Electron Beam Ionization Mass Fragmentographic Analysis of Tricyclic Antidepressants in Human Plasma

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Abstract □ A method for the measurement, in human plasma, of all tertiary and secondary tricyclic antidepressants prescribed in the United States is described. The method uses electron beam ionization GLC–mass spectrometry, employing a computer-controlled multiple-ion detector. This method, mass fragmentography, is used with internal standards for each drug. Plasma levels to as low as 10 ng/ml of the following drugs can be measured: amitriptyline, nortriptyline, doxepin, desmethyldoxepin, imipramine, desipramine, and protriptyline. Deuterium-labeled amitriptyline and imipramine are used as internal standards for those two drugs; for the other drugs, deuterated amitriptyline, nortriptyline, desmethyldoxepin, or desipramine is used. The method can measure up to 15 samples/hr, making it practical for large-scale studies of these drugs in patients. Spectra of each drug and examples of their analysis are given.

Keyphrases □ Antidepressants, tricyclic—analysis, GLC–mass spectrometry (mass fragmentography), plasma □ GLC–mass spectrometry—analysis, tricyclic antidepressants, plasma □ Mass fragmentography—analysis, tricyclic antidepressants, plasma

One aspect of the rational treatment of depression with tricyclic antidepressants is a knowledge of the plasma level attained by the patient with a given dose. While methods for the measurement of the secondary tricyclic amines (*e.g.*, desipramine and nortriptyline) have been utilized for several years, the tricyclic antidepressants bearing tertiary amine groups on the side chain are more difficult to analyze. This paper describes studies using electron beam ionization mass fragmentography that have resulted in a rapid and general method for the measurement of all tertiary amine tricyclic antidepressants and their demethylated active metabolites used in the United States.

The use of individual mass spectral ions, selected because of their specificity for a molecule, as detectors for the gas chromatograph has become one of the most powerful modern analytical techniques. The essentials of the method were introduced in 1966 (1), and the power of the technique in the study of drugs was first demonstrated in 1968 through the identification of chlorpromazine and its metabolites in human plasma (2).

The ease of acquiring data by this method has been greatly enhanced by computer methods (3–6). The improvement of the signal by computer processing has extended the sensitivity of the method (3). In this system, the signal is sampled up to 400 times/sec, the findings are averaged to improve the signal to noise ratio, and the resulting data are stored and displayed. Finally, the increased accuracy of focusing on ions by computer control and the ability to monitor the ions sampled using the computer display have made it possible to make measurements using baseline resolution, with minimal contribution from adjacent interfering ions.

By the judicious choice of ions, selected for their intensity (sensitivity), their uniqueness to the drug being measured (selectivity), and their absence from plasma during the elution of the particular drug, the simultaneous measurement of each tricyclic drug and its internal standard is possible at the rate of 15 samples/hr. All data are stored on-line on magnetic tape and can be recalled for graphic or alphanumeric output at any time (7).

EXPERIMENTAL

GLC–Mass Spectrometry—All analyses were carried out on a gas chromatograph–mass spectrometer[1] computer[2] system, which was described previously (3). The mass spectrometric conditions were: ionizing energy, 70 ev; trap current, 60 μamp; source temperature, 270°; separator temperature, 250°; and helium carrier flow, 30 ml/min. Resolution was set to 0% valley at the masses monitored.

Silanized glass columns, 50 cm × 6 mm o.d., were packed with either 3% OV-17 or 1% SE-30 on 100–120-mesh Gas Chrom Q[3]. An injection port temperature of 230° was maintained, and column oven temperatures were varied from 180 to 220° to attain a retention time for the drugs analyzed of 2–3.5 min. The analysis of desipramine and protriptyline was carried out on SE-30, and the other drugs were chromatographed on OV-17. The electron multiplier preamplifier used in the mass fragmentography mode was modified to give approximately 30-fold greater amplification by substitution of a 10,000-Mohm resistor[4] for the standard input resistor of the preamplifier.

Reagents and Standards—Pure samples of each drug were provided by their manufacturers: amitriptyline[5], *N,N*-di-C^2H_3-amitriptyline[5], clomipramine[6], desipramine[7], desmethyldoxepin[8], doxepin[8], imipramine[6], nortriptyline[9], and protriptyline[5]. Solvents and reagents used were: nanograde petroleum ether[10], spectranalyzed carbon disulfide[11], analyzed reagent grade chloroform[12], and trifluoroacetic anhydride[13].

Derivatization of the secondary tricyclic amines (desipramine, desmethyldoxepin, nortriptyline, and protriptyline) was carried out by dissolving the drug in chloroform containing 10% trifluoroacetic anhydride. The reaction was complete in 30 min and was immediately subjected to GLC–mass spectrometry. Both desipramine and protriptyline underwent decomposition after standing in the 10% trifluoroacetic anhydride overnight. Tertiary tricyclic antidepressants were analyzed without derivatization.

Determination of Tricyclic Antidepressant Levels in Plasma—To 4 ml of heparinized plasma is added the appropriate internal standard (Table I) followed by 1 ml of 0.5 *M* NaOH. The plasma is then extracted with 10 ml of petroleum ether by shaking

[1] LKB-9000, LKB Produkter AB, Stockholm-Bromma 1, Sweden.
[2] PDP-12, Digital Equipment Corp., Maynard, Mass.
[3] Applied Science Laboratories, State College, Pa.
[4] Victoreen.
[5] Merck Sharp and Dohme, West Point, Pa.
[6] Ciba-Geigy Corp., Summit, N.J.
[7] Lakeside Laboratories, Milwaukee, Wis.
[8] Chas. Pfizer & Co., Brooklyn, N.Y.
[9] Eli Lilly and Co., Indianapolis, Ind.
[10] Mallinckrodt Chemical Co., St. Louis, Mo.
[11] Fisher Scientific Co., Fair Lawn, N.J.
[12] Baker Chemical Co., Philadelphia, Pa.
[13] Pierce Chemical Co., Rockford, Ill.

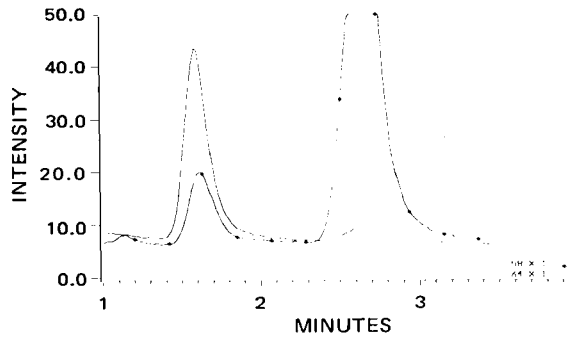


Figure 1—*Mass fragmentogram from patient plasma showing the ion intensities of amitriptyline,* m/e *58, and* N,N-*di*-C^2H_3-*amitriptyline,* m/e *64,* versus *elution time (4 ng of amitriptyline injected). The electron multiplier was operated at 2.7 kv. See text for other conditions.*

for 10 min in a shaker[14]. Occasional emulsions result, which are broken by sonication or freezing in dry ice–acetone. After centrifugation at 850×*g*, 8 ml of the organic phase is transferred to a 5-ml centrifuge tube, in two steps, and taken to dryness under a stream of nitrogen in a 60° water bath. The tubes are cooled to room temperature and rinsed down with 3 ml of petroleum ether. In the measurement of secondary amines, 0.1 ml of trifluoroacetic anhydride is added and the samples are allowed to stand for 30 min. The tubes are then rinsed down an additional three times with 0.1–0.25 ml of petroleum ether and are taken to dryness as before.

Samples are stable when stored at −20° for at least 1 week prior to analysis. At the time of analysis, the dry residue is taken up in 80 μl of carbon disulfide or chloroform and placed on ice to minimize evaporation prior to injection into the GLC–mass spectrometer. Depending on the plasma concentration of the drugs, 0.5–10 μl of the solution is chromatographed.

Dilution curves for each drug to be measured are prepared by adding various amounts of the test drug and a constant amount of the internal standard (Table I). Dilutions of 10, 50, 100, 200, and 300 ng/ml are made, in triplicate, of the free base form of the drug in 4 ml of pooled, non-drug-treated human plasma. Patient samples are similarly prepared by adding the same amount of internal standard. For overdose studies, a 1500-ng/ml sample is also prepared.

As an example, a patient treated with amitriptyline would have both that drug and its demethylated metabolite nortriptyline measured. To the patient's plasma would be added 50 ng/ml of deuterated amitriptyline and 100 ng/ml of desmethyldoxepin (Table I). After extraction and evaporation partially to dryness, trifluoroacetic anhydride would be added to derivatize the nortriptyline and desmethyldoxepin. Mass fragmentography would be carried out in two separate analyses, one for the amitriptyline–deuterated

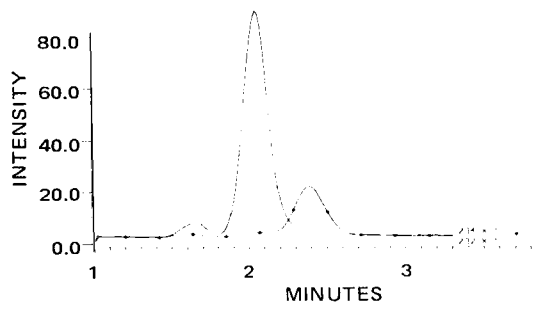


Figure 2—*Mass fragmentogram from patient plasma showing the ion intensities of* N-*trifluoroacetyl derivatives of nortriptyline,* m/e *232, and desmethyldoxepin,* m/e *234, the internal standard,* versus *elution time (8 ng of the nortriptyline derivative injected). The electron multiplier was operated at 2.5 kv. See text for other conditions.*

[14] Equipoise.

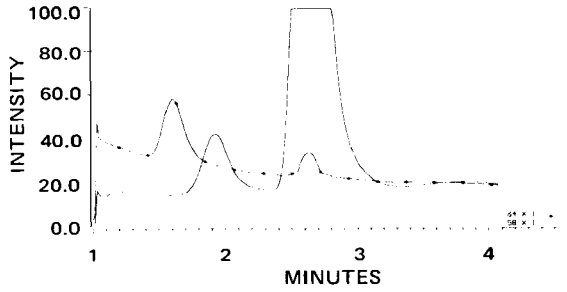


Figure 3—*Mass fragmentogram from patient plasma showing the ion intensities of doxepin,* m/e *58, and* N,N-*di*-C^2H_3-*amitriptyline,* m/e *64, the internal standard,* versus *elution time (4 ng of doxepin injected). The electron multiplier was operated at 3.5 kv. See text for other conditions.*

amitriptyline (Fig. 1) and the other for the desmethyl drugs (Fig. 2).

The determination of the concentration of each drug is made by reference to the internal standard, using the ratio of peak heights of the mass fragmentograms of the drug and internal standard. The use of area measurements did not improve the precision. Examples of these mass fragmentograms are given in Figs. 1–6. When this ratio of ion intensities is related to a dilution curve prepared as already described, the drug concentration in plasma is obtained directly. A typical standard dilution curve is illustrated in Fig. 7.

Whenever patients are on combinations of tricyclic antidepressants, internal standards for the desmethyl drugs cannot be used since the patient may be ingesting the compound used as an internal standard. In such cases, all injection and sample volumes must be carefully controlled throughout the procedure and the measurement must be made using the dilution curve and the measurement of mass fragmentographic peak heights instead of ratios to an internal standard.

RESULTS

GLC Retention Times—Table II shows the relative retention times on 3% OV-17 of the tricyclic antidepressants measured in this study. All tertiary tricyclic amines have shorter retention times than their *N*-trifluoroacetylmonomethyl counterparts, with the exception of clomipramine. For practical reasons, the *N*-trifluoroacetyl derivatives of desipramine and protriptyline are measured in patient samples using a 1% SE-30 column, resulting in better separation from plasma contaminants. Although retention times on SE-30 are shorter than on OV-17, the relative retention of these two drugs remains the same on the two columns.

Mass Spectra—*Amitriptyline (I)*—Under electron beam ionization, this drug gives one major fragment ion, *m/e* 58 (Fig. 8). In the spectrum of the *N,N*-di-C^2H_3-analog of amitriptyline, *m/e* 58 shifts to *m/e* 64. Thus, this ion results from chain fragmentation and has the structure $[CH_2{=}N(CH_3)_2]^+$ (I).

Doxepin (II)—Doxepin fragments similarly, giving even less abundant species at higher *m/e* values than amitriptyline.

Nortriptyline—As the *N*-trifluoroacetate (III), nortriptyline

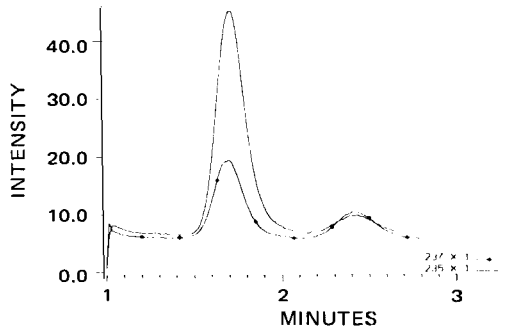


Figure 4—*Mass fragmentogram from patient plasma of imipramine,* m/e *235, and 10,10-di-*$^2H_2$-*imipramine,* m/e *237,* versus *elution time (4 ng of imipramine injected). The electron multiplier was operated at 3.5 kv. See text for other conditions.*

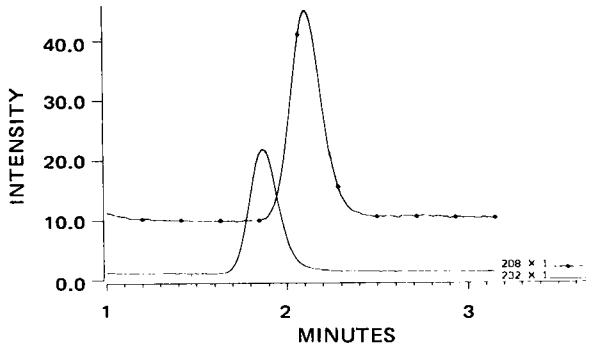


Figure 5—*Mass fragmentogram from patient plasma showing the ion intensities of the* N-*trifluoroacetyl derivatives of desipramine,* m/e *208, and nortriptyline,* m/e *232, the internal standard,* versus *elution time from the SE-30 column (4 ng of the desipramine derivative injected). The electron multiplier was operated at 2.5 kv. See text for other conditions.*

gives *m/e* 232 as the base peak (Fig. 9), which results from the loss of *N*-trifluoroacetylmethylamine from the molecular ion observed to a small extent at *m/e* 359.

Desmethyldoxepin (IV)—As the *N*-trifluoroacetyl derivative, desmethyldoxepin fragments in the same way as the nortriptyline derivative, losing *N*-trifluoroacetylmethylamine to give the base peak ion *m/e* 234 (Fig. 10).

Imipramine (V) and N-*Trifluoroacetyldesipramine (VI)*—These compounds fragment in a similar manner, as a result of the tertiary nitrogen in the tricyclic nucleus. Imipramine eliminates dimethylamine to give *m/e* 235, again the base peak. The molecular ion (*m/e* 280) is present in an abundance of 25% (Fig. 11). The spectrum of *N*-trifluoroacetyldesipramine (Fig. 12) also shows an abundant molecular ion (*m/e* 362). The ion used for analysis, *m/e* 208, results from α–β chain cleavage with charge retention, again by the tricyclic nucleus. In the spectrum of 10,10-$^2H_2$-imipramine, *m/e* 235 is shifted to *m/e* 237 as expected.

Protriptyline—As the *N*-trifluoroacetyl derivative (VII), protriptyline cleaves principally at the ring–chain juncture to give *m/e* 191, the dibenzocycloheptene ion, not surprising in its abundance (Fig. 13).

In experiments performed with the mass spectrometer source, the separator, and the chromatographic system at 180°, no noteworthy changes were seen in the spectra of amitriptyline or imipramine or of their trifluoroacetylated desmethyl counterparts. Thus, thermal effects on fragmentation, if present, are not abated at 180°.

Plasma Analysis of Tricyclic Antidepressants—*Amitriptyline*—This compound is measured by mass fragmentography monitoring *m/e* 58, and the deuterium-labeled internal standard is measured using *m/e* 64. Figure 1 shows an example of the analysis of a patient sample. The early (1.65 min) peaks are the drug and standard; the following peaks are from a plasma contaminant, well resolved chromatographically from the drug. The reliability of this analysis is surprising considering the possible substances that might have fragment ions at *m/e* 58–64. However, as the dilution curve (Fig. 7) shows from its linearity and extrapolation to zero concentration, no interfering substances are present. Typically, the standard curve has a slope of 0.011 and intercepts the ordinate at −0.072, with a correlation coefficient of 0.998. That this analysis, using electron beam ionization with its apparently unfavorable

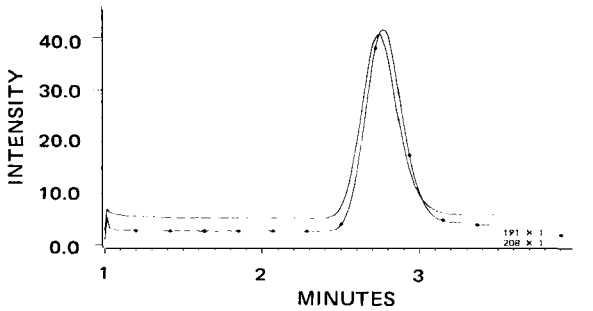
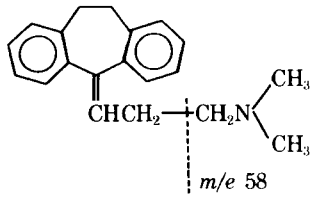
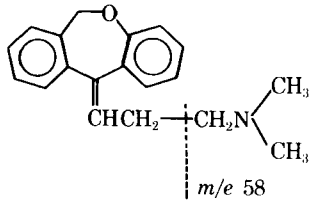


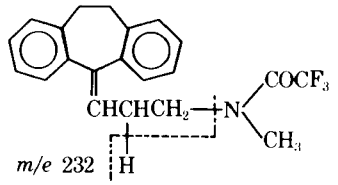
Figure 6—*Mass fragmentogram from patient plasma showing the ion intensities of the* N-*trifluoroacetyl derivatives of protriptyline,* m/e *191, and desipramine,* m/e *208, the internal standard,* versus *elution time (4 ng of the protriptyline derivative injected). The electron multiplier was operated at 2.5 kv. See text for other conditions.*



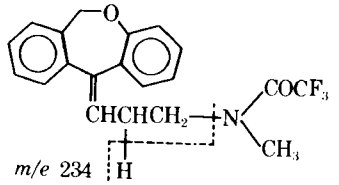
I: amitriptyline



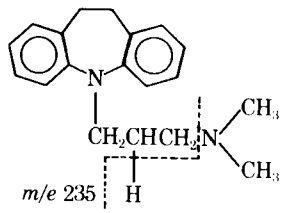
II: doxepin (*cis*-form)



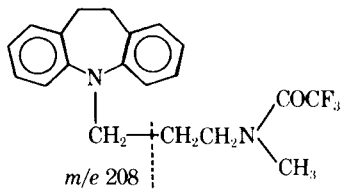
III: *N*-trifluoroacetylnortriptyline



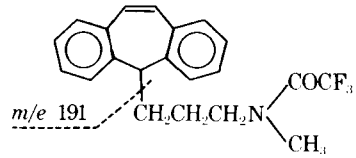
IV: *N*-trifluoroacetyldesmethyldoxepin (*cis*-form)



V: imipramine



VI: *N*-trifluoroacetyldesipramine



VII: *N*-trifluoroacetylprotriptyline

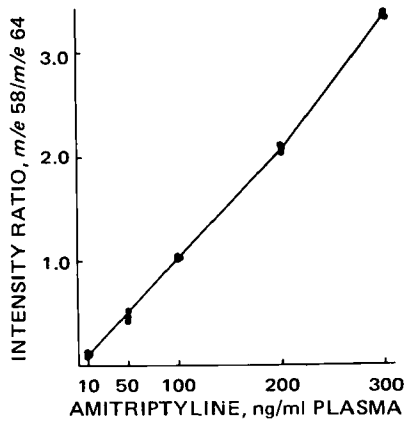


Figure 7—*Amitriptyline standard curve from pooled human plasma.*

fragmentation mode, is practical has been shown many times in this laboratory (*e.g.*, Fig. 14).

These analyses would not be possible without the use of the deuterium-labeled internal standard. Amitriptyline, as is true of imipramine and doxepin, is a difficult substance to analyze by GLC. All columns and phases tested in this laboratory show variable adsorption of these amines. This problem is overcome by making eight to 10 injections of 100 ng of the amine to prime the chromatographic column. The priming is seen as an increase in the peak height of the drug on sequential injections.

Even after the tertiary amines achieve a maximal chromatographic peak height, they show much greater variability in analysis than do the trifluoroacetyl secondary amines. However, the deuterated internal standards undergo the same vagaries in the analytical process as do the drugs themselves; thus, the ratio of the two is a very precise measure of the drug, as can be seen by the agreement of triplicate analyses in Fig. 7. Surprisingly, even immediately following the priming procedure, the injection of volumes of carbon disulfide or chloroform comparable to those used in the analysis does not displace the tricyclic amines from the column as a ghost peak. Nevertheless, the column does not remain saturated with the drug over extended periods and must be primed on each day of use.

Table I—Mass Fragmentography Internal Standards

Compound	*m/e*	Internal Standard	*m/e*	Amount of Internal Standard, ng/ml of Plasma
Amitriptyline	58	*N,N*-Di-C^2H_3-amitriptyline	64	50
Nortriptyline	232[a]	Desmethyldoxepin	234[a]	100
Doxepin	58	*N,N*-Di-C^2H_3-amitriptyline	64	50
Desmethyldoxepin	234[a]	Nortriptyline	232[a]	100
Imipramine	235	10,10-Di-$^2H_2$-imipramine	237	50
Desipramine	208[a]	Nortriptyline	232[a]	100
Protriptyline	191[a]	Desipramine	208[a]	100

[a] Measured as *N*-trifluoroacetyl derivatives.

Nortriptyline—Nortriptyline is measured with desmethyldoxepin as an internal standard, both as the *N*-trifluoroacetyl derivatives. Ions *m/e* 232 and 234 are measured, respectively. Figure 2 shows a mass fragmentogram of these two drugs from patient plasma, with *N*-trifluoroacetylnortriptyline having a retention time of 2 min and trifluoroacetyldoxepin eluting at 2.4 min. A small plasma contaminant frequently precedes the nortriptyline peak. A five-point standard dilution curve of nortriptyline, using desmethyldoxepin as the internal standard, typically has a slope of 0.02, an ordinate intercept of −0.035, and a correlation coefficient of 0.999. As is typical of the other secondary amine tricyclics, *N*-trifluoroacetylnortriptyline can be analyzed by GLC without priming effects or other complications.

Doxepin—This compound has been analyzed successfully by mass fragmentography using *N,N*-di-C^2H_3-amitriptyline as the internal standard. The analysis is much the same as that of amitriptyline. Again a plasma contaminant is detected by *m/e* 58 (Fig. 3), but the chromatographic separation is sufficient for the analysis. The standard curve follows the equation for a straight line be-

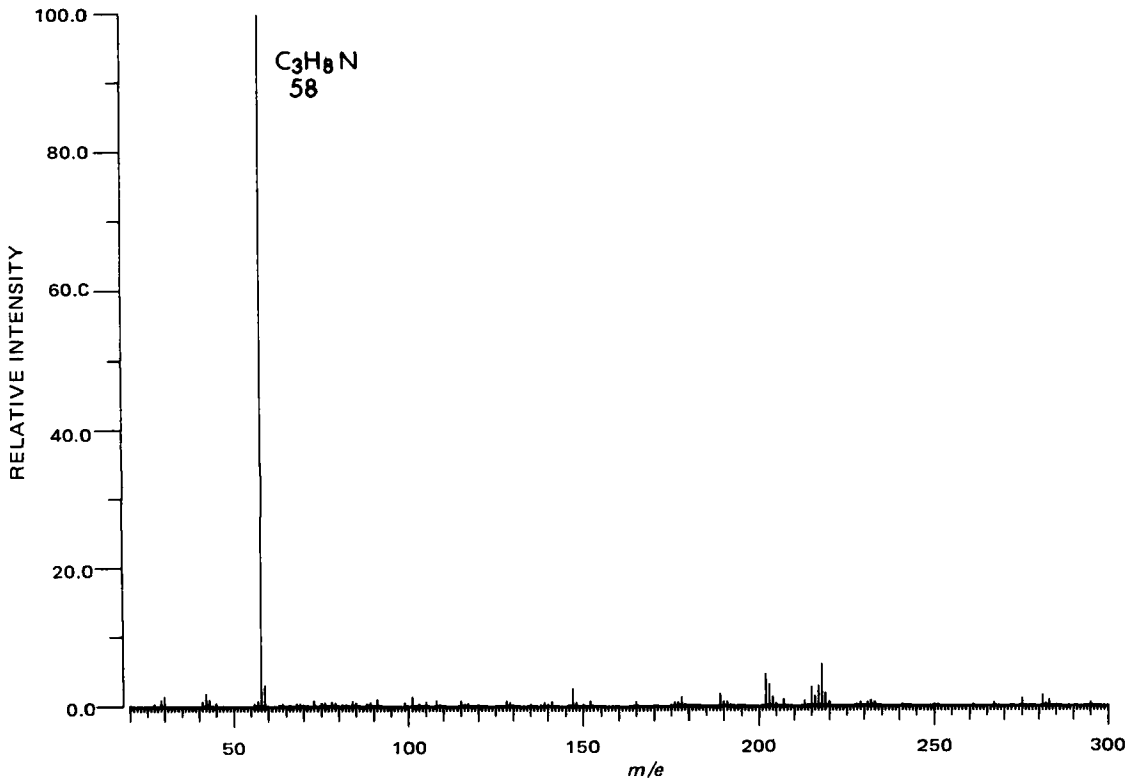


Figure 8—*The 70-ev mass spectrum of amitriptyline.*

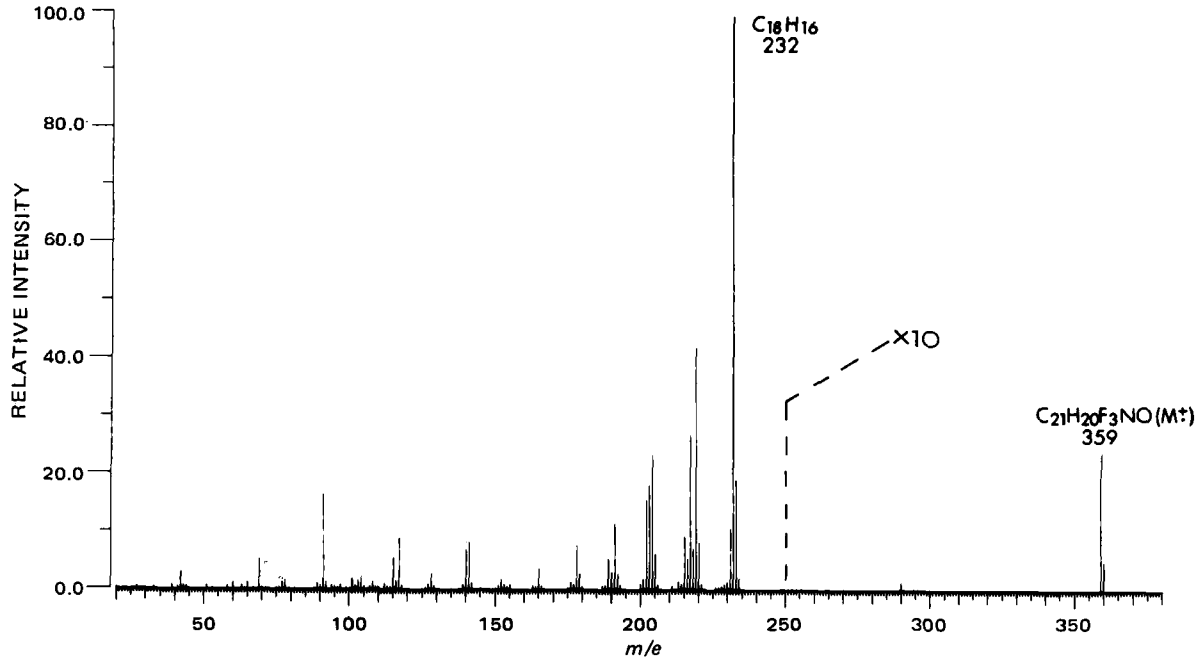


Figure 9—*The 70-ev mass spectrum of* N-*trifluoroacetylnortriptyline.*

tween 10 and 300 ng/ml, with a slope of 0.008, an ordinate intercept at 0.078, and a correlation coefficient of 0.989. As with amitriptyline, doxepin usually requires priming of the GLC column prior to a series of analyses.

Desmethyldoxepin—This derivative is measured using nortriptyline as an internal standard (Fig. 2). A typical five-point standard dilution curve for this procedure follows the equation for a straight line between 10 and 300 ng/ml, with a slope of 0.004, an ordinate intercept of 0.001, and a correlation coefficient of 0.997.

Imipramine—This drug, analyzed using 10,10-$^2H_2$-imipramine as the internal standard, presents problems similar to amitriptyline and doxepin chromatographically. However, following priming, a symmetrical chromatographic peak is obtained, resolved from a small plasma contaminant (Fig. 4). As with each of the other drugs studied, analyses can be carried out to levels of 10 ng/ml of plasma. The standard curve is linear to 1500 ng/ml, with a slope of 0.026, an ordinate intercept of 0.385, and a correlation coefficient of 0.995.

Desipramine—Desipramine can be measured conveniently using nortriptyline as the internal standard (Fig. 5). When analyzed on a 1% SE-30 column, the drug is separated better from the plasma contaminants, which precede the trifluoroacetyldesipramine peak (a trace may be seen eluting at 1 min in Fig. 5). A typical six-point standard curve for this procedure is linear between 10 and 1500 ng/ml, with a slope of 0.016, an ordinate intercept of 0.016, and a correlation coefficient of 0.998.

With proper attention to solvent volumes and chromatographic injection size, the desipramine analyses can be carried out readily without an internal standard. This procedure is necessary when a patient is being treated with amitriptyline or nortriptyline as well as desipramine. A significant peak in the spectrum of *N*-trifluoroacetyldesipramine, *m/e* 193 (Fig. 12), is unreliable for quantitative

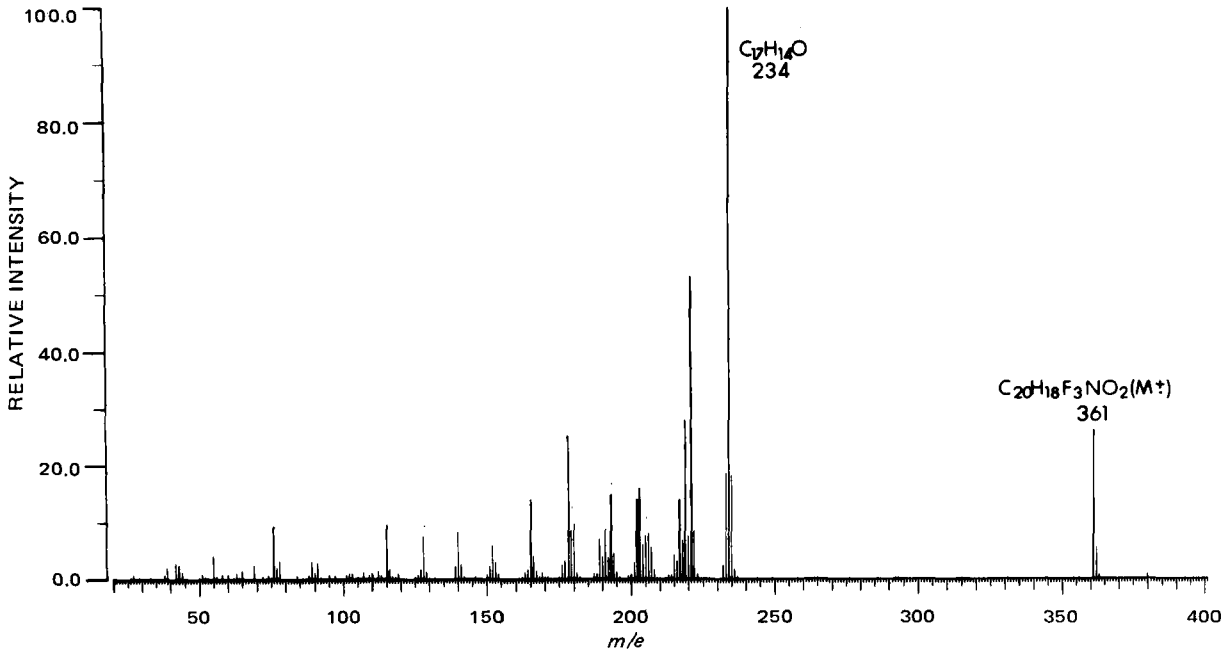


Figure 10—*The 70-ev mass spectrum of* N-*trifluoroacetyldesmethyldoxepin.*

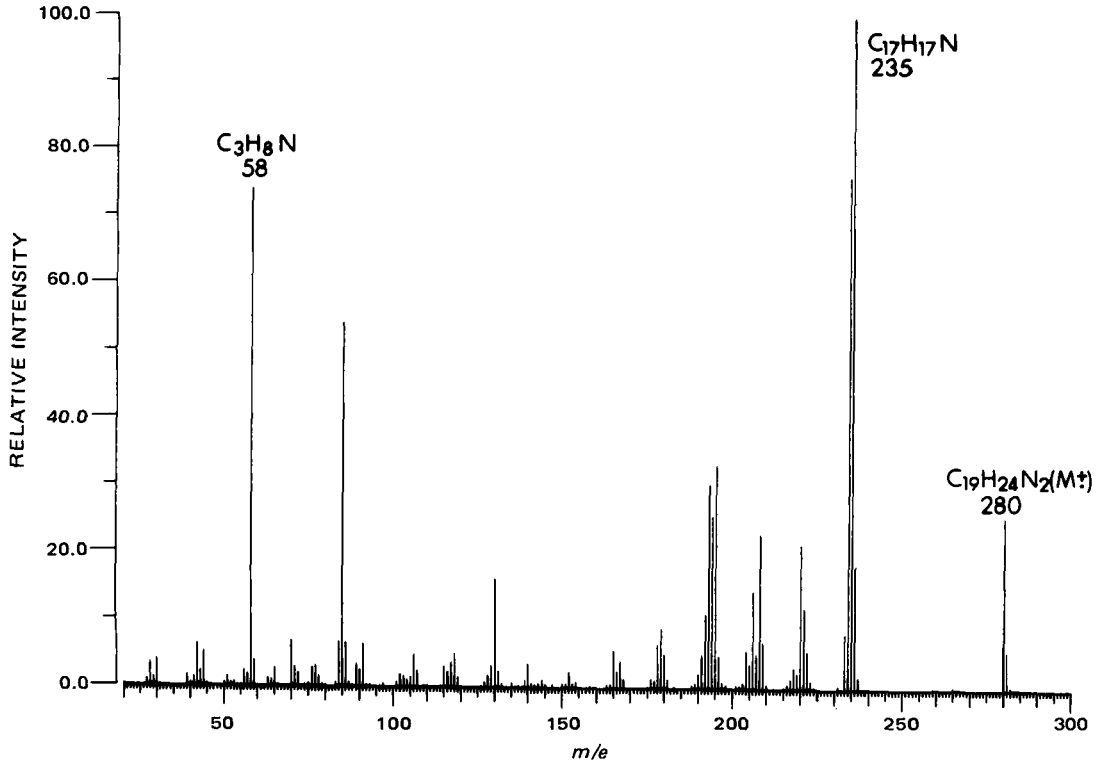


Figure 11—*The 70-ev mass spectrum of imipramine.*

measurements, probably due to plasma contaminants.

Protriptyline—This compound is similar to desipramine in that chromatography of the *N*-trifluoroacetyl derivative is best carried out on SE-30. Figure 6 shows a mass fragmentogram of the protriptyline derivative measured with trifluoroacetyldesipramine as the internal standard. Occasionally, plasma contaminants elute following the protriptyline derivative, but they are well resolved from it. A five-point standard dilution curve for this procedure is linear between 10 and 300 ng/ml in plasma, with a slope of 0.011, an ordinate intercept of −0.027, and a correlation coefficient of 0.996.

DISCUSSION

The measurement of plasma tricyclic antidepressant levels in patients is becoming generally accepted as a desirable component of treatment. However, major difficulties still exist in the measurement of the tertiary tricyclic amines, amitriptyline, doxepin, and imipramine, although these drugs are the most widely used clinically to treat depression. One fluorometric method (8) has not gained widespread use, nor have other indirect methods, due to a lack of sensitivity and/or specificity (9, 10).

GLC analysis of amitriptyline in this laboratory by the method of Braithwaite and Widdop (11) was complicated by variable chromatographic recovery as measured by inconsistent detector response for repetitive analysis of a standard. In addition, the column liquid phase described by these workers had a short useful life, necessitating frequent replacement. Even this column, which uses an amine liquid phase, required priming before it could be

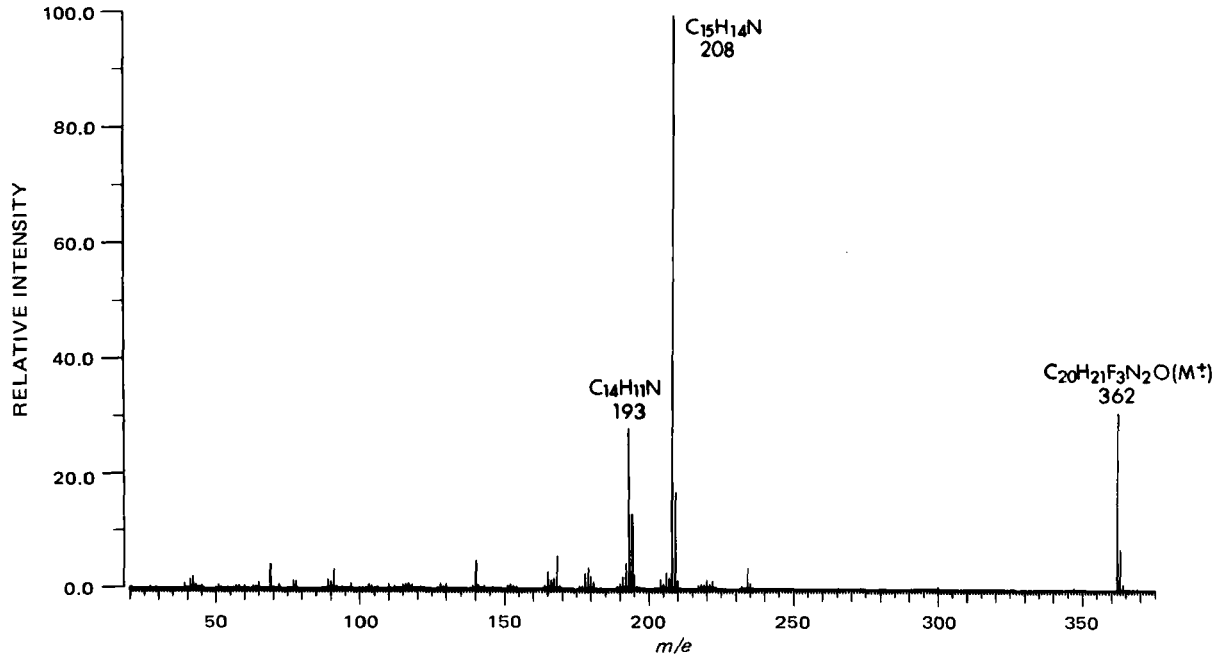


Figure 12—*The 70-ev mass spectrum of* N-*trifluoroacetyldesipramine.*

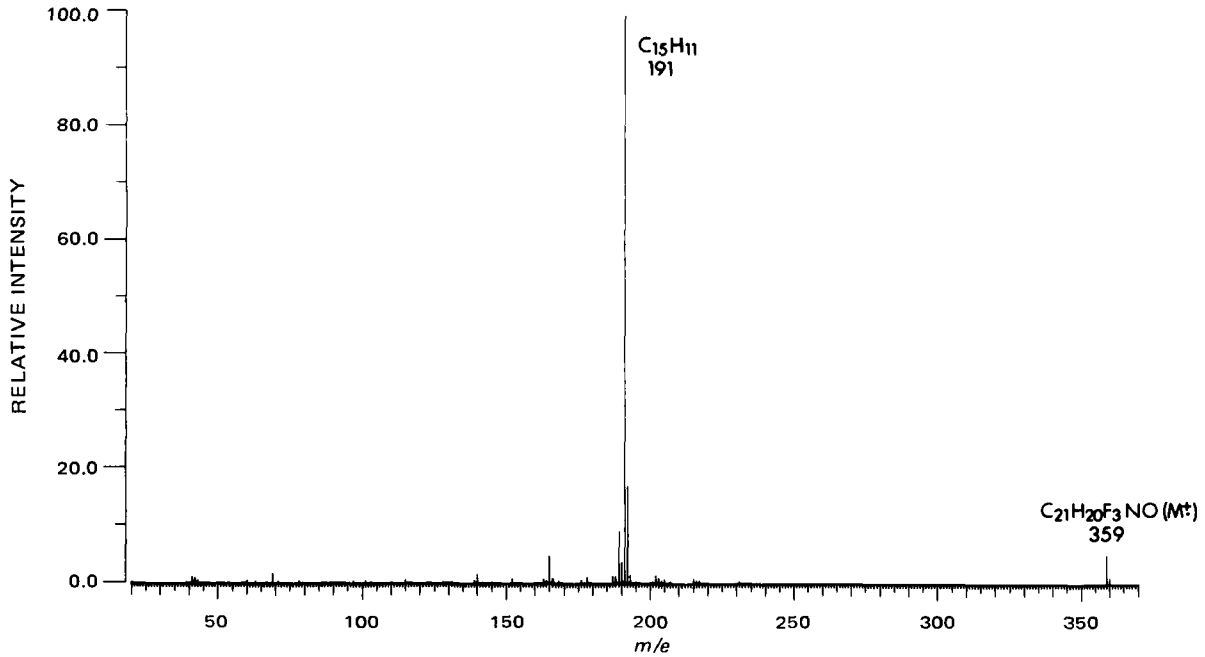


Figure 13—*The 70-ev mass spectrum of* N-*trifluoroacetylprotriptyline.*

used. And at best, only one or two analyses per hour could be carried out because of interference by late-eluting plasma substances which had to be cleared from the column prior to another analysis. It was also found, as reported by Hucker and Stauffer (12), that OV-17 columns served as well as the Braithwaite and Widdop column but without rapid degradation. However, the slowness of the GLC method using flame-ionization detection makes it impractical for clinical studies where many samples must be examined.

The most successful method for the measurement of the tertiary tricyclic amines has been mass fragmentography. Frigerio *et al.* (13), using promazine as an internal standard, measured imipramine in human plasma by this method. In this study, deuterium-labeled internal standards were employed wherever possible. They appear to be the ideal internal standards in that they most closely mimic the parent drugs with respect to plasma protein binding as well as analytical variables such as the partition coefficient in the extraction procedure and adsorption to glassware and chromatographic columns.

The secondary tricyclic amines (*e.g.*, nortriptyline, desipramine, desmethyldoxepin, and protriptyline) are much more readily measured in plasma, a consequence of having an available functional group for derivatization. The original method of Hammer and Brodie (14), wherein ^{3}H-acetic anhydride is reacted with the secondary amine to form a radioactive derivative, has been most widely used but is not applicable to the measurement of the tertiary amines. Borgå and Garle (15) showed that the heptafluorobutyryl derivative could be chromatographed as readily as the trifluoroacetate (11). Hucker and Stauffer (12) showed that the secondary amines can be analyzed by GLC without derivatization.

By using mass fragmentography, *N*-trifluoroacetylnortriptyline was measured in cerebrospinal fluid, plasma, and urine (16). The metabolism of nortriptyline was studied in humans using nortriptyline and deuterium-labeled nortriptyline (17). By this means the metabolites of the drug are easily discerned in chromatograms by their mass spectra, which show a "signature," seen as doublet spectra separated by three mass units whenever a GLC peak contains a metabolite.

Only one clinical study has been published in which a plasma tertiary tricyclic antidepressant level was compared with the therapeutic effect (18). This study involved the measurement of amitriptyline in 15 patients. Several studies of the relationship between nortriptyline plasma levels and antidepressant effect have been carried out with conflicting results (19–22). This correlation remains controversial (23); before the question can be resolved, much larger studies must be conducted and the tertiary tricyclic antidepressants must be included. Furthermore, in this center the availability of routine tricyclic plasma assays for the clinic service has, in many cases, improved patient care, from the trivial, but essential, case of ensuring patient compliance with ingesting the pre-

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

Compound	Retention Time Relative to *N*-Trifluoroacetylnortriptyline
Amitriptyline	0.47
N,N-Di-C^2H_3-amitriptyline	0.46
N-Trifluoroacetylnortriptyline	1.00 (5.75 min)
Doxepin	0.56[b]
N-Trifluoroacetyldesmethyldoxepin	1.26[b]
Imipramine	0.51
10,10-Di-$^2H_2$-imipramine	0.50
Clomipramine	1.01
N-Trifluoroacetyldesipramine	1.21
N-Trifluoroacetylprotriptyline	1.20

[a] Obtained on an F&M model 810 gas chromatograph using 50-cm × 6-mm o.d. silanized glass columns packed with 3% OV-17 on Gas Chrom Q, with a helium flow of 100 ml/min and an oven temperature of 195°. [b] Doxepin was obtained as a mixture containing 15% of the *cis*-isomer and 85% of the *trans*-isomer, which did not separate under the chromatographic conditions used. Desmethyldoxepin was obtained as the *trans*-isomer.

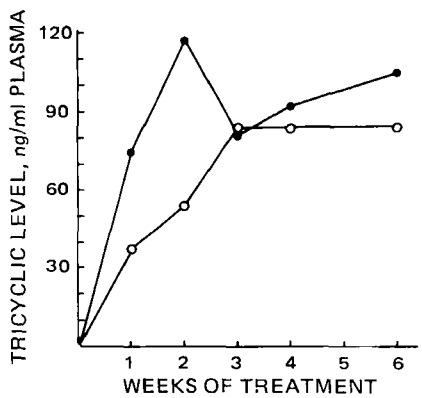


Figure 14—*Weekly measurement of amitriptyline* (●) *and nortriptyline* (○) *in plasma from a patient ingesting amitriptyline (50 mg three times daily).*

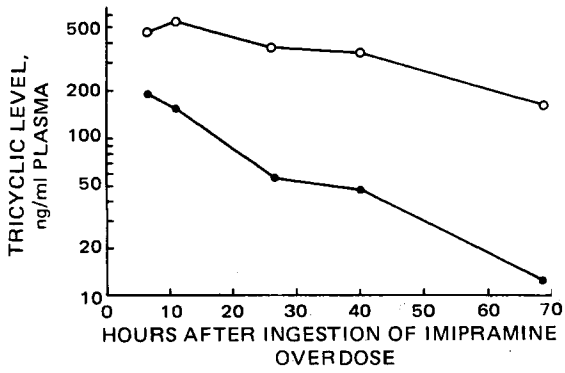


Figure 15—*Disappearance curve of imipramine (●) and desipramine (○) from patient plasma following an imipramine overdose.*

scribed drug to the question of attaining an "adequate" plasma tricyclic level. In addition to such applications, in which several hundred patient samples have been analyzed during the past year, plasma levels in overdose patients have also been studied.

Figure 14 illustrates the measurement of amitriptyline and nortriptyline in a patient treated with 50 mg of amitriptyline three times daily. Samples were collected weekly at 8 am prior to the morning dose. This patient essentially reached a steady-state plasma level in 3 weeks. Figure 16 illustrates the disappearance of doxepin and desmethyldoxepin from a patient who, after several weeks of treatment with doxepin, discontinued the medication. As can be seen, both drugs were measured over 50 hr. The last two data points in this figure were obtained by extrapolation of the standard curve.

Figure 15 illustrates the measurement of imipramine and desipramine in patients who ingested an imipramine overdose of an unknown amount. In this case, plasma samples were collected for up to 70 hr following the overdose. The data from patients presented in Figs. 14–16 were assayed in duplicate, with differences in all cases being less than 10%. Data involving patients treated with protriptyline were presented elsewhere (24).

In conclusion, a method has been developed that can be applied to the rapid and accurate measurement of all tricyclic antidepressants currently available in the United States. The use of internal standards and of computerized mass fragmentography has given the analysis unsurpassed precision and speed.

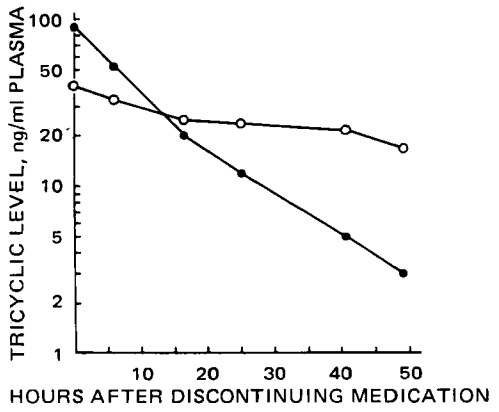


Figure 16—*Multiple analysis of doxepin (●) and desmethyldoxepin (○) after a subject discontinued doxepin (100 mg three times daily).*

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