Isotope Dilution Gas Chromatographic-Mass Spectrometric Measurement of Tricyclic Antidepressant Drugs. Utility of the 4-Carbethoxyhexafluorobutyryl Derivatives of Secondary Amines

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Abstract

Stable isotope dilution gas chromatographic-mass spectrometric (GC-MS) measurement of tricyclic antidepressants (TCA) is a useful alternative to high-performance liquid chromatography (HPLC) methods when interfering substances prevent accurate quantitation by HPLC. For satisfactory GC-MS analysis, secondary amine TCA must be derivatized. Commonly employed trifluoroacetyl and heptafluorobutyryl derivatives are relatively unstable and cause rapid deterioration of capillary GC columns. Therefore we examined 4-carbethoxyhexafluorobutyryl chloride (CHFB-CI) as an alternative derivatizing agent and developed a stable isotope dilution GC-MS method employing ring-labeled [2H₄]-desipramine and [2H₄]-imipramine internal standards, which permits measurement of desipramine, nortriptyline, imipramine, and amitriptyline in plasma samples containing one or all of these analytes. The GC-MS assay is linear for each analyte from the lower limit of quantitation (25 ng/mL) up to 1500 ng/mL and correlates well with HPLC measurements. The GC-MS analytic coefficient of variation was 9.7 ± 1.3% for all analytes considered together. Although interferences are observed in the HPLC assay, thioridazine, perphenazine, cyclobenzaprine, and norcyclobenzaprine do not interfere with GC-MS measurements of the TCA examined here. The stability of the CHFB derivative of secondary amine TCA was found to be superior to that of the trifluoroacetyl derivatives of these compounds.

Introduction

Tricyclic antidepressants (TCA) are psychoactive drugs that are widely used to treat depression (1). Antidepressant efficacy may be optimized if plasma TCA concentrations are maintained within a target concentration range (1,2). The most important adverse side effects of tricyclic antidepressants involve the central nervous and cardiovascular systems (3–5). Central nervous system toxicity, including delirium and convulsions, and cardiovascular effects, including conduction delays and arrhythmias, may occur more frequently at plasma TCA concentrations considerably above the target concentration range (1,4). Laboratory measurement of the plasma TCA concentrations is therefore commonly used for therapeutic monitoring and may provide adjunctive information for assessment of the potential severity of acute overdosage.

Several analytic methods have been developed for measuring plasma levels of TCA. These methods include thin-layer chromatography with densitometry (6), radioimmunoassay (7), high-performance liquid chromatography (HPLC) with UV detection (8), and gas chromatography-mass spectrometry (GC-MS) (8–13). GC-MS can provide accurate quantitation of TCA when other substances such as cyclobenzaprine (14) or phenothiazines interfere with alternate measurement methods. When tertiary amine TCA (e.g., amitriptyline and imipramine) are administered, it is customary to measure both parent drugs and desmethyl metabolites because such metabolites are pharmacologically active (1).

The desmethyl metabolites are secondary amines and require derivatization for satisfactory GC or GC-MS analysis. Several methods for the derivation of secondary amine TCA for GC-MS analysis have been described (8–13). Derivatization reagents used for this purpose include heptafluorobutyric anhydride (9,11), and trifluoroacetic anhydride (TFAA) (8,10,12,13). The resultant derivatives are somewhat moisture-sensitive, and residual traces of derivatizing reagent can adversely affect capillary GC column lifetime.

The reagent 4-carbethoxyhexafluorobutyryl chloride (CHFB-CI) has been used to form stable derivatives of drugs with secondary amine groups, such as methamphetamine (15), and the stability of the resultant derivatives permits removal of excess derivatizing agent by the addition of protic solvents. The CHFB derivatives are also less volatile than other commonly employed derivatives and...
tend to yield mass spectra with more abundant ions at high mass-to-charge ratios. These properties facilitate elimination of interference from extraneous low molecular weight contaminants.

We examined the suitability of CHFB derivatives of TCA with secondary amine groups for isocele dilution GC-MS measurements and have developed an assay to measure desipramine and nortriptyline and the tertiary amine TCA imipramine and amitriptyline in plasma samples. Ring-labeled \([^{3}H_4]\)-desipramine and \([^{3}H_4]\)-imipramine were used as the internal standards.

Materials and Methods

Reagents

Desipramine (10,11-dihydro-N-methyl-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine), nortriptyline (3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine), imipramine (10,11-dihydro-N,N-dimethyl-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine), and amitriptyline (3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine), and trimipramine (5-G-(3-dimethylamino-2-methylpropyl)-10,11-dihydro-5H-dibenz[b,f]azepine) were obtained from Alltech-Applied Science (Deerfield, IL). The deuterium-labeled compounds (2,4,6,8)-\(^{3}H_4\)-imipramine and (2,4,6,8)-\(^{3}H_4\)-desipramine, each labeled on the ring structure, were obtained from Cambridge Isotope Laboratories (Andover, MA) for use as internal standards. The derivatizing agent 4-carbethoxyhexafluorobutyryl chloride (CHFB-Cl) was obtained from PCR (Gainesville, FL). Toluene, acetonitrile, and methanol were from Burdick & Jackson (Muskegee, MI). Isoamyl alcohol and \(n\)-hexane were from Mallinckrodt-Baker, Inc. (Paris, KY). Ethyl acetate, mono- and dibasic potassium phosphate were from Fisher Scientific (Pittsburgh, PA). Sodium tetraborate decahydrate was from Aldrich Chemical (Milwaukee, WI). Control media (Lyphochek Benzo/TCA Controls 1 and 2) were from BioRad (Anaheim, CA). These media contain desipramine, nortriptyline, imipramine, and amitriptyline at defined concentrations. A calibrator solution (Tricyclic Serum Control) was obtained from Quality Assurance Service Corp. (Augusta, GA). This solution contains desipramine, nortriptyline, imipramine, and amitriptyline (250 ng/mL each). Thioridazine, perphenazine, cyclobenzaprine, norcyclobenzaprine, trifluoroacetic anhydride (TFAA), sodium hydroxide, and fetal bovine serum were from Sigma Chemical Co. (St. Louis, MO).

Preparation of samples for GC–MS

For determination of GC retention times and full-scan electron impact mass spectra, 1000 ng of each tricyclic antidepressant (desipramine, nortriptyline, imipramine, or amitriptyline) was prepared by dilution from stock ethanolic solutions of drug (1 mg/mL). Each drug was placed in a separate silanized 125 x 16-mm screw-cap test tube for derivatization. For all other experiments, fetal bovine serum was used as a surrogate for drug-free plasma, and sample volume was 2 mL. Patient plasma specimens were collected in containers that did not contain plasma-separator gel. Specimens were diluted with fetal bovine serum to achieve a final volume of 2 mL for the methods comparison. Internal standard (100 {\mu}L of 10 mg/L solutions of \([^{3}H_4]\)-imipramine and \([^{3}H_4]\)-desipramine) was added to each 2-mL sample to achieve a final concentration of 500 ng/mL. After addition of internal standard, samples were extracted by addition of 2 mL of borate buffer (pH 11) and 8 mL of organic extraction solvent (toluene/n-hexane/isoamyl alcohol, 77:20:3). Samples were rotated for 10 min to permit mixing and centrifuged (5 min, 2500 g). The upper (organic) layer containing the extracted TCA was then subjected to derivatization and GC–MS analysis, as described.

Derivatization of samples with CHFB-Cl

Sample extracts were transferred to silanized 10 mL screw-cap tubes and concentrated to dryness under air at 40°C. Derivatization with CHFB-Cl was performed by reconstituting the sample in 1-chlorobutane (1 mL), followed by the addition of 0.1 mL of a 1:10 dilution of CHFB-Cl in 1-chlorobutane. Each tube was capped, vortex-mixed, and then incubated for 30 min at 60°C. Ethanol (0.25 mL, 100%) was then added, followed by a second 30-min incubation at 60°C. The samples were then concentrated to dryness under air at 40°C and reconstituted (ethyl acetate, 25 {\mu}L) for GC analysis.

GC–MS

Aliquots (5 {\mu}L) of the derivatized samples were injected (model 7673A autosampler, Hewlett-Packard) in splitless mode as ethyl acetate solutions into a GC–MS system consisting of a Hewlett-Packard GC (model 5890) interfaced with a 5970A electron-impact quadrupole MS controlled via a Hewlett-Packard RTE-A data system. GC was performed on a Hewlett-Packard Ultra-1 capillary column (cross-linked methyl-silicone, 8 m x 0.31-mm i.d., 0.17-mm film thickness). The carrier gas was helium with a total flow rate of 28 cm/s and a head pressure of 5 psi. The initial GC oven temperature was 150°C, and injector and interface temperatures were 265°C. After injection, the oven temperature was increased at 30°C/min to 240°C, held for 7 min, then increased to 300°C at 10°C/min, and held at 300°C until the completion of analyses. Source temperature was 200°C, and the MS was operated in full-scan mode (100–600 amu) for acquisition of mass spectra of pure derivatized standards and in selected ion monitoring mode for all other experiments. Quantitation of drug content was achieved by selected monitoring of ions arising from unlabeled compounds and from the internal standards (\([^{3}H_4]\)-imipramine or \([^{3}H_4]\)-desipramine-CHFB).

Preparation of samples for HPLC

Patient samples which were submitted for the measurement of tricyclic antidepressants by an established HPLC method (14) were split into two aliquots, and one was used for analysis by GC–MS. For HPLC analysis, 1 mL of patient sample, 0.5 mL of internal standard (trimipramine), 1.0 mL of 0.5N NaOH, and 4.0 mL of extraction solvent were added to silanized 15 x 16-mm screw-cap tubes. Each tube was rotated for 10 min to permit mixing of contents and then centrifuged (2500 x g, 5 min). The upper (organic) phase was concentrated to dryness under air at 40°C and reconstituted with methanol (0.2 mL). Aliquots (50 {\mu}L) were then injected into an HPLC system consisting of a Waters model 6000 A solvent delivery system, a Supelcosil LC-PCN column (15 cm x 4.7 mm i.d., cyano-propyl bonded 5-mm silica packing), a Waters model 481 variable wavelength UV detector (254 nm), and a Hewlett-Packard HP 3392A integrator. Mobile phase for HPLC was prepared by mixing 600 mL HPLC-grade...
acetonitrile, 150 mL HPLC-grade methanol, and 240 mL 0.01M phosphate buffer and filtering under vacuum. A minimum of 20 samples of each was analyzed for desipramine, nortriptyline, imipramine, and amitriptyline both by HPLC and GC-MS. The same calibrator and tricyclic antidepressant control materials were used for both methods.

Linearity and stability of isotope dilution GC-MS quantitation of desipramine, nortriptyline, imipramine, and amitriptyline

[2H₄]-Labeled imipramine or [2H₄]-labeled desipramine (1 µg) was added to a series of samples (2 mL fetal bovine serum) containing known and varied amounts (0 to 1500 ng/mL) of each of the following tricyclic antidepressant drugs: desipramine, nortriptyline, imipramine, and amitriptyline. These samples were extracted and derivatized with CHFB-CI as described here previously. The samples were analyzed by GC-MS in EI mode, and the linearity and lower limit of detection were determined. In order to determine the stability of the derivative, duplicate samples containing 250 ng/mL each of desipramine, nortriptyline, imipramine, and amitriptyline were derivatized using CHFB-CI or TFAM and analyzed immediately and 24 h and 48 h later.

Interference studies

Samples containing 250 ng/mL each of desipramine, nortriptyline, imipramine, and amitriptyline plus added thioridazine, perphenazine, cyclobenzaprine, or norcyclobenzaprine (500 ng each) were split into two aliquots and analyzed by both HPLC and GC-MS as described here previously.

Results

The structures of desipramine, nortriptyline, imipramine, amitriptyline, and 4-carbethoxyhexafluorobutyl chloride (CHFB-Cl) are illustrated in Figure 1. The EI mass spectra (for mass-to-charge ratios >100) of the CHFB derivatives of desipramine and [2H₄]-desipramine and the proposed fragmentation sites are illustrated in Figure 2. For the nondeuterated compound, the molecular ion (m/z 516) and an ion reflecting loss of an ethoxy radical (m/z 471) are visualized. The most abundant ion (m/z 208) reflects a fragment that retains the ring structure but has lost most of the side chain. Because [2H₄]-desipramine is ring labeled, the analogous ion occurs at m/z 212 in the mass spectrum of its CHFB derivative.

The EI mass spectrum of CHFB-nortriptyline and the proposed major fragmentation site are illustrated in Figure 3. The molecular ion (m/z 513) and an ion reflecting loss of an ethoxy radical
(m/z 468) are visualized at low abundance. The base ion (m/z 232) likely reflects loss of the substituted amine moiety from the side chain as a neutral secondary amine.

The tertiary amine compounds imipramine and amitriptyline do not form derivatives when treated with CHFB-CI. EI mass spectra of those compounds and of ring-labeled [2H4]-imipramine (Figure 4) correspond well to published spectra (16). For imipramine, the molecular ion (m/z 280) is visualized, and the most abundant ions (m/z 234 and m/z 235) may arise from losses of methyl amine and methyl radical and from loss of dimethyl amine, respectively, from the side chain. For [2H4]-imipramine, the corresponding ions are observed at m/z 284 (M+), m/z 238, and m/z 239, respectively. For amitriptyline, the molecular ion (m/z 277) is visualized, and the most abundant ion (m/z 202) may reflect loss of dimethylamine and ethane from the side chain.

For quantitative analysis, the most abundant ion with a mass-charge ratio exceeding 100 in the spectrum of each target analyte and of the two [2H4]-labeled internal standards were chosen for selected ion monitoring. Two additional ions in the spectrum of each compound were also monitored and were demonstrated to co-elute with the quantitator ions to ensure accurate identification of the peaks representing the target analytes and the internal standards (17). The mass-to-charge ratio values for the quantitator and qualifier ions selected for each compound are summarized in Table I. For routine therapeutic drug level monitoring measurements, monitoring qualifier ions is optional and is not generally required for accurate quantitation. Useful qualifier ions are nonetheless identified in Table I for

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**Figure 3.** Proposed major fragmentation site (A) and EI mass spectrum (B) of the 4-carbethoxyhexafluorobutyryl derivative of nortriptyline.

**Figure 4.** EI mass spectra of imipramine (A), (2,4,6,8)-2H4-imipramine (B), and amitriptyline (C).
circumstances under which identification of the analyte in question might have forensic significance (17).

The GC retention times (RT) under the conditions described in Materials and Methods are illustrated in Figure 5. The underivatized tertiary amines amitriptyline (RT, approximately 9.15 min), imipramine (RT, approximately 9.55 min), and [2H₄]-imipramine (RT, approximately 9.55 min) elute before the CHFB derivatives of the secondary amines nortriptyline (RT, approximately 18.7 min), desipramine (RT, approximately 19.2 min), and [2H₄]-desipramine (RT, approximately 19.2 min). The absolute retention times varied with column age, but the order of elution illustrated in Figure 5 was preserved.

In order to place a constraint on the lower limit of quantitation of the assay, internal standards were added to ten blank samples containing no TCA, and the specimens were extracted. The extracts were then subjected to the derivatization reaction and analyzed by GC–MS. The blank signal plus 10 standard deviations was found to be ≈ 25 ng/mL for each target analyte. Ten specimens that contained 25 ng/mL each of desipramine, nortriptyline, imipramine, and amitriptyline were then prepared. After addition of internal standards, these specimens were processed as described and analyzed by GC–MS. The mean within-run precision was similar for each analyte at the concentration of 25 ng/mL, and the coefficient of variation was 9.7 ± 1.3% for all analytes considered together. Both secondary amine compounds

<table>
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<th>Analyte</th>
<th>Quantitator Ion</th>
<th>Qualifier Ions</th>
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<tbody>
<tr>
<td></td>
<td>m/z (ra)</td>
<td>m/z (ra)</td>
</tr>
<tr>
<td>CHFB-desipramine</td>
<td>208 (08)</td>
<td>234 (08)</td>
</tr>
<tr>
<td>CHFB-[2H₄]-desipramine</td>
<td>212 (08)</td>
<td>238 (08)</td>
</tr>
<tr>
<td>CHFB-nortriptyline</td>
<td>232 (26)</td>
<td>217 (26)</td>
</tr>
<tr>
<td>Imipramine</td>
<td>234 (43)</td>
<td>193 (43)</td>
</tr>
<tr>
<td>[2H₄]-Imipramine</td>
<td>238 (35)</td>
<td>197 (35)</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>202 (52)</td>
<td>215 (52)</td>
</tr>
</tbody>
</table>

Table 1. Ions Monitored for Quantitation and Qualitative Identification in GC–MS Analyses of Tertiary Amine TCA Drugs and of CHFB Derivatives of Secondary Amine TCA Drugs

Figure 5. GC–MS-selected ion monitoring analysis of amitriptyline (A), imipramine (B), (2,4,6,8)-2H₄-imipramine (C), CHFB-nortriptyline (D), CHFB-desipramine (E), and CHFB-(2,4,6,8)-2H₄-desipramine (F).
(nortriptyline and desipramine) were quantitated relative to \[^{2}H_{4}\]-desipramine. Both tertiary amine compounds (amitriptyline and imipramine) were quantitated relative to \[^{2}H_{4}\]-imipramine. At a concentration of 25 ng/mL, the signal-to-noise ratios for the quantitator ion were 22.9, 96.2, 3.70, and 28.6 for amitriptyline, nortriptyline, imipramine, and desipramine, respectively.

As illustrated in Figure 6, the GC–MS assay was linear within the concentration range of 25–1500 ng/mL for each of the four target analytes. In the experiment summarized in the figure, a constant amount of the internal standards (1 µg each of \[^{2}H_{4}\]-desipramine and \[^{2}H_{4}\]-imipramine) and varied amounts of desipramine, nortriptyline, imipramine, and amitriptyline were added to aliquots (2 mL) of fetal bovine serum, and the samples were processed and analyzed by GC–MS as described.

Reproducible values for the concentrations of each of the four target analytes in commercial control materials were obtained by the GC–MS method, as summarized in Table II. After storage for two days at room temperature, CHFB-Cl-derivatized samples yielded TCA concentration values that were identical to those obtained immediately after derivatization for each of the four

| Table II. Comparison of GC–MS and HPLC Measurements of Tricyclic Antidepressant Drug Concentrations in Quality Control Materials |
|---|---|---|---|---|
| | GC–MS |  | HPLC |  |
| | Low QC CV High QC CV Low QC CV High QC CV CV Low QC High QC Target |
| **Analyte** | (ng/mL) (%) (ng/mL) (%) | (ng/mL) (%) (ng/mL) (%) | | |
| Desipramine | 106 ± 12.7 12.0 | 315 ± 15.8 5.0 | 89 ± 9.9 11.2 | 357 ± 39.0 10.9 | 98 ± 11.4 336 ± 26.7 |
| Nortriptyline | 95 ± 5.2 5.5 | 319 ± 27.2 8.5 | 95 ± 10.9 11.5 | 357 ± 42.7 11.0 | 95 ± 8.1 338 ± 33.0 |
| Imipramine | 92 ± 8.2 8.9 | 327 ± 24.2 7.4 | 93 ± 9.3 11.2 | 363 ± 36.3 10.0 | 93 ± 8.5 345 ± 30.0 |
| Amitriptyline | 105 ± 10.7 10.2 | 372 ± 16.0 4.3 | 86 ± 9.4 11.0 | 325 ± 32.6 11.0 | 96 ± 10.2 349 ± 24.9 |

Figure 6. Linearity of stable isotope dilution GC–MS measurement of CHFB-desipramine (A), CHFB-nortriptyline (B), imipramine (C), and amitriptyline (D).
target analytes, although this was not the case for trifluoroacetyl derivatives of desipramine or nortriptyline (not shown). HPLC analyses of specimens containing 250 ng/mL of each of the target analytes and added thioridazine, perphenazine, cyclobenzaprine, or norcyclobenzaprine (500 ng), revealed interfering peaks that prevented HPLC quantitation of amitriptyline and imipramine (reference 14 and data not shown). When these specimens were analyzed by GC–MS, interference was not observed.

The interference of cyclobenzaprine and its metabolite norcyclobenzaprin with HPLC measurement of amitriptyline and nortriptyline are illustrated in Figure 7, which represents HPLC analyses of standard TCA (panel A) and of a specimen from a patient who had taken a large quantity of cyclobenzaprine but had not taken TCA (panel B). Analysis of the TCA standards (panel A) illustrated the typical HPLC elution profile of internal standard trifluoropramine (peak 1, RT, 2.28 min), amitriptyline (peak 2, RT, 3.10 min), imipramine (peak 3, RT, 3.55 min), nortriptyline (peak 4, RT, 5.38 min), and desipramine (peak 5, RT, 5.83 min). The patient specimen (panel B) exhibited a large peak with the retention time (3.10 min) of amitriptyline and a smaller peak with a retention time (5.29 min) very similar to that of cyclobenzaprine. GC–MS analysis of this sample, however, revealed no amitriptyline or nortriptyline, but both cyclobenzaprine and norcyclobenzaprine were identified.

As illustrated in Figure 8, standard amitriptyline is clearly resolved from cyclobenzaprine on GC–MS analysis (upper panel), and standard CHFB-nortriptyline is clearly resolved from CHFB-norcyclobenzaprine (lower panel). Amitriptyline and cyclobenzaprine have been previously reported to co-elute in the HPLC system used in Figure 7 but to resolve on GC–MS (14). Nortriptyline and norcyclobenzaprine are also incompletely resolved in this HPLC system (14). The TFA derivatives of nortriptyline and norcyclobenzaprine can be resolved by GC–MS (14), and Figure 8 (lower panel) indicates that the CHFB derivatives of these two compounds are also resolved by GC–MS.

Values obtained by GC–MS corresponded well (by least-squares regression analysis) to those obtained by HPLC in a series of patient specimens submitted for TCA analysis, and there was no statistically significant difference (by Student's t-test) between the two sets of values (Table III). These specimens included at least 20 samples each for desipramine, nortriptyline, imipramine, and amitriptyline.

**Table III. Comparison of GC-MS and HPLC Measurements of Tricyclic Antidepressant Drug Concentrations in Patient Specimens.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>r</th>
<th>SY*</th>
<th>p</th>
<th>HPLC mean (ng/mL)</th>
<th>GC-MS mean (ng/mL)</th>
<th>Equation</th>
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</thead>
<tbody>
<tr>
<td>CHFB-Desipramine</td>
<td>25</td>
<td>0.97</td>
<td>19.9</td>
<td>0.82</td>
<td>149</td>
<td>148</td>
<td>y = 0.97x + 3.8</td>
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<tr>
<td>CHFB-Nortriptyline</td>
<td>32</td>
<td>0.97</td>
<td>15.8</td>
<td>0.96</td>
<td>137</td>
<td>136</td>
<td>y = 0.93x + 9.9</td>
</tr>
<tr>
<td>Imipramine</td>
<td>20</td>
<td>0.98</td>
<td>14.1</td>
<td>0.38</td>
<td>126</td>
<td>123</td>
<td>y = 0.99x – 0.9</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>21</td>
<td>0.97</td>
<td>30.3</td>
<td>0.15</td>
<td>203</td>
<td>193</td>
<td>y = 0.97x – 3.4</td>
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</table>

**Discussion**

Treatment of the secondary amine TCA desipramine and nortriptyline with 4-carbethoxyhexafluorobutyryl chloride (CHFB-Cl) yields stable CHFB-derivatives of these compounds which are suitable for isotope dilution GC–MS quantitation. Although the tertiary amine TCA imipramine and amitriptyline do not form derivatives with this reagent, they do not undergo decomposition upon exposure to it and can be quantitated simultaneously with the secondary amine TCA in specimens that contain both classes of compounds. This is important because tertiary amine TCA are metabolically demethylated to the corresponding secondary amine TCA, and the sum of the plasma concentrations of the parent drug and desmethyl metabolite is used to guide dosage adjustments (2).

Most routine measurements of TCA concentrations in plasma can be performed by HPLC methods (8,14) that are more facile and less labor-intensive than GC–MS. The principle utility of GC–MS methods is to provide an alternate, more structurally specific means to measure TCA in cases in which interfering substances co-elute with or are poorly resolved from TCA on HPLC analysis and prevent accurate HPLC measurements. GC–MS analyses combine a separation method with structurally informative mass fragmentographic detection which permits distinction of TCA from even very closely related compounds which, for example, may differ from TCA only by...
the presence of a single double bond (14). When heavy-isotope-labeled internal standards are included in the analyses, GC–MS methods can also achieve accurate quantitation of TCA, which correlates well with HPLC methods, as demonstrated both here and previously (8,14).

In this study we used ring-labeled, rather than side-chain-labeled (14), deuterated internal standards. Because most fragment ions of higher mass-to-charge ratio values in the mass spectra of TCA and their derivatives retain the ring structure but have suffered losses from the side chain, the use of ring-labeled internal standards permits ions of higher mass-to-charge ratio values to be monitored for quantitation and qualitative identification. With side-chain-labeled internal standards, most fragment ions with higher mass-to-charge ratio values are common to the mass spectra of labeled and unlabeled compounds. This requires that ions of low mass-to-charge ratios be monitored. For example, with [3H3]-amitriptyline or [3H3]-imipramine labeled in a methyl group on the tertiary amine, the most suitable ion for quantitation is m/z 61 (CH2=N+-CH3C[3H3]) (14). The analogous ion in the unlabeled compounds is m/z 58, so that the ion pair m/z 58 and m/z 61 is monitored for quantitation (14). Ions with such low mass-to-charge ratio values contain much less structural information than ions with higher mass-to-charge ratio values and may be produced by a variety of extraneous compounds other than the target analyte. This can cause interferences which prevent accurate quantitation. In the assay described here, the mass-to-charge ratio values of ions selected for quantitation exceed 200 for each analyte, and in each case the target ion has suffered loss of that portion of the side chain which bears label in commercially available, side-chain labeled standards.

In our laboratory, GC–MS is used to quantitate TCA when our primary HPLC assay fails to yield accurate measurements because of interfering substances such as phenothiazines, cyclobenzaprine and its desmethy metabolite (14), or unidentified compounds which distort the usual chromatographic profile. Although we have used the trifluoroacetyl (TFA) derivative of secondary amine TCA for this purpose (14), we found that this derivative is relatively labile and must be analyzed shortly after processing. In addition, capillary GC column lifetime is dramatically shortened when analyzing TFA derivatives, possibly because of liberation of trifluoroacetic acid from the derivative itself during GC analysis or to residual traces of derivatizing reagent. Because we encountered similar problems with the TFA derivatives of amphetamine and methamphetamine and because these problems were circumvented by the use of CHFB derivatives of amphetamine and methamphetamine (15), we explored the use of CHFB derivatives of secondary amine TCA for GC–MS analyses. The findings reported here and our experience to date with this assay in routine practice indicate that the CHFB derivatization procedure is suitable for the quantitative analysis of TCA by GC–MS and offers advantages over the trifluoroacetylation procedure.

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