Journal of Chromatography, 104 (1975) 165–169

CHROM. 7971

Note

Determination of sulfinpyrazone in serum by high-performance liquid chromatography

TADANOBU INABA, MARY E. BESLEY and EVA J. CHOW
Department of Pharmacology, University of Toronto, Toronto M5S 1A8 (Canada)
(Received August 5th, 1974)

Sulfinpyrazone is a widely used drug in the treatment of gout[1]. Presently, there is considerable interest in the use of sulfinpyrazone for the management of thromboembolic complications in man[2]. To investigate drug effects, it is essential to have a specific method for measuring drug levels in blood or serum[3]. Since the spectrophotometric assay of sulfinpyrazone developed by Burns *et al.*[4] is not specific enough when a patient is on multiple medications, it was decided to develop a new chromatographic method for this drug. Because of the labile nature of sulfinpyrazone, liquid chromatography (LC) was chosen. Radio-labeled sulfinpyrazone served as an internal standard to correct any loss during the analysis.

EXPERIMENTAL

Liquid chromatography

A Varian Model 4010-01 liquid chromatograph equipped with a Micro-Pak SI-10 column (silica gel 10-μm particle, Varian, 25 cm $\times$ 2.2 mm I.D.) was utilized[5,6]. The ultraviolet (UV) detector of the instrument was operated at 254 nm and a Hewlett-Packard Model 3370 A electronic integrator was used. The solvent, dioxane–methanol (65:35), was delivered at 35 atm by compressed helium gas. The flow-rate of solvent through the column was 0.7 ml/min. Samples were applied to the column through a septum with a Unimetrics 10-μl microsyringe. The sensitivity of the UV detector was set at 0.04 absorbance units full scale (a.u.f.s.).

Reagents

Sulfinpyrazone (Anturan) was supplied by Ciba-Geigy Canada (Montreal, Canada) and radio-labeled sulfinpyrazone (phenyl-UL-^{14}C) with a specific activity of 2.4 μCi/mg by Geigy (Basle, Switzerland). A stock solution for the labeled drug in dioxane was prepared at a concentration of 0.1 mg/ml, or 5.3 $\times$ 10^5 dpm/ml. The stock solution was kept at $-20°$ in the dark and used within three months. All solvents were reagent grade and used without further purification. The radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation counter using butyl-PBD in toluene (7 g/l).

Standard curve

Standard solutions were prepared from the stock solution of radioactive sulfin-

pyrazone and a freshly dissolved solution of pure sulfinpyrazone in methanol. The standard solutions (2–8 μl containing 0.1–8.0 μg) were injected into the liquid chromatograph. The eluate from the column was collected for the time period corresponding to the peak and the radioactivity determined. Thus, the exact volume of injection was obtained. The area of the sulfinpyrazone peak was plotted against the amount of drug injected. The retention time for sulfinpyrazone was 3.7 min.

Extraction and LC analysis

To 0.5-ml aliquots of serum, including blank, in 15-ml conical glass tubes were added 0.5 ml 1 *N* HCl, 2.0 ml 1-chlorobutane and 20 μl [^{14}C]sulfinpyrazone stock solution using a Lang Levy micropipet (Fisher Scientific). The liquids were mixed with the aid of a vortex mixer. One millilitre of the supernatant was transferred to a 5-ml conical tube and evaporated to dryness under a stream of nitrogen in a water-bath below 25°. Immediately before the LC analysis, the residue was dissolved in 100 μl of methanol and 8 μl were injected. The eluate was collected as described above, and its radioactivity determined to monitor the exact injection volume. From the blank sample, the peak area due to radio-labeled sulfinpyrazone in the chromatogram was computed and subtracted from the area of the other samples. The serum levels of sulfinpyrazone were calculated from the standard curve. Possible interference of other drugs was checked. Each of the following drugs (10 μg/ml) was added to serum containing sulfinpyrazone (10 μg/ml): acetylsalicylic acid, sodium salicylate, phenylbutazone, warfarin, diphenylhydantoin, amobarbital, and phenobarbital. The whole procedure was carried out as described above.

RESULTS AND DISCUSSION

The choice of a proper column and solvent is not an easy task in LC. From our experience, a column comprised of small-particle silica gel[5] was found to be very versatile and offered excellent resolution for the analysis of lipid-soluble drugs and drug metabolites. Selection of a suitable solvent system for this column was achieved by direct extrapolation of thin-layer chromatographic results[6].

Fig. 1 illustrates chromatograms from sulfinpyrazone analysis. A linear response curve for LC peak areas was obtained in the range from 0.1 to 8.0 μg of injected sulfinpyrazone. A trace amount of [^{14}C]sulfinpyrazone was added to samples as an internal standard. Therefore, the exact volume of injection was obtained from the radioactivity in the LC effluent.

Blank serum samples from several healthy subjects were found to be free from any interfering endogenous substances in this LC assay (Fig. 1c). Next, recovery studies were carried out. Known amounts of sulfinpyrazone were added to pooled serum samples at concentrations of 20, 40, and 60 μg/ml. As shown in Table I, the recovery ranged from 80 to 94% after correction with the radio-labeled internal standard. Higher recovery was obtained with increasing concentration of sulfinpyrazone. Extraction of the drug from serum was best accomplished with 1-chlorobutane, although other solvents such as 1,2-dichloroethane, diisopropyl ether, benzene and heptane were also tried. The minimum quantitatively detectable concentration of sulfinpyrazone was 3 μg/ml using 0.5 ml serum.

Possible interference of other acidic drugs in this LC assay was examined.

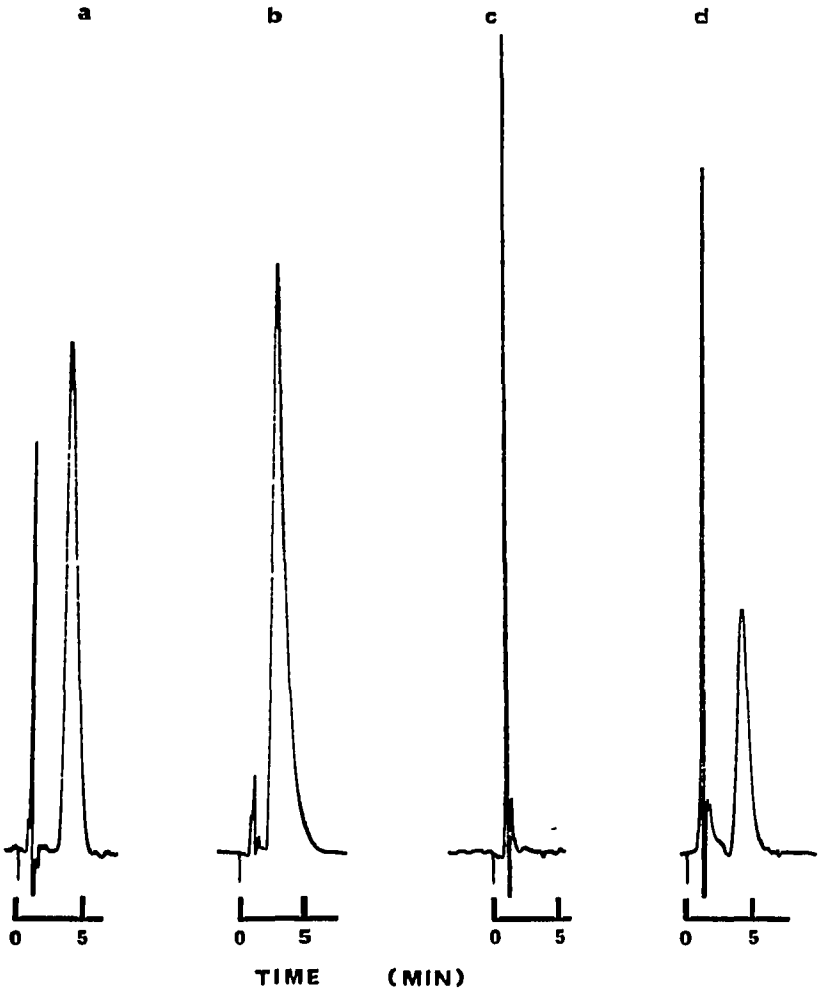

Fig. 1. Chromatograms from sulfinpyrazone analysis. Column, Micro-Pak (small-particle-size silica gel), 25 cm × 2.2 mm I.D.; carrier, dioxane–methanol (65:35); flow-rate, 0.7 ml/min; pressure, 35 atm; temperature, 22°. (a) A standard —0.3 μg sulfinpyrazone; UV sensitivity, ×0.04 (a.u.f.s.). (b) A standard —3.0 μg sulfinpyrazone; UV sensitivity, ×0.32 (a.u.f.s.). (c) Blank serum extract; UV sensitivity, ×0.04 (a.u.f.s.). (d) A standard added to serum —5 μg sulfinpyrazone in 0.5 ml serum— and extracted; UV sensitivity, ×0.04 (a.u.f.s.).

Some drugs, such as acetylsalicylic acid, sodium salicylate, phenylbutazone, warfarin, diphenylhydantoin, amobarbital and phenobarbital, might be administered concomitantly with sulfinpyrazone in clinical situations. These drugs under normal therapeutic concentrations were found not to interfere with the present sulfinpyrazone assay. Sulfinpyrazone had a longer retention time and/or higher UV response than the acidic drugs mentioned above.

The method has been applied to the determination of sulfinpyrazone in dog serum following its intravenous administration. The concentration of sulfinpyrazone

TABLE I

RECOVERY OF SULFINPYRAZONE FROM SERUM

Amount added (μg/ml)	*Found by LC (μg/ml)*	*Recovery (%)*
20	17.0	85
20	16.0	80
20	16.5	83
40	36.5	91
40	36.5	91
60	56.5	94
60	54.5	91
60	56.5	94

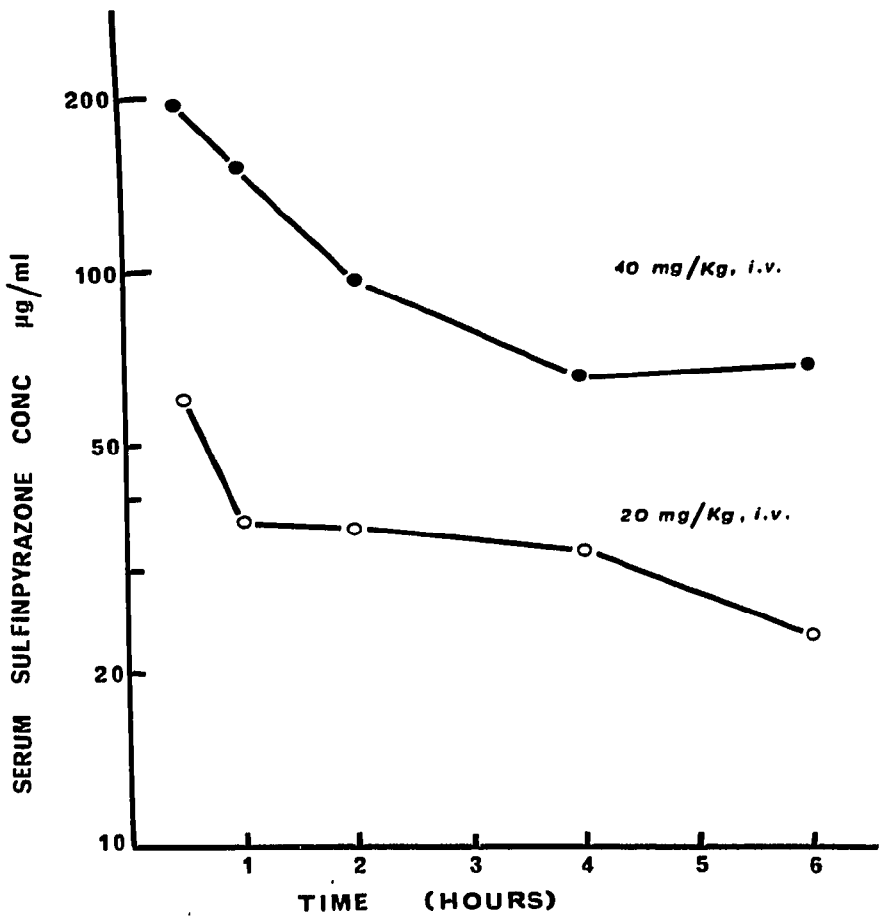

Fig. 2. Serum sulfinpyrazone levels following its intravenous administration (40 and 20 mg/kg body weight) in the dog.

ranged from 20 to 200 μg/ml, as shown in Fig. 2. Similar concentration ranges were found in human plasma following an intravenous administration of sulfinpyrazone[4].

ACKNOWLEDGEMENT

This work was supported in part by Ciba-Geigy Canada Ltd., Montreal, Canada, and by the Medical Research Council, Ottawa, Canada (Grant No. MA-4214).

REFERENCES

1 L. S. Goodman and A. Gilman (Editors), *The Pharmacological Basis of Therapeutics*, MacMillan, New York, 4th ed., 1970, p. 889.
2 J. F. Mustard and M. A. Packham, *Biochem. Pharmacol.*, 22 (1973) 3151.
3 B. B. Brodie and W. D. Reid, in B. N. LaDu, H. G. Mandel and E. L. Way (Editors), *Fundamentals of Drug Metabolism and Drug Disposition*, Williams and Wilkins, Baltimore, 1971, p. 328.
4 J. J. Burns, T. F. Yü, A. Ritterband, J. M. Perel, A. B. Gutman and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 119 (1957) 418.
5 T. Inaba and J. F. Brien, *J. Chromatogr.*, 80 (1973) 161.
6 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.