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Note

Isotachophoretic determination of sulbactam in rat serum

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Sulbactam, the penicillanic acid sulphone (Fig 1), is a relative new and frequently used specific irreversible β -lactamase inhibitor. Unlike other drugs of the penicillin type, sulbactam is not normally administered alone for its low and restricted anti-bacterial activity but in combination with other β -lactamase antibiotics (penicillins or cephalosporins) in order to increase substantially their activity against β -lactamase-producing bacteria [1]. The most common and commercially available is the sulbactam-ampicillin mixture (Unasyn, Pfizer).

Several analytical methods have been utilised for the evaluation of sulbactam in biological material for the purpose of pharmacokinetic studies. In ad-

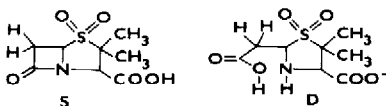


Fig 1 Structures of sulbactam (S) and its hydrolytic degradation product (D)

dition to the commonly used bioassay, most of the pharmacokinetic data published have been derived from chromatographic methods, involving gas chromatography (GC) or gas chromatography-mass spectrometry [2,3], and also high-performance liquid chromatography (HPLC) [2,4-7]

The isotachophoretic (ITP) evaluation of sulbactam purity, as an alternative supplementary method, has been published recently [8] The experiments presented in this paper show that this technique, which combines sufficient sensitivity with high selectivity, could be well used for the quantitative determination of sulbactam in rat serum, and may serve as a way of providing initial analytical data for pharmacokinetic studies of sulbactam

EXPERIMENTAL

Materials

All ITP separations were performed with an LKB 2127 Tachophor device equipped with a conductivity detector and a poly(tetrafluoroethylene) (PTFE) capillary The statistical computations were processed on DS-15 data station (Varian, Mulgrove, Australia)

The chemicals used for the preparation of the electrolyte system were of at least analytical grade and dissolved in doubly distilled deionized water β -Alanine (Merck, Darmstadt, F R G) was recrystallized from water-methanol (1:1), caproic acid (Lachema, Brno, Czechoslovakia) was refined by extraction into water and hydroxypropylmethylcellulose (HPMC) (Dow Chemical, Midland, MI, U S A) was deionized on a Zerolit DM-F mixed-bed ion-exchanger (BDH, Poole, U K) Hydrochloric acid (30%), methanol (Merck), β -cyclodextrin (Chinoin, Budapest, Hungary), butyl acetate (Lachema) and water-ammonia 25% (BDH) were used without further purification Lyophilizate of sulbactam sodium salt was produced in the Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia)

Methods

Sulbactam in serum cannot be analysed by direct injection of the sample into the Tachophor The low concentration of sulbactam, together with the number of ionic compounds from the rat serum, make a straight ITP analysis in a one-column apparatus impossible Therefore it is necessary to pre-concentrate and purify the serum sample The proposed extraction procedure, described below, ensures an optimal composition of the sample without significant loss of sulbactam

The fresh serum sample (0.5 ml) was acidified with 0.5 ml of 0.25 M hydrochloric acid and extracted with 4 ml of butyl acetate After centrifugation, 3 ml of the organic phase were evaporated in vacuum to dryness The modified sample was diluted in 5 mM ammonia (40 μ l), and aliquots were injected with

TABLE I

ELECTROLYTE SYSTEMS AND CONDITIONS FOR ITP

Leading electrolyte	5 mM HCl containing 0.08% hydroxypropylmethylcellulose with β -alanine to pH 3.50, plus 5 mM β -cyclodextrin
Terminating electrolyte	5 mM Caproic acid with ammonium solution to pH 4.50
Capillary	370 mm \times 0.55 mm I.D.
Current	100 μ A, for detection 50 μ A
Detection	Conductivity
Temperature	18°C
Injection volume	5 μ l of each aliquot

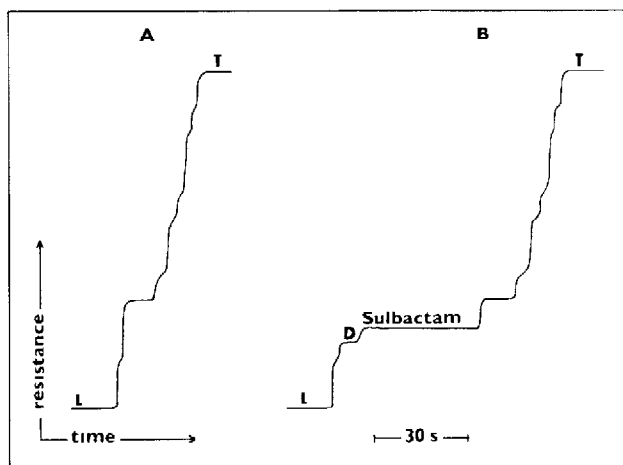


Fig. 2. Isotachopherograms of extracted blank serum (A) and sulbactam-serum model mixture (B) (35 μ g of sulbactam sodium salt per 1 ml of serum). D = Hydrolytic degradation product of sulbactam, L and T = leading and terminating zone, respectively.

a 10- μ l Hamilton syringe into the ITP column. For ITP conditions see Table I.

RESULTS

A typical isotachopherogram of an extracted serum sample is shown in Fig. 2. It may be concluded that the introduction of one-step extraction, together with the use of an acidic electrolyte system, significantly simplifies the ITP spectrum. Sulbactam, which was previously detectable only as a serum microcomponent, now forms the main and readily visible zone. The addition of β -cyclodextrin, as a selective complex-forming agent, ensures the resolution of sulbactam and its hydrolytic degradation product D (Fig. 1), which has an opened β -lactam ring [8].

The calibration curve was obtained by plotting the sulbactam zone lengths against the injected amount of standard sulbactam solution. The parameters of the calibration are given in Table II.

The overall recovery of the method was determined by adding various amounts of sulbactam to 0.5-ml aliquots of standard rat serum. After the extraction procedure, 5 μ l of the sample solution were injected into the Tachophor. The amount of sulbactam in the serum was calculated from the calibration parameters. The analytical recovery, at ca. 94%, was almost quantitative. The limit of the correct quantitative determination was taken to be the amount of sample that produced a 1-s zone. In agreement with this definition, experiments with model sulbactam-serum mixtures proved that the method can quantify ca. 1 μ g of sulbactam in 1 ml of serum.

The pharmacokinetic applicability of the assay was verified by the measurement of serum samples collected in defined time intervals (5, 15, 30, 45, 60 and

TABLE II

CALIBRATION RESULTS FOR SULBACTAM SODIUM SALT STANDARD SOLUTION

Calculated for $\alpha=0.05$, number of calibration points 8

Parameter	Value
Amount injected	1.242–9.936 μ g (1–8 μ l) ^a
Standard deviation	$s=0.729$
Intercept	$a=-0.11 \pm 1.39$ (s)
Slope	$b=22.06 \pm 0.22$ (s/ μ g)
Correlation coefficient	$R=0.999$

^aSulbactam standard solution was prepared by dissolving 124.2 mg of sulbactam sodium salt in 100 ml of water.

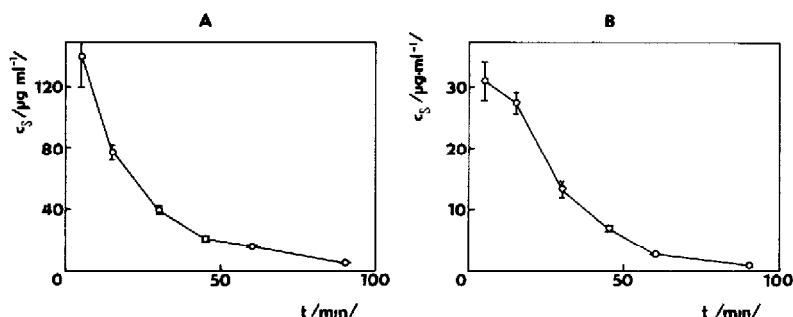


Fig. 3. Time-based measurement of rat serum samples after the intramuscular application of sulbactam to rats. (A) 50 mg of sulbactam sodium salt per kg, (B) 25 mg of sulbactam sodium salt per kg. Samples taken at 5, 15, 30, 45, 60 and 90 min after injection.

90 min) after the intramuscular application of sulbactam to rats (Wistar, average weight 200 g). Time-based sulbactam serum levels, obtained for two different initial doses of sulbactam, are shown in Fig. 3. Each point in the diagram represents an average of six replicate measurements, marked confidence intervals (Dean-Dixon, $\alpha=0.05$) confirm that the method is sufficiently reliable for pharmacokinetic purposes.

DISCUSSION

Capillary ITP is a separation technique with an "empty" separation compartment without a stationary phase. It has proved to be suitable for analyses of crude biological materials without special pre-treatment of samples or time-consuming rinsing of the separation compartment in order to release and wash out strongly bonded or precipitated components.

The problem of poor resolution of sulbactam and some of its degradation products in acidic electrolyte systems was solved by the addition of β -cyclodextrin to the leading electrolyte. This results in much better resolution, especially of sulbactam and its hydrolytic degradation product.

The sensitivity and selectivity of the proposed method correspond well with the sensitivity of available HPLC methods, and the extraction procedure ensures even higher recovery [2,4-7]. The ITP technique does not require a derivatization step, and therefore it is simpler than GC methods [2].

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