Analysis of Tricyclic Antidepressants in Human Plasma by GLC–Chemical-Ionization Mass Spectrometry with Selected Ion Monitoring

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Abstract □ A method is described for the analysis of amitriptyline, doxepin, imipramine, nortriptyline, desmethyldoxepin, desipramine, and protriptyline in human plasma utilizing GLC–chemical-ionization mass spectrometry with selected ion monitoring. The assay is highly specific and is quantitative to at least 1 ng/ml with a standard error typically less than 5%. Representative concentrations of the parent compounds and their monodemethylated metabolites, as measured in plasma samples from patients under treatment with tertiary amine tricyclic antidepressants, are given.

Keyphrases □ Antidepressants, various tricyclic—GLC–chemical-ionization mass spectrometric analyses, human plasma □ GLC–chemical-ionization mass spectrometry—analysis, various tricyclic antidepressants in human plasma □ Mass spectrometry, chemical ionization—analysis, various tricyclic antidepressants in human plasma

Tricyclic antidepressants are the most commonly used medications for the treatment of depression. Approximately 60–70% of patients treated with these substances respond favorably. The lack of response in the remaining 30–40% may be attributed, in part, to interpatient differences in drug absorption, distribution, metabolism, and elimination, resulting in nontherapeutic blood levels of the drugs and their active metabolites (1–6).

To evaluate the importance of these pharmacokinetic processes on the response of depression to tricyclic antidepressant treatment, selective and sensitive assay methods for these compounds and their metabolites must be utilized. Previous approaches include TLC (7–10), photometry (11–16), radioisotopic derivatization (17, 18), GLC (9, 13, 19–28), and GLC–mass spectrometry with selected ion monitoring (4, 29–39), which is the most accurate and among the most sensitive. To date, assays with this method have utilized electron-impact ionization techniques.

The purpose of the present study was to develop GLC–mass spectrometric assays with selected ion monitoring for the most commonly used tricyclic antidepressants utilizing the more recently developed chemical-ionization method.

EXPERIMENTAL

Blood samples, 5–10 ml, were drawn in heparinized tubes[1] from patients under treatment for depression. The plasma was separated by centrifugation at 2000 rpm for 10 min, transferred to tubes with polytef-lined screw caps, and stored frozen at −20° until analysis. The following were used as reference compounds: amitriptyline[2], protriptyline[2], nortriptyline[3], doxepin[4], desmethyldoxepin[4], imipramine[5], and desipramine[6].

All solvents were either high purity[7] or glass distilled[8]. Trifluoroacetic, heptafluorobutyric[9], and pentafluoropropionic[10] anhydrides were reagent grade; perfluorotributylamine[10] was mass spectrometric grade. All other reagents were ACS reagent grade. Glassware was acid washed, and silanization of glassware with dimethylchlorosilane or the inclusion of an alcohol such as isobutyl in the extraction solvent was essential to prevent serious drug adsorption on the extraction glassware. Addition of isobutyl alcohol was preferred as a matter of convenience.

A gas chromatograph–mass spectrometer system[11] interfaced with a data system[12] was equipped with a differentially pumped chemical-ionization source that received the total effluent from the gas chromatograph.

A series of standards was prepared daily and analyzed along with each set of unknowns. Standards were prepared by spiking 2-ml aliquots of drug-free human plasma with known concentrations of the drugs of interest along with the appropriate internal standards as aqueous solutions of their hydrochloride salts. (These solutions were stable for several weeks when prepared in 0.01 *N* HCl and stored at 4°.) Tertiary and secondary amine analogs from the tricyclic class were chosen as internal standards. A range of 5–150 ng/ml in the spiked standards generally coincided with the range of actual blood levels measured in most patients. Internal standards were added at a concentration falling approximately in the center of the standard range. The standards were extracted simultaneously and identically with the unknowns.

The unknown plasmas were thawed, and 2.0-ml aliquots of each were pipetted into 8-ml screw-capped tubes with polytef-lined caps[13]. The same quantities of the internal standards were added to these as were added to the known standards, again as aqueous solutions of their hydrochloride salts. The pH of the samples was adjusted to 10.5–11.5 by dropwise addition of 1 *N* NaOH. Control of the pH with bicarbonate or other buffers was unnecessary, and dilution of the plasma with these buffers generally resulted in excessive emulsion formation with shaking.

Samples were extracted twice with 3 ml of 1.5% (v/v) isobutyl alcohol–heptane by shaking vigorously for 2 min and then centrifuging at 1500 rpm for 10 min to effect complete phase separation. The aqueous phases were discarded, and the combined organic phases were back-extracted into 1 ml of 0.1 *N* H_2SO_4, again with 2 min of shaking followed by centrifugation. The heptane phases were discarded, and the acidic aqueous phases were washed once with 4 ml of fresh extraction solvent; again the organic phases were discarded after centrifugation.

It was necessary to include this back-extraction step to eliminate interfering substances that gave mass fragments at identical *m/e* values to those being selectively monitored as well as late eluting contaminants that excessively prolonged the chromatography time. Three common contaminants, other than neutral lipids, substantially removed by this step were identified from their mass spectra as tributoxyethyl phosphate,

[1] Vacutainer, Becton-Dickenson, Rutherford, N.J.
[2] Merck Sharp and Dohme, West Point, Pa.
[3] Eli Lilly and Co., Indianapolis, Ind.
[4] Pfizer Research Laboratories, Groton, Conn.
[5] Ciba-Geigy Corp., Summit, N.J.
[6] Lakeside Laboratories, Milwaukee, Wis.
[7] Nanograde, Mallinckrodt, St. Louis, Mo.
[8] Burdick & Jackson Laboratories, Rockford, Mich.
[9] Pierce Chemical Co., Rockford, Ill.
[10] PCR, Gainesville, Fla.
[11] Model 3200E, Finnigan Corp., Sunnyvale, Calif.
[12] Model 6100, Finnigan Corp., Sunnyvale, Calif.
[13] Corning Glass Works, Corning, N.Y.

dibutyl phthalate, and dioctyl phthalate. The vacuum blood collection tubes[1] or their stoppers contain these compounds (40).

The samples were then readjusted to pH 10.5–11.5 by dropwise addition of 1 *N* NaOH and extracted twice with 2 ml of extraction solvent, followed by shaking and centrifuging as before. The upper organic phases were then transferred to clean, dry, screw-capped tubes and evaporated under a stream of dry gaseous nitrogen at 40°. Imipramine samples had to be analyzed at this point due to degradation of this tertiary amine by the derivatization reaction conditions of the next step. Samples were then derivatized to convert the secondary amine metabolites to products more easily analyzed by GLC.

To the dried residues in each tube were added 25 μl of ethyl acetate and 50 μl of heptafluorobutyric anhydride. The tubes were tightly capped and heated at 60° in a block-type heater. The maximal reaction yield was achieved in 30–60 min. The samples were dried again under gaseous nitrogen at 40°, with a few drops of toluene added near the end of drying to facilitate complete removal of unreacted heptafluorobutyric anhydride and residual heptafluorobutyric acid. The residues were taken up in 20–50 μl of ethyl acetate just prior to analysis.

Samples were stable for several days when stored either dry or in ethyl acetate at 4°. Samples, 1–5 μl, were then analyzed by GLC–chemical-ionization mass spectrometry with selected ion monitoring.

Reference Spectra—Aliquots of 100–200 ng of each pure reference compound (or the heptafluorobutyramide derivative of the secondary amines) were injected into the gas chromatograph–mass spectrometer–computer system and chromatographed using the following conditions. The silanized glass column, 6 mm o.d., 2 mm i.d. × 1.5 m, was packed with 3% SP-2250DB on 100–120-mesh Supelcoport[14]. The column oven was maintained at 205° isothermal, and the injector temperature was 235° isothermal. The carrier gas was methane, and the flow rate was adjusted to give a source pressure of 1000 μm (equivalent to a flow rate of 20 ml-atm/min).

The mass spectrometer conditions were: interface temperature, 170°; ion source temperature, at equilibrium (130–135°); emission current, 500 μamp; electron multiplier, 2100 v; electron energy, 105 ev; and preamplifier range, 10^{-9} amp/v.

The computerized data system produced an internal mass calibration table by scanning a spectrum of perfluorotributylamine for its known mass peaks. To the two or four masses selected, 0.1 or 0.2 amu was added for mass defect compensation. The integration time was automatically selected by the data system to optimize the signal-to-noise ratio, and the scan time was 1 sec. The mass spectrometer was operated under computer control in the scan mode (over the appropriate mass range).

Scans were taken at 2–3-sec intervals using an integration time of 8 msec/amu and stored on the computer disk memory. A reconstructed gas chromatogram was generated by the data system in real time from these scans as they were acquired. Each reference spectrum represents the scan corresponding to the chromatogram peak apex minus a background scan taken prior to the peak elution. The spectra were normalized on the base peak and plotted on a digital plotter[15].

GLC–Chemical-Ionization Mass Spectrometry—The selected ions monitored were chosen from the reference spectra (Table I). Up to four ions were monitored simultaneously by the data system. Although the ions as listed in Table I are of integer mass, they were actually monitored with +0.1 or +0.2 amu offset to compensate for mass defect. The quadrupole mass set voltages corresponding to these ions were automatically set by the computer referenced to a mass calibration table, with perfluorotributylamine[16] as the calibration compound. Integration times were determined by a computer algorithm to maximize the signal-to-noise ratio.

The standard curves were computer constructed from a multiple linear regression analysis of the mass chromatogram area ratios (standard drug–internal standard) *versus* the corresponding concentration ratios (nanograms per milliliter).

The final drug concentrations of the samples were determined mathematically from the ratios of the mass chromatogram peak areas (unknown drug–internal standard) using the slope and intercept of the standard curve.

RESULTS

Reference Mass Spectra—Methane chemical-ionization mass spectra of pure reference compounds, or their heptafluorobutyramide derivatives where appropriate, are shown in Figs. 1–7. The structures indicated are not those of the molecular ion(s) because the adduct protons or alkyl chains are not shown. The left ordinates represent the relative intensity normalized on the base peak (100%) of the mass range indicated. The right ordinates represent the percent of the sum of the total ions over the indicated mass range.

Table I—Selected Ions Monitored for Analysis of Tricyclic Antidepressants[a]

Compound	Base Peak (*m/e*)	Reference Peaks (*m/e*)
Amitriptyline	278 (MH^+)	233, 276
Nortriptyline heptafluorobutyramide	233	460 (MH^+)
Doxepin	280 (MH^+)	235, 278
Desmethyldoxepin heptafluorobutyramide	235	462 (MH^+)
Imipramine	281 (MH^+)	208, 279, 280
Desipramine bis(heptafluorobutyryl) derivative	228	430, 657 (MH^+)
Protriptyline heptafluorobutyramide	191	233, 460 (MH^+)

[a] Mass spectra were prepared from solutions of pure reference compounds (Figs. 1–7). The base peak in each spectrum was chosen for quantitation, and one or more other major peaks were selected as reference peaks for qualitative verification.

The spectra (Figs. 1–7) include the significant ions above *m/e* 100, extending approximately from the mass of the intact tricyclic ring system to the $(M + 41)^+$ ion. The most abundant ions found below this range and, therefore, not included were those at *m/e* 58 of the tertiary amines. The relative abundances of *m/e* 58 were 100, 95, and 30% for amitriptyline, doxepin, and imipramine, respectively, and represented 75, 30, and 8%, respectively, of the sum of the total ions between 50 and 350 amu for these compounds.

Amitriptyline, mol. wt. 277, gave a relatively simple spectrum (Fig. 1), with the base peak at *m/e* 278 from the protonated molecular ion MH^+. Also present were the molecular adduct ions at *m/e* 306 $(M + 29)^+$ and 318 $(M + 41)^+$ from the addition of $C_2H_5^+$ and $C_3H_5^+$, respectively. The $(M - 1)^+$ ion at *m/e* 276 was derived from hydride abstraction.

The spectrum of doxepin, mol. wt. 279, was analogous to that of amitriptyline, with the base peak at *m/e* 280 from the MH^+ ion and associated adduct ions at *m/e* 308 and 320 (Fig. 2). The $(M - 1)^+$ ion at *m/e* 278 was much less prominent relative to the MH^+ ion in this spectrum than were the corresponding ions in the spectrum of amitriptyline, probably due to the oxygen in the ethylene bridge of the doxepin ring system.

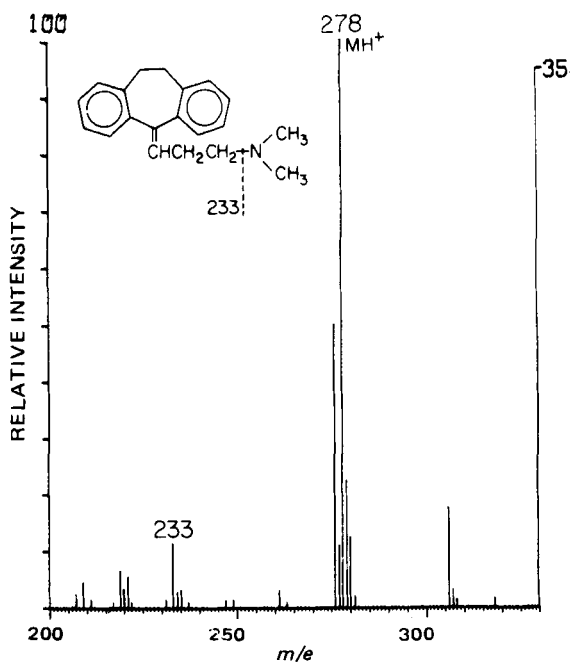

Figure 1—*Chemical-ionization mass spectrum and structure of amitriptyline.*

[14] Supelco, Inc., Bellefonte, Pa.
[15] Zeta Research Inc., Lafayette, Calif.
[16] FC-43.

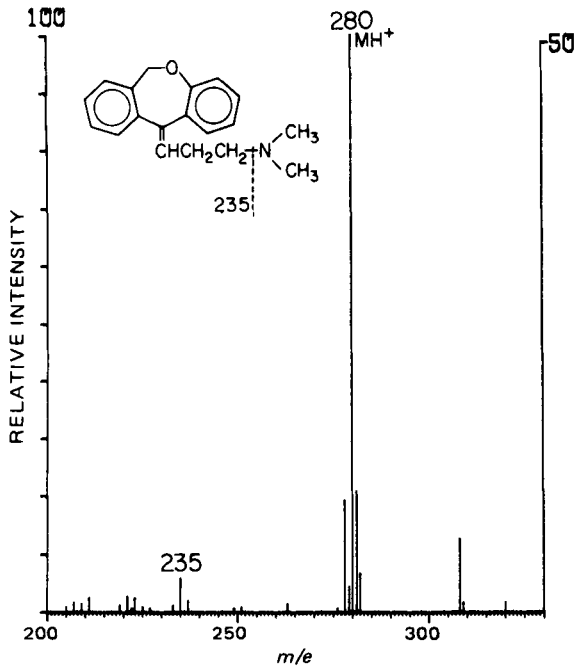

Figure 2—*Chemical-ionization mass spectrum and structure of doxepin.*

Imipramine, mol. wt. 280, yielded a spectrum analogous to spectra of amitriptyline and doxepin, with the MH^+ ion at *m/e* 281 as the base peak and the expected adduct ions at *m/e* 309 and 321 (Fig. 3). A relatively large M^+ ion at *m/e* 280 derived from charge exchange ionization and a relatively small $(M - 1)^+$ ion from hydride abstraction are the reverse of the relative intensities seen for amitriptyline and doxepin.

Nortriptyline heptafluorobutyramide, mol. wt. 459, gave a more complex spectrum than that of amitriptyline, with a fragment ion at *m/e* 233 as the base peak (Fig. 4). This ion (identical to *m/e* 233 of amitriptyline) was derived from inductive cleavage of the MH^+ ion with neutral loss of $C_3F_7CONHCH_3$. The MH^+ ion at *m/e* 460 and the associated ions at *m/e* 488 and 500 were much less stable than the analogous ions in the spectrum of amitriptyline and were, therefore, comparatively low in relative abundance (note that the intensities of all ions above 400

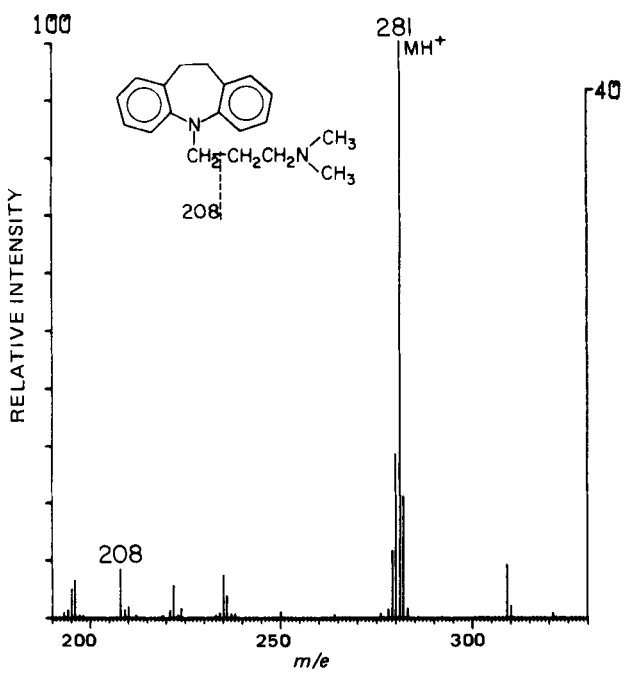

Figure 3—*Chemical-ionization mass spectrum and structure of imipramine.*

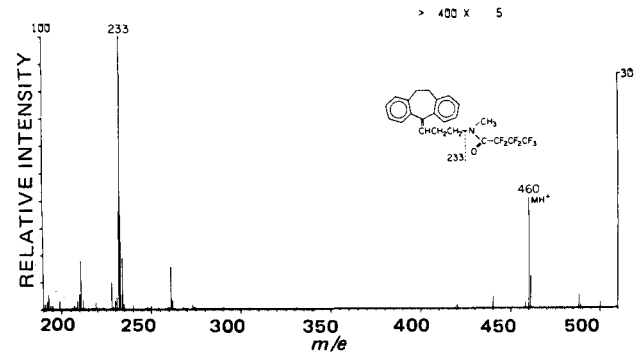

Figure 4—*Chemical-ionization mass spectrum and structure of nortriptyline heptafluorobutyramide.*

were magnified five times). Ions at *m/e* 440 and 420 were from single and double loss of HF, respectively. Fragments at *m/e* 261 and 273 were probably derived from *m/e* 388 $(M + 29)^+$ and 500 $(M + 41)^+$ by the same cleavage that produced *m/e* 233 from *m/e* 460.

Desmethyldoxepin heptafluorobutyramide, mol. wt. 461, gave a spectrum (Fig. 5) analogous to that of nortriptyline heptafluorobutyramide, with the base peak at *m/e* 235 formed from the MH^+ ion as described for the base peak of nortriptyline heptafluorobutyramide. The MH^+ ion was present at *m/e* 462 along with the accompanying adduct ions at *m/e* 490 and 502, all of low relative abundance. Ions at *m/e* 263 and 275 were probably from cleavage of *m/e* 490 $(M + 29)^+$ and 502 $(M + 41)^+$, as described for the analogous ions of nortriptyline heptafluorobutyramide.

Desipramine was converted by reaction with heptafluorobutyric anhydride to a diacyl derivative, mol. wt. 656, of uncertain structure. The spectrum of desipramine bis(heptafluorobutyramide) (Fig. 6) gave an MH^+ ion at *m/e* 657. The base peak at *m/e* 228 was structurally identified as $C_3F_7CON^+H_2CH_3$. Another prominent ion at *m/e* 430 $(MH - 227)^+$ was derived from loss of $C_3F_7CONHCH_3$ from the MH^+ ion. Reaction of desipramine with trifluoroacetic anhydride or pentafluoropropionic anhydride produced diacyl derivatives analogous to desipramine diheptafluorobutyramide. The yields of these reactions were essentially quantitative.

Protriptyline heptafluorobutyramide, mol. wt. 459, gave a spectrum significantly different from that of nortriptyline heptafluorobutyramide, mol. wt. 459 (Fig. 7). The base peak of protriptyline heptafluorobutyramide was at *m/e* 191, derived from loss of the side chain from the tricyclic ring system. A significant ion at *m/e* 268 probably represented charge retention by the cleaved side chain. The MH^+ ion at *m/e* 460 and accompanying ions at *m/e* 488 and 500 were of much greater relative abundance than the analogous ions in the spectra of the other secondary amine tricyclic heptafluorobutyramide derivatives (spectrum is shown with no vertical expansion). Prominent ions at *m/e* 233, 261, and 273 were probably from cleavage of MH^+, $(M + 29)^+$, and $(M + 41)^+$ ions as described for nortriptyline heptafluorobutyramide.

Relative Retention Times—Relative GLC retention times of the tricyclic antidepressants are listed in Table II. The values were normalized relative to amitriptyline (first column) and nortriptyline heptafluorobutyramide (second column), since these compounds were used as

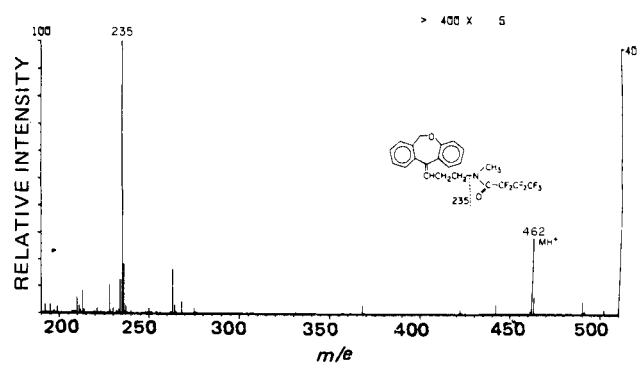

Figure 5—*Chemical-ionization mass spectrum and structure of desmethyldoxepin heptafluorobutyramide.*

Table II—Relative GLC Retention Times of Tricyclic Antidepressants[a]

Compound	Relative Retention Time
Amitriptyline	1.00
Doxepin (*cis-trans*)	1.70
Imipramine	1.40
Nortriptyline heptafluorobutyramide	3.25, 1.00
Desmethyldoxepin heptafluorobutyramide	
cis	3.90, 1.20
trans	4.52, 1.39
Desipramine bis(heptafluorobutyryl) derivative	1.23, 0.38
Protriptyline heptafluorobutyramide	4.33, 1.30

[a] The GLC effluent containing the solvent front was vacuum diverted ahead of the mass spectrometer ion source for 3.0 min from the point of injection until the start of data acquisition. The values were normalized relative to amitriptyline in the first column and nortriptyline heptafluorobutyramide in the second column.

the internal standards for the analysis of the other tricyclics. Doxepin and desmethyldoxepin were used as internal standards for the analysis of amitriptyline and nortriptyline. Doxepin and imipramine eluted later than, and were completely resolved from, amitriptyline under the conditions described. The *cis*- and *trans*-isomers of doxepin were not separable on this stationary phase.

Nortriptyline heptafluorobutyramide and desmethyldoxepin heptafluorobutyramide were separated, eluting much later than their tertiary amine counterparts, amitriptyline and doxepin. The *cis*- and *trans*-isomers of desmethyldoxepin heptafluorobutyramide were well resolved, in contrast to those of doxepin, eluting in that order after nortriptyline heptafluorobutyramide. Desipramine diheptafluorobutyramide eluted well ahead of nortriptyline heptafluorobutyramide, having the shortest retention time of any of the secondary amine acyl derivatives. Protriptyline heptafluorobutyramide was completely separated from nortriptyline heptafluorobutyramide.

GLC–Chemical-Ionization Mass Spectrometry—Typical mass chromatograms of four different ions (*m/e* values) monitored during a doxepin and desmethyldoxepin analysis are shown in Figs. 8 and 9. A slight separation of the *cis–trans*-isomers of doxepin was observed in Fig. 8. The *cis*-isomer appeared as a small shoulder on the leading edge of the much larger *trans*-isomer peak (the isomer ratio of the reference compound as supplied was 15:85 *cis–trans*). This was apparent in both peak 1 from the MH^+ ion (*m/e* 280) and the smaller peak from the $(M - 1)^+$ ion (*m/e* 278).

The *cis–trans*-isomers of desmethyldoxepin heptafluorobutyramide are separated in Fig. 9 (peaks 5 and 3). No *cis*-isomer is seen in Fig. 8 because the pure reference sample of desmethyldoxepin contained only the *trans*-isomer (verified by melting-point analysis). That peak 5 in Fig. 9 truly represents *cis*-desmethyldoxepin heptafluorobutyramide was confirmed by analyzing plasma from a doxepin overdosed patient. Identical mass spectra were obtained from both isomer peaks. The unnumbered fragmentogram peaks present at the same scan number (retention time) as a numbered peak were all generated from the single drug component eluting at that retention time. These smaller peaks represent ions with different *m/e* values and lower relative abundances than the base peak ion (producing the numbered peak) in the mass spectrum of the particular drug component.

Efficiency of Extraction—The extraction efficiency was measured by comparing samples containing internal standards with those containing external standards. Typical recoveries were greater than 95%.

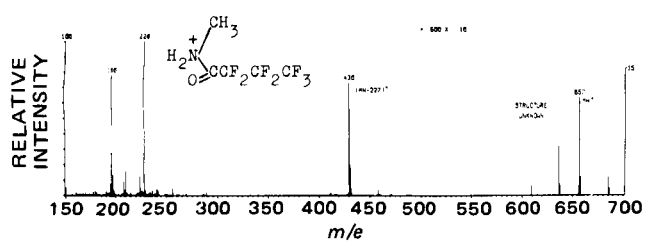

Figure 6—*Chemical-ionization mass spectrum and structure of desipramine bis(heptafluorobutyramide).*

Table III—Assay Precision[a]

Plasma Standard	Plasma Doxepin[a], ng/ml	*x* Area[b] Ratios	*n*	*SE*	*SE*, %
A	10	0.26	5	0.012	4.6
B	25	0.76	5	0.026	3.5
C	50	1.61	5	0.077	4.8
D	100	3.56	5	0.119	3.4
E	150	5.88	4	0.145	2.5
Plasma Standard	**Plasma Desmethyl-doxepin, ng/ml**	***x* Area[c,d] Ratios**	***n***	***SE***	***SE*, %**
A	10	0.29	5	0.014	4.8
B	25	0.81	5	0.030	3.7
C	50	1.59	5	0.124	7.8
D	100	3.55	5	0.148	4.2
E	150	5.04	3	0.254	5.0

[a] Forty nanograms per milliliter each of amitriptyline and nortriptyline were used as internal standards with doxepin and desmethyldoxepin, respectively. [b] Mass chromatogram peak area ratio = doxepin (*m/e* 280)/amitriptyline (*m/e* 278). [c] Mass chromatogram peak area ratio = desmethyldoxepin (*m/e* 235)/nortriptyline (*m/e* 233). [d] Combined area of *cis*- and *trans*-peaks.

Assay Precision—The precision of the described method is illustrated by the data in Table III from an analysis of doxepin and desmethyldoxepin. The standard error was generally less than 5% and is typical of results obtained for the other tricyclic compounds assayable by this method.

Standard Curve Linearity—Statistical analysis of 16 standard curves from the analyses of the three tertiary amine tricyclics gave a mean regression coefficient of 0.996 ± 0.003 ($\bar{r} \pm SD$). Similar analysis of 16 curves from the analyses of the four secondary amine tricyclic heptafluorobutyramide derivatives yielded a slightly better mean coefficient of 0.998 ± 0.002.

Patient Plasma Samples—The data in Table IV are representative of the plasma concentrations of the parent compounds and their monodemethylated metabolites as measured in samples from patients under treatment with the tertiary amine tricyclics. The dosages represent the total daily amount regardless of administration schedule. Indicated dosages were maintained for at least 4–5 days prior to sampling.

DISCUSSION

The measurement of blood tricyclic antidepressant levels is useful in the evaluation of the treatment of patients with these agents (1–5, 29). However, the routine clinical measurement of these compounds has only recently become feasible because of the sensitivity and specificity required.

Many techniques have been applied to the analyses of these substances. Qualitative and some semiquantitative analyses were reported using conventional TLC (7, 9). Quantitative application of TLC with 5–10-ng/ml sensitivity (4–5 ml of plasma required) was demonstrated using both UV and fluorometric direct densitometry (8, 10). Interference by other concurrently administered drugs is possible because qualitative accuracy is based upon R_f values and spot color tests.

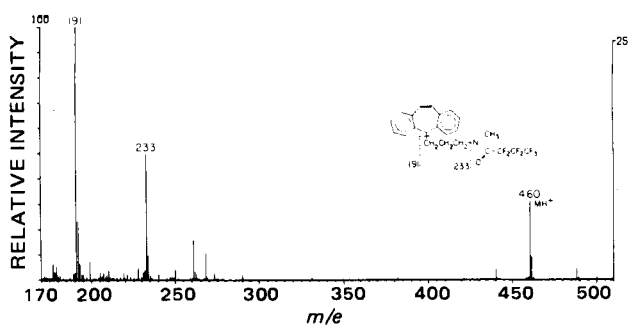

Figure 7—*Chemical-ionization mass spectrum and structure of protriptyline heptafluorobutyramide.*

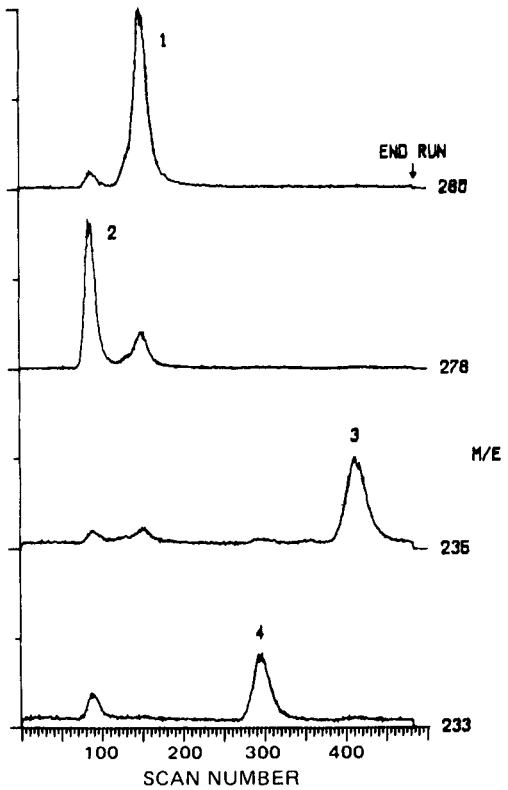

Figure 8—*Representative mass fragmentograms from doxepin and desmethyldoxepin analysis of a spiked plasma standard. Amitriptyline (2) and nortriptyline (4) were used as internal standards (40 ng/ml) for* cis-trans-*doxepin (1) and* trans-*desmethyldoxepin (3), respectively.*

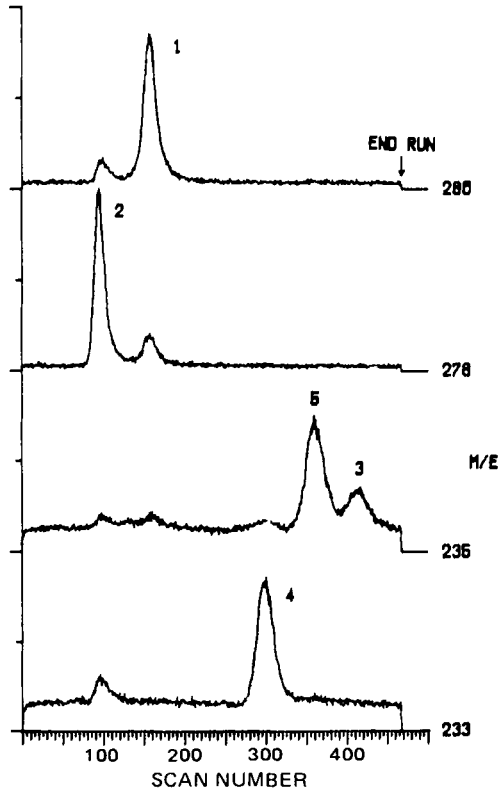

Figure 9—*Representative mass fragmentograms from doxepin and desmethyldoxepin analysis of plasma from a patient receiving doxepin. Amitriptyline (2) and nortriptyline (4) were used as internal standards (40 ng/ml) for* cis-trans-*doxepin (1) and* cis-*desmethyldoxepin (5), respectively.*

Photometric methods, including visible and UV spectrophotometry and fluorometry, were developed (11–16). Reported sensitivities ranged from 2 ng/ml to 2 μg/ml. Photometric methods lacking chromatographic separations generally showed poor selectively because of interferences by many compounds including metabolites and other drugs.

Radioisotopic derivatization methods were described (4, 17) for some primary and secondary amine tricyclics by acylation with ^{3}H-acetic anhydride. A method using ^{14}C-methyl iodide to convert imipramine to a radiolabeled quaternary amine has been the only application of isotopic techniques to the tertiary amine compounds (26). The reported sensitivities varied from 5 to 20 ng/ml. These methods are difficult to reproduce and are subject to interference by any compounds with derivatizable functional groups (including many concurrently administered drugs). These methods are unable to differentiate between the primary and secondary amine tricyclics, and only the method using ^{14}C-methyl iodide is applicable to the tertiary amine parent compounds.

Many GLC methods were described, differing primarily in the type of detector used. The flame-ionization detector yielded reported sensitivities ranging from 2 to 50 ng/ml (9, 20, 21, 28, 35). Qualitative accuracy necessarily was based upon relative retention times because of the nonspecificity of the flame-ionization detector. Cochromatography of contaminants with compounds of interest and prolonged chromatography times due to late eluting contaminants were common difficulties. Correction factors are required to compensate for variable flame-ionization detector responses to different compounds.

The electron-capture detector achieved sensitivities of 5–10 ng/ml for derivatives of some tricyclics (13, 19, 22, 26), but conversion of the tricyclics to products capable of capturing electrons was mandatory for detectability. Two approaches were used: acylation to polyfluorinated amides and oxidation to polyaromatic carbonyl derivatives. The first method was applied to desipramine (22), protriptyline (25), and nortriptyline (27) and its metabolites (19) but was not applicable to the tertiary amine tricyclics. Amitriptyline and nortriptyline were analyzed by the second method as anthraquinone, the product of their oxidation with ceric sulfate–sulfuric acid (13, 26). The oxidation of amitriptyline and nortriptyline yielded the same product, so only combined values were obtained. Protriptyline and cyproheptadine are also converted to anthraquinone and are potential sources of interference.

Difficulties associated with the electron-capture detector in general are: lack of sensitivity to nonelectrophilic compounds (*e.g.*, unaltered tricyclics), limited dynamic range, nonlinearity of response, foil contamination, and susceptibility to interference from electron-capturing contaminants. A nitrogen-specific detector was applied (24) to assays of amitriptyline and nortriptyline, with reported sensitivities of 5 and 10–15 ng/ml, respectively. Of several benzodiazepines and metabolites tested, only 3-hydroxydiazepam interfered. However, many other nitrogen-containing compounds and drugs cannot be ruled out as potential sources of interference.

Table IV—Typical Plasma Tricyclic Antidepressant Levels

Patient	Dose, mg/day	Plasma Concentration[a], ng/ml		
	Imipramine	Imipramine	Desipramine	Total
1	150	18	8	26
2	150	12	28	40
3	200	54	100	154
4	200	15	66	81
5	300	66	97	163
	Amitriptyline	Amitriptyline	Nortriptyline	Total
6	100	24	69	93
7	150	52	37	89
8	200	46	61	107
9	225	97	87	184
10	300	198	125	323
	Doxepin	Doxepin	Desmethyldoxepin	Total
11	75	22	24	46
12	100	17	27	44
13	150	26	40	66
14	225	103	118	221
15	300	122	115	237

[a] Blood was drawn 8–10 hr after the preceding dose of drug.

The application of the mass spectrometer as a gas chromatograph detector (41) made possible the direct identification of compounds as they eluted from the gas chromatograph. Initially, the sensitivity of the gas chromatograph–mass spectrometer was much less than some of the previously mentioned techniques due to the conventional mode of mass spectrometer operation by repetitive scanning over wide mass ranges. Selected ion monitoring (36, 42–44) increased the sensitivity of GLC–mass spectrometry up to 10,000-fold relative to the conventional scanning techniques.

Selective monitoring of single mass ions allowed detection of as low as 10^{-12} g of many compounds in some GLC–mass spectrometer systems (45). Single mass monitoring does not yield the qualitative information obtained by scanning complete mass spectra. However, partial mass spectra may still be obtained by simultaneous selective monitoring of several ions characteristic of the compound(s) of interest (chosen from complete spectra).

GLC–mass spectrometry with and without selected ion monitoring was applied to the analysis of nortriptyline and its metabolites (4, 29, 31, 35–38) and imipramine and desipramine (32–34, 39). Recently, a method was reported for the analysis of all commonly prescribed tricyclic antidepressants by GLC–electron-impact mass spectrometry with selected ion monitoring (30) with reported sensitivities of generally 10 ng/ml or less. An important feature common to all of these studies was the use of electron-impact ionization mass spectrometer systems.

Some additional problems are common to GLC as well as GLC–mass spectrometric analyses of the tricyclics. Column adsorption due to the chemical characteristics and small quantities of these compounds frequently occurs. Biggs *et al.* (30) reported the necessity of "priming" the chromatographic column with eight to 10 100-ng injections of drug prior to actual sample analyses. The present results support this finding, especially with new or infrequently used columns. Injection of 10–20 μl of the silylation reagent[17] also is beneficial. Adsorption is most serious for the free secondary amine tricyclics. However, their acyl derivatives (acetyl, trifluoroacetamide, pentafluoropropionamide, and heptafluorobutyramide) are chromatographed with comparative ease, showing little adsorption.

Imipramine and desipramine react differently to the acid anhydrides in the presence of ethyl acetate than any of the other tricyclics. Imipramine is degraded, and desipramine is converted to a diacyl derivative of uncertain structure. This problem was observed by several investigators (30, 46, 47), and a tentative structure for the diacyl derivative was proposed (47). Molecular weight, spectroscopic, and other data indicate that the compound is an enamine (1,2- or 2,3-). In the absence of ethyl acetate, using pure anhydride, only a monoacyl derivative is obtained, as with the other secondary amine tricyclics (47).

In the present report, a two-part analysis for imipramine and its desmethyl metabolite and utilization of the enamine derivative of the latter for quantitation are proposed. Under the reported reaction conditions, quantitative yields of the diacyl enamine product were obtained; with nortriptyline as an internal standard, the analytical accuracy and precision were equal to those achieved for the other secondary amine tricyclics forming monoacyl derivatives. Although it may be possible to eliminate the two-step analysis of imipramine and desipramine by use of a different solvent system or straight anhydride, a single consistent procedure applicable to the entire tricyclic class was desired, and some of these alternatives were not evaluated thoroughly. The use of *N*-perfluoroacylimidazoles was evaluated using nortriptyline and desmethyldoxepin and was unacceptable. The reaction by-products such as imidazole caused severe chromatographic tailing and interference at some of the masses monitored. A new reagent, *N*-methylbis(trifluoroacetamide), has not been tried. The acid anhydrides are preferred due to the ease of removal of the unreacted excess anhydride and free acid by evaporation prior to GLC analysis, thereby presenting no interferences.

The chromatography time required for separation of sample components sometimes is quite long and can be a problem when the number of samples is large. The method described generally requires 8–12 min/injection, depending on column length and compounds analyzed. This requirement can be complicated by late eluting contaminants such as plastisizers and cholesterol. Dioctyl phthalate and cholesterol are not completely removed during extraction, and it has been necessary to wait between injections for their elution (retention times of both are slightly longer than all of the compounds in Table II under the described conditions). Cholesterol specifically interferes at *m/e* 233 and 235, and dioctyl phthalate interferes at *m/e* 280.

[17] Silyl-8, Pierce Chemical, Rockford, Ill.

The methane chemical-ionization mass spectra of the tricyclics reported here differ significantly from those obtained by electron-impact ionization methods (30–32, 34, 36). A brief comparison of the characteristics of chemical ionization and electron-impact ionization of importance to their applicability to the assay of tricyclics should be noted. More comprehensive treatment is given elsewhere (40, 45, 48–51).

In chemical ionization, the energy transfer during ionization is much lower than in electron-impact ionization; consequently, less fragmentation occurs, resulting in simpler spectra with a predominance of intense high mass ions. The most characteristic ions of a compound are those of a high mass, including parent ion(s), since low mass fragment ions may originate from many compounds. For example, in the quantitative analysis of amitriptyline by chemical ionization, the intense parent ion at *m/e* 278 MH^+ is monitored; in electron-impact analysis, the base peak ion at *m/e* 58 is monitored (30).

No molecular ion (*m/e* 277) is observed in the electron-impact mass spectrum at the nanogram level. It is readily apparent that *m/e* 278 is much more diagnostic of the tertiary amine tricyclic amitriptyline than is *m/e* 58, an ion common to many amines. Also, background from column bleed and other sources is significant at low masses but is generally low or absent at high masses (*i.e.*, molecular ion region). A greater measure of selectivity is possible with chemical ionization by the monitoring of its characteristically abundant high mass ions while achieving sensitivity equivalent with electron-impact ionization.

The described method was applicable to the quantitative analysis of all commonly prescribed tricyclic antidepressants to a level of 1 ng/ml, with only a single minor modification necessary for imipramine and desipramine. The use of the members of this class of drugs as internal standards for each other eliminated the necessity of obtaining stable isotopically labeled compounds for this purpose.

With these selective and sensitive GLC–mass spectrometric assays for tricyclic antidepressants now available, it seems reasonable to expect that the other less expensive and more available assay methods for these substances be validated against these norms. Even when this is done, however, the lack of selectivity of these other techniques, as already noted, must be considered a serious limitation. It is hoped that the problem will be resolved by the development of inexpensive, selective, and sensitive radioimmunoassay methods. The major difficulty with the radioimmunoassay approach will be in developing a degree of selectivity sufficient to distinguish between the tricyclics as well as their metabolites. To date, no assay meeting these criteria has been reported.

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In Vitro Studies on Drug–Antibiotic Interactions I: Analgesics, Antipyretics, Antimalarials, and Tranquilizers

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Abstract □ The antimicrobial effects of some analgesics, antipyretics, antimalarials, and tranquilizers were determined. The phenothiazines were the most active group. The effect of the chosen drugs when combined with a selected number of antibiotics was studied on *Staphylococcus aureus* and *Escherichia coli* to determine the type of interaction. Most analgesics, antipyretics, and antimalarials showed either no effect or a synergistic action. However, some exhibited antagonistic effects. All tested tranquilizers were synergistic. Preliminary studies, using electronic absorption spectrometry, indicated that the antagonistic action may be attributed to a physical interaction.

Keyphrases □ Antimicrobial activity—evaluated in various analgesics, antipyretics, antimalarials, and tranquilizers, effect of combination with various antibiotics □ Drug–antibiotic interactions—various analgesics, antipyretics, antimalarials, and tranquilizers, effect of combination with various antibiotics □ Antibiotics, various—effect of combination with various analgesics, antipyretics, antimalarials, and tranquilizers □ Analgesics, various—antimicrobial activity, effect of combination with various antibiotics □ Antipyretics, various—antimicrobial activity, effect of combination with various antibiotics □ Antimalarials, various—antimicrobial activity, effect of combination with various antibiotics □ Tranquilizers, various—antimicrobial activity, effect of combination with various antibiotics

The bioavailability of drugs at their sites of action can be enhanced or reduced by interaction with other drugs. Several studies concerned the biochemical and pharmacological effects of antimicrobial agents when given with other drugs (1, 2). The type of interactions reported involved competition for renal tubular excretion, displacement from carrier sites, increased metabolism by stimulation of hepatic enzymes, decreased protein synthesis, and increased tissue toxicity (3, 4).

Analgesics, antipyretics, antimalarials, and tranquilizers generally are prescribed along with antibiotics for the treatment of infectious diseases. The pharmacological and