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Note

High-performance liquid chromatographic analysis of the sulfide metabolite of sulfinpyrazone in plasma

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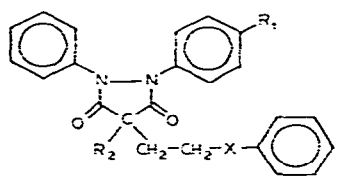
The sulfide G 25671 [1,2-diphenyl-3,5-dioxo-4-(2'-phenylsulfethyl)pyrazolidine] (Fig. 1) has recently been detected as a major plasma metabolite of sulfinpyrazone in animals [1—3] and man [4—6]. This compound, formed by reduction of the parent sulfoxide, is cleared from plasma predominantly by direct glucuronidation. Only trace amounts are detectable in urine after sulfinpyrazone administration [4].

The appearance of the sulfide in plasma may be clinically important since this metabolite shows a strong inhibitory effect on platelet aggregation in



Compound	R_1	R_2	X
G 28 315 Sulfinpyrazone (SO)	H	H	SO
G 25 671 Sulfide (S)	H	H	S
G 31 442 Sulfone (SO_2)	H	H	SO_2
G 32 642 *p*-hydroxy-sulfinpyrazone (OH-SO)	OH	H	SO
G 33 378 *p*-hydroxy-sulfide (OH-S)	OH	H	S
CGP 17 395 *p*-hydroxy-sulfone (OH-SO_2)	OH	H	SO_2
GP 52 097 4-hydroxy-sulfinpyrazone (4-OH-SO)	H	OH	SO

Fig. 1. Sulfinpyrazone and its unconjugated metabolites.

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various in vitro experimental systems [4, 5] and appears to be responsible for the prolonged inhibition of prostaglandin-synthesis-dependent platelet functions after sulfinpyrazone administration [1, 7].

The investigation of its pharmacokinetics after administration of either sulfinpyrazone or the sulfide itself required a specific analytical method. Gas chromatographic and high-performance liquid chromatographic (HPLC) methods for the assay of sulfinpyrazone, either alone [8—11] or together with oxidation metabolites — sulfone (SO_2) and *p*-hydroxy-sulfinpyrazone (OH-SO) (see Fig. 1) — [12, 13] have already been reported, but so far only a gas chromatographic—mass spectrometric method is available for the assay of this new metabolite in plasma [6, 14].

We report a specific and sensitive method for the assay of this sulfide in plasma by HPLC.

EXPERIMENTAL

Reagents and chemicals

Sulfinpyrazone and its metabolites were supplied by Ciba-Geigy, Basle, Switzerland.

The acetonitrile was of spectroscopy quality (Uvasol) and was purchased from Merck (Darmstadt, G.F.R.). All other solvents and reagents were of analytical grade.

All glassware used during sample preparation was silanized. The silanizing agents, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), were purchased from Pierce (Rockford, IL, U.S.A.). They were used as a 1% solution in toluene containing 1% of pyridine. After immersion for 15 min, the glassware was rinsed twice with methanol and dried.

Sample preparation

Plasma (1 ml) is placed in a 10-ml conical glass tube and 250 μl of 2 *N* hydrochloric acid are added. The tube is shaken for a few seconds on a Vortex mixer; 100 μl of 0.05 *N* sodium hydroxide or of calibration solutions (prepared in 0.05 *N* sodium hydroxide) and 2 ml of 1-chlorobutane are added. The stoppered tube is shaken mechanically for 10 min at 350 rpm (Infors shaker) and centrifuged at 2000 *g* for 10 min. A 1-ml aliquot of the organic phase is transferred to another tube, then 500 μl of 0.01 *N* sodium hydroxide are added. After shaking for 10 min at 300 rpm and centrifugation at 2000 *g* for 5 min, a 250-μl aliquot of the aqueous phase is diluted with 250 μl of distilled water; 100 μl of this mixture are injected on to the column.

Chromatography

Chromatography was performed on Hewlett-Packard instruments: Model 1082A equipped with a fixed-wavelength detector (254 nm) and a loop injector (Rheodyne, Model 7120), or Model 1081A equipped with the same detector and a variable-volume injector and connected to a Hewlett-Packard 3353E data system.

The column was a stainless-steel tube (25 cm × 4.6 mm I.D.) filled with 10-μm LiChrosorb RP-8 using the balanced-density-slurry packing technique.

The degassed mobile phase (2.22 m*M* orthophosphoric acid—acetonitrile—ethanol, 50:35:15, v/v) was used at a flow-rate of 3 ml/min.

The mobile phase and the column were at room temperature.

Calibration

Calibration samples were prepared by adding 100 μl of diluted calibration solutions to 1 ml of control plasma. The added amounts ranged from 50 to 5000 ng/ml. The calibration curves were constructed by plotting the peak area against the amount of compound added, and verified daily.

RESULTS AND DISCUSSION

Specificity

As shown in Fig. 2A, the sulfide metabolite is well separated from the parent drug and the metabolites listed in Fig. 1. The plasma components did not interfere in the assay (Fig. 2B).

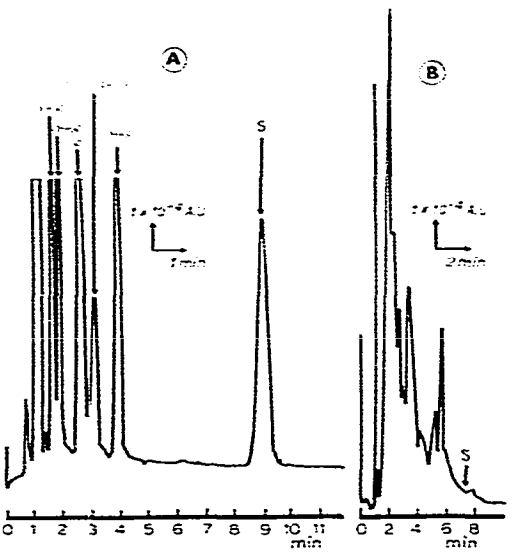

Fig. 2. HPLC determination of the sulfide metabolite of sulfinpyrazone. (A) Separation of sulfinpyrazone and its unconjugated metabolites (Fig. 1) in pure solution (column 1). (B) Chromatogram corresponding to an extract of 1 ml of blank human plasma (column 2).

The specificity of the present method was further assessed by a cross-check using isotope dilution analysis. Plasma samples spiked with the ^{14}C-labeled sulfide as well as with the ^{14}C-labeled parent drug were analyzed by both methods. The results given in Table I show the reliability of the described HPLC method.

Accuracy, precision and sensitivity

Spiked plasma samples were prepared and analyzed several times. The results

TABLE I

SPECIFICITY OF THE HPLC ASSAY FOR THE SULFIDE METABOLITE OF SULFINPYRAZONE: COMPARISON WITH ISOTOPE DILUTION ANALYSIS (IDA)

Plasma samples were spiked with ^{14}C-labeled sulfide and sulfinpyrazone.

Amount added (μmol/l)*	Recovery (%)	
	HPLC**	IDA
3.1	110.6	102.5
13.3	103.7	96.1
22.6	102.5	95.7
27.7	102.7	—

*1 μmol/l = 0.388 μg/ml.
**Mean of duplicate determinations.

summarized in Table II show that the described method permits the accurate and precise determination of the sulfide in plasma at concentrations down to 50 ng/ml (130 nmol/l). The simultaneous determination in a single run of sulfinpyrazone and all its metabolites by HPLC did not appear feasible with an acceptable sensitivity.

TABLE II

PRECISION AND ACCURACY OF THE HPLC ASSAY FOR THE SULFIDE METABOLITE OF SULFINPYRAZONE IN PLASMA (SPIKED SAMPLES)

Amount added (μmol/l)*	Amount found (μmol/l)	No. of replicates	Coefficient of variation (%)	Mean recovery (%)
0.13	0.13	12	11.9	100
1.29	1.31	8	3.25	102
3.27	3.12	6	3.8	95

*1 μmol/l = 0.388 μg/ml.

Application

The application of the present method to the determination of the sulfide metabolite in plasma from three healthy volunteers given successive single oral doses of 200 mg and 400 mg of sulfinpyrazone is demonstrated in Fig. 3. The concentrations of the parent drug in these samples were also determined by an HPLC method [10].

The sulfide metabolite, which is present in concentrations much lower than the parent drug in the first hours after dosing, is slowly eliminated from plasma.

CONCLUSION

The proposed method permits the specific assay of the sulfide metabolite of sulfinpyrazone with a sensitivity that appears sufficient for pharmacokinetic investigations in man.

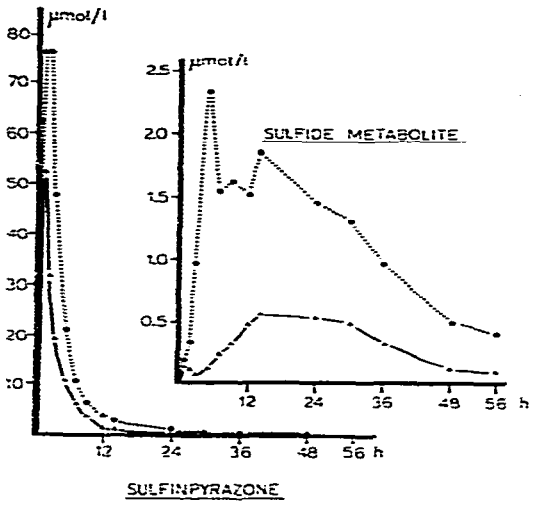

Fig. 3. Plasma concentrations of sulfinpyrazone (1 µmol/l = 0.404 µg/ml) and its sulfide metabolite (1 µmol/l = 0.388 µg/ml) after administration of single oral doses of 200 mg (▲———▲) and 400 mg (● - - - - - - ●) of sulfinpyrazone (mean values for three healthy volunteers).

NOTE ADDED IN PROOF

An HPLC method for the simultaneous determination of sulfinpyrazone and four of its metabolites has recently been published [15].

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