Determination of Serum Desipramine and 2-Hydroxydesipramine for Pharmacokinetic Applications by HPLC with Ultraviolet Detection

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This procedure for measuring desipramine and its 2-hydroxy metabolite in serum at a minimum concentration of 1 µg/L involves high-performance liquid chromatography (HPLC), with ultraviolet detection at 214 nm. After desipramine and 2-hydroxydesipramine are extracted from alkalinized serum by a single-step solvent extraction, they are separated by HPLC and quantified with amitriptyline as the internal standard. Desipramine, 2-hydroxydesipramine, and amitriptyline are separated in 6 min. The standard curve is linear (r = 1.000) for both desipramine and 2-hydroxydesipramine concentrations over the range of 1 to 100 µg/L, and the assay demonstrates an excellent precision profile, even at low concentrations. Between-run CVs for 20 and 60 µg/L controls (n = 20) were 3.9% and 3.6% for desipramine and 3.4% and 3.8% for 2-hydroxydesipramine, respectively. In a pharmacokinetic evaluation of patients with depression, we examined single-dose elimination curves before and after a four-week regimen of desipramine treatment. The results showed that the method's simplicity and high precision render it ideal for pharmacokinetic studies of desipramine.

Additional Keyphrases: tricyclic antidepressants - chromatography, liquid

The efficacy of tricyclic antidepressants for treating depression has been substantiated by more than 30 years of experience (1), but even with refinements in diagnosis (2), progress in identifying those patients most likely to respond to these drugs (3), and more aggressive use of therapeutic drug monitoring (4, 5), as many as 40% of patients treated with these medications still fail to achieve adequate clinical response. Concentrations of antidepressant in serum after a test dose have been shown to predict patient-specific steady-state pharmacokinetic parameters (6). Unfortunately, there is still no convincing demonstration that using such predictive dosing techniques significantly accelerates or improves clinical response. One reason for the delay in applying predictive dosing to clinical practice is a lack of suitable analytical methods having both the speed demanded to initiate clinical intervention and the sensitivity to reliably quantify the low concentrations (<50 µg/L) of antidepressant in serum after safe test doses. As a part of ongoing studies in our laboratory, we developed a procedure for rapidly analyzing for desipramine and 2-hydroxydesipramine at the low concentrations necessary for pharmacokinetic studies. We chose desipramine as the first drug to study because it had a simpler pharmacological and metabolic profile, less interindividual variation in metabolism, and a more favorable side-effect profile.

Quantitative enzyme immunoassay methods for desipramine are rapid enough to use in clinical settings and can be easily automated (7–9), but do not reliably measure concentrations low enough for pharmacokinetic evaluations, nor do they measure 2-hydroxydesipramine at all. Previous studies evaluating single-dose predictive techniques have used gas-chromatographic or high-pressure liquid-chromatographic (HPLC) methods that require much time and technical skill. Of the many published methods for desipramine analysis by HPLC, few measure 2-hydroxydesipramine, and those that do (10, 11) require more sophisticated electrochemical or fluorescence detectors. None combine the speed, precision, sensitivity, and simplicity achieved with the method we describe here. This method for simultaneously determining desipramine and 2-hydroxydesipramine in serum involves ultraviolet detection at 214 nm and is sensitive enough to measure each compound at concentrations as low as 1 µg/L.

Materials and Methods

Apparatus. For development and evaluation of this method, we used a Model 590 pump, a WISP 710B automatic sample injector, a Model 481 LC spectrophotometric detector, and a QA-1 Data System—all available from Waters, Division of Millipore, Milford, MA 01757.

Reagents. All chemicals were "HPLC grade," where available, or reagent grade. We used acetonitrile, methanol, hexane, and ethyl acetate from American Burdick and Jackson, Muskegon, MI 49442. We also used ammonium hydroxide (1 mol/L), isoamyl alcohol, and potassium phosphate buffer (10 mmol/L, pH 7.0) that we prepared by mixing 0.67 mL of concentrated (14.8 mol/L) phosphoric acid into 1 L of water and adjusting the pH with 1.0 mol/L potassium hydroxide. These latter reagents were from Fisher Scientific, Pittsburgh, PA 15219. Desipramine hydrochloride and amitriptyline hydrochloride were purchased as 1.0 mg/mL free-base standards from Supelco, Bellefonte, PA 16823; 2-hydroxydesipramine was prepared as a 1.0 mg/mL free-base standard from its hydrochloride salt (Ciba-Geigy Medical Division, Summit, NJ). We made a 2 mg/L aqueous solution of amitriptyline as the internal standard solution; an aqueous solution containing 10 mg/L each of desipramine and 2-hydroxydesipramine was used to prepare 0, 2, 5, 10, 25, 50, and 100 µg/L standards in horse serum (Arnel Products, New York, NY 10014).

Sample collection and storage. Blood samples were drawn into red-top Vacutainer Tubes (no. 6432; Becton-Dickinson & Co., Rutherford, NJ 07070) and centrifuged within 4 h of collection. Serum was separated and stored in polypropylene tubes at −20 °C until assayed. To demonstrate the reliability of this procedure for studies of desipramine pharmacokinetics, we obtained specimens from two volun-

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Received May 5, 1989; accepted July 17, 1989.
ter patients during the 48 h after a single oral test dose of 100 or 150 mg of desipramine prior to a four-week treatment with desipramine; each subject had given informed consent before participating in the study. We also conducted a pharmacokinetic evaluation after the last dose of medication in the four weeks of treatment.

**Extraction procedure.** Desipramine and 2-hydroxydesipramine are extracted from serum by a single-step solvent extraction from alkalinized serum. Transfer 2 mL of standard, control, or patient’s serum to a 16 × 100 mm screw-capped sample tube and add 100 μL of the 2 mg/L internal standard (amitriptyline) solution. Add 3 mL of ethyl acetate/hexane/isoamyl alcohol (50/49/1 by vol) and alkalinize the serum with 200 μL of 1.0 mol/L ammonium hydroxide. Shake the two phases for 10 min, then centrifuge for 5 min at 2000 × g. Transfer the organic (top) layer to a fresh 12 × 75 mm test tube; discard the aqueous phase. Evaporate the samples under nitrogen at 40 °C (we used the N-Evap; Organamation Associates Inc., Northborough, MA 01532). Reconstitute the residue with 200 μL of mobile phase, vortex-mix thoroughly, and inject 150 μL of this solution for chromatographic analysis.

**Chromatography.** For chromatographic separation of extracted desipramine, 2-hydroxydesipramine, and amitriptyline, we used a cyanopropyl column (Supelco no. 5-8377) on HPLC apparatus described above. Each liter of mobile phase contained 600 mL of acetonitrile, 180 mL of methanol, and 220 mL of potassium phosphate buffer. The mobile phase was mixed and degassed under reduced pressure for 10 min before use. Mobile-phase flow was 3.0 mL/min, and the detector wavelength was set at 214 nm. The data module, set to a chart speed of 0.4 cm/min, reported peak heights. The assay was run at ambient temperature.

To quantify concentrations, we measured the peak-height ratios of desipramine and 2-hydroxydesipramine to the internal standard, plotted the ratios vs concentration, and constructed a calibrator curve, using least-squares regression analysis. Sample concentrations were determined by comparison with this curve.

**Results and Discussion**

The simple, inexpensive solvent extraction used in this procedure yields consistent analytical recoveries throughout the assay range. Analytical recovery was about 85% for both desipramine and 2-hydroxydesipramine, and all interfering substances were removed. The practical detection limit of the method, as governed by sample size, injection volume, and losses in recovery, is about 1 μg/L (about four times the signal-to-noise ratio), well below the lowest concentration, 5 μg/L, observed in patients 48 h after a single 100-mg dose. Figure 1 illustrates representative chromatograms of a human serum blank supplemented with internal standard, a standard extracted from serum, and a patient’s sample extracted from a physically healthy but depressed man. No interference from endogenous substances was encountered in 100 samples assayed; however, imipramine, nortriptyline, and other drugs frequently administered to psychiatric patients do interfere with this analytical method. Thus, this procedure is designed for pharmacokinetic evaluations in which desipramine is the only drug present.

We verified linearity by adding known amounts of desipramine and 2-hydroxydesipramine to serum (2–100 μg/L) and subjecting these to the extraction procedure and chromatography. For eight standard curves assayed over two weeks, the mean (and SD) slope and intercept for desipramine were 4.41 (0.27) and −0.160 (0.87); they were 4.51 (0.26) and −0.527 (1.19) for 2-hydroxydesipramine. Figure 2 illustrates the precision profile of the peak-height ratios observed for each calibrator concentration in the eight standard curves. A plot of the mean peak-height ratio for each calibrator concentration yielded $y = 4.41x - 0.41$ ($r = 1.000, \text{SEE} = 0.183$) for desipramine; $y = 4.51x - 0.35$ ($r = 1.000, \text{SEE} = 0.255$) for 2-hydroxydesipramine.

Within-run precision was determined by analyzing, on three different occasions spanning two weeks, three aliquots from each of two serum pools, the first containing 20 μg and the second containing 60 μg of each compound per liter. The average CVs were 3.3% (range 2.7–4.5%) and 3.9% (range 1.8–5.9%), respectively, for desipramine, and 3.6% (range 2.7–5.3%) and 3.9% (range 3.3–4.2%) for 2-hydroxydesipramine. Between-run CVs were obtained by analyzing aliquots of the above serum pools with each of eight analytical runs over a two-week period. The CVs were 3.9% and 3.6%, respectively, for desipramine at 20 and 60 μg/L, and 3.4% and 3.8%, respectively, for 2-hydroxydesipramine.

Figure 3 illustrates the concentrations observed in the serum of a typical patient as a function of time, both before and after a four-week treatment regimen of desipramine. Preliminary analysis of two patients’ desipramine and 2-hydroxydesipramine serum concentration–time profiles

![Figure 1](image1.png)  
**Figure 1.** Representative chromatograms of (A) a human serum blank supplemented with internal standard, (B) a 10 μg/L standard extracted from horse serum, and (C) a patient’s sample containing 5 μg of 2-hydroxydesipramine and 18 μg of desipramine per liter.

![Figure 2](image2.png)  
**Figure 2.** Precision profile for calibrator peak-height ratios from eight standard curves.
as assayed by this specific HPLC method demonstrated several interesting findings. [All serum concentration-time data were analyzed by using "R-Strip," a computerized pharmacokinetic data-analysis program which uses both curve stripping and non-linear least squares fitting and is a modification of the Levenberg-Marquardt technique (12).]

First, the shape of the serum concentration-time profiles was not as smooth as expected on the basis of previous reports (13, 14). Both patients displayed two unexpected "bumps" at 12 and 36 h, after either the initial or a steady-state dose of desipramine. These increased concentrations in serum occurred 2 h after the evening meal and perhaps represent either a redistribution phenomenon or enterohepatic recirculation. Again, this phenomenon has not been observed previously (15). These changes were not seen with the metabolite, 2-hydroxydesipramine.

Second, and perhaps more important, comparison of the terminal elimination phase from the first dose with that after steady-state dosing of desipramine showed that both patients had an increase in elimination half-life of 4 and 7 h, respectively. Similarly, the elimination half-life of 2-hydroxydesipramine was increased by 17 and 5 h in the two patients, again comparing first dose with steady-state dosing. This change in half-life may represent nonlinearity in desipramine pharmacokinetics (13–15).

These preliminary findings suggest that desipramine and its metabolite pharmacokinetics need to be examined by classical means to define an appropriate pharmacokinetic model.

This work was supported in part by a Mental Health Clinical Research Center grant (MH-41115) to the Department of Psychiatry, University of Texas Southwestern Medical Center, and by grants from the Waters Division of Millipore, Milford, MA. We thank Ms. Sarah Shigley, R.N., M.S., for her assistance in this project and Ms. Louisa McBee for secretarial help.

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