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Note

Analysis of sulfinpyrazone and its metabolites in human plasma and urine by high-performance liquid chromatography

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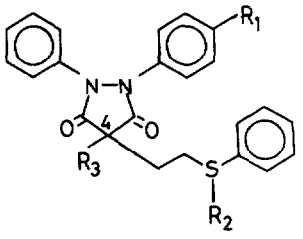
Sulfinpyrazone {1,2-diphenyl-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione} (Anturane®) (Fig. 1, 1) was used initially as an uricosuric drug [1]. Recently it has been studied as a platelet-aggregation inhibitor and in the prevention of sudden death after myocardial infarction [2, 3]. Sulfinpyrazone is transformed into a series of metabolites (2—6) of which the sulfide (2) is thought to be the pharmacologically active compound [4]. Clinical pharmacological studies on drug interactions [5] and on the induction of drug-metabolizing enzymes by sulfinpyrazone after single and chronic administration [6] have prompted us to develop a high-performance liquid chromatographic (HPLC) method for the determination of the parent drug (1) and metabolites



	R_1	R_2	R_3
1	H	O	H
2	H	—	H
3	H	O_2	H
4	OH	O	H
5	OH	—	H
6	OH	O_2	H
7	H	O	OH

Fig. 1. Structure of sulfinpyrazone (1) and metabolites: (2) sulfide, (3) sulfone, (4) *p*-hydroxysulfinpyrazone, (5) *p*-hydroxysulfide, (6) *p*-hydroxysulfone, (7) 4-hydroxysulfinpyrazone.

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2 to 6 in human plasma and urine; solvent extraction of the samples, followed by HPLC gradient elution on a C_{18} reversed-phase column and UV detection was used. Colorimetric [7], gas chromatographic [8, 9] as well as liquid chromatographic methods [10—17] have been described but none of these has measured simultaneously sulfinpyrazone and its metabolites in plasma and urine with non-radioactive techniques.

MATERIALS AND METHODS

Apparatus

A system consisting of two Model 6000A pumps, a WISP Model 710B automatic injector, a Model 660 solvent programmer, a Model 440 UV detector (all from Waters, Königstein, F.R.G.) and a Model C-R1B data processor plotter (Shimadzu, Düsseldorf, F.R.G.) were used for chromatography and calculations.

Reagents

All test substances were of analytical grade; sulfinpyrazone (1) and substances 2 to 7 were gifts from Ciba Geigy (Basel, Switzerland) and Ciba Geigy Pharmaceutical Division (Horsham, U.K.). Naproxen was from Chemie Grünenthal (Stolberg/Rhdl., F.R.G.). Acetonitrile (for spectroscopy, Merck, Darmstadt, F.R.G.) was used without purification; deionized water was purified with Norganic cartridges (Millipore, Neu-Isenburg, F.R.G.). Standard solutions of sulfinpyrazone (1), metabolites 2 to 6 and naproxen (1 g/l) in methanol were kept at 4°C in the dark.

Procedure

Plasma and urine from healthy male volunteers were collected at specified time intervals, after 400-mg doses of sulfinpyrazone [18]. Blood was collected in heparinized tubes, centrifuged and the plasma was frozen at −23°C. Sodium bisulfite (1 mg/ml) was added to urine samples and kept at the same temperature; they were thawed and centrifuged before analysis.

Plasma extraction. A 1-ml aliquot of plasma was spiked with 10 μg of naproxen, and then 1 ml of 1 *N* hydrochloric acid, 0.5 ml of sodium sulfite (20 mg/ml) and 4 ml of solvent [1-chlorobutane—dichloromethane (1:3, v/v)] were added. After 15 min shaking and 10 min centrifugation at 2000 *g*, 3 ml of the organic phase were transferred to a 15-ml conical test tube and evaporated under a stream of nitrogen at 37°C. The residue was dissolved in 100 μl of 2% sodium sulfite with vortexing, and 10 μl were injected in the HPLC system.

Urine extraction. A 0.5-ml volume of urine was mixed with 0.5 ml of water; 25 μg of naproxen were added and extracted as for plasma.

Calibration curves. For each analysis series a five-point calibration curve for each of substances 1 to 6 was run in parallel with the samples. To 15-ml extraction tubes known amounts of the substances (for concentration ranges see Table I) dissolved in methanol were added and evaporated under nitrogen. Blank plasma (1 ml) or urine (0.5 ml) was added and the extraction procedure was performed as described above. The slope and *y* intercept values were

evaluated for each substance and further used for calculations of the concentrations of the samples.

Chromatographic conditions

The mobile phase consisted of: eluent A, 0.1 *M* ammonium acetate—acetonitrile (780:220, v/v); eluent B, acetonitrile. The solvents were filtered and degassed before use. For the gradient elution, convex curve 7 program from 0% to 100% of eluent B in 15 min was used. The solvent rate was 2.0 ml/min and the pressure varied from 70 to 140 bar (7—14 mPa). A reversed-phase C_{18} column (μBondapak C_{18}, Waters; 300 × 3.9 mm I.D.; 10-μm irregular particles) and a guard column (Bondapak C_{18} Corasil, Waters; 25 × 4 mm I.D.; 35—50-μm spherical particles) were used for the separation. After a series of analyses the columns were washed with water and methanol. UV detection was at 254 nm and a sensitivity of 0.1 a.u.f.s. was used. Quantitation was effected by electronic area integration and the calibration curves were calculated from the ratio of peak area of the substance (1 to 6)/area of internal standard vs. added concentration (mg/l). Sample analysis time was 15 min followed by 5 min reequilibration before the next sample injection. Chromatograms were run at room temperature.

RESULTS

Fig. 2a shows a chromatogram of plasma spiked with sulfinpyrazone (1) and metabolites 2 to 6. Fig. 2b displays a HPLC separation of a plasma extract and Fig. 2c is of a urine extract obtained from volunteers after drug administration. Peaks were sharp and well separated, and blank plasma or urine did not show interfering peaks in the chromatogram. Several drugs were also tested: anti-

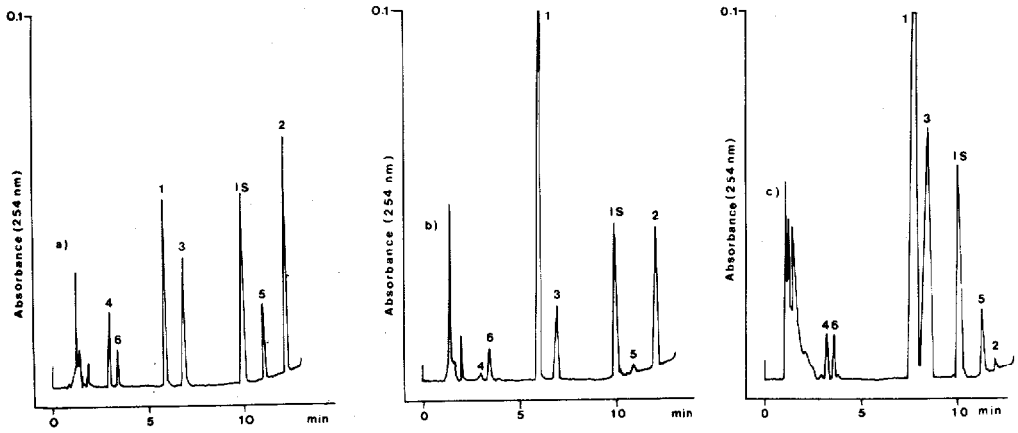

Fig. 2. (a) Chromatogram of an extract from blank plasma spiked with sulfinpyrazone (1) (5 mg/l, retention time, RT, 5.86 min, k' 3.34), (4) (0.5 mg/l, RT 2.91 min, k' 1.16), (6) (0.5 mg/l, RT 3.37 min, k' 1.50), (3) (4 mg/l, RT 6.86 min, k' 4.08), IS (internal standard) (10 mg/l, RT 9.85 min, k' 6.30), (5) (1 mg/l, RT 10.81 min, k' 7.01), and (2) (5 mg/l, RT 12.06, k' 7.93). The number above each peak corresponds to the formula in Fig. 1. (b) Chromatogram of plasma extract from a volunteer 2 h after ingestion of a 400-mg sulfinpyrazone tablet. (c) Extract of 2—4 h urine fraction after 400-mg oral sulfinpyrazone administration.

pyrine and metabolites (nor-antipyrine, 4-hydroxyantipyrine, 3-hydroxymethylantipyrine [19]), cimetidine, digoxin, acetylsalicylic acid, ampicillin, azlocillin, cotinine, heparin, hippuric acid, 1-(β-hydroxypropyl)theobromine, lidocaine, metoprolol, methaqualone, mezlocillin, nicotine, neostigmine bromide, pindolol, procainamide, propranolol, quinidine, salicylic acid, salicylamide, secobarbital, theobromine, theophylline, uric acid, vitamin B complex, vitamin C, and warfarin did not interfere. Phenprocoumon interfered with the internal standard and antipyrine with metabolite 6. 4-Hydroxysulfinpyrazone (7) which is considered an artefact [16] was not determined and eluted after the sulfide (2) (k' = 8.63). Treatment of urine with β-glucuronidase—arylsulfatase yielded concentration values which did not differ significantly from those of untreated samples.

Accuracy and precision of the analysis for plasma and urine are shown in Table I. The calibration curves were found to be linear in the ranges indicated in Table I. Sensitivity was 0.1 mg/l for plasma and 0.5 mg/l for urine. Calculated recoveries were 90—100% for all substances except for 4 which was 60%.

TABLE I

REPRODUCIBILITY OF PLASMA AND URINE ANALYSIS FOR SULFINPYRAZONE AND METABOLITES

Substance	Conc. added (mg/ml)	Conc. found (mg/ml)	C.V.* (%)	Conc. added (mg/ml)	Conc. found (mg/ml)	C.V.* (%)
1	0.50	0.58	1.5	5.0	5.2	6.3
	5.0	4.9	2.2	50.0	48.7	1.2
	30.0	30.0	3.4	100.0	100.1	1.6
2	0.50	0.53	2.1	2.0	2.1	6.3
	2.00	1.93	2.3	10.0	9.8	2.9
	4.0	4.0	3.9	50.0	50.1	0.8
3	1.00	0.94	3.5	0.50	0.46	6.1
	4.0	4.0	3.9	2.0	2.0	1.7
4	0.50	0.52	9.8	5.0	4.7	7.5
	1.50	1.49	6.4	20.0	20.0	2.7
5	1.00	0.89	8.3	0.50	0.53	9.6
	4.0	4.0	6.0	2.0	2.0	1.0
6	0.20	0.20	10.4	5.0	4.7	8.6
	2.0	1.9	3.9	20.0	20.0	2.0

*C.V. = coefficient of variation (n = 5).

DISCUSSION

Different extraction procedures, stationary phase packings and elution procedures have been published [11—17]. The method described in this paper

simplifies the extraction step, allows the simultaneous determination of sulfinpyrazone (1) and metabolites 2 to 6 in both plasma and urine, and possesses higher peak resolution and sharpness. The chromatographic system is very stable (more than one thousand injections can be done with the same column) and endogenous compounds and numerous drugs do not interfere. Up to 50 extractions and analyses can be done in 24 h. The precision and accuracy (Table I) are adequate.

After drug administration, sulfinpyrazone (1) is the main substance in plasma, the sulfone (3) and the sulfide (2) are the main metabolites and 4, 5 and 6 minor ones. In some volunteers 5 could not be detected (Fig. 3). The decline of the plasma concentrations of the metabolites parallels that of the parent compound with the exception of the sulfide whose maximum concentration appears much later [14, 15, 20] (Fig. 3). The delay in the peak plasma concentration of the sulfide is probably due to reduction of sulfinpyrazone by the gut microflora following enterohepatic circulation [21]. The 4-C glucuronides of 1, 2 and 3 which have been described [16, 17] in urine were not quantified as these derivatives were unavailable, and also because of their highly polar character which precludes their extraction in the solvents used. Dieterle and co-workers [16, 17] detected them after administration of radioactively labeled sulfinpyrazone and using radioactivity detection techniques.

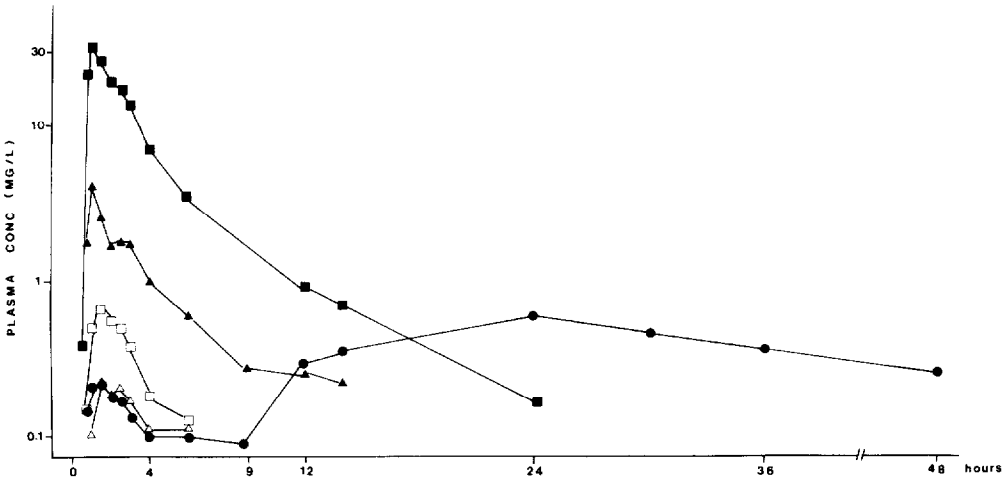

Fig. 3. Plasma concentration—time curve for sulfinpyrazone (1, ▪) and metabolites 2 (●), 3 (▲), 4 (□) and 6 (△) after a single oral dose of 400 mg of sulfinpyrazone.

The described method is simple, specific and sensitive for the analysis of sulfinpyrazone and metabolites in human blood and urine, and may be used in human pharmacokinetic studies, in the drug level monitoring of patients and in animal exprimentation. It has been used routinely during the last six months and the results of these investigations will be published elsewhere.

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