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Note

Reversed-phase high-performance liquid chromatographic assay for the determination of mezlocillin in human and rat biological samples

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Mezlocillin is a semi-synthetic broad-spectrum penicillin antibiotic which is effective against a wide range of Gram-negative and Gram-positive bacteria Pharmacokinetic studies recently conducted in newborn infants [1] and in rats [2] required a sensitive assay method for mezlocillin that utilized small sample volumes of 50 μl or less Published methods involve high-performance liquid chromatographic (HPLC) analysis but either following complex extraction procedures or utilizing large sample volumes [3,4] To circumvent these problems, an isocratic reversed-phase HPLC assay was developed for the determination of mezlocillin concentrations in small sample volumes This method is applicable to human and rat serum urine and rat bile with the advantages of direct injection of specimens after protein precipitation or dilution using 50 μl of sample or less

EXPERIMENTAL

Equipment

The HPLC system consisted of a Model M45 pump, Model 710B intelligent sample processor, and Model 441 absorbance detector with a zinc lamp using a 214-nm filter (Waters Assoc , Milford, MA, U S A.). Peak heights were integrated using an HP 3392A integrator (Hewlett-Packard, Avondale, PA, U S A). Chromatographic separation was obtained on an Altex Ultrasphere

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(neonatal samples) [1] or an Alltech Econosphere (rat samples) [2] ODS, 5 μm particle size, C_{18} column (25 cm $\times$ 4 6 mm) after passing samples through a guard column packed with C_{18} Bondapak, 30–38 μm particle size (Whatman, Clifton, NJ, U S A)

Chemicals and reagents

Mezlocillin sodium was a gift from Miles Pharmaceuticals (Division of Miles Labs , West Haven, CT, U S A) and piperacillin sodium (P-8396) was obtained from Sigma (St Louis, MO, U S A) KH_2PO_4 and Na_2HPO_4 were reagent grade (Fisher Scientific, Fair Lawn, NJ, U S A) and acetonitrile and 1,2-dichloromethane were of HPLC grade (Burdick and Jackson Labs , Muskegon, MI, U S A)

The mobile phase consisted of 23% (v/v) acetonitrile in 0 1 *M* phosphate buffer (3 5 g KH_2PO_4 and 11 05 g Na_2HPO_4 per liter of distilled water, adjusted to pH 7 0 with concentrated hydrochloric acid) The mixture was filtered and degassed, and the flow-rate was 1 3 ml/min

Sample preparation

For the assay of mezlocillin in human neonatal serum, 50 μl of serum were added to 100 μl of acetonitrile and mixed on a vortex mixer for 5 s to precipitate proteins A 50-μl volume of piperacillin internal standard solution (50 mg/l piperacillin in 0 1 *M* pH 7 phosphate buffer) was then added, mixed on a vortex mixer for 30 s, shaken for 5 min, and centrifuged at 800 *g* for 5 min The aqueous portion was then added to 200 μl of 1,2-dichloromethane, mixed on a vortex mixer, shaken, and centrifuged as before A 10-μl volume of the aqueous (top) layer was assayed Human neonatal urine samples were diluted with 0 1 *M* pH 7 phosphate buffer between 5- and 81-fold A 250-μl volume of diluted urine was combined with 1 ml of acetonitrile and 250 μl of piperacillin internal standard solution, mixed on a vortex mixer, shaken, and centrifuged as before The aqueous portion was then combined with 2 ml of 1,2-dichloromethane as described above Standards were prepared in serum or phosphate buffer in concentrations between 1 25 and 100 mg/l Serum samples with higher concentrations were diluted into the standard curve range

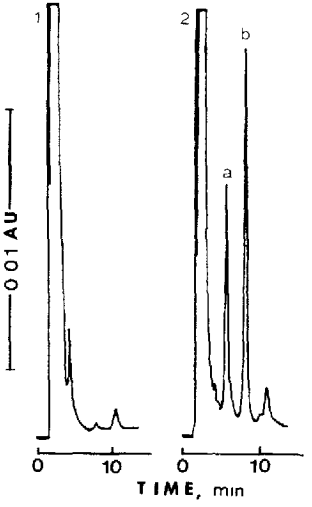
Rat samples containing mezlocillin were prepared similarly, except 40 μl of sample were added to 100 μl of acetonitrile, 40 μl of 0 1 *M* pH 7 phosphate buffer, and 200 μl of piperacillin internal standard solution (8 μg/ml in 0 1 *M* pH 7 phosphate buffer) Rat urine and bile were diluted with distilled water between 10- and 300-fold A 300-μl volume of the supernatant was combined with 150 μl of 1,2-dichloromethane, mixed on a vortex mixer, shaken, and centrifuged as described before, and 20–50 μl of the aqueous (top) layer were assayed Standards were prepared in concentrations between 1 25 and 80 μg/ml All standards and samples were stored at $-70\,^{\circ}C$, at which temperature mezlocillin has been found to be stable for at least three months [5]

RESULTS AND DISCUSSION

Fig 1 shows chromatograms from drug-free adult human serum and a neonatal serum sample containing 60 μg/ml mezlocillin The retention times for piperacillin and mezlocillin are 5 6 and 7 8 min, respectively The selectivity for mezlocillin is shown by the sharp resolution of the peaks and no interfering peaks in the drug-free chromatogram at the times of the internal standard and mezlocillin peaks Endogenous compounds, particularly in neonatal serum, elute shortly after mezlocillin

Fig 2 shows representative chromatograms for drug-free serum and urine samples taken from a rat dosed intravenously with 50 mg/kg mezlocillin Chromatograms from all fluids showed no interferences at the times of the mezlocillin and piperacillin peaks (6.3 and 4 5 min) However, bile, unlike serum and urine, contained many endogenous substances that continued to elute after mezlocillin for as long as 20 min

Mezlocillin concentrations were determined by linear regression of calibration curves prepared from the peak-height ratio of mezlocillin to internal standard versus mezlocillin standard concentrations The assay was linear over the range 1.25–80 μg/ml, with regression correlation coefficients typically equal



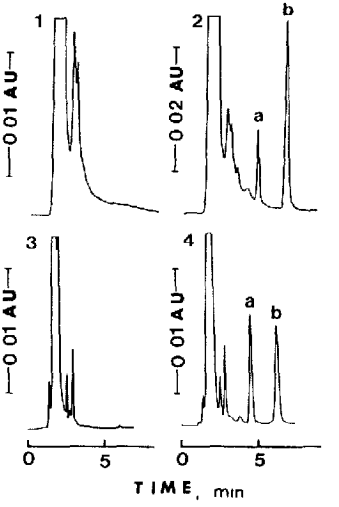


Fig 1 Chromatograms of drug-free human serum (1) and a human neonate serum sample (2) containing a mezlocillin concentration of 60 mg/l Piperacillin (a) and mezlocillin (b) retention times are 5 6 and 7 8 min, respectively The third peak in (2) is an endogenous compound in neonatal serum

Fig 2 Chromatograms of drug-free rat serum (1), a rat serum sample (2) containing 62 mg/l mezlocillin, a drug-free rat urine (3), and a rat urine sample (4) Piperacillin (a) and mezlocillin (b) retention times are usually 4 5 and 6 3 min, respectively

TABLE I

PRECISION AND ACCURACY FOR THE ASSAY OF MEZLOCILLIN IN RAT SERUM

Concentration added (mg/l)	Intra-assay				Inter-assay			
	Concentration found (mg/l)	C V (%)	*n*	Accuracy[a]	Concentration found (mg/l)	C V (%)	*n*	Accuracy[a]
1 25	1 25	4 6	3	100	1 22	5 3	7	97 6
20 0	19 9	1 9	3	99 5	20 0	2.1	8	100 0
80 0	80 0	1 1	3	100	79 6	1 1	8	99 5

[a] (Concentration found/concentration added) × 100%

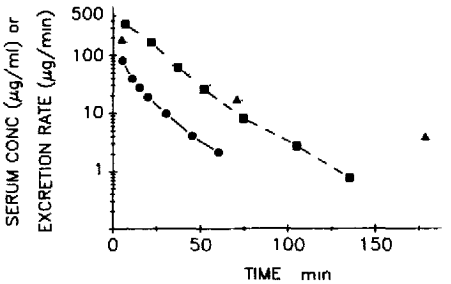


Fig 3 Mezlocillin serum concentrations (●) and biliary (■) and urinary (▲) excretion rate versus time profiles in rat dosed intravenously with 50 mg/kg mezlocillin

to 0 99 or better The detection limit was 0 1 μg/ml at a signal-to-noise ratio of 3 0 although the usual standard curve employed a limit of quantitation of 1 25 μg/ml The range of the standard curve was intentionally limited to gain better accuracy and reproducibility for the clinical and animal samples [1,2] Table I shows the intra- and inter-assay coefficients of variation (C V) for three mezlocillin concentrations, all of which were ⩽5 3%

Fig 3 shows a representative profile of mezlocillin serum concentrations, and urinary and biliary excretion rates versus time for a rat dosed intravenously with 50 mg/kg mezlocillin This dose is similar to 4 g per 70 kg as used in humans The quantitation of mezlocillin in all three fluids yielded useful pharmacokinetic data [2]

Previous HPLC procedures for mezlocillin have the disadvantages of inefficient double extraction of samples [3], solid phase extraction [4,6], or anion-exchange column extraction [7] using up to 1 ml of sample with a detection limit of only 5 μg/ml Another assay does not remove proteins from plasma before injection [5], which will decrease analytical column life The use of 200 μl of sample quantitated by ion-paired chromatography has been reported, but this method lacks precision at low concentrations [8] Our assay procedure

has the advantages of simple and rapid sample preparation for small volumes of sample, followed by rapid quantitation using isocratic reversed-phase chromatography In addition, the sensitivity and reproducibility of this approach are superior to previously published methods

In summary, a simple and selective assay for mezlocillin in several biological fluids from humans and rats has been described. By precipitating proteins with acetonitrile, direct injection of the resultant sample is then possible. The assay is precise and accurate for concentrations of mezlocillin seen in human and rat pharmacokinetic studies Quantitation by this reversed-phase HPLC method allows analysis in under 10 min

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