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QUANTITATIVE ASSAY OF SULPHINPYRAZONE IN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method for the quantitative determination of sulphinpyrazone in plasma and urine is described. The drug is extracted from the acidified aqueous phase with 1-chlorobutane–ethylene dichloride (4:1) and separated from its metabolites by high-performance liquid chromatography on 5-μm LiChrosorb using dichloromethane–ethanol–water–acetic acid (79.1:19:1.9:0.002) as the mobile phase.

The sensitivity limit is 0.2 μg/ml using a 1-ml sample. Examples of applications are given.

INTRODUCTION

Sulphinpyrazone (Anturan, Ciba-Geigy, Basle, Switzerland) has long been known as a uricosuric agent. It was more recently found to be effective in preventing the aggregation of blood platelets, and it is now being intensively investigated as an antithrombotic agent in humans.

There are only two methods available for the quantitative assay of sulphinpyrazone (SP) in biological fluids. Burns *et al.*[1] described a UV assay in 1957, but it is neither specific nor sensitive. In 1975, Inaba *et al.*[2] reported a high-performance liquid chromatographic (HPLC) technique, but it requires the use of a radioactively labelled internal standard and its sensitivity is limited to 3 μg/ml. Its specificity was not demonstrated.

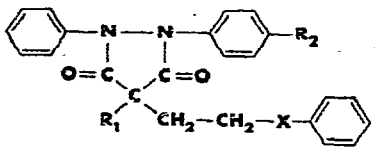
This paper describes a simple, sensitive and specific method for the quantitative assay of SP in plasma and urine at the concentrations found when therapeutic doses are administered.

EXPERIMENTAL

Materials

SP and its metabolites G. 31 442, G. 32 642 and GP. 52 097 (Fig. 1) were supplied by Ciba-Geigy.

The solvents used were all of analytical grade: dichloromethane (E. Merck, Darmstadt, G.F.R.; Cat. No. 6050), ethylene dichloride (Merck; Cat. No. 955),



Compound	R_1	R_2	X
Sulphinpyrazone	H	H	SO
GP. 52 097	OH	H	SO
G. 31 442	H	H	SO_2
G. 32 642	H	OH	SO

Fig. 1. Sulphinpyrazone and its main metabolites in man.

1-chlorobutane (Merck; Cat. No. 801640), acetic acid (Merck; Cat. No. 90063) and ethanol (Prolabo, Paris, France; Cat. No. 2082129).

Instruments

Chromatography was performed on a Model 1010 high-performance liquid chromatograph (Hewlett-Packard) equipped with an LA 102 septum injector (Reeve Angel) and a fixed-wavelength (254 nm) UV absorbance detector (Laboratory Data Control). The detector was connected to a CRS 204 electronic integrator (Infotronics) and to a 3012 potentiometric recorder (W + W Kontron Verwaltungs AG)

Column

The chromatographic column was a stainless-steel tube (12 cm × 4.7 mm I.D.) filled with 5-μm LiChrosorb Si60 (Merck) using the high-pressure slurry technique[3]. A 1.5-g amount of LiChrosorb was suspended in 6.5 ml of dichloromethane–ethanol–water–acetic acid (79.1:19:1.9:0.002). The slurry was rapidly pumped with the same solvent under high pressure (up to 350 bar) into the column, which was terminated with a 2-μm, $\frac{1}{4}$-in. porous metal disk frit (Reeve Angel) and an LDV 4-16 connector of low dead volume (Reeve Angel). The column was ready for use as soon as it was filled.

In order to obtain good column efficiency (1500–2000 theoretical plates for the SP peak), it was found necessary to inject the sample about 2 mm inside the column filling. In order to prevent blockage of the needle of the high-pressure syringe (Hamilton No. 88212 and No. HP 305) with the filling material, its tip was modified so as to make it sharper, with an angle of about 10° to its axis.

Glassware

The normally washed glass tubes were immersed for 30 min in a methanol ultrasonic bath and then dried at 100°. They were then immersed in a 1% aqueous solution of Siliciad (Clay Adams Parsippany, N.J., U.S.A.), rinsed with distilled water and dried at 100°.

Assay procedure

To 1 ml of plasma or urine in a 10-ml conical glass tube are added 1 ml of 1 *N*

hydrochloric acid and 0.5 ml of 1-chlorobutane–ethylene dichloride (4:1). The tube is sealed with Parafilm (American Can Co.) and shaken for 30 sec on a Vortex mixer. The tube is centrifuged for 5 min at 2500 *g*, then 300 μl of the supernatant organic phase are collected with an automatic pipette (Oxford) through the seal and transferred into a 2-ml stoppered glass tube. A 40-μl volume of this extract (4 μl when the SP concentration is higher than 20 μg/ml) is injected into the chromatographic column.

The dichloromethane–ethanol–water–acetic acid (79.1:19:1.9:0.002) mobile phase is pumped at a constant flow-rate of about 2 ml/min under a pressure of about 70 bar at room temperature. The retention time of SP is about 3 min.

Two injections of each extract are performed; the integrated areas of the SP peaks must agree to within $\pm$ 3%.

Calibration

Calibration samples are prepared by adding 100 μl of suitable SP solutions in 0.05 *N* sodium hydroxide solution to 1 ml of plasma or urine. Three or four calibration samples containing 0.2–20 μg of SP are prepared. The calibration graph (peak area *versus* concentration on a log–log graph) must be a straight line. A calibration is performed every day using the assay procedure on unknown samples.

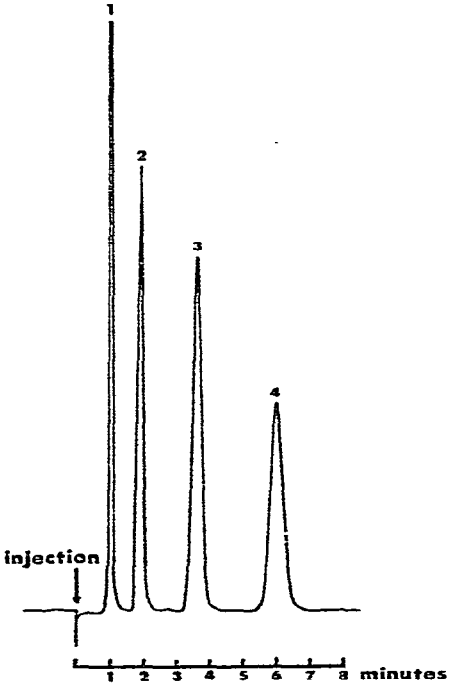


Fig. 2. Separation of a synthetic mixture of sulfinpyrazone and its metabolites by HPLC. 1, GP. 52 097 + solvent; 2, G. 31 442; 3, Sulphinpyrazone; 4, G. 32 642. Column: 5-μm LiChrosorb Si60, 125 mm × 4.7 mm. Eluent: dichloromethane–ethanol–water–acetic acid (79.1:19:1.9:0.002, v/v). Flow-rate: 2 ml/min. Temperature: ambient. Pressure: 70 bar. Detection: UV 254 nm, 0.08 absorbance unit full-scale. Volume injected: 40 μl of the extraction solvent solution.

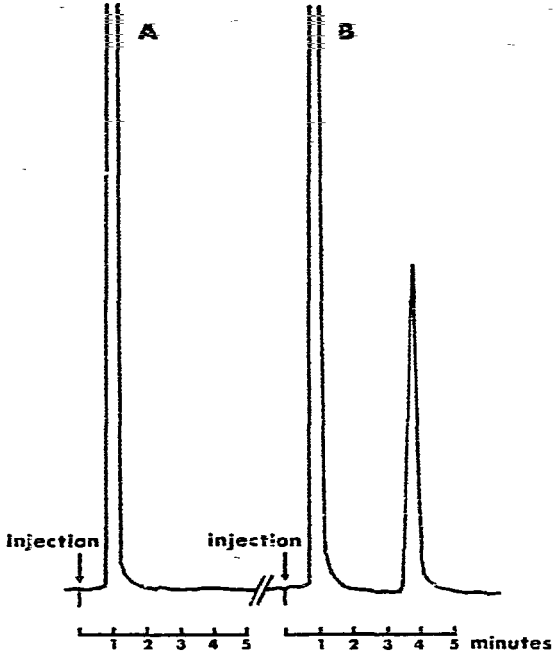


Fig. 3. A, Chromatography of a blank plasma extract; B, chromatography of an extract of plasma spiked with sulphinpyrazone (4 μg/ml). HPLC conditions as in Fig. 2.

RESULTS AND DISCUSSION

Specificity

SP is conveniently separated from its three known metabolites[4] as shown in Fig. 2. Plasma or urine components do not interfere (see Figs. 3 and 4). Also, the following drugs which may be associated with SP, and their metabolites, were not found to interfere: paracetamol, diazepam, methyldopa, flurazepam, propoxyphen, digoxin and dicloxacillin.

Reproducibility and accuracy

Various spiked plasma and urine solutions were analyzed repeatedly .The results given in Table I show that the proposed procedure permits the quantitative assay of SP down to a concentration of 0.2 μg/ml with satisfactory precision and accuracy. The lowest limit of quantitative determination is about 50 ng/ml, which corresponds to a peak height that is about ten times the background noise.

Table II demonstrates the excellent reproducibility, confirming that no internal standard is needed. The extraction yield (standard deviation) is 81 ± 3% from plasma and 83 ± 3% from urine. It is not dependent on the SP concentration in plasma or urine as the log–log calibration graphs are straight lines with a slope 1.01–1.05.

From time to time, the pressure increases at constant flow-rate, owing to small pieces of the septum accumulating at the entrance of the column. It is then necessary to remove the first 3 mm of the filling and to replace it with a LiChrosorb paste,

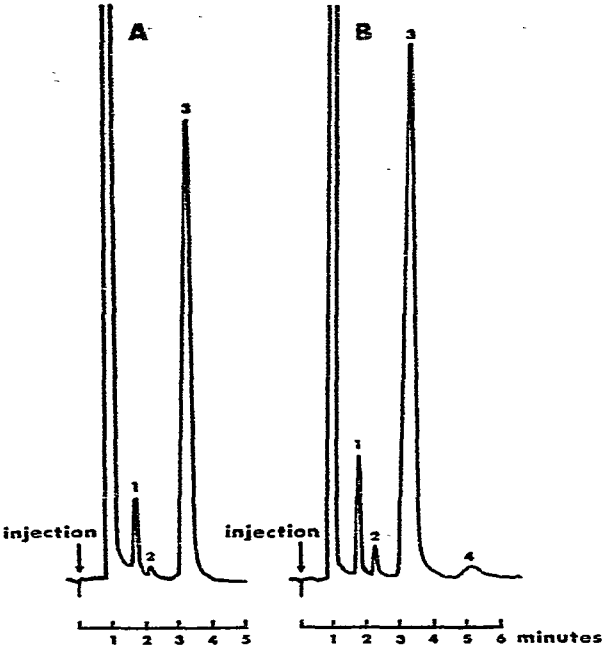


Fig. 4. A, Chromatography of a plasma extract of a subject given an oral dose of sulphinpyrazone. 1, G. 31 442; 2, unknown peak; 3, sulphinpyrazone. B, Chromatography of a urine extract from the same subject. 1, G. 31 442; 2, unknown peak; 3, sulphinpyrazone; 4, G. 32 642. HPLC conditions as in Fig. 2.

using a spatula. As the preparation of a new column takes about 1 h, it is recommended that it should be changed as soon as its performance appears to decrease.

Stability of sulphinpyrazone

As the assay procedure is extremely simple and does not involve an evaporation step, it avoids the degradation of SP, which is very easily oxidized when taken to dryness. The silicone treatment of the glassware prevents the loss by adsorption which occurs when small volumes of SP organic solutions are handled in untreated glass containers.

SP calibration solutions in 0.05 *N* sodium hydroxide solution are stable for more than 1 month at 5°. Human plasma and urine samples containing SP must be frozen as soon as they are obtained. SP is stable for at least 2 months when these samples are stored at −20°.

Speed of analysis

The proposed technique is very rapid: one assay (with duplicate injection) takes 10 min for chromatography, so that a large number of samples can be analyzed per day.

Application

The procedure was applied to the quantitative assay of SP in the plasma of

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE ANALYTICAL PROCEDURE

Sample	*SP added (μg)*	*SP found (μg)*		*95% confidence interval*		*Standard deviation (%)*	*Standard error of the mean (%)*
		Individual determinations	*Average*	*Min.*	*Max.*		
Human plasma (1 ml)	0.05	0.05, 0.05, 0.06, 0.06, 0.08, 0.08	0.06	0.05	0.08	21.5	8.8
	0.09	0.10, 0.10, 0.10, 0.10, 0.11, 0.11	0.10	0.10	0.11	4.9	2.0
	0.15	0.12, 0.13, 0.13, 0.14, 0.14, 0.16	0.14	0.12	0.15	12.3	4.1
	0.20	0.19, 0.19, 0.20, 0.20, 0.21, 0.22	0.20	0.19	0.21	5.8	2.4
	0.30	0.30, 0.28, 0.30, 0.28, 0.27, 0.28	0.29	0.27	0.30	4.3	1.7
	0.50	0.47, 0.47, 0.48, 0.49, 0.50, 0.52	0.49	0.47	0.51	4.0	1.6
	0.80	0.75, 0.85, 0.75, 0.75, 0.75, 0.80	0.78	0.73	0.82	5.4	2.2
	4.00	3.80, 3.90, 4.00, 4.05, 4.20, 4.25	4.03	3.85	4.21	4.2	1.7
	15.0	15.3, 15.6, 16.1 14.8, 14.9, 15.8	15.42	14.88	15.95	3.3	1.3
	56.0	56.0, 56.5, 56.7, 57.2, 58.2, 61.7	57.72	55.52	59.91	3.6	1.5
Human urine (1 ml)	15.0	14.9, 15.1, 14.5, 14.7, 15.0, 15.5	14.95	14.58	15.31	2.3	0.9
	40.0	40.7, 40.0, 42.2, 40.7, 40.1, 40.7	40.73	39.90	41.55	1.9	0.8
	80.0	81.5, 80.5, 75.0, 81.0, 79.5, 82.0	79.92	77.23	82.60	3.2	1.3

TABLE II

INJECTION OF TEN SUCCESSIVE SAMPLES OF 40 μl OF THE SAME SULPHINPYRAZONE DICHLOROMETHANE SOLUTION

No. of injection	*SP peak area (arbitrary units)*
1	128238
2	126450
3	126540
4	126727
5	126371
6	125823
7	128313
8	126407
9	123411
10	127547
Average	126582
Standard error (%)	0.34
Standard deviation (%)	1.1

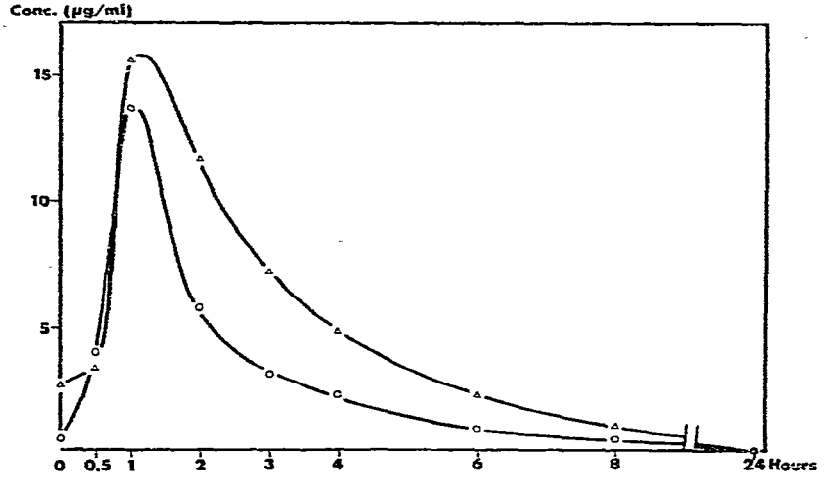


Fig. 5. Sulphinpyrazone plasma concentrations in two volunteers dosed with 200 mg of sulphinpyrazone twice daily for 1 week, at the seventh day of administration. ○, Volunteer A; △, volunteer B.

TABLE III

CROSS-CHECK OF THE UV SPECTROPHOTOMETRIC AND HPLC METHODS

Duplicate analyses of plasma samples of volunteer B at the seventh day.

Time (h)	*SP (µg/ml)*	
	*UV results**	*HPLC results*
0	1.7– 1.7	2.55– 2.77
0.5	2.1– 2.5	3.35– 3.40
1	14.2–11.8	15.40–15.50
2	10.5–11.8	11.40–11.60
3	7.5– 8.1	7.00– 7.05
4	4.1– 5.3	4.55– 4.82
6	4.7– 3.5	2.15– 2.20
8	0 – 0	0.93– 0.93
24	0 – 1.3	0.16– 0.13

* The 24-h plasma was used as a blank.

two volunteers dosed twice daily with 200 mg of SP as Anturan tablets for 1 week. Fig. 5 shows the plasma concentration curves at the seventh day after the last dose. The same plasma samples were also assayed by the UV method of Burns *et al.*[1] and the results are given in Table III. The UV assay appears to give unsatisfactory results, especially at low SP concentrations.

CONCLUSION

Sulphinpyrazone can be assayed in plasma and urine of patients receiving single or multiple therapeutic doses of the drug. The speed of analysis permits the routine monitoring of sulphinpyrazone every time it is needed for clinical pharmacokinetic purposes.

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