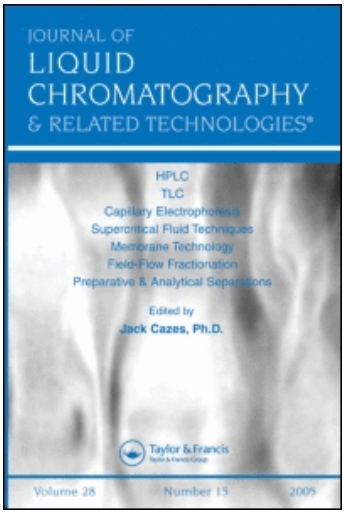

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High-Pressure Liquid Chromatographic Assay of Azlocillin and Mezlocillin with an Anion-Exchange Extraction Technique

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HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF AZLOCILLIN AND MEZLOCILLIN WITH AN ANION-EXCHANGE EXTRACTION TECHNIQUE

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ABSTRACT

An anion-exchange column deproteination technique has been employed with the high-pressure liquid chromatographic (LC) assay for azlocillin and mezlocillin. The anion-exchange extraction gave excellent (97%-99%) drug recovery. Quantitation of antibiotics using the LC method compares favorably to the traditional biological assay technique with correlation coefficients for azlocillin = 0.998 and mezlocillin = 0.988. The anion-exchange extraction provides an interference-free chromatogram which aids in the LC assay of these drugs.

INTRODUCTION

Azlocillin and mezlocillin, ureidopenicillins, belong to a new class of semisynthetic penicillins possessing an extended spectrum of antimicrobial activity. Several methods for the LC assay of these agents have been described in the literature using organic extraction and Sep-Pak column (Waters Associates, Milford, MA.) protein separation techniques (1,2). We have developed an anion-exchange column procedure that works well for several cephalosporins (3,4) and have employed

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this method for the LC assay of azlocillin and mezlocillin. The purpose of this report is to (i) apply anion-exchange deproteination techniques to the assay of azlocillin and mezlocillin, (ii) define drug recovery using this method, and (iii) compare quantitative results of LC method to the biological assay method.

EXPERIMENTAL

Antibiotic Extraction Technique

Anion-exchange columns were prepared by packing 6-ml syringes with DEAE A-25 Sephadex (Pharmacia Fine Chemical, Piscataway, N.J.) as described previously (3). Samples were processed by placing 0.5 ml serum on the column, followed by 0.5 ml phosphate buffered saline pH 7.2 (PBS) to rinse sample completely into column. Proteins were removed with a 4.0 ml PBS column wash, and antibiotics were then eluted from the column with 5.0 ml of 1.0 M sodium chloride. The final eluent is collected for LC analysis.

LC Assay

The chromatographic system used was a Varian (Walnut Creek, CA.) model LC 5020 liquid chromatograph with a Varichrom variable wavelength detector and a CDS 111L peak integrator with strip chart recorder. Sample eluates (100 µl) were chromatographed using an analytical octadecylsilane column (Waters Associates) of 30-cm length, 4 mm internal diameter, and 10 µm particle size. The mobile phase had a flow rate of 2.0 ml/min at isocratic conditions of 60% 0.01 M acetate buffer at pH 4.8 and 40% methanol. The UV detector was set at 220 nm and 0.1 A full scale. The calculations were based on an external standard of 20 µg/ml for both azlocillin and mezlocillin assays. Each sample was injected in duplicate and results averaged to obtain the concentration value.

Microbiological Assay

Antibiotic bioassays were performed using a standard well diffusion method with a 24-hour incubation at 37°C (5). The assay used *Micrococcus luteus* ATCC #9431 as the indicator organism in antibiotic medium no. 1 (Difco Laboratories, Detroit, MI.) at pH 6.6. Antibiotic concentrations were determined by reading zone diameters to the nearest 0.1 mm and comparing them to a standard line from 40 µg/ml to 1.25 µg/ml of either azlocillin or mezlocillin. All samples exceeding 40 µg/ml were diluted so as to fall within the standard line range. Bioassay of unknowns were run on three separate plates for each sample and then averaged.

Samples

Azlocillin and mezlocillin were added to pooled human serum *in vitro* to provide assay samples. LC assay linearity was determined for both drugs at concentrations of 100, 80, 60, 40, 20, 10, and 5 µg/ml. Peak area of antibiotic was plotted on a linear graph against drug concentration. Antibiotic recovery, using anion-exchange columns for sample preparation, was performed at 2 concentrations, 50 and 25 µg/ml. Recovery was calculated by comparing the antibiotic peak of a column eluate to one representing total drug. The described protein removal process dilutes original serum sample 10-fold, therefore saline samples containing 50 µg/ml and 25 µg/ml were diluted 1 to 10 in 1.0 M sodium chloride and used in drug recovery determination. Peak areas from serum samples were divided by the saline dilution peak areas and multiplied by 100 to give percent drug recovered. Each concentration's recovery was determined using two column extractions for both drugs. Antibiotic concentrations of approximately 100, 75, 50, 25, 10, and 5 µg/ml were prepared for each drug and si-

multaneous LC and biological ssays were performed to compare methodologies.

RESULTS

The retention times for azlocillin and mezlocillin were 4.0 and 4.4 minutes respectively, near the reported retention time of 5.25 minutes for piperacillin using the same mobile phase (6). The chromatograms from human serum samples are shown in Figure 1. The azlocillin and mezlocillin had linear detector response through range tested as shown in Figure 2. Quantitation of less than 5 µg/ml could be achieved by increasing the sensitivity of UV detector. The anion-exchange column extraction gave recoveries of 98% at 50 µg/ml for both azlocillin and mezlocillin, and at 25 µg/ml, 99% and 97% respectively. Comparison of bioassay and LC assay are shown in Figure 3 for azlocillin and Figure 4 for mezlocillin. Azlocillin comparison had a correlation coefficient of 0.998 and the mezlocillin comparison had a correlation coefficient of 0.988.

DISCUSSION

The anion-exchange column extraction provides excellent results for both quantitative drug recovery and serum interference removal for the azlocillin and mezlocillin assays, as previously found for cephalorporins in our reports using this extraction method (3,4). Since organic extractions have not given 100% antibiotic recovery (1) and involve several steps for sample preparation, a simpler and faster deproteination method is preferred. A Sep-Pak column preparation technique has also been described (2) and reports good antibiotic recovery, but at the present time Sep-Paks are costly and are used only once. The anion-exchange columns are made quickly and can be used at least ten times (3), which markedly reduces cost of sample preparation.

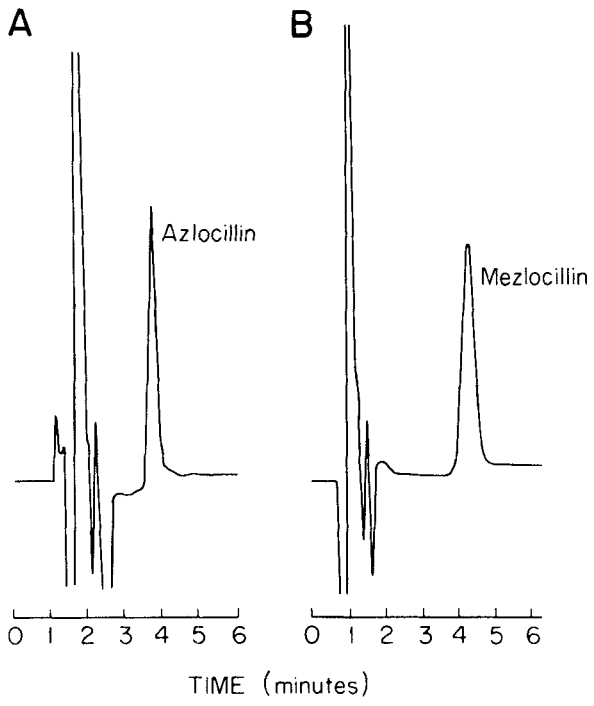

Figure 1. Sample chromatogram of azlocillin, 25 µg/ml (a) and mezlocillin, 40 µg/ml (b) from serum samples.

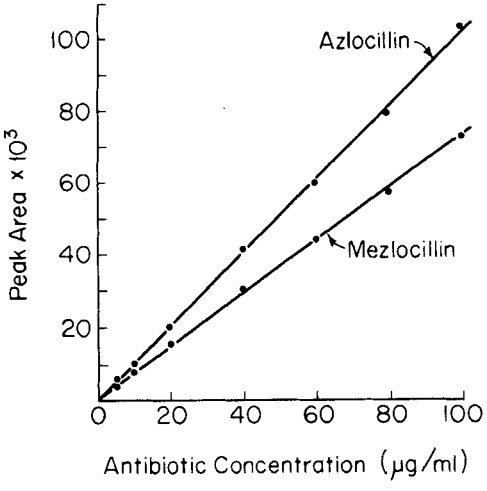

Figure 2. Comparison of detector responses (in peak area) to antibiotic concentration in sample for azlocillin and mezlocillin.

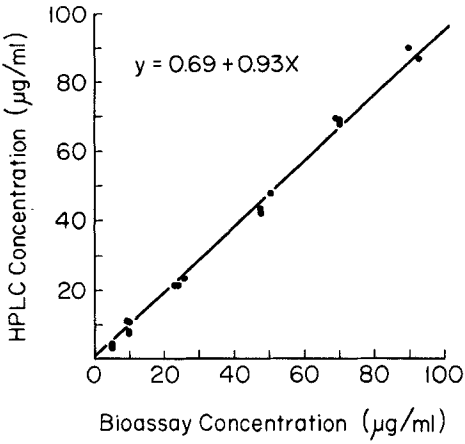

Figure 3. Linear regression of LC and bioassay for azlocillin.

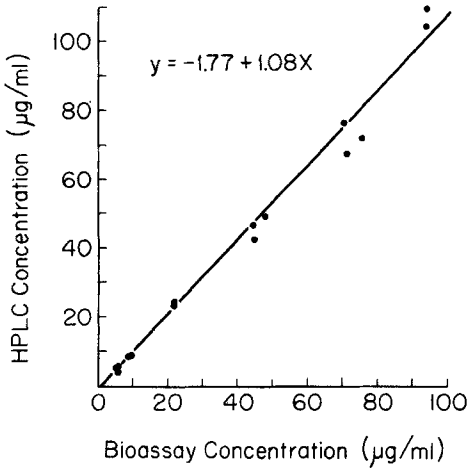

Figure 4. Linear regression of LC and bioassay for mezlocillin.

The results of linear regression analysis indicate that for both azlocillin and mezlocillin slope values obtained were near a perfect direct relationship of b=1.0. The correlation coefficient calculations represent the close agreement of the two groups of assay data obtained for each drug and reflects a favorable comparison for the two methods. Anion-exchange column extraction is a rapid, inexpensive technique for the deproteination of serum samples for use with the LC assay of azlocillin and mezlocillin. The LC method provides a sensitive, specific, and quantitative antibiotic assay.

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