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# Positive and Negative Ion Mass Spectrometry of Tricyclic Antidepressants

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**Summary.** Positive electron impact (EI), positive chemical ionization (CI), and negative CI mass spectra of eight tricyclic antidepressants are presented. In the positive EI mode, peak(s) at m/z 193 and/or 195, which corresponded to the tricyclic nucleus, appeared for five compounds; a peak at m/z 58 was common to compounds having a γ-dimethylaminopropyl group as their side chain. Molecular ions appeared for all compounds though they were very small in some compounds in the positive EI mode. In the positive CI mode, $[M+H]^+$ quasi-molecular peaks appeared together with $[M+C_2H_5]^+$ peaks in five compounds; the ion at m/z 196, which corresponded to the tricyclic nucleus also appeared in five compounds. In the negative CI mode with 1 Torr chamber pressure, $[M-1]^-$ quasi-molecular ions were observed for all compounds except for lofepramine; $[M+43]^-$ anions, which probably corresponded to $[M+C_3H_4]^-$, appeared in five compounds; strong $[M+Cl]^-$ ions appeared in carpipramine and clocapramine. The anions due to the tricyclic nucleus also appeared in this mode. In the negative CI mode at low pressure (0.01 Torr), the spectra were generally similar to those in the 1 Torr negative CI mode. However, the cluster anions never appeared at the low pressure. Some data on extraction for some antidepressants from human urine and plasma, and their separation by gas chromatography, are also presented.

**Key words:** Tricyclic antidepressants, mass spectrometry – Mass spectrometry, negative ion chemical ionization

**Zusammenfassung.** Von acht trizyklischen Antidepressiva werden die Massenspektren nach Elektronenstoß-Ionisation (EI) und positiver und negati-

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ver chemischer Ionisation (CI) vorgestellt. Bei der Elektronenstoß-Ionisations-Technik (EI) fanden sich bei fünf Substanzen die Massen m/z 193 und/oder 195, die dem trizyklischen Grundgerüst entsprechen; eine Masse m/z 58 trat häufig bei Substanzen mit einer γ-Dimethylaminopropyl-Gruppe in der Seitenkette auf. Molekülionen fanden sich bei allen Substanzen, obwohl sie bei manchen in der EI-Technik sehr klein ausfielen. In der positiven CI-Technik traten bei fünf Substanzen $[M-1]^-$ Quasi-Molekülionen zusammen mit $[M+C_2H_5]^+$ Peaks auf, auch erschien die Masse m/z 196, die dem trizyklischen Grundgerüst entspricht. In der negativen CI-Technik wurden bei einem Kammerdruck von 1 Torr $[M-1]^-$ Quasi-Molekülionen für alle Substanzen mit Ausnahme des Lofepramins beobachtet. $[M+43]^-$ Anionen, die möglicherweise $[M+C_3H_4]^-$ entsprechen, traten bei fünf Substanzen auf. Ein $[M+Cl]^-$ Ion war besonders stark bei Carpipramin und Clocapramin ausgeprägt. Mit dieser Technik ließen sich auch die Anionen nachweisen, die dem trizyklischen Grundgerüst entsprechen. Die Spektren waren bei der negativen CI-Technik bei unterschiedlichem Kammerdruck (0,01 oder 1 Torr) grundsätzlich ähnlich. Jedoch fanden sich niemals Anlagerungskomplexe bei niedrigem Druck. Weiterhin werden Daten zur Extraktion einiger Antidepressiva aus menschlichem Urin und Plasma sowie ihre Auftrennung durch Gaschromatographie vorgestellt.

**Schlüsselwörter:** Trizyklische Antidepressiva, Massenspektrometrie – Massenspektrometrie

## Introduction

Tricyclic antidepressants are frequently encountered in actual forensic science practice. In a therapeutic point of view, their analyses with blood of patients are also essential to provide them with optimal effective doses [1–6]. In this paper, we present positive electron impact (EI), positive chemical ionization (CI), and negative CI mass spectra of eight tricyclic antidepressants; and also methods for their extraction from human samples and their separation by gas chromatography (GC) to serve for their actual identification by GC/mass spectrometry (MS).

## Materials and Methods

### *Materials*

Tricyclic antidepressants investigated were imipramine-HCl (Compound I), desipramine-HCl (Compound II), trimipramine maleate (Compound III), chlorimipramine-HCl (Compound IV), lofepramine-HCl (Compound V), carpipramine-HCl (Compound VI), clocapramine-HCl (Compound VII), and amitriptyline-HCl (Compound VIII). Compounds I, II, and IV were obtained from Ciba-Geigy, Basel, Switzerland; Compound III from Shionogi & Co., Ltd., Osaka, Japan; Compound V from Daiichi Seiyaku Co., Ltd., Tokyo, Japan; Compounds VI and VII from Yoshitomi Pharmaceutical Industries Ltd., Osaka, Japan; and Compound VIII from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan. Extrelut was pur-

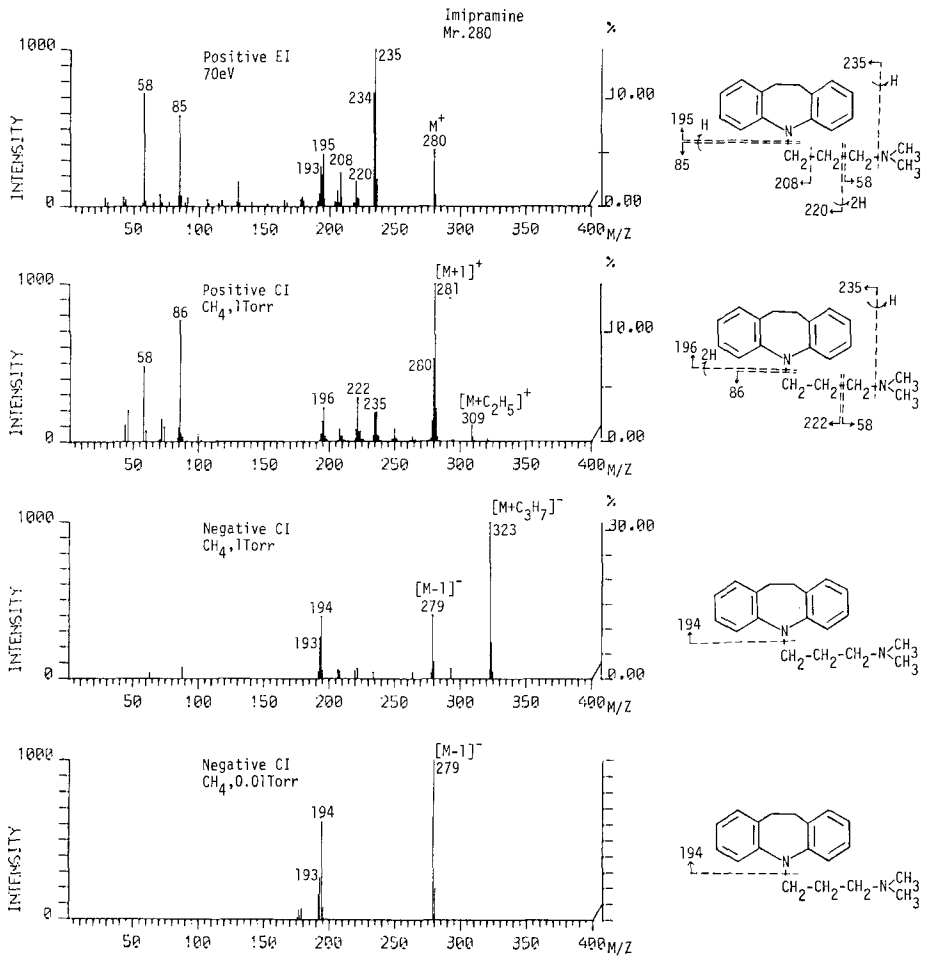


**Fig. 1.** Positive EI, positive CI, medium pressure negative CI, and low pressure negative CI mass spectra of imipramine (Compound I) and its probable fragmentation modes

chased from E. Merck, Darmstadt, FRG; and 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh) from Gasukuro Kogyo Inc., Tokyo, Japan. Other common chemicals used were of the highest purity commercially available.

The urine and serum obtained from healthy subjects were also used for the extraction experiments.

### *MS Conditions*

Mass spectra in the positive EI, positive CI, and medium pressure (1 Torr) negative CI modes were recorded on a JMS-D300 (GC) MS instrument with a JMA-2000E computer-controlled data analysis system by the direct inlet method in Hamamatsu. Less than 1 μg of each antidepressant dissolved in methanol was applied to the instrument. MS conditions were: accelerating voltage 3.0 kV, ionization current 300 μA, separator temperature 280°C, and ion source temperature 220°C; in the positive EI mode, electron energy 70 eV; in the positive and negative CI modes, electron energy 200 eV, reagent gas methane, and chamber pressure 1 Torr.

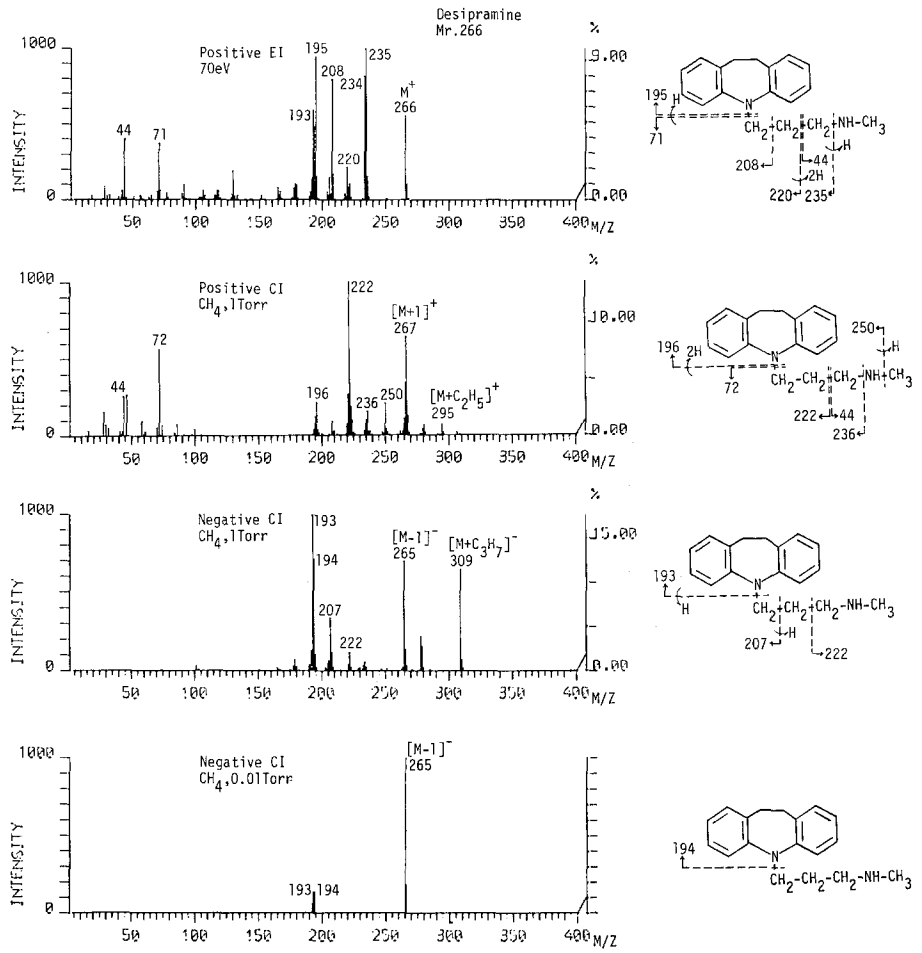


**Fig. 2.** Positive and negative mass spectra of desipramine (Compound II) and its probable fragmentation modes

Mass spectra in the low pressure negative CI mode were recorded in Zürich on a LKB 2091 MS instrument, modified for CI MS as described by Ryhage [7]. Its MS conditions were: accelerating voltage 3.5 kV, ionization current 250 μA, electron energy 100 eV, separator temperature 270°C, ion source temperature 300°C, and chamber pressure 0.01 Torr.

*Extraction and GC Separation*

To 1 ml urine or serum containing antidepressants, were added 1 ml water and 0.25 ml 10*N* NaOH. It was applied to 3 g Extrelut packed in a 10-ml glass syringe. After standing for 30 min, the compounds were eluted with 10 ml chloroform and evaporated to dryness under the stream of nitrogen. The residue was mixed with 1 ml 1*N* HCl and 2 ml hexane, shaken vigorously and centrifuged (3,000 rpm, 5 min). The organic layer was discarded by aspiration; to the aqueous layer, were added 1.5 ml 1*N* NaOH and 2 ml chloroform, shaken vigorously and centrifuged (3,000 rpm, 5 min). The chloroform layer was again evaporated to dryness under the stream of nitrogen. The residue was dissolved in 50–100 μl methanol and subjected to GC analysis. GC was carried out on a Shimadzu GC-4CM instrument with a 1.0 m × 3 mm (i.d.)

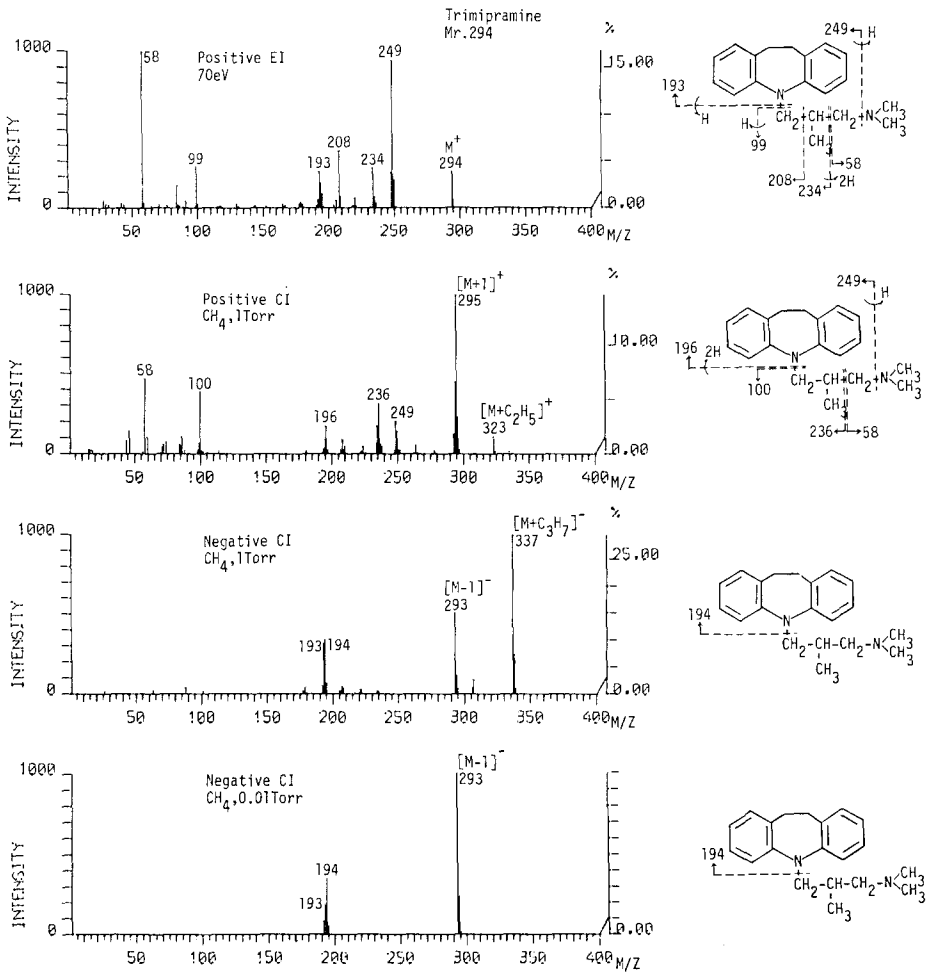


**Fig. 3.** Positive and negative mass spectra of trimipramine (Compound III) and its probable fragmentation modes

glass column packed with 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh). The GC conditions were: injection temperature 250 or 300°C, column temperature 220 or 280°C and nitrogen flow rate 40 ml/min. The peaks appearing in the gas chromatogram were identified with the above JMS-D300 GC/MS instrument.

## Results

*Positive EI Mass Spectra.* Positive EI, positive CI, medium pressure (1 Torr) negative CI, and low pressure (0.01 Torr) negative CI mass spectra of eight tricyclic antidepressants, and each probable fragmentation mode, are shown in Figs. 1–8.

In the positive EI mode, peak(s) at m/z 193 and/or 195, which corresponded to the tricyclic (iminodibenzyl) nucleus of the antidepressants, appeared in

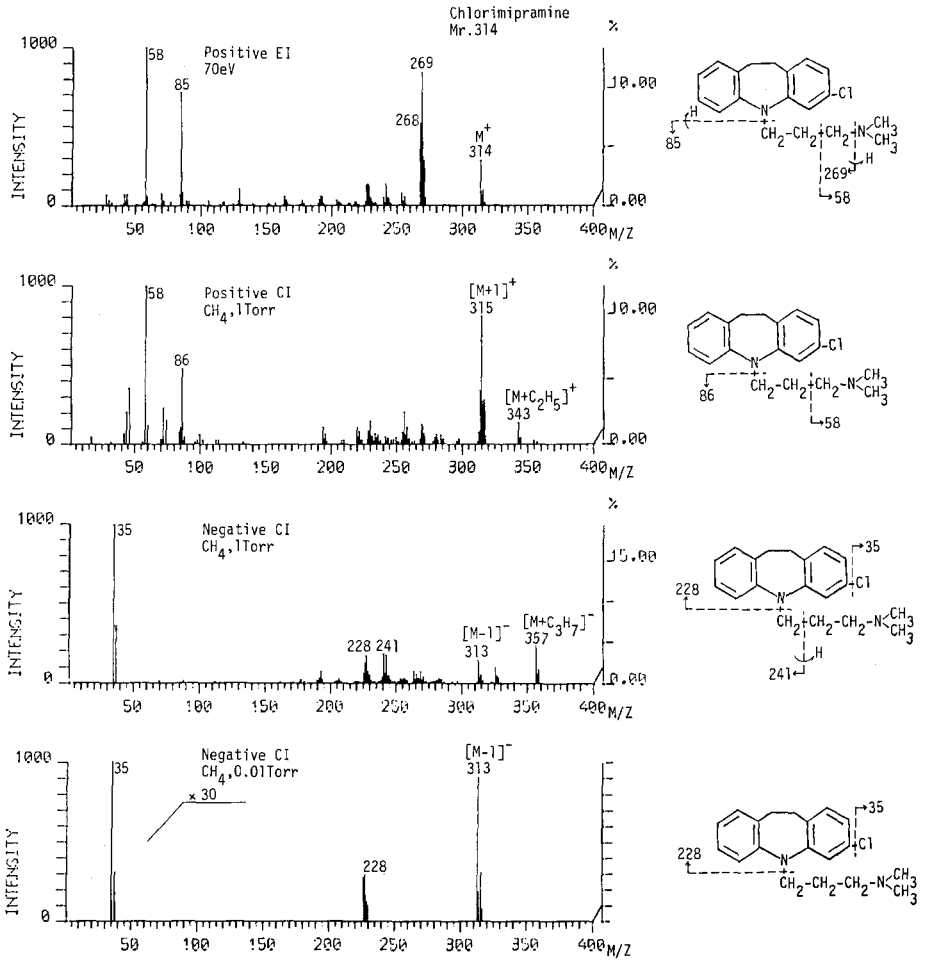


**Fig. 4.** Positive and negative mass spectra of chlorimipramine (Compound IV) and its probable fragmentation modes

Compounds I–III, V, and VI. A peak at m/z 58, due to the dimethylaminomethyl group liberated, was observed in Compounds I, III–V, and VIII. Molecular cations were observed for all compounds, though they were small in Compounds V–VIII.

*Positive CI Mass Spectra.* In the positive CI mode, a $[M+1]^+$ quasi-molecular peak appeared for all compounds except for Compound VII. A $[M+C_2H_5]^+$ ion also appeared in Compounds I–IV and VIII. An ion at m/z 196, due to the tricyclic nucleus, was observed in Compounds I–III, V, and VI. The fragment peak at m/z 58, which corresponded to the dimethylaminomethyl group, appeared in Compounds I, III, IV, and VIII.

*Negative CI Mass Spectra at 1 Torr.* In the medium pressure (1 Torr) negative CI mode, a $[M-1]^-$ quasi-molecular ion appeared for all compounds except for

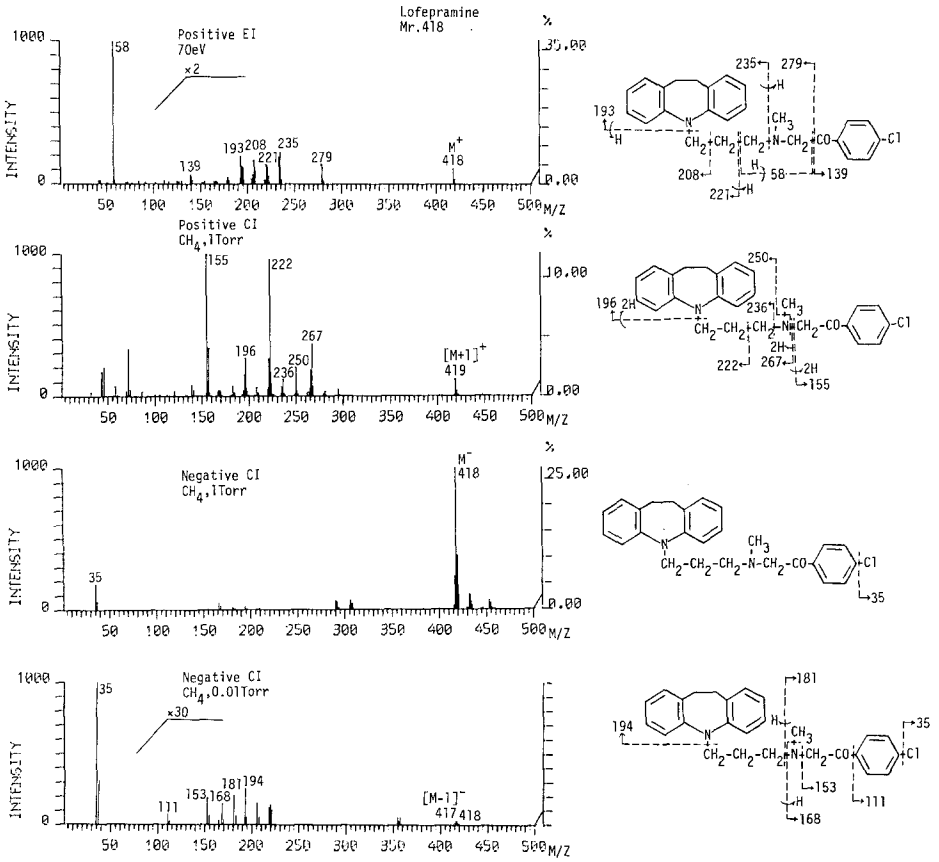


**Fig. 5.** Positive and negative mass spectra of lofepramine (Compound V) and its probable fragmentation modes

Compound V. A cluster anion at m/z M + 43 was observed in Compounds I–IV and VIII. This ion seems due to addition of $C_3H_7$ to the molecules. The anions at m/z 193 and 194 due to the tricyclic structure appeared in Compounds I–IV, VI, and VII. A peak at m/z M + 35, due to addition of chlorine, appeared in Compounds VI and VII. A peak due to liberated chlorine appeared in Compounds IV, V, and VII.

*Negative CI Mass Spectra at 0.01 Torr.* In the negative CI mode at 0.01 Torr of the chamber pressure, the spectra were generally similar to those at 1 Torr. However, the cluster anions never appeared at the low pressure; thus the $[M-H]^-$ anions gave the highest mass numbers in the spectra.

*Separation by GC.* To actually identify tricyclic antidepressants in human samples by GC/MS, the drugs, which had been added to urine or plasma, were extracted with Extrelut columns and an organic solvent, and applied to a GC column (1 m × 3 mm) of 5% SP-2100 on Chromosorb W at 220 or 280°C. An isothermal gas chromatogram at 220°C for Compounds I–IV and VIII is shown

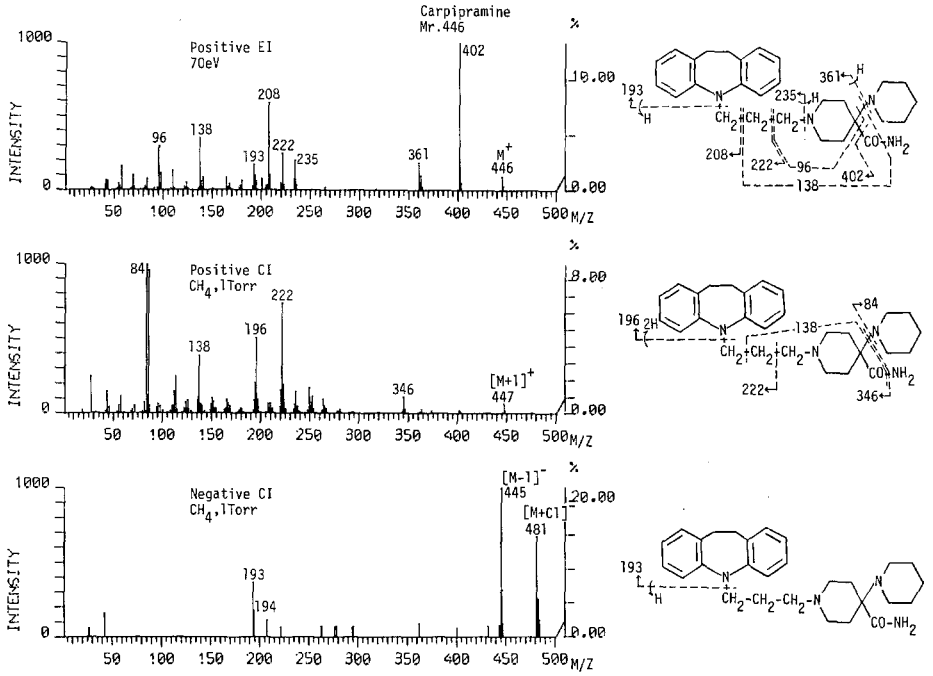


**Fig. 6.** Positive and negative mass spectra of carpipramine (Compound VI) and its probable fragmentation modes

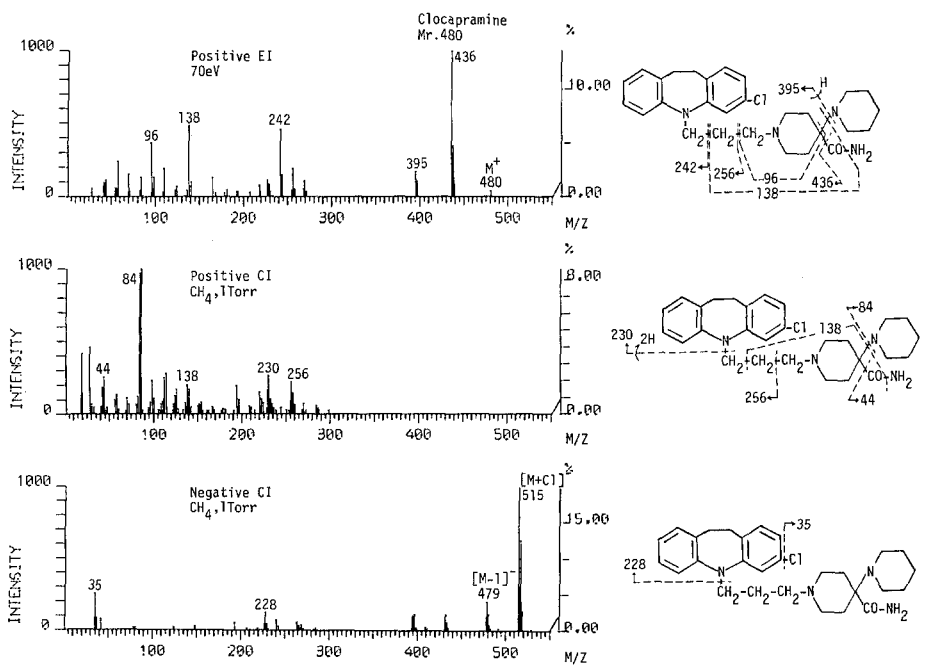


**Fig. 7.** Positive and negative mass spectra of clocapramine (Compound VII) and its probable fragmentation modes

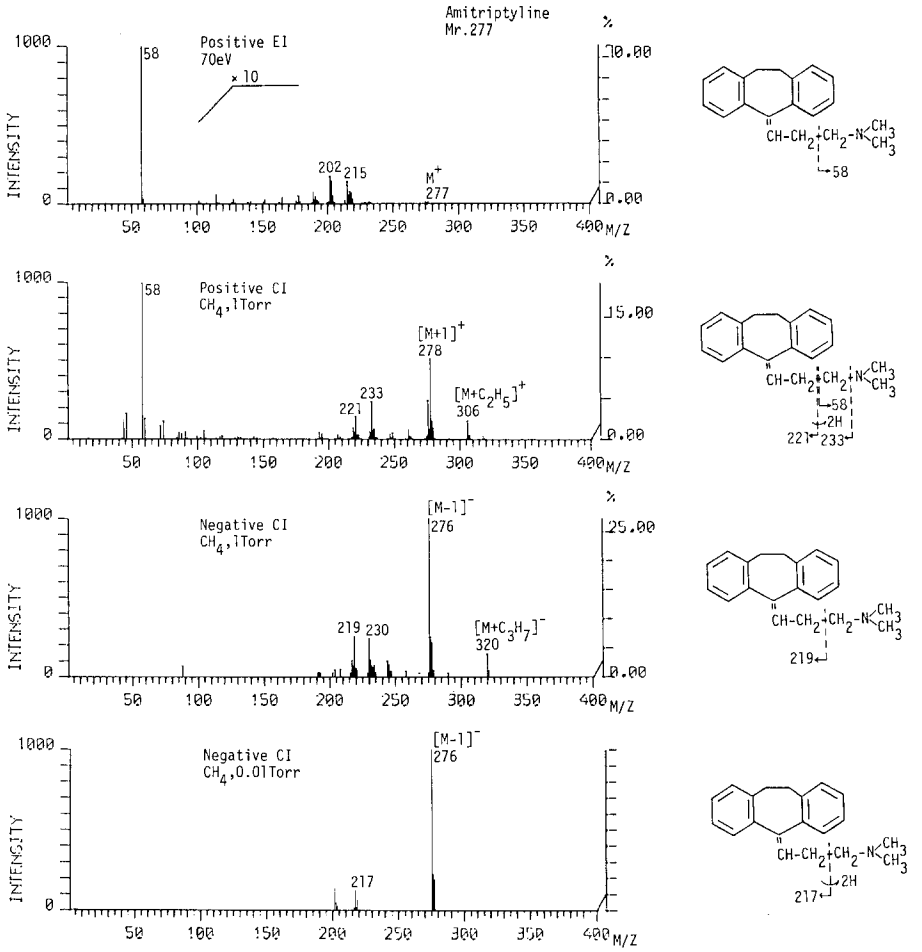


**Fig. 8.** Positive and negative mass spectra of amitriptyline (Compound VIII) and its probable fragmentation modes

in Fig. 9. Lofepramine (Compound V) had to be separated at 280°C of the column temperature. The retention times for the six compounds are listed in Table 1. The separation of the compounds from biologic impurities was satisfactory (Fig. 9). The recovery of the six antidepressants added to either urine or plasma was around 70%. Carpipramine (Compound VI) and clocapramine (Compound VII) could not be analyzed by GC because they easily broke down on the GC column even with the temperatures being close to their boiling points.

## Discussion

In this paper, we have presented positive EI, positive CI, and negative CI mass spectra of eight tricyclic antidepressants. Such systematic studies including

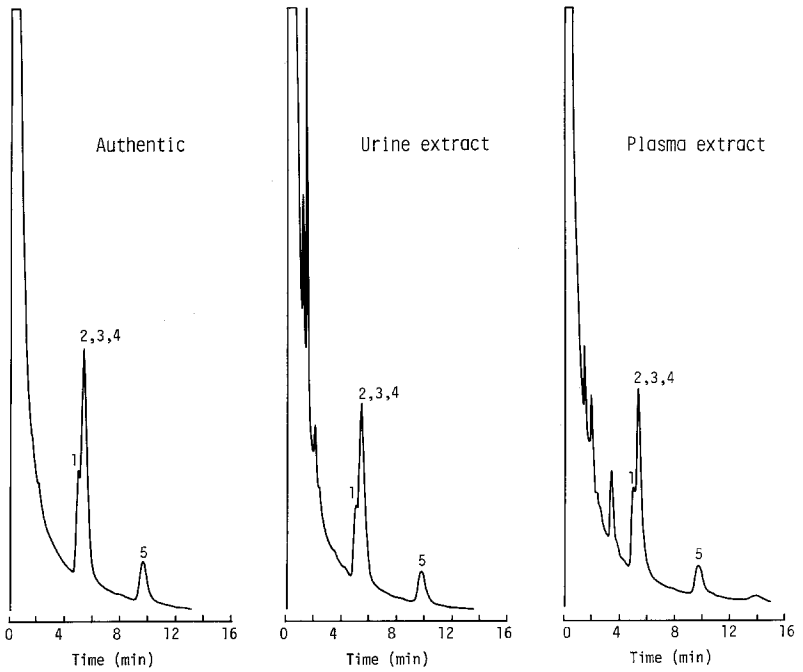


**Fig. 9.** GC for five tricyclic antidepressants extracted from human urine or plasma. *Key:* amitriptyline, 1; imipramine, 2; desipramine, 3; trimipramine, 4; and chlorimipramine, 5. GC was isothermally carried out with a 1.0 m × 3 mm (i.d.) glass column packed with 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh). Its conditions were: column temperature 220°C and nitrogen flow rate 40 ml/min. The mixture of five antidepressants, 10 μg each, was added to 1 ml urine or plasma prior to the extraction with Extrelut (procedure see text)

**Table 1.** Retention times of six tricyclic antidepressants by GC with a SP-2100 column[a]

| Compound | Retention time (min) |
|---|---|
| Column temperature: 220°C | |
| Amitriptyline | 4.98 |
| Imipramine | 5.30 |
| Trimipramine | 5.30 |
| Desipramine | 5.50 |
| Chlorimipramine | 9.40 |
| Column temperature: 280°C | |
| Lofepramine | 10.7 |

[a] The 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh) packed in a 1.0 m × 3 mm (i.d.) glass column was used. The injection temperatures were 20°C higher than the column temperatures. The nitrogen flow rate was 40 ml/min

negative CI data, to our knowledge, have never been reported before, although positive EI and positive CI mass spectra of only a few tricyclic antidepressants [8], and quantitation of some antidepressants by selected ion monitoring (SIM) in the positive EI [2, 4, 6] or positive CI [1, 3, 5] mode, have appeared.

We have presented negative CI mass spectra at both low (0.01 Torr) and medium (1 Torr) pressures in six compounds (Figs. 1–5, 8). The low pressure mode was first introduced by Ryhage and Brandenberger [9] in 1978, and is successfully being used in their laboratory [10] in actual forensic science practice. The negative spectra at both pressures were generally similar to each other, but cluster anions never appeared at the low pressure. There are some advantages in the use of the negative mass spectrometry [10, 11]; the negative mode gives simpler spectra than do the positive EI and CI modes, as shown in the present study (Figs. 1–8), and thus the peaks are easily explicable. Since every impurity does not give anions, the background in the spectra or in the SIM is generally much lower than that in the positive modes [10, 11]. The negative mode often gives much higher sensitivity than do the positive EI and CI modes. Although we did not make quantitative experiments in this study, higher sensitivity can be expected also for the antidepressants because base peaks in the 1 Torr negative CI mode showed high percentages of total abundance (Figs. 1–8).

Our interest is focused on the identification of a drug in samples obtained from victims by GC/MS. The peak at m/z 58, due to the dimethylaminomethyl group liberated, observed in both positive EI and CI modes (Figs. 1, 3–5, 8) can be used for screening. The peaks, at m/z 193 or 195 in the positive EI mode, at m/z 196 in the positive CI mode, and at m/z 193 or 194 in the negative CI mode, which are all due to the tricyclic nucleus (Figs. 1–3, 5, 6), can also be used as indication of the presence of a tricyclic antidepressant. To test the presence of a halogen in the structure, the negative CI spectra are most useful (Figs. 4, 5, 7).

A strong cluster anion at m/z M+43 appeared for imipramine in the medium pressure negative CI mode (Fig. 1). Such anions were also observed in some other compounds (Figs. 2–4 and 8). The peak seems interpretable as the addition of $C_3H_7$, although such examples have never been reported before as far as we know. The anions observed at m/z M+35 (Figs. 6, 7) seem due to the addition of chlorine derived from the HCl salt of the antidepressants used in this study. Such chloride attachment is not rare in the negative CI mode [12].

We have added a method for extraction of the drugs from human urine and plasma, and also their GC conditions (Fig. 9, Table 1). These studies together with the mass spectra (Figs. 1–8) seem very useful especially in forensic chemistry and clinical toxicology.

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