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Note

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Gas–liquid chromatographic analysis of fluphenazine and fluphenazine sulfoxide in the urine of chronic schizophrenic patients

A number of methods for the identification and analysis of phenothiazines from biological fluids include thin-layer chromatography (TLC)[1], spectrophotofluorometry[2], and gas–liquid chromatography (GLC)[1,3]. Recently CIMBURA[4] has reviewed all the methods available for analysis of phenothiazine drugs, and has discussed the relative merits of each method. Although these methods are useful for detection and analysis in cases of drug abuse[5], the problem of monitoring the urinary excretion of patients maintained on therapeutic levels of these drugs requires methods of greater sensitivity and selectivity. Other investigators have claimed that GLC is the most satisfactory method to date for separating and identifying phenothiazines and their metabolites, but use of this method has been limited to those compounds of lower molecular weight[1,4].

This report describes the GLC analysis of urinary fluphenazine (FPZ) and fluphenazine sulfoxide (FPZSO) as their trimethylsilyl ethers, which provides a new method for the determination of the excretion of these psychoactive drugs in schizophrenic patients.

Methods and materials

Reagents. Ammonium chloride buffer, pH 8.5 (200 ml of 0.2 *N* ammonium hydroxide was adjusted to pH 8.5 with 6 *N* hydrochloric acid). Silylating reagents: bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane and anhydrous pyridine were purchased from Pierce Chemical in hypo vials. Internal standard: 2.28 mg of cholesterol propionate (CHP) (Sigma) was dissolved in 10 ml of chloroform–methanol (90:10). Other reagents: nanograde chloroform and methanol were glass-distilled before use.

Preparation of phenothiazine standards. 200 mg of fluphenazine·2HCl (generous gift of Schering Corp.) were dissolved in 50 ml of water, and the pH was adjusted to 9.5 with 10 *N* NaOH. Free base fluphenazine was extracted with 200 ml of chloroform (three times) as suggested by TOMPSETT[2], and evaporated to dryness *in vacuo* at 40°. The crude residue was fractionated on a Unisil column (2.5 × 28.5 cm) by eluting with 20-ml mixtures of 100:0, 98:2, 95:5, 90:10 and 80:20 of chloroform–methanol. Most of the mass was located in fractions of chloroform–methanol (95:5) as determined by TLC assay in the chloroform–ethanol–ammonium hydroxide (80:10:1) system described by DREYFUSS *et al.*[6]. After removal of the solvent, pure fluphenazine (183.7 mg) was obtained and was judged pure by TLC, spectrophotofluorometry[6], ultraviolet (UV) spectroscopy[1] and GLC.

Fluphenazine sulfoxide was prepared by oxidation of 100 mg of FPZ·2HCl according to a modification of the method of RAGLAND AND KINROSS-WRIGHT[7], in which the oxidation mixture is adjusted to pH 9.5 with 10 *N* NaOH and extracted three times with chloroform–methanol (80:20). After removal of the organic solvent,

the residue was purified on 0.50-mm Silica Gel G plates which were developed in chloroform–methanol–ammonium hydroxide (80:20:1). The major sulfoxide band was located by UV light (320–380 nm), and was eluted with methanol. The purified FPZSO (13.9 mg) was characterized by TLC[6], fluorescence maximum[7] and GLC.

Resin. Amberlite XAD-2, 20–50 mesh (Mallinckrodt) was washed before use as described by WEISSMAN *et al.*[8].

Gas chromatograph. A Hewlett-Packard Model 5750 gas chromatograph with flame ionization detector (FID) was used. Two 6-ft., glass, coiled columns (Supelco, Inc.) packed with 2% SE-30 coated by the filtration method[9] on resilanized Gas-Chrom Q (100–200 mesh) were run under the following conditions: column oven temperature, 225°; injection port temperature, 265°; FID temperature, 310°. Helium was used as the carrier gas at a flow-rate of approximately 70 ml/min. Peak areas were measured by triangulation, and the amount of each compound present was calculated using the area of the internal standard and relative detector responses (RDR).

Procedure. A sample of urine collected for 12 h prior to analysis is filtered, and, if necessary, the volume is adjusted to 500 ml by addition of water. A 200-ml aliquot of this sample is passed through a 1 × 25 cm column of Amberlite XAD-2 resin previously washed as described by WEISSMAN *et al.*[8]. After the sample of urine has drained into the resin bed, the column is washed with 100 ml of ammonium chloride buffer, pH 8.5 (see *Reagents*), and 50 ml of deionized water. Excess water is removed from the resin by gently aspirating the column with a water aspirator, and the phenothiazines and other urinary pigments are eluted with 200 ml of methanol[10]. The solvent is removed at 40° under reduced pressure, and the residue is redissolved in 20 ml of chloroform–methanol (3:1) followed by 25 ml of chloroform. Each solution is mixed for 30 sec on a Vortex-Geni with 2.0 ml of 0.2 *N* ammonium hydroxide which removes most of the urinary pigments. Evaporation of the combined organic layers at 40° under reduced pressure gives a crude residue which is transferred to a 5-ml centrifuge tube with methanol. CHP (22.8 μg) is added as the internal standard, and the solution is evaporated to dryness under nitrogen at 40°. Desiccation *in vacuo* at 40° for 15–30 min yields a crude residue which is suitable for silylation.

Preparation of trimethylsilyl ethers of FPZ and FPZSO. A mixture of 80 μl of BSTFA and 20 μl of anhydrous pyridine is added to the residue of the urinary extract containing CHP, and silylation is achieved by heating the stoppered tube at 40° for 10–15 min prior to injection of 1.0–1.5 μl of the mixture into the gas chromatograph for quantitative analysis. The detector response is calibrated separately for each phenothiazine relative to CHP. Calibration standards are prepared by silylating 8.25 μg of FPZ and 20.6 μg of FPZSO individually with 11.4 μg of CHP using 10 μl of pyridine and 40 μl of BSTFA. After heating as described above, approximately 1.0 μl is injected for GLC analysis.

Results and discussion

Typical retention times and detector responses of FPZ and FPZSO are summarized in Table I and show clearly that the FID is more sensitive to FPZ than to its sulfoxide. These results indicate the importance of determining the RDR for each compound studied.

The GLC analysis of a urinary extract from a patient receiving 20 mg of

TABLE I

GLC PROPERTIES OF FLUPHENAZINE AND FLUPHENAZINE SULFOXIDE

Conditions for GLC analysis are given in the text.

Compound	*Absolute retention time (min)*	*Relative retention time*	*Relative detector response*
Fluphenazine	24.7	0.60	0.61
Cholesterol propionate	40.9	1.00	1.00
Fluphenazine sulfoxide	54.1	1.35	0.41

fluphenazine·2HCl tablets daily is shown in Fig. 1. Although FPZ was present in this chromatogram (this region is indicated by an arrow), quantitative estimations of this compound were not possible under these conditions due to the presence of endogenous urinary components. As has been typical of these studies, FPZSO was well separated and measurable. The area of this peak corresponds to 441 ng and a total excretion of 29 μg per 200 ml of urine.

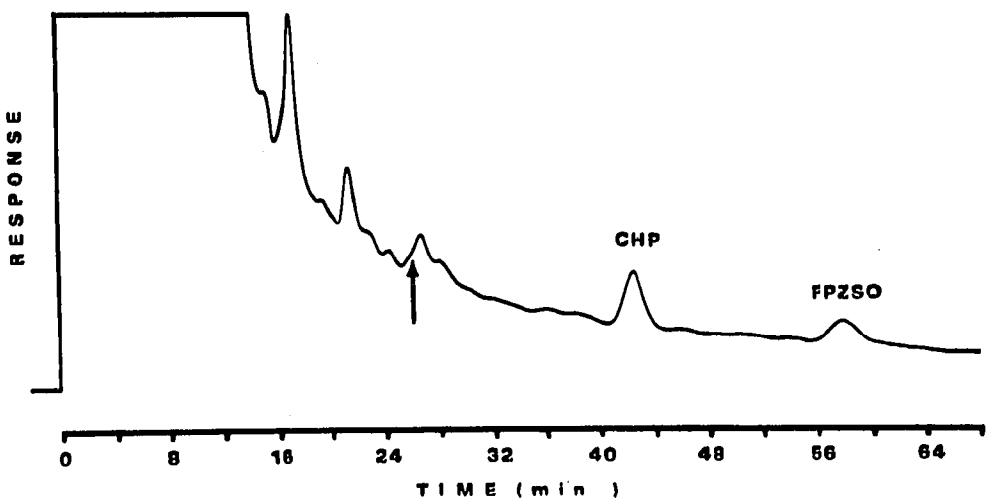

Fig. 1. Urine extract of a patient receiving 20 mg of fluphenazine daily. Amount injected, 1.5 μl; attenuation, 160. FPZ retention time is indicated by the arrow.

Preliminary results in an ongoing study (5 patients) indicate that it is more typical to find FPZSO only in urinary extracts. These data are similar to those reported by VAN KEMPEN[11] who showed that perphenazine sulfoxide was the major metabolite of the parent phenothiazine compound. The absence of FPZ in the majority of these extracts may be due to complete metabolism of the phenothiazine to the sulfoxide and/or other unidentified metabolites[12], which were unobtainable as standards. Clinical studies are now in progress and will be reported at a later date.

Addition of FPZ and FPZSO at physiological concentrations of approximately 0.1 μg/ml to duplicate 200-ml aliquots of urine from a patient gave recoveries of 93 and 102% for FPZSO and 132 and 156% for FPZ. The variable high values obtained for FPZ are most probably due to the endogenous peaks found in the urine. In a

separate recovery experiment, 20 μg of FPZ and FPZSO were added to 10 ml of control urine and 156% and 100% recoveries were obtained for FPZ and FPZSO, respectively. Better resolution of the FPZ from the endogenous contaminants can be obtained presumably by lowering the oven temperature, but this was not done since FPZSO was of major interest in this investigation. Thus the method is quantitatively useful for FPZSO and can qualitatively detect the presence of FPZ under the conditions used.

Preparation of the trimethylsilyl ethers of fluphenazine and its sulfoxide offers a new clinical method for analyzing these and other related phenothiazines and metabolites which have not been amenable to GLC analysis.

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Missouri Institute of Psychiatry,
University of Missouri School of Medicine,
5400 Arsenal Street, St. Louis, Mo. 63139 (U.S.A.)

M. I. KELSEY
A. KESKINER
E. A. MOSCATELLI

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