High-Performance Liquid Chromatographic Assay for Imipramine, Desipramine, and Their 2-Hydroxylated Metabolites

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Abstract □ A high-performance liquid chromatographic method is presented for the simultaneous determination of imipramine, desipramine, and their 2-hydroxylated metabolites in plasma. The method involves a simple plasma extraction at basic pH with organic solvent, chromatography on a silica gel column, and fluorescence detection. Correlation with a GLC–mass spectrometric method for imipramine and desipramine is illustrated. The method can detect 1 ng of each component/ml of plasma, sufficient sensitivity for pharmacokinetic studies.

Keyphrases □ High-performance liquid chromatography—analysis, imipramine, desipramine, 2-hydroxylated metabolites in plasma □ Desipramine—analysis in plasma, high-performance liquid chromatography □ Imipramine—analysis in plasma, high-performance liquid chromatography, metabolites □ Antidepressants, tricyclic—imipramine, desipramine, high-performance liquid chromatographic analysis in plasma

Clinical monitoring of plasma imipramine is complicated by biotransformation of the tricyclic antidepressant into another therapeutic entity, desipramine, and by their low plasma concentrations (1). Recent studies also identified hydroxylated metabolites in the brain following acute intoxication (2) and demonstrated biological activity of the 2-hydroxy metabolites of both compounds (3–7). An appropriate assay is needed to assess the potential importance of these metabolites in psychopharmacological and pharmacokinetic studies.

Several methods for tricyclic antidepressant analysis by TLC (8–10), GLC (11, 12), and high-pressure liquid chromatography (HPLC) (13–16) have been published. However, few were applied to imipramine and desipramine determination in biological specimens, and none dealt with the 2-hydroxy metabolites. The imipramine and desipramine assays required either sophisticated instrumentation not readily available or multistep extraction and/or derivatization. The sensitivity achieved generally was not adequate for pharmacokinetic studies, even when large samples were used.

An HPLC method for the simultaneous determination of plasma imipramine, 2-hydroxyimipramine, desipramine, and 2-hydroxydesipramine was developed. The procedure involves a simple extraction followed by separation of all four components and an internal standard in a single injection. High fluorescence sensitivity permits detection of 1 ng of each drug and metabolite in 1 ml of plasma.

EXPERIMENTAL

Reagents—Acetonitrile[1], ammonium hydroxide[2], *n*-butyl alcohol[1], hexane[1], and methanol[1] were spectroanalytical grade.

Equipment—A high-performance liquid chromatograph[3] with a syringe-loading sample injector[4], a 10-mv recorder[5], and a fluorescence detector[6] were used. An excitation wavelength of 240 nm and a 370-nm emission filter gave optimum fluorescence under the assay conditions. Detector range settings of 0.05–0.2 μamp were sufficient for most analyses; limits of the range settings were 0.01–1.0 μamp. Chromatography was done on a 0.46 × 25-cm, stainless steel column packed with 5-μm (particle diameter) silica B/5[7].

Assay Standards—Stock methanol solutions of desipramine hydrochloride[8] (113 mg/100 ml), imipramine hydrochloride[9] (113 mg/100 ml), 2-hydroxydesipramine free base[10] (10 mg/10 ml), and 2-hydroxyimipramine free base[10] (10 mg/10 ml) were prepared.

Plasma standards were prepared by spiking drug-free plasma with the methanol solutions to give final concentrations of 5–500 ng of imipramine and desipramine/ml and 5–150 ng of the 2-hydroxy metabolites/ml. The internal standard was 200 μg of *N*-desmethylclomipramine hydrochloride[9]/10 ml of methanol.

Extraction—To 1 ml of plasma standard or unknown, 50 μl of internal standard solution and 300 μl of ammonium hydroxide were added in polypropylene test tubes. The mixtures were extracted with 1 ml of 20% *n*-butyl alcohol in hexane for 15 min on a reciprocating shaker. After centrifugation, the upper organic layers were transferred to clean polypropylene tubes and evaporated to dryness at ambient temperature under nitrogen. Each extract was reconstituted just prior to chromatography with 100 μl of methanol, and 50 μl was injected.

Chromatography—All chromatography was done at ambient temperature. The mobile phase was methanol–acetonitrile (1:5) plus am-

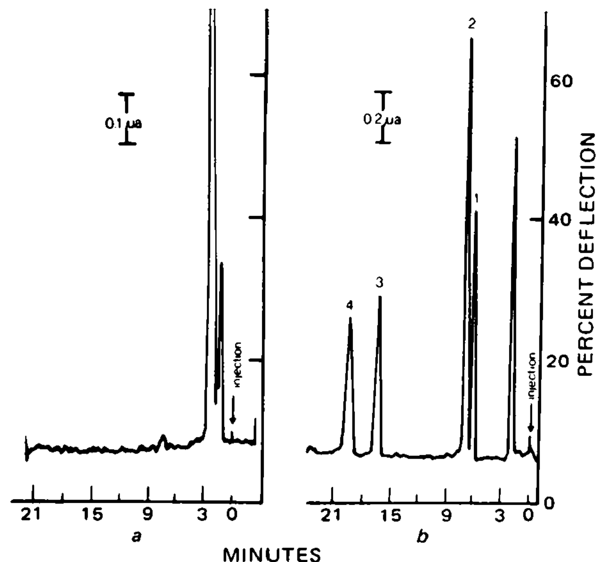

Figure 1—(a) *Chromatogram of an extract of 1 ml of blank plasma. The extract was reconstituted with 100 μl of methanol and the entire extract was injected.* (b) *Chromatogram of 100 μl of a methanol solution containing 50 ng/100 μl each of imipramine (1), 2-hydroxyimipramine (2), desipramine (3), and 2-hydroxydesipramine (4).*

[1] Burdick & Jackson Laboratories, Muskegon, Mich.
[2] "Baker Analyzed" reagent, VWR, Rochester, N.Y.
[3] DuPont model 848, Wilmington, Del.
[4] Rheodyne model 7105 injection valve, Perkin-Elmer, Norwalk, Conn.
[5] Linear Instruments Corp., Scientific Products, Rochester, N.Y.
[6] F. S. 970 L. C. fluorometer, Schoeffel Instrument Corp., Westwood, N.J.
[7] Perkin-Elmer Instrument Division, Norwalk, Conn.
[8] USV Pharmaceutical Corp., Tuckahoe, N.Y.
[9] Ciba Pharmaceutical Co., Summit, N.J.
[10] Gift of Dr. Albert A. Manian, National Institute of Mental Health (NIMH), Rockville, Md.

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Table I—Reproducibility of HPLC Standards

Concentration, ng/ml	n	Imipramine Mean Peak Height Ratio	Imipramine Slope[a]	Imipramine CV, %	2-Hydroxyimipramine Mean Peak Height Ratio	2-Hydroxyimipramine Slope[a]	2-Hydroxyimipramine CV, %	Desipramine Mean Peak Height Ratio	Desipramine Slope[a]	Desipramine SD, %	2-Hydroxydesipramine Mean Peak Height Ratio	2-Hydroxydesipramine Slope[a]	2-Hydroxydesipramine CV, %
5	6	0.17	0.034	5.8	0.32	0.064	9.4	0.18	0.036	11.1	0.11	0.022	13.6
50	6	1.57	0.031	10.8	3.12	0.062	10.6	1.09	0.022	8.3	0.91	0.018	8.8
150	6	5.07	0.034	5.5	9.61	0.064	7.4	3.15	0.021	6.0	2.84	0.019	2.5
300	3	10.01	0.033	7.2	—	—	—	6.34	0.021	7.3	—	—	—

[a] Slope = mean peak height ratio/concentration.

Table II—Recovery and Within-Run Precision

Concentration, ng/ml	n	Imipramine %	Imipramine SD	Imipramine CV, %	Desipramine %	Desipramine SD	Desipramine CV, %
10	5	68.6	1.8	2.6	64.0	1.4	2.2
50	10	69.5	2.3	3.3	64.8	2.5	3.9
150	10	68.3	2.6	3.8	66.1	2.6	3.9
300	6	67.6	1.5	2.2	67.2	1.7	2.5
Mean		68.5	2.0	3.0	65.5	2.0	3.1
		2-Hydroxyimipramine			2-Hydroxydesipramine		
10	5	84.6	2.5	2.9	94.3	2.8	2.9
50	4	79.2	1.3	1.6	76.9	3.2	4.2
100	5	81.4	2.1	2.6	84.6	3.1	3.7
Mean		81.7	2.0	2.4	85.3	3.0	3.6

monium hydroxide (4 ml/liter). The flow rate was 1.8 ml/min (~1100 psig).

Quantitation—Peak height ratios of the drug or metabolite to the internal standard were plotted against concentration to obtain standard calibration curves for each compound. Plasma standards were included with each assay.

RESULTS AND DISCUSSION

Imipramine, desipramine, and their 2-hydroxy metabolites were separated and eluted in ~21 min (Fig. 1*b*). Blank plasma extracts yielded no interference from endogenous plasma components (Fig. 1*a*).

Detector response was linear to plasma imipramine and desipramine concentrations of 5–500 ng/ml and to plasma 2-hydroxyimipramine and 2-hydroxydesipramine concentrations of at least 5–150 ng/ml. All four lines passed through the origin. At least 1 ng of each component/ml could be detected by injecting the entire 1-ml plasma extract.

Standard curve reproducibility for 1 month is indicated by Table I. The mean peak height ratio variation coefficients were approximately 5–10% for each compound. Plasma with added imipramine and desipramine stored in polypropylene tubes at −4° was stable for at least 3 months. Similarly stored clinical serum specimens also were stable for at least 3 months.

Drug and metabolite recoveries from plasma were estimated by comparing the peak heights from spiked plasma extracts directly with those of methanol solutions. The entire plasma extract was injected, and the methanol solution concentrations were calculated to permit injections of the same volumes. Table II shows reproducible recoveries of each compound over the concentration ranges studied and within-run variability of <5%. Studies of different concentrations were performed on separate days.

Several drugs were tested chromatographically in methanol solutions for use as internal standards and for interference (Table III). Drugs with relative retention times within 10% of the relative retention time of the experimental components could interfere with the assay. Therefore, complete medication histories are recommended before clinical or pharmacokinetic studies.

Two other imipramine and desipramine metabolites, 2-hydroxyiminodibenzyl and didesmethylimipramine, were chromatographed; only the former metabolite was incompletely separated. Previous evidence suggests that this metabolite is present only in small amounts (17) and that its potential interference is insignificant.

Other drugs that did not give fluorescent peaks within the analysis time were nortriptyline, protriptyline, diazepam, flurazepam, clorazepate, chlordiazepoxide, and scopolamine.

N-Desmethylclomipramine was chosen as the internal standard because it eluted near, but was completely separated from, the experimental components and did not add to total analysis time. It is reproducibly extracted from plasma by the method presented but is unlikely to be present in specimens from patients receiving other tricyclics. Figure 2*a* illustrates a chromatogram of a patient specimen with added internal standard. The patient was receiving a daily (150 mg) imipramine main-

Table III—Relative Chromatographed Drug Retention Times

Drug	Relative Retention Time
Fluphenazine	0.05
Perphenazine	0.65
Amitriptyline	0.68
Chlorpromazine	0.68
Mepazine	0.70
Doxepin	0.70
Promazine	0.93
Imipramine	1.00
Prochlorperazine	1.10
2-Hydroxyiminodibenzyl	1.14
2-Hydroxyimipramine	1.15
Didesmethylimipramine	1.32
Triflupromazine	1.35
Trifluoperazine	1.80
Mesoridazine	1.90
Thioridazine	2.10
N-Desmethylclomipramine	2.15
Desipramine	2.40
2-Hydroxydesipramine	2.90

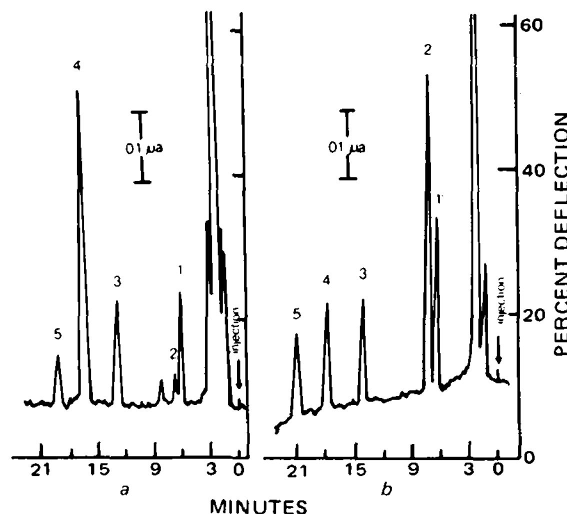

Figure 2—(a) *Chromatogram of the extract of 1 ml of serum from a patient receiving imipramine. The extract was reconstituted with 100 µl of methanol; 50 µl was injected. Concentrations measured were: imipramine (1), 32 ng/ml; 2-hydroxyimipramine (2), 5 ng/ml; internal standard (3); desipramine (4), 134 ng/ml; and 2-hydroxydesipramine (5), 29 ng/ml.* (b) *Chromatogram of a 1-ml blank plasma extract spiked with 50 ng/ml each of the drugs and metabolites. The extract was reconstituted with 100 µl of methanol; 50 µl was injected. Peak identification is the same as in Fig. 2*a.

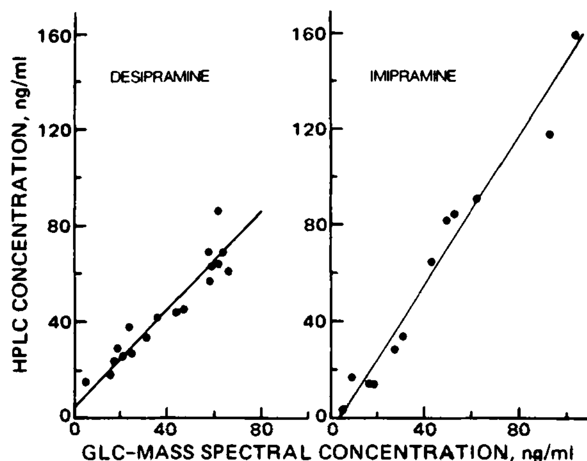

Figure 3—*Correlation between HPLC and GLC–mass spectral methods for plasma imipramine and desipramine analysis. For desipramine, the slope = 1.01, the intercept = 4.7, and the correlation coefficient = 0.943. For imipramine, the slope = 1.55, the intercept = −7.2, and the correlation coefficient = 0.960.*

tenance dose. Figure 2b shows a chromatogram of a plasma standard containing 50 ng/ml of each drug and metabolite and added internal standard. One milliliter each of the plasma standard and patient sample were extracted, and one-half of the extract was injected according to the procedure.

Eighteen plasma specimens from seven patients participating in an NIMH study (5) and receiving imipramine or desipramine were assayed by the HPLC method and a GLC–mass spectrometric method (18) (Fig. 3). The two assays showed excellent correlation, with a slope of nearly unity (1.01) for desipramine. The HPLC assay yielded higher results than the GLC–mass spectral method for imipramine. Imipramine and desipramine are stable at least 3 months in plasma stored at −4°. The NIMH samples were 6–15 months old at the time of HPLC analysis; therefore, interference in the fluorescence assay due to some unidentified degradation component(s) may explain the discrepancy between the methods for imipramine. Further comparison studies with fresh patient samples and spiked plasma are in progress. Data for the 2-hydroxy metabolites quantitated by GLC–mass spectra were not available for comparison.

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Chemistry of 8-Chloroberberine

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Abstract □ 8-Chloroberberine (V), obtained by treatment of oxyberberine (I) with phosphorus oxychloride, is a reactive intermediate. Treatment with ammonia, methylamine, n-propylamine, aniline, and p-toluidine furnished the 8-berberinylidene derivatives IV and VII–X. Reaction of V with malononitrile, ethyl acetoacetate, and ethyl malonate anions yielded the 8-berberinylidene derivatives XII–XIV. Acid hydrolysis of XIV gave 8-berberinylacetic acid (XV) whose reduction provided 8-canadinylacetic acid (XVI). Grignard reagents react readily with V. Methylmagnesium iodide, ethylmagnesium iodide, and benzylmagnesium iodide led to 8,8-dimethyldihydroberberine (XVII), 8,8-diethyldihydroberberine (XIX), and the benzyl derivative XX, respectively. Sodium borohydride reduction of XX gave rise to 8-benzylcanadine (XXI).

Keyphrases □ Chloroberberine—synthesis, derivatives □ Protoberberine salts—chloroberberine, synthesis, derivatives □ Antibacterials—chloroberberine, synthesis, derivatives □ Antiarrhythmics—chloroberberine, synthesis, derivatives □ Tranquilizers—chloroberberine, synthesis, derivatives

Protoberberine salts and tetrahydroprotoberberine bases have wide pharmacological potency including tranquilizing, antimicrobial, antifungal, hypotensive, antiulcer, uterine, antiarrhythmic, neuroleptic, and antipsychotic effects. Protoberberine salt pharmacology was reviewed recently (1, 2).

The present report describes the structure of 8-chloroberberine (V), a compound characterized partially by

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