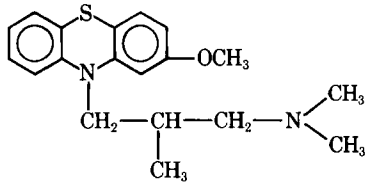
Identification and Quantitative Determination of Phenothiazine Drugs in Urine Samples of Psychiatric Patients

A. P. De LEENHEER

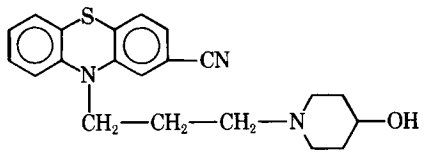
Abstract □ A GLC method for the specific identification and quantitation of phenothiazine drugs excreted in urine at the microgram level is described. The phenothiazine drugs and less polar metabolites are isolated by two-phase extraction with ether as an organic extractant. Quantitative determination at the microgram level is done on an acetylated fraction of the alcoholic extract by GLC using a suitable internal standard. The compounds are also identified by examining an aliquot of the final alcoholic extract by TLC for supporting evidence of the GLC method's specificity.

Keyphrases □ Phenothiazines—identification and determination in urine of psychiatric patients, GLC □ Antipsychotic agents—GLC identification and determination of phenothiazine drugs in urine samples from psychiatric patients □ GLC—identification and determination, phenothiazine drugs in urine, psychiatric patients

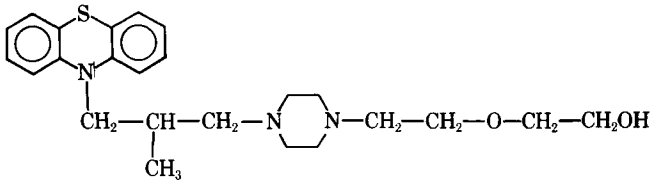
Medicinal chemistry has led to the development of phenothiazine drugs containing a piperidyl or piperazinyl group in their structure. Because these more potent drugs are administered in lower amounts, the analyst faces the problem of their specific identification and quantitation at the microgram level. This report describes a GLC method for detecting and assaying propericiazine[1], dixyrazine[2], perazine[3], trifluoperazine[4], prothipendyl[5], and analogs in urine samples taken from psychiatric patients under controlled drug therapy.



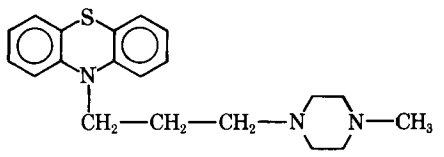
levomepromazine



propericiazine



dixyrazine



perazine

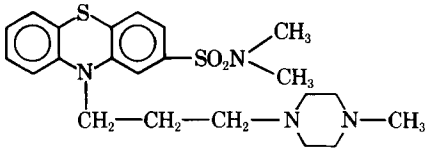
EXPERIMENTAL

Collection of Urine Samples—Twenty-four-hour urine outputs were collected separately from seven male patients under drug therapy (Table I) over 4 successive days following drug dosage. The samples were kept in the refrigerator at 4° to the moment extraction was started.

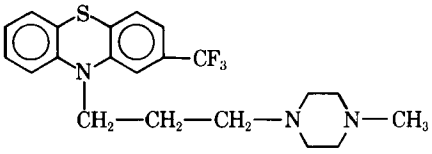
Extraction Procedure—A 200-ml volume of urine sample was acidified with 2 *N* HCl and extracted under a nitrogen atmosphere with 400- and 200-ml aliquots of ether (peroxide free, freshly distilled over hydroquinone, reagent grade) which was discarded.

The acidic urine layer was made alkaline with 10 *N* NaOH, saturated with nitrogen, and extracted twice with 400 ml of ether. The collected ether phases were washed with 5-ml fractions of 0.001 *N* NaOH until the aqueous layer remained colorless. The ether extract was evaporated at 40° in a rotory evaporator to a 100-ml final volume. The 100-ml ether phase was washed again with 5-ml fractions of 0.001 *N* NaOH, and the ether phase was then extracted twice with 10 ml of 0.1 *N* HCl.

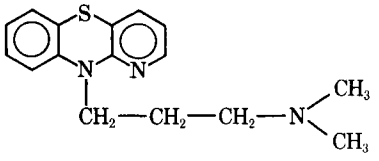
The acidic extract was made basic with 1 ml of 10 *N* NaOH



thioproperazine



trifluoperazine



prothipendyl

[1] Also known as periciazine, the chemical name is cyano-2[(hydroxy-4''-piperidino)-3'-propyl]-10-phenothiazine.

[2] The chemical name is [(B-hydroxyethoxyethylpiperazinyl)-3'-methyl-2'-propyl]-10-phenothiazine.

[3] The chemical name is *N*-{[methyl-4'-piperazinyl-(1')]-3-propyl}-phenothiazine.

[4] The chemical name is trifluoromethyl-3{[methyl-4''-piperazinyl-(1'')]-3'-*N*-propyl}-10-phenothiazine.

[5] The chemical name is (dimethylamino-3-*N*-propyl)-10-aza-1-phenothiazine.

Table I—Psychiatric Patients Receiving Phenothiazine Drug Medication

Patient	Age, Years	Drug Treatment: Type	Drug Treatment: Daily Dose Administered Orally, mg	Time Medication Went on before First Urine Collection
1	31	Propericiazine	10	3 years
2	42	Dixyrazine	3 × 25	3 weeks
3	25	Perazine	5 × 100	2.25 years
4	30	Trifluoperazine	2 × 20	7 months
5	44	Prothipendyl	3 × 20	1.5 years
6	44	Propericiazine	20	3 years
		Carbamazepine	2 × 200	
		Diphenylhydantoin[b]	100	
		Phenobarbital[c]	200	
7	60	Dixyrazine (levomepromazine)	3 × 25 —[a]	8 months

[a] Uncontrolled self-medication. [b] Diphantoine. [c] Luminal.

Table II—GLC Conditions, Retention Times (R_t), and Calibration Data ($\bar{k}$) of Phenothiazine Drugs

Phenothiazine Drug	Internal Standard	Oven Temperature	R_t (Drug), min	R_t (Standard), min	$\bar{k}$	Linearity over Weight Ratio Range, $\Delta(w_x/w_s)$
Column System: 5% OV-1 on Diatoport S, 80–100 mesh						
Propericiazine	Dixyrazine	260°	12.7	19.6	1.94	0.25/1–1.00/1
Dixyrazine	Thioproperazine	260°	18.5	21.4	1.06	0.25/1–0.67/1
Perazine	Trifluoperazine	240°	7.7	5.8	0.69	1.00/1–4.00/1
		240°	6.0	4.5	0.67	1.00/1–4.00/1
Trifluoperazine	Perazine	230°	6.9	9.3	1.28	0.11/1–0.67/1
Trifluoperazine sulfoxide	Perazine	230°	15.6	9.3	0.91	0.11/1–0.67/1
Prothipendyl	Levomepromazine	230°	2.7	4.6	0.92	0.43/1–1.50/1
Column System: 2% FFAP on Diatoport S, 80–100 mesh						
Perazine	Trifluoperazine	240°	18.5	8.0	0.55	1.00/1–4.00/1
		240°	17.8	8.0	0.53	1.00/1–4.00/1
Trifluoperazine	Perazine	230°	13.6	30.0	1.60	0.11/1–0.67/1
Prothipendyl	Levomepromazine	230°	6.1	10.6	0.75	0.43/1–1.50/1

and twice extracted with 40 ml of ether. The combined ether layers were dried on sodium sulfate, and the ether was evaporated with a nitrogen stream in a water bath at 40°.

The residue obtained was dissolved in 10.0 ml of ethanol, and the alcoholic extract was kept in a deep freeze for further analysis.

GLC—A research gas chromatograph[6] equipped with two dif-

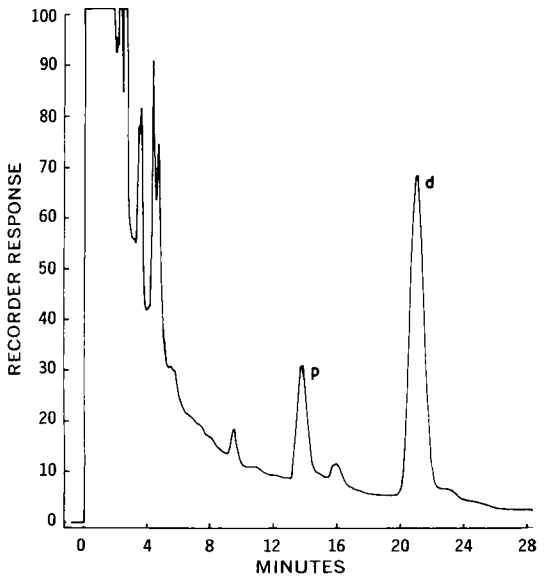


Figure 1—*Gas chromatogram of a urine extract from Patient 1. The column was 5% OV-1; quantitative determination (acetylation) of propericiazine (p) with internal standard dixyrazine (d).*

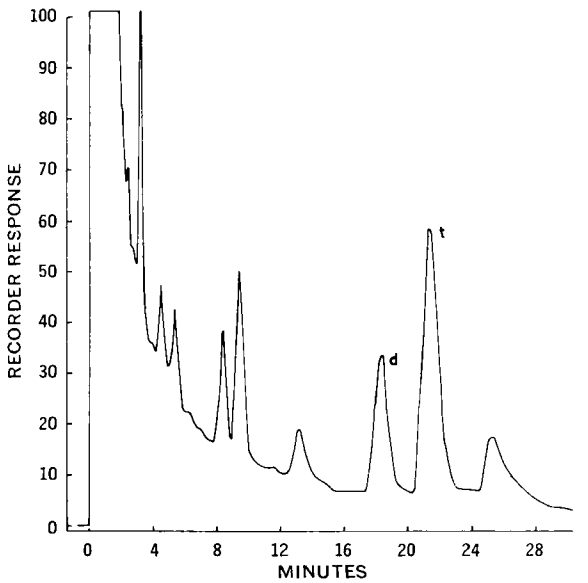


Figure 2—*Gas chromatogram of a urine extract from Patient 7. The column was 5% OV-1; quantitative determination (acetylation) of dixyrazine (d) with internal standard thioproperazine (t).*

[6] Hewlett-Packard 5750.

Table III—Recovery Data of Phenothiazine Drugs Added to Blank Urine Samples and Determined by Quantitative GLC[a]

Phenothiazine Drug	Amount Added to 200 ml of Blank Urine Sample, μg	Recovery of Drug							
		5% OV-1				2% FFAP			
		First Sample		Second Sample		First Sample		Second Sample	
		μg	%	μg	%	μg	%	μg	%
Propericiazine	400	344	86	352	88	—	—	—	—
Dixyrazine	400	376	94	376	94	—	—	—	—
Perazine	248	241	97	223	90	245	99	223	90
		233	94	226	91	241	97	236	95
Trifluoperazine	339	315	93	325	96	315	93	319	94
Prothipendyl	336	319	95	329	98	323	96	323	96

[a] Identical operating conditions as indicated in Table II.

Table IV—Quantitative Results of GLC Determination of Phenothiazine Drugs in Urine Samples Collected from Psychiatric Patients Receiving Drug Therapy

Patient	Phenothiazine Drug	Sample	$F(U)$[a], ml (ml)	I[b], μg	v[c], μl	f[d], μl	C_u, μg/100 ml Urine		Urinary Excretion[e], %
							5% OV-1	2% FFAP	
1	Propericiazine	1	2.0 (40)	20	40	0.9	4.2		1.01
		2	2.0 (40)	20	40	1.3	7.1		3.12
		3	2.0 (40)	20	40	1.4	7.5		1.80
		4	2.0 (40)	20	40	1.4	7.3		0.70
2	Dixyrazine	1	1.8 (36)	15	15	1.4	6.2		0.10
		2	1.8 (36)	15	15	1.4	4.7		0.12
		3	1.8 (36)	15	15	1.5	5.9		0.15
		4	1.8 (36)	15	15	1.4	3.5		0.08
3	Perazine	1 (a)	1.0 (20)	10	160	0.5	190		1.33
					40	0.9		216	1.51
		(b)	1.0 (20)	10	40	1.0	208		1.46
					40	1.0		211	1.48
		2 (a)	1.0 (20)	10	200	0.4	313		1.50
			1.0 (20)	10	40	0.6		307	1.47
		(b)	1.0 (20)	10	120	0.7	307		1.47
			1.0 (20)	10	40	0.9		306	1.47
		3 (a)	1.0 (20)	10	120	0.5	55.2		0.22
			1.0 (20)	10	20	0.6		50.2	0.20
		(b)	1.0 (20)	10	40	0.9	55.9		0.22
			1.0 (20)	10	40	1.4		50.0	0.20
		4 (a)	1.0 (20)	10	200	1.0	98.3		0.24
			1.0 (20)	10	20	1.0		104	0.25
		(b)	1.0 (20)	10	80	0.9	96.8		0.23
			1.0 (20)	10	40	1.0		107	0.26
4	Trifluoperazine (sulfoxide)	1	2.0 (40)	20	40	0.7	5.0 (6.8)		0.09 (0.15)
			2.0 (40)	20	20	1.9		5.4	0.10
		2	2.0 (39)	20	40	0.7	8.8 (18.3)		0.24 (0.57)
			2.0 (39)	20	20	1.4		8.1	0.22
		3	2.0 (40)	20	40	0.8	—(−)		
			2.0 (40)	20	20	1.4		—	
		4	2.0 (40)	20	40	0.8	16.3 (27.5)		0.45 (0.88)
			2.0 (40)	20	20	1.4		14.5	0.40
5	Prothipendyl	1	3.0 (60)	10	40	0.8	4.2		0.24
			3.0 (60)	10	20	0.6		4.1	0.24
		2	3.0 (60)	10	40	0.7	2.3		0.24
			3.0 (60)	10	20	0.8		1.7	0.18
		3	3.0 (50)	10	40	0.5	1.3		0.22
			3.0 (50)	10	20	0.9		0.9	0.15
		4	3.0 (60)	10	40	0.7	1.7		0.22
			3.0 (60)	10	20	0.8		1.4	0.18
6	Propericiazine	1	2.0 (40)	30	60	1.5	19.7		1.87
		2	2.0 (40)	30	60	1.5	19.8		1.58
		3	2.0 (40)	30	60	1.4	13.6		0.75
		4	2.0 (40)	30	60	1.5	4.9		0.37
7	Dixyrazine	1	1.7 (34)	20	20	1.3	24.0		0.51
		2	2.0 (40)	20	20	1.5	27.0		0.68
		3	1.8 (36)	20	20	1.4	18.9		0.07
		4	2.0 (40)	20	20	1.3	5.9		0.12

[a] F = aliquot of alcoholic extract in milliliters taken for evaporation; (U) = corresponding volume in milliliters of original urine sample. [b] I = amount of internal standard in micrograms added to fraction F of alcoholic extract for evaporation. [c] v = volume in microliters of ethyl acetate in which residue was dissolved. [d] f = fraction in microliters of end volume v injected on top of GLC column. [e] Urinary excretion or percent of administered dose recovered as unchanged drug.

Table V—TLC Results of Reference Substances and Extracts of Urine Samples Collected from Psychiatric Patients Receiving Drug Therapy[a]

Patient	Material Spotted (Sample and Reference Substance)	Number of Samples Analyzed	$F(U)$[b]	Rel. R_f[c]	(R_f)[d]	Detection[e]
1	Propericiazine			1.00	(0.41)	I, orange
	Propericiazine sulfoxide			0.46		I, orange
		1	2.0 (40)	0.46		I, orange
				0.90		I, orange
				1.22		I, orange
				1.48		I, orange
2	Dixyrazine			1.00	(0.44)	I, pink
	Dixyrazine sulfoxide			0.39		I, pink
		4	2.0 (40)	0.35		I, pink
				0.86		I, blue
				1.00		I, pink
3	Perazine dimalonate			1.00	(0.39)	I, pink
	Perazine sulfoxide			0.28		I, pink
		3	1.0 (20)	0.18		I, pink
				0.31		I, pink
				0.49		I, pink
				1.02		I, pink
				1.64		I, pink
4	Trifluoperazine dihydrochloride			1.00	(0.49)	I + III, brown
	Trifluoperazine sulfoxide			0.39		I + III, brown
		4	2.0 (40)	0.18		I + III, brown
				0.41		I + III, brown
				1.00		I + III, brown
				1.23		I + III, brown
5	Prothipendyl hydrochloride monohydrate			1.00	(0.50)	I + II, yellow-brown
	Prothipendyl sulfoxide			0.52		I + II, yellow
		1	2.0 (40)	—		
		2	2.0 (40)	—		
		3	2.0 (40)	—		
		4	2.0 (40)	—		
6	Propericiazine			1.00	(0.38)	I, pink
	Propericiazine sulfoxide			0.50		I, pink
		2	1.0 (20)	0.50		I, pink
				1.08		I, pink
				1.74		I, yellow
				2.02		I, yellow
7	Dixyrazine			1.00	(0.53)	I, pink
	Dixyrazine sulfoxide			0.38		I, pink
		1	2.0 (40)	0.09		I, pink
				0.38		I, pink
				0.98		I, pink
		2	2.0 (40)		(0.11)	I, blue
					(0.55)	I, blue

[a] Drug therapy as described in Table I. [b] F = aliquot of alcoholic extract in milliliters taken for evaporation and spotting on the plate; (U) = corresponding volume in milliliters of original urine sample. [c] Rel. R_f = R_f of compound/R_f of reference substance. [d] (R_f) = R_f of reference substance as measured on chromatogram. [e] I = ferri reagent; II = vanadium reagent; III = iodoplatinate reagent.

ferent columns for simultaneous single-column operation was used. The columns consisted of 5% OV-1 (methyl silicone polymer, apolar) and 2% FFAP (reaction product of Carbowax 20M and 2-nitroterephthalic acid, polar) coated on Diatoport S[7], 80–100 mesh (acid washed and silane treated), and packed in 1.80-m × 4-mm i.d. spiralized Pyrex columns.

Nitrogen was used as the carrier gas at 60–80 ml/min (bubble flowmeter), and a double flame-ionization detector was used as the detection system. The hydrogen flow rate was 50 ml/min; air was optimized for good sensitivity and stability.

Both columns were conditioned for 72 hr, at 280° for the 5% OV-1 and at 245° for the 2% FFAP column. Analyses were done isothermally with 20° higher temperature settings for the injector and detector blocks and at an electrometer setting range of ×1 and an attenuation of ×32.

Fixed volumes of alcoholic solutions of the compound to be determined and of internal standard were transferred with a 50-μl syringe[8] into conical, silylated (left in contact for 1 hr with 5% dichlorodimethylsilane in benzene, rinsed with benzene, washed three times with water, and dried in the air) centrifuge tubes of 15-ml capacity and thoroughly mixed. For each calibration series, four or five different solutions of equal weight ratio increments ($\Delta w_x/w_s$) were prepared. After the mixtures were evaporated in a water bath at 40° with the aid of a slow stream of nitrogen, acetylation was performed by dissolving residues in 0.2 ml of pyridine (refluxed and redistilled from potassium hydroxide) and 0.2 ml of acetic acid anhydride (refluxed and redistilled from calcium carbide), mixing thoroughly with a mixer[9], and leaving for a 3-hr reaction time in a phosphorus pentoxide desiccator. Reagents were evaporated at 40° with the aid of a nitrogen stream, and the residue was redissolved in a small volume of ethyl acetate. Volumes varying from 1 to 3 μl were injected with a 10-μl syringe[10] on top of the column(s).

An appropriate aliquot of the alcoholic extract of the urine sample was mixed with a known amount of internal standard and treated in a manner similar to the calibration solutions. Accord-

[7] Hewlett-Packard.
[8] Hamilton 705N.
[9] Vortex.
[10] Hamilton 701N.

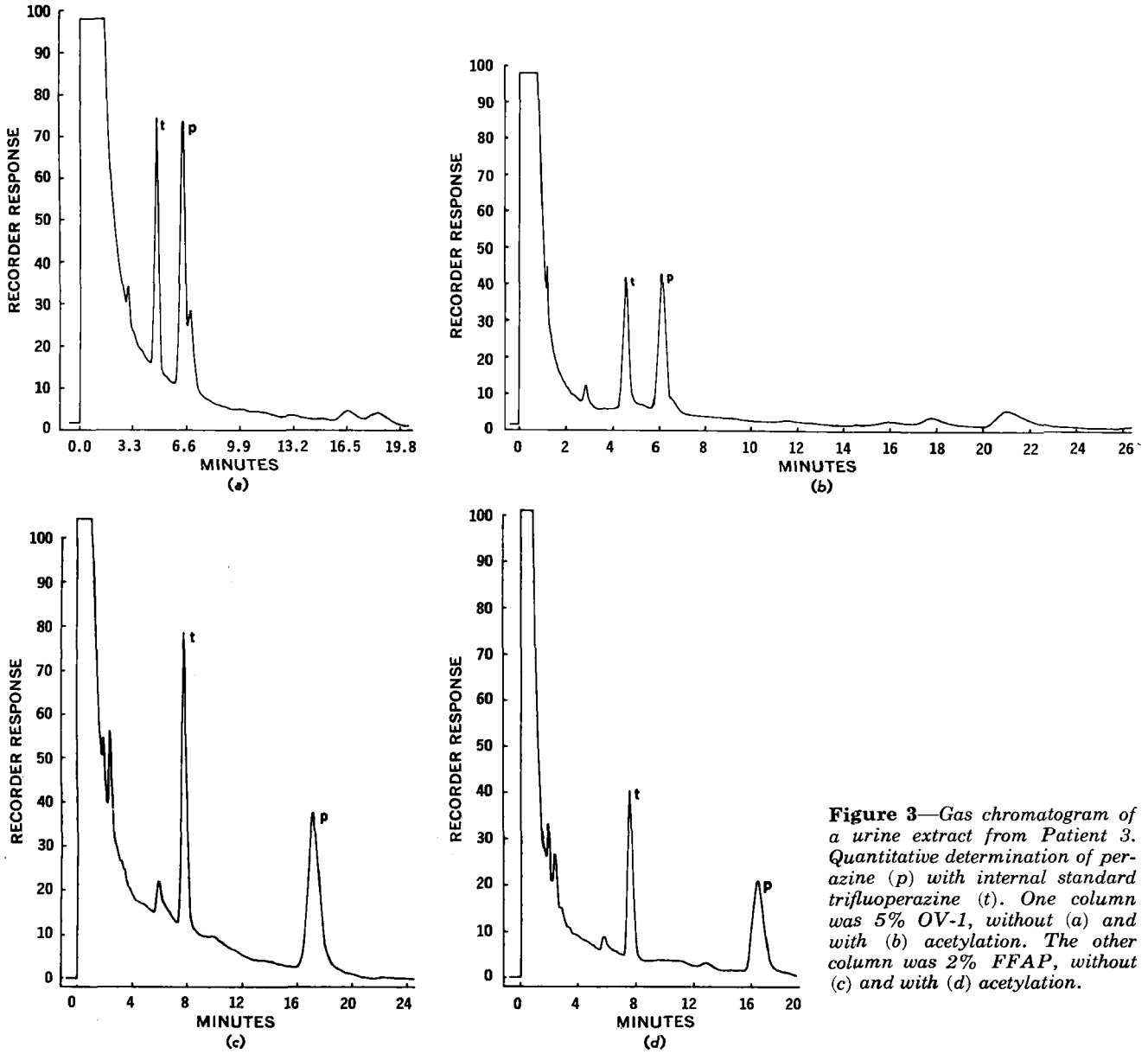


Figure 3—*Gas chromatogram of a urine extract from Patient 3. Quantitative determination of perazine (p) with internal standard trifluoperazine (t). One column was 5% OV-1, without (a) and with (b) acetylation. The other column was 2% FFAP, without (c) and with (d) acetylation.*

ing to their chemical structure relationship and retention properties, dixyrazine, thioproperazine, trifluoperazine, perazine, and levomepromazine served as suitable internal standards.

GLC Calculations—Calibration factors k_i were determined by measuring the corresponding peak areas on chromatograms from the calibration series:

$$k_i = A_x w_s / A_s w_x \qquad \text{(Eq. 1)}$$

where A_x and A_s and w_x and w_s are peak areas and weights of the compound to be determined and the internal standard, respectively. Peak areas are measured by the method of peak height times peak width at half-height.

For each calibration series, the mean calibration factor $\bar{k}$ was calculated using four or five measurements.

Results for urine samples C_u (in μg/100 ml) were found by the equation:

$$C_u = \frac{5}{v} \frac{A_x'}{A_s'} w_s \qquad \text{(Eq. 2)}$$

where $5/v$ is the conversion factor for the volume v of the alcoholic extract recalculated for 100 ml of original urine sample; A_x' and A_s' are peak areas of the compound to be determined as present in the urine extract and the internal standard, respectively; and w_s is the weight of the internal standard added to a fraction of the alcoholic extract prior to evaporation.

TLC—TLC plates were prepared by mixing 12.5 g of silica gel HF_{254}[11] and 12.5 g of cellulose MN300[12] with 90 ml of water and using the slurry for five 20 × 20-cm plates of 250-μm thickness. The plates were air dried and used without further temperature activation.

A 1–2-ml aliquot of the alcoholic extract was transferred into conical centrifuge tubes of 15-ml capacity and evaporated with a nitrogen stream in a water bath at 40°. The residue was dissolved in 10 μl of ethanol and quantitatively spotted with a micropipet in a 2-cm line on 3 cm of the lower edge of a TLC plate. A 10-μl volume of a solution containing 10 mg of a reference substance in 10 ml ethanol was applied in the same way on each plate.

Elution was performed over a 10-cm distance taken from the point of the applied spots and using a mixture of chloroform, acetone, and 25% ammonia (50:50:1 ml).

Substances were visualized by inspection under 254- and 350-nm UV light and by spraying with ferri, vanadium, and iodoplatinate reagents. After spraying with one of the reagents, stains were intensified by blowing a hot air stream over the TLC plate for a few moments.

For the ferri reagent (1), 500 mg of ferric nitrate was dissolved in 80 ml of concentrated sulfuric acid and the solution was diluted with water to 1000 ml. For the vanadium reagent, 500 mg of

[11] Merck.
[12] Macherey, Nagel and Co.

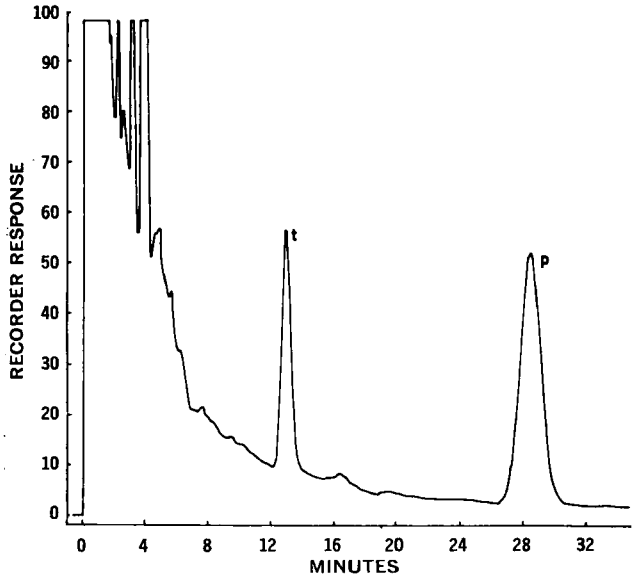


Figure 4—*Gas chromatogram of a urine extract from Patient 4. The column was 2% FFAP; quantitative determination of trifluoperazine (t) with internal standard perazine (p).*

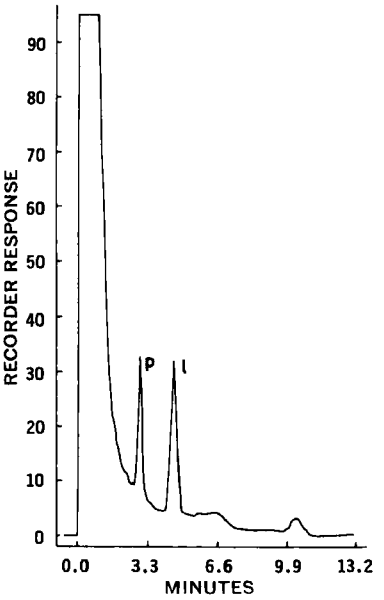


Figure 5—*Gas chromatogram of a urine extract from Patient 5. The column was 5% OV-1; quantitative determination of prothipendyl (p) with internal standard levomepromazine (l).*

vanadium pentoxide was dissolved in 80 ml of concentrated sulfuric acid and the solution was diluted with water to 1000 ml. For the iodoplatinate reagent (2), 5 ml of 5% platinum chloride was mixed with 45 ml of 10% KI and 100 ml water was added.

Recovery Experiments—To fractions of 200 ml of urine, collected from persons under no drug medication, propericiazine, dixyrazine, perazine, trifluoperazine, and prothipendyl were added in amounts of 400, 400, 248, 339, and 336 μg, respectively, and taken through the complete procedure.

RESULTS AND DISCUSSION

GLC operating conditions, retention times (R_t) of phenothiazine compounds and internal standards, calibration data ($\bar{k}$), and linear ranges of standard curves [$\Delta(w_x/w_s)$] are presented in Table II. Substances with a free polar alcohol group in their structure, such as propericiazine and dixyrazine, had first to be derivatized to the corresponding acetyl esters. In this way, excessive tailing effects were eliminated while separation efficiency and analytical precision increased. Calibration mixtures of perazine–trifluoperazine were examined with and without acetylation treatment. From the results, it appeared that no selective loss of one of the compounds mentioned occurred during the derivatization step. Acetyl esters of propericiazine, dixyrazine, and their sulfoxide metabolites are high molecular weight compounds and showed long retention times on apolar liquid phases. They could not have been chromatographed on the more polar and selective 2% FFAP column system within a reasonable time.

Results for recovery experiments of the phenothiazine compounds added to blank urine samples are given in Table III. Figures obtained on two different samples using both GLC columns correlate well. These data indicate an extraction efficiency of 86–99% and a method reproducibility of about 3%. They also are considered representative over the entire range of the standard curves. The described procedure for these phenothiazine drugs has a low detection limit of 5–10 ng/ml urine and an overall precision of about 7%.

Actual concentrations of drugs excreted in urine and expressed as μg/100 ml or percent of administered dose recovered as unchanged drug are tabulated in Table IV. Some examples of quantitative chromatograms are given in Figs. 1–5. Chromatographic patterns of urine extracts determined on both GLC columns show extraneous peaks produced by unidentified metabolites, which were also detected by TLC. Only small amounts or rather low percentages of the phenothiazine drugs were excreted in urine; regardless of the compound, the absolute dose administered, and the physiological condition of the patient, urinary excretion varied from 0.1 to 1.9%. These data demonstrate that this GLC method is very selective and sensitive for the quantitation of phenothiazine drugs in urine following the administration of low therapeutic doses of these drugs. Furthermore, as might be evaluated from the retention characteristics described in Table II, this GLC procedure should enable one to separate and measure more than one phenothiazine drug from the urine of a patient receiving a combination of these drugs.

TLC results were expressed as Rel. R_f values, *i.e.*, ratio of actual R_f to R_f of phenothiazine compound, and some typical examples are given in Table V. As indicated by underlined figures, the presence of free drug administered, sulfoxides, and other unidentified, structurally related metabolites was established in urine extracts of all patients except Patient 5. In this case, negative results were obtained because of the low dose of administered drug. Both separation efficiency and a high sensitivity of 25–50 ng/ml urine make TLC a valuable tool for following low therapeutic levels of phenothiazine drugs and apolar metabolites as excreted in urine for the verification of the specificity of the GLC procedure used for the quantitative measurement of these drugs.

REFERENCES

(1) H. Leach and W. R. C. Crimmin, *J. Clin. Pathol.*, **9**, 164, (1956).

(2) A. Stolman, "Progress in Chemical Toxicology," vol. 2, Academic, New York, N.Y., 1965, p. 357.

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