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Note

Determination of moxalactam by high-performance liquid chromatography

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Moxalactam (Eli Lilly Company, Indianapolis, IN (U.S.A.) is a new oxa-β-lactam antibiotic in which oxygen is substituted for sulfur in the cephem nucleus. Due to its marked antimicrobial activity against most gram-negative bacterial pathogens, including anaerobes, and activity against some gram-positive cocci, it promises to become an important broad-spectrum antibiotic for clinical use [1, 2]. Published studies [3,4] to date have employed a microbiological assay to measure moxalactam in biological samples. However, this assay is relatively cumbersome and susceptible to error when used in the presence of other antibiotics. The purpose of this report is to describe a high-performance liquid chromatographic (HPLC) assay which is rapid, requires small sample volume, and is not subject to interference from other commonly used antibiotics, such as ampicillin, chloramphenicol, gentamicin, kanamycin and penicillin-G, which may be administered concurrently with moxalactam.

MATERIALS AND METHODS

Chromatography

The chromatographic system consisted of a Perkin-Elmer Series II HPLC (Perkin-Elmer, Norwalk, CT, U.S.A.) equipped with a LC75 UV—VIS variable-wavelength detector and interfaced to a Sigma 10B data system (Perkin-Elmer). The data system provided a print-out of the digitally integrated area under the peaks and the retention times for moxalactam and the internal standard. A 25 × 0.46 cm Perkin-Elmer C18-ODS—HC-SIL-X 10-μm particle size reversed-phase column, maintained at ambient temperature, was used. A guard column packed with C-18 10-μm size pellicular material (Supelco, Bellefonte, PA, U.S.A.) was installed between the injection port and the analytical column. The flow-rate was 1.0 ml/min and the eluent was monitored at 230 nm.

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Reagents

Moxalactam disodium salt, used as the analytical standard, was provided by Eli Lilly. The internal standard was 8-chlorotheophylline (K&K Chemicals, Plainview, NY, U.S.A.). Methanol, HPLC grade, was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). All other chemicals were analytical grade. Citrate buffer (0.1 *M*), pH 6.5, was prepared by titrating 0.1 *M* potassium citrate monohydrate with 0.1 *M* citric acid monohydrate.

The mobile phase consisted of 4% methanol in 10 m*M* ammonium phosphate titrated to pH 6.5 by the drop-wise addition of concentrated orthophosphoric acid. This solution was freshly prepared and degassed under vacuum just prior to use.

A stock solution of disodium moxalactam was prepared in de-ionized water to yield a final concentration of 1.0 g/l moxalactam acid. The stock solution was then diluted with drug-free serum, urine, or cerebrospinal fluid (CSF) to provide assay standards over a concentration range of 1.0—50 mg/l. A 12 mg/l solution of 8-chlorotheophylline, used as the internal standard, was prepared in methanol and stored at −10°C.

Procedure

A 200-μl aliquot of standard, control, patient serum, CSF, or urine, diluted 1:20 with distilled water, was placed in a 10 × 75 mm disposable glass tube and kept on ice. Ice cold methanol (200 μl) containing the internal standard was added. The tubes were then vortexed for 30 sec and returned to the ice bath for 5 min. All tubes were centrifuged for 15 min at 1640 *g* in a refrigerated centrifuge. A 200-μl aliquot of the clear supernatant was transferred to a clean 10 × 75 mm disposable tube and an equal amount of citrate buffer was added. The tubes were gently mixed and returned to the ice bath until analyzed. A 20-μl aliquot of the final mixture was injected onto the column.

RESULTS

Fig. 1A shows a typical chromatogram for blank serum containing the internal standard. Fig. 1B illustrates a chromatogram obtained from drug-free serum to which 20 mg/l of moxalactam was added. Fig. 1C is a chromatogram obtained upon analysis of a patient sample in which the determined concentration of moxalactam was 40.9 mg/l. Moxalactam eluted in two peaks with retention times of 2.16 and 2.63 min, respectively. The internal standard had a retention time of 5.97 min.

The concentration of moxalactam was calculated from the sum of the areas under both peaks and was linearly related to the sum of the peak areas over the concentration range of 1.0—50 mg/l. Standard curves were identical for serum, urine, or CSF. Table I shows the recovery data for different concentrations of moxalactam from prepared serum samples. The mean recovery of moxalactam was 102% over the concentration range studied. Within-run precision was evaluated by processing fifteen separate aliquots of a prepared standard serum pool containing 20 mg/l of moxalactam. The mean ± S.D. was 20.0 ± 1.8 mg/l. Stability studies were conducted using drug-free sera spiked with 20 mg/l of moxalactam. There was a 30% and 10% loss of detectable

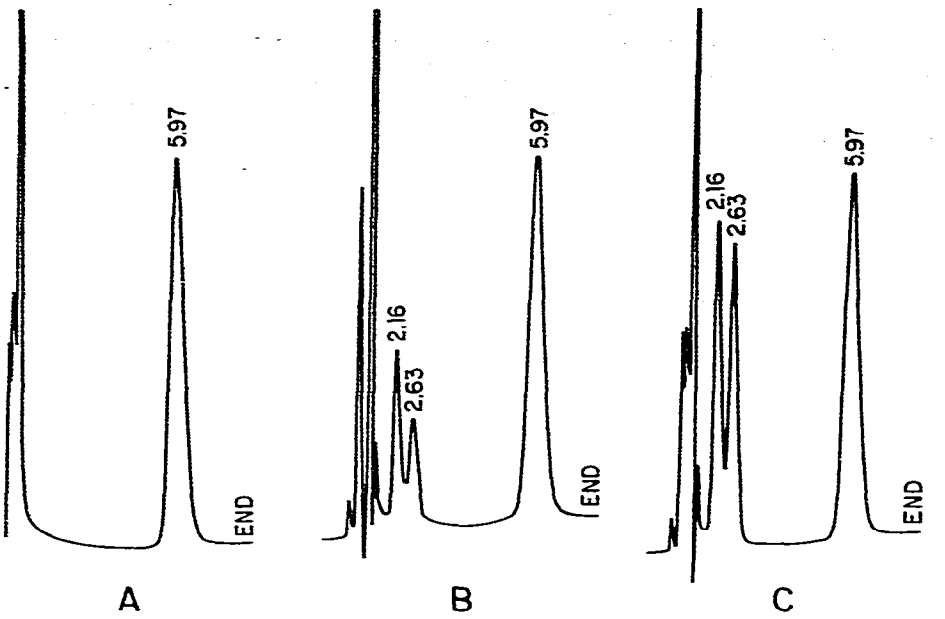

Fig. 1. Typical chromatograms of (A) blank serum containing internal standard; (B) drug-free serum reconstituted with 20 mg/l of moxalactam; and (C) patient's serum determined as 40.9 mg/l of moxalactam. Retention times: moxalactam peak I, 2.16 min; moxalactam peak II, 2.63 min; internal standard (8-chlorotheophylline), 5.97 min. Moxalactam eluted as two peaks, presumably the D and L isomers.

TABLE I

ANALYTICAL RECOVERY OF KNOWN AMOUNTS OF MOXALACTAM ADDED TO HUMAN SERUM

Five samples were analyzed at each concentration.

Added (mg/l)	Measured (mg/l)	S.D.	Recovery (%)
50	45.0	± 2.4	90
30	29.9	± 0.5	99
20	20.0	± 1.8	100
10	11.0	± 0.2	112
5	5.4	± 0.4	108

moxalactam at room temperature and at 3°C, respectively, during a 24-h period. However, there was no significant change in concentration (the mean ± S.D. of nine samples was 21.1 ± 1.9 mg/l) when stored at −70°C over a 3-week period.

Samples (25 mg/l) of ampicillin, chloramphenicol, gentamicin, kanamycin and penicillin-G were individually prepared in drug-free serum. These samples were then processed by our procedure. These commonly co-administered antibiotics did not chromatograph under the conditions of the assay procedure.

Various concentrations of moxalactam ranging from 0–50 mg/l were prepared in drug-free CSF ($n = 8$) and serum ($n = 16$). The samples were split and blindly analyzed by this HPLC method and a microbiological assay. The results of the two assay methods were highly correlated with a coefficient (r) of 0.99 [5].

DISCUSSION

HPLC technology offers many advantages over previously reported assays [3,4] of moxalactam which rely on antimicrobial activity to estimate concentration in biological fluids. Disadvantages of antimicrobial assays include long turn-around time, a propensity for random error due to unavoidable biological variables inherent in the assay system, possible decomposition of the antibiotic during the 37°C incubation, and interference by concurrently administered antibiotics. In contrast, our assay provides relatively rapid turn-around time and the capability of measuring moxalactam in the presence of other commonly used antibiotics. Furthermore, it can be applied to serum, urine, or CSF without modification.

The stability of moxalactam in aqueous solution is temperature dependent. This relative instability of moxalactam dictates that all specimens be stored at −70°C and kept at 3°C during sample preparation for optimal accuracy. For best results, stored samples should be analyzed for moxalactam within three weeks of collection.

Moxalactam, as supplied for clinical use, consists of approximately equal amounts of the D and L isomers of the drug [6]. Under the conditions of this assay, moxalactam eluted as two peaks, presumably the D and L isomers. Consequently, quantitation of moxalactam was determined from the sum of the areas under both peaks. The first and second moxalactam peaks consistently constituted 54% and 46%, respectively, of the sum of the areas of the two peaks. It was impossible to identify which peak represented the D or L form because the pure D and L isomers were not available to us. The two moxalactam peaks are consistent with respect to retention time and relative areas as long as conditions of the assay are strictly controlled. However, the variations in pH and relative composition of the mobile phase tend to alter the ratio of the two peaks and may lead to erroneous results.

In conclusion, a practical HPLC method to measure moxalactam in biological fluids is described. The assay is rapid, requires small sample volume, and can be used for serum, urine, or CSF. The simplicity and accuracy of this method makes it attractive for routine use in clinical laboratories which have the required HPLC equipment.

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