# High-Performance Liquid Chromatographic Assay of Perhexiline Maleate in Plasma

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**Abstract** □ A sensitive assay is described for the calcium antagonist perhexiline maleate. Alkalinized plasma was extracted with *n*-hexane, the organic phase was evaporated, and the residue was dansylated prior to analysis by reversed-phase high-performance liquid chromatography using a fluorescence detector. Perhexiline was resolved from its mono- and dihydroxylated metabolites, and the limit of sensitivity was 5 ng of perhexiline/ml. This limit represents approximately 100 times the sensitivity of the previously described GLC assay. Single-dose pharmacokinetic studies were performed with 150- and 300-mg oral doses of perhexiline maleate in five patients with severe angina pectoris and impaired left ventricular function. Peak plasma perhexiline levels occurred 3–6 hr after drug ingestion in four patients and after 12–18 hr in the fifth patient. The mean elimination half-life, measured 24 hr after drug ingestion, varied with plasma perhexiline concentration. It was 11.2 ± 2.1 hr after the 150-mg dose and 19.1 ± 2.8 hr after the 300-mg dose. The mean ratio of areas under the concentration–time curve for the 300- *versus* 150-mg doses was 5.3:1, suggesting that hepatic metabolism of perhexiline may be saturable and that the bioavailability of perhexiline is dose dependent.

**Keyphrases** □ Perhexiline maleate—high-performance liquid chromatographic assay, human plasma □ High-performance liquid chromatography—analysis, perhexiline in human plasma □ Vasodilators, coronary—perhexiline, high-performance liquid chromatographic analysis in human plasma

Perhexiline maleate, a calcium antagonist, is used as a prophylactic antianginal drug (1, 2) and shows promise as an antiarrhythmic agent (3). In spite of the impressive therapeutic efficacy of perhexiline, its clinical use has remained limited largely due to the occurrence, with long-term use, of severe adverse effects such as peripheral neuropathy and hepatotoxicity (4).

This paper reports the development of a sensitive high-performance liquid chromatographic (HPLC) assay for perhexiline maleate in plasma and its application to the study of single-dose pharmacokinetics of perhexiline in patients with severe angina pectoris.

The data suggest that the bioavailability of perhexiline is dose dependent. This information may be of value in avoiding toxic drug levels.

## EXPERIMENTAL

**Instrumentation**—Assays were carried out using a constant-flow, high-performance liquid chromatograph[1] equipped with a solvent delivery system[2], a universal injector[3], and a filter fluorescence detector[4] operating with standard excitation[5] and emission[6] filters corresponding to nominal wavelengths of 360 and 510 nm, respectively. The stainless steel column (30 cm long × 3.7 mm i.d.) was prepacked[7].

**Reagents**—Perhexiline maleate [2-(2,2-dicyclohexylethyl)piperidine maleate, I], the internal standard hexadiline hydrochloride [1,1-dicyclohexyl-2-(2-piperidyl)ethylene hydrochloride, II], and a mixture of the 4-monohydroxy and 4,4′-dihydroxy metabolites of perhexiline were used[8]. The derivatizing reagent was dansyl chloride[9]. *n*-Hexane, cyclohexane, benzene, methylene chloride, ethyl acetate[10], ether[11], and dimethyl sulfoxide[12] were analytical reagent grade, and methanol was HPLC grade[1].

**Standard Solutions**—Standard solutions of perhexiline maleate (2 μg/ml) and hexadiline hydrochloride (1.4 μg/ml) were prepared by dissolving pure drug in dimethyl sulfoxide (2 ml) and diluting the resultant solutions to 250 ml with 0.1 *N* HCl. All solutions were stable when stored at −20° for 30 days.

**Extraction**—Tris(hydroxymethyl)aminomethane buffer (0.5 ml, 2 *M*, pH 8.75), hexadiline (0.1 ml), and *n*-hexane (7 ml) were added to 1.0 ml of plasma in a polypropylene tube[13]. Each sample then was mixed[14] for 1 min and centrifuged at 3000 rpm for 15 min. The upper *n*-hexane layer was transferred to a polypropylene tube and evaporated to dryness under nitrogen at 60°. Sodium bicarbonate solution (0.1 ml, 0.1 *M*, pH 10) and dansyl chloride (0.1 ml, 0.01 *M*) in acetone were added to the remaining residue and mixed[14].

The derivatization was allowed to proceed for 20 min at 37°. Each tube was extracted with 3 ml of *n*-hexane by mixing[14] for 20 sec. Following a quick centrifugation (1 min) to separate the phases, the *n*-hexane layer was transferred to another clean polypropylene tube and evaporated to dryness at 60° under nitrogen. The dry residue was stable for 7 days when stored at −20°.

Each sample was reconstituted in 0.1 ml of mobile phase prior to chromatography.

**Chromatography**—The injection volumes were 50 μl. The mobile phase was methanol–water (92.5:7.5) maintained at a flow rate of 1.2 ml/min with a back pressure of 1000 psi. The retention time for both the mono- and dihydroxylated metabolites was 5.3 min whereas the retention times for hexadiline and perhexiline were 13.7 and 15.7 min, respectively.

**Quantitation**—Standard curves for the determination of perhexiline maleate were prepared by spiking drug-free plasma samples. The curve used for the estimation of perhexiline levels following single-dose pharmacokinetic studies was in the range of 0–5000 ng/ml.

Recoveries from spiked plasma samples were obtained by comparing the peak height of perhexiline after extraction with the corresponding peak height obtained on the same amount of perhexiline chromatographed from a dansylated stock solution.

The perhexiline maleate concentrations in unknown plasma were determined from a graph in which the peak height ratios of perhexiline to the internal standard (hexadiline) were plotted against the concentrations of known standards.

**Patients and Sampling Procedure**—Five patients (68–75 years old), suffering from severe angina pectoris for which perhexiline administration was clearly indicated, were studied after obtaining written consent. After an overnight fast, 150 mg of perhexiline maleate was given as a single dose. Patients continued to fast for 3 hr after ingestion. Immediately prior to the 150-mg dose and at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, and 24 hr, 10-ml blood samples were taken from an indwelling catheter in a forearm vein and placed in heparinized tubes for assay of plasma perhexiline concentrations. Samples were centrifuged immediately to separate the plasma, which was stored frozen at −20° until it was assayed.

Forty-eight hours after the first dose, a second dose of 300 mg of per-

[1] Waters Associates, Chippendale, Sydney, Australia, 2008.
[2] Model 6000A, Waters Associates.
[3] Model U6K, Waters Associates.
[4] Fluorichrom, Varian Pty. Ltd., Victoria, Australia, 3170.
[5] No. 7-54 and 7-50, Varian Pty. Ltd.
[6] No. 4-76 and 3-71, Varian Pty. Ltd.
[7] μBondapak $C_{18}$, Waters Associates.
[8] Merrell-National Laboratories, Cincinnati, Ohio.
[9] Sigma Chemical Co., St. Louis, Mo.
[10] Merck, Darmstadt, West Germany.
[11] May and Baker, Melbourne, Australia.
[12] Mallinckrodt, St. Louis, Mo.
[13] Size 15P, Disposable Products, Melbourne, Australia.
[14] Vortex mixer.



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**Table I—Recovery of Perhexiline Maleate from Plasma with Various Solvents**

| Solvent | Mean Recovery, % |
|---|---|
| *n*-Hexane | 40 |
| Cyclohexane | 23 |
| Benzene | 26 |
| Methylene chloride[a] | 40 |
| Ether[b] | 60 |
| Ethyl acetate[b] | 75 |

[a] Peaks interfered with detection of perhexiline metabolites. [b] Peaks interfered with determination of perhexiline.

hexiline maleate was given under the same conditions, and the study was repeated.

**Pharmacokinetic Analysis**—For single-dose pharmacokinetic studies, plasma concentration–time data were fitted to a polyexponential equation using a nonlinear least-squares regression analysis computer program (5). Pharmacokinetic parameter estimates were obtained for the computer program AUTOAN 2 NONLIN (6), assuming first-order absorption and elimination. Equations generated by this program contained two or more exponentials and included a possible lag time.

The area under the concentration–time curve was calculated by the trapezoidal method and extrapolated to infinity by dividing the final concentration point by the slope of the terminal phase.

## RESULTS AND DISCUSSION

Previous investigators experienced difficulty with the development of a sensitive and specific assay for perhexiline. Studies with radiolabeled perhexiline did not differentiate between perhexiline and its hydroxylated metabolites (7). Because of the low recovery of perhexiline due to extraction and the tendency of perhexiline, a weakly basic lipophilic drug, to adsorb to laboratory glassware, the sensitivity of the GLC assay was limited to plasma drug concentrations above 500 ng/ml (8).

The HPLC assay reported here resolved perhexiline from its mono- and dihydroxylated metabolites with a lower limit of sensitivity of 5 ng/ml. Perhexiline has no natural absorption properties in the UV or visible regions. However, under suitable reaction conditions, derivatization of the secondary amine nitrogen in the piperidine ring of perhexiline by the highly reactive fluorophore dansyl chloride for 20 min at 37° resulted in complete conversion of perhexiline to a highly absorptive dansylated compound. Free dansyl chloride elutes with the solvent front on HPLC. However, preliminary separation of the perhexiline dansylate from most of the unreacted dansyl chloride using hexane extraction reduced the solvent front and resulted in a cleaner chromatogram.

Figure 1 illustrates typical chromatograms obtained after extraction of blank plasma (Fig. 1*a*) and of plasma from a patient treated with perhexiline (Fig. 1*b*). The peak at 15.7 min in Fig. 1*b* represents a plasma perhexiline concentration of 200 ng/ml. Endogenous peaks in blank plasma emerged before the hexadiline and perhexiline peaks. The mono- and dihydroxylated metabolites of perhexiline were not resolved under the conditions employed. However, by altering the mobile phase to methanol–water (80:20, v/v), adequate separation of these metabolites was obtained. Thus, simultaneous assay of individual metabolites and perhexiline also would be feasible under nonisocratic conditions using a solvent programmer.

Lignocaine, procainamide, digoxin, propranolol, verapamil, disopyramide, hydralazine, prazosin, and metoclopramide were examined for possible interference. None of these drugs produced peaks that emerged simultaneously with perhexiline, hexadiline, or the hydroxylated metabolites of perhexiline.

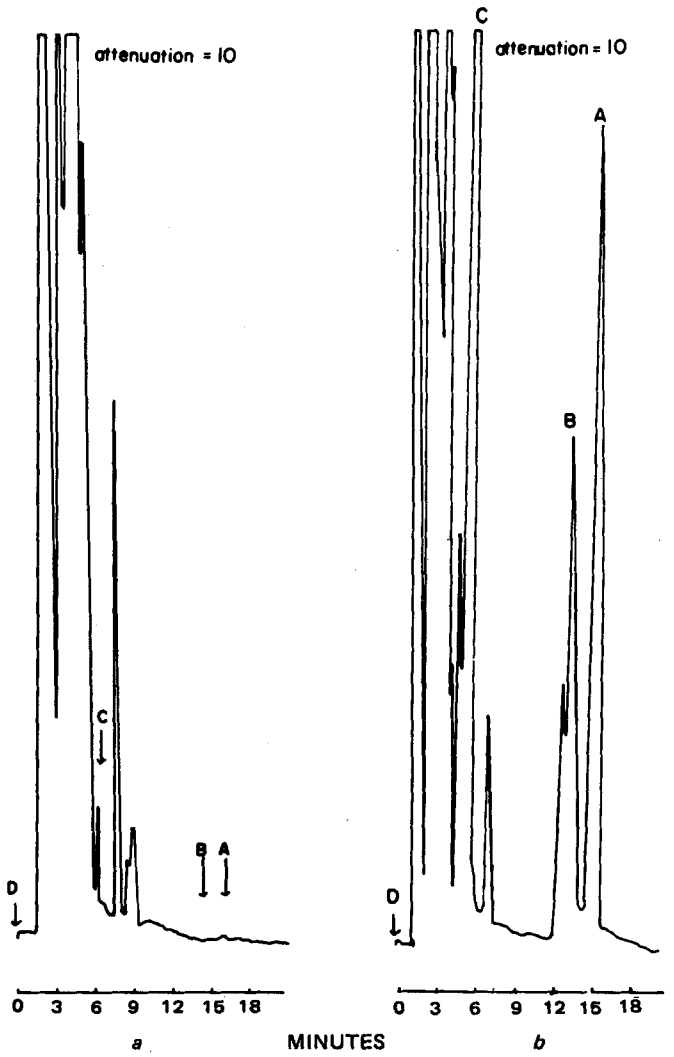


**Figure 1**—*High-performance liquid chromatograms for a blank extract (a) and for an extract of a plasma sample obtained from a patient receiving a daily dose of 100 mg of perhexiline (b). (Perhexiline concentration = 200 ng/ml.) Key: A, perhexiline; B, hexadiline; C, hydroxylated perhexiline metabolites; and D, injection.*

The component eluting just before the internal standard is believed to be a contaminant of hexadiline since it appears when only hexadiline is chromatographed and is not present in chromatograms devoid of hexadiline. The height of this additional peak was a constant fraction of that of the hexadiline peak and did not affect quantitation of perhexiline.

Of a number of solvents selected to extract perhexiline from alkalinized plasma (Table I), only *n*-hexane was satisfactory. This solvent gave an extraction efficiency, measured throughout the assay, of 40 ± 3%. Other

**Table II—Summary of Perhexiline Pharmacokinetics**

| | | 150-mg Dose | | | | 300-mg Dose | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age, years | Time to Peak, hr | Peak Concentration, ng/ml | *AUC*, (ng hr)/ml | $t_{1/2}$[a], hr | Time to Peak, hr | Peak Concentration, ng/ml | *AUC*, (ng hr)/ml | $t_{1/2}$[a], hr | $\frac{AUC_{300}}{AUC_{150}}$ |
| 1 | 73 | 2.5 | 82 | 810 | 14.0 | 4.2 | 348 | 5260 | 18.0 | 6.49 |
| 2 | 68 | 4.5 | 22 | 490 | 12.0 | 4 | 144 | 1740 | 23.5 | 3.55 |
| 3 | 75 | 6 | 116 | 1190 | 4.5 | 3 | 438 | 7110 | 9.5 | 5.97 |
| 4 | 68 | 12 | 65 | 1850 | 16.5 | 18 | 234 | 6240 | 26.0 | 3.37 |
| 5 | 70 | 6 | 49 | 690 | 9.0 | 6 | 204 | 5020 | 18.5 | 7.27 |
| Mean | 70.7 | 6.2 | 66.8 | 1010 | 11.2 | 7.0 | 274 | 5110 | 19.1 | 5.33 |
| *SE* | 1.1 | 1.6 | 15.8 | 240 | 2.1 | 2.8 | 52.8 | 920 | 2.8 | 0.79 |

[a] At 24 hr.

solvents gave better recoveries (ether and ethyl acetate), but they also extracted other compounds from the plasma eluting in the region of perhexiline and the hydroxylated metabolites. This effect precluded their use as alternative extraction solvents.

The threshold of detection of perhexiline maleate in plasma corresponding to a peak height three times that of baseline noise was 5 ng/ml. The coefficient of variation for replicate assays of a pooled plasma sample containing 150 ng of perhexiline/ml was studied over 21 days and was 8.5% ($n$ = 8) while that for replicate assays performed on the same day was 2.8% ($n$ = 5).

Calibration curves for the peak height ratio of perhexiline to hexadiline were linear over a perhexiline concentration range of 0–5000 ng/ml. The use of disposable polypropylene tubes minimized inaccuracies due to adsorption of perhexiline onto glass surfaces.

Limited pharmacokinetic treatment of the single-dose studies in patients with angina pectoris was performed (Table II). There was a lag time of 1.0–2.5 hr before detectable concentrations of perhexiline appeared in plasma. Absorption rates of perhexiline varied, and peak drug concentrations occurred 3–6 hr after ingestion of the tablets in four of the five patients. In Patient 4, peak drug concentrations were delayed, occurring 12 hr after ingestion of the 150-mg dose and 18 hr after the 300-mg dose. However, peak levels for this patient were close to the mean of all five subjects for both the 150- and 300-mg doses. Peak concentrations of perhexiline after ingestion of the 300-mg doses were 4.3 times greater than those after the 150-mg dose.

The elimination half-life of perhexiline as determined 24 hr after drug ingestion appeared to be concentration dependent, increasing from ~18 hr with the 150-mg doses to 24 hr after the 300-mg dose. Patient 4, who had delayed absorption, also exhibited a prolonged elimination half-life at 24 hr compared to the other subjects. This patient was not excluded from the analysis since the data merely highlight the variable absorption of this drug and do not modify the conclusions concerning nonlinear kinetics. Because the mean ratio of the areas under the plasma concentration–time ($AUC$) curve for the 300- *versus* 150-mg doses in all patients was 5.3:1, nonlinear elimination kinetics clearly applied.

Possible contributions of variable plasma protein binding to the nonlinearity of perhexiline elimination kinetics are currently under investigation. Preliminary results do not reveal saturation of protein binding within the perhexiline concentration range encountered in these studies.

The results suggest that the elimination pharmacokinetics of perhexiline are nonlinear and that hepatic hydroxylation is saturable, resulting in variable systemic availability of orally administered perhexiline. Similar saturability of metabolism previously was demonstrated for alcohol (9), lorcainide (10), phenytoin (11), and fluorouracil (12). Drugs that demonstrate this phenomenon need great attention to dosage since it is often easy to move from therapeutic to toxic plasma levels with small changes in dose.

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