Z Rechtsmed (1988) 99:253–261

Zeitschrift für
Rechtsmedizin

Positive and Negative Ion Mass Spectrometry of Antiepileptic Hydantoins and Their Analogs

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Summary. Positive ion electron impact (PIEI), positive ion chemical ionization (PICI), and negative ion chemical ionization (NICI) mass spectra of seven compounds of hydantoins and their analogs are presented; their probable fragmentation modes are also presented. In the PIEI mode, intensities of molecular ions differed according to different compounds. Cleavage at outsides of both carbonyl groups was commonly observed for all compounds. In the PICI mode, all compounds showed $[M+1]^+$ quasi-molecular ions constituting the base peaks. In the NICI mode, $[M-1]^-$ quasi-molecular anions were the base peaks except for trimethadione and paramethadione. All negative spectra showed anions at m/z 42 due to $[NCO]^-$; these peaks seem useful for screening of antiepileptics. An extraction procedure for the antiepileptics from human urine or plasma, and their separation by gas chromatography (GC), are also presented to serve for their actual identification by GC/mass spectrometry.

Key words: Antiepileptics, mass spectrometry – Mass spectrometry, negative ion chemical ionization – Hydantoins, mass spectrometry

Zusammenfassung. Es werden Positiv-Ionen-EI-(PIEI)-, Positiv-Ionen-CI-(PICI)- und Negativ-Ionen-CI-(NICI)-Massenspektren von sieben Hydantoinderivaten und Analogen vorgestellt, daneben die wahrscheinlichen Fragmentierungsmuster. Beim PIEI-Modus unterschieden sich die Molekularionen im Hinblick auf die verschiedenen Verbindungen. Eine Spaltung an der Außenregion der beiden Carbonylgruppen war allen Verbindungen gemeinsam. Beim PICI-Modus zeigten alle Verbindungen quasi-molekulare

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$[M+1]^+$-Ionen. Beim NICI-Modus waren quasi-molekulare $[M-1]^-$-Anionen Basispeaks mit Ausnahme von Trimethadion und Paramethadion. Alle Negativ-Spektren zeigten Anionen bei m/z 42, verursacht durch $[NCO]^-$; diese Peaks erscheinen nützlich zum Screening von Antiepileptika. Weiterhin werden ein Extraktionsverfahren für Antiepileptika aus Humanurin oder -plasma sowie die gaschromatographische Trennung (GC) beschrieben. Diese Verfahren gestatten die Identifizierung mittels GC/MS.

Schlüsselwörter: Antiepileptika, Massenspektrometrie – Massenspektrometrie, Negativspektren – Hydantoine, Massenspektrometrie

Introduction

The toxicity of antiepileptic hydantoins and their analogs is comparable to that of barbiturates. The antiepileptics sometimes cause death in suicide and accidents; and thus encountered in actual forensic science practice. The present paper deals with positive ion electron impact (PIEI), positive ion chemical ionization (PICI), and negative ion chemical ionization (NICI) mass spectra of seven compounds of hydantoins and their analogs; 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. Ethotoin, phenytoin, and trimethadione were obtained from Dainippon Pharmaceutical Co., Ltd., Osaka (Japan); paramethadione from U.S.P.C., Inc., Rockville, MD (USA); phensuximide and methsuximide from Warner-Lambert Company, Ann Arbor, MI (USA); ethosuximide from Eisai Co., Ltd., Tokyo (Japan); and 5% Poly-I-110 on Chromosorb WAW DMCS (80/100 mesh) from Gasukuro Kogyo Inc., Tokyo (Japan). Other common chemicals used were of the highest purity commercially available.

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

MS Conditions. Mass spectra in the PIEI, PICI, and NICI modes were recorded on a JMS-D300 (GC)MS instrument with a JMA-2000E computer-controlled data analysis system by both GC/MS and direct inlet methods. Less than 1 μg of each antiepileptic drug 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 PIEI mode, electron energy 70 eV; in the PICI and NICI modes, electron energy 200 eV, reagent gas methane, and chamber pressure 1 Torr.

Extraction and GC Separation. To 0.5 ml urine or plasma containing antiepileptics, three drops of 3N HCl and 5 ml dichloromethane were added in a glass-stoppered centrifuge tube. It was shaken vigorously and centrifuged (3,000 rpm, 5 min). The organic layer, after addition of 100 μl isoamyl acetate, was evaporated to about 100 μl under the stream of nitrogen. The 2-μl aliquot of the concentrated solution was subjected to GC analysis.

GC was carried out on a Shimadzu GC-4CM instrument with a 1.0 m × 3 mm (i.d.) glass column packed with 5% poly-I-110 on Chromosorb WAW DMCS (80/100 mesh). The GC conditions were: injection temperature 280°C, column temperature 80–260°C (10°C/min), and nitrogen flow rate 50 ml/min. The identity of peaks appearing in the gas chromatogram were confirmed with the above JMS-D300 GC/MS instrument.

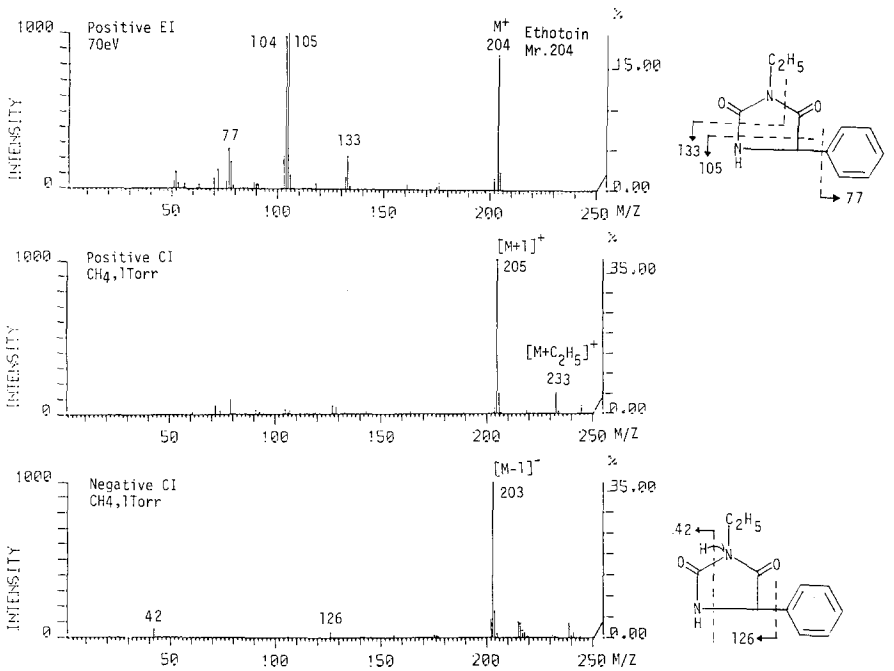

Fig. 1. PIEI, PICI, and NICI mass spectra of ethotoin and its probable fragmentation modes

Results

PIEI Mass Spectra. PIEI, PICI, and NICI mass spectra of the seven antiepileptics and each probable fragmentation mode are shown in Figs. 1–7.

In the PIEI mode, intensities of molecular ions differed in different compounds; the molecular ion of trimethadione was the base peak (Fig. 3), but those of paramethadione and ethosuximide were very small (Figs. 4, 7). For compounds with phenyl groups, peaks at m/z 77 appeared (Figs. 1, 2, 5, 6). Peaks due to fragmentation at outsides of both carbonyl groups were observed for all compounds.

PICI Mass Spectra. All compounds showed $[M + 1]^+$ quasi-molecular ions constituting the base peaks together with small $[M + C_2H_5]^+$ peaks. Fragment ions were generally very few and small; thus, the quasi-molecular base peaks showed high percentages of total ion current.

NICI Mass Spectra. The $[M - 1]^-$ quasi-molecular anions were the base peaks, except for trimethadione and paramethadione (Figs. 3, 4), which showed fairly complicated fragment anions. All spectra showed peaks at m/z 42, which correspond to $[NCO]^-$.

Separation by GC. To enable actual identification of antiepileptics in human samples by GC/MS, the mixture of the seven drugs, 20 μg of each, was added to 0.5 ml urine or plasma, extracted with an organic solvent, and applied to the GC column. The oven temperature was programmed from 80° to 260°C. The gas

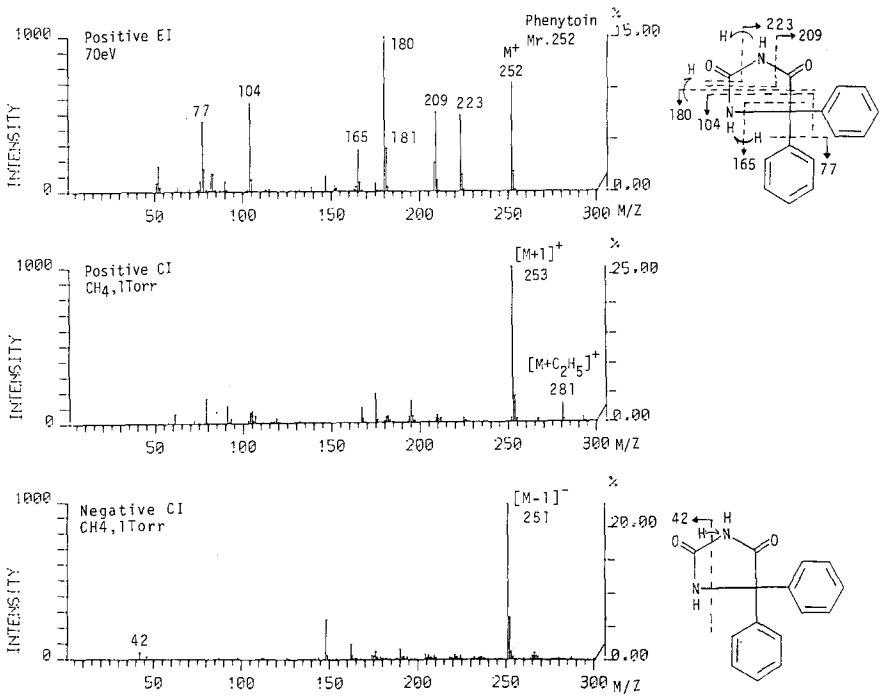

Fig. 2. PIEI, PICI, and NICI mass spectra of phenytoin and its probable fragmentation modes

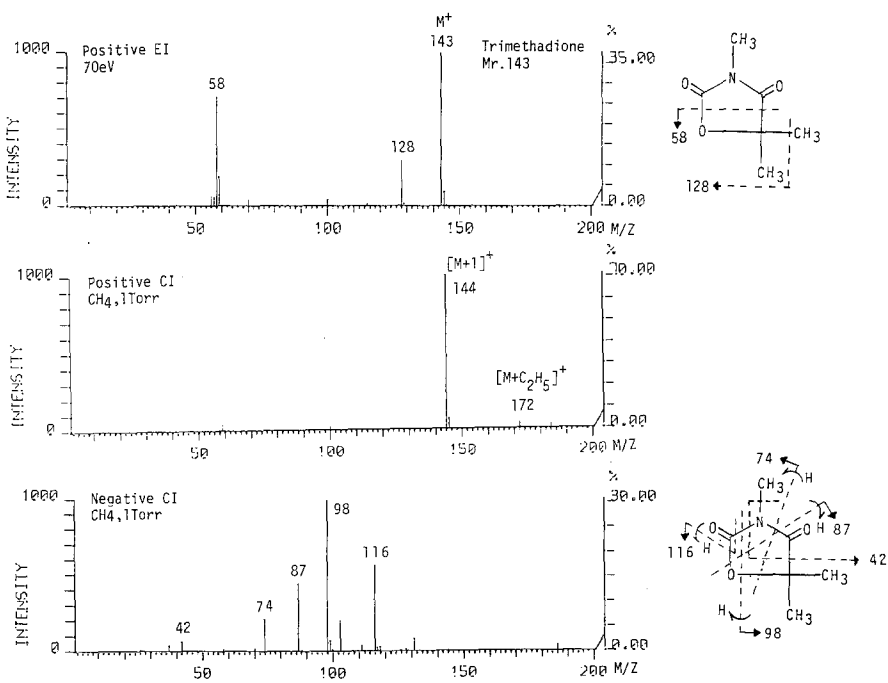

Fig. 3. PIEI, PICI, and NICI mass spectra of trimethadione and its probable fragmentation modes

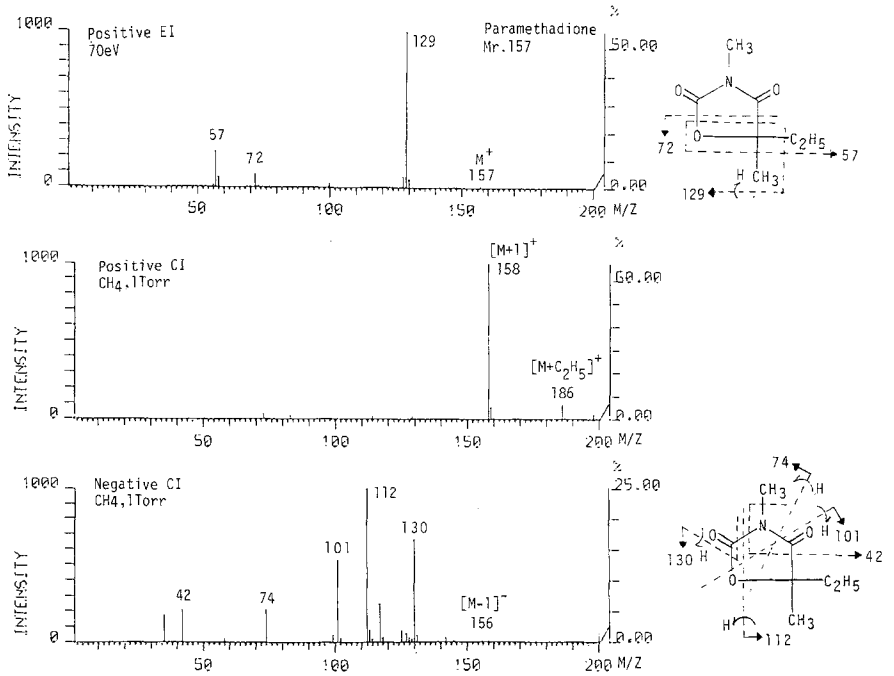

Fig. 4. PIEI, PICI, and NICI mass spectra of paramethadione and its probable fragmentation modes

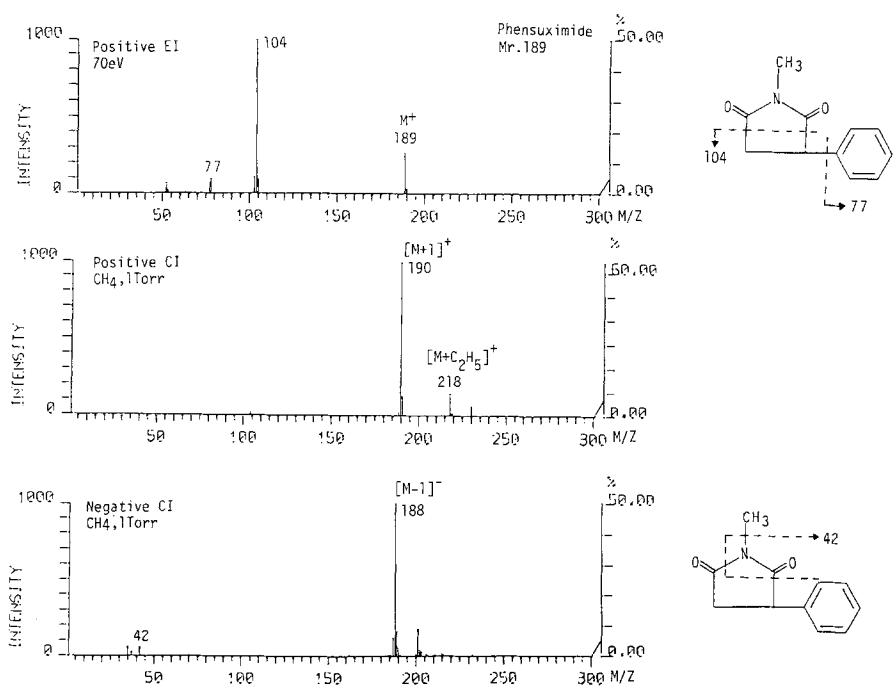

Fig. 5. PIEI, PICI, and NICI mass spectra of phensuximide and its probable fragmentation modes

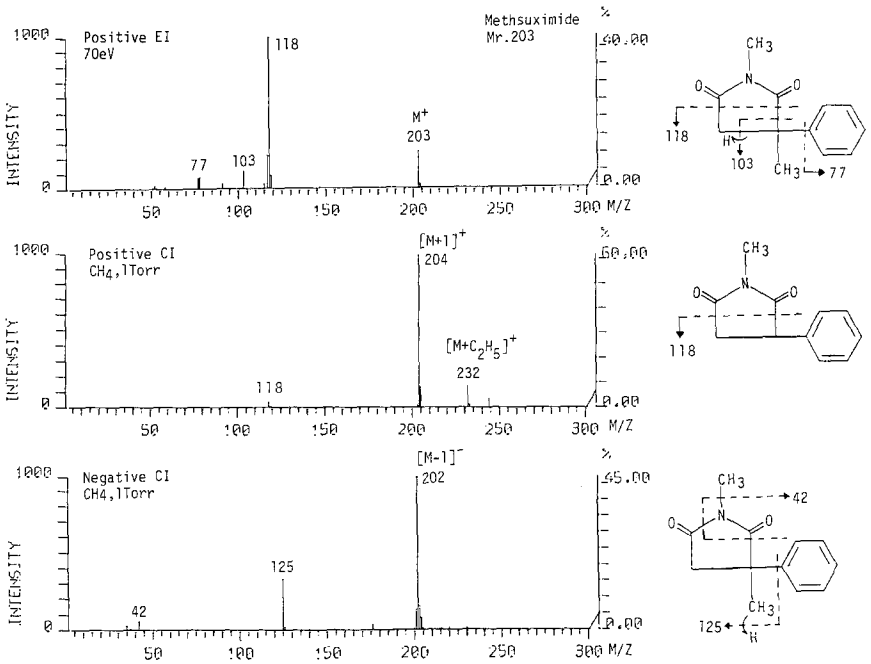

Fig. 6. PIEI, PICI, and NICI mass spectra of methsuximide and its probable fragmentation modes

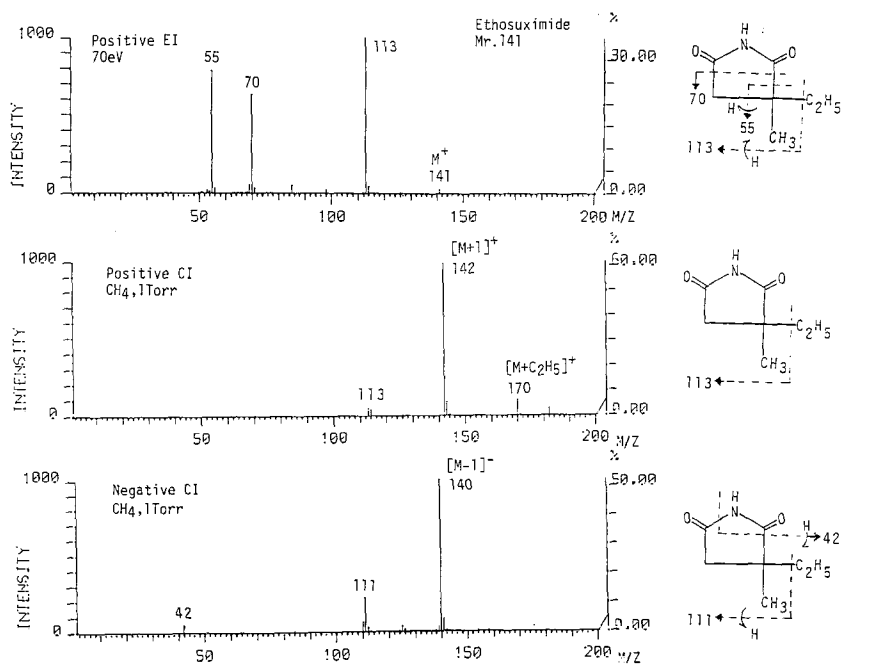

Fig. 7. PIEI, PICI, and NICI mass spectra of ethosuximide and its probable fragmentation modes

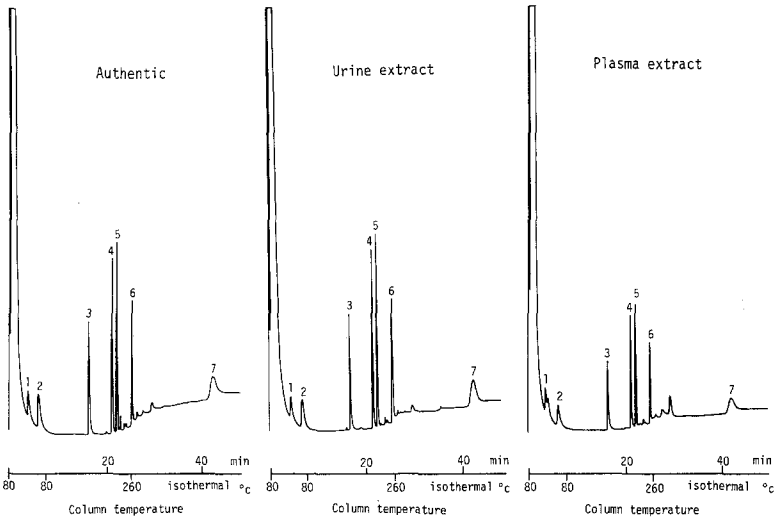

Fig. 8. GC for the seven antiepileptics extracted from human urine or plasma. *Keys:* trimethadione, 1; paramethadione, 2; ethosuximide, 3; methsuximide, 4; phensuximide, 5; ethotoin, 6; phenytoin, 7. GC was carried out with a 1.0 m × 3 mm (i.d.) glass column packed with 5% Poly-I-110 on Chromosorb WAW DMCS (80/100 mesh). Its conditions were: column temperature 80–260°C (10°C/min) and nitrogen flow rate 50 ml/min. The mixture of seven hydantoins and analogs, 20 μg of each, was added to 0.5 ml of urine or plasma prior to extraction

chromatograms are shown in Fig. 8. Separation of all drugs from biologic impurities was satisfactory for both urine and plasma. The recoveries of drug added to urine or plasma were more than 90% and about 60%, respectively.

Sensitivity in the PIEI, PICI, and NICI Modes. To check sensitivity by the present GC/MS method, phenytoin and methsuximide were subjected to selected ion monitoring (SIM) in the PIEI, PICI, and NICI modes with use of each intense or base peaks. The detection limits for methsuximide in the PIEI, PICI, and NICI modes were about 100 pg, 1 ng, and 10 ng, respectively, in an injected volume. The relative sensitivity among the different modes for phenytoin were similar to that for methsuximide; but the sensitivity for phenytoin in each mode were one or two orders of magnitude lower than that for methsuximide, probably due to strong adsorption of phenytoin to the GC column.

Discussion

In this paper, we have presented PIEI, PICI, and NICI mass spectra of seven compounds of hydantoins and their analogs. Such systematic studies including NICI data, to our knowledge, have never been reported before. All previous papers were limited to phenytoin, its metabolites [1–5] and mephenytoin [6] in the PIEI and PICI modes.

Derivatization is usually undesirable for analyses of unknown toxic substances, because it makes analyses much more complicated. The antiepileptics

contain many functional groups in their structures and are generally not suitable for GC analysis in the underivatized forms. Phenytoin and its metabolites were methylated or silylated prior to GC/MS analyses in all previous reports [1–5]. To avoid such derivatization, we tested various GC columns, such as 5% SP-2100 and GP 2% SP-2510-DA. These columns showed marked adsorption and tailing of the drugs, which made their detection impossible at low concentrations. The only column usable for the drugs without derivatization was 5% Poly-I-110, which gave fairly satisfactory gas chromatograms (Fig. 8).

Various conditions for extraction of antiepileptics were also tested. Extrelut columns and Sep-Pak C_{18} cartridges resulted in low recoveries of the drugs added to urine and plasma (unpublished observation). It was noticed that appreciable amounts of trimethadione and paramethadione were lost during evaporation of the organic extracts because the two drugs are relatively volatile. The addition of isoamyl acetate [7] was very effective to prevent such loss.

NICI MS has been shown to have some advantages over the traditional PIEI mode [8, 9]. The first advantage is that the NICI MS sometimes gives high sensitivity, but this was not true for the present antiepileptics. The second advantage is the appearance of a group-specific peak in the spectrum. The peaks, at m/z 42 due to $[NCO]^-$, and at m/z 198 due to a phenothiazine nucleus, appear group-specifically for barbiturates [10] and phenothiazines [11], respectively. Such group-specific peaks also appeared for the antiepileptics at m/z 42 (Figs. 1–7), which correspond to $[NCO]^-$ as in the case of barbiturates. These peaks seem very useful for narrowing down the candidates of an unknown poison. The peaks at m/z 77 observed in some PIEI spectra (Figs. 1, 2, 5, 6) can also be used for screening of antiepileptics with phenyl groups.

Our present mass spectral data on antiepileptics and methods for their extraction and GC separation seem very useful in forensic chemistry and also in clinical toxicology.

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Received August 10, 1987