Determination of Mianserin and Metabolites in Plasma by Liquid Chromatography with Electrochemical Detection

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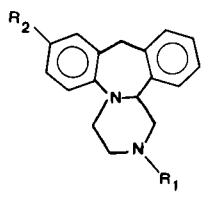
Abstract □ A procedure for the determination of mianserin, desmethylmianserin, and 8-hydroxymianserin in plasma at therapeutic concentrations by liquid chromatography with electrochemical detection is described. Following a multiple-step extraction from alkaline plasma into methyl-*tert*-butyl ether, the reconstituted extract was injected onto a reversed-phase trimethylsilyl-packed column and eluted with an acetate–acetonitrile mobile phase containing an ion-paired reagent. The method provides an absolute recovery of 71–76% and a day-to-day precision of 5.4–9.1% for each compound at 25 ng/ml. The minimum quantifiable level for all three compounds was 5 ng/ml (*RSD* > 11%), and the detector response was linear up to 500 ng/ml. Fixed-dose steady-state plasma level data for 34 patients are reported.

Keyphrases □ Mianserin—determination in plasma, liquid chromatography, electrochemical detection, metabolites □ Liquid chromatography—determination of mianserin in plasma, electrochemical detection, metabolites □ Electrochemical detection—determination of mianserin in plasma by liquid chromatography, metabolites

High-pressure liquid chromatography (HPLC) with electrochemical detection is becoming an increasingly popular and useful combination for the quantitation of a wide variety of biologically important compounds (1). The sensitivity and selectivity of this detector makes it an important alternative tool to UV and fluorescence detection in biological science. The use of reversed-phase columns and ion-pairing reagents facilitate the examination of metabolite profiles in that rapid and efficient separation can be achieved in relatively short time periods. A method was recently described using ion-pair reversed-phase electrochemical detection for the determination of imipramine as well as its metabolites in plasma (2).

Mianserin hydrochloride (1,2,3,4,10,14b-hexahydro-2-methyldibenzo[*c*,*f*]pyrazino[1,2-*a*]azepine monohydrochloride) is a new tetracyclic antidepressant currently undergoing clinical evaluation in the United States. The chemical structures of this compound and known pharmacologically active metabolites are shown in Structure I. The pharmacology and therapeutic efficacy of mianserin in depressive illness have been reviewed (3) and the identification of its major urinary metabolites in various species including humans have been reported (4). The determination of mianserin in biofluids has been performed using GC with nitrogen detection (5) and by mass fragmentography (6). Neither assay included the simultaneous determinations of the metabolites found in plasma. To date HPLC has only been used for sample clean-up prior to mass fragmentography (7) and not for quantitation of mianserin.



	R_1	R_2
MIANSERIN	CH_3	H
DESMETHYLMIANSERIN	H	H
8-HYDROXYMIANSERIN	CH_3	OH

In the present report, a liquid chromatographic method using electrochemical detection is described, which simultaneously quantitates mianserin, desmethylmianserin, and 8-hydroxymianserin in plasma. In addition, results of actual patient samples are reported.

EXPERIMENTAL

Apparatus—Chromatography was performed by a dual piston solvent delivery pump[1] connected to an automatic sampler[2]. Separations were achieved with either a 15-cm × 4.6-mm i.d. trimethylsilyl 5-μm particle size column[3] or a 25-cm × 4.6-mm i.d. octadecylsilyl 10-μm particle size column[4]. The detector system consisted of a thin-layer flow-through electrochemical cell[5] with glassy carbon as the working and auxiliary electrodes and a silver–silver chloride reference electrode. The potential and current response was monitored by an amperometric controller[6] and

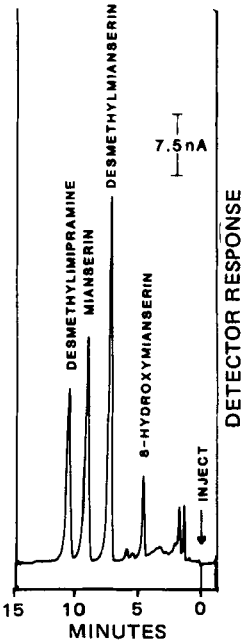


Figure 1—*Sample chromatogram of a 1-ml spiked plasma extract containing 25 ng of 8-hydroxymianserin and 100 ng each of mianserin and desmethylmianserin. One-half (50 μl) of the reconstituted extract was injected.*

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Wisp 710B, Waters Associates, Milford, Mass.
[3] LC-1, Supelco, Bellafonte, Pa.
[4] Partisil-10 ODS-3, Whatman Inc., Clifton, N.J.
[5] TL-5A, Bioanalytical Systems Inc., West Lafayette, Ind.
[6] Metrohm model E-611 V/A Detector, Brinkman Instruments, Inc., Westbury, N.Y.

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Table I—Summary of the Linear Regression Data for the Standard Curve [a]

Compound	Slope ±*SD*, ng/ml	*x* Intercept ±*SD*, ng/ml	Correlation coefficient ±*SD*	Standard error of the estimate ±*SD*
Mianserin	76.28 ± 1.52	−0.41 ± 1.74	0.9999 ± 0.0001	1.93 ± 0.90
Desmethylmianserin	47.96 ± 1.29	0.99 ± 2.68	0.9999 ± 0.0002	1.72 ± 1.27
8-Hydroxymianserin	44.78 ± 4.16	4.10 ± 1.09	0.9986 ± 0.0012	1.50 ± 0.62

[a] Data computed from five consecutive standard curves.

recorder[7] and interfaced with a laboratory data acquisition system[8]. Aluminum column temperature control blocks were devised to fit columns of either size, and the temperature was controlled by a circulating water bath[9].

Reagents—Acetic acid[10], sodium acetate[10], sodium carbonate[10], and sodium bicarbonate[10] were all analytical reagent grade. Sodium heptane sulfonate[11] was used as received. Acetonitrile-UV[12] and methyl-*tert*-butyl ether[12] were used without further purification. Distilled water was passed through a water purification system[13] before use.

Standards—One milligram (free base) per milliliter of stock solution of mianserin hydrochloride[14], desmethylmianserin maleate[14], 8-hydroxymianserin maleate[14], and desmethylimipramine hydrochloride[15] were prepared in 0.1 *N* HCl. A stock solution of 1 mg/ml of 2-hydroxyimipramine[16] was prepared in methanol. For spiking plasma, the mianserin and desmethylmianserin stock solutions were diluted with 0.01 *N* HCl to give a working solution of 2 ng/μl of each. The stock solution of 8-hydroxymianserin was diluted with 0.1 *N* HCl to give working solutions of 1 ng/μl and 0.1 ng/μl. Desmethylimipramine was diluted with 0.01 *N* HCl to 2 ng/μl and 2-hydroxyimipramine was diluted with 0.1 *N* HCl to give 1 ng/μl to provide working solutions for the internal standard.

Standard curves were prepared containing five levels of spiked samples: 25, 50, 100, 200, and 400 ng/ml of mianserin and desmethylmianserin and 5, 10, 25, 50, and 100 ng/ml of 8-hydroxymianserin. Each set of standards included a blank.

Extraction—Internal standard, desmethylimipramine (25 μl, 50 ng), and 1.0 ml of 0.6 *M* carbonate buffer (pH 9.7) were added to 1.0 ml of plasma standard or unknown sample in specially washed glassware[17]. Eight milliliters of methyl-*tert*-butyl ether was added and the mixture was shaken for 15 min and centrifuged at 1500×*g* for 10 min. The organic layer was then transferred to a 15-ml tapered centrifuge tube containing 1.2 ml of 0.1 *N* HCl. After mixing for 10 min and centrifuging at 1500×*g* for 10 min, the organic layer was aspirated, the aqueous portion transferred to a 3-ml tapered glass-stoppered minicentrifuge tube, and neutralized with 0.5 ml of 0.6 *M* carbonate buffer (pH 9.7). Methyl-*tert*-butyl ether (0.5 ml) was added and the tube was stoppered, shaken, and centrifuged for 5 min at 1500×*g*. The lower aqueous layer was discarded and the organic layer transferred to a small glass vial[18].

The vial was placed within a 4-ml vial assembly (containing the adapter spring) and placed in a vacuum centrifuge[19]. The ether was evaporated under vacuum at 45°. The extract was then reconstituted with 100 μl of mobile phase, capped, and mixed.

Chromatographic Conditions—The mobile phase consisted of 0.1 *M* acetate buffer (pH 4.2)–acetonitrile (67:33) with 0.005 *M* sodium heptane sulfonate. The mixture was filtered and degassed prior to use.

[7] Houston Omniscribe model B5217 B-2, Houston Instruments, Austin, Tex.
[8] PDP 11/34 "Peak II" System, Digital Equipment Co., Maynard, Mass.
[9] Model FE, Haake Co., Saddlebrook, N.J.
[10] Fisher Scientific Co., Fairlawn, N.J.
[11] Eastman Kodak Co., Rochester, N.Y.
[12] Burdick and Jackson Laboratories, Muskegon, Mich.
[13] Milli-Q, Millipore Corp., Bedford, Mass.
[14] Organon, Inc., West Orange, N.J.
[15] USV Pharmaceutical Corp., Tuckahoe, N.Y.
[16] A gift from Dr. A. A. Manian, National Institute of Mental Health, Rockville, Md.
[17] All glassware (including disposable pipets) were soaked in detergent, washed, and then immersed in dichromate cleaning solution for 16–24 hr. The thoroughly rinsed glassware was neutralized with dilute ammonium hydroxide solution, thoroughly rinsed again with deionized double distilled water, and dried overnight at 160°.
[18] Limited Volume Insert, Waters Associates, Milford, Mass.
[19] Model SVC-100M Speed Vac Concentrator, Savant Instruments, Inc., Hicksville, N.Y.

Table II—Reproducibilty of Assays for Mianserin, Desmethylmianserin, and 8-Hydroxymianserin

Within Run [a] Concentration in Plasma, ng/ml	*RSD*,% Mianserin	Desmethylmianserin	8-Hydroxymianserin
100	3.3	2.9	7.2
50	6.9	5.9	8.5
10	4.7	4.3	11.8
5	8.1	10.4	8.2
Day-to-day [b]			
25	6.6	5.4	9.1

[a] $n = 6$. [b] $n = 5$.

Table III—Recovery of Mianserin, Desmethylmianserin, and 8-Hydroxymianserin from 1 ml of Plasma [a]

Compound	Recovery, %	*SD*, %	*RSD*, %
Mianserin, 50 ng	76	4.9	5.4
Desmethylmianserin, 50 ng	71	4.9	7.0
8-Hydroxymianserin, 25 ng	72	2.5	3.4

[a] $n = 6$.

The flow rate was 1.5 ml/min and temperature set at 30° resulting in an inlet pressure of ~1000 psi. The effluent was monitored through the detector cell at a potential of +1.05 V *versus* silver–silver chloride reference electrode.

Quantitation—All determinations were performed by calculating the peak height and/or area ratios of each compound to the internal standard. A linear regression analysis for each of the standard curves was performed by a computer program resulting in the calculation of slope, *x* intercept, correlation coefficient, and standard error.

RESULTS AND DISCUSSION

Mianserin, desmethylmianserin, and 8-hydroxymianserin were separated in a single chromatogram within 12 min. A typical chromatogram of spiked plasma is shown in Fig. 1. A blank plasma extract showed no endogenous interfering peaks (Fig. 2).

The absolute sensitivity (S/N ~3) of this method was checked by injecting a standard solution containing 1 ng of all three compounds. In practical terms the minimum quantifiable levels were 5 ng/ml of plasma. The electrochemical detector cell with glassy carbon as the working and auxilliary electrodes opposite each other permitted a linear detector response over a range from 5 to at least 500 ng. A summary of the data for the regression curves appears in Table I.

The precision of this method was determined by spiking six 1.0-ml aliquots of drug-free plasma with various levels of drug and metabolites. After the addition of 50 ng of internal standard, the sample was processed as described. The percent relative standard deviation for various levels are reported in Table II.

The absolute recovery was checked by preparing a solution of 50 ng of mianserin and desmethylmianserin, and 25 ng of 8-hydroxymianserin. The internal standard was added to each solution and the sample injected into the chromatograph. One milliliter of plasma was added to each of

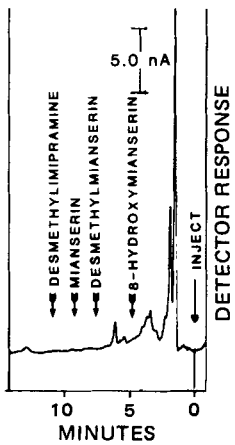

Figure 2—*Sample chromatogram of a 1-ml blank sample extract. The entire reconstituted extract (100 μl) was injected.*

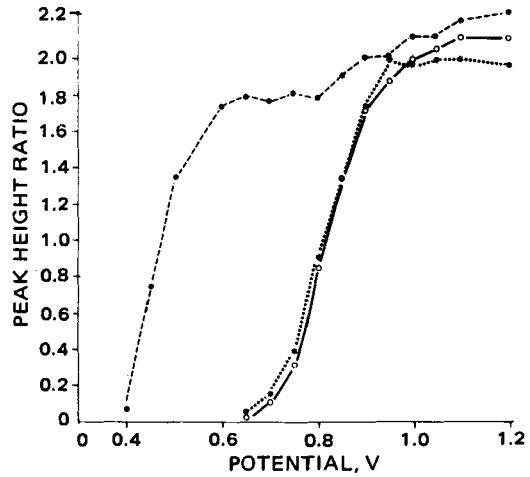

Figure 3—*A plot of the peak height ratio* (i.e., *detector response*) *against the oxidation potential* (versus *silver–silver chloride reference electrode*) *under the same chromatographic conditions described in the text:* (--●--) *8-hydroxymianserin,* (···*···) *desmethylmianserin,* (—o—) *mianserin.*

the same number of aliquots and processed routinely except for the internal standard. When the final extract was dried down, the internal standard was added (50 ng) with mobile phase and injected. The difference between the ratios of standards to internal standard in the processed samples *versus* direct injection samples gave a measure of the overall recovery (Table III).

The determination of the optimum oxidation potential for these compounds was done by injecting a standard solution of these compounds in methanol into the chromatograph at various potentials. A fixed wavelength UV detector[20] at 254 nm connected in series preceding the electrochemical cell, was used as an internal standard.

The ratios of the peak heights from the electrochemical and UV detectors were plotted against the various potentials. Figure 3 shows that the 8-hydroxymianserin undergoes oxidation at a more negative potential than either mianserin or its desmethyl metabolite. The lower oxidation potential of this metabolite is due to the presence of the ring hydroxyl group. A possible mechanism of electrochemical oxidation of hydroxylated derivatives could be explained by the formation of a quinone imine (8). Similar current–potential curves were observed with imipramine,

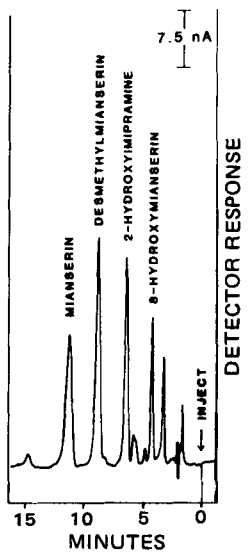

Figure 4—*Sample chromatogram of a 1-ml spiked plasma extract containing 50 ng each of mianserin and desmethylmianserin and 25 ng of 8-hydroxymianserin with 50 ng of 2-hydroxyimipramine as the internal standard. The column was octadecylsilane and the mobile phase was 0.1* M *acetate buffer (pH 4.2)–acetonitrile (65:35) with 0.005* M *heptane sulfonate. The flow rate was 1.3 ml/min.*

[20] Model 440, Waters Associates, Milford, Mass.

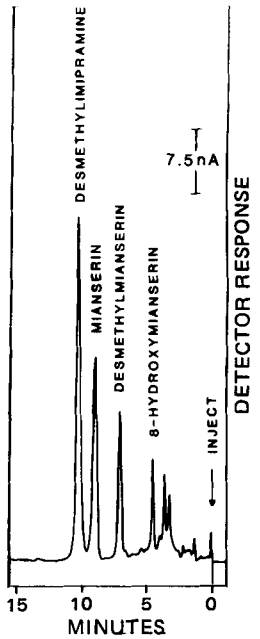

Figure 5—*Sample chromatogram of 1 ml of plasma sample from a patient receiving 120 mg/day of mianserin. Seventy percent reconstituted extract was injected. The plasma levels were calculated to be 13 ng of 8-hydroxymianserin, 21 ng of desmethylmianserin, and 45 ng of mianserin.*

desmethylimipramine, and their 2-hydroxy metabolites under similar experimental conditions (2). A mechanism of electrochemical oxidation of imipramine and chemically related dibenzazepines was recently described by a two-step three-electron ECE process[21] (9). Mianserin and its metabolites, which contain the dibenzazepine nucleus, may undergo similar mechanisms. The optimum potential found for electrochemical detection of mianserin and metabolites was +1.05 V *versus* silver–silver chloride which was compatible with that found previously for the internal standard, desmethylimipramine.

Interfering peaks from other pharmacologic agents, that may be administered concomitantly with this antidepressant, appeared from chlorpromazine and its metabolites and the hydroxylated metabolites of loxapine. Other low dose phenothiazine major tranquilizers such as fluphenazine, perphenazine, and haloperidol are generally found in much lower quantities in plasma and are not detected or elute beyond 12 min. Some commonly used benzodiazepines (flurazepam, chlordiazepoxide, and diazepam) are frequently administered anxiolytic and hypnotic adjuvants. These compounds, as well as their metabolites, would interfere

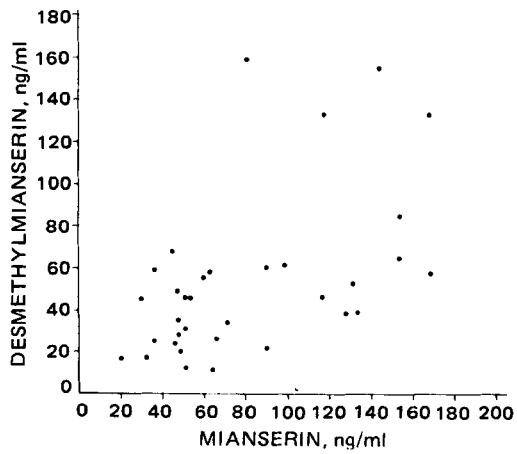

Figure 6—*Relationship between steady-state plasma mianserin and desmethylmianserin level in 34 patients on a fixed dose of 150 mg/day* (r = *0.54*).

[21] An electron-transfer step followed by a chemical reaction and a second electron-transfer step.

if a UV detector (at 254 nm) was used. However none of these drugs or metabolites were electrochemically responsive under the described conditions. A knowledge of the patient drug profile can be very helpful in the identification of interfering peaks.

The multistep extraction procedure used in this method was developed for the recovery of mianserin and its desmethylated and 8-hydroxy metabolites from plasma. A relatively clean chromatogram resulted with no endogenous interfering peaks but with an acceptable recovery of all compounds. Ether may be used in place of methyl-*tert*-butyl ether with a similar rate of recovery. However, it was found that methyl-*tert*-butyl ether required no prior distillation, was less likely to form peroxides, and was easier to handle in the laboratory. Mianserin and desmethylmianserin may also be extracted from plasma by either increasing the plasma pH to 12 and/or using a more nonpolar solvent such as *n*-heptane with 1.5% isoamyl alcohol thus eliminating the 8-hydroxymianserin from the assay. The other major metabolite found in human urine was mianserin-*N*-oxide. This metabolite may be present in plasma but is not extracted from a basic plasma medium.

This method was suitable for automatic sample processing. The use of the automatic injector was compatible with the electrochemical detector and the data acquisition systems. The primary concern in automating this system was the stability of the baseline with respect to drift. It was found that at a detector attenuation of 30 nA full scale or higher, the baseline remained virtually drift-free during a typical 6–8-hr run. In addition, there was no evidence of electrode contamination due to the possible oxidation products forming at the electrode during continuous operation.

If necessary, chromatography can be performed with an octadecylsilyl reversed-phase column with only a minor modification in mobile phase and a change in internal standard. However, several interfering endogenous plasma peaks were present in some samples, which may prove troublesome when low levels of drug are encountered. A spiked plasma sample using this column appears in Fig. 4.

Plasma samples from 34 different patients on a 150-mg/day fixed dose were analyzed for mianserin, desmethylmianserin and its 8-hydroxy metabolite. A representative chromatogram of a patient plasma sample appears in Fig. 5. The mean mianserin plasma level was found to be 81 ng/ml with a range of 20–169 ng/ml. The mean desmethyl metabolite was found to be 53 ng/ml with a range of 11–150 ng/ml. Of these samples, only four had measurable levels (5 ng/ml) of the 8-hydroxy metabolite and the remainder had traces (<5 ng) or none detected. Two patients receiving <150 mg/day (one 120 mg/day, and another 90 mg/day) were found to have 12 and 13 ng/ml of the 8-hydroxy metabolite, respectively. There was no apparent correlation between the plasma level of the nonconjugated 8-hydroxymianserin and the plasma levels of mianserin or desmethylmianserin. Figure 6 demonstrates the relatively weak correlation between mianserin and its desmethyl metabolite.

In three studies, where patients received 60 mg/day of mianserin, steady-state blood levels of mianserin were found in a 6–120-ng/ml range (10), 4–98 ng/ml (mean 36 ng/ml) (6), and a mean of 50 ng/ml (11). The presence of a significant amount of 8-hydroxymianserin as well as the parent compound has been reported (4), although desmethylmianserin could not be detected in the urine of two female volunteers. This was in contrast to earlier findings and rather unusual.

This method provides a means for the simultaneous determination of mianserin, desmethylmianserin, and 8-hydroxymianserin in plasma. The procedure is reliable and sensitive enough for routine plasma monitoring and single dose pharmacokinetic studies. In the single-dose studies, larger volumes of plasma (~3 ml) will be required to determine time points late in the pharmacokinetic curve.

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