Assay Procedures for Thioridazine, Trifluoperazine, and Their Sulfoxides and Determination of Urinary Excretion of These Compounds in Mental Patients

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Abstract □ Assay procedures for thioridazine, trifluoperazine, and their sulfoxides are described which are rapid, simple, and relatively specific (about 0.5 μg of drug or sulfoxide/ml). Chlorpromazine interferes with the determination of these drugs whereas imipramine, desipramine, biperidin, and haloperidol do not. The urinary concentrations of these four compounds were determined in 24-hr samples and timed-interval collections obtained from patients with schizophrenic reactions. Thioridazine and trifluoperazine seem to be extensively metabolized, and less than 1% of the unchanged drugs and 2–6% of the corresponding sulfoxides were observed in 24-hr urine samples. The ratio between the parent drug and its respective sulfoxide was fairly constant. Urinary excretions of thioridazine and its sulfoxide were dose independent, whereas those of trifluoperazine and its sulfoxide showed a strong dose dependency. Timed-interval collections during an 8-hr period showed rather consistent excretions of unchanged trifluoperazine and thioridazine but wide intra- and interpatient variations in the sulfoxide excretions.

Keyphrases □ Thioridazine, trifluoperazine, and their sulfoxides —spectrophotofluorometric analysis in patient urine, interference of chlorpromazine, imipramine, desipramine, biperidin, and haloperidol considered □ Trifluoperazine, thioridazine, and their sulfoxides—spectrophotofluorometric analysis in patient urine, interference of chlorpromazine, imipramine, desipramine, biperidin, and haloperidol considered □ Spectrophotofluorometry—analysis, thioridazine, trifluoperazine, and their sulfoxides in patient urine

Phenothiazines such as chlorpromazine, thioridazine (I), and trifluoperazine (III) find wide clinical use, but most pharmacological studies on the fate of phenothiazines in humans have been limited to chlorpromazine (1–3). A possible reason is the lack of rapid and simple, yet relatively sensitive and specific, methods for measuring trifluoperazine, thioridazine, and some of their metabolites. Therefore, assay procedures were developed for trifluoperazine, thioridazine, and their sulfoxides, and the 24-hr and timed-interval urinary excretion of these drugs and metabolites from mental patients was determined.

EXPERIMENTAL

Materials—Standards of chlorpromazine[1], chlorpromazine sulfoxide[1], trifluoperazine[1], and thioridazine[2] and its sulfoxide[2] were received from commercial sources. Trifluoperazine sulfoxide was synthesized according to the method of Huang and Bhansali (4) and found to be identical to trifluoperazine sulfoxide obtained from these investigators. All reagents used were of the highest purity commercially available.

Patients—Urinary excretion studies were performed with male schizophrenic patients who had been hospitalized for at least 1 year at the Veteran's Administration Hospital in Coatesville, Pa. All urines were collected, stored at 4°, and frozen after the collection. Assays were performed within 4 weeks.

[1] Smith Kline and French.

[2] Sandoz Pharmaceuticals.

Table I—Fluorescence of Thioridazine (I), Thioridazine Sulfoxide (II), Trifluoperazine (III), and Trifluoperazine Sulfoxide (IV) as a Function of Concentration of the Compound during the Fluorometric Determinations[a]

Amount Added, μg	Fluorescence, Relative Units	
I and II	I	II
0	10 ± 5	15 ± 5
0.5	35 ± 5	40 ± 5
1.0	60 ± 10	80 ± 15
10.0	600 ± 100	750 ± 110
50.0	3200 ± 450	4000 ± 590
III and IV	III	IV
0	30 ± 10	10 ± 5
0.1	45 ± 15	20 ± 5
0.5	90 ± 15	60 ± 10
1.0	120 ± 25	105 ± 20
10.0	1000 ± 75	950 ± 100
50.0	5500 ± 600	5000 ± 450

[a] Values represent the mean ± standard deviations of at least three determinations.

Determination of Chlorpromazine and Its Sulfoxide—Chlorpromazine and its sulfoxide were determined according to the method of Salzman and Brodie (5), and creatinine values were obtained by routine clinical laboratory procedures.

Determination of Thioridazine (I) and Its Sulfoxide (II)—A 10-ml sample of urine was adjusted to pH 12 by the addition of 10 *N* NaOH and extracted with 25 ml of heptane containing 1.5% isoamyl alcohol. After shaking for 5 min and centrifugation for 5 min, 22.5 ml of the heptane phase was transferred to a second tube containing 2.0 ml of 1 *M* acetate buffer (pH 5.6), shaken, and centrifuged. The buffer contained II. Twenty-one milliliters of the heptane phase was washed with 2.0 ml of the same acetate buffer. After shaking and centrifugation, 20 ml of heptane was extracted with 2.0 ml of 0.1 *N* HCl; the acid fraction contained I. For quantitation of both compounds, 0.15 ml of 10 *N* H_2SO_4 was added to 1.5 ml of the first buffer and 1.5 ml of the acid fraction was combined with 0.02 ml of 10 *N* H_2SO_4. Both samples received 0.1 ml of 0.1% potassium permanganate and were then mixed and allowed to stand for 5 min at room temperature. The reactions were stopped by the addition of 0.1 ml of 0.6% hydrogen peroxide. The samples were read within 30 min on a spectrophotofluorometer[3] using excitation and emission wavelengths of 350 and 440 nm, respectively. Urine from individuals who did not receive drug treatment served as "blank" specimens; after addition of a specified amount of thioridazine or thioridazine sulfoxide, they served as "internal" standards.

The recovery by this method was approximately 80% (70–85%) for both compounds, and standard curves of extracted thioridazine and thioridazine sulfoxide were linear from 0.5 to 50 μg (Table I).

Separation and specificity of the methods were checked as follows. Addition of thioridazine (20 μg) and thioridazine sulfoxide (20 μg) to control urine resulted in complete separation of both compounds by the extraction method described. Fluorescent spectra of extracts from control urines with added thioridazine or thioridazine sulfoxide were identical to those seen with the corresponding fractions from urines of patients receiving thioridazine.

[3] Aminco-Bowman.

Table II—Concentrations of Chlorpromazine (V), Thioridazine (I), Trifluoperazine (III), and Their Sulfoxides (II and IV) in 24-hr Urine Samples Obtained from Schizophrenic Patients

Compound	Patient	Dose, mg	Volume, ml[a]	Drug		Sulfoxide		Ratio[c]
				μg/24 hr	%[b]	μg/24 hr	%[b]	
V	B	100	1520	486	0.4	2,736	2.7	5.6
	M	500	1310	4323	0.8	12,838	2.6	3.0
	H	600	3220	1030	0.2	3,542	0.6	3.4
	D	800	830	2822	0.4	7,636	1.0	2.7
I	T	100	1305	76	0.1	1,005	1.0	13.0
	F	100	1330	1205	1.2	12,600	12.6	10.5
	R	200	1110	1232	0.6	9,350	4.7	7.6
	S	400	1010	1120	0.2	9,560	2.4	8.6
	M	400	1465	1465	0.4	9,950	2.5	6.8
	W	600	470	1280	0.2	1,805	0.3	1.4
	B	600	1550	1220	0.2	10,200	1.7	8.4
	S	600	1060	1520	0.3	15,700	2.6	11.2
III	H	5	825	12[d]	0.3	84[d]	1.7	6.8
	R	5	1670	16	0.3	215	4.3	12.9
	H	10	1240	34	0.4	127	1.3	3.6
	D	10	3980	83	0.8	374	3.7	4.5
	J	15	2375	135	1.0	505	3.4	3.8
	F	20	780	100	0.5	660	3.3	6.6
	B	30	1045	196	0.6	1,870	6.2	9.5
	M	40	1025	205	0.5	1,670	4.2	8.2
	L	50	4000	300	0.6	1,970	3.9	6.6

[a] Urine volume of 24-hr collection. [b] Percent of total dose. [c] Ratio of sulfoxide to unchanged drug. [d] Excretion of III and IV was found to be significantly dose dependent using a Student t test ($p < 0.001$).

Urine extracts from patients receiving thioridazine were subjected to TLC on 250 μm silica gel G in two solvent systems [acetic acid–ethanol–water (30:50:20) or methanol–25% ammonia (95:5)]. Methods of visualization were fluorescence under 253- and 320-nm UV light and color reactions with sprays of iodoplatinate, 2% ferric chloride, or 50% sulfuric acid in ethanol (4:1) (2, 6). The thioridazine fraction contained only one spot corresponding to standard thioridazine. The thioridazine sulfoxide fraction contained three spots; the major spot corresponded to standard thioridazine sulfoxide whereas the two minor spots fluoresced at different wavelengths and did not react with the sprays. Thus, it can be concluded that the thioridazine and thioridazine sulfoxide fractions were essentially free of interfering compounds. Nevertheless, the metabolism of phenothiazines is extensive; it is possible that small quantities of minor metabolites might still be present, but they should not significantly interfere with the determination of thioridazine or thioridazine sulfoxide.

The addition of various drugs (amounts equal to thioridazine) frequently used in combination with thioridazine medication showed that chlorpromazine and trifluoperazine interfered with the determination of thioridazine and thioridazine sulfoxide, whereas imipramine, desipramine, biperidin, and haloperidol did not. Thioridazine and thioridazine sulfoxide were stable when added to normal urine, left at room temperature for up to 24 hr, frozen, and stored for at least 4 weeks.

Determination of Trifluoperazine (III) and Its Sulfoxide (IV)—A 10-ml sample of urine was adjusted with 10 N NaOH to pH 12 and extracted with 25 ml of heptane containing 1.5% isoamyl alcohol. The mixture was shaken for 5 min and then centrifuged for 5 min. Twenty-two milliliters of the heptane was transferred to a tube containing 1.5 ml of 1 M acetate buffer, pH 5.6. After shaking and centrifuging, the buffer phase contained the trifluoperazine sulfoxide and was subjected to fluorescence analysis as described later. Twenty milliliters of the remaining heptane was extracted with 1 ml of 0.1 N HCl. After shaking and centrifuging, 0.8 ml of the hydrochloric acid phase containing trifluoperazine was combined with 0.6 ml of 1 M sodium acetate and 0.05 ml of 0.1% potassium permanganate at room temperature. After mixing, the oxidation was stopped after 30 sec with 0.05 ml of 0.6% hydrogen peroxide. Both samples were read within 30 min on a spectrophotofluorometer[3] using excitation and emission wavelengths of 350 and 405 nm, respectively. Again, urines from drug-free individuals served as blanks or, after addition of trifluoperazine or trifluoperazine sulfoxide, as internal standards. The recovery of the method is approximately 75% (68–80%), and standard curves of extracted drug and its sulfoxide were linear from 0.5 to 50 μg (Table I).

Separation and specificity of the assay procedure were checked as follows. Addition of trifluoperazine (10 μg) and its sulfoxide (10 μg) to normal urine was quantitatively separated by the extraction procedure. Spectral scans of both fractions obtained from urines of patients receiving trifluoperazine were identical to those obtained from blank urines to which either trifluoperazine or trifluoperazine sulfoxide had been added. TLC of the trifluoperazine fraction obtained from the urine of patients receiving the drug and standard trifluoperazine on 250-μm silica gel G plates [acetic acid–ethanol–water (30:50:20) or methanol–25% ammonia (95:5)] produced identical results. The trifluoperazine sulfoxide fraction showed three fluorescent spots, one major spot corresponding to trifluoperazine sulfoxide and two very faint spots which fluoresced at the same wavelength (less than 10% of the total fluorescence after elution from the plate) but did not respond to iodoplatinate spray, 2% ferric chloride, or 50% sulfuric acid in ethanol

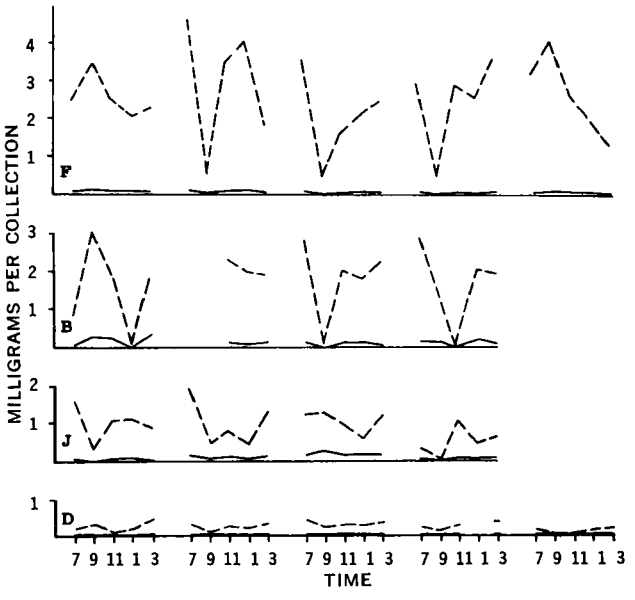

Figure 1—*Timed-interval urinary excretion of thioridazine (—) and its sulfoxide (- - -) in patients taking 100 mg (D), 600 mg (J and B), and 800 mg (F) of thioridazine daily. The abscissa indicates the time of day collection periods ended.*

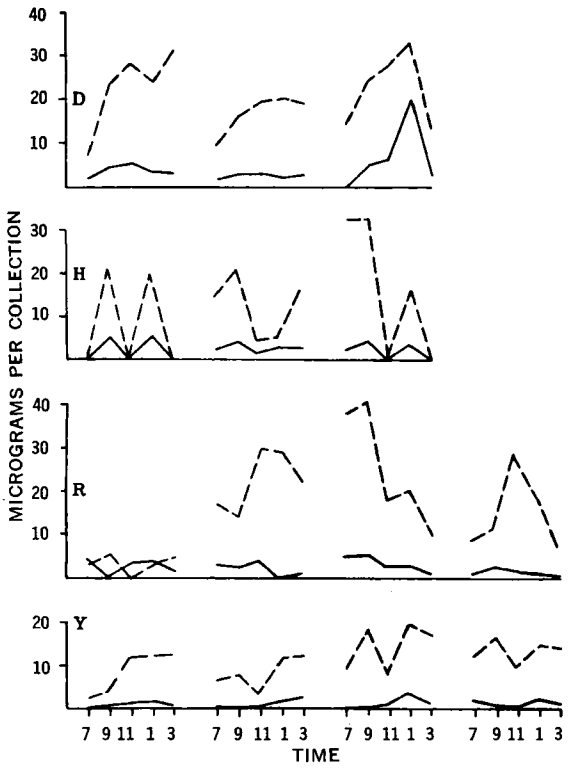

Figure 2—*Timed-interval urinary excretion of trifluoperazine (—) and its sulfoxide (- - -) in patients taking 5 mg (Y and R) and 10 mg (H and D) of trifluoperazine daily. The abscissa indicates the time of day collection periods ended.*

(4:1) (8). Since the concentrations of trifluoperazine and its sulfoxide had been measured in rat plasma and brain with a sensitive and specific radioisotope procedure (9), these experiments were repeated using the extraction and determination procedure. Results obtained for both plasma and brain levels of trifluoperazine and trifluoperazine sulfoxide were in excellent agreement with reported values. Thus, the trifluoperazine fraction seems to be free of interfering compounds, whereas the trifluoperazine sulfoxide fraction might contain some interfering metabolites which, however, do not account for more than 10%. Again, trifluoperazine is probably extensively metabolized in the body, and the presence of minor amounts of other metabolites in both fractions is likely.

The addition of equal amounts of chlorpromazine and thioridazine interfered strongly with the determination of trifluoperazine, whereas imipramine, desipramine, biperidin, and haloperidol did not. Again, trifluoperazine and its sulfoxide remained unchanged when added to normal urine, kept at room temperature for 24 hr, frozen, and stored for at least 4 weeks.

RESULTS AND DISCUSSION

Analytical Procedures—Methods based on published analytical procedures (5, 7, 8) were developed for the determination of thioridazine, trifluoperazine, and their sulfoxides. The objective was to find relatively specific, rapid, and easy methods, so that they could be performed in the average clinical laboratory. The developed methods do fulfill these criteria and are indeed quite specific for the particular drug and its sulfoxide; impurities resulting from the extensive metabolism of phenothiazines are either absent or present in negligible concentrations, except in the case of trifluoperazine sulfoxide fraction which contained an impurity accounting for 5–10% of the reading. The procedures are easy and rapid, and a laboratory technician can readily determine 20 samples in 4 hr. Preliminary results indicate that the assay procedures can also be used to determine the drugs and their sulfoxides in plasma or biological tissues.

Twenty-Four Hour Urine Concentrations of Thioridazine, Trifluoperazine, and Their Sulfoxides—The patients studied were males who had been taking either thioridazine or trifluoperazine for at least 4 months. For comparison, a few patients taking chlorpromazine were also included. Collections were made under the supervision of the particular ward physician, and several urine samples were assayed for creatinine to ensure proper 24-hr specimens; values obtained for creatinine were as expected for 24-hr specimens.

Table II shows the results of the 24-hr collection. As reported previously (2), unchanged chlorpromazine was found only in small, dose-unrelated quantities in the urine and the sulfoxide accounted for 1–3% of the total dose. The ratio of both compounds in this study was fairly constant, ranging between 2.7 and 5.6.

Thioridazine also was metabolized extensively, and a similar small, dose-unrelated quantity was found in the urine. The sulfoxide accounted for approximately 1–5% of the dose, with one patient excreting 12%. The ratio of the sulfoxide and thioridazine was fairly constant and, with one exception, ranged between 7 and 13.

Unchanged trifluoperazine accounted for less than 1% of the dose, indicating extensive metabolism of the drug. The sulfoxide was 1–6% of the total dose, and the ratio of sulfoxide to drug was between 4 and 13. In contrast to the other two drugs, the excretion of this drug and its sulfoxide was strongly dose dependent.

Timed-Interval Urine Concentrations of Thioridazine, Trifluoperazine, and Their Sulfoxides—The patients studied were males who had been taking thioridazine or trifluoperazine for at least 4 months. Urine was collected in the morning (7:00 am), and then the medication was given by mouth and urine was collected until 9:00 am and from 9:00 to 11:00 am, 11:00 am to 1:00 pm, and 1:00 to 3:00 pm. Another dose of the medication was given in the evening.

The timed-interval urinary excretion of thioridazine of four patients proved to be rather consistent and constant (Fig. 1). The excretion of its sulfoxide varied among patients and within the same patient on different days; it was rather uniform in Patient D but varied widely in each of the other three patients. The ratios between sulfoxide and unchanged drug were markedly higher than those found for 24-hr samples. In the 8-hr collection period, the patients excreted about 30% of I and about 75% of II of the expected daily excretion of the morning dose. No correlation between urine volume and drug excretion was noted.

The timed-interval urinary excretion of unchanged trifluoperazine was rather constant among the four patients studied and on different days within one particular patient, whereas the sulfoxide excretion showed wide inter- and intrapatient variations (Fig. 2). During the collection periods, the patients excreted 30–50% of the expected 24-hr amounts of both the parent compound and its sulfoxide. No correlation between drug excretion and urine volume was noted.

The methods and the urinary excretion data reported, particularly for trifluoperazine excretion which is dose dependent, should be useful in screening patient populations as to the proper intake of the prescribed doses and in studying possible correlations between the fate of thioridazine or trifluoperazine and the clinical response of the patient.

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GLC Determination of Medroxyprogesterone Acetate in Plasma

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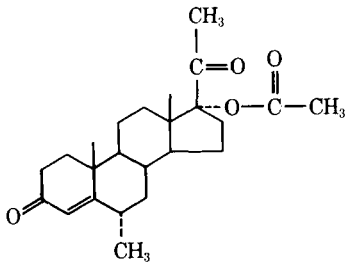
Abstract □ To study the absorption, metabolism, and excretion of medroxyprogesterone acetate in animals and man, a method was developed for measurement of the intact drug in plasma based on: *(a)* cyclohexane extraction of the specimen, *(b)* formation of the 3-enol heptafluorobutyrate ester, and *(c)* quantification *via* GLC using an electron-capture detector. The assay is quantitative above 1 ng medroxyprogesterone acetate/ml of plasma, and overall precision is approximately ±10% *(SD)* in the range of 5–20 ng/ml. The procedure was successfully applied to absorption studies in dogs after intramuscular drug administration. Plasma drug concentrations, as measured by radioimmunoassay, were five to 10 times greater as compared to the GLC assay, indicating that drug-related materials in addition to intact medroxyprogesterone acetate were in peripheral circulation.

Keyphrases □ Medroxyprogesterone acetate—GLC analysis in plasma, compared to radioimmunoassay technique, absorption in dogs after intramuscular administration □ GLC—analysis, medroxyprogesterone acetate in plasma, compared to radioimmunoassay technique

Medroxyprogesterone acetate[1] (17α-acetoxy-6α-methylprogesterone, I), a synthetic progestogen (1, 2), is an effective agent for the control of fertility (3) and for the treatment of malignancies. Investigations in animals (4, 5) and humans (6–8) indicated that, after subcutaneous or intramuscular drug administration, I and drug-related materials were slowly absorbed from the injection site, resulting in prolonged activity.

To study the absorption, metabolism, and excretion of I in animals and man as well as to aid in determining the specificity of the radioimmunoassay (7) for measurement of I, a simple, sensitive, and specific GLC method of analysis was developed for the intact drug in plasma.



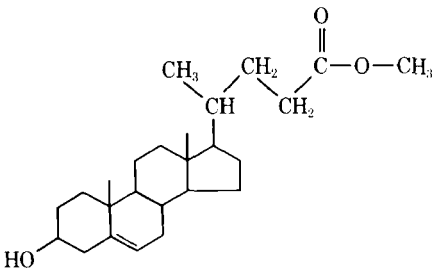
I

EXPERIMENTAL

Reagents and Materials—Compound I and the methyl ester of 3β-hydroxy-5-cholenic acid[2] (II), used as internal standard, were synthesized. Acetone, benzene, and cyclohexane, distilled in glass, were used as supplied[3]. Spectral grade acetonitrile[4] and heptafluorobutyric anhydride (1-ml ampuls)[5] were used without further purification. Aqueous stock solutions of I (10 μg/ml) were prepared by dissolving 1 mg of drug in 1 ml of acetone. The solution was diluted to a final volume of 100 ml with distilled water. Aliquots were diluted with distilled water to final concentrations of 12.5, 25, 50, and 100 ng/ml. The stock solution of II (10 μg/ml) was prepared in 50% (v/v) acetonitrile in acetone. All stock solutions were stored in glass containers. Silicone gum rubber (OV-17) on 80–100-mesh Gas Chrom Q (10% w/w) was used as supplied[6].

Instrumentation—A two-speed reciprocating shaker[7] was used for shaking the samples in the horizontal position. A mixer[8] was used to aid in preparing the heptafluorobutyrate esters. GLC measurements were made with a gas chromatograph[9] equipped with a hydrogen flame-ionization detector, a nickel-63 electron-capture detector, and a −0.2 to 1.0-mv recorder[10]. All cylinders of gases for chromatography (*i.e.*, helium, hydrogen, oxygen, and 10% methane in argon) were fitted with filters containing molecular sieve 4A.

Chromatographic Conditions—All analytical chromatography was conducted on U-shaped glass columns [0.61-m (2-ft) × 3-mm i.d.] of 10% (w/w) OV-17 on 80–100-mesh Gas Chrom Q. All newly



II

[1] Provera, The Upjohn Co.

[2] Methyl ester of 3β-hydroxy-5-cholenic acid, supplied to The Upjohn Co. by Dr. L. C. King, Evanston, Ill.

[3] Burdick and Jackson Labs., Muskegon, Mich.

[4] Matheson, Coleman and Bell, Milwaukee, Wis.

[5] Pierce Chemical Co., Rockford, Ill.

[6] Applied Science Labs., State College, Pa.

[7] Eberbach and Sons, Ann Arbor, Mich.

[8] Vortex model K-500, Scientific Industries, Queen's Village, N.Y.

[9] F & M model 400, Hewlett-Packard Co., Avondale, Pa.

[10] Honeywell Electronik 15, Honeywell, Philadelphia, Pa.