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Note

Determination of sulbactam in biological fluids by high-performance liquid chromatography

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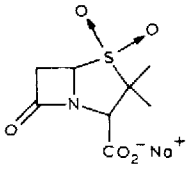
Sulbactam, or penicillanic sulphone acid, has the following structural formula:



Sulbactam is a β-lactamases inhibitor without real antibacterial activity, which can inhibit β-lactamases degradation. The levels of sulbactam in biological fluids [blood, urine, cerebrospinal fluid (CSF)] are determined by microbiological [1–3] and high-performance liquid chromatographic (HPLC) methods [4, 5].

This paper presents a reversed-phase HPLC technique with UV detection, the advantage of which is a rapid, easy extraction preceding a rapid separation. It can be used readily to determine the level of sulbactam even when it is administered with other β-lactamins.

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EXPERIMENTAL

Chemicals and drugs

The following substances were used: sulbactam (or penicillanic sulphone acid) (Pfizer, U.S.A.); internal standard (I.S.), pyrogallol (1,2,3-trihydroxybenzene) (Pfizer); hydrochloric acid R.P. (UCB, Brussels, Belgium); diethyl ether R.P. (Normapur, Prolabo, Paris, France); methanol R.P. (Carlo Erba, Milan, Italy); glacial acetic acid R.P. (UCB); phosphate buffer (pH 7.4).

Standard solutions of sulbactam and I.S. were prepared by dissolution in mobile phase at concentrations of 100 and 10 μg/ml.

Apparatus and technique

The chromatographic system consisted of a high-performance liquid chromatograph equipped with a 600 A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.) and a Rheodyne injector fitted with a 50-μl loop. The column was a μBondapak C_{18} (30 cm × 4.6 mm I.D., 10 μm) (Waters Assoc.), maintained at room temperature (20 ± 2°C). The detector was a variable-wavelength Schoeffel spectrophotometer. The detection wavelength was 225 nm (0.04 a.u.f.s.). All chromatograms were recorded on a CSA 10-mV recorder (CSA, Paris, France) at a chart-speed of 5 mm/min.

Mobile phase. The mobile phase was methanol–water (20:80, v/v) with 0.5% of glacial acetic acid. The flow-rate was 0.75 ml/min. This mobile phase was thoroughly degassed for 10 min by ultrasound.

Extraction. To 1 ml of human plasma or CSF were added 200 μl of hydrochloric acid R.P., 100 μl of an aqueous I.S. solution (10 mg/l) and 6 ml of doubly distilled diethyl ether. The mixture was shaken for 1 min in a vortex mixer (Bioblock, France). The solution was then centrifuged for 10 min at 900 *g* at 4°C, and the supernatant was discarded. The upper phase was transferred to a clean glass tube and then evaporated to dryness in a vortex evaporator (Buchler, NY, U.S.A.). The residue was dissolved in 100 μl of the mobile phase, and a 50-μl aliquot was injected into the chromatograph with a Hamilton syringe.

Calibration. The standard curve for plasma was obtained by adding sulbactam to drug-free plasma, to achieve concentrations of 2, 5, 10 and 20 μg/ml. Standard plasmas were extracted under experimental conditions as described above, and the peak-height ratios of sulbactam to I.S. were plotted versus concentrations.

The standard curve for CSF was obtained under the same conditions, but sulbactam was added to phosphate buffer (pH 7.4) to obtain concentrations of 2, 5, 10 and 20 μg/ml.

RESULTS

Fig. 1A shows a typical chromatogram of a 50-μl injection obtained with 1 ml of a human plasma extract from a patient to whom 1 g of sulbactam was administered.

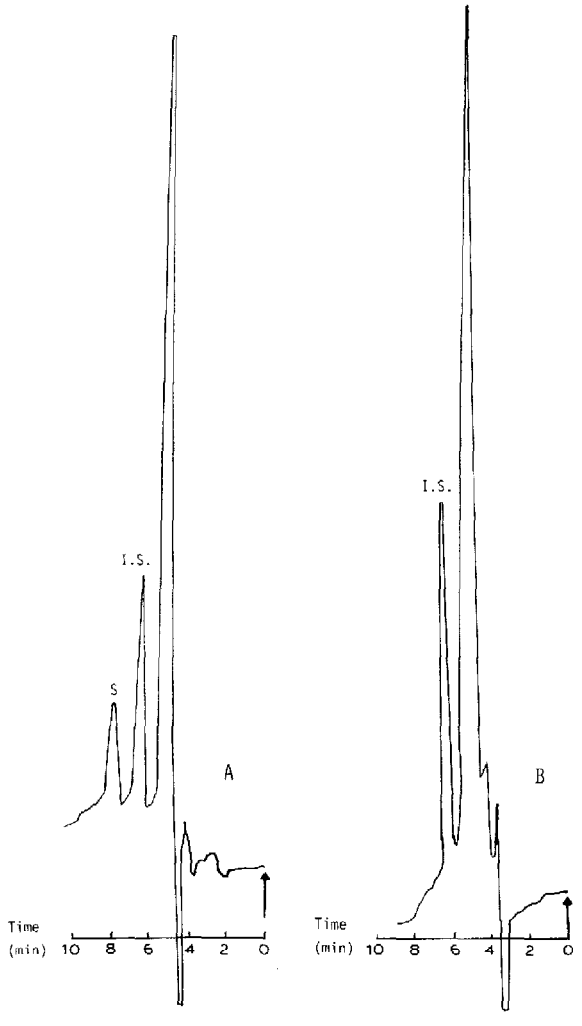

Fig. 1. (A) Typical chromatogram obtained from a plasma extract of a patient following a 1-g injection of sulbactam (S) 1.5 h before (I.S. = internal standard). (B) Blank plasma.

Assay technique

The calibration curves were linear from 1 to 20 μg/ml in plasma and CSF. Their equations were: blood: $y = 0.012 + 0.029x$ ($r = 0.998$); CSF: $y = 0.082 + 0.044x$ ($r = 0.985$), where y is the peak-height ratio of sulbactam to I.S., x is the concentration of sulbactam (in μg/ml), and r is the correlation coefficient.

Precision

The reproducibility of the method was checked for two plasma concentrations (5 and 10 μg/ml), and the coefficients of variation were 7 and 5%, respectively ($n = 20$).

Recovery

The recovery of sulbactam was assessed by comparing the peak height after

an injection of a pure solution of sulbactam with that obtained after an injection of extracted plasma containing the same amount of sulbactam. The mean recovery of the extraction procedure for the 5 μg/ml sample was 82.0 ± 5.0%.

Sensitivity

The threshold of sensitivity of this technique (defined as a signal-to-noise ratio of 2) was 0.5 μg/ml of plasma or CSF.

Application

The HPLC—UV method described gives reproducible results and is sensitive enough for the determination of sulbactam in human plasma and CSF. It is easy to perform, cheap, and does not suffer from interference from amoxycillin, ampicillin or cefoxitin, the retention times of which were 15, 17 and 20 min, respectively (Fig. 2). It has been used to compare sulbactam plasma concentration with levels of this drug in CSF in patients with bacterial meningitis. All patients were receiving intravenous ampicillin therapy, 4-h doses. Six patients were receiving intravenous bolus injections of sulbactam and twelve received multiple doses of sulbactam. The concentrations of sulbactam and ampicillin detected in CSF and in the serum of some patients are listed in Table I.

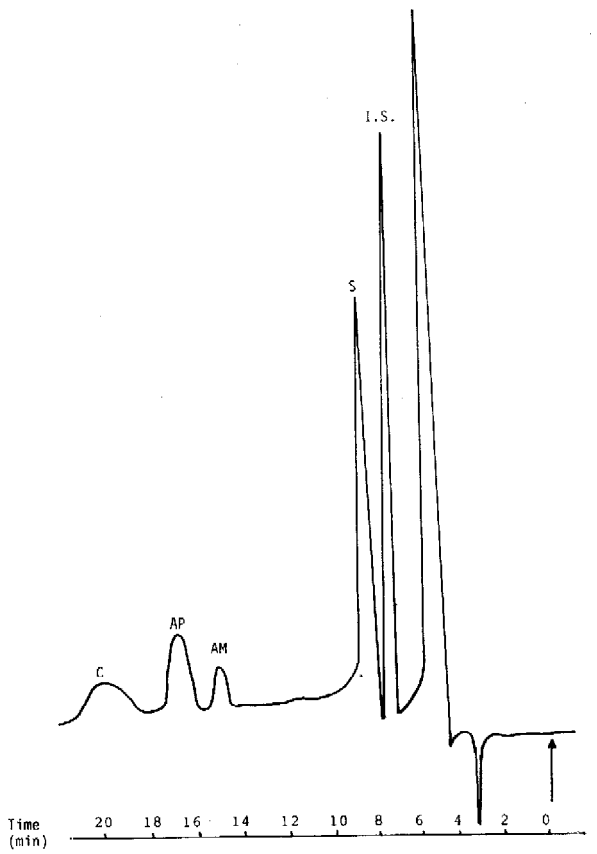

Fig. 2. Typical chromatogram obtained from a plasma extract containing 5 μg of I.S., 10 μg of amoxycillin (AM), 10 μg of ampicillin (AP), and 10 μg of cefoxitin (C); S = sulbactam.

TABLE I

SULBACTAM AND AMPICILLIN CONCENTRATIONS IN MENINGITIS PATIENTS AFTER A SINGLE DOSE OF SULBACTAM AND MULTIPLE DOSES OF AMPICILLIN

Patients with mild inflammation of the mininges (CSF protein < 1 g/l).

Patient number	Time after dose (h)	Intravenous dose administered*	Sulbactam concentration		Ampicillin concentration		CSF protein (normal range, 0.3—0.5 g/l)
			CSF (mg/l)	Serum (mg/l)	CSF (mg/l)	Serum (mg/l)	
14	1.5	1 g S + 1.5 g A	0.65	9.00	<0.30	7.60	0.70
14	1.5	1 g S + 1.5 g A	1.20	11.00	0.30	17.00	0.24
18	1.5	1 g S + 1.5 g A	3.50	9.00	2.20	12.80	0.60
17	4	1 g S + 1.5 g A	<0.50	2.10	<0.30	2.60	0.38
17	4	1 g S + 1.5 g A	N.D.**	<0.5	N.D.**	<0.30	0.35

*S = sulbactam; A = ampicillin.
**N.D. = not determined.

CONCLUSION

In this note we have discussed a simple and rapid technique for determination of sulbactam in plasma and CSF. Sensitivity and reproducibility are sufficient to allow us to elucidate the pharmacokinetics of this drug in patients treated by ampicillin and sulbactam.

REFERENCES

1 C. Cornel and G. Fredj, J. Antimicrob. Chemother., in press.
2 R. Labia, V. Lelievre and J. Peduzzi, Biochim. Biophys. Acta, 611 (1980) 351.
3 R. Wise, J.M. Andrews and K.A. Bedford, J. Antimicrob. Chemother., 6 (1980) 197.
4 H.J. Rogers, I.D. Bradkbrook, P.J. Morison, R.G. Spector and D.A. Cox, J. Antimicrob. Chemother., 11 (1983) 435.
5 J. Haginaka, J. Wakai, H. Yasuda, T. Uno and T. Nakagawa, J. Chromatogr., 341 (1985) 115.