

The connection time of the extraction column to the analytical column was optimized by stepwise reduction until the peak area of cefodizime started to decrease. An optimum yet safe connection time was found to be 2,5 min. A longer connection time increased only the retention time of cefodizime because the extraction eluent did not elute the drug in the analytical column. After the analyte has eluted from the primary column and has been transferred onto the secondary column, the pre-column was back-flushed to remove the components of the matrix that are strongly retained. Therebe, the analytical column is protected from contamination by late-eluting matrix components. The back-flush also minimize peak-broadening. Figure 2 shows chromatograms obtained after injecting both a blank and a spiked plasma. As can be seen cefodizime peak is well separated from the other detectable

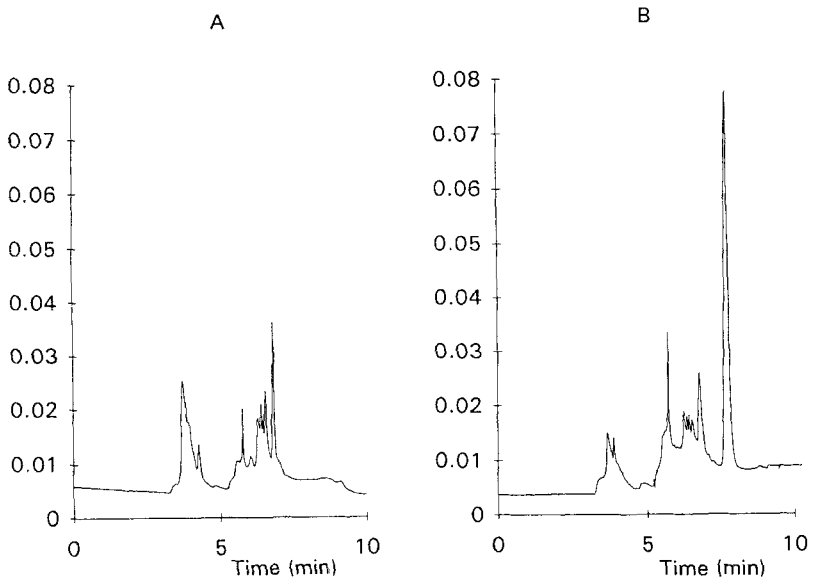

FIGURE 2. Chromatograms of (A) drug-free plasma and (B) plasma spiked with 15 µg/ml of cefodizime.

components in human plasma at the selected wavelenght. The sensitivity of the proposed method can be further improved by increasing the sample volume and it can therefore be used to analyse samples containing very low amounts of cefodizime with acceptable precision and recovery.

Linearity, precision and accuracy

To investigate the linearity of the procedure, blank plasma samples were spiked with amounts varying

TABLE 1

Precision, accuracy and linearity for cefodizime (spiked plasma)

Y=(39,58 ± 0,10) X + (-6,70 ± 3,33)

Nominal (μg/ml)	Actual value (Mean ± S.D.,n=8) (μg/ml)	Precision (%)	Accuracy (%)
1	1.06 ± 0.04	3.77	6.00
5	4.72 ± 0.22	4.66	-5.60
10	10.39 ± 0.45	4.33	3.90
25	25.34 ± 0.76	3.00	1.36
50	49.1 ± 2.34	4.77	-1.80
100	102.34 ± 2.21	2.16	2.34
250	253.17 ± 4.01	1.58	1.27
400	394.45 ± 4.78	1.21	-1.39

from 1 to 400 μg/ ml of cefodizime (Table 1). The regression line obtained obeyed the equation: $y = (39.58 \pm 0.10)\ x + (-6.70 \pm 3.33)$, the correlation coefficient being $r^2 = 0.998$. Accuracy was between 1.27 and 6.0 % for the concentration range 1-400 μg/ml. Values for precision (coefficient of variation, C.V.) were between 4,77 and 1,21 % (Table 1).

Recovery and detection limit

Mean recoveries (± S.D.) of the investigated drug from spiked plasma samples (calculated by comparison

with standards dissolved in mobile phase 1) are shown in Table 1. As can be seen, the recovery of cefodizime was found to be 96% in plasma.

Using the criterion of minimum detectability as 3 times the system noise, the detection limit was 0.1 μg/ml using a 100-μl loop.

Application

The method described was applied to determine plasma concentrations of cefodizime after 2 g i.v. administration in 3 male patients. Plasma levels of the drug were fitted to a three compartment open model with elimination from the central compartment by means of an iterative, nonlinear least-squares technique. Plasma levels of cefodizime from a patient and the fitted curve are shown in Figure 3. The main pharmacokinetic parameters (means ± SD) were obtained by standard methods(6) and include: terminal half life ($t_{½\beta}$), area under the plasma concentration time curve (AUC), serum clearance (Cls), volume of distribution of the central compartment (V_1), and mean residence time in the central compartment (MRT_1). Our results, summarized in Table 2, are in agreement with those previously reported (7).

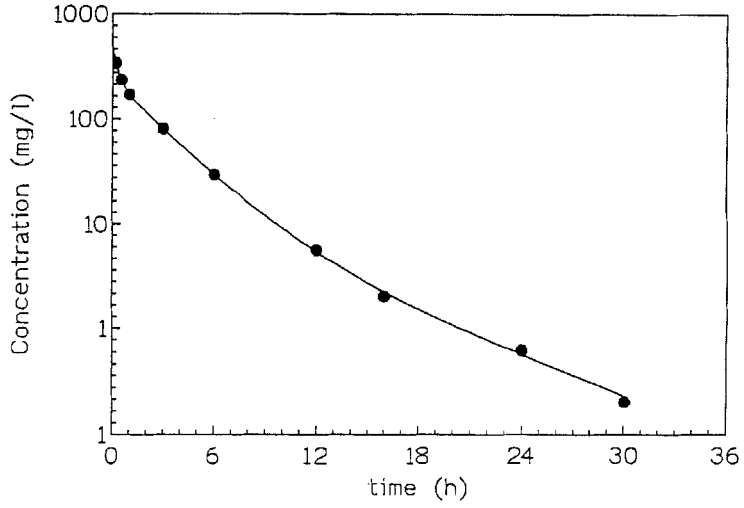

FIGURE 3. Plasma concentration-time curve of cefodizime in a patient after a 2 g i.v. dose.

TABLE 2

Pharmacokinetic parameters of cefodizime after intravenous administration of a 2 g dose(means ± SD).

$t_{1/2\beta}$ (h)	AUC (mg.h/L)	Cls (ml/min)	V_1 (L)	MRT_1 (h)
3.37	756.97	44.04	4.62	1.75
±1.02	±7.11	±0.41	±0.20	±0.09

CONCLUSIONS

In conclusion, a simple, sensitive, selective and validated HPLC method for the analysis of cefodizime in plasma has been developed. The sample preparation of the proposed method is extremely simple since it requires no extraction or clean-up steps, only dilution of the sample and direct injection onto the chromatographic column. Good precision and accuracy in plasma was achieved without the need for an internal standard. The assay is suitable for human pharmacokinetic studies.

ACKNOWLEDGEMENT

This work was supported by a grant of MURST 60%.

REFERENCES

1. B. Barradell, R.N. Brogden, Drugs, 44(5):800-834 (1992).

2. A. Boccazzi, G. Fusi, A.M. Mezzopane, M. Maretti, P.Careddu, J.Antimicrob., 26 (Suppl.C), 83-87 (1990)

3. T.Marunaka, E. Matsushima, M. Maniwa, J.Chromatogr., 420: 329-339 (1987)

4. A. Bryskier, T. Procyk, D. Tremblay, B. Lenfant, J.B. Fourtillan, J. Antimicrob. Chemother., 26 (Suppl.C), 65-70 (1990)

5. P. Mendes, N. Lameire, B. Rosenkranz. B. Malerczyk, D. Damm, J. Antimicrob. Chemother., 26 (Suppl.C), 89-93 (1990)

6. M. Gibaldi, D. Perrier, Pharmacokinetics, Marcel Dekker, Inc., New York, 1982.

7. J. Barrè, J. Antimicrob. Chemother., 26 (Suppl.C), 95-101 (1990)

Received: March 1, 1995
Accepted: March 16, 1995