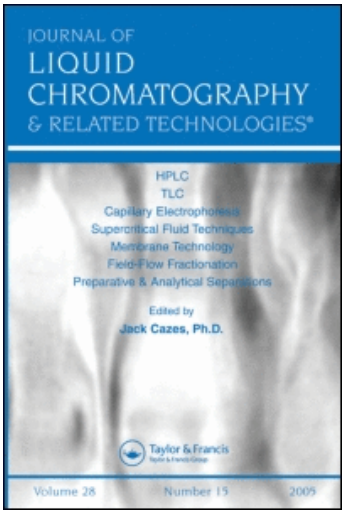

Journal of Liquid Chromatography & Related Technologies

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

High-Performance Liquid Chromatographic Determination of Ceftazidime in Serum, Urine, CSF, and Peritoneal Dialysis Fluid

Philip T. R. Hwang[a]; P. Gregory Drexler[a]; Marvin C. Meyer[a]

[a] Department of Pharmaceutics, College of Pharmacy University of Tennessee Center for the Health Sciences, Memphis, Tennessee

To cite this Article Hwang, Philip T. R. , Drexler, P. Gregory and Meyer, Marvin C.(1984) 'High-Performance Liquid Chromatographic Determination of Ceftazidime in Serum, Urine, CSF, and Peritoneal Dialysis Fluid', Journal of Liquid Chromatography & Related Technologies, 7: 5, 979 — 987

To link to this Article: DOI: 10.1080/01483918408074020

URL: http://dx.doi.org/10.1080/01483918408074020

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(5), 979-987 (1984)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFTAZIDIME IN SERUM, URINE, CSF AND PERITONEAL DIALYSIS FLUID

Philip T.R. Hwang, P. Gregory Drexler and Marvin C. Meyer*

Department of Pharmaceutics
College of Pharmacy
University of Tennessee Center for the Health Sciences
Memphis, Tennessee 28163

ABSTRACT

A rapid, sensitive and specific high performance liquid chromatographic method is described for the determination of ceftazidime in serum, urine, CSF and peritoneal dialysis fluid. The procedure employs reversed-phase chromatography, using hydrochlorothiazide as an internal standard. The assay only requires 100 µl of sample with direct injection of diluted urine, CSF, peritoneal dialysis fluid or protein precipitated serum. Stability studies indicate good drug recovery if urine and serum are stored under proper conditions. The method is specific for ceftazidime in the presence of amikacin, gentamicin, kanamycin, tobramycin, methicillin, penicillin G, ampicillin, chloramphenicol and caffeine. The method has been successfully employed in the assay of over 700 samples obtained during human clinical trials.

INTRODUCTION

Ceftazidime (Fortaz™), developed by Glaxo Group Research, is a beta-lactamase resistant cephalosporin antibiotic for parenteral administration. Ceftazidime exhibits good in vitro activity

*Author to whom correspondence should be addressed.

 0148-3919/84/0705-0979$3.50/0

against a wide range of gram negative pathogens and most gram positive organisms. Particularly noteworthy is its excellent activity against Pseudomonas aeruginosa (1).

The present study concerns the development of a HPLC method for the determination of ceftazidime, using hydrochlorothiazide as an internal standard. The method employs the direct injection of diluted urine, CSF, peritoneal dialysis fluid or protein precipitated serum into the HPLC, and is based on an earlier method developed for cefoperazone (2).

EXPERIMENTAL

Reagents and Materials

HPLC grade acetonitrile and methanol and ACS grade sodium hydroxide and glacial acetic acid were from Fisher Scientific Company (Fair Lawn, NJ). The peritoneal dialysis fluid was Dianeal 137 with 1.5% dextrose from Travenol Laboratories, Inc. (Deerfield, IL). Glaxo Inc. (Research Triangle Park, N. C.) supplied the ceftazidime pentahydrate. Hydrochlorothiazide was supplied by Merck Sharp and Dohme (West Point, PA). Methicillin, ampicillin, penicillin G, kanamycin, tobramycin and gentamicin were obtained from Pfizer Quality Control (Brooklyn, NY). Chloramphenicol was supplied by Parke Davis (Detroit, MI) and amikacin was supplied by Bristol Labs (Syracuse, NY). Caffeine was purchased from Sigma (St. Louis, MO).

Chromatography Equipment and Conditions

The HPLC system (Waters Assoc., Milford, MA) consisted of a model 6000A solvent delivery system; a U6K loop injector; a model

440 UV absorbance detector with a 254 nm filter; a guard column containing Bondapak phenyl/corasil; and a prepacked 30 cm x 3.9 mm i.d. stainless steel column containing 10 µm C_{18} µ-Bondapak. The chromatograms were recorded by a 10 mv strip chart recorder (Fisher Recordall, Series 500, Fairlawn, NJ) at a chart speed of 0.25 cm/minute. The chromatographic system was operated at ambient temperature with a flow rate of 2.0 ml/minute and a column pressure of approximately 2000 psi. The detector was employed at 0.02 or 0.05 a.u.f.s., and a 10-15 µl sample injection volume was used.

The mobile phase was prepared by combining 20 ml of glacial acetic acid, 200 ml deionized water, and 120 ml of acetonitrile. The resultant solution was thoroughly mixed before bringing the final volume to 2000 ml with deionized water. The pH of the solution was adjusted to pH 4.0 with a 6N sodium hydroxide solution. The mobile phase was filtered through a Millipore filter, type HA, pore size 0.45 µm (Millipore, Bedford, MA) prior to use.

Standard Solutions

Four different concentration ranges of ceftazidime in methanol were prepared for the analysis of serum, urine, CSF and peritoneal dialysis fluid samples. Standard curves for serum employed methanol solutions containing 25, 50, 100 and 200 µg/ml of ceftazidime. For urine assays 100, 200, 400 and 800 µg/ml methanol standards were employed. For CSF assays 1.25, 2.5, 5 and 10 µg/ml methanol standards were used, and 50, 100, 200 and 400 µg/ml methanol standards were used for the peritoneal dialysis assays. These standards were prepared fresh each day. The internal standard, hydrochloro-

thiazide was also prepared in methanol at concentrations of 100, 400 and 5 µg/ml for the assay of plasma, urine and peritoneal dialysis fluid, and CSF, respectively. Methanol solutions of hydrochlorothiazide were stable for at least 6 months, stored at 4°C.

Standard Curves and Patient Samples

A 100 µl aliquot of each sample was transferred to a 15ml conical centrifuge tube with a micropipet, along with 100 µl of ceftazidime methanol standard and 100 µl of internal standard. The mixture was vortexed (Vortex-Genie, Ace Scientific Supply, Linden, NJ) for 30 seconds, then centrifuged at 1400 xg and 0°C in a refrigerated centrifuge (Beckman, Model J6, Palo Alto, CA) for 15 minutes. Serum and urine standard curves employed pooled, drug free human serum and urine. Standard curves for CSF were prepared using deionized water. Dianeal 137 with 1.5% dextrose was employed to prepare standard curves for peritoneal dialysis fluid. Blank samples for the standard curves were prepared using 200 µl of methanol instead of the drug and internal standard solutions, and patient samples were assayed by substituting 100 µl of methanol for the drug standard solution. Quantitation of ceftazidime concentrations employed least squares regression of plots of peak height ratio (ceftazidime/internal standard) versus ceftazidime concentration.

Assay Specificity

Samples containing 1000 µg/ml of gentamicin, kanamycin, tobramycin, penicillin G, methicillin, amikacin, ampicillin, chloramphenicol and caffeine were assayed to determine the possibility of interference should these compounds be present in patient samples.

Stability Studies

Pooled human serum, human urine of pH 5 and pH 8, and Dianeal 137 with 1.5% dextrose (pH 5.1) were fortified with ceftazidime in concentrations of 100, 400, 400 and 100 µg/ml, respectively. The samples were then assayed after storage for 0, 0.5, 1, 2, 4, 8, 24 and 48 hours at ambient temperature.

Individual 1 ml aliquots of serum and pH 5.5 urine, containing 100 µg/ml and 400 µg/ml of ceftazidime, respectively, were stored at -15°C and -70°C for 30 days. These samples were assayed after 1, 3, 14 and 30 days of storage, and the results were compared to those from freshly prepared samples.

RESULTS AND DISCUSSION

Figure 1 illustrates typical chromatograms for the assay of patient serum, urine and CSF samples. The retention times for the internal standard and the ceftazidime were about 6 min and 11 min, respectively. Blank samples containing no drug or internal standard were free from any interfering peaks in the vicinity of the drug and internal standard. Peaks observed with serum samples fortified with 1000 µg/ml of other antibiotics and caffeine were well resolved from both the drug and internal standard. The retention times were 5, 10, 18 and 37 minutes for amikacin, ampicillin, caffeine and chloramphenicol, respectively. No peaks were found in samples containing kanamycin, gentamicin, tobramycin, penicillin G or methicillin, and these drugs presumably eluted with the solvent peak or were retained on the column.

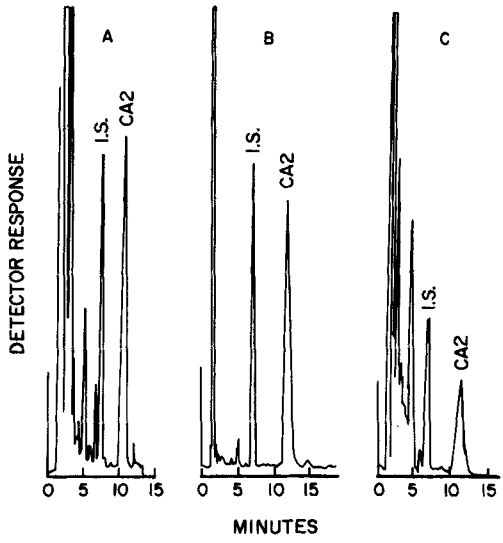

FIGURE 1 - Chromatograms from the assay of samples obtained from patients receiving intravenous ceftazidime (CA2). (A) urine containing 417 µg/ml. (B) serum containing 97 µg/ml. (C) CSF containing 4.7 µg/ml. I.S. is internal standard.

Calibration curves for peak height ratio versus ceftazidime serum, urine, CSF and dialysis fluid all exhibited excellent linearity, with negligible intercepts and correlation coefficients which were consistently in the range of 0.998-0.999. The lower limit of detection was 0.5 µg/ml, and the recovery of drug was consistently at least 95 percent. Table I summarizes the results of the precision studies for four serum, urine and CSF standards assayed on a single day, or individually on four separate days.

The ambient temperature stability studies exhibited ceftazidime recoveries after 24 and 48 hr of 100 and 99 percent for dialysis fluid, 101 and 96 percent for pH 5 urine, 86 and 80 percent

TABLE I

WITHIN-DAY AND BETWEEN-DAY PRECISION OF CEFTAZIDIME ASSAY*

Within-Day Precision

Serum Standards		Urine Standards		CSF Standards	
Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)
21.5	20.8±0.6	86.2	85.1±3.4	1.08	0.97±0.04
43.1	42.5±0.9	172.4	172.3±2.7	2.16	2.00±0.03
86.2	86.1±1.2	344.8	342.4±3.3	4.31	4.26±0.29
172.4	168.7±2.3	689.6	686.0±8.4	8.62	8.42±0.35

Between-Day Precision

Serum Standards		Urine Standards		CSF Standards	
Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)
21.5	20.4±1.1	86.2	84.1±2.5	1.08	0.95±0.05
43.1	42.9±1.3	172.4	168.4±5.5	2.16	2.03±0.04
86.2	85.4±1.9	344.8	338.9±8.6	4.31	4.10±0.10
172.4	170.4±4.5	689.6	683.8±9.7	8.62	8.86±0.55

*N = 4 replicates for serum, urine and CSF (±S.D.)

for pH 8 urine, and 29 and 4 percent for serum, respectively. The marked deterioration of ceftazidime in serum samples at ambient temperature precludes the storage of serum samples containing ceftazidime for any appreciable length of time at ambient temperature. The stability of ceftazidime in peritoneal dialysis fluid over 48 hr at ambient temperature makes possible the advance preparation of ceftazidime dialysis fluids for intraperitoneal admin-

istration. Ceftazidime recovery after storage at -15°C for 3, 14 and 30 days was 105, 94 and 88 percent for pH 5.5 urine, and 99, 108 and 86 percent for serum, respectively. The stability of the samples increased when stored at -70°C, with ceftazidime recoveries of 99 and 101 percent for the urine, and 97 and 91 percent for the serum, respectively, after 3 and 30 days of storage.

Assay of Patient Samples

This assay method has been employed for over one year in the analysis of human serum, urine, CSF and peritoneal dialysis fluid from patients participating in clinical trials. Serum ceftazidime concentrations have usually been in the range of 10-200 µg/ml, in agreement with earlier work (3). Urine drug concentrations were usually between 100-800 µg/ml. Urine concentrations above this range were easily diluted prior to assay. The CSF and peritoneal dialysis fluid drug concentrations have usually been in the range of 1-10 µg/ml and 10-100 µg/ml, respectively.

It is concluded that the method is rapid, sensitive and does not exhibit interferences from a number of other drugs which may be present in samples obtained from patients. The primary advantage of this method is the use of hydrochlorothiazide, which is quite stable, as the internal standard. An earlier assay (4) employed cephalexin as the internal standard, and this drug is considerably less stable (5,6) than is hydrochlorothiazide.

ACKNOWLEDGEMENTS

This study was supported by Glaxo, Inc., Research Triangle Park, N.C.

REFERENCES

1. Neu, H.C. and Labthavikul, P., Antibacterial activity and β-lactamase stability of ceftazidime, an aminothiazolyl cephalosporin potentially active against Pseudomonas aeruginosa, Antimicrob. Agents Chemother., 21, 11-18, 1982.

2. Hwang, P.T.R. and Meyer, M.C., High-performance liquid chromatographic micro-assay for cefoperazone in human plasma, urine and CSF, J. Liq. Chromatogr., 6, 743-754, 1983.

3. Wittman, D.H. and Schassan H.H., Penetration of eight β-lactam antibiotics into the peritoneal fluid, Arch. Surg., 118, 205-212, 1983.

4. Ayrton, J., Assay of ceftazidime in biological fluids using high-pressure liquid chromatography, J. Antimicrob. Chemother., 8, 227-231, 1981.

5. Lecaillon, J.B., Rowan, M.C., Souppart, C., Febvre, N. and Juge, F., Determination of cefsulodin, cefotiam, cephalexin, cefotaxime, desacetyl-cefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography, J. Chromatogr., 228, 257-267, 1982.

6. Yamana, T. and Tsuji, A., Comparative stability of cephalosporins in aqueous solution: kinetics and mechanisma of degradation, J. Pharm. Sci., 65, 1563-1574, 1976.