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Note

High-performance liquid chromatographic analysis of azthreonam in tissues and body fluids

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Azthreonam is a new antibacterial agent. It is a synthetic monocyclic β-lactam antibiotic displaying high activity in vitro against aerobic Gram-negative bacteria, including β-lactamase-positive *Haemophilus influenzae* and many aminoglycoside-resistant members of the enterobacteriaceae family [1, 2].

The assay of azthreonam in plasma or body fluids has many important applications: the study of penetration of this molecule in cerebrospinal fluid or in various body fluids at therapeutic plasma levels [3]; monitoring patients with various degrees of renal dysfunction [4] or simply the pharmacokinetic parameters of the drug [5, 6].

Recently, high-performance liquid chromatography (HPLC) [7] has been used for azthreonam determinations in serum and urine, but this assay lacks sensitivity (1 μg/ml) for other body fluids. This report deals with an HPLC system for the quantitative analysis of azthreonam in plasma, cerebrospinal

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fluid, aqueous humour, bronchial secretion and biopsies of organs, with a limit of sensitivity which enables patients to be monitored during clinical studies.

EXPERIMENTAL

Apparatus

Analyses were performed on a Waters liquid chromatograph (Waters, Paris, France) equipped with a Model 440 absorbance detector, a Model 6000A pump, an automatic injector (WISP) and a 10-mV strip chart recorder. Separations were performed using a μBondapak C_{18} column (300 × 3.9 mm I.D., particle size 10 μm) at 20°C.

Solvents and standards

Freshly bidistilled water was used throughout the procedure. Propanol-2, chloroform and isoamyl alcohol were analytical grade (Merck). Azthreonam disodium salt was a gift from Squibb (Neuilly S/Seine, France).

Chromatographic separation

The mobile phase was a mixture of acetonitrile (17%) and water [Pic A (0.005 *M* tetrabutylammonium phosphate; Waters), pH 2.5, 83%]. Eluent was pumped through at a flow-rate of 2 ml/min. The detector was set at 280 nm. Under these conditions, the retention time of azthreonam was 6.3 min with the μBondapak C_{18} column.

Extraction

All specimens were extracted in the following manner: 0.5 ml of various physiological liquids or organ extracts was mixed with 2.5 ml of propanol-2. After agitation and centrifugation (4°C, 1000 *g*), 2 ml of the supernatant were added to 2.5 ml of chloroform—isoamyl alcohol (100:4). After agitation and centrifugation, 0.02 ml of the supernatant was diluted with 0.02 ml of a phosphate buffer (pH 6, 0.04 *M*) and injected directly into the chromatograph.

Preparation of standards and specimens

Standards were prepared in pooled human plasma for plasma determinations and in physiological saline with phosphate buffer (pH 7.4, 0.005 *M*) for cerebrospinal fluid, aqueous humour, mucus and organ extracts; 0.5-ml aliquots of physiological fluid were extracted directly. Organs (brain, lungs and others) were weighed after thawing (a few milligrams depending on surgery), put into 1 ml of physiological saline with phosphate buffer and crushed with an ultra-thurax apparatus; 0.5 ml of the supernatant was extracted. All standards and specimens must be stored at −80°C until use to avoid degradation. Degradation occurs rapidly at room temperature (a few hours). All centrifugations must be done in refrigerated apparatus (4°C).

RESULTS

The retention time of azthreonam under the described conditions enabled its detection without interference from endogenous compounds in the plasma,

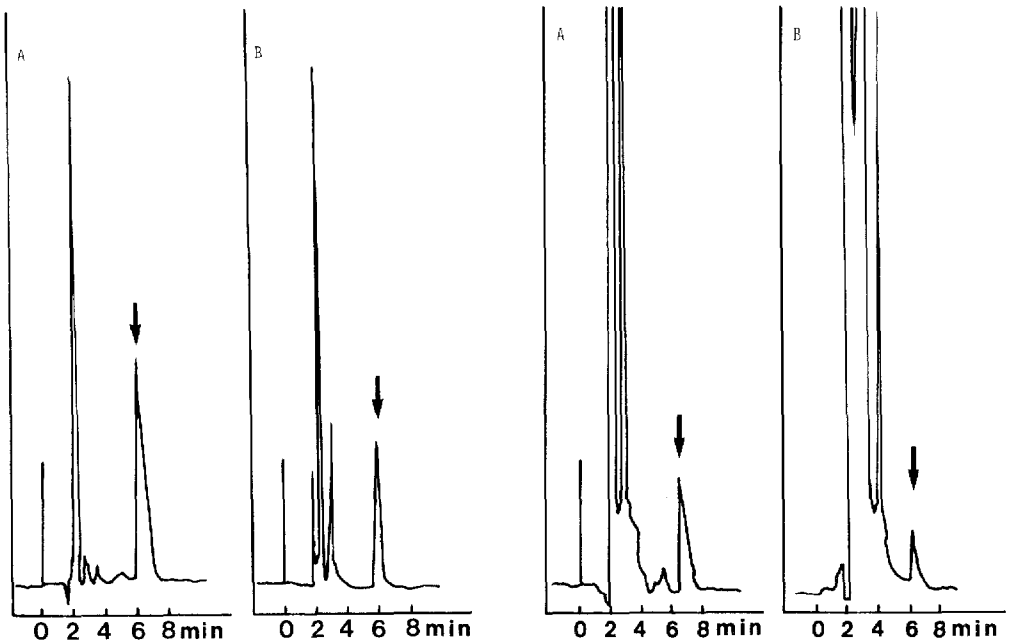

Fig. 1. (A) Chromatogram of a plasma sample containing 153 μg/ml azthreonam at 0.02 a.u.f.s. (B) Chromatogram of cerebrospinal fluid (CSF) containing 16 μg/ml azthreonam at 0.05 a.u.f.s.

Fig. 2. (A) Chromatogram of aqueous humour containing 2.5 μg/ml azthreonam at 0.01 a.u.f.s. (B) Chromatogram of a mucus sample containing 0.85 μg/ml azthreonam at 0.01 a.u.f.s.

cerebrospinal fluid and other fluids and extracts. Fig. 1 shows plasma and cerebrospinal fluid extracts. Fig. 2 presents aqueous humour and bronchial secretion extracts and Fig. 3 shows lung and brain extracts. The plasma standard curves and the phosphate buffer standard curves showed good linearity in the range studied: 0.1—300 μg/ml. The accuracy of these assays was tested using the dilution test and by adding known amounts of drug to known samples. For this assay, the range of recovery was between 97 and 103%. The limit of sensitivity of the method was determined with pooled plasma or dilution of known samples for other fluids. This limit is equal to 0.1 μg/ml under the described conditions, with a signal-to-noise ratio of 2; but it can be lowered by changing the sample or extract volume by a factor of 10. This limit is sufficient for clinical monitoring. Intra-assay variation and inter-assay variation were studied on five plasma and five cerebrospinal samples tested ten times on the same day and on five different days. These two coefficients were below 7.5% by this method. Fig. 4 shows the mean plasma concentrations of aztreonam after an intravenous bolus injection of 1 or 2 g in man (ten male patients for each point); these results agreed with previously described pharmacokinetic data. A correlation of 50 plasmas with a standardized microbiological assay [8] gave a correlation coefficient of 0.991. The same good correlation was obtained with cerebrospinal fluid, aqueous humour and mucus in ten assays.

Fig. 3. (A) Chromatogram of a brain sample containing 0.12 μg/ml azthreonam at 0.005 a.u.f.s. (B) Chromatogram of a lung sample containing 0.5 μg/ml azthreonam at 0.005 a.u.f.s.

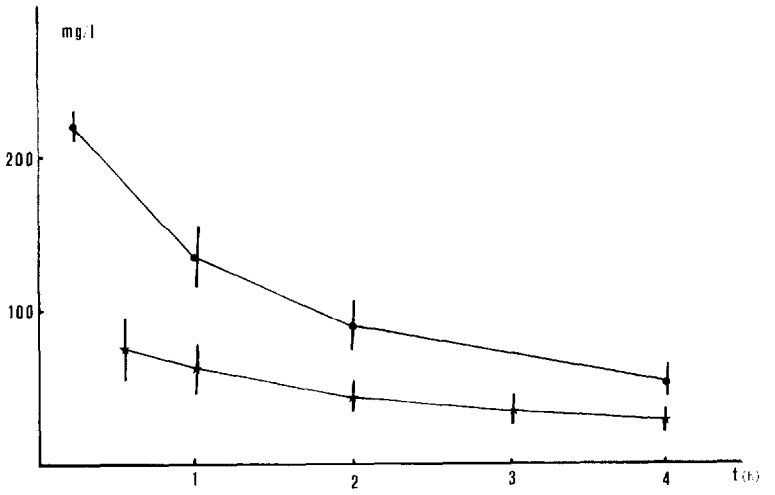

Fig. 4. Plasma levels of azthreonam after a bolus injection of 1 g (★) and 2 g (●) in man (n = 10 for each point).

DISCUSSION

The procedure described above enables azthreonam concentration to be monitored in various fluids and tissues during clinical practice. This assay correlates with microbiological assay. Specimens of plasma or other fluids or biopsies can be stored at −80°C until analysis for a few months. Degradation

of the drug occurs after one week of storage at −30°C. Manipulations of the specimen before freezing and extraction at room temperature must take a maximum of a few hours to avoid loss of product; with these precautions, this new HPLC assay of azthreonam gives reliable results for clinical trials in human chemotherapy.

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