Journal of Chromatography, 225 (1981) 488—492
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 955

Note

Liquid chromatographic analysis of an antimicrobial 5-nitroimidazolyl-2-sulphide derivative in biological fluids

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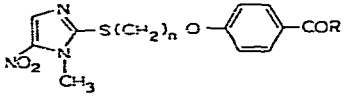
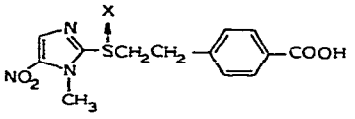
(First received March 16th, 1981; revised manuscript received April 28th, 1981)

SC-28538 [Fig. 1 (1a); sodium 4-[2-(1-methyl-5-nitro imidazolylthio)ethoxy] benzoate] is a novel antimicrobial nitroimidazole. The compound has high activity against obligatory anaerobic bacteria, including several strains of Bacillus fragilis [1, 2]. SC-28538 is also active against several Trichomonads, and penicillin resistant Neisseria gonorrhoeae [2].

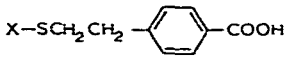
Further study of this compound required an assay for the unchanged drug in biological fluids. Preliminary investigations indicated poor gas chromatographic properties of the methyl esters of SC-28538 and some analogues selected as possible internal standards. High-performance liquid chromatography (HPLC) was therefore selected as an alternative method.



1. (a) R = ONa, n = 2
   (b) R = ONa, n = 3
   (c) R = NH.CH_2CO_2H, n = 2
   (d) R = NH.$CH(CH_2CONH_2).CO_2H$, n = 2



2. (a) X = O
   (b) X = O_2



3. (a) X = CN
   (b) X = $CONH_2$

Fig. 1. Structures of SC-28538 and related compounds.

0378-4347/81/0000—0000/$02.50

EXPERIMENTAL

Apparatus

Chromatography was performed with a system comprised of an Applied Chromatography (Luton, Great Britain) Model 750/03 pump, Deci-Linear gradient programmer, solvent composition optimising unit and a Model 750/11 UV detector (254 nm). The analysis was carried out in a stainless-steel column (100 mm × 5 mm I.D.) fitted with a syringe injector (Shandon Southern Products, Runcorn, Great Britain), and slurry packed with a C_{18} alkyl silylated silica (5-μm diameter, ODS-Hypersil, Shandon Southern Products). The output from the UV detector was linked to a 10-mV potentiometric recorder (Servoscribe IS, Smiths Industries, London, Great Britain).

Materials

SC-28538 (1a) was obtained from G.D. Searle, and the homologue (1b) was synthesized by a modification of the method for SC-28538 as described by Tweit and co-workers [2, 3], and purified by column chromatography on silica gel in chloroform—methanol (97:3, v/v). All other reagents, and solvents which were redistilled before use, were purchased from Hopkin & Williams (Chadwell Heath, Great Britain).

Metabolite synthesis

The glycine and glutamine conjugates (1c and 1d) were synthesised by the reaction of the acid chloride derivative of SC-28538 (0.500 g, 1.46 mmole) with the corresponding amino acid (1.40 mmole) in dioxan (30 ml) and 1 *M* sodium hydroxide solution (4 ml). The crude product obtained by acidifying the reaction mixture was purified by column chromatography on silica gel in chloroform—methanol (96:4, v/v), to give the pure conjugates (0.63 mmole).

The sulphone (2a) and sulphoxide (2b) were synthesised by a modification of the method used by Tweit et al. [3], and compound (3a) was prepared by the reaction of potassium thiocyanate (0.396 g, 4.082 mmole) with 4-(2′-bromoethoxy)-benzoic acid (1.00 g, 4.082 mmole) in dimethylformamide (12 ml) at 90°C for 18 h. The crude product (0.898 g) was crystallised from chloroform (30 ml) to give white crystals of 3a (0.505 g, 2.25 mmole).

Compound 3a (0.400 g, 1.79 mmole) was converted to 3b by hydrolysis in concentrated sulphuric acid (5 ml) at 5°C for 1.25 h. The crude product (0.391 g) obtained by dilution with water (25 ml) was chromatographed on a silica gel column in chloroform—methanol (90:10, v/v), to give the purified product (0.050 g, 0.21 mmole).

The structures of these compounds were confirmed by NMR, IR and mass spectrometry and their purity was established by thin-layer chromatography (TLC) on silica gel plates ($60F_{254}$, E. Merck, Darmstadt, G.F.R.) in the solvent systems: ethylene dichloride—methanol (9:2, v/v), *n*-butyl acetate and ethyl acetate—toluene (1:1, v/v) with detection of components under UV light (254 nm).

Selection of HPLC conditions

The separation of SC-28538 and its homologue (1b) from endogenous components of urine and plasma was optimised by using different proportions of 0.01 *M* potassium dihydrogen phosphate buffer, at different pH values, and methanol monitoring absorbance at 254 nm. The optimal solvent mixture of 50% methanol—0.01 *M* potassium dihydrogen phosphate buffer adjusted to pH 3.5 with phosphoric acid was used.

Assay procedures

The internal standard (1b; 1.5 μg) was added in aqueous methanol (15 μl) to plasma or urine (1 ml) in acid-washed glass test tubes. Saturated aqueous potassium dihydrogen phosphate solution (1 ml), adjusted to pH 4 with phosphoric acid, was added to plasma samples and mixed on a Whirlimixer. The urine and acidified plasma were extracted for 10 min with 1 ml or 2 × 2 ml of ethyl acetate respectively on a partitioning extractor and centrifuged. The separated organic phases were evaporated to dryness under a nitrogen stream, the residues dissolved in 0.01 *M* sodium hydroxide solution (20 μl) and methanol—water (80 μl, 40:60, v/v) and 10-μl aliquots were analysed on ODS-Hypersil (5 μm, 100 mm × 5 mm I.D.) in 50% methanol—0.01 *M* potassium dihydrogen phosphate (pH 3.5) at 2 ml/min, pressure 70—80 bar with UV detection at 254 nm. The peak height ratio of SC-28538 to internal standard was measured and concentrations were calculated from a calibration curve.

The standard curves and the accuracy and precision of the assay methods were obtained by the analysis of plasma and urine samples containing added SC-28538.

RESULTS AND DISCUSSION

The chromatographic conditions efficiently resolved SC-28538 and the internal standard from the co-extracted components of urine and plasma (Fig. 2). The extraction procedures recovered 96.4 ± 2.60% (S.D.) and 88.2 ± 3.17% of SC-28538 and the internal standard respectively from the biological fluids.

There was a linear correlation between SC-28538 concentration and SC-28538/internal standard peak height ratio for urine or plasma samples over the concentration range 0—2.5 μg/ml. Analysis of quality control samples showed an acceptable level of accuracy and precision (Table I) with a detection limit of about 0.05 μg/ml. The HPLC retention times of several potential SC-28538 metabolites (Table II) showed that they or closely related compounds would not interfere in the assay.

The assay was used to analyse plasma and urine samples from a monkey that had received a single intravenous dose of SC-28538. The plasma results (Fig. 3) showed a fast initial decline in SC-28538 concentrations over the first 1.5 h. They declined more slowly over the next 8 h with an apparent elimination half-life of 3.75 h. Some 14% of the administered SC-28538 was excreted unchanged in urine during the first 24 h after dosing, and a metabolite with a retention time similar to that of the glutamine conjugate (1d) was detected in the urine.

These results indicate that the HPLC method will be suitable for the analysis of SC-28538 in biological fluids.

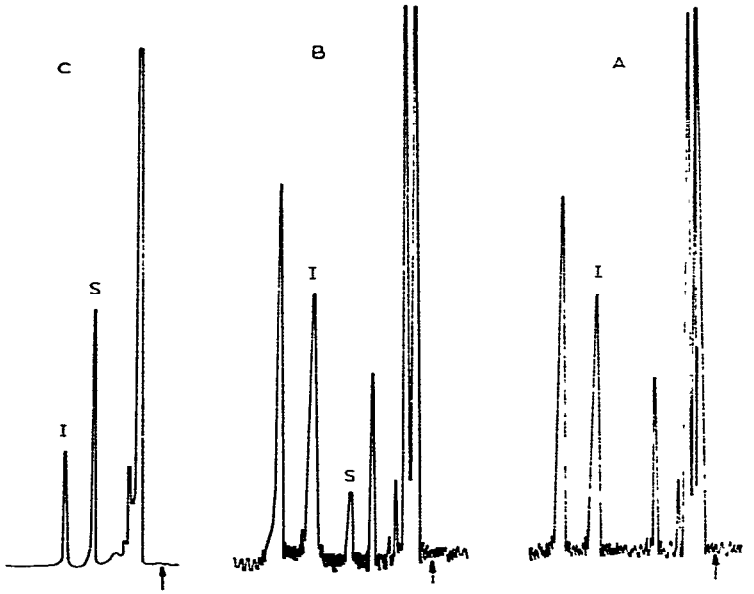


Fig. 2. Chromatograms of extracts from (A) plasma containing 0 μg/ml SC-28538, (B) plasma containing 0.25 μg/ml SC-28538, (C) urine containing 2.5 μg/ml SC-28538. Peaks: S = SC-28538; I = internal standard.

TABLE I

ACCURACY AND PRECISION OF ASSAY FOR SC-28538 IN PLASMA AND URINE

SC-28538 concentration (μg/ml)			Recovery (%)	
Theory	Measured ± S.D. (*n*)		Plasma	Urine
	Plasma	Urine		
2.50	2.56 ± 0.23 (16)	2.67 ± 0.27 (9)	102	107
0.25	0.24 ± 0.03 (15)	0.25 ± 0.01 (9)	96.4	100
0.10	0.09 ± 0.02 (16)	0.10 ± 0.01 (9)	90.0	100
Mean recovery (± S.D.)			96.1 ± 6.0	102 ± 4.0

TABLE II

RETENTION TIMES OF SC-28538 AND POTENTIAL METABOLITES

Compound	Retention time (min)
1a	4.30
1b	6.10
1c	2.00
1d	1.80
2a	1.80
2b	1.30
3a	1.70
3b	0.94

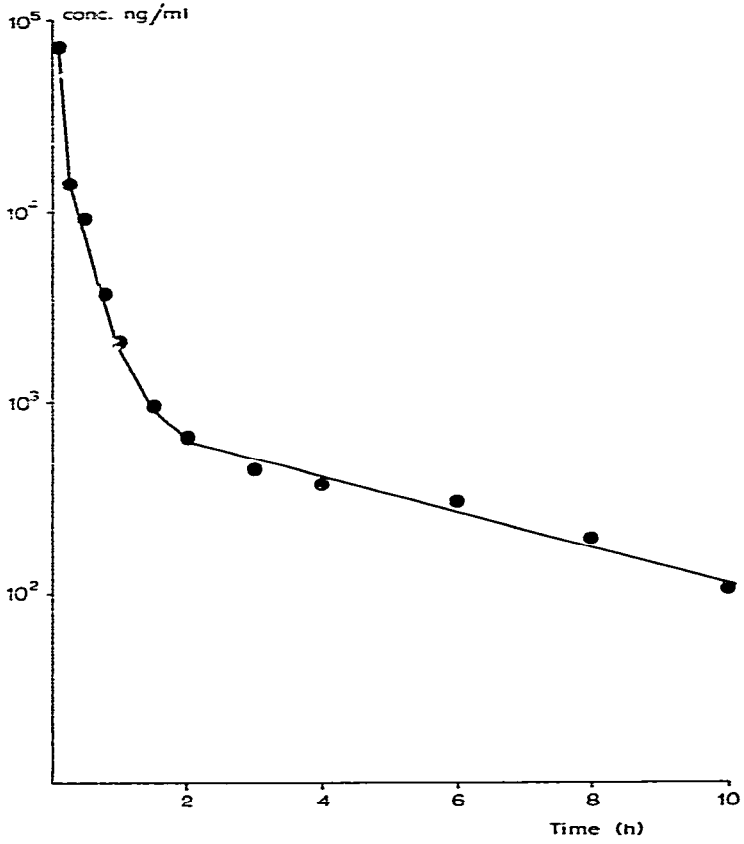


Fig. 3. Plasma levels of SC28538 in a monkey after a single intravenous dose of the compound.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. A. Barrow and Mr. T.J. Forrest for the biological samples from the monkey and Mrs. D. Morris for typing the manuscript.

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