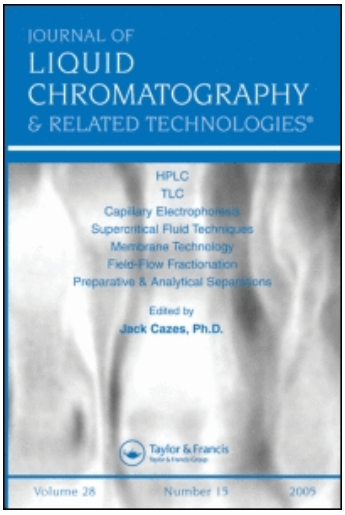

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of LY217332, A New 3'-Quaternary Ammonium Cephalosporin, In Plasma by Solid Phase Column Extraction and HPLC

G. W. Whitaker[a]; T. D. Lindstrom[a]
[a] Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

To cite this Article Whitaker, G. W. and Lindstrom, T. D.(1988) 'Determination of LY217332, A New 3'-Quaternary Ammonium Cephalosporin, In Plasma by Solid Phase Column Extraction and HPLC', Journal of Liquid Chromatography & Related Technologies, 11: 4, 901 — 912
To link to this Article: DOI: 10.1080/01483918808068353
URL: http://dx.doi.org/10.1080/01483918808068353

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(4), 901-912 (1988)

DETERMINATION OF LY217332, A NEW 3'-QUATERNARY AMMONIUM CEPHALOSPORIN, IN PLASMA BY SOLID PHASE COLUMN EXTRACTION AND HPLC

G. W. Whitaker and T. D. Lindstrom
Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana 46285

ABSTRACT

A sensitive and rapid assay has been developed for the determination of LY217332, a 3'-imidazolo[4,5-c]pyridinium cephalosporin, in plasma. The method utilizes cyano solid phase column extraction and HPLC with ultraviolet detection. The lower limit of detection is 5 ng/ml plasma and the relative standard deviation for precision and accuracy was 5% or less from 50-500 ng/ml. The method is applicable to the assay of ceftazidime, cephaloridine, cefpirome and BMY-28142 with minor modification of the mobile phase and the detection wavelength.

INTRODUCTION

A large number of 3'-quaternary ammmonium cephalosporins have been evaluated for use as injectable broad-spectrum antibiotics (1-4). Of these, 7-[2(2-amino-4-thiazolyl)-2-(Z-methoximino)acetamido]-3-[3-methyl-3H-imidazolo[4,5-c]pyridinium-

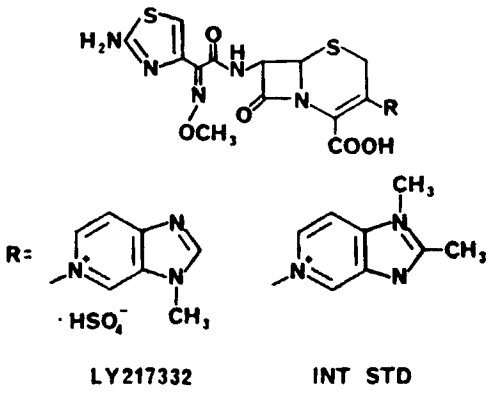

FIGURE 1. Chemical Structure of LY217332 and Internal Standard (LY137428)

5-yl methyl]-3-cephem-4-carboxylate, inner sulfate salt (LY217332) is especially active. Compound LY217332 exhibits in vitro activity against gram negative bacteria better than ceftazidime and cefotaxime. Gram positive activity of LY217332 is similar to HR810 and better than ceftazidime having very good activity against Staphylococcus. LY217332 is currently undergoing preclinical studies in several animal species. In this paper we report the development of a rapid, selective and sensitive assay for LY217332 in plasma using cyano solid phase column extraction and HPLC. Additionally, minor modification of the mobile phase and detection wavelength allows for the simultaneous assay of ceftazidime, cephaloridine, cefpirome and BMY-28142.

MATERIALS AND METHODS

Reagents

All reagents were analytical grade or higher quality. Acetonitrile and methanol (ChromAr HPLC grade) were obtained from

Mallinckrodt. Water was purified through a Milli-Q water purification system (Millipore). Ammonium acetate (Fischer) was A.C.S. certified and sodium heparin (USP Lilly) was derived from porcine intestinal mucosa. Cyano 10-SPE solid phase extraction columns were obtained from Baker Chemical Co. Compounds LY217332 (98% pure), LY137428 (internal standard), and cephaloridine (99% pure) were synthesized at the Lilly Research Laboratories. Cefpirome was obtained from Hoechst, BMY-28142 was supplied by Bristol-Meyers and ceftazidime was obtained from Glaxo. Solvents were of HPLC grade quality (Burdick and Jackson).

Dosing and Plasma Collection

LY217332 in 0.9% saline was administered by intravenous bolus injection to female mongrel dogs (18-21 kg) and male Fischer 344 rats (150 $\pm$ 5 g) at a dose of 20 mg/kg. Drug was administered to dogs by cephalic vein injection and blood was collected from the contra-lateral cephalic vein. Drug was administered to rats by tail vein injection and blood samples were collected by tail vein drip. Blood samples were collected in heparinized Vacutainer tubes (Becton/Dickinson) or heparinized centrifuge tubes and spun at 2000 rpm for 10 min in an I.E.C. Model CL centrifuge. Plasma was removed and stored at -4°C until analyzed.

Solid Phase Extraction

A disposable 1 ml cyano 10-SPE column (Baker Chemical) was attached to a Vac-Elut apparatus (Analytichem International) connected to a vacuum system. The column was conditioned by successively passing 2 ml of methanol and 2 ml of water through the column under a 20 in. Hg vacuum. Internal standard (50 ng, 5

μl of a 10 μg/ml aqueous solution) was added to a 100 μl sample of plasma and the mixture was applied to the column. For plasma samples containing drug concentrations greater than 500 ng/ml, samples were diluted with control plasma, processed and analyzed again. The sample was aspirated through the column until the column was clear of liquid using a 5 in. Hg vacuum. The column was washed with 500 μl of water aspirated through the column at 15 in. Hg vacuum. Compounds LY217332 and internal standard were eluted into a 1.5 ml polyethylene centrifuge tube (Sarstedt #72.690) by washing the column with a 500 μl aliquot of acetonitrile/13 mM ammonium acetate (1:1) under a 15 in. Hg vacuum. The eluate was reduced in volume on a Buchler Vapomix under reduced pressure and the remaining aqueous eluate was reconstituted to 500 μl with HPLC mobile phase (acetonitrile/13 mM ammonium acetate buffer, 10/90).

HPLC Chromatography

Chromatography was accomplished with an HPLC RP-8 10 μm, 25 cm x 4.6 mm (i.d.) column (Alltech) protected with a Spheri-5 RP-8 3 cm x 4.6 mm (i.d.) guard column (Brownlee). Solvent flow was maintained at 1 ml/min with a Beckman Model 110A pump. A Spectroflow Model 773 UV detector (Kratos) was used to monitor eluate absorbance at 210 nm and was interfaced to a Hewlett Packard model 2177F computer for peak analysis. Samples were applied to the system by an HPLC autosampler (Dupont Model 834) equipped with a 250 μl injection loop. The mobile phase was acetonitrile/13 mM ammonium acetate (10:90) and the K' values for LY217332 and internal standard were 3.17 and 4.83, respectively. Alternatively, a mobile phase mixture of acetonitrile/13mM ammonium acetate buffer (5:95) was utilized for the simultaneous chromatography of ceftazidime (K'=2.67), BMY-28142 (K'=5.83),

LY217332 (K'=8.50), internal standard LY137428 (K'=15.17), cefpirome (K'=19.16) and cephaloridine (K'=24.00). Absorbance was monitored at 260 nm.

Calibration curves were generated over the concentration range of 20-500 ng/ml by admixing control plasma with known concentrations of LY217332 and internal standard. Drug concentration of unknowns were calculated by comparing LY217332/internal standard peak height ratios to LY217332 concentration using least squares linear regression.

RESULTS

Cyano solid phase column extraction and recovery were found to be consistent and quantitative over a range of at least 2-50 ng LY217332 on column (corresponding to the standard curve range of 20-500 ng/ml plasma) as shown in Table 1.

Figure 2 shows an HPLC chromatogram of control dog plasma and control dog plasma admixed with 50 ng/ml of LY217332 plus internal standard which was subjected to cyano solid phase extraction and chromatographed as described in Methods. The chromatography time is less than 8 min per sample. Instrument precision was 1.6% (relative standard deviation) as determined by repetitive autoinjection (n=10) of 5 ng samples of LY217332. Six

TABLE 1
Cyano 10-SPE Column Extraction and Recovery

LY217332 Applied	Recovery (%)	RSD (%)
2 ng (n=3)	100.9	0.0
10 ng (n=3)	98.9	1.8
50 ng (n=4)	100.2	1.5

20-500 ng samples of LY217332 were prepared in 1 ml of dog plasma and 0.1 ml was subjected to solid phase extraction.

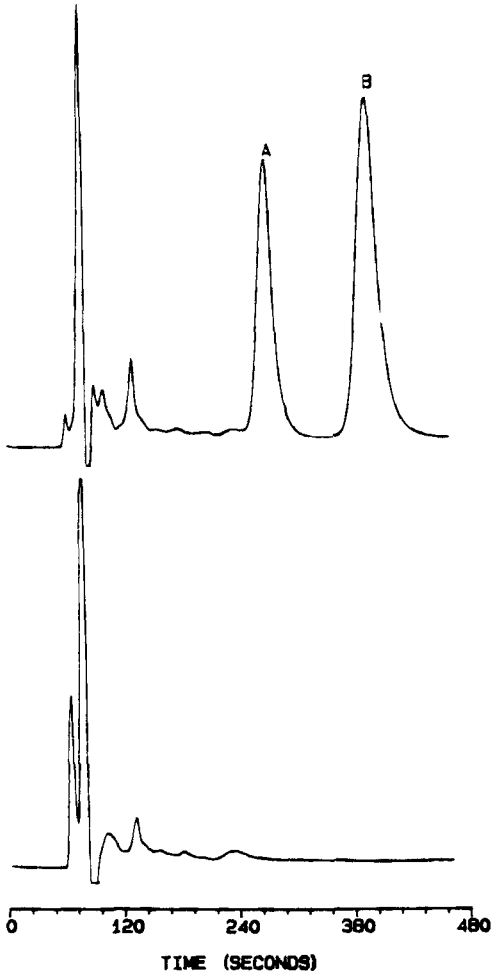

FIGURE 2. HPLC chromatogram of control dog plasma admixed with 50 ng of LY217332 (A) and internal standard (B) in upper panel and control dog plasma only in lower panel. The HPLC solvent system was acetonitrile/13 mM ammonium acetate (10/90) with detection at 210 nm.

point calibration curves of LY217332 in control dog plasma were highly linear (correlation coefficient 0.998-0.999) over the nominal range of 20-500 ng/ml (Table 2). The curves were also highly reproducible even when subjected to multiple determinations on alternating days as indicated by the low variation in the relative standard deviations (3.3% -7.7%). Method accuracy and precision is shown in Table 3. The relative standard deviation of five calibration curves using freshly prepared samples ranged from 1.6% to 3.0%. Samples of control plasma admixed with drug were also prepared and stored at 0°C for five days. On the fifth day, the samples were thawed, subjected to cyano solid phase extraction and chromatographed identical to freshly prepared samples. The accuracy of the assay for these stored samples remained very good as did the precision (relative standard deviation 2.7% - 5.1%).

Plasma concentrations and half-lives of LY217332 were determined in dogs and rats dosed with 20 mg/kg of LY217332 and assayed as described previously. Plasma concentrations of drug in rats ranged from 32.3 μg/ml 15 min after injection to 0.2 μg/ml after 3 hr (Figure 3). Plasma concentrations of drug in dogs ranged from 67.4 μg/ml 15 min after injection to 0.3 μg/ml after 8 hr. The plasma elimination half-life of LY217332 in rats and dogs was 18 ± 1 min and 70 ± 7 min, respectively.

TABLE 2
Linearity and Precision of Calibration Curves

Range (ng/ml)	20 - 500
Slope	0.0491 - 0.0527
Y-Intercept	0.0079 - 0.0128
Relative Std. Dev. (%)	3.3 - 7.7
Correlation Coefficient	0.9981 - 0.9998

Six point calibration curves (n=3) were prepared, processed and analyzed on alternating days over a five day period. Values are the range of values for each parameter over the five day period.

TABLE 3
Accuracy and Precision of LY217332 Determination

LY217332 (ng/ml)	Fresh Samples[a] ng/ml	RSD(%)[c]	Stored Samples[b] ng/ml	RSD(%)[c]
50	53.0	1.6	49.1	3.0
200	195.5	2.8	201.3	2.7
500	501.5	3.0	499.6	5.1

[a] Samples were prepared, process and analyzed on the same day (n=5).
[b] Drug was added to control plasma and samples were stored at 0°C for five days. On day five, samples were thawed, extracted and analyzed identical to fresh samples (n=5).
[c] RSD(%) = (standard deviation/mean)x100.

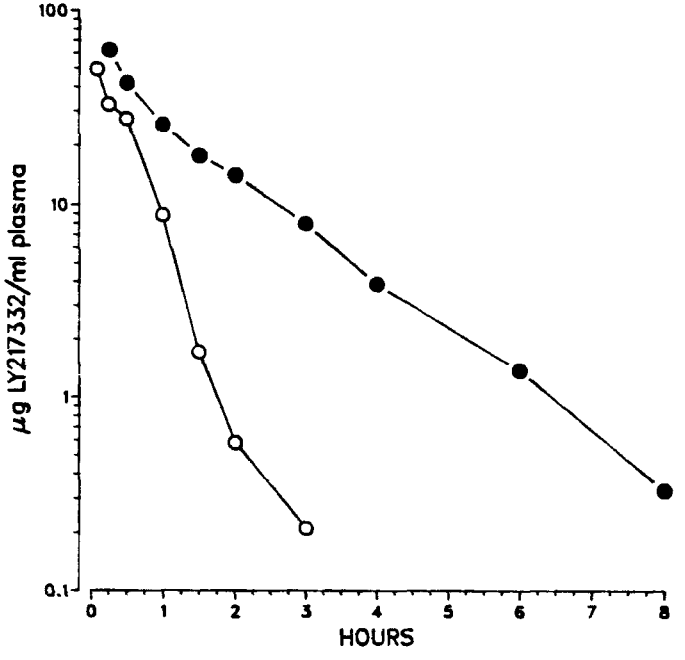

FIGURE 3. Plasma levels of LY217332 following a single intravenous bolus injection of 20 mg/kg of LY217332 to rats (o, n=3) and dogs (o,n=3).

Additional 3'-quaternary ammonium cephalosporins used in clinical trials or in clinical use can be assayed by this methodology with minor modification of the HPLC mobile phase. The detection wavelength was changed to 260 nm since the maximum ultraviolet absorbance of cephtazidime, cefpirome and BMY 28142

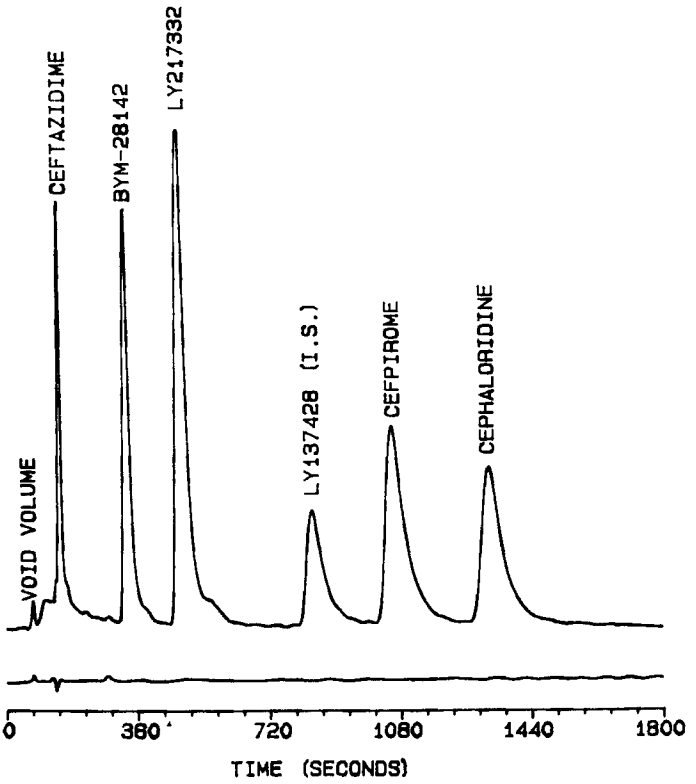

FIGURE 4. HPLC chromatogram of standards of ceftazidime (300 ng), BYM-28142 (500 ng), LY217332 (500 ng), cefpirome (1200 ng) and cephaloridine (1200 ng) admixed with 1 ml of control dog plasma and subjected to cyano 10-SPE column extraction (upper tracing). A chromatogram of control dog plasma subjected to cyano 10-SPE column extraction is also shown (lower tracing). The HPLC solvent system was acetonitrile/13 mM ammonium acetate (5/95) with detection at 260 nm.

occurred at 260 nm. LY217332, LY137428 (internal standard), and cephaloridine also had appreciable absorbance at this wavelength. An admixture of ceftazidime, BMY-28142, LY217332, cefpirome, cephaloridine and internal standard (LY137428) were prepared in control dog plasma and subjected to solid phase extraction and HPLC. Cyano solid phase extraction of control dog plasma rendered a very clean background and all 6 quaternary ammonium cephalosporins were well resolved by the HPLC system (Figure 4).

DISCUSSION

The method reported herein allows for a more sensitive and selective analysis of LY217332 than other previously reported methods for cephalosporin antibiotics (1,5,6). The small volume of plasma needed for analysis (0.1 ml or less) is beneficial for use in small laboratory animal models. Thus, serial blood collections from mice, rats and guinea pigs can be made allowing assessment of plasma elimination half-life within individual animals.

The advantages of solid phase extraction techniques have been demonstrated for many xenobiotics (7,8). The data reported herein have shown the cyano 10-SPE column to be very efficient for the extraction of the new 3'-quaternary ammonium cephalosporin LY217332 from plasma. The cyano 10-SPE column also allows a clean extraction of other 3'-quaternary ammonium cephalosporins such as cephaloridine, ceftazidime, cefpirome and BMY-28142 from plasma. Indeed, other cephalosporins should be amenable to solid phase extraction utilizing the wide range of bonded phases available. In addition, the low ultraviolet absorption of the cyano 10-SPE column plasma eluates greatly increased the signal to noise ratio allowing high sensitivity analysis of the samples. Solid phase extraction should also aid

in the analysis of other cephalosporins with absorption maximums at 190-260 nm as compared to classical protein precipitation or liquid extraction techniques. The combination of cyano solid phase extraction and the HPLC chromatographic system used herein should be applicable to a very wide range of quaternary cephalosporin antibiotics.

The plasma elimination half-life of LY237216 in rats and dogs has been determined. The half-life of the compound in rats subsequent to a 20 mg/kg intravenous bolus dose was 18 $\pm$ 1 min whereas the half-life of the compound in dogs after an identical dose was almost 4 times longer than in rats. These data are in very good agreement with the half-lives in rats and dogs as determined by bioassay of 36 substituted and unsubstituted pyridinium, furopyridinium, thienopyridinium and quinolinium 3'-quaternary ammonium cephalosporins derived from the same series as the imidazolopyridinium cephalosporin LY217332 (2).

REFERENCES

1. Bawdon, R.E., Lu, Y.S. and Brater, D.C. 1985. High-pressure liquid chromatography assay and pharmacokinetics of HR810 after intramuscular injection in rabbits. Antimicrob. Agents Chemother. 27:436-438.

2. Counter, F.T., Eudaly, J.A., Quay, J.F., Ruffolo, R.F., Stucky, J.F. and Wright, W.E. 1983. Program Abst. 23rd Intersci. Conf. Antimicrob. Agents Chemother., Abst no. 996.

3. Brown, R.F., Counter, F.T., Ensminger, P.W., Katner, A.S., Kinnick, M.D., Kurz, K.D., Morin, J.M., Jr., Ott, J.L., Preston, D.A. and Steinberg, M.I. 1985. Program Abst. 25th Intersci. Conf. Antimicrob. Agents Chemother., Abst no. 365.

4. O'Callaghan, C.H., Acred, P., Harper, P.B., Ryan, D.M., Kirby, S.M. and Harding, S.M. 1980. GR 20263, a new broad-spectrum cephalosporin with pseudomonal activity. Antimicrob. Agents Chemother. 17:876-883.

5. McCormick, E.M., Echols, R.M. and Rosano, T.G. 1984. Liquid chromatographic assay of cefitzoxime in sera of normal and uremic patients. Antimicrob. Agents Chemother. 25:336-338.

6. Nygard, G. and Wahba Khalil, S.K. 1984. An isocratic HPLC method for the determination of cephalosporins in plasma. J. Liquid Chromatogr. 7:1461-1475.

7. Good, T.J. and Andrews, J.S. 1981. The use of bonded-phase extraction columns for rapid sample preparation of benzodiazepines and metabolites from serum for HPLC analysis. J. Chromatogr. Sci. 19:562-566.

8. Goto, J., Goto, N. and Nambara, T. 1982. Separation and determination of naproxen enantiomers in serum be high performance liquid chromatography. J. Chromatogr. 239:559-564.