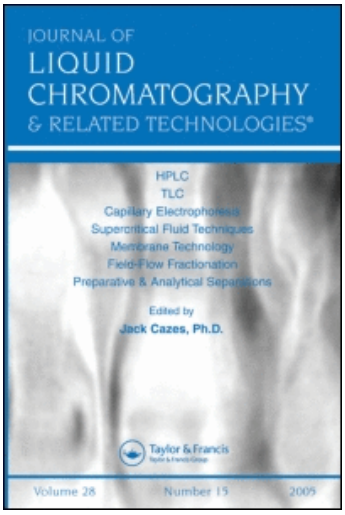

Journal of Liquid Chromatography & Related Technologies

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

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To cite this Article Fasching, Claudine E. , Peterson, Lance R. and Gerding, Dale N.(1986) 'High-Pressure Liquid Chromatographic Analysis for Quantitation of BMY-28142 and Ceftazidime in Human and Rabbit Serum', Journal of Liquid Chromatography & Related Technologies, 9: 8, 1803 — 1814
To link to this Article: DOI: 10.1080/01483918608076719
URL: http://dx.doi.org/10.1080/01483918608076719

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(8), 1803-1814 (1986)

HIGH PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS FOR QUANTITATION OF BMY-28142 AND CEFTAZIDIME IN HUMAN AND RABBIT SERUM

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ABSTRACT

The quantitative measurement of BMY-28142 and ceftazidime in serum after protein precipitation were compared using a reverse-phase liquid chromatographic (LC) method. Detection using ultraviolet absorbance at 275 nm gave a sensitivity of 1.0 mcg/ml and 1.5 mcg/ml for BMY-28142 and ceftazidime respectively, and both gave linear response to concentration of at least 80 mcg/ml. Drug recovery after sample deproteination was 104% for BMY-28142 in rabbit serum and 93% in human serum. Results indicate a precise and accurate assay for this drug can be obtained using a simple LC method.

INTRODUCTION

High-pressure liquid chromatography is in wide use for analytical determination of many antimicrobials

 0148-3919/86/0908-1803$3.50/0

(1, 2, 3). BMY-28142 and ceftazidime, semisynthetic cephalosporins with close structural similarity, are broad spectrum antibiotics under current evaluation (Figure 1). Quantitative analysis of ceftazidime is available (4, 5, 6) for pharmacokinetic testing (7, 8, 9, 10) and in vivo susceptibility studies (11, 12). This quantitation is required for proper correlation of results to in vitro data (13, 14, 15, 16). Use of the LC for BMY-28142 is desirable because of the sensitivity, specificity and high degree of precision of the LC method. For new agents, an LC assay is generally preferred over the standard microbiological assay which lacks specificity, often cannot be used when multiple antibiotics are present in samples, and does not distinguish biologically active metabolites from the parent compound. The purpose of this paper is to i) describe a LC methodology for BMY-28142, ii) to compare the LC assay to a microbiological assay for BMY-28142, and iii) to describe a similar LC assay for ceftazidime.

MATERIALS AND METHODS

Test samples.

Stock solutions of BMY-28142 and ceftazidime were made from reference powders combined with sterile phosphate buffered saline (PBS) pH 7.2 to make a concentration of 1000 mcg/ml and stored in 0.5 ml aliquots at -70°C. Serum used for analysis development was either pooled human serum (PHS) collected in our laboratory or commercial rabbit serum (CRS) (Gibco) to which antibiotics were added in vitro. Current supplies of BMY-28142 were not sufficient for in vivo human studies or extended animal investigation.

Microbiological assay.

A standard agar diffusion technique (17,18,19) employing E. coli ATCC #10536 as indicator organism was used. Volumes of 10 ml of antibiotic agar #1 (Difco Laboratories) which contained a 0.15% suspension of organism, which had been grown overnight in tryptic soy

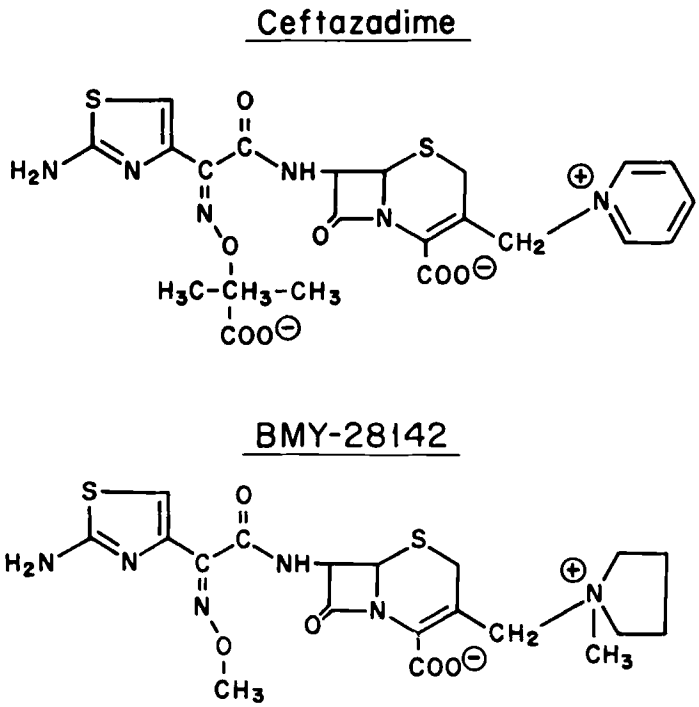

FIGURE 1. Structure of BMY-28142 and ceftazidime.

broth (Difco), were dispensed into 15 X 100 mm Petri dishes (Falcon) and left to harden on a level surface. Twenty mcl of serum sample containing BMY-28142 were placed into wells that had been punched into prepared plates and then the plates were incubated at 37°C for 24 hours. Resultant zone diameters were read to the nearest 0.1 mm and sample concentrations were determined by comparison to a standard curve.

Sample preparation for LC.

Serum samples were precipitated using one of two methods. Method A used a combination of 0.5 ml serum sample, 0.5 ml methanol (Fisher Scientific) and 0.5 ml dilute acetic acid pH 2.8 (1). Method B used a

combination of 0.5 ml serum sample and 1.0 ml methanol. Methods A and B both use a ten-minute room temperature incubation after vortex mixing. Supernatants were removed and were ready for direct injection after a ten-minute centrifugation at 12,000 X g using an Eppendorf Microfuge Model 5414.

LC assay.

The chromatographic system used was a Varian (Walnut Creek, CA.) Model 5020 liquid chromatograph with a Varichrom variable wavelength detector and CDS 111L peak integrator with strip chart recorder. Deproteinated samples were injected using a fixed loop injector (100 mcl) and chromatographed using a micro-Bondapak-C18 column (Waters Associates, Inc.). Mobile phase was dilute acetic acid pH 2.8 (1) with acetonitrile (Fisher Scientific) as the organic modifier. BMY-28142 required 5% acetonitrile and ceftazidime required 10% acetonitrile for optimal peak separation and shape. Assay for both drugs used a 2.0 ml/min flow rate and ultraviolet absorbance detection at 275 nm with detector set at 0.02 aufs.

Statistical analysis.

Linear regression using least squares method and correlation coefficient were calculated using a Hewlett-Packard 41-C programmable calculator with Stat-Pac.

RESULTS

BMY-28142 and ceftazidime gave retention times of 5.3 and 6.4 minutes respectively when using sample precipitation method A, at their appropriate mobile phase composition. Figures 2-5 represent chromatograms of drugs for each preparation method. Linear regression analysis, determined by plotting drug concentration (x) against peak area (y), gave correlation coefficients of 0.996 for BMY-28142 and 0.999 for ceftazidime when tested in CRS at concentrations from 0.625 mcg/ml to 80 mcg/ml. Drug detection in CRS was sensitive to 1.0

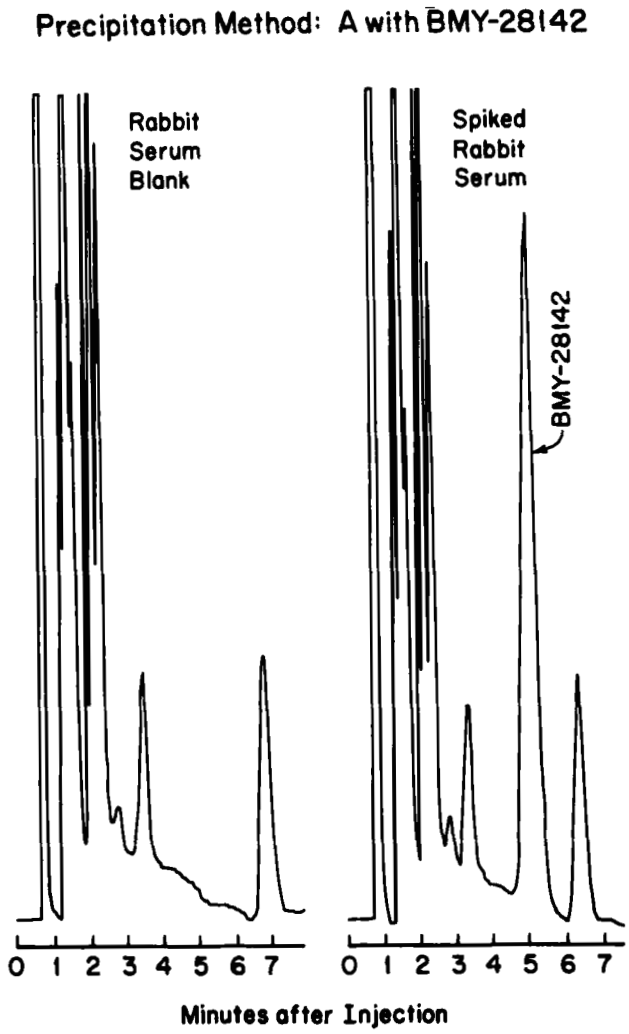

FIGURE 2. Chromatogram of BMY-28142 at 10 mcg/ml.

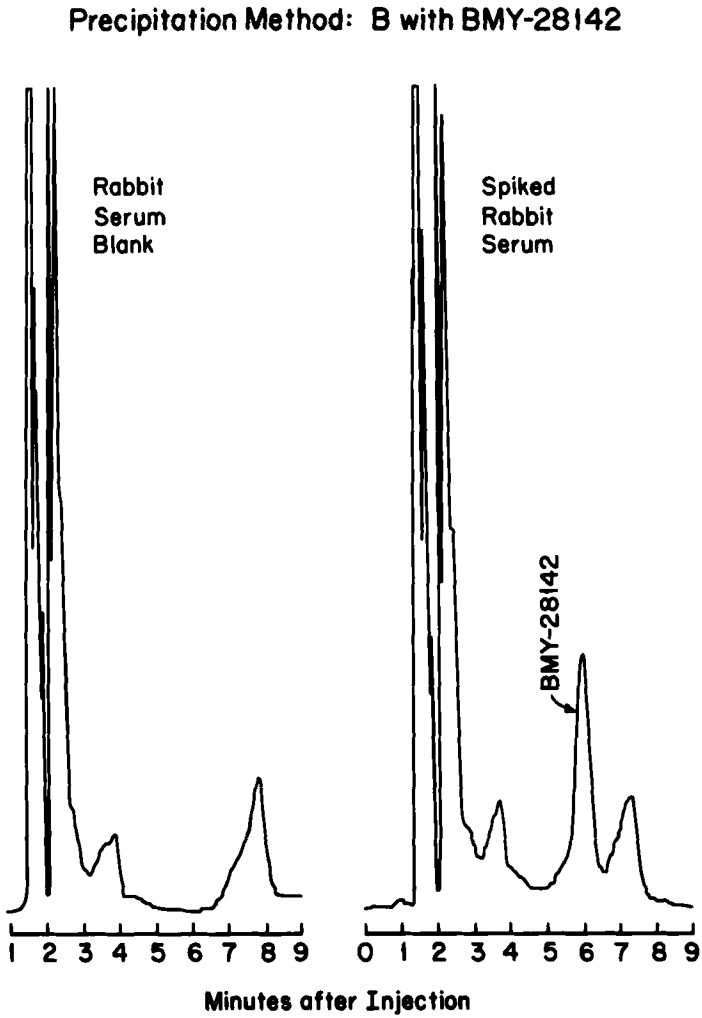

FIGURE 3. Chromatogram of BMY-28142 at 10 mcg/ml.

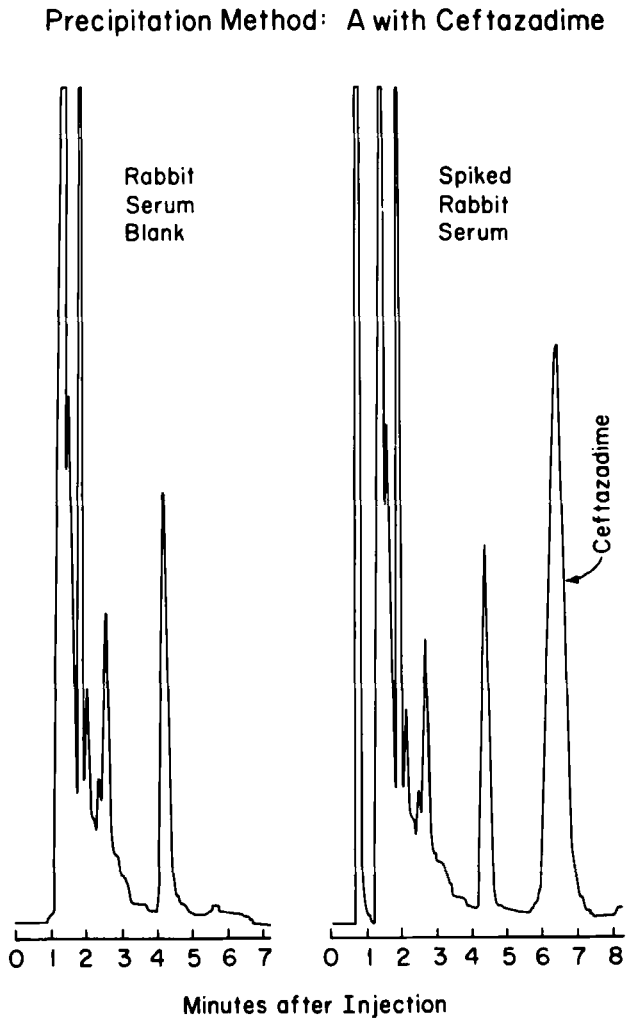

FIGURE 4. Chromatogram of Ceftazidime at 10 mcg/ml.

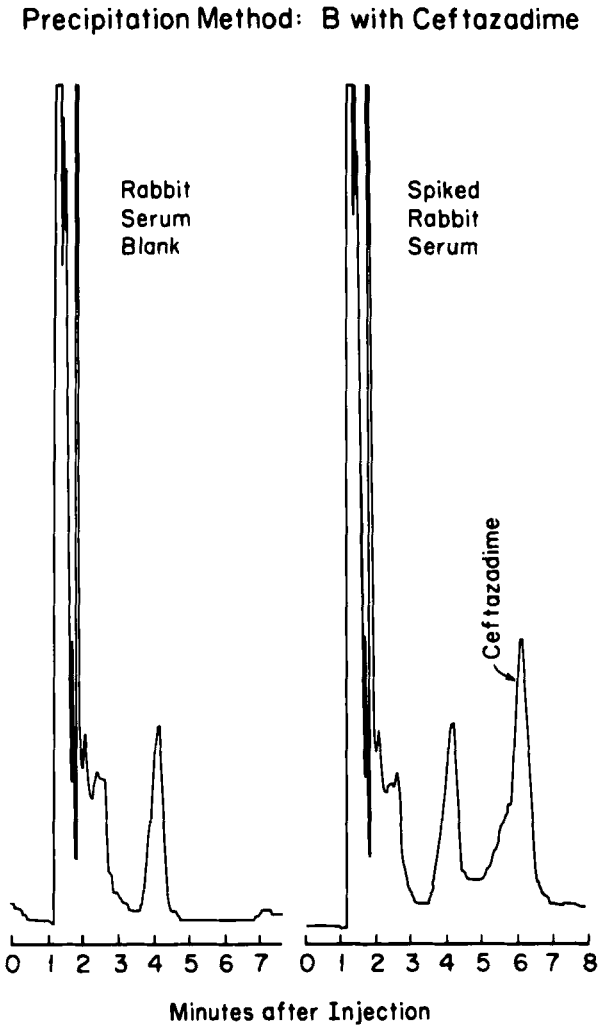

FIGURE 5. Chromatogram of ceftazidime at 10 mcg/ml.

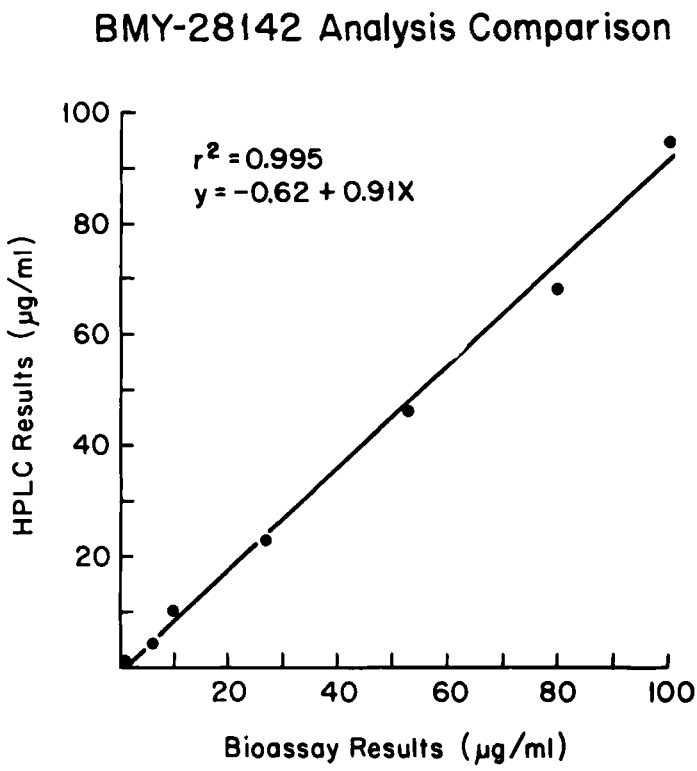

FIGURE 6. Linear regression results for assay of BMY-28142.

mcg/ml for BMY-28142 and 1.5 mcg/ml for ceftazidime using detector settings listed, and precipitation method A. Drug recoveries determined by comparing peak areas using method A to precipitate serum to peak areas from PBS samples equally diluted were 104% for BMY-28142 in CRS and 93% PHS, and 91% for ceftazidime in CRS.

Accuracy (% deviation from mean) determined using 2.5 mcg/ml antibiotic concentration in CRS assayed ten times was 4.2% and 4.0% for BMY-28142 and ceftazidime respectively. Precision (coefficient of variation) determined using 10.0 mcg/ml antibiotic concentration in CRS assayed ten times was 2.5% and 4.5% for BMY-28142 and ceftazidime respectively.

Comparison of LC to microbiological assays was performed in CRS spiked with concentrations of BMY-28142 (1.0 - 100.0 mcg/ml). Linear regression analysis determined by plotting bioassay (x) against LC (y) gave equation y = -0.617 + 0.907 x and a correlation oefficient of 0.995, and is represented in Figure 6.

DISCUSSION

Chromatograms (Figures 2 and 4) show good separation using the described techniques. Peak shape and drug recovery were improved by using precipitation method A, which included both the acetic acid mobile phase and methanol as opposed to method B which used only methanol. Drug recoveries of greater than 90% were obtained with Method A, presumably as a result of the influence of pH on dissociation of drug from protein (2). The enhancement of drug recovery by precipitation with acetic combination is preferable to methanol alone. Linearity was good for both drugs in the range tested as shown by correlation coefficients close to 1.00. The sensitivity and range can be further manipulated by dilution of samples and/or changing the detector sensitivity setting. Both the accuracy and precision of the LC method is statistically acceptable.

Comparison analysis, shown in Figure 6, demonstrates that the LC method has a statistically equivalent direct relationship with the standard microbiological assay for BMY-28142 spiked into CRS.

Indications for use of LC are the accuracy and precision of the method and the ability to quantitate only the drug of interest. Such an assay will be of importance in forthcoming investigations of the newly synthesized, extended spectrum agent BMY-28142.

ACKNOWLEDGMENTS

Tnis work was supported by the Veterans Administration and Bristol Laboratories.

The authors thank Evelyn C. Glatt for typing the manuscript.

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