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Note

Spiroarsoranes, a new class of antiparasitical compounds: quantitative analysis by high-performance liquid chromatography

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Many arsenicals were formerly used in pharmacology, but were progressively replaced from 1940 by modern antibiotics. The only area where they are still important is in antiparasitical drugs. We recently synthetized over 100 new compounds containing hypervalent arsenic moieties, especially penta-coordinated spiroarsoranes. Among them, about 50 showed interesting filaricidal and trypanocidal activity [1, 2]. In particular, octamethyl-2,2,3,3,7,7,8,8-arsa-5-anilino-5-spiro-(4,4)-nonane (I, Fig. 1) showed an important filaricidal activity and minimal toxicity in animals. It was therefore important to know its diffusion in living organisms and a specific and sensible analytical method was also necessary.

To our knowledge, no liquid chromatographic method was published in this

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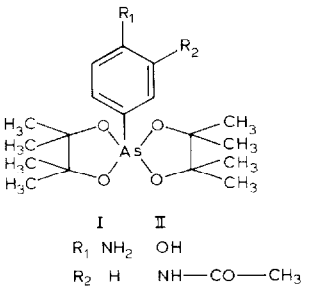

	I	II
R_1	NH_2	OH
R_2	H	NH—CO—CH_3

Fig. 1. Chemical structure of octamethyl-2,2,3,3,7,7,8,8-arsa-5-anilino-5-spiro-(4,4)-nonane (I) and octamethyl-2,2,3,3,7,7,8,8-arsa-5-*p*-hydroxy-*m*-acetamidophenyl-5-spiro-(4,4)-nonane (II).

field. Nevertheless, a highly sensitive high-performance liquid chromatographic (HPLC) method with atomic absorption detection was recently described in mineral and organo-arsenic trace determination [3, 4]. Moreover, only one gas chromatographic (GC) determination of spiroarsoranes is known which uses a capillary column and an electron-impact mass spectrometric (EI-MS) detector [5].

In this paper, we propose a HPLC method using a C_{18} reversed-phase column showing high sensitivity in plasma.

EXPERIMENTAL

Reagents

Spiroarsoranes I and octamethyl-2,2,3,3,7,7,8,8-arsa-5-*p*-hydroxy-*m*-acetamidophenyl-5-spiro-(4,4)-nonane (II, internal standard) showed no impurities in two different thin-layer chromatographic (TLC) systems. All reagents were of analytical grade. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Merck (Darmstadt, F.R.G.); methanol and ethyl acetate from Prolabo (Paris, France). All reagents were used without further purification.

Equipment

A Model 6000A liquid chromatograph and a U6K injector (Waters Assoc., Paris, France) were used with a UV spectrometer (Lambda Max 481) as detector. The absorbance maximum for I and II was 275 nm. A 250 mm × 4 mm I.D. column (Hibar prepacked column RT 250-4, Merck) was used, packed with LiChrosorb RP-18 with an average particule size of 10 μm. The chromatographic solvent was methanol—0.05 *M* Tris buffer, pH 8.1 (75:25), delivered at a flow-rate of 1.5 ml/min at room temperature.

Preparation of standard solutions

Standard solutions of 1 mg/ml I and II were prepared in methanol for each series of analyses. The standard solutions of I were then dissolved in drug-free plasma to give final concentrations of 25—4000 ng/ml. The internal standard solutions were diluted to a final concentration of 2000 ng/ml.

Assay procedure

Plasma (0.4 ml) and 50 μl of the solution of internal standard were pipetted

into a 20-ml glass-stoppered centrifuge tube. After gentle shaking, 0.4 ml of 0.2 *M* Tris buffer (pH 8.1) was added and mixed carefully. Ethyl acetate (4 ml) was added and the tube was shaken for 1 min using a multi-tube Vortexer (Corning, Paris, France). After centrifugation (20 min at 1500 *g*), the organic phase was transferred to a 5-ml glass tube and carefully evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of the chromatographic solvent with vigorous shaking (Vortex mixer). A 20-μl volume of this solution was then injected into the column.

Recovery

For recovery experiments, I and II dissolved in methanol were added to a serum blank to give final concentrations of 500 and 1000 ng/ml, respectively. After mixing and incubating for 30 min, the compounds were extracted as described above.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms obtained under various conditions: (A) blank plasma extract containing only the internal standard, (B) drug-free plasma plus I (1000 ng/ml) and II, and (C) plasma obtained 11 h after intravenous administration of 15 mg/kg of I to a rabbit.

The retention times for II and I are, respectively, 3.2 and 3.9 min. Calibration graphs are obtained by the least-squares method by fitting the peak-height ratio of the sample substance and the internal standard (y) versus the amount of added substance (x). The equation of this regression line was: $y = 1.11 \cdot 10^{-3}x + 2.30 \cdot 10^{-3}$, with a correlation coefficient of 0.9998. This indicates reasonable linearity between the detector response and amounts added to plasma in the range of tested concentrations, i.e. 25–4000 μg/l.

Analytical recoveries were calculated from ten independent determinations by comparison of peak heights from extracted serum standards with those from non-extracted standards of the compounds prepared in methanol. The recovery was 95.6 ± 2.8 for I and 98.2 ± 3.1 for II.

The detector sensitivity for I is 2 ng. Taking into account the recovery, the overall sensitivity is 25 ng/ml I when 20 μl of the extracted sample solution are injected.

Replicate analyses of plasma samples to which known amounts of I were added demonstrated that the method has acceptable accuracy and precision (Table I).

The method was applied to the determination of I in plasma and urine using five animals (rabbits). None of them showed any contaminant in the zero-time plasma sample corresponding to the retention times of the tested drug (Fig. 2). A plasma profile of I over 24 h for one animal after a single intravenous dose (15 mg/kg) is shown in Fig. 3. This profile shows that the described method is suitable for pharmacokinetic studies. In particular, its sensitivity is well above the concentration measured 24 h after injection, which allows a good estimation of the pharmacokinetic parameters. Preliminary results in the rabbit show a double-phase decay after intravenous administration, with a terminal half-life of 4.7 h. Total body clearance is 2.3 l/h (mean) and the overall distri-

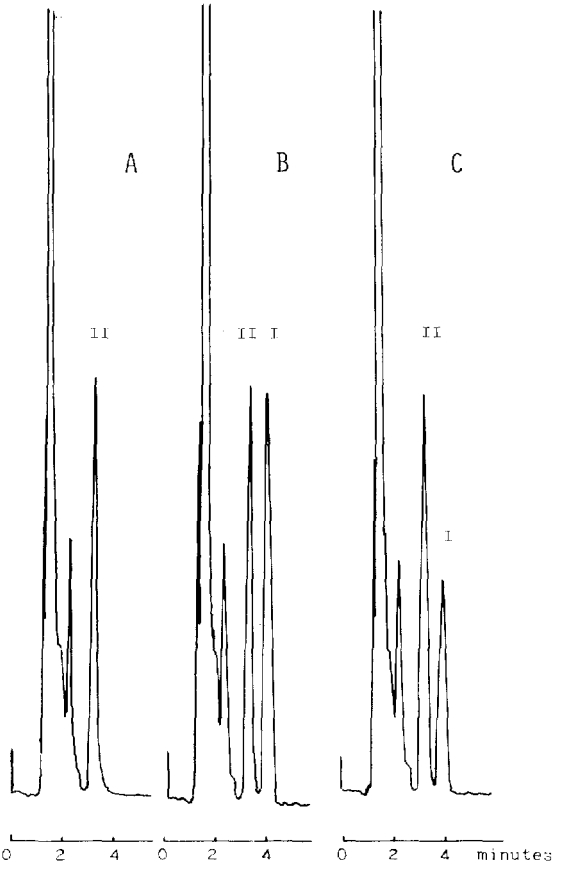

Fig. 2. Chromatograms of I and the internal standard (II). (A) Plasma extract prior to drug administration (containing internal standard II); (B) plasma extract for standard calibration curve containing 1000 ng/ml I; (C) plasma extract of I obtained 11 h after intravenous administration of I to a rabbit (containing 480 ng/ml I).

TABLE I

PRECISION AND ACCURACY OF ANALYSIS OF I

The mean standard deviations in ng/ml for ten determinations are given.

Amount of I added (ng/ml)	Amount of I found (ng/ml)	Coefficient of variation (%)	Accuracy (%)
50	51.4 ± 5.00	9.74	102.8
100	102.4 ± 7.34	7.17	102.4
250	249.3 ± 12.35	4.95	99.7
500	492.9 ± 20.95	4.25	98.6
1000	1013 ± 20.60	2.03	101.3
2000	2022.7 ± 31.89	1.58	101.1
4000	3986 ± 70.13	1.76	99.7

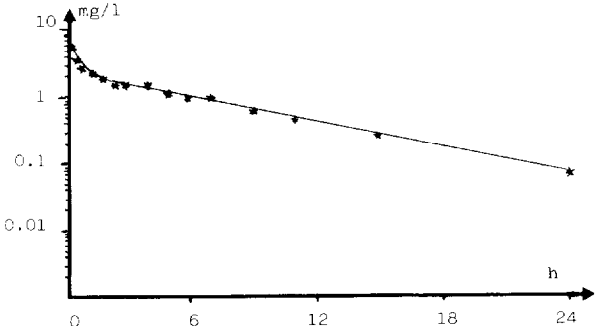

Fig. 3. Plasma profile of I obtained in a rabbit following a 15 mg/kg intravenous administration.

bution volume is 15.9 l. For each animal, rebounds can be observed during the decay, which may indicate some enterohepatic recycling; this is under study for confirmation.

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