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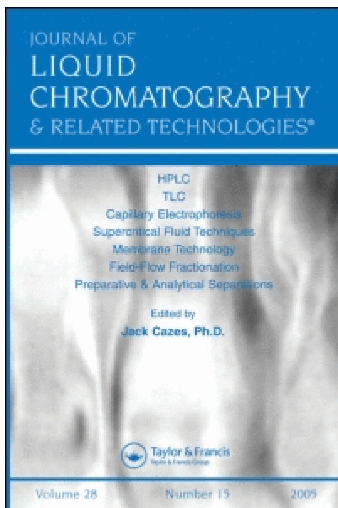

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
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Determination of Ticarcillin in Serum by Reversed Phase High Performance Liquid Chromatography

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To cite this Article Aravind, M. K. , Miceli, J. N. and Kauffman, R. E.(1984) 'Determination of Ticarcillin in Serum by Reversed Phase High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 7: 14, 2887 — 2893

To link to this Article: DOI: 10.1080/01483918408067053

URL: http://dx.doi.org/10.1080/01483918408067053

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(14), 2887-2893 (1984)

DETERMINATION OF TICARCILLIN IN SERUM BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ticarcillin is a semi-synthetic penicillin useful against several *Pseudomonas* species. In order to easily quantitate this drug, a new procedure was developed whereby ticarcillin in serum is converted to its free acid form by the addition of citric acid and, subsequently, extracted into ethyl acetate. The organic extract which contains the nonionized form of ticarcillin is dried under nitrogen, the sample is reconstituted with mobile phase and analyzed by high performance liquid chromatography. Elution is completed in less than five minutes. The assay is linear from 1 mg/L through 100 mg/L. The correlation coefficient of ticarcillin concentration to peak area (r) was 0.999 over this concentration range. The small sample volume (100 µl) makes this assay particularly suitable for pediatric patients.

INTRODUCTION

Ticarcillin is a α-carboxypenicillin which is used predominately for the treatment of serious infections caused by gram-negative organisms, particularly *Pseudamonas aeruginosa*. Bacteriocidal efficacy is dependent on the maintenance of relatively high concentrations of the antibiotic during the dosing interval. It is frequently desirable, therefore, to be

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0148-3919/84/0714-2887$3.50/0

able to measure the concentration of ticarcillin in biological fluids during treatment to ensure adequate dosing.

During the past decade, a number of studies have employed bioassays of ticarcillin (1-5). However, the bioassays are subject to substantial random error, require a relatively long incubation time, and provide limited sensitivity. Furthermore, ticarcillin is invariably administered in combination with an aminoglycoside antibiotic which interfers with the bioassays, particularly at low ticarcillin concentrations (4).

The high performance liquid chromatographic method described here was designed to provide an accurate, sensitive, and rapid ticarcillin assay which can be performed in the presence of concurrently administered antibiotics. The small sample volume (100 µl) makes the assay readily applicable to pediatric patients.

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MATERIALS AND METHODS

Reagents

Ticarcillin disodium (analytical grade) was a gift from Beecham Laboratories, Bristol, Tennessee, U.S.A. 8-chlorotheophylline was from ICN Pharmaceuticals, Plainview, New York, U.S.A. HPLC-grade acetonitrile, ethyl acetate, and methanol and reagent grade citric acid monohydrate, sodium acetate, and glacial acetic acid were obtained from Fisher Scientific Company (Fairlawn, New Jersey, U.S.A.).

Standards

A stock standard solution of ticarcillin, equivalent to 1 g/L in methanol, was prepared and stored in the freezer at -20°C. Working standards were prepared daily in drug-free plasma to yield concentrations of 1 through 100 mg/L. The internal standard (8-chlorotheophylline) was added to the extraction solvent (ethyl acetate) to yield a concentration of 1 mg/L.

Instrumentation

Chromatography was performed on a Perkin-Elmer Series II HPLC system equipped with a LC 75 UV/VIS variable wavelength

detector interfaced to a Sigma-10 data system. A 4.6 mm x 12.5 cm HC ODS 5 μ-particle size reversed phase column (Perkin-Elmer Corporation, Norwalk, CT, U.S.A.) was used for the chromatography. The data system provided a readout of the digitally integrated area under the peaks, determined the retention times, calculated the response factors for ticarcillin and the internal standard and printed the results in the appropriate concentration units.

Chromatographic Conditions

The mobile phase consisted of 9% acetonitrile in 0.2 M sodium acetate containing 0.35% glacial acetic acid (pH = 2.7) which was filtered and thoroughly degassed prior to use. The flow rate was 1.5 ml/min, and the effluent was monitored at 220 nm. All analyses were performed at ambient temperature.

Procedure

One hundred μl of standard, control, or patient serum was placed in a 1.5 ml Eppendorf centrifuge tube. To each tube 200 μl of 1 M citric acid monohydrate (pH = 2.2, prepared fresh weekly and stored refrigerated) was added and mixed. This was followed by the addition of 1.0 ml of ethyl acetate containing the internal standard. The tubes were vortexed vigorously for at least 1 minute and then centrifuged for 3 minutes at 21,000 g in a Brinkman table-top microcentrifuge. The organic layer was then transferred to a 10 x 75 mm disposable glass tube and evaporated to a dryness at 40°C under a stream of nitrogen. The dried samples were reconstituted with 50 μl of mobile phase; 10 μl of which was injected onto the column.

RESULTS

Ticarcillin was extracted into ethyl acetate as its free acid after the addition of citric acid. Figure 1 shows typical chromatographs of (A) drug-free serum extracted with ethyl acetate containing the internal standard; (B) drug-free serum

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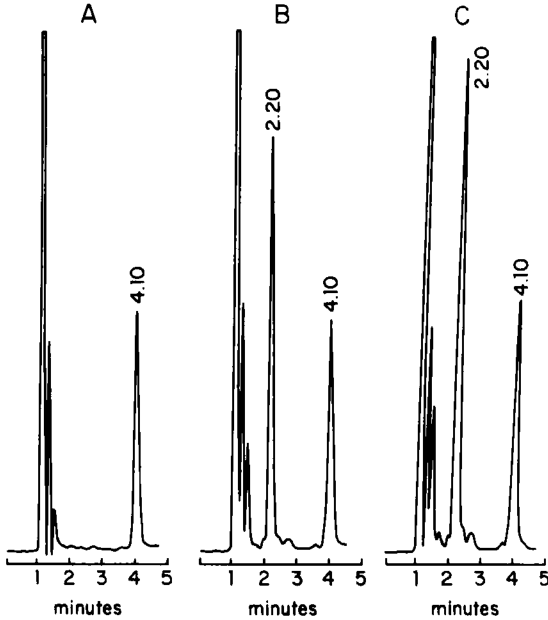

FIGURE 1. Chromatogram of blank serum, standard, and a patient serum. (See text for details.)

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reconstituted with 100 mg/L of ticarcillin and the internal standard; and (C) a chromatogram of a patient serum extract containing 127 mg/L of ticarcillin. The retention times for internal standard and ticarcillin were 2.2 and 4.1 min., respectively.

The concentration of ticarcillin calculated from the integrated area under the peaks was linearily related to the internal standard area over a concentration range from 1.0 to 100 mg/L. The correlation coefficient (r) was 0.999 and the equation for the regression line was $y = 0.969\ x + 1.019$. Samples with concentrations greater than 100 mg/L were appropriately diluted with normal saline and re-analyzed. The mean recovery of ticarcillin from serum was 89%.

Table 1 shows the results of within-run and day-to-day precision studies conducted on spiked serum.

TABLE 1

Precision of Ticarcillin Assay

	Within Run	Day-to-Day
Concentration (Added) mg/L	50	20
Concentration (Obtained) mg/L		
Mean	49.7	21.4
SD	1.8	1.7
CV%	3.6	7.9
n	9	8

TABLE 2

Stability of Ticarcillin in Serum

Week	Value mg/L	Week	Value mg/L
1	46.9	5	51.9
2	50.4	6	50.7
3	46.3	7	49.6
4	52.9	8	49.7
Mean (mg/L)	50.1		
± SD	1.9		

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Stability studies were conducted using a serum pool to which a known quantity of ticarcillin was added. Aliquots of this pool were frozen at -20°C and analyzed over a period of 8 weeks. Ticarcillin was stable for up to 8 weeks under these conditions (Table 2).

DISCUSSION

The ultraviolet absorption and solubility characteritistics of ticarcillin proved to be unusually problematic during the development of a satisfactory HPLC assay. The extinction coefficient of ticarcillin in the U.V. range is relatively low with an absorption maximum less than 200 nm. This region of the U.V. spectrum is

unsuitable for most analyses with U.V. detection because most solvents and many endogenous chromogens absorb below this wavelength, providing unacceptable background absorption. By monitoring the effluent at 220 nm we were able to reduce these background interferences while retaining satisfactory sensitivity in the detection of ticarcillin.

Because of less than optimal U.V. absorbance, it became necessary to extract ticarcillin from serum and concentrate it prior to chromatography in order to achieve satisfactory sensitivity. Ticarcillin is a highly water soluble dicarboxylic acid with a pKa of approximately 2.7, making it difficult to extract efficiently from an aqueous medium. We found that ticarcillin could be satisfactorily extracted into ethyl acetate as the free acid. The free acid was then concentrated by evaporating the ethyl acetate and reconstituting the drug with the acidic mobile phase.

Kwan, et al. (6) employed a back-extraction from ethyl acetate into 0.04 M pH 6.8 phosphate buffer to concentrate ticarcillin and separate it from interfering substances. However, we were unable to duplicate the recovery reported by Kwan and found the extra back extraction step to be cumbersome as well as unnecessary. In addition, we found the strong acidic mobile phase conditions used by Kwan to unacceptably shorten the functional life span of the C-18 column and to be unnecessary for optimal chromatography.

The method described here is specific and sensitive, may be carried out with a simple single extraction step and requires less than five minutes for elution of each sample. The sample size of 100 μl makes it an ideal assay for use in infants and young children where sample volume is frequently restricted.

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