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Simultaneous Measurement of Imipramine and Desipramine by Selected Ion Recording with Deuterated Internal Standards†

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Abstract—A gas chromatographic mass spectrometric method for the quantitative determination of imipramine and its *N*-demethylated metabolite desipramine in plasma samples at the nanogram level is reported. The method involves derivatization of the extracted drugs with trifluoroacetylimidazole, a mild derivatizing reagent. Specificity is provided by selected ion recording of the $[M + H]^+$ ions, formed upon chemical ionization with methane as reagent gas. Quantitation is achieved by stable isotope dilution techniques, using deuterium labeled analogs, prepared by acid-catalyzed exchange, as internal standards. Data on patient samples are presented.

Introduction

IMIPRAMINE, amitriptyline and their *N*-demethylated homologs, desipramine and nortriptyline are tricyclic antidepressants currently used in the treatment of primary affective disorders. The importance of monitoring antidepressants in plasma samples at a therapeutic level has been stressed by several investigators.[1–6] Most clinical data are on nortriptyline, for which Kragh-Sörensen *et al.*[4] reported clinical response for therapeutic levels below 175 ng ml^{-1}. Because of individual variation in the metabolism of drugs, patients on identical dosage may develop subtherapeutic or excessive and toxic levels. Therefore, the measurement of circulating blood levels provides useful clinical information in adjusting dosage schedules of antidepressants.

Methods on plasma level determinations of the secondary amines, nortriptyline and desipramine have been documented extensively in the literature.[7–10] Less information is available on methods allowing measurement of the tertiary amines, imipramine and amitriptyline.[11–13] This may be attributed to the complication that the tertiary amines, imipramine and amitriptyline are metabolized to *N*-demethylated and pharmacologically active compounds, desipramine and nortriptyline, resulting in the fact that both compounds have to be assessed in order to correlate circulating drug levels with clinical outcome.

A variety of methods have been described for the determination of tricyclic antidepressants; however, very few allow simultaneous measurement of imipramine and desipramine. Harris *et al.*[14] extended the technique of Hammer and Brodie,[15] who used coupling of secondary amines to radioactive acetic anhydride for measurement of desipramine, by adding a coupling of tertiary amines to radioactive methyl iodide for measurement of imipramine. A densitometry method has been described by Nagy and Treiber.[16] The relatively low sensitivity of these methods precludes their use for measurement in CSF, where low levels can be expected. Our interest in pharmacokinetic studies with single doses of imipramine and steady state levels in small plasma and CSF samples, has prompted us to develop a specific and sensitive method for simultaneous measurement of imipramine and desipramine. (Since the manuscript of this paper was prepared another g.c.m.s. method for simultaneous measurement of imipramine and desipramine using promazine as internal standard has been published by Belvedere, G.; Burti, L.; Frigerio, A.; Pantarotto, C. *J. Chromatogr.* **1975**, 111, 313.) In the present work, details on the method and data on steady state plasma levels of patients with primary affective disorders are reported.

The method utilizes selected ion recording, a technique introduced by Hammar[17] and stable isotope dilution procedures, first used by Samuelsson *et al.*[18] and Gaffney *et al.*,[19] who demonstrated their usefulness in biological work.

Experimental

MATERIAL AND GLASSWARE

The tricyclic antidepressants were supplied by the following companies: imipramine by Geigy (Ardsley, N.Y.), desipramine by USV Pharm. (Tuckahoe, N.Y.) and ^{14}C-imipramine by Amersham (Arlington Heights, Il.). The derivatizing reagents TFAA and TFAI were from Pierce Chem. Co. (Rockford Il.). Deuterium oxide was from Aldrich Chem. Co. (Milwaukee, Wi.). All other solvents or reagents were of analytical grade.

All glassware was washed with hot nitric acid, distilled water and ethanol. Pipettes used for the trans-

† Abbreviations used: TFA = trifluoroacetyl; TFAI = trifluoroacetylimidazole; TFAA = trifluoroacetic anhydride; CSF = cerebrospinal fluid.

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fer of extracts and the conical tubes used in their evaporation were prerinsed with a solution of 5% triethylamine in hexane.

PREPARATION AND ISOTOPIC DISTRIBUTION OF THE DEUTERIUM LABELED INTERNAL STANDARDS

d_4-Imipramine (2,4,6,8-tetradeutero-10,11-dihydro-5 (3,3-dimethylaminoaminopropyl)-5H-dibenz-[*b*,*f*]-azepine) and d_4-desipramine (2,4,6,8-tetradeutero-10,11-dihydro-5(3-methylaminopropyl)-5H-dibenz-[*b*,*f*]-azepine) were prepared by two subsequent exchanges in a 10% solution of deuterium chloride and acetic acid-*d* (CH_3COOD) in deuterium oxide, made by adding acetyl chloride to deuterium oxide.

Hydrochloride salts of the amines (200 mg) were dissolved in 30 ml of the exchanging medium and reacted at 80 °C. After 8 h the solution was cooled and lyophilized, and the exchange repeated. After cooling, the reaction mixture was extracted with ethyl acetate; the aqueous layer was alkalinized with 1 M sodium hydroxide and was twice extracted with hexane. Dried extracts were redissolved in 10 ml hexane and refrigerated until use. Calculation of the isotropic distributions was facilitated with a computer program, (Labdet) developed by Hammer and Vlietstra at Georgetown University, Washington, D.C. Data were obtained by selected ion recordings of ions in the molecular ion regions, formed by methane c.i. of the deuterated and the nondeuterated compounds. The isotopic distributions were: for d_4-imipramine: d_4, 55.5%; d_3, 33.3%; d_2, 6.2%; d_1, 0.8%; d_0, 0.2%: for d_4-desipramine: d_4, 55.6%; d_3, 35.6%; d_2, 7.8%; d_1, 0.7%; d_0, 0.1%.

STANDARD SOLUTIONS, CALIBRATION CURVES AND CALCULATION OF UNKNOWN CONCENTRATIONS

Standard solutions of imipramine and desipramine were made by dissolving the hydrochloride salts of the amines in 0.1 M HCl to give approximately 0.1 mg ml^{-1} calculated as free base. From these solutions appropriate dilutions, 1/250 for imipramine and 1/100 for desipramine were prepared in each series of experiments. The exact concentrations were taken into account when the calibration curves were made.

Solutions of d_4-imipramine and d_4-desipramine were prepared in methanol from the hexane extracts obtained in the acid-catalyzed deuterium exchange, to give approximate concentrations of 6 μg ml^{-1} for d_4-imipramine and of 10 μg ml^{-1} for d_4-desipramine.

Standards for the calibration curve were prepared with drug-free plasma by adding 20–200 ng ml^{-1} imipramine and 25–250 ng ml^{-1} desipramine, and by carrying these samples through extraction and derivatization procedures.

Quantitative calculations were based on peak area ratios of the $[M + H]^+$ ion records of the compounds of interest versus those of the d_4-labeled standards. Ratios for the standard mixtures were plotted against the concentrations in ng ml^{-1}, and an unweighted least squares linear regression analysis was performed. Using the regression characteristics of the calibration curve, the unknown drug concentrations were estimated and their associated standard errors were calculated.

EXTRACTION AND DERIVATIZATION PROCEDURE

After addition of 1 ml 0.1 M sodium hydroxide and 100 μl of the d_4-imipramine and d_4-desipramine to standard solutions which contained approximately 0.6 μg and 1 μg, respectively, 2 ml of plasma was extracted twice with 3 ml of hexane by horizontal shaking for 5 min in 15 ml glass-stoppered tubes. Hexane layers were transferred to 6 ml conical tubes and evaporated to dryness under a nitrogen stream. Emulsion did occur occasionally; in this case, the emulsion was broken by cooling with dry ice, and the water and the hexane layers were separated by centrifugation. The recovery of this extraction procedure was estimated by using 200 ng [^{14}C]-imipramine to be 38.1% (standard deviation = 1.7%; N = 3).

The dried extracts were dissolved in 20 μl of hexane, 2 μl TFAI was added and the mixture was reacted for 30 min at room temperature prior to analysis. Usually 1–2 μl of this solution was injected for g.c.m.s. analysis.

INSTRUMENTAL CONDITIONS

A dual e.i.c.i. Finnigan 3200 gas chromatograph mass spectrometer interfaced with a Finnigan 6000 data system has been used in this study.

Gas chromatography was performed on a 6 ft × 2 mm i.d. glass column, containing 1% OV-17 on Supelcoport 100/120 mesh with a helium flow of 30 ml/min^{-1}. Temperatures were: injector, 230 °C; column, 220 °C; g.c.m.s. interface, 230 °C.

The m.s. conditions for e.i. were: electron energy, 70 eV; emission current, 1 ma; continuous dynode electron multiplier voltage, 1.5 kV; pre-amp. range, 10^{-7} AV^{-1}. The m.s. conditions for c.i. were: electron energy, 150 eV; emission current, 1 mA; continuous dynode electron multiplier voltage, 1.7 kV; pre-amp. range 10^{-8} AV^{-1} for analysis plasma samples and 10^{-7} AV^{-1} for recording of reference spectra.

All selected ion recordings were performed by using the data system and the revision G software.

HUMAN SAMPLES

Patients

Ten hospitalized depressed patients were studied on a metabolic research unit at the NIMH, specifically designed for the collection of behavioral and biochemical data on a longitudinal basis. These patients were diagnosed by two psychiatrists and a social worker, according to Winokur's[20] criteria for primary affective disorders. The patients were drug-free for at least two weeks prior to the beginning of the study.

Drug regimen

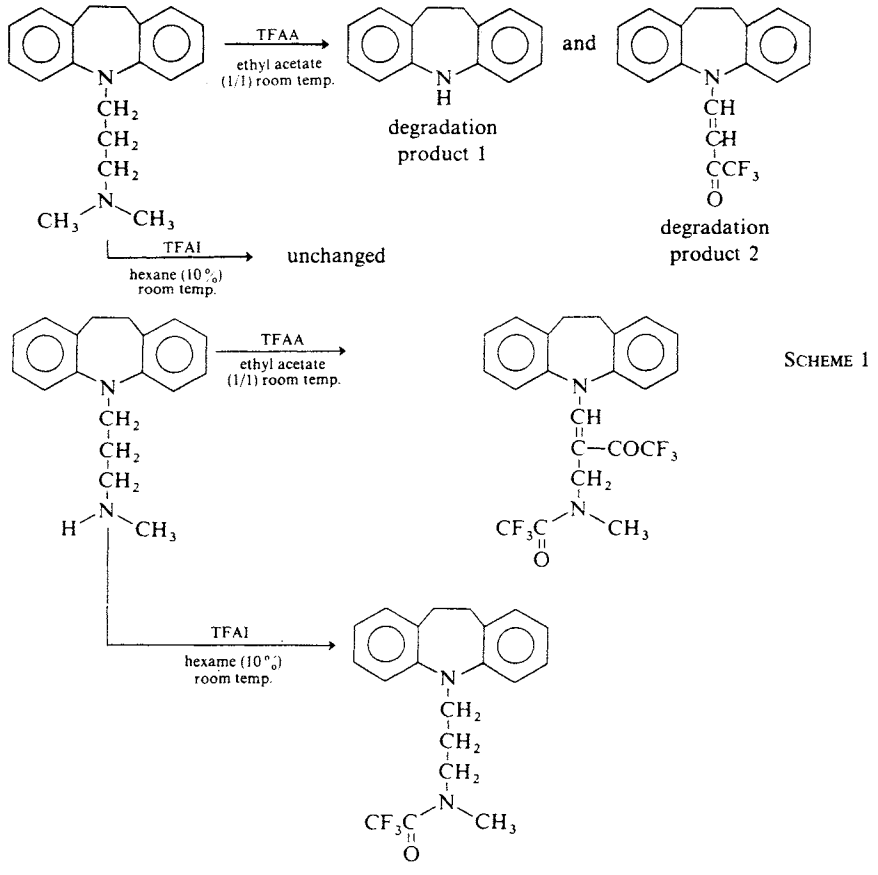
Imipramine was administered orally for at least four weeks in four divided doses ranging from 150–350 mg day^{-1}. Other drugs, including tranquilizers and barbiturates were carefully avoided before reaching the steady state level. To all patients except for two (one for cardiological problems, the other discharged against medical advice), the maximum therapeutic dosage (250–350 mg day^{-1}) was given without any significant side effect. Blood samples were drawn immediately before the administration of the morning dose. Plasma was obtained by centrifugation and was stored frozen (−20 °C) until analyzed.

Results

A gas chromatographic study was necessary in order to determine adequate g.c. behaviour for desipramine. While imipramine gives rise to a symmetrically shaped peak on a 1% OV-17 column, desipramine, a more polar secondary amine, tails when analyzed as such. Therefore, volatilization by making TFA derivatives was evaluated. Derivatization with TFAA alone or in combination with a solvent, ethyl acetate or hexane, was unsuccessful in our hands. Reaction of the amines with the system TFAA + ethyl acetate (1 : 1) for 15 min at room temperature yielded a bis-TFA derivative for desipramine and totally degraded imipramine. The structure of the bis-TFA derivative of desipramine has been studied extensively by Walle *et al.*[21] The structures of the degradation products of imipramine were elucidated on the basis of c.i. (methane) and e.i. mass spectral characteristics and are presented in Scheme 1. The mass spectra are listed in Table 1.

Reaction of the amines with the system TFAA + hexane (1 : 1) for 15 min at room temperature gave rise to a mixture of 31% degradation products and 69% unchanged product for imipramine and to 92% mono-TFA derivative and 8% bis-TFA derivative for desipramine. These data show that the reaction conditions for trifluoroacetylation must be controlled. By a careful choice of the reaction conditions Biggs *et al.*[22] were able to achieve satisfactory results for the analysis of desipramine. Reaction with a 10% solution of TFAI in hexane resulted in the desired products: formation of one derivative for desipramine, desipramine-mono-TFA and no degradation for imipramine (Scheme 1). This mild derivatizing method has been suggested by Franken and Trijbels,[23] who used it for derivatizing catecholamines and indolealkylamines. The method has the advantage that no elimination of the reagent is necessary prior to g.c.m.s. analysis and that the derivative of desipramine is stable in the reaction medium for at least two days. Retention indices of imipramine, desipramine, the degradation products of imipramine and the mono-and bis-TFA derivatives of desipramine are listed in Table 1.

In order to ensure maximum sensitivity for selected ion recording and to select a suitable internal standard for quantitative purposes, we compared the m.s. characteristics of the two compounds of interest, imipramine and desipramine-mono-TFA for several



SCHEME 1

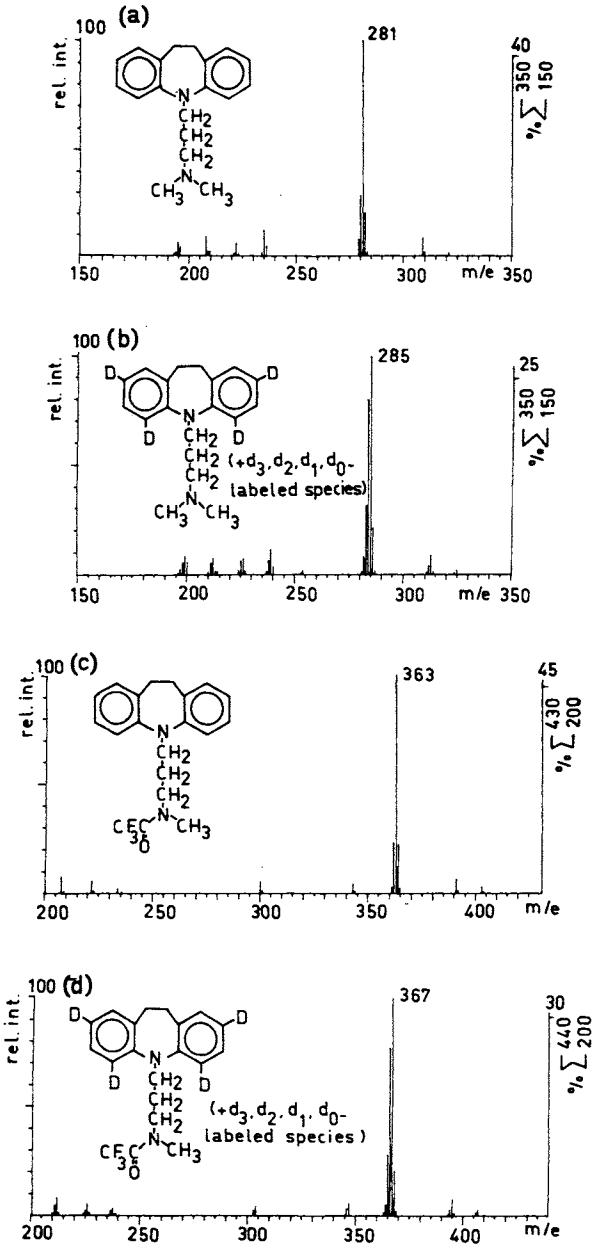
TABLE 1. Retention indices and m.s. data of imipramine, degradation products of imipramine, desipramine and desipramine-TFA derivatives

Compound name[a] (IUPAC name)	Retention index	m.s. data *m/e* (% rel. int.)[b]	$[M]^{+\cdot}$ (% rel. int.)[c]
Imipramine (10,11-dihydro-5-(3,3-dimethylamino-propyl)-5*H*-dibenz [*b*,*f*]-azepine)	2489	e.i.: 58 (100), 85 (50), 173 (17), 193 (17), 194 (14), 195 (15), 234 (22), 235 (20) c.i. methane: 85 (17), 86 (76), 222 (20), 235 (13), 279 (14), 280 (51), 281 (100), 309 (11) c.i. isobutane: 85 (13), 86 (23), 222 (4), 280 (3), 281 (100), 337 (14) c.i. ammonia: 86 (5), 196 (3), 222 (8), 280 (4), 281 (100)	280 (3)
Imipramine degradation product 1 (10,11-dihydro-5*H*-dibenz [*b*,*f*]-azepine)	2263	e.i.: 63 (15), 77 (15), 89 (18), 90 (16), 180 (49), 193 (25), 194 (100), 195 (99) c.i. methane: 194 (7), 195 (59), 196 (100), 224 (10)	195 (99)
Imipramine degradation product 2 (10,11-dihydro-5-(2-trifluoroacetylvinyl)-5H-dibenz [*b*,*f*]-azepine)	2582	e.i.: 51 (58), 63 (65), 65 (57), 69 (83), 77 (100), 89 (94), 102 (64), 248 (88) c.i. methane: 115 (3), 248 (21), 270 (3), 298 (11), 317 (16), 318 (100), 346 (24), 358 (12)	317 (14)
Desipramine (10,11-dihydro-5-(3-methylamino-propyl)-5H-dibenz [*b*,*f*]-azepine)	2572	e.i.: 42 (50), 70 (39), 71 (100), 85 (59), 130 (40), 193 (55), 194 (35), 195 (52) c.i. methane: 100 (8), 208 (8), 222 (8), 265 (13), 266 (39), 267 (100), 295 (9)	266 (2)
Desipramine-mono-TFA (10,11-dihydro-5-[3-(trifluoroacetyl-methylamino)propyl]-5H-dibenz [*b*,*f*]-azepine)	2735	e.i.: 42 (16), 69 (43), 91 (23), 140 (25), 168 (16), 192 (15), 193 (76), 208 (100) c.i. methane: 128 (21), 168 (39), 170 (20), 343 (5), 362 (27), 363 (100), 391 (10), 403 (5) c.i. isobutane: 168 (10), 362 (12), 363 (100) c.i. ammonia: 208 (3), 362 (11), 363 (100)	362 (6)
Desipramine-bis-TFA (10,11-dihydro-5-[3-trifluoracetyl-amino)-2-trifluoro-acetyl-1-propenyl]-5H-dibenz [*b*,*f*]-azepine)	2771	e.i.: 42 (29), 57 (27), 69 (100), 91 (35), 110 (33), 140 (36), 195 (50), 232 (34) c.i. methane: 128 (12), 310 (4), 316 (4), 330 (100), 358 (3), 371 (5), 437 (5), 457 (7)	not present

[a] Trivial name or descriptive name used in text.
[b] Maximum 8, no isotope and only peaks above 2% listed.
[c] Only listed for e.i.

modes of ionization. The mass spectra of these compounds are presented in Table 1. The e.i. spectrum of imipramine exhibits an intense peak at *m/e* 58 and ions of lower intensity at *m/e* 234 and 235. Fragmentation mechanisms for the formation of these ions have been rationalized by Frigerio *et al.*[13] Desipramine-mono-TFA gives rise to an e.i. spectrum showing two intense and characteristic ions at *m/e* 193 and *m/e* 208, corresponding to a rearrangement process and a C—C cleavage in the trifluoroacetylmethylaminopropyl sidechain. The c.i. methane spectrum of imipramine yielded an intense $[M + H]^+$ ion at *m/e* 281 and an intense ion at *m/e* 86, due to C—N cleavage in the sidechain. In the molecular ion region $[M]^{+\cdot}$ and $[M - H]^+$ ions could be detected too. In the c.i. isobutane spectrum the ion at *m/e* 86 is less intense and only $[M + H]^+$ ions were present in the molecular ion region. The c.i. ammonia spectrum exhibited only $[M + H]^+$ ions. The mass spectra in Table 1 indicate that the c.i. behaviour of desipramine-mono-TFA is parallel to that of imipramine. Comparing the e.i. and c.i. spectra it is clear that both e.i. and c.i. yield intense ions for determination of imipramine and desipramine. Taking into account the structures of these ions it is evident that e.i. requires the use of labeled analogs with the label at a specific position if stable isotope dilution techniques are to be employed. This limitation directed our quantitative work towards chemical ionization. In the c.i. mode four additive reagent gases were evaluated. Ammonia was not considered for routine purposes because of gas handling problems. Experiments with the use of ethylenediamine, a liquid, as a substitute for ammonia,[24] gave rise to problems with maintaining high sensitivity. Finally methane was selected on the basis of a sensitivity test; compared to isobutane, methane resulted in a seven-fold increase in sensitivity when the $[M + H]^+$ ion was recorded for 100 ng imipramine. It should be stressed that relative ion abundance in a mass spectrum, recorded in any ionization mode, is not the determining factor for sensitivity of selected ion recording in that particular ionization mode. The important criteria are the absolute ion currents, which can be compared by measuring a fixed amount of the compound of interest in the different modes of ionization. The partial c.i. methane spectra of imipramine, d_4-imipramine, desipramine-mono-TFA and d_4-desipramine-mono-TFA are presented in Figs. 1(a)–1(d). The $[M + H]^+$ ions at *m/e* 281, 285, 363 and 367 were chosen for selected ion recording. Selected ion records from a typical analysis are presented in Fig. 5.

From Figs. 1(b) and 1(d) it is apparent that in the spectra of the d_4-labeled analogs, there is a small response on the d_0-channel, corresponding to the loss of a hydrogen molecule from $[M + H]^+$ ions of d_2-labeled species. The presence of ions at the *m/e* value of the $[M + H]^+$ ions of the nondeuterated compounds does not complicate calculations of unknown concentrations, since a calibration curve was made in each series of experiments and the regression characteristics



FIGS. 1(a)–1(d). Partial c.i. methane mass spectra of imipramine, d_4-imipramine, desipramine-mono-TFA and d_4-desipramine-mono-TFA. The ions at *m/e* 281, 285, 363 and 367 were chosen for selected ion recording.

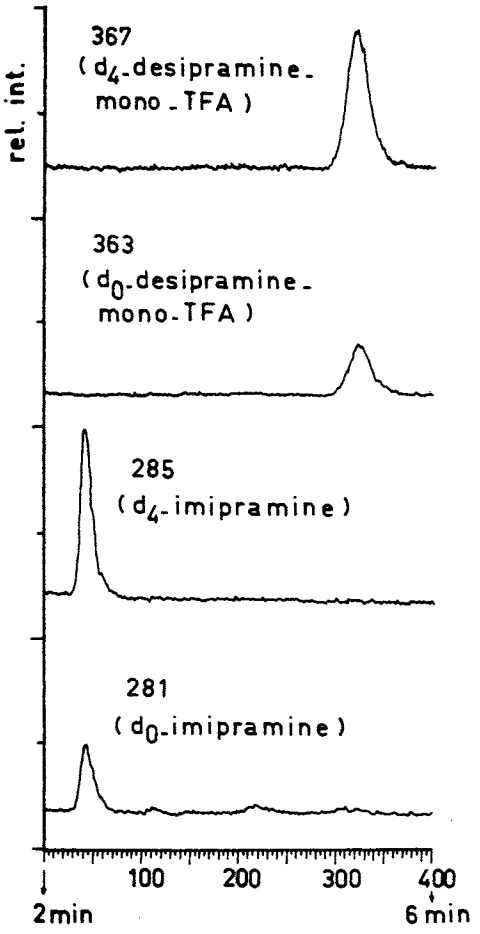
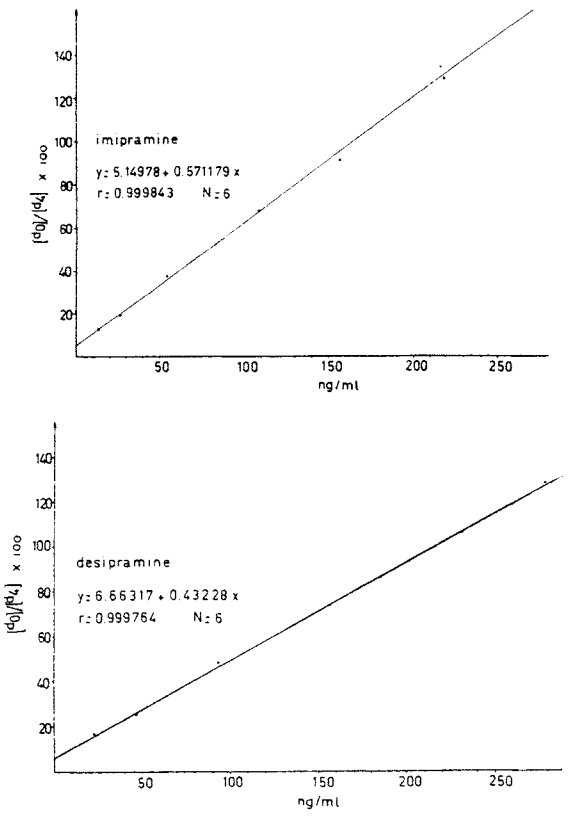


FIG. 2. Selected ion records obtained from a patient sample.

of this curve were used for calculating the unknown concentrations. Since deuterium-labeled standards, prepared by acid-catalyzed exchange are used, back-exchange of these products could occur in the extraction and derivatization procedures and in the source of the mass spectrometer during the ionization process. Acid-catalyzed exchange at activated positions in aromatic systems is discussed extensively by Thomas.[25] Experiments revealed that no back-exchange occurred during the extraction and derivatizing procedures: d_4-imipramine, carried through these steps showed the same isotopic pattern as untreated d_4-imipramine. Comparison of isotopic distributions, calculated from c.i. methane and c.i. ammonia data, gave no evidence that a deuterium atom gets lost preferentially in one of the two ionization processes and is involved in the loss of a hydrogen molecule in the c.i. methane mode. For the calculation of the isotopic distributions from c.i. methane data it was assumed that none of the deuterium atoms was involved in the loss of a hydrogen molecule from $[M + H]^+$ ions. The c.i. ammonia ionization only gave rise to a $[M + H]^+$ ion in the molecular ion region and calculated isotopic distributions based on these data, resulted in values which were within 1% of values obtained with methane. The fact that a deuterium atom is not lost in either ionization mode indicates that exchange does not occur or occurs reproducibly during the c.i. process and does not affect the precision of the method since a calibration curve is used in each series of experiments. Figs. 3(a) and 3(b) present the calibration curves and their regression characteristics for imipramine and desipramine in a typical experiment. Table 2 is a list of calculated results, estimates of concentrations and associated standard errors, for the unknown plasma samples from the same experiment.

From a review of the literature dealing with quantitation by selected ion recording, it is apparent that a variety of statistical methods have been applied. We believe the correct procedure is the use of the regression line in reverse; that is, the regression line of response ratio on concentration is used to estimate unknown



FIGS. 3(a) and 3(b). Calibration curves and regression characteristics for imipramine and desipramine quantitation in a typical experiment.

concentrations from measured response ratios. This procedure is not generally available on preprogrammed calculators or as a function in software statistical packages, and is not to be confused with the normal use of the regression line, predicting y-values from known x-values. Unweighted least squares linear regression analysis seemed appropriate as the absolute errors in peak ratio determination were approximately constant. A more careful investigation of errors using variance analysis is in progress. A short computer program in BASIC (available from the authors upon request) was written in order to facilitate calculations.

Two different solvents, hexane and benzene were evaluated for the extraction of the drugs from alkalinized plasma. From the work of Walle and Ehrsson[27] it is evident that benzene is a superior solvent for the extraction of desipramine. In our g.c.m.s. assay, however, benzene extraction gave rise to an interference peak, having an ion at *m/e* 281 and coeluting with imipramine. Hexane did not result in this interference and was used subsequently. A large contamination peak did occur on the *m/e* 367 record and was identified as cholesterol-TFA on the basis of its retention time and typical ions in its c.i. methane spectrum. The long retention time (retention index = 3059) of this substance resulted in a relatively long analysis time (10 min) for each sample in order to avoid its appearance in later runs. Preliminary results on patient samples are given in Fig. 4, summarizing the maximum daily dosage of imipramine, the steady state plasma levels of

TABLE 2. Estimated concentrations and associated standard errors obtained from $[d_0]/[d_4]$ ratio data[a] of patients samples in a typical experiment

Sample number	Imipramine			Desipramine		
	$[d_0]/[d_4]$ × 100	Concentration (ng ml^{-1})	Standard error (ng ml^{-1})	$[d_0]/[d_4]$ × 100	Concentration (ng ml^{-1})	Standard error (ng ml^{-1})
1	27.75	39.6	1.8	15.38	20.2	3.0
2	47.48	74.1	1.8	16.70	23.2	3.0
3	47.57	74.3	1.8	28.87	51.4	2.9
4	65.96	106.5	1.8	43.02	84.1	2.8
5	7.46	4.1	1.8	5.23	not detectable	
6	38.27	58.0	1.8	60.40	123.5	2.7
7	11.95	11.9	1.9	6.49	not detectable	
8	55.15	87.5	1.7	22.29	36.1	3.0

[a] Mean value, obtained from 3 injections.

imipramine, desipramine and their total, and the mean steady state ratio imipramine/desipramine for eight patients who had established a steady state in the time period studied.

From Fig. 4 it becomes evident that a large variability exists for steady state plasma levels in this small patient group. The steady state plasma levels range from 54.8 ng ml^{-1}–293.0 ng ml^{-1} for imipramine, from 64.8 ng ml^{-1}–180.0 ng ml^{-1} for desipramine, from 139.0 ng ml^{-1}–473.0 ng ml^{-1} for the (imipramine + desipramine)-total and from 0.48–1.85 for the imipramine/desipramine ratio. The clinical response of each of the patients and their diagnosis are also included in Fig. 8. There was no indication for a correlation between plasma drug levels and clinical outcome. More work is in progress to illuminate this important clinical issue.

Discussion

The most characteristic features of the g.c.m.s. method presented in this work are (1) the mild trifluoroacetylation procedure, (2) specific and sensitive detection provided by selected ion recording of the $[M + H]^+$ ions formed upon c.i. with methane, and (3) precise quantitation, achieved by the use of d_4-labeled analogs. Since only $[M + H]^+$ ions are used for the detection of the compounds, it should be possible to extend the method to related amines, e.g. amitriptyline and nortriptyline.

The method as it stands now offers adequate sensitivity for steady state plasma determinations. The clinical relevance of continuous monitoring of the plasma levels of antidepressant drugs has been stressed in numerous studies. In this work the large individual variability in the steady state plasma levels has been confirmed. The finding of large variations in the imipramine/desipramine ratios ranging from 0.48–1.85, further demonstrates the variable metabolic pattern of the patients studied. Systematic and continuous monitoring of the tricyclic antidepressants in plasma from

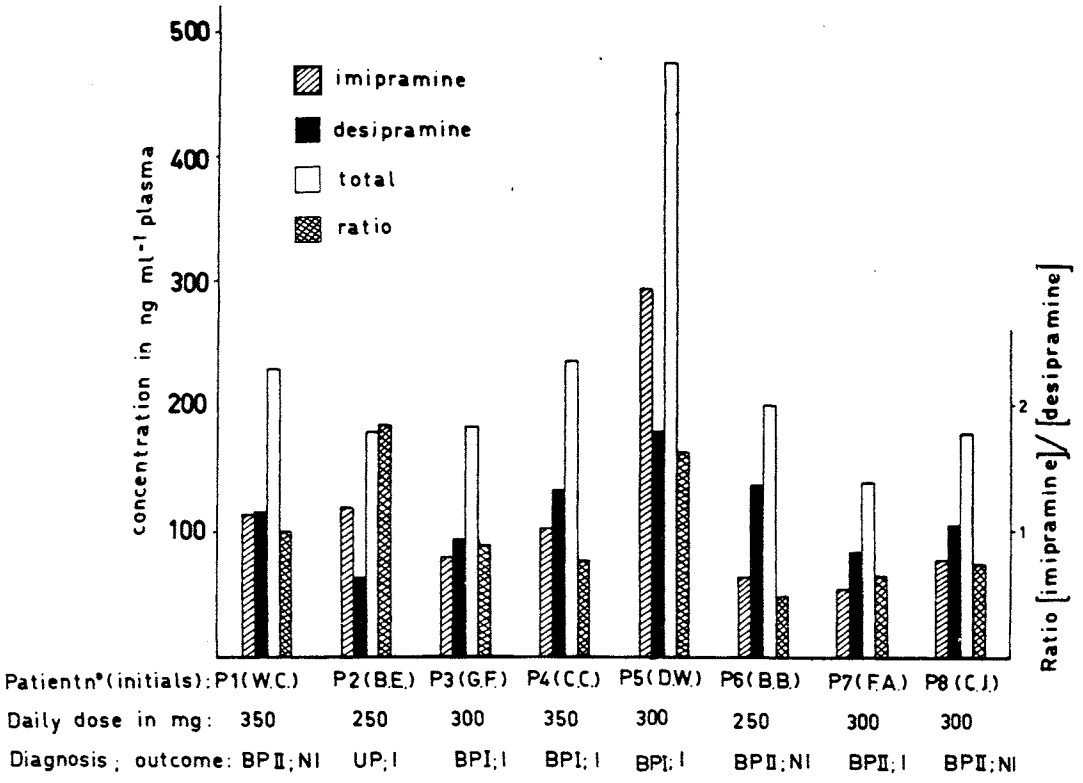


FIG. 4. Mean steady state data for the eight patients out of the ten who achieved a steady state: imipramine, desipramine (imipramine + desipramine)—total levels and imipramine/desipramine ratios. Clinical diagnosis and outcome of the patients studied. Abbreviations: UP = unipolar; BP = bipolar; I = improved; NI = not improved.

the beginning of the therapy could offer the possibility of more rational adjustment of the dosage, a better way of reaching a stable steady state and favorable clinical outcome.

This g.c.m.s. assay for the simultaneous determination of imipramine and desipramine must be considered as a preliminary method, open to improvement in sensitivity, for determination of levels in CSF samples or in plasma samples obtained from single dose loading of imipramine.

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