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Note

Determination of cephaloridine in serum and tissue by high-performance liquid chromatography

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Antibiotics in serum or tissue samples have traditionally been determined by bioassay rather than by using chemical methodology. Cephalosporins, including cephaloridine, are usually assayed by the disc-plate, cylinder-plate or agar-well-diffusion methods that measure the degree of inhibition of growth of specific bacteria.

Cephaloridine is a broad spectrum antibiotic used intramuscularly against susceptible organisms in the treatment of infection. The administration of cephaloridine to laboratory animals can produce dose-related nephrotoxicity, and renal damage has been reported to occur in man. Cephaloridine nephrotoxicity has been thoroughly reviewed by Foord[1].

Studies in our laboratory designed to investigate the mechanism of cephaloridine nephrotoxicity required a rapid, reliable method for the determination of cephaloridine applicable to serum and tissue from experimental animals.

This report describes the development of a method employing high-performance liquid chromatography (HPLC) to quantify cephaloridine in serum and renal cortex of rabbits. The method should also be applicable to serum samples from patients receiving cephaloridine.

MATERIALS AND METHODS

Cephaloridine (Loridine®) standard solutions were prepared in rabbit serum at concentrations of 2–100 μg/ml for analysis of serum and 25–300 μg/ml for analysis of tissue.

Homogenates (1:10, w/v) of rabbit renal cortex were prepared in water using a Potter-Elvehjem type homogenizer equipped with a PTFE pestle. Equal volumes of 6% aqueous trichloroacetic acid were added to blank, standard or experimental serum or tissue homogenates and the solutions were placed in ice for 15 min. The solutions were centrifuged (1800 rpm, Model K Laboratory Centrifuge, International Equipment Co., Needham Heights, Mass., U.S.A.) for 10 min, the supernatant solution was removed and 10–20 μl aliquots were injected onto the HPLC column.

HPLC analyses were performed on a Model ALC202 chromatograph, equipped with a Model U6K injector (Waters Assoc., Milford, Mass., U.S.A.) at ambient temperature. A 60 cm × 2 mm stainless-steel column packed with phenyl Corasil®

(37–50 μm) reversed-phase column packing material (Waters Assoc.) was used. The mobile phase consisted of 18–20% methanol in 0.2 *M* aqueous ammonium acetate maintained at a flow-rate of 2.0 ml/min, pressure 1500–1700 p.s.i.

The concentration of methanol in the mobile phase was adjusted periodically to provide optimum peak shape and separation as the condition of the column changed slightly from day to day. Effluent absorbance was monitored at 254 nm and recorded at 0.02–0.04 absorbance units full scale. Peak areas were determined by triangulation.

RESULTS

Chromatograms from blank, standard and experimental samples are presented for both serum (Fig. 1) and renal cortical tissue (Fig. 2). The chromatography provided adequate separation of the cephaloridine peak from other ultraviolet-absorbing components of serum or the tissue homogenate. A standard curve prepared from triplicate samples of serum containing cephaloridine at concentrations of 2–100 μg/ml is shown in Fig. 3. The measurement of peak areas results in a linear, reproducible relationship to cephaloridine serum concentrations.

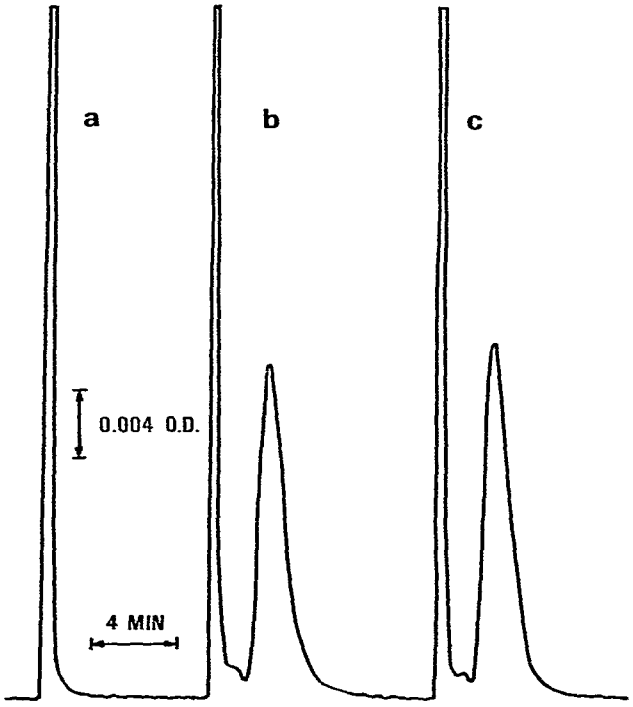

Fig. 1. HPLC of cephaloridine in rabbit serum. (a) Blank serum; (b) blank serum with cephaloridine added at 100 μg/ml; (c) serum from a rabbit 3 h after the administration of cephaloridine, 100 mg/kg. Serum was prepared as described in text. O.D. = Absorbance units.

The reproducibility of the method was evaluated by the addition of known amounts of cephaloridine to serum and renal cortex homogenate and analysis of eight aliquots by comparison to a single standard curve prepared in serum. The results are shown in Table I.

The recovery of cephaloridine added to a tissue homogenate was quantitative (103% of actual) and the variability was essentially identical to that observed in the

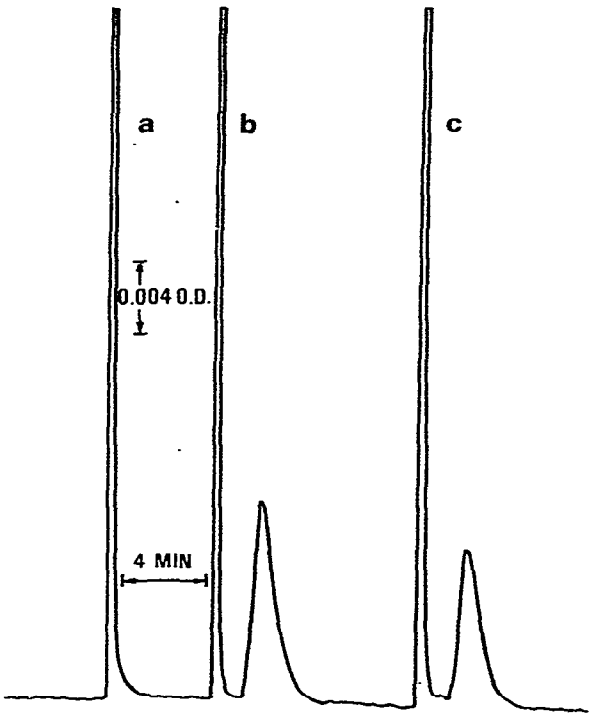

Fig. 2. HPLC of cephaloridine in homogenates of rabbit renal cortical tissue. (a) Blank tissue homogenate; (b) blank tissue homogenate with cephaloridine added at 160 μg/ml; (c) homogenate of renal cortex from a rabbit 3 h after the administration of cephaloridine, 100 mg/kg. Homogenates were prepared as described in text. O.D. = Absorbance units.

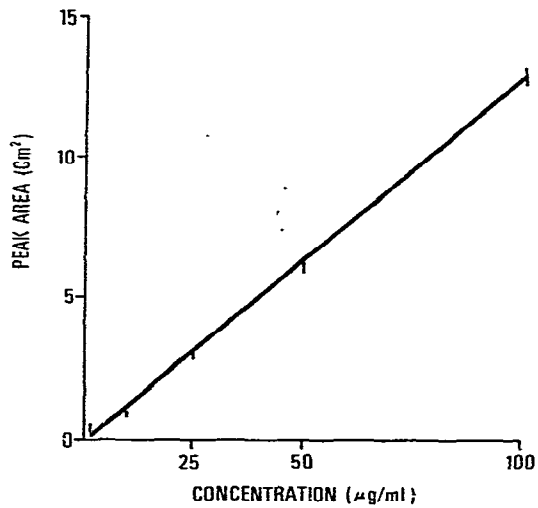

Fig. 3. Standard curve in rabbit serum for HPLC determination of cephaloridine. Vertical lines show the range of peak areas at each concentration. Regression line (shown) is from triplicate analyses with slope = 0.130, intercept = −0.14, correlation coefficient = 0.999.

TABLE I

REPRODUCIBILITY OF CEPHALORIDINE DETERMINATION IN SERUM AND HOMOGENATE OF RABBIT RENAL CORTICAL TISSUE

	Serum	*Tissue homogenate*
Actual cephaloridine concn. (μg/ml)	35.0	1600
Number	8	8
Mean analyzed concn. (μg/ml)	34.3	1643
% of actual concn.	98.0%	102.7%
S.D.	1.3	46.3
Range (μg/ml)	31.6–36.1	1566–1704
Coefficient of variation	3.9%	2.8%

replicate analysis of serum (coefficient of variation 3.9% *vs.* 2.8%). The accuracy and reproducibility of the assay were considered to be thoroughly adequate for use in further investigations of serum and tissue concentrations of cephaloridine in experimental animals.

DISCUSSION

The use of HPLC for the analysis of cephaloridine in serum or tissue homogenates offers the marked advantage of speed over the conventional microbiological assays. Microbiological determination of cephaloridine involves an overnight incubation whereas the HPLC procedure described here allows determination of serum levels within 30 min or less. Other chemical methods for the determination of cephaloridine in biological samples are considerably more complex than the HPLC procedure and involve fluorometric or gas chromatographic determination of pyridine after hydrolysis of cephaloridine[2,3].

HPLC has been used previously for the determination of the antibiotics tetracycline and cephalothin in serum[4,5]. We have described the use of HPLC for the determination of cefazolin in dog or human serum and have compared the HPLC results with conventional microbiological assays[6]. HPLC results, although occasionally somewhat different from microbiological assay results, produced data that would lead to identical conclusions regarding pharmacokinetics or achievement of therapeutic serum levels.

The procedure described here for the determination of cephaloridine in tissue allows the disruption of tissue binding by trichloroacetic acid precipitation which is not accomplished by conventional microbiological assay procedures. This report, extending the usefulness of HPLC determination of cephalosporins to tissue as well as serum, accentuates the utility of HPLC for the rapid determination of antibiotics in biological samples.

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