Journal of Chromatography, 130 (1977) 361–367

CHROM. 9575

Note

Quantitative determination of sulpyrid in biological samples from rats by gas–liquid chromatography and chemical ionization–mass fragmentography

A. FRIGERIO and C. PANTAROTTO

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan (Italy)

(Received July 6th, 1976)

Sulpyrid, N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-sulphamoylbenzamide, is a psychotropic drug used as a behaviour regulator in the psychopathology of senescence in depression and schizophrenia, and it may also have an important application in gastroenterology. The pharmacological properties of sulpyrid have been described in several papers[1–8].

The lack of a satisfactory analytical method for the assessment of its body concentrations prompted us to develop a sensitive and specific gas–liquid chromatographic (GLC) and chemical ionization–mass fragmentographic (CI–MF) method that has been applied to the determination of sulpyrid in the urine, plasma, liver and brain of rats.

EXPERIMENTAL

Standards and reagents

Sulpyrid was supplied by Ravizza (Milan, Italy) and chlorthalidone (used as the internal standard) by Ciba-Geigy (Milan, Italy).

The following reagents were used: N,N-dimethylaniline, methyl iodide and silver oxide (Merck, Darmstadt, G.F.R.); sodium hydroxide, hydrochloric acid, methanol, ethanol, ethyl acetate and diethyl ether (Carlo Erba, Milan, Italy).

A 1 *M* solution of trimethylbenzylammonium hydroxide in methanol was prepared according to the procedure of Brochmann-Hanssen and Oke[9], with minor modifications.

Apparatus

Gas–liquid chromatography. GLC was carried out on a Carlo Erba Fractovap Model G-1 gas chromatograph, equipped with a flame-ionization detector (FID).

The column was a glass tube (2 m × 4 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, Pa., U.S.A.), conditioned for 1 h at 250° with a nitrogen flow-rate of 30 ml/min, 4 h at 310° with no nitrogen flow-rate and 24 h at 280° with a nitrogen flow-rate of 35 ml/min. The column was operated at 280° with an injector port temperature of 300° and a carrier gas (nitrogen) flow-rate of 35 ml/min. Air and hydrogen flow-rates were adjusted so as to provide maximum response.

Gas chromatography–chemical ionization–mass fragmentography. A Finnigan

Model 3100 quadrupole mass spectrometer, equipped with a gas chromatograph and a Model 6000 computer system programmed for mass fragmentography was used. The GLC conditions were as given above and the carrier gas [helium for electron impact (EI)–mass spectrometric (MS) and methane for CI–MS analysis] flow-rate was 35 ml/min. The mass spectrometer was operated under the following conditions: molecular separator temperature, 250°; ion source temperature, 100°; energy of the ionization beam, 70 eV; ionization current, 200 μA. CI–MF measurements were performed by focusing the instrument on the "quasimolecular ions" $(M + 1)^+$ of the permethylated derivatives of sulpyrid and chlorthalidone.

Extraction of sulpyrid from water, plasma and urine

The procedure reported was designed to measure sulpyrid in the range from nanograms to micrograms. A 1-ml volume of 0.1 *N* sodium hydroxide solution and 5 ml of ethyl acetate were added to 1 ml of water, plasma or urine. The test-tube was shaken mechanically for 20 min and, after centrifugation at 9000 *g* at 4° for 5 min, 4.5 ml of the organic phase were transferred into a second test-tube and evaporated to dryness at 60° (in a water-bath) under a gentle stream of nitrogen. The alkaline aqueous layer was extracted again with a further 5 ml of ethyl acetate and, after shaking and centrifugation as before, 5 ml of the organic phase were transferred into the corresponding test-tube and evaporated to dryness.

Then 25 μl of 1 *M* trimethylbenzylammonium hydroxide solution and 25 μl of a methanolic solution of chlorthalidone (containing 100 ng or 5 μg of the drug, depending on the position on the calibration graph and the detector utilized) were added to the dry residue.

The tube was capped, passed for 20 sec over a mixer, and 1 or 2 μl were injected on to the GLC column. The recoveries of sulpyrid from water, plasma and urine are reported in Table I.

Extraction of sulpyrid from liver and brain

Because of the large amount of interfering endogenous substances present in liver and brain tissues, the extraction procedure described above was modified as follows. To 0.5 g of rat liver or brain (after homogenization with 2 ml of a 1.15% solution of potassium chloride), 0.1 ml of 2 *M* sodium hydroxide solution and 5 ml of ethyl acetate were added. The test-tube was shaken gently for 30 min and then centrifuged at 9000 *g* at 4° for 10 min. A 4.5-ml volume of the organic layer was transferred into a second test-tube and evaporated to dryness under a gentle stream of nitrogen. The aqueous phase was extracted a second time with a further 5 ml of ethyl acetate, and 5 ml of the ethyl acetate extract were removed and added to the dry residue from the first extraction.

After mixing, 2 ml of 0.1 *N* hydrochloric acid were added and the capped tubes shaken vigorously for 20 min, then centrifuged at 9000 *g* at 4° for 5 min. A 1.9-ml volume of the acidic aqueous phase was transferred into a third test-tube and extracted three times with 5 ml of diethyl ether. After discarding the organic phase, 0.1 ml of 6 *N* sodium hydroxide solution was added to the acidic aqueous phase and the mixture extracted twice with 5 ml of ethyl acetate. After centrifugation, the combined ethyl acetate extracts were transferred into a fourth test-tube and evaporated to dryness under a gentle stream of nitrogen in a water-bath at 60°.

Then 25 μl of 1 *M* trimethylbenzylammonium hydroxide solution and 25 μl

of a methanolic solution of chlorthalidone (containing 100 ng or 5 μg of the drug, depending on the position on the calibration graph and the detector utilized) were added to the dry residue.

The tube was capped, passed for 20 sec over a mixer and 1 or 2 μl were injected on to the GLC column. The recoveries of sulpyrid from liver and brain are reported in Table I.

TABLE I

RECOVERY OF SULPYRID FROM WATER, URINE, PLASMA, LIVER AND BRAIN

Sample	*Recovery (% ± standard error)*
Water	81 ± 3
Urine	78 ± 5
Plasma	83 ± 5
Liver	50 ± 4
Brain	52 ± 5

RESULTS AND DISCUSSION

The "flash methylation" reaction[9] of sulpyrid and chlorthalidone renders these substances suitable for GLC analysis. The gas chromatograms of sulpyrid and the internal standard obtained from water and biological extracts of rats are shown in Fig. 1.

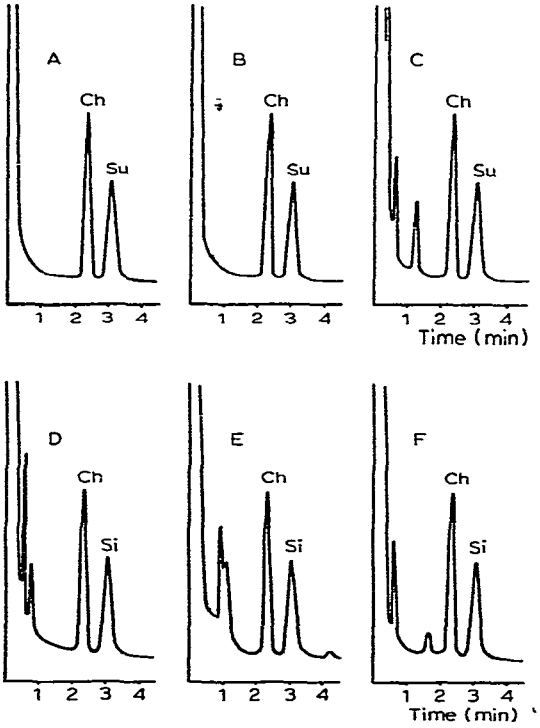

Fig. 1. Gas chromatograms of (A) standards; (B) water extract; (C) plasma extract; (D) urine extract; (E) liver extract; (F) brain extract. Ch = chlorthalidone (internal standard); Su = sulpyrid. Other peaks present on the chromatograms are due to endogenous substances.

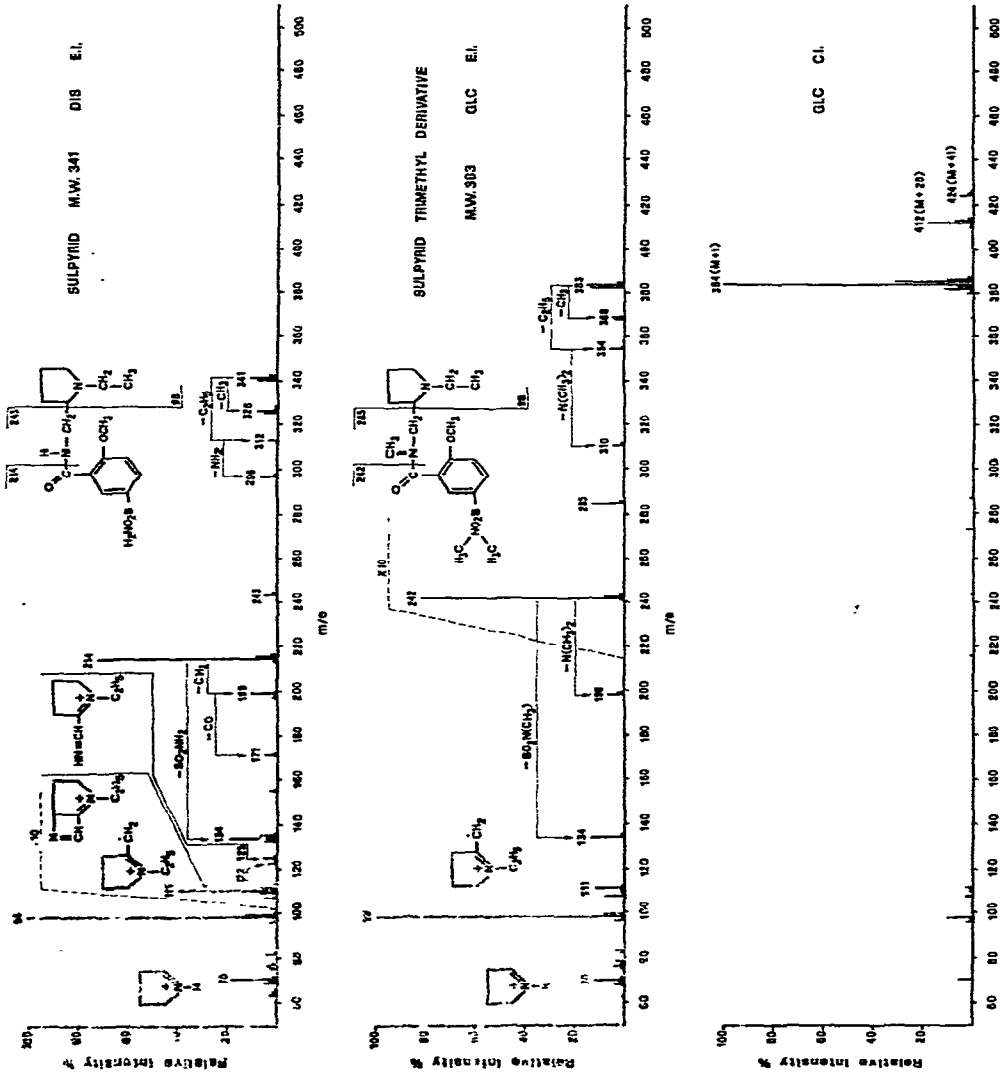

Fig. 2. Mass spectra of sulpyrid obtained by direct inlet system (DIS) or GLC, by EI or CI ion sources.

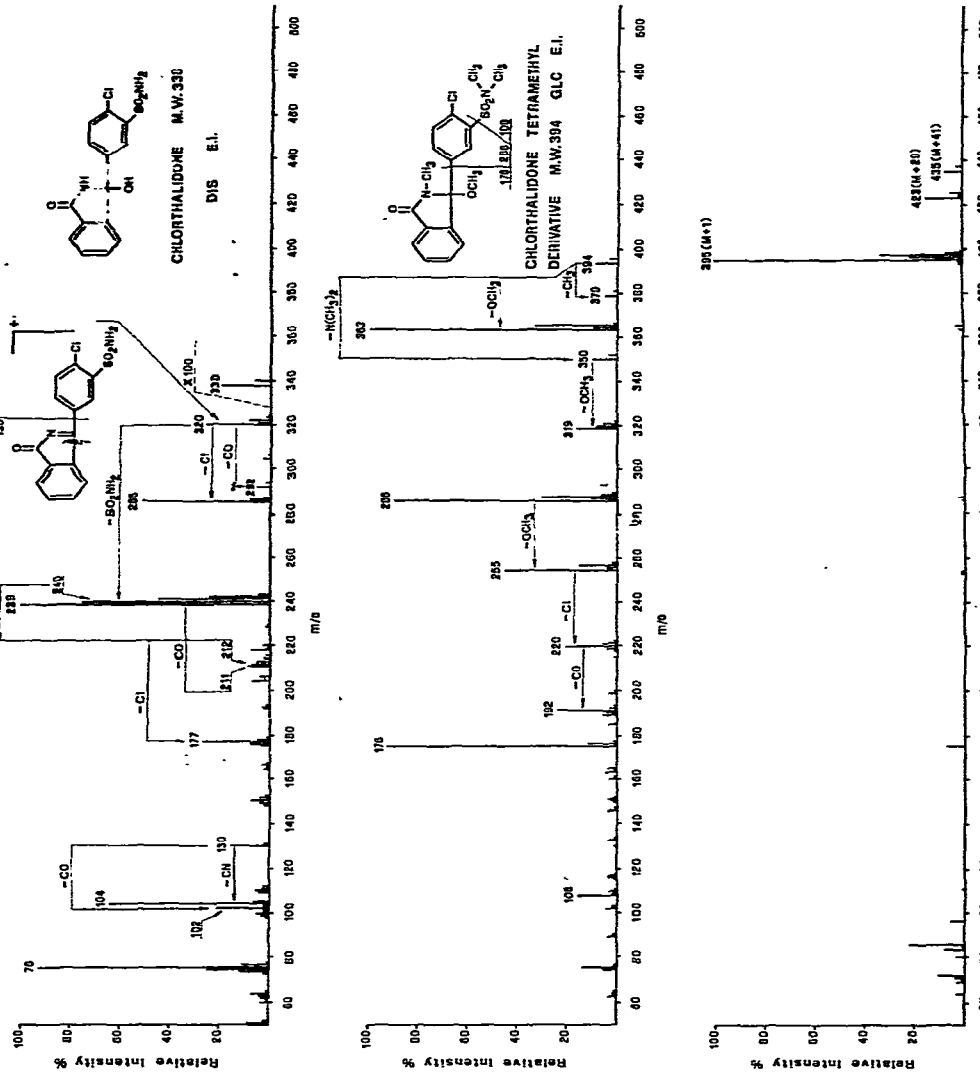

Fig. 3. Mass spectra of chlorthalidone obtained by direct inlet system (DIS) or GLC, by EI or CI ion sources.

The identities of the GLC peaks were checked by means of GC–MS, and the structure of the derivatives was elucidated by comparing the mass spectra obtained with either EI or CI ion sources (Figs. 2 and 3).

It can be seen that the two types of mass spectra clearly differ in the degree of fragmentation, and significantly so in relation to the intensity of the molecular ion (in the case of EI) and the "quasimolecular ion" (in the case of CI). According to the fundamentals of mass fragmentography[10], in order to obtain the highest sensitivity, it is generally advisable to focus the instrument on ions with an high relative abundance (preferably the base peak). Especially in the case of the sulpyrid trimethyl derivative, fragmentation by EI is not useful for quantitative determinations, because the main fragment ($m/e = 98$) falls in a low mass unit range and, therefore, EI–MF detection and quantification would not be able to achieve the required level of specificity necessary for its application to pharmacological problems. Conversely, in CI–MS, the base peak of the permethylated derivatives of sulpyrid and chlorthalidone corresponds to the "quasimolecular ion", which is highly specific and satisfies the requirements of MF detection[10].

Fig. 4 shows the mass fragmentogram of sulpyrid and the internal standard chlorthalidone obtained from water or biological extracts of rats. Detection was performed by focusing the instrument on the $(M + 1)^+$ ion for both substances, and no interferences from endogenous substrates were found.

Internal calibration graphs (Figs. 5 and 6) were obtained by adding sulpyrid in various amounts from 50 ng/ml or ng/g to 25 μg/ml or μg/g to water, plasma, urine,

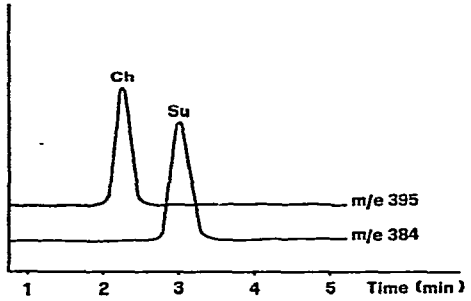

Fig. 4. Mass fragmentogram of sulpyrid (Su) and the internal standard chlorthalidone (Ch) obtained from biological extracts of rats. Interferences from endogenous substances were never observed.

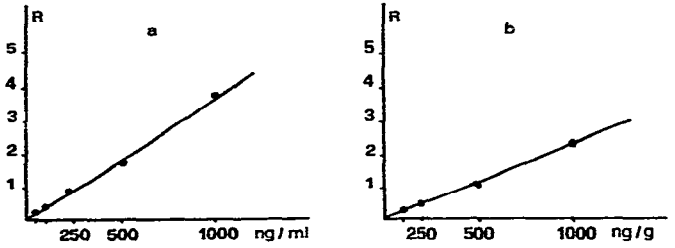

Fig. 5. Internal calibration graphs for sulpyrid (MF detection): (a) water, urine and plasma extracts; (b) liver and brain extracts. Only one graph is shown in each instance because the recoveries were identical.

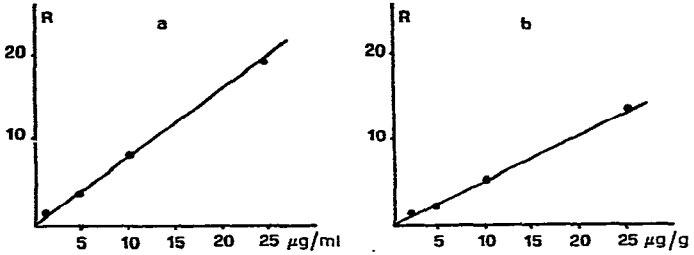

Fig. 6. Internal calibration graphs for sulpyrid (FID): (a) water, urine and plasma extracts; (b) liver and brain extracts. Only one graph is shown in each instance because the recoveries were identical.

liver homogenate and brain homogenate, and then processing the samples as described above.

The linearity of the method ranges from concentrations of 50 ng/ml or ng/g to 1 µg/ml or µg/g when MF detection is used (the minimum detectable amount for injection being 200 pg), and from 1 µg/ml or µg/g to 25 µg/ml or µg/g when the FID is employed (the minimum detectable amount for injection being 20 ng).

The high sensitivity and specificity of the method described appear to be satisfactory for measurements during chronic therapy with sulpyrid, and for pharmacokinetic studies in both humans and animals.

REFERENCES

1 L. Justin-Besançon, M. Thominet, C. Laville and J. Margarit, *C.R. Acad. Sci., Paris*, 265 (1967) 1253.
2 J. C. Benoit, G. Delagrange, A. Richou, G. Souchon, I. Sow and P. Sivadon, *Sem. Hôp.*, 45 (1969) 958.
3 P. Borenstein, C. Champion, P. Cujo, F. Gekière, C. Olivenstein and P. Kramarz, *Sem. Hôp.*, 45 (1969) 1301.
4 C. Laville and J. Margarit, *Pathol. Biol.*, 17 (1969) 71.
5 J. Collard, *Thérapeutique*, 46 (1970) 503.
6 E. Marmo, A. Caputi, A. Imperatore and L. Vuolo, *Gazz. Int. Med. Chir.*, 76 (1971) 758.
7 L. Valzelli and S. Bernasconi, *Psychopharmacologia*, 26 (1972) 255.
8 A. Benakis, M.-A. Pongis, F. Sugnaux, B. Glasson, H. Jirounek, M. Redard and J. Vitus, *Eur. J. Drug Metab. Pharmacokinet.*, 1 (1976) 51.
9 E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, 58 (1969) 370.
10 C.-G. Hammar, G. Pettersson and P. T. Carpenter, in A. Frigerio and N. Castagnoli (Editors), *Advances in Mass Spectrometry in Biochemistry and Medicine*, Vol. I, Spectrum, New York, 1976 p. 421.