Gas-Chromatographic Analysis for Therapeutic Concentrations of Imipramine and Desipramine in Plasma, with Use of a Nitrogen Detector

David N. Bailey and Peter I. Jatlow

We describe a method for the simultaneous gas-chromatographic analysis of imipramine and desipramine (the active N-desmethyl metabolite) at therapeutic concentrations in human plasma, with use of a nitrogen detector. Imipramine is measured as the unchanged base and desipramine as its N-trifluoroacetyl derivative. Promazine is the internal standard. The lower limit of sensitivity is 5 \( \mu \text{g/liter} \) for each drug, and the within-day coefficients of variation for the determination of imipramine and desipramine, respectively, are 6.0% and 3.3%. We measured concentrations of these drugs both in patients receiving therapeutic doses of imipramine and in those who had ingested an overdose.

Additional Keyphrases: tricyclic antidepressants • monitoring drugs in plasma • values after overdose • toxicology

Imipramine (“Tofranil,” Geigy; “Presamine,” USV Pharmaceutical) is one of the most frequently prescribed tricyclic compounds used for the treatment of depression. Its N-desmethyl metabolite desipramine, also pharmacologically active, is always found after imipramine ingestion and is itself administered as an antidepressant (“Norpramin,” Lakeside; “Pertofran,” USV Pharmaceutical). The structures of these two compounds are shown in Figure 1.

Recent studies have reported relationships between therapeutic response and plasma concentrations of imipramine and desipramine (1, 2), although considerable individual variation has been noted in plasma concentrations among patients receiving the same therapeutic dose of the tricyclic antidepressants (1, 3–7). While optimal therapeutic ranges for plasma concentrations of the tricyclics have not been definitively established, therapeutic doses of imipramine have been reported to produce concentrations of 15 to 500 \( \mu \text{g/liter} \) for the unchanged drug and its active metabolite (1, 2, 6, 8–12). Gram et al. (1) have suggested that concentrations above 45 and 75 \( \mu \text{g/liter} \) for imipramine and desipramine, respectively, are consistent with satisfactory therapeutic response. There is considerable interest in measuring plasma concentrations of the tricyclic drugs for pharmacologic studies and potentially for therapeutic monitoring.

Fig. 1. Structures of (A) imipramine, (B) desipramine, and (C) promazine (internal standard)

Imipramine and desipramine have been measured in biological fluids by various techniques, including isotope-dilution (13, 14), spectrofluorometry (10), thin-layer chromatography (15), and colorimetry (16). However, these methods are either very laborious or lack sufficient sensitivity or specificity for optimal measurement over the entire range expected after therapy. More recently, mass fragmentography has afforded sufficient specificity and sensitivity (10 \( \mu \text{g/liter} \)) for the measurement of imipramine and desipramine (8, 9, 17), but the instrumentation, for reasons both technical and economical, is not widely available, particularly in clinical laboratories.

Weder and Bickel (18) and Gillette et al. (19) reported the use of flame-ionization gas–liquid chromatography for measurement of imipramine and desipramine, but these procedures were developed only for the analysis of rat tissue homogenates, required involved extractions, and were not applied to plasma. In our opinion, detection by flame-ionization is not sufficiently sensitive for the accurate measurement of therapeutic concentrations of tricyclic antidepressants in plasma. For this reason we have used a nitrogen detector (20) for the measurement of these compounds. We previously reported the gas-chromatographic analysis for amitriptyline and nortriptyline (the active N-desmethyl metabolite) at therapeutic concentrations in plasma with use of this detector (21).

Recently, Gifford et al. (22) used gas chromatography with a nitrogen detector to measure tricyclic antidepressants, including imipramine and desipramine. However, little data relative to biological fluids were presented, and most of the data related to imipramine alone. The assay was applied only to plasma supplemented in vitro with imipramine, and patients receiving this drug were not studied. In addition, imipramine was not measured in the presence of its active metabolite, desipramine.
We report here a procedure for the simultaneous determination of imipramine and desipramine in human plasma at concentrations as low as 5 ng/liter. The procedure has been applied both to patients receiving therapeutic doses of imipramine and to patients who had ingested an overdose.

Materials and Methods

Apparatus

We used a Model 3920 gas-liquid chromatograph equipped with a nitrogen/phosphorus detector (Perkin-Elmer Corp., Norwalk, Conn. 06582) and a coiled glass column (2 mm i.d., 1.8 m long) containing 3% OV-17 on “Gas Chrom Q,” 100/120 mesh (Applied Science Laboratories, Inc., State College, Pa. 16801). The carrier-gas flow rate was kept constant with an auxiliary flow controller (“Dial-A-Flow,” Perkin-Elmer Corp.) Relative retention times and peak areas were measured with a “PEP-1” data processor (Perkin-Elmer Corp.)

Reagents and Standards

All reagents were analytical (AR) grade.

Hexane. This was redistilled and then passed through a 4 x 26 cm column of alumina A-540, 80/200 mesh (Fisher Scientific Co., Pittsburgh, Pa. 15219). Subsequently, we found a commercial source of hexane, distilled in glass, to be satisfactory (Burdick & Jackson Laboratories, Inc., Muskegon, Mich. 49442).

Isoamyl alcohol. This compound was redistilled.

Methanol. This was redistilled.

Hexane/isooamyl alcohol, 98.5/1.5 by vol.

Na2CO3, anhydrous.

Na2SO4, saturated aqueous solution. This reagent was washed with hexane before use.

HCl, 0.1 mol/liter. The distilled water used to prepare this solution was washed with hexane. This reagent was stored away from nitrogenous bases to avoid absorption of possibly interfering compounds.

Trifluoroacetic anhydride (Sigma Chemical Co., St. Louis, Mo. 63178). This reagent was stored in a desiccator at −15°C.

Stock Standards in Methanol

Store all standards in the freezer (−15°C). Prepare at least once per month.

Promazine hydrochloride (internal standard), 1.00 g/liter. Dissolve 10 mg of promazine hydrochloride (Wyeth Laboratories, Inc., Philadelphia, Pa. 19101) in 10 ml of methanol.

Imipramine, 1.00 g/liter. Dissolve 11.30 mg of imipramine hydrochloride (CIBA Pharmaceutical Co., Div. CIBA-GEIGY Corp., Summit, N.J. 07901), which is equivalent to 10 mg of imipramine base, in 10 ml of methanol.

Desipramine, 1.00 g/liter. Dissolve 11.37 mg of desipramine hydrochloride (USV Pharmaceutical Corp., Tuckahoe, N.Y. 10707), which is equivalent to 10 mg of desipramine base, in 10 ml of methanol.

Dilute Methanolic Standards

Mixed imipramine and desipramine standard, 100 mg of each per liter. Combine 100 μl of each of the 1.00 g/liter stock solutions of imipramine and desipramine with 0.8 ml of methanol. Prepare at the time of analysis.

Column-equilibration solution, 333 mg/liter. Mix 100 μl of each of the 1.00 g/liter stock solutions of imipramine, desipramine, and promazine hydrochloride.

Dilute internal standard in hexane/isooamyl alcohol, 100 μg/liter. Add 10 μl of the 1.00 g/liter stock promazine hydrochloride solution to each 100 ml of hexane/isooamyl alcohol used for the initial extraction. This provides 333 ng of internal standard per milliliter of plasma extracted. Prepare at the time of analysis.

Working Standards in Plasma

Standard in plasma, 500 μg/liter. Add 100 μl (10 μg) of the dilute mixed standard of imipramine and desipramine to 20 ml of drug-free plasma. While we prepared this standard with each run, it is stable for at least six weeks if frozen. For working standards in plasma, appropriately dilute the 500 μg/liter standard with drug-free plasma to produce the concentration(s) desired (usually 10–500 μg/liter).

Procedure

All glassware was washed with HCl (1 mol/liter) and rinsed with hexane. Blood from patients receiving therapeutic doses of imipramine was drawn into glass syringes coated with heparin (Vitarine Co., Inc., New York, N.Y. 11413). Blood from patients who had ingested an overdose of imipramine was collected routinely into evacuated tubes containing no anticoagulant (“Vacutainers,” Becton-Dickinson, Div. Becton, Dickinson and Co., Rutherford, N.J. 07070). The samples were centrifuged and the plasma (serum in overdose cases) frozen (−15°C) until analyzed.

Pipet 3 ml of plasma (samples, drug-free plasma, and plasma standards) into 15-ml glass culture tubes equipped with Teflon-lined screw caps. Adjust the pH to about 10.5 by addition of 0.5 ml of saturated Na2CO3. Add 10 ml of hexane/isooamyl alcohol containing the internal standard and extract for 3 min. Centrifuge (3000 rpm for 3 min) and transfer the solvent (upper) layer to a second 15-ml screw-capped tube. Add 1 ml of HCl (0.1 mol/liter), extract for 3 min, and, after centrifugation, aspirate and discard the solvent (upper) phase. Wash the acid for 1 min with 2 ml of hexane (no internal standard or isoamyl alcohol), centrifuge, and discard the solvent (upper) layer. Add about 500 mg (with a spatula) of anhydrous Na2CO3 to saturate the aqueous phase, mix well, and extract for 3 min with 2 ml of hexane (no internal standard or isoamyl alcohol). After centrifugation, transfer the solvent (upper) layer to a 10-ml test tube and dehydrate it over anhydrous Na2SO4. Transfer the dried solvent to a 5-ml glass centrifuge tube, add 100 μl of trifluoroacetic anhydride, and vortex-mix for 30 s. Evaporate the solvent at room temperature under a gentle stream of nitrogen. Be
certain that the odor of trifluoroacetic anhydride is no longer detectable after evaporation.

Dissolve the extract residue in 20 µl of methanol and chromatograph 4 µl under the following conditions: column temperature program, 240 °C (initial temperature, 2 min hold), 32 °C/min to 265 °C (final temperature, 8 min hold); injector temperature, 280 °C; detector temperature, 300 °C (detector current setting of 5.9–6.9, corresponding to rubidium glass bead temperatures of approximately 350–400 °C); carrier gas (helium) flow rate, 17 ml/min; air flow, 400 ml/min; hydrogen, 1–2 ml/min; amplifier setting, ×1. Calculate the peak-area ratio (peak area of imipramine or desipramine to that of the promazine internal standard) for each sample and determine the plasma concentration by comparison to the peak-area ratios (relative peak areas) of the extracted standards. Alternatively, peak heights may be used.

Before analysis of samples and standards, it is desirable to equilibrate the column with at least two 3-µl injections of the column-equilibration solution (equivalent to 2 µg each of imipramine, desipramine, and promazine). Then methanol should be chromatographed to prevent carryover or “ghosting.”

Results

A standard curve was prepared by supplementing drug-free plasma with imipramine and desipramine. When relative peak areas were plotted vs. the concentrations of imipramine and desipramine, a linear relationship (r = 0.99) was observed for each drug over the range studied (5–500 µg/liter).

The analysis of 11 aliquots of plasma supplemented to a concentration of 200 µg/liter with imipramine and desipramine gave coefficients of variation of 6.0% and 3.3% within-day, respectively. Eleven measurements of the same plasma during six weeks showed coefficients of variation of 2.6% and 5.0% between-day, respectively. The stability of both drugs in plasma was thus at least six weeks when frozen (–15 °C). The extract residues were stable at least overnight when stored in a desiccator at 4 °C.

The uncorrected analytical recovery was 60% for both imipramine and desipramine, calculated by comparison of peak areas obtained from supplemented plasma with those of nonextracted standards. This incomplete recovery, as well as variations in aliquoting, are corrected by use of extracted plasma standards and an internal standard (promazine).

Ten different drug-free heparinized plasma, analyzed by the proposed procedure, gave “background” responses averaging 0 µg/liter for imipramine and 2 µg/liter for desipramine (range 0–3). Thus the reliable lower limit of sensitivity was estimated to be about 5 µg/liter; for accurate and reliable quantitation, it is about 10 µg/liter. No interfering peaks were found at the retention time of the internal standard, although a small peak at retention time 0.88 relative to the internal standard was usually seen (peak b, Figure 2). This peak did not interfere, being separated by 0.8 min from imipramine (peak a, Figure 2) and promazine (peak c, Figure 2). Whenever blood was collected in “Vacutainers,” this contaminant was much more pronounced.

Forty-six common basic drugs (Table 1) were screened for interference in the proposed assay by comparing the retention times of their nonextracted methanolic solutions with that of imipramine, the N-trifluoroacetyl derivative of desipramine, and promazine, all after treatment with trifluoroacetic anhydride. Acidic drugs were not screened because they would not be extracted under the alkaline conditions of the assay. Amitriptyline ("Elavil," Merck Sharp & Dohme) and trihexyphenidyl ("Artane," Lederle) had the same retention time as imipramine, and, when drug-free plasma was supplemented with these drugs and extracted, the chromatograms showed a peak corresponding in retention time to imipramine. No drugs with the same retention time as desipramine or the internal standard were found. Thus, plasma from patients receiving either amitriptyline or trihexyphenidyl would give “false-positive” results for imipramine, but, in these cases, desipramine, the N-desmethyl metabolite of imipramine, would be absent (an unlikely occurrence if imipramine were really present). No other interfering drugs were found although three drugs, which we have indicated in Table 1, had retention times approaching that of imipramine.

Plasma from patients receiving therapeutic doses of
Table 1. Forty-Six Common Basic Drugs Screened for Interference

<table>
<thead>
<tr>
<th>Drug</th>
<th>Fluorazepam</th>
<th>Haloperidol</th>
<th>Prochlorperazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>Fluphenazine</td>
<td>Hydrochlorothiazide</td>
<td>Propoxyphene</td>
</tr>
<tr>
<td>Atropine</td>
<td>Methadone</td>
<td>Methamphetamine</td>
<td>Sulfamethoxazole</td>
</tr>
<tr>
<td>Benztropine</td>
<td>Meperidine</td>
<td>Methyprylline</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Methaqualone</td>
<td>Methylphenidate</td>
<td>Thiothixene</td>
</tr>
<tr>
<td>Chlorzoxazone</td>
<td>Morphine</td>
<td>Nortriptyline</td>
<td>Trihexyphenidyl^a</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Nicotine</td>
<td>Perhexazine</td>
<td>Tranlcypromine</td>
</tr>
<tr>
<td>Codeine</td>
<td>Nicotine</td>
<td>Trihexyphenidyl^a</td>
<td>Tranlcypromine</td>
</tr>
<tr>
<td>Dextroamphetamine</td>
<td>Nicotine</td>
<td>Trihexyphenidyl^a</td>
<td>Tranlcypromine</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Nortriptyline</td>
<td>Trihexyphenidyl^a</td>
<td>Tranlcypromine</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>Oxazepam</td>
<td>Trihexyphenidyl^a</td>
<td>Tranlcypromine</td>
</tr>
<tr>
<td>Doxepin^b</td>
<td>Phenacetin</td>
<td>Promazine</td>
<td>Desipramine</td>
</tr>
<tr>
<td></td>
<td>Phenylpropanolamine</td>
<td>Promazine</td>
<td>Desipramine</td>
</tr>
</tbody>
</table>

^a Extracts and chromatograms as imipramine.

^b Retention time of extracted drug within 0.5 min of that of imipramine.

Table 2. Plasma Concentrations of Imipramine and Desipramine in Eight Patients Chronically Treated with Imipramine

<table>
<thead>
<tr>
<th>Daily dose, mg</th>
<th>Other medication</th>
<th>Imipramine, µg/liter</th>
<th>Desipramine, µg/liter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>dextroamphetamine, phenobarbital</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>50</td>
<td>none</td>
<td>27</td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>75</td>
<td>none</td>
<td>98</td>
<td>130</td>
<td>228</td>
</tr>
<tr>
<td>150</td>
<td>none</td>
<td>45</td>
<td>425</td>
<td>470</td>
</tr>
<tr>
<td>200</td>
<td>perphenazine, benztropine</td>
<td>142</td>
<td>295</td>
<td>437</td>
</tr>
<tr>
<td>250</td>
<td>none</td>
<td>91</td>
<td>258</td>
<td>349</td>
</tr>
<tr>
<td>250</td>
<td>none</td>
<td>120</td>
<td>580</td>
<td>700</td>
</tr>
<tr>
<td>250</td>
<td>perphenazine</td>
<td>363</td>
<td>458</td>
<td>821</td>
</tr>
<tr>
<td>250</td>
<td>none</td>
<td>160</td>
<td>218</td>
<td>378</td>
</tr>
</tbody>
</table>

^a Sample drawn two weeks later.

Discussion

There has been increasing interest in optimizing drug therapy by monitoring concentrations in plasma. This is also true for the tricyclic antidepressants, including imipramine and desipramine, although the relationship between plasma concentration and clinical response is still being defined (1–7).

Values for our eight patients receiving therapeutic doses of imipramine on a long-term basis (Table 2) were in the range reported by others (1, 2, 6, 8–12). As expected, both imipramine and its active N-demethyl metabolite desipramine were found in patients who had received imipramine, and concentrations of both generally increased with increasing dose. After long-term treatment with imipramine, the concentration of desipramine in plasma usually exceeded that of imipramine, while the opposite was true after acute overdose. This may be a reflection of insufficient time for mono-N-demethylation of the parent drug in acute overdose. Total tricyclic concentrations (sum of imipramine and desipramine) ranged up to 1732 µg/liter for our six patients who had ingested an overdose of imipramine. Spiker et al. (23) in their series of overdoses with tricyclic antidepressants noted electrocardiographic evidence of cardiac toxicity when the total tricyclic concentration exceeded 1000 µg/liter.

Because promazine is a reasonably good structural homolog of imipramine (Figure 1) and is itself infrequently prescribed, it was used as the internal standard. Promazine has also been used by others as an internal standard for the analysis of imipramine and desipramine (8, 9). Extraction of these drugs from plasma at alkaline pH followed by back-extraction into dilute acid and subsequent recovery into solvent eliminates neutral lipids and fatty acids, affording essentially "clean" chromatograms.

Although we used hexane/isooamy alcohol for the initial plasma extraction, hexane was substituted for the extraction of the alkalinized acid phase, because isooamy alcohol is chemically incompatible with trifluoroacetic anhydride, which is used in the subsequent step. Some of the samples were also analyzed after extraction with hexane/acetone, and the same results were obtained. However, these extracts were less free of contaminants, and hexane/isooamy alcohol was selected as the preferable solvent.

Derivatization of desipramine with trifluoroacetic anhydride was required to enhance the sensitivity and to improve the chromatographic behavior of this drug, in contrast to our determination of nortriptyline (the N-demethyl metabolite of amitriptyline), for which derivatization was not necessary (21). The completeness of derivatization was monitored by looking for underivatized desipramine, which has a retention time of 0.85 relative to the internal standard. Such monitoring is particularly desirable because the internal standard, promazine, is a tertiary and thus nonderivatizable amine and will not serve as a control for completeness of derivatization. In all analyses performed in this study, 100 µl of trifluoroacetic anhydride afforded complete

the following drugs (often administered concomitantly with tricyclics) were analyzed to see whether their metabolites interfered: benztropine, chlorpromazine, fluphenazine, perphenazine, and thioridazine. No interferences were found.

We analyzed nine plasma samples from eight different patients who had been on long-term treatment with therapeutic doses of imipramine (Table 2). The chromatogram of one such analysis is shown in Figure 2. In addition, we analyzed seven samples from six different patients who had ingested a nonfatal overdose of drugs including imipramine; their plasma concentrations of imipramine and desipramine ranged up to 1515 and 217 µg/liter, respectively.
derivatization over the range of 5–500 μg/liter. Samples above 500 μg/liter were diluted and re-analyzed. Desipramine has also been measured as its N-trifluoroacetyl derivative by others (17); our daily use of the nitrogen detector also has been previously reported (24).

As indicated, amitriptyline (“Elavil,” Merck Sharp & Dohme) and trihexyphenidyl (“Artane,” Lederle) interfered with the analysis of imipramine, but the metabolite desipramine (always present after imipramine ingestion) would not be seen after the ingestion of amitriptyline or trihexyphenidyl. Amitriptyline is not administered concurrently with imipramine, although the two drugs could be combined in an overdose. The analysis of plasma from patients receiving other drugs often administered with imipramine showed no interferences from either the parent drug or metabolites.

Although we did not study the more polar hydroxylated and conjugated metabolites of imipramine and desipramine, it is unlikely that they would be extracted under the conditions of the assay. The noninterfering contaminant peak (peak b, Figure 2), separated by 0.8 min from imipramine and the internal standard, was much more pronounced in samples that had been collected in “Vacutainers,” and may represent the tri(2-butoxyethyl)phosphate contaminant reported previously (21, 25). When blood was drawn into heparinized glass syringes, this peak was small.

This work was supported in part by Research Grant DA 01294-06 from the National Institute on Drug Abuse. We thank Drs. Craig Van Dyke, Donald Sweeney, and Craig Nelson for providing plasma samples. Imipramine hydrochloride was a gift from CIBA Pharmaceutical Co., Div. CIBA-GEIGY Corp., Summit, N.J.; desipramine hydrochloride from USV Pharmaceutical Corp., Tuckahoe, N.Y.; and promazine hydrochloride from Wyeth Laboratories, Inc., Philadelphia, Pa.

References