Aminoglutethimide Bioavailability, Pharmacokinetics, and Binding to Blood Constituents

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Received December 1, 1980, from the *Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Ardsley, NY 10502.* Accepted for publication March 2, 1981.

Abstract □ The bioavailability of aminoglutethimide tablets was examined using a spectrophotometric assay. For six subjects receiving 500 mg of aminoglutethimide as an oral solution, the average peak concentration was 6.2 μg/ml with a median time of 0.8 hr. The corresponding average peak concentration for tablet administration was 5.9 μg/ml with a median time of 1.5 hr. Average values for the area under the curve (*AUC*) extrapolated to infinity were 89.0 and 96.8 μg hr/ml for the solution and tablets, respectively. The tablets had a 9% larger mean for the *AUC* than the solution and a 5% lower value for the mean maximum concentration. The bioavailability of the tablets is considered equal to that of oral solution. Data for individual concentration *versus* time curves were treated by nonlinear least-squares curve fitting. A two-compartment model with first-order absorption gave an acceptable fit for most data sets, but the individual absorption rate coefficients were not reliably determined. Values were estimated for plasma clearance, renal clearance, and volume of distribution. The distribution of aminoglutethimide between plasma and cells of human blood was examined *in vitro*; the drug concentration in cells was 1.4–1.7 times the concentration in plasma. The binding of aminoglutethimide to plasma proteins of human blood was measured by equilibrium dialysis at starting concentrations of 5 and 10 μg/ml. The binding ranged from 21.3 to 25.0% without concentration dependence.

Keyphrases □ Aminoglutethimide—bioavailability, pharmacokinetics, binding to blood constituents □ Bioavailability—aminoglutethimide, pharmacokinetics, binding to blood constituents □ Pharmacokinetics—aminoglutethimide, bioavailability, binding to blood constituents

Aminoglutethimide, α-(*p*-aminophenyl)-α-ethylglutarimide, is an inhibitor of adrenal corticosteroid biogenesis (1) used for the treatment of Cushing's syndrome (2). The urinary excretion of aminoglutethimide in humans was measured and accounted for 39 and 54% of 250- and 500-mg oral doses, respectively (3). Acetamidoglutethimide was reported as a metabolite in human urine (4). An assay procedure for aminoglutethimide in plasma and information about its half-life, clearance, and compliance in patients were reported previously (5). The purposes of the present study were to evaluate the bioavailability of a commercial dosage form[1], to explore the pharmacokinetics, and to measure the binding of aminoglutethimide to blood constituents.

EXPERIMENTAL

Clinical Study Protocols—Eight healthy adult male volunteers, 25–54 years of age, were given a copy of the appropriate protocol and full information about the drug; they signed statements of informed consent. Each subject was screened regarding general health and history of drug hypersensitivity and underwent a physical examination, urine analysis, and a hematologic and blood chemistry analysis. The subjects ranged in height from 173 to 188 cm and in weight from 64 to 91 kg. Each subject was instructed to avoid any medication during the study and during the preceding week. The consumption of alcoholic beverages was prohibited during and for 3 days preceding the study.

Two subjects participated in a pilot study, and six others participated in a random crossover bioavailability study with a washout period of 14 days.

[1] Cytadren, 250-mg tablets, E-10447, Ciba-Geigy Corp.

Aminoglutethimide (500 mg) was administered in the morning after an overnight fast. No food or liquid other than water was permitted until 2 hr after drug administration, when a simple standard breakfast was allowed. Samples (10 ml) of venous blood were collected[2] at 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 12, 24, and 48 hr in the pilot study and at 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 15, and 24 hr in the bioavailability study. The heparinized blood samples were centrifuged, and the plasmas were separated. Saliva production was stimulated during the bioavailability study by having the subjects chew a small piece of paraffin[3], and 2-ml saliva samples were obtained at the same time that the 0-, 1-, 2-, 3-, 6-, 9-, and 12-hr blood samples were drawn. Total urines were collected during the pilot study at intervals ending at 2, 4, 6, 12, 24, and 48 hr. Samples were frozen until analysis.

Assay Methodology—Plasma, urine, and saliva samples were analyzed for aminoglutethimide by the quantitative spectrophotometric method described previously, which involves extraction with chloroform and reaction with diazotized *p*-dimethylaminobenzaldehyde (5). The method was tested before use by analysis of control samples to which aminoglutethimide had been added. Frozen samples of human control plasma were thawed, pooled, and centrifuged to remove solids. Syringes[4] were used to add 0, 23, 30, 52, 57, 80, and 160 μl of a 1-μg/μl solution of aminoglutethimide in ethanol to seven 40-ml centrifuge tubes. The alcohol was evaporated, and 10.0 ml of plasma was added to each tube. Polytef-lined screw-capped tubes were rotated for 15 min. Approximately 2.5 ml of each concentration of aminoglutethimide in plasma was placed in triplicate into vials. Two blanks were added, and the vials then were number coded in random sequence. All samples were frozen as soon as prepared and stored at −10° pending analysis.

The specificity of the method was examined with respect to the metabolite acetamidoglutethimide and three other metabolites isolated from rat urine[5]. Aliquots (8, 25, and 40 μl) of a 1-μg/μl solution of each metabolite were placed in duplicate into test tubes, evaporated, redissolved in ethanol, and analyzed without extraction.

The stability of aminoglutethimide in plasma during frozen storage at −10° was examined. Aminoglutethimide was added to plasma from control volunteers to provide concentrations of 5, 10, and 15 μg/ml. Replicate aliquots of each concentration and blank were placed into separate vials. One set was analyzed immediately; the other sets were stored in a freezer pending analysis after 1, 5, 15, and 30 days.

Binding Methodology—For the whole blood distribution experiments, a 2.0-mg/ml stock solution of aminoglutethimide in ethanol was diluted 10-fold with 0.9% saline. The resulting 0.2-mg/ml solution was added to heparinized whole blood obtained within 2 hr from three healthy human volunteers. Concentrations of 0, 5, and 10 μg/ml were prepared and equilibrated in a shaking water bath for 30 min at 37°. Plasma was prepared by centrifugation and stored overnight in a freezer pending analysis. The concentration of drug in cells was calculated as follows:

$$\mu g/ml \text{ in cells} = \frac{\mu g/ml \text{ in blood} - \mu g/ml \text{ in plasma}}{\text{hematocrit}} + \mu g/ml \text{ in plasma} \quad \text{(Eq. 1)}$$

Binding of aminoglutethimide by plasma proteins was measured by equilibrium dialysis against pH 7.4 isotonic buffer. Aminoglutethimide in ethanol (1.0 mg/ml) was added to heparinized plasma from different individuals to produce 5- and 10-μg/ml concentrations. The dialysis buffer was prepared by mixing 8.7 ml of 5.4 g/liter KH_2PO_4 with 30.4 ml of 10.7 g/liter $Na_2HPO_4 \cdot 7H_2O$, adding 4.5 g of NaCl, and diluting to 500 ml. The pH was adjusted to 7.4. Dialysis membranes were prepared for

[2] Becton-Dickinson green-topped Vacutainers were used.
[3] Parafilm M, American Can Co., Greenwich, CT 06830.
[4] Hamilton.
[5] Metabolites of aminoglutethimide were obtained from Mr. Hp. Egger, Ciba-Geigy Corp., Ardsley, NY 10502.

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Table I—Number of Subjects Having Treatment Emergent Signs and Symptoms during Aminoglutethimide Administration

Sign or Symptom	Ethanolic Solution			Tablets		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Dizziness	1	4	—	1	—	—
Light headedness	—	—	—	2	—	—
Sedation	1	—	—	2	1	—
Headache	1	—	—	—	—	—

use by boiling in distilled water for 5 min and rinsing thoroughly. The membranes were stored under water in a refrigerator. Dialysis was carried out for 18 hr using 5-ml cells in a shaking water bath at 37°. Aliquots (2.0 ml) of both the dialyzed plasma and buffer were analyzed for aminoglutethimide.

Data Evaluation—The bioavailability of the aminoglutethimide tablets was evaluated by comparison of the area under the curve (*AUC*) extrapolated to infinity for plasma concentration curves resulting from two 250-mg tablets and from 500 mg of aminoglutethimide dissolved in a special vehicle containing 10% ethanol, 30% propylene glycol, and 10% glycerin. The *AUC* values were obtained by application of the trapezoidal rule. Peak plasma concentrations (C_{max}), times of peak concentration (T_{max}), and half-lives ($T_{1/2}$) also were examined.

Pharmacokinetic Analysis—Nonlinear least-squares curve fitting was carried out using AUTOAN and NONLIN computer programs. Values for clearance and the volume of distribution were calculated as follows, where $t_1 - t_2$ is elapsed time:

$$\text{renal clearance} = \frac{\text{amount excreted } t_1 - t_2}{AUC\ t_1 - t_2} \quad \text{(Eq. 2)}$$

$$\text{plasma clearance} = \text{dose}/AUC \quad \text{(Eq. 3)}$$

$$V_c = \frac{\text{dose}}{(AUC)\,(K_{el})} \quad \text{(Eq. 4)}$$

$$V_\beta = 1.44 V_c K_{el} T_{1/2\beta} \quad \text{(Eq. 5)}$$

$$V_{ss} = V_c \frac{K_{21} + K_{12}}{K_{21}} \quad \text{(Eq. 6)}$$

RESULTS AND DISCUSSION

Assay Validation—The assay procedure was tested by blind analysis of a group of 20 samples of human plasma to which known amounts of aminoglutethimide from 2.3 to 16 μg/ml had been added. Replicates and blank samples were included. The samples were analyzed in random sequence, and the concentrations were unknown to the analyst until later. The mean and standard deviation for the percent recovery in the blind validation experiment were 97.4 ± 3.8, demonstrating good accuracy and precision. Plasma calibration standards of three different concentrations were freshly prepared and analyzed on 14 occasions during several months. The mean of slopes for the standard curves and also the mean analytical response for each drug level had relative deviations of 2%, showing excellent agreement.

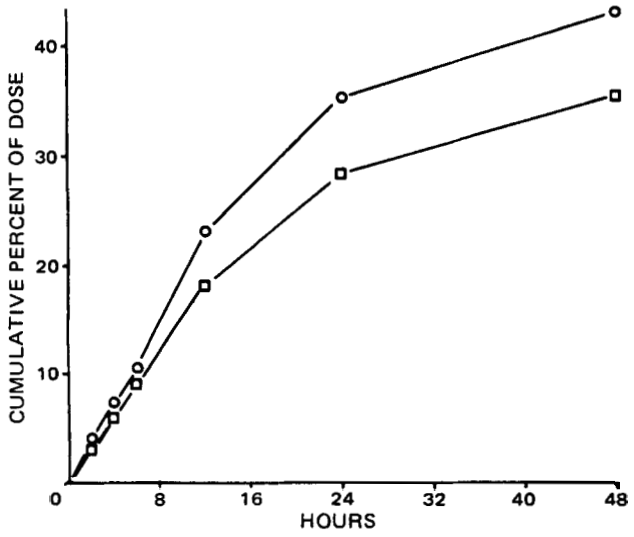

Figure 1—*Urinary excretion of aminoglutethimide by two subjects after two 250-mg tablets.*

Table II—Renal Clearance of Aminoglutethimide

Subject	Interval, hr	R_c, ml/min	Mean ± *SD*
1	0–2	24	30.4 ± 5.9
	2–4	25	
	4–6	31	
	6–12	38	
	12–24	34	
2	0–2	33	37.6 ± 8.4
	2–4	28	
	4–6	36	
	6–12	50	
	12–24	41	

The stability of aminoglutethimide in plasma during frozen storage was examined. Aminoglutethimide was added to human control plasma to obtain concentrations of 5.0, 10.0, and 15.0 μg/ml. Aliquots were analyzed after 0, 1, 5, 15, and 30 days of frozen storage, and the percent recoveries ranged from 96 to 103% with no apparent trend. All samples in the bioavailability study were analyzed before 30 days of storage had elapsed.

The assay is applicable to urine and saliva as well as to plasma. Five 2.0-ml aliquots of human urine to which 2.5–15.0 μg/ml of aminoglutethimide had been added were analyzed to test the procedure. The average recovery and standard deviation were 91.4 ± 3.2%. A similar recovery experiment with saliva gave 99.4 ± 1.1%.

Acetamidoglutethimide and three metabolites of unknown structure from rat urine did not cause any response in the assay.

Clinical Observations—Each subject completed the study as planned. Table I shows that no severe signs or symptoms appeared, although four subjects complained of moderate dizziness after receiving aminoglutethimide in ethanolic solution. This effect probably resulted from ingestion of ethanol while fasting. In no case did the symptoms prevent the subjects from performing their usual activities.

Cumulative Percent of Dose Excreted—The cumulative percent of aminoglutethimide excreted unchanged in urine was measured for the two subjects who ingested two 250-mg tablets in the pilot experiment (Fig. 1). Renal excretion of unchanged drug proceeded slowly and accounted for 35.0–42.6% of the dose during 48 hr. These values are in accord with those reported previously (3). Urine was not obtained in the six-subject bioavailability experiment.

Renal Clearance—Renal clearance was calculated for the two subjects for whom both urine and plasma samples were obtained (Table II). The values suggest that aminoglutethimide may be processed renally by glomerular filtration with passive tubular reabsorption of some filtered drug.

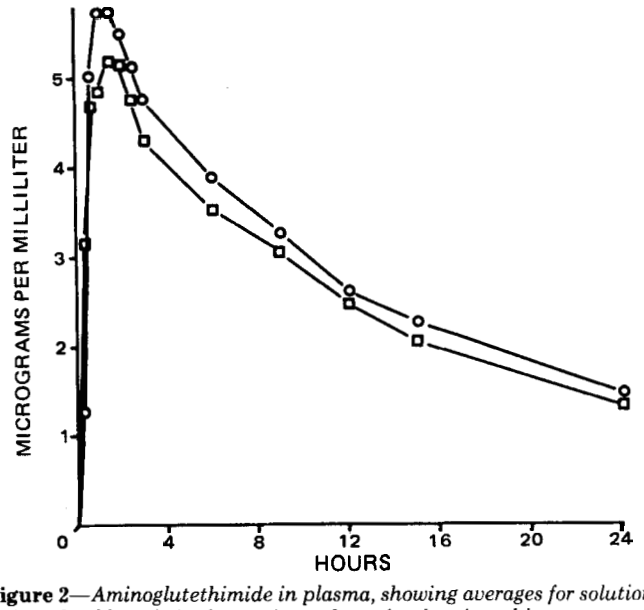

Figure 2—*Aminoglutethimide in plasma, showing averages for solution (□) and tablets (○) after 500-mg doses in the six-subject crossover study.*

Table III—Concentration Maximum, Half-Life, and Area under the Curve for Aminoglutethimide in Plasma

Subject	500 mg in Solution				Two 250-mg Tablets			
	C_{max}, μg/ml	T_{max}, hr	$T_{1/2\beta}$, hr	$AUC_{0-\infty}$, μg hr/ml	C_{max}, μg/ml	T_{max}, hr	$T_{1/2}$, hr	$AUC_{0-\infty}$, μg hr/ml
3	5.3	2.0	9	70	5.9	1.5	10	88
4	5.0	2.0	12	88	5.8	1.5	12	93
5	7.4	0.7	14	101	5.8	0.7	13	97
6	7.2	0.3	16	96	5.6	1.5	15	101
7	4.9	1.0	12	74	6.1	1.0	13	95
8	7.1	0.7	12	105	6.3	1.5	12	107
Mean	6.2	1.1	12.5	89	5.9	1.3	12.5	97
SD	1.2	0.7	2.3	14	0.2	0.3	1.6	7
Median	—	0.8	—	—	—	1.5	—	—

Table IV—Pharmacokinetic Parameters from Computer Modeling of Aminoglutethimide Plasma Data

Dosage Form	Subject	r^2	K_A	K_{21}	K_{el}	K_{12}	C_0	Lag Time
Tablet	3	1.000	2.800	1.917	0.1097	1.053	9.6	0.3
	4	1.000	2.212	0.334	0.0719	0.085	6.8	0.1
	5	0.999	10.750	1.284	0.0676	0.284	6.4	0.3
	6	0.999	2.718	0.477	0.0747	0.297	7.6	0.2
	7	0.999	2.841	0.494	0.0839	0.298	8.1	0.3
	8	0.999	4.341	0.097	0.0590	0.026	6.9	0.3
Solution	3	0.835	0.395	0.115	0.2496	0.266	22.6	0.8
	4	0.998	2.632	0.283	0.0609	0.009	5.4	0.2
	5	0.999	9.659	0.359	0.0797	0.249	8.3	0.3
	6	0.991	8.590	0.336	0.0810	0.173	6.8	None
	7	0.999	3.440	0.678	0.0816	0.291	6.2	0.1
	8	0.998	3.906	0.210	0.0690	0.064	7.6	None

Plasma Concentration–Time Curves—The concentration–time curves were similar for all subjects. Maximum concentrations of 4.9–7.4 μg/ml were found 0.3–2.0 hr after ingestion of 500 mg of aminoglutethimide in solution, and two 250-mg tablets resulted in peak concentrations of 5.6–6.3 μg/ml occurring 0.7–1.5 hr after ingestion (Table III). An initially rapid decline was followed by a slower decline having a half-life of 9–16 hr for the solution and 10–15 hr for the tablets.

A graph of the average plasma concentrations (Fig. 2) shows similar, but not superimposable curves. The tablets had a 9% larger average for the *AUC* than the solution (Table III). A difference in *AUC* of this magnitude has little practical significance, and the tablet formulation may be considered bioequivalent to the solution. It is common practice to assess the absorption efficiency of tablets relative to an oral solution by calculating a ratio of average *AUC* values. According to pharmacokinetic theory, the ratio of areas equals a ratio of the fraction of each dose absorbed if the plasma clearance (C_p) is the same (6). The tablets and solution had average C_p values of 86.2 and 95.7 ml/min, respectively. Since the tablets and solution were administered at the same dose level in a random crossover pattern, the apparent differences in C_p and *AUC* values probably are artifacts of the data. The C_{max} value for the solution was 5% larger than for the tablets, and the average terminal half-life value was 12.5 hr for both the tablets and solution. This value for aminoglutethimide half-life agrees well with the average 13.3 hr reported for an initial dosage of six patients (6). The time of peak concentration was slightly earlier for solution, 0.8 hr *versus* 1.5 hr for tablets.

Table V—Plasma Clearance and Volumes of Distribution for Aminoglutethimide

Dosage Form	Subject	C_p, ml/min	V_c, liters	V_β, liters	V_{ss}, liters
Tablet	3	94	51.8	82.4	80.2
	4	89	74.8	92.7	93.8
	5	86	76.2	97.2	93.2
	6	82	66.3	107.7	107.6
	7	88	62.7	99.2	100.5
	8	78	79.2	80.6	100.6
	Mean	86.2	68.50	93.30	95.98
	SD	5.6	10.30	10.37	9.36
Solution	3	119	28.6	92.7	94.8
	4	94	93.3	98.0	96.3
	5	82	62.1	99.0	105.2
	6	86	64.3	121.1	97.4
	7	114	82.8	116.5	118.3
	8	79	69.0	82.1	90.0
	Mean	95.7	66.68	101.57	100.33
	SD	17.0	22.14	14.71	10.09

Nonlinear least-squares curve fitting of plasma data using AUTOAN and NONLIN computer programs with equal weighting of data gave equations of acceptable fit using a two-compartment model with first-order absorption. Values for the squared correlation coefficient were >0.99 in all but one instance (Table IV). However, the standard deviations for the individual absorption rate constants were too great for meaningful evaluation of this parameter.

Values for C_p are shown in Table V. The observed average C_p values of 86.2 and 95.7 ml/min indicate a moderate clearance rate. Table V also shows values for principal volume of distribution parameters. In the absence of intravenous data, the dose was assumed to be completely absorbed. The values for clearance and volumes of distribution are overestimated if this assumption is incorrect.

Binding to Blood Cells and Plasma Proteins—The distribution of aminoglutethimide between plasma and cells of human blood was calculated after *in vitro* equilibration of drug with heparinized blood and analysis of the drug in plasma. The concentration of aminoglutethimide in cells was 1.4–1.7 times the concentration in plasma. The binding of aminoglutethimide *in vitro* to plasma proteins of three subjects was measured by equilibrium dialysis at 37°. Initial drug concentrations of 5 and 10 μg/ml showed binding of 21.3–25.0%. No concentration-dependent trend was apparent.

Saliva Concentrations—Saliva samples were obtained to determine whether saliva assay is an acceptable alternative to plasma assay. Saliva production was stimulated by chewing a small piece of paraffin. Paraffin was reported to absorb significant amounts of propranolol and indomethacin (7), and a preliminary test for glutethimide absorption was carried out as described previously (7). Little or no absorption was found. However, the concentrations of aminoglutethimide found in saliva of the bioavailability subjects were too low for the analytical method. Measurable concentrations were found in only a few samples, and no correlation with plasma concentrations was apparent.

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High-Performance Liquid Chromatographic Assay for Nanogram Determination of Chlorpromazine and Its Comparison with a Radioimmunoassay

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Received June 6, 1980, from the **College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada*, the ‡*Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario K1A 0L2, Canada*, and the §*Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.* Accepted for publication February 17, 1981.

Abstract □ A specific and sensitive high-performance liquid chromatographic (HPLC) method for the quantitative determination of plasma chlorpromazine concentrations is described. The procedure is capable of determining 1 ng of chlorpromazine/ml and is adequate for following plasma concentration–time profiles after 7-mg single intravenous doses. After a simple organic extraction of the drug and an internal standard (mesoridazine) from plasma, the organic layer was transferred to a vial and evaporated to dryness at 55° under nitrogen. The residue was dissolved in 200 μl of HPLC grade acetonitrile. Aliquots (70–100 μl) were chromatographed, and the drug was quantitated in the range of 1–15 ng/ml of plasma using a fixed-wavelength UV detector. Plasma concentrations determined by the method were compared with those obtained by a previously reported radioimmunoassay specific for chlorpromazine and *N*-desmethylchlorpromazine. The two methods agreed favorably with a correlation coefficient of 0.993 and a slope of 0.994.

Keyphrases □ Chlorpromazine—high-performance liquid chromatographic analysis in plasma and comparison with a radioimmunoassay □ Psychotropic agents—chlorpromazine, high-performance liquid chromatographic analysis □ Antipsychotic phenothiazines—chlorpromazine, high-performance liquid chromatographic analysis and radioimmunoassay comparison

Chlorpromazine is widely used in the treatment of certain psychiatric disorders (1). It undergoes extensive metabolism, and several of its metabolites (2) are considered to be psychoactive. Some of the metabolites can be quantitated in plasma; in some studies, the ratio of plasma concentrations of active to inactive metabolites was correlated with clinical improvement in schizophrenic patients (3–5).

The various chemical methods of quantitation that have been used include GLC with electron-capture detection (6), GLC with mass spectrometric detection (7, 8), fluorescent labeling with dansyl chloride (9), labeled derivative formation (10), and TLC of a quaternary ammonium derivative formed by reaction with 9-bromomethylacridine, followed by UV photolysis and spectrofluorometric determination (11). These methods may have adequate sensitivity to determine plasma concentrations following therapeutic dosage regimens, but they are generally cumbersome and not easily amenable to routine clinical monitoring.

The radioimmunoassay procedures are generally simple, sensitive, and readily applicable to routine analysis. Although sensitive, radioimmunoassays generally are suspect from the aspect of specificity. In the case of chlorpromazine radioimmunoassay (12–14), this specificity concern is augmented by the extremely large number of identified metabolites. To verify the specificity of the radioimmunoassay procedure reported previously (14), which was based on proper designing of the antibody (15), high-performance liquid chromatographic (HPLC) assay, specific and sensitive to 1 ng/ml of plasma, was developed. This HPLC assay is described and compared with the radioimmunoassay reported earlier (14).

EXPERIMENTAL

Materials—Chlorpromazine hydrochloride[1], prochlorperazine[1], mesoridazine besylate[2], 2-chlorophenothiazine[3], chlorpromazine sulfoxide[4], 7-hydroxychlorpromazine sulfoxide[4], *N*-monodesmethylchlorpromazine[4], *N*-monodesmethylchlorpromazine sulfoxide[4], *N*-didesmethylchlorpromazine[4], *N*-didesmethylchlorpromazine sulfoxide[4], and chlorpromazine *N*-oxide[4] were used. All solvents were HPLC grade[5], and all other chemicals were commercial analytical reagent grade.

Apparatus—A liquid chromatographic pump[6] and a valve-loop injector[7] fitted with a 1000-μl loop were connected to a fixed-wavelength detector[8] operated at 254 nm. The detector was attenuated to 0.005 aufs for chlorpromazine and to 0.5 aufs for the internal standard.

Column—A 250 × 3.2-mm i.d. column, packed with 5-μm cyano-bonded column packing[9], was used at ambient temperature with a mobile phase flow rate of 1.6 ml/min.

Mobile Phase—The mobile phase consisted of 10% aqueous 0.015 *M* sodium acetate–acetic acid buffer (pH 6.5) and 90% acetonitrile. It was degassed by refluxing for 5 min and transferred to the solvent reservoir.

Internal Standard—A stock solution of mesoridazine besylate (1000 μg/ml, calculated as free base) was prepared in double-distilled water, stored in the absence of UV light at 4°, and used throughout the experiments. Dilutions of 50 μg/ml were prepared weekly and used for analysis.

Preparation of Standard Curves—An aqueous solution of chlorpromazine hydrochloride (100 μg/ml, calculated as free base) was prepared weekly in double-distilled deionized water and stored in the ab-

[1] Poulenc Ltd., Montreal, Quebec, Canada.
[2] Sandoz Pharmaceuticals, Dorval, Quebec, Canada.
[3] Aldrich Chemical Co., Montreal, Quebec, Canada.
[4] Donated by Dr. A. A. Manian, National Institute of Mental Health, Rockville, Md.
[5] Fisher Scientific Co., Montreal, Quebec, Canada.
[6] Model 110A, Altex, Beckman Instruments, Toronto, Ontario, Canada.
[7] Model 7120, Rheodyne, Technical Marketing Associates, Ottawa, Ontario, Canada.
[8] Model 440, Waters Associates, Mississauga, Ontario, Canada.
[9] Spherisorb CN, Altex, Beckman Instruments, Toronto, Ontario, Canada.

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