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Note

Simple and rapid micro-determination of sulfinpyrazone (Anturan) in biological fluids by reversed-phase high-performance liquid chromatography

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Sulfinpyrazone (Anturan) is a uricosuric agent[1] which has been used for the treatment of gout for more than a decade. Recently, it has been demonstrated that it is also an effective drug for the management of thromboembolic complications since it acts on platelets by prolonging their survival and decreasing their turnover[2,3].

For the analysis of sulfinpyrazone in biological fluids, the UV method described by Burns *et al.*[1] appears to lack both sensitivity and specificity[4]. In 1975, Inaba *et al.*[5] reported a high-performance liquid chromatographic (HPLC) method for the quantitation of sulfinpyrazone in plasma using micro-particle size (10 μm) silica as the absorption phase, but the technique requires the use of [^{14}C]sulfinpyrazone as the internal standard. A more sensitive and specific HPLC assay using the same stationary phase but without the use of an internal standard was recently described by Lecaillon and Souppart[6].

In our investigation of the interactions between analgesics (salicylate, phenylbutazone) and sulfinpyrazone in laboratory animals, it was necessary to have a sensitive and specific micro-method for the analysis of sulfinpyrazone. This paper describes the use of reversed-phase HPLC for its quantitation with warfarin serving as an internal standard. The technique is simple, rapid, requires only 50–100 μl of samples and has a sensitivity limit of about 2 μg/ml. The method has been employed in our laboratory in the past two years for the pharmacokinetic analysis of sulfinpyrazone in animal models and *in vitro* protein-binding studies of sulfinpyrazone.

EXPERIMENTAL

Liquid chromatography

A Varian-Aerograph Series 4100 liquid chromatograph equipped with a positive displacement pump capable of developing a pressure of 5000 p.s.i., a stop-flow injection port and a variable-wavelength UV absorbance detector (Vari-Chrom) operated at 275 nm was utilized. The column was a stainless-steel tube (25 cm × 2.8 mm I.D.) packed with 10 μm LiChrosorb RP-2 using the high-pressure (5000 p.s.i.) balanced-density slurry packing technique[7]. A pre-column (5 cm × 2.8 mm I.D.) packed with Vydac-RP (30–44 μm) was used to trap eluent insoluble materials. The mobile phase was 0.1 *M* ammonium acetate in acetonitrile–water (30:70 or

35:65, v/v) adjusted to pH 5 with acetic acid. The mobile solvent was degassed by applying vacuum to the solvent reservoir for 2 min before use. All analyses were performed at ambient temperature.

Reagent and materials

Sulfinpyrazone and *p*-hydroxysulfinpyrazone were supplied by Ciba-Geigy (Montreal, Canada) and [^{14}C]sulfinpyrazone (phenyl-UL-^{14}C) with a specific activity of 14.55 μCi/mg by Geigy (Basle, Switzerland). Sodium warfarin was obtained from Merck-Frosst Labs. (Montreal, Canada) and [^{14}C]warfarin (74 μCi/mg) from Amersham/Searle (Oakville, Canada). Ammonium acetate was analytical grade supplied by BDH (Toronto, Canada); acetonitrile and *n*-butyl chloride were obtained from Caledon Labs. (Georgetown, Canada).

Treatment of animals

Male albino Wistar rats (300–350 g) and male New Zealand white rabbits (3.0–3.5 kg) were used in the studies. The rats were dosed with sulfinpyrazone (50 mg/kg, intraperitoneally) and duplicate blood samples (0.25 ml) were collected from the tail into heparinized Natelson pipets at 1, 3, 6, 10, 24, 31 and 48 h after injection. The rabbits were dosed with sulfinpyrazone (20 mg/kg, intravenously) and blood samples were similarly collected from the ear veins at 15, 30, 45, 60, 90, 120 and 180 min after drug administration.

Extraction and analysis procedure

To 50–100 μl of plasma or urine were added 0.25 ml of internal standard (25 μg or 8.3 μg sodium warfarin per ml water), 0.2 ml of 1 *N* HCl and 0.50 ml of *n*-butyl chloride. The sample was agitated in a Vortex mixer (30 sec) and centrifuged for 2 min at 2000 *g* to separate the phases. About 0.4 ml of the organic phase was taken and partitioned into 0.1 ml of 0.1 *N* NaOH by vortexing for 30 sec. After centrifugation for 2 min at 750 *g*, 5–50 μl of the NaOH solution were injected into the liquid chromatograph.

Evaluation of extraction efficiency from plasma

Drug-free plasma was spiked with [^{14}C]sulfinpyrazone to give concentrations of 10, 25, 50, 75 and 100 μg/ml and extracted with *n*-butyl chloride containing warfarin internal standard (25 μg/ml) as previously described. In another experiment, plasma was spiked with sulfinpyrazone to give concentrations of 10, 25, 50, 75 and 100 μg/ml and extracted with *n*-butyl chloride containing [^{14}C]warfarin internal standard (25 μg/ml) as before. The organic phase (0.4 ml) was partitioned into 0.1 ml of 0.1 *N* NaOH solution and duplicate samples (20 μl) of the aqueous layer were quantitated for recoveries of radioactivity by counting in a liquid scintillation counter.

RESULTS AND DISCUSSION

Sulfinpyrazone is susceptible to oxidation. Evaporating a solution to dryness may cause extensive decomposition especially when heated. For this reason, the analysis procedure has to be simple and rapid and should not contain an evaporation step. *n*-Butyl chloride and ethylene dichloride were found to be suitable extraction solvents, but ether and ethyl acetate caused decomposition of the drug. After extrac-

tion into *n*-butyl chloride, sulfinpyrazone was partitioned back into 0.1 *N* NaOH and the alkaline solution was injected into the liquid chromatograph. The drug in NaOH solution was stable for at least a week at room temperature.

Fig. 1 shows the chromatographic separation of sulfinpyrazone (t_R = 5 min) and warfarin internal standard (t_R = 10 min) using the system described above. The internal standard is well resolved from and elutes behind the sulfinpyrazone peak.

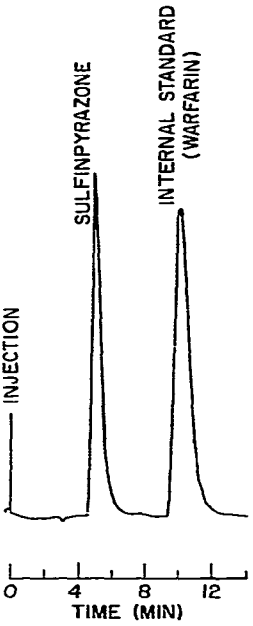

Fig. 1. Chromatogram of a plasma extract containing sulfinpyrazone and warfarin internal standard. Column: 25 cm × 2.8 mm I.D. packed with 10 μm LiChrosorb RP-2. Mobile phase: 0.1 *M* ammonium acetate in acetonitrile–water (35:65, v/v), pH 5. Flow-rate: 30 ml/h at 1600 p.s.i.

The standard curves for the analysis are shown in Figs. 2A and 2B. These results were obtained from three or more determinations of each sulfinpyrazone concentration. The peak height ratio of sulfinpyrazone and the internal standard was used as an index for quantitation. A linear response was obtained with sulfinpyrazone concentrations ranging from 0–100 μg/ml (Fig. 2A). For assay in the lower range (0–20 μg/ml), a standard curve constructed with a lower amount of the internal standard was used in order to increase the accuracy of the analysis (Fig. 2B). The overall coefficient of variation of the technique was 4.3%.

Table I shows the efficiency of the extraction procedure as quantitated using ^{14}C radioactivity. The average recoveries of sulfinpyrazone and warfarin were 78.52 ± 4.04% and 79.69 ± 2.97%, respectively. The recovery of sulfinpyrazone was independent of its concentration.

The technique was specific for sulfinpyrazone since blank plasma and urine gave no interfering peaks in the assay. *p*-Hydroxysulfinpyrazone, the major metabolite of sulfinpyrazone[8,9], also did not interfere as it elutes ahead ($t_R \approx 3$ min) of the sulfinpyrazone peak.

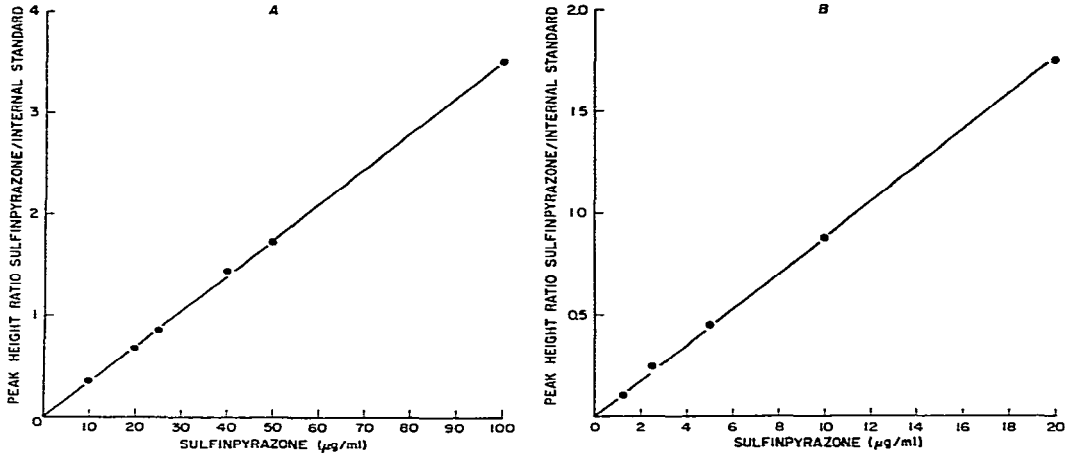

Fig. 2. Standard curves for sulfinpyrazone analysis. (A) High range (0–100 μg/ml) with 25 μg/ml of warfarin solution as internal standard. (B) Low range (0–20 μg/ml) with 8.3 μg/ml of warfarin solution as internal standard.

TABLE I

RECOVERY OF [^{14}C]SULFINPYRAZONE AND [^{14}C]WARFARIN FROM PLASMA

μg [^{14}C]sulfinpyrazone added to 1 ml plasma	*n*	*Mean [^{14}C]sulfinpyrazone (μg/ml) recovered ± S.D. (% recovery)*	*[^{14}C]Warfarin (μg) added for quantitation*	*n*	*Mean [^{14}C]warfarin recovered ± S.D. (% recovery)*
10	3	7.52 ± 0.75 (75.21)	6.25	3	4.94 ± 0.12 (79.06)
25	3	18.51 ± 1.19 (74.02)	6.25	3	5.15 ± 0.11 (82.36)
50	3	40.99 ± 0.76 (81.98)	6.25	3	5.02 ± 0.21 (80.70)
75	3	62.40 ± 1.56 (83.20)	6.25	3	4.68 ± 0.63 (74.85)
100	3	78.20 ± 5.55 (78.20)	6.25	3	5.09 ± 0.17 (81.50)

Application of the technique for the micro-analysis of plasma sulfinpyrazone levels in laboratory animals is depicted in Fig. 3. The chromatographic analysis of a sample took approximately 12 min. The short analysis time, together with the rapid and simple extraction step, made possible the assay of a large number of samples per day using this technique. The sensitivity limit was 2 μg/ml with a 50-μl sample. The plasma profile of sulfinpyrazone in rats is shown in Fig. 3A. The profile is bi-phasic, with a half-life of elimination measured between 10 and 48 h to be 13.78 h which is quite a contrast to the short half-life found in rabbits (approx. 30 min, Fig. 3B). In humans, the reported elimination half-lives of sulfinpyrazone ranged from 2 to 6 h[1,4,9]. These data demonstrate the wide species variations in the elimination of sulfinpyrazone.

One of the advantages of "reversed-phase" LC over "forward-phase" LC is the possibility of analyzing water-soluble organic materials in predominantly aqueous systems, thus precluding the requirement that the compound of interest be extracted prior to analysis. This is demonstrated in the assay of sulfinpyrazone in urine samples. Because sulfinpyrazone is not extensively metabolized[8,9], its concentration in urine is

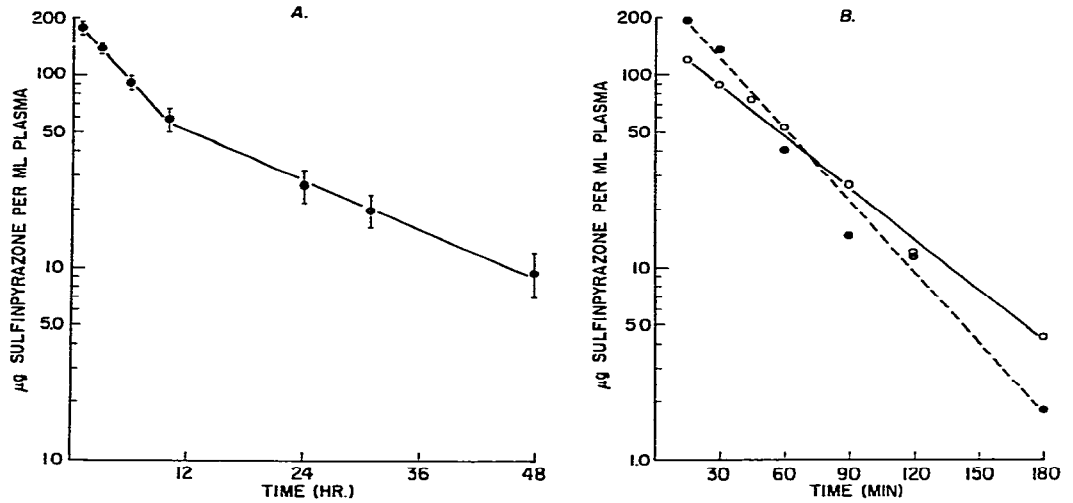

Fig. 3. Plasma sulfinpyrazone levels from (A) a group of 5 rats dosed intraperitoneally with 50 mg/kg of sulfinpyrazone, and (B) two rabbits dosed intravenously with 20 mg/kg of sulfinpyrazone.

usually high after therapeutic doses and therefore can be rapidly determined by direct injection into the liquid chromatograph. The preclusion of the extraction step further shortens the analysis time and decreases the uncertainty in the analytical results due to the labile nature of sulfinpyrazone. Fig. 4 illustrates the chromatograms for the

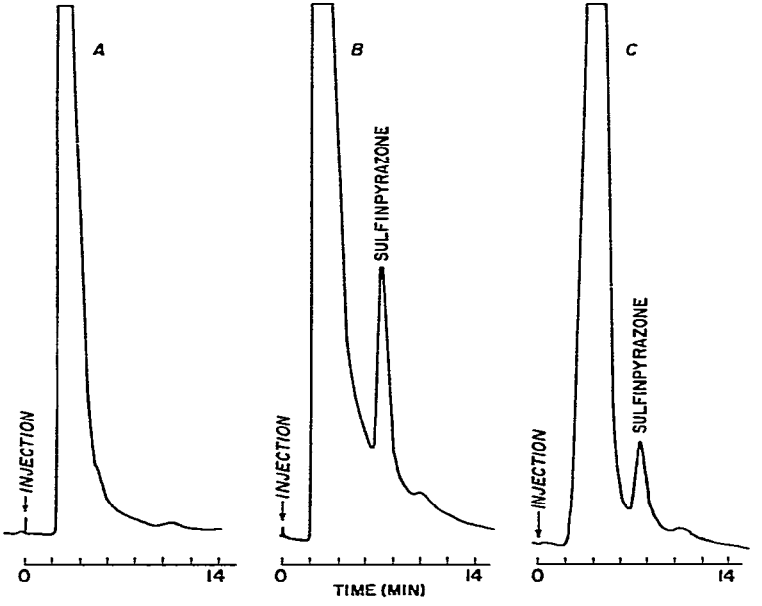

Fig. 4. Chromatograms from sulfinpyrazone analysis in rat urine. (A) Control urine (3 μl). (B) Control urine spiked with sulfinpyrazone to give a final concentration of 50 μg/ml (2 μl). (C) Rat urine containing 20 μg/ml of sulfinpyrazone (2 μl). Column: 25 cm × 2.8 mm I.D. packed with 10 μm LiChrosorb RP-2. Mobile phase: 0.1 *M* ammonium acetate in acetonitrile–water (30:70, v/v), pH 5. Flow-rate: 30 ml/h at 1600 p.s.i.

analysis of sulfinpyrazone in rat urine. The standard curve for quantitation was obtained by plotting sulfinpyrazone concentrations *vs.* peak heights ranging from 20 to 200 μg/ml. Due to the high background of endogenous UV absorbing materials contained in urine, only 2–3 μl of undiluted sample could be injected without causing interference with the sulfinpyrazone peak and this limits the sensitivity of the analysis to about 20 μg/ml. Below this concentration, the urine was extracted and quantitated as in the plasma.

In summary, a procedure for the analysis of sulfinpyrazone in biological fluids by reversed-phase HPLC has been developed. Several appealing features of this method compared with previously reported HPLC techniques[5,6] for the quantitation of sulfinpyrazone may be noted. Sample preparation is rapid, simple and does not involve an evaporation step. A readily available internal standard is employed to increase the accuracy of the analysis. The minimum sample volume required is 50 μl with a sensitivity limit of 2 μg/ml. Finally, the technique also allows for the direct analysis (without prior extraction) of urine samples containing a sufficiently high concentration of the drug.

REFERENCES

1 J. J. Burns, T. F. Yü, A. Ritterbrand, J. M. Perel, A. B. Gutman and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 119 (1957) 418.
2 H. A. Smythe, M. A. Ogryzlo, E. A. Murphy and J. F. Mustard, *Can. Med. Ass. J.*, 92 (1965) 818.
3 P. P. Steele, H. S. Weily and E. Genton, *New Engl. J. Med.*, 288 (1973) 1148.
4 D. Kadar, T. Inaba, L. Endrenyi, G. E. Johnson and W. Kalow, *Clin. Pharmacol. Ther.*, 14 (1973) 552.
5 T. Inaba, M. E. Besley and E. J. Chow, *J. Chromatogr.*, 104 (1975) 165.
6 J.-B. Lecaillon and C. Souppart, *J. Chromatogr.*, 121 (1976) 227.
7 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974, p. 190.
8 A. Aldridge and G. E. Johnson, *Can. J. Physiol. Pharmacol.*, 50 (1972) 1061.
9 W. Dieterle, J. W. Faigle, H. Mory, W. J. Richter and W. Theobald, *Eur. J. Clin. Pharmacol.*, 9 (1975) 135.