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Jpn. J. Forensic Toxicol. Vol.12, No.3, 1994

原　　報

POSITIVE- AND NEGATIVE-ION MASS SPECTROMETRY OF PYRAZOLONES AND PYRAZOLIDINES

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Received August 6, 1994
Accepted August 29, 1994

ピラゾロンならびにピラゾリジン系薬剤の正イオン・負イオン質量分析

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Summary

Positive-ion electron impact (PIEI), positive-ion chemical ionization (PICI) and negative-ion chemical ionization (NICI) mass spectra are presented for six pyrazolones and four pyrazolidines, and their fragmentation modes have been analyzed. In the PIEI mode, molecular ions were relatively intense for most compounds. Peaks at m/z 56 and 77 appeared in common for pyrazolones and peaks at m/z 77 and 183 in common for pyrazolidines. In the PICI mode, $[M + 1]^+$ quasi-molecular ions constituted base peaks for most compounds. Common peaks at m/z 56 and 120 appeared for pyrazolones and peaks at m/z 120 for pyrazolidines. In the NICI mode, pyrazolones gave both molecular and $[M - 1]^-$ quasi-molecular anions except dipyrone, and common peaks at m/z 106. For pyrazolidines, molecular or quasi-molecular anions were small or missing except for ketophenylbutazone. A rapid extraction procedure with Sep-Pak C_{18} cartridges from human body fluids of these compounds and their detection by wide-bore capillary gas chromatography (GC) were also presented.

Key words: Pyrazolones; Antipyrine; Dipyrone; Pyrazolidines; Phenylbutazone; Mass spectrometry; Sep-Pak C_{18} cartridge; Wide-bore capillary GC; Negative ion

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 0915−9606/94/$05.00

Introduction

Pyrazolones and pyrazolidines are used as analgesic, antipyretic and anti-inflammatory drugs; they are sometimes encountered in forensic science practice. In this paper, we present positive-ion electron impact (PIEI), positive-ion chemical ionization (PICI) and negative-ion chemical ionization (NICI) mass spectra for pyrazolones and pyrazolidines. A method for their rapid clean-up with Sep-Pak C_{18} cartridges from biological fluids, and wide-bore capillary gas chromatography (GC) are also presented.

Experimental

Materials

Pyrazolones used were antipyrine, dipyrone, aminopyrine, 4-aminoantipyrine, isopyrin and isopropylantipyrine. Pyrazolidines were phenylbutazone, oxyphenbutazone, ketophenylbutazone and sulfinpyrazone. Antipyrine, aminopyrine, isopyrin-HCl, phenylbutazone, oxyphenbutazone and sulfinpyrazone were obtained from Sigma Chemical Co. (St. Louis, MO, USA); dipyrone from Daiichi Seiyaku Co., Ltd. (Tokyo); 4-aminoantipyrine from Daiichi Pure Chemicals Co., Ltd. (Tokyo); isopropylantipyrine from Yoshida & Co., Ltd. (Tokyo); and ketophenylbutazone from Kyowa Hakko Kogyo Co., Ltd. (Tokyo). Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA). Other common chemicals used were of the highest purity commercially available. Human urine, plasma and whole blood were obtained from healthy subjects.

MS conditions

Mass spectra were recorded on a JMS-D300 (GC) MS instrument with a JMA-2000E computer-controlled data analysis system by direct inlet method. MS conditions were: accelerating voltage 3.0 kV, ionization current 300 μA, separator temperature 280 ℃, and ion source temperature 220 ℃; 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 procedure

For urine or plasma, 9 ml of distilled water was mixed with 1 ml of a sample containing 10 μg of each drug; in the case of whole blood, 19 ml of distilled water was mixed with 1 ml of a sample for complete hemolysis.

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform/methanol (9:1), 10 ml of acetonitrile and 10 ml of distilled water. The sample solution was loaded onto the pretreated cartridge at a flow rate not greater than 5 ml/min. The cartridge was washed with 20 ml of

distilled water. Finally, 3 ml of chloroform/methanol (9:1) was passed through it, and the eluate was collected in a vial. The upper aqueous layer was discarded by aspiration with a Pasteur pipette, and the organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of methanol, and a 1-2 μl aliquot of it was subjected to GC analyses.

GC conditions

GC was carried out on a Shimadzu GC-4CM instrument with a flame ionization detector and a wide-bore SPB-1 fused silica capillary column (15 m x 0.53 mm i.d., film thickness 1.5 μm, Supelco Inc., Bellefonte, PA, USA). GC conditions were: injection temperature 280 ℃, column temperature 120−180 ℃ (10 ℃/min), and nitrogen flow rate 20 ml/min.

Results and discussion

Figures 1−6 and 7−10 show PIEI, PICI and NICI mass spectra and each probable fragmentation mode of six pyrazolones and four pyrazolidines, respectively. There are some reports about mass spectrometry for some drugs of these groups, but they were limited to PIEI or PICI mass spectra [1−4]. To our knowledge, this is the first one that deals with NICI mass spectra.

In the PIEI mode, many compounds gave relatively intense molecular ions. Pyrazolones gave common peaks at m/z 56 and at m/z 77 due to $[N_2CO]^+$ and the phenyl group, respectively; the peak at m/z 56 constituted base peaks for dipyrone, aminopyrine, 4-aminoantipyrine and isopyrin. Pyrazolidines gave common peaks at m/z 77 and at m/z 183 probably due to the phenyl group and [phenyl-N_2-phenyl + H]$^+$, respectively, except for oxyphenbutazone.

In the PICI mode, $[M + 1]^+$ quasi-molecular ions together with small peaks at m/z M + C_2H_5 appeared for most compounds, and constituted base peaks for antipyrine, aminopyrine, 4-aminoantipyrine, isopyrin, isopropylantipyrine, oxyphenbutazone and ketophenylbutazone. For pyrazolones, common peaks at m/z 56, which had been observed in the PIEI mode, also appeared in this mode except for antipyrine; they also gave common peaks at m/z 120 which was probably due to [phenyl-NCO + H]$^+$. Pyrazolidines showed peaks at m/z 120 except oxyphenbutazone; for the latter compound, the same fragmentation mode gave a peak at m/z 136 which was due to [hydroxyphenyl-NCO + H]$^+$.

In the NICI mode, pyrazolones except dipyrone gave M^- and $[M - 1]^-$, and common peaks at m/z 42 due to $[NCO]^-$. The peak at m/z 106, which was probably due to [phenyl-N_2 + H]$^-$, appeared in common for all pyrazolones. For pyrazolidines, $[M - 1]^-$ anion was intense

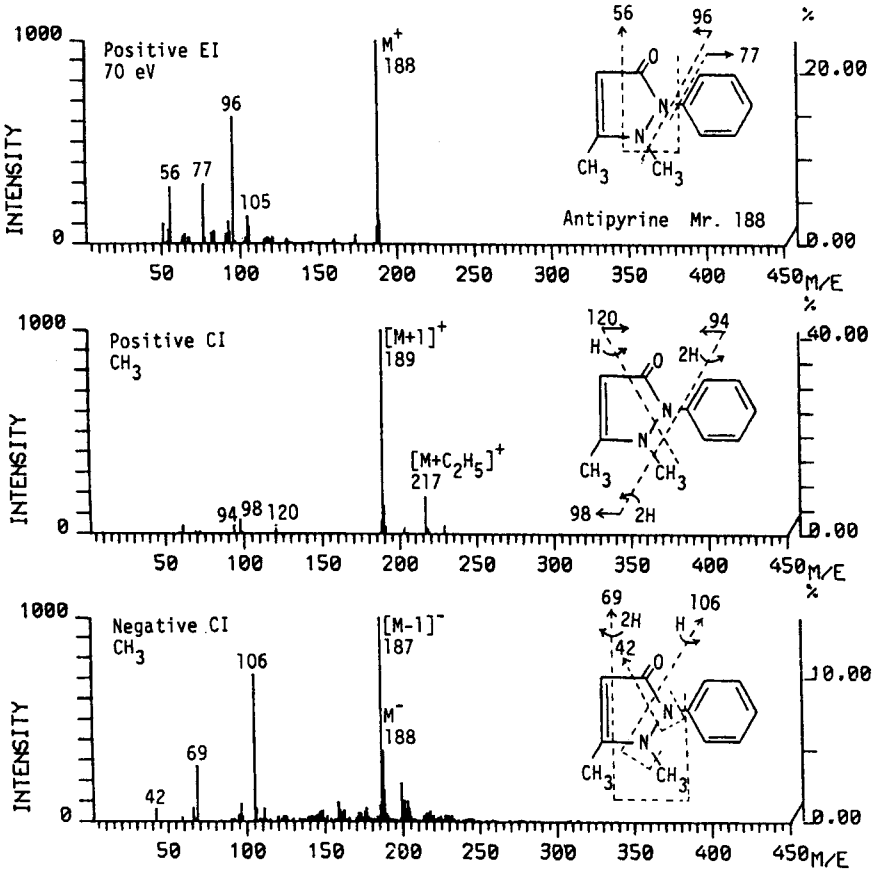

Fig. 1. PIEI, PICI and NICI mass spectra of antipyrine and its probable fragmentation modes.

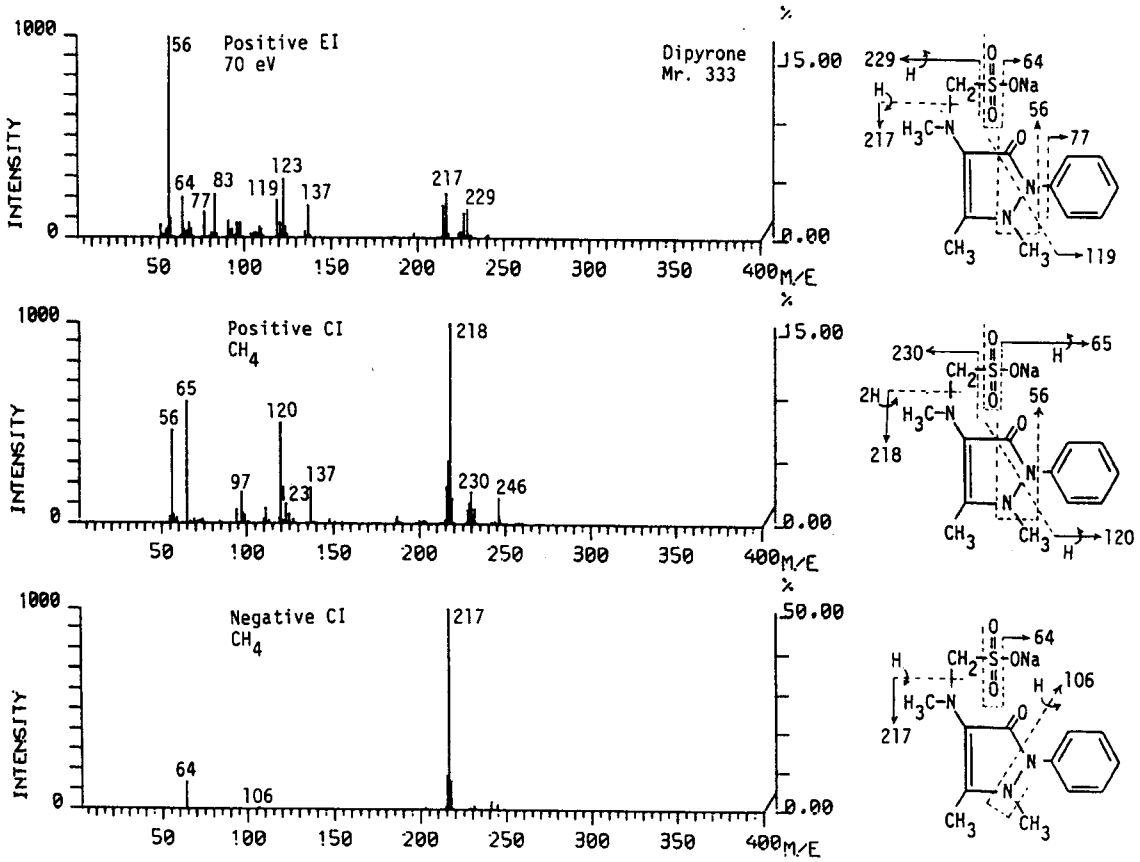

Fig. 2. PIEI, PICI and NICI mass spectra of dipyrone and its probable fragmentation modes.

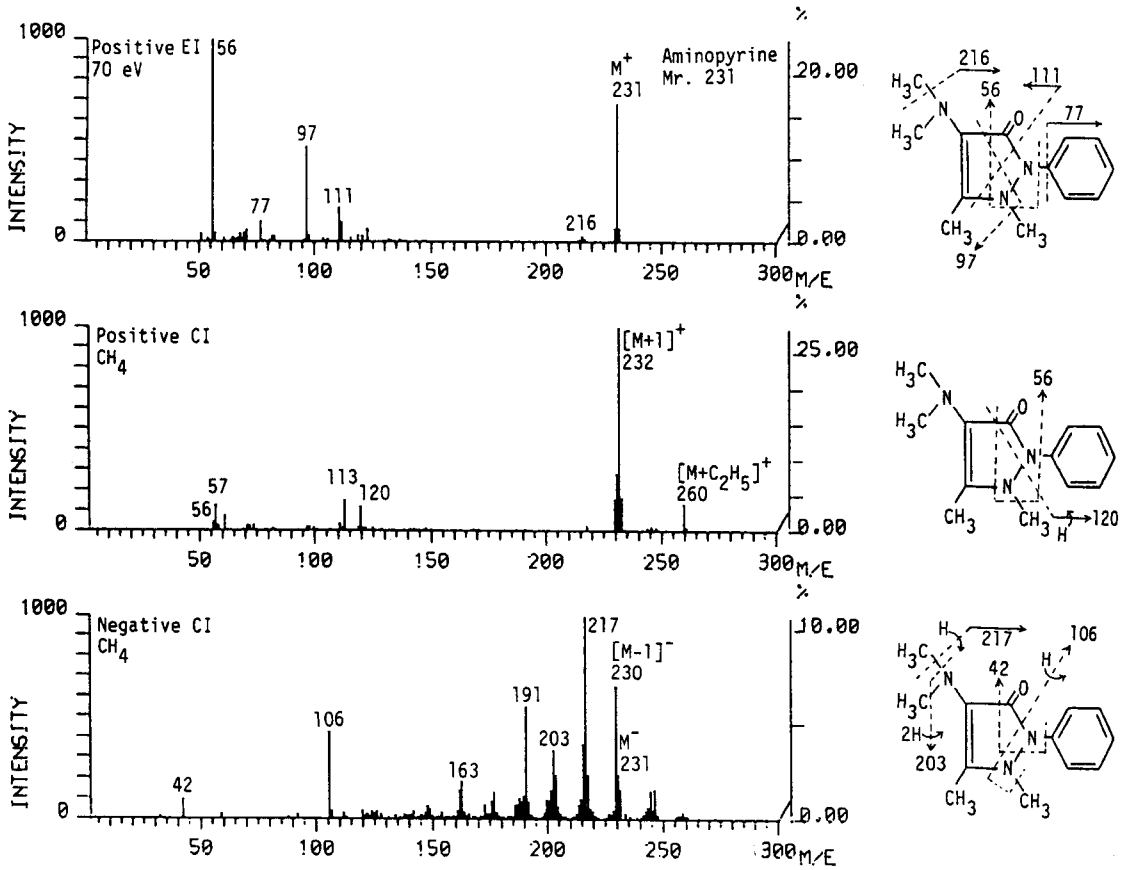

Fig. 3. PIEI, PICI and NICI mass spectra of aminopyrine and its probable fragmentation modes.

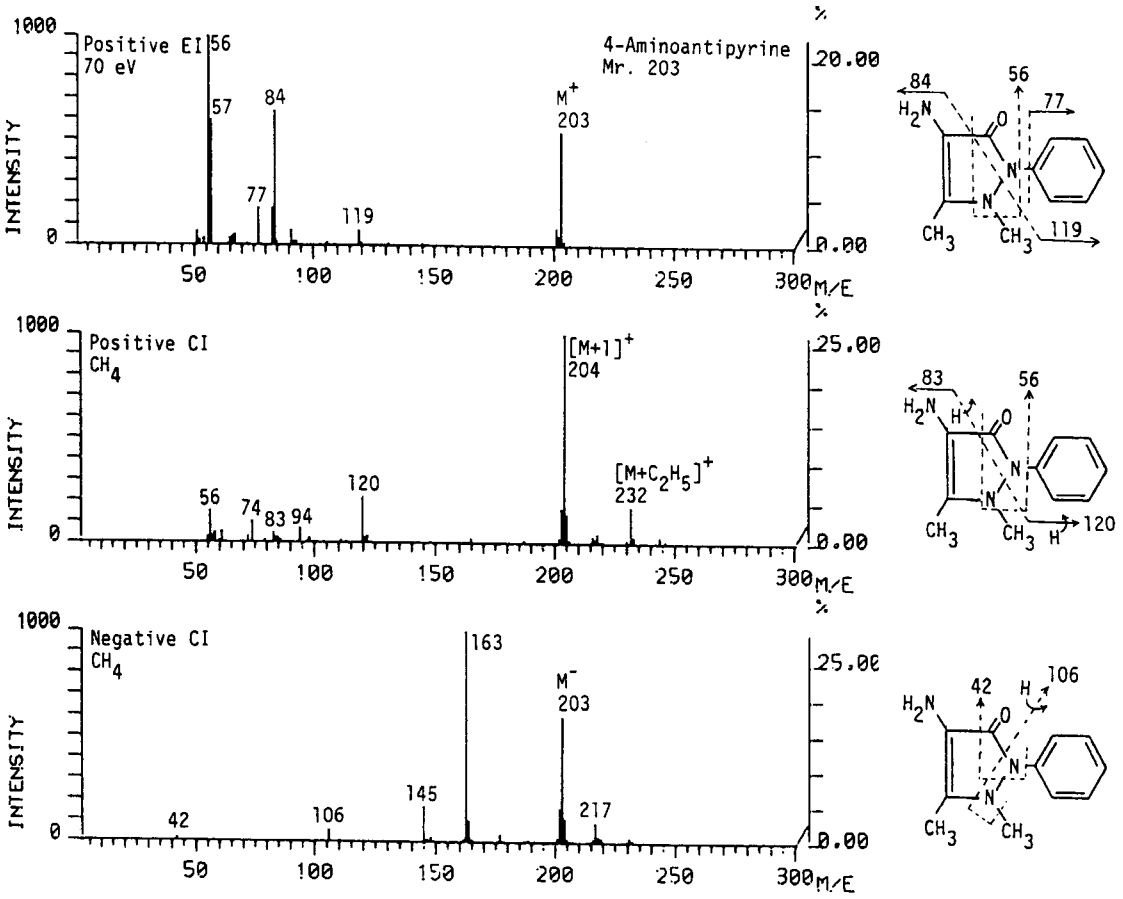

Fig. 4. PIEI, PICI and NICI mass spectra of 4-aminoantipyrine and its probable fragmentation modes.

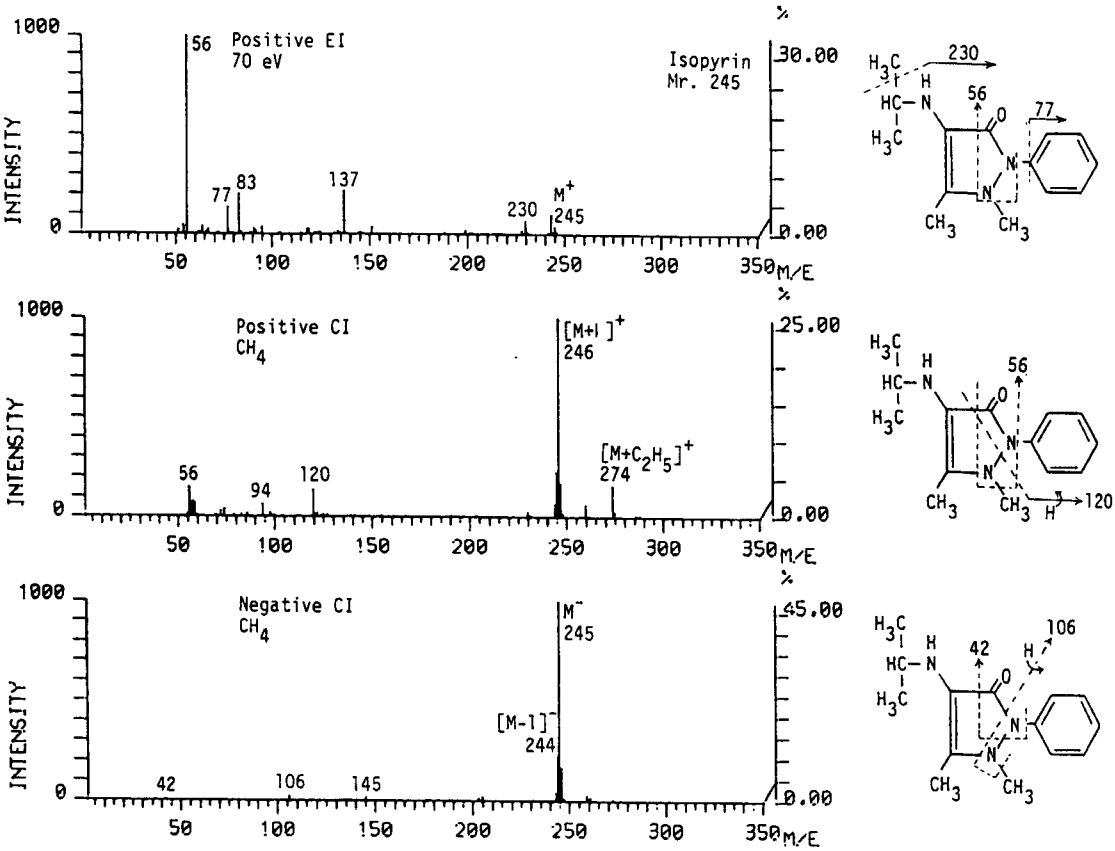

Fig. 5. PIEI, PICI and NICI mass spectra of isopyrin and its probable fragmentation modes.

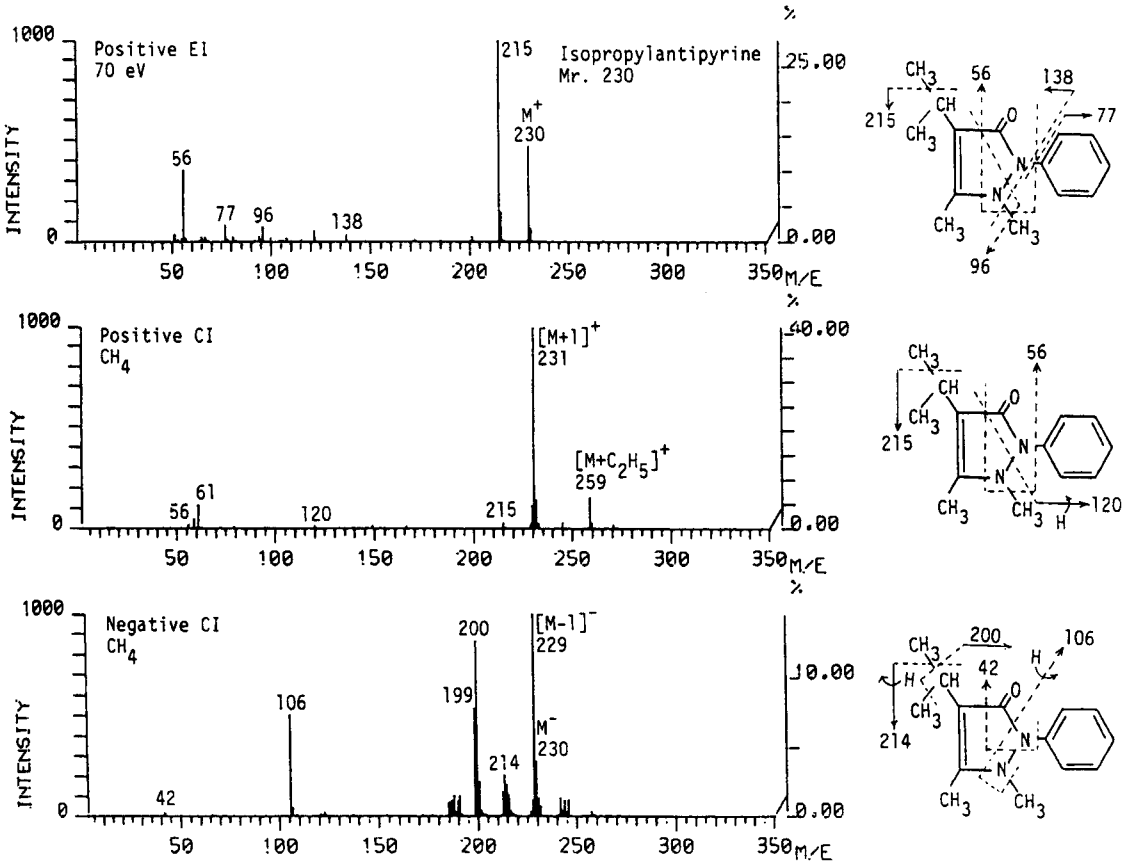

Fig. 6. PIEI, PICI and NICI mass spectra of isopropylantipyrine and its probable fragmentation modes.

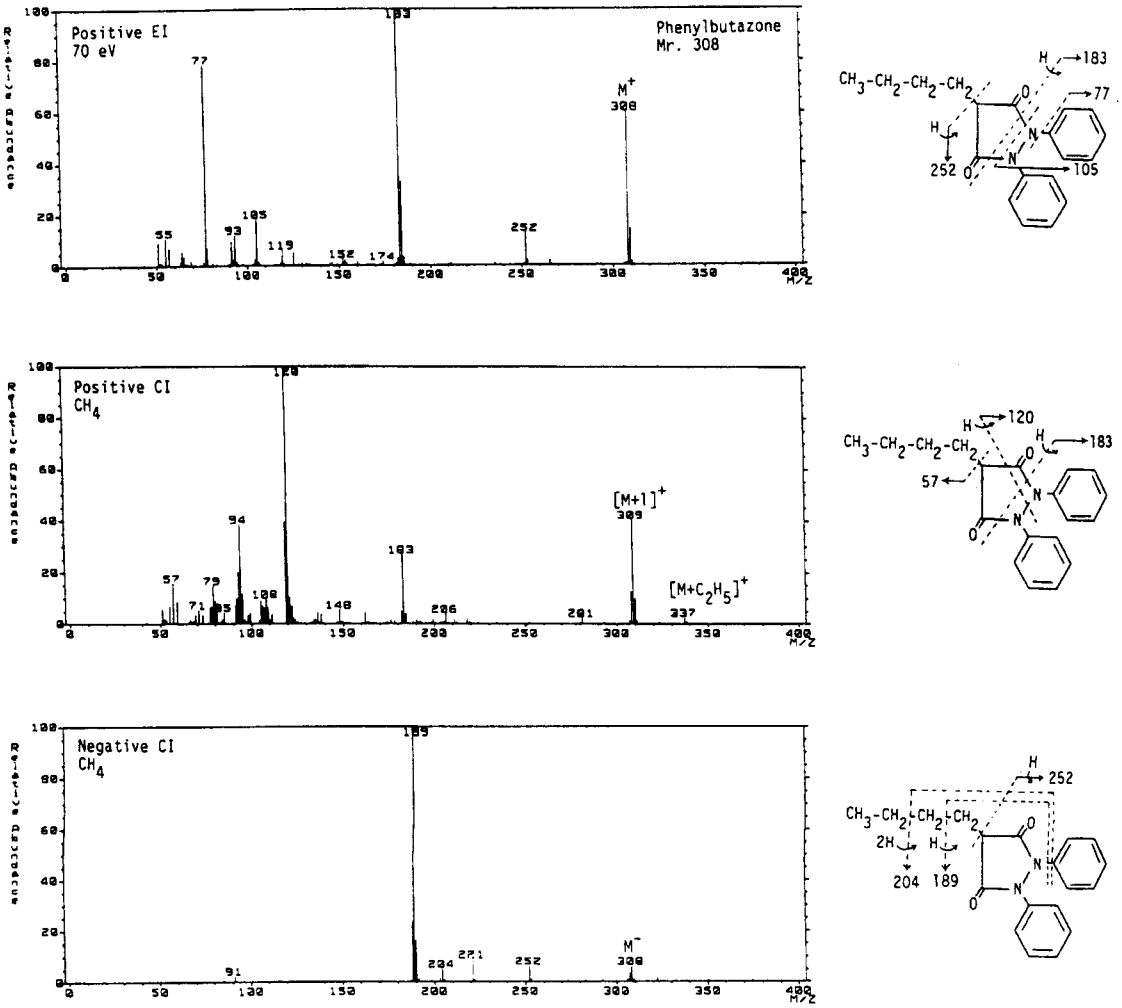

Fig. 7. PIEI, PICI and NICI mass spectra of phenylbutazone and its probable fragmentation modes.

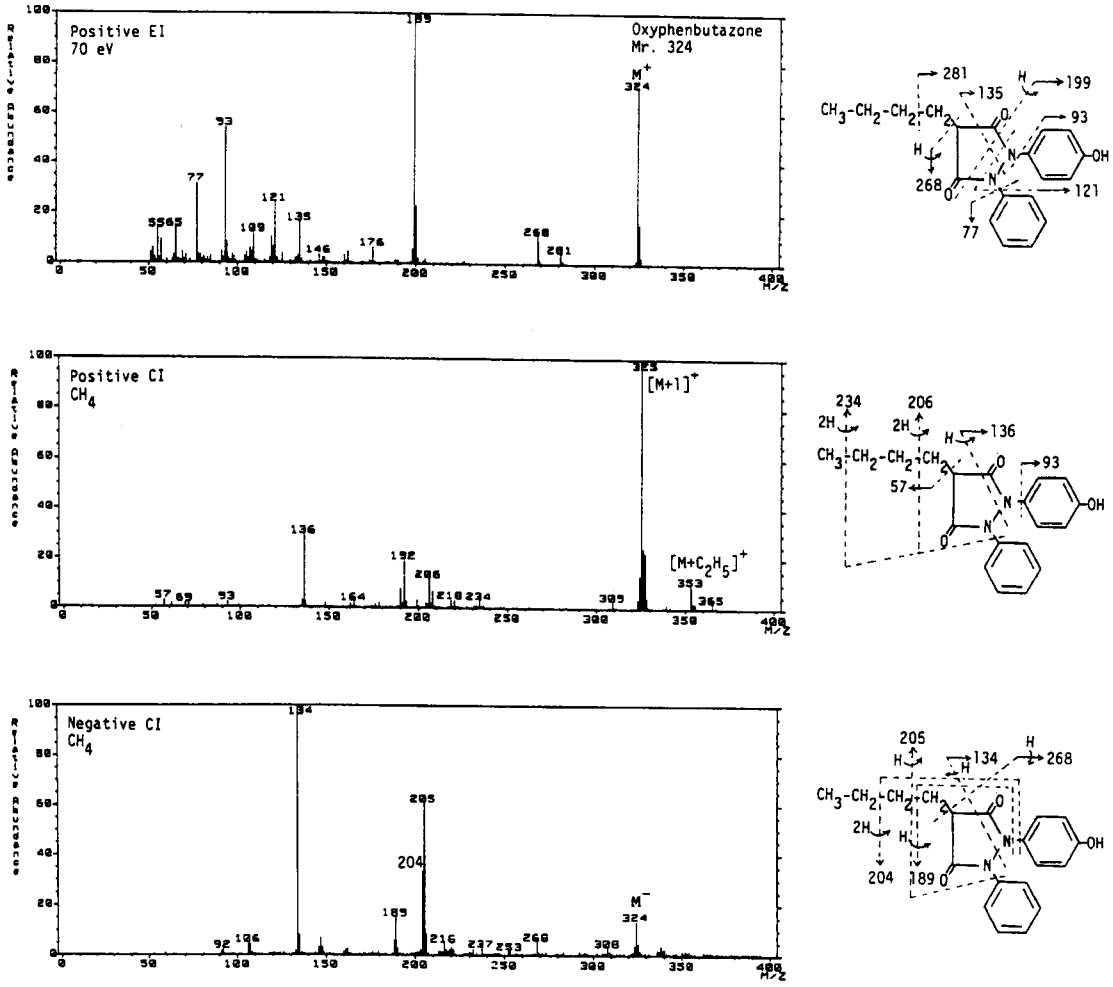

Fig. 8. PIEI, PICI and NICI mass spectra of oxyphenbutazone and its probable fragmentation modes.

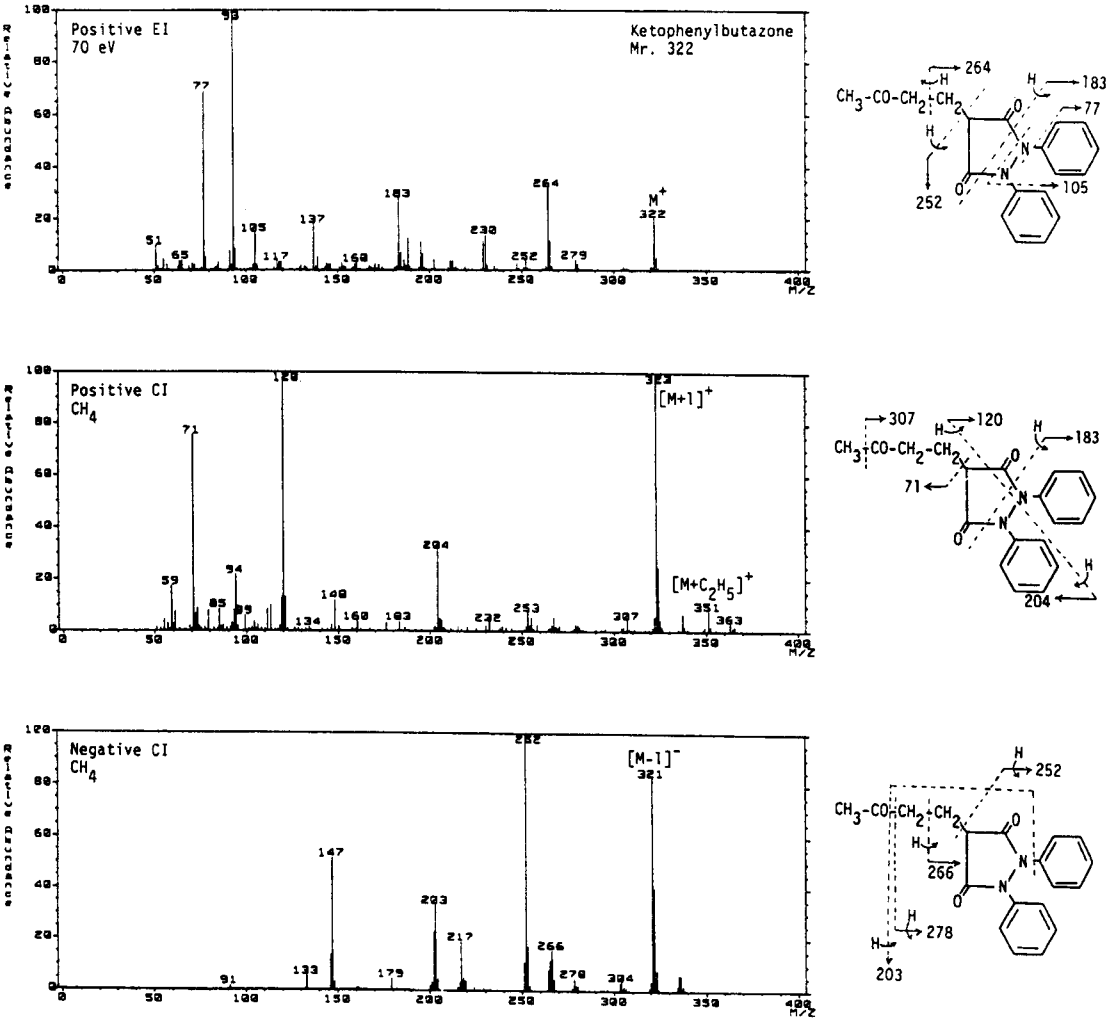

Fig. 9. PIEI, PICI and NICI mass spectra of ketophenylbutazone and its probable fragmentation modes.

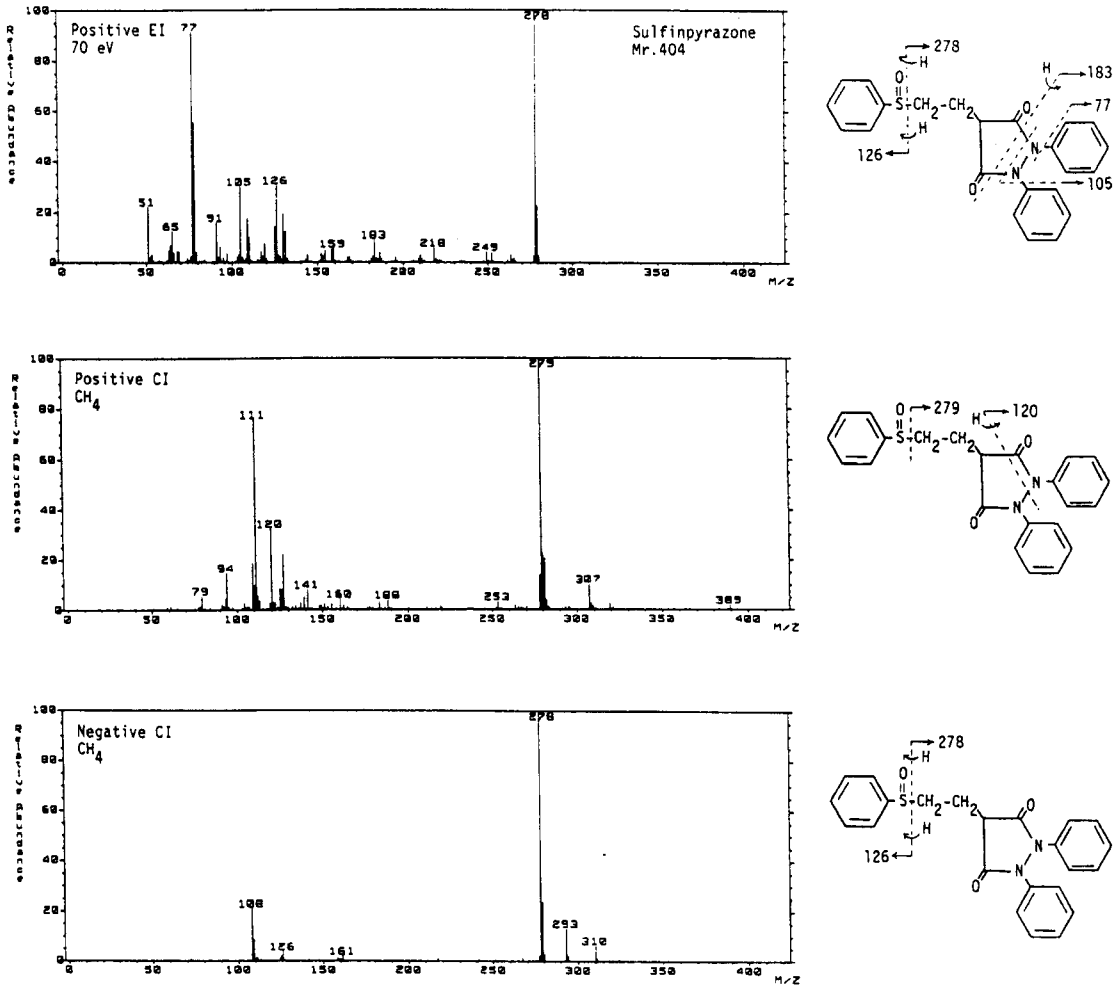

Fig.10. PIEI, PICI and NICI mass spectra of sulfinpyrazone and its probable fragmentation modes.

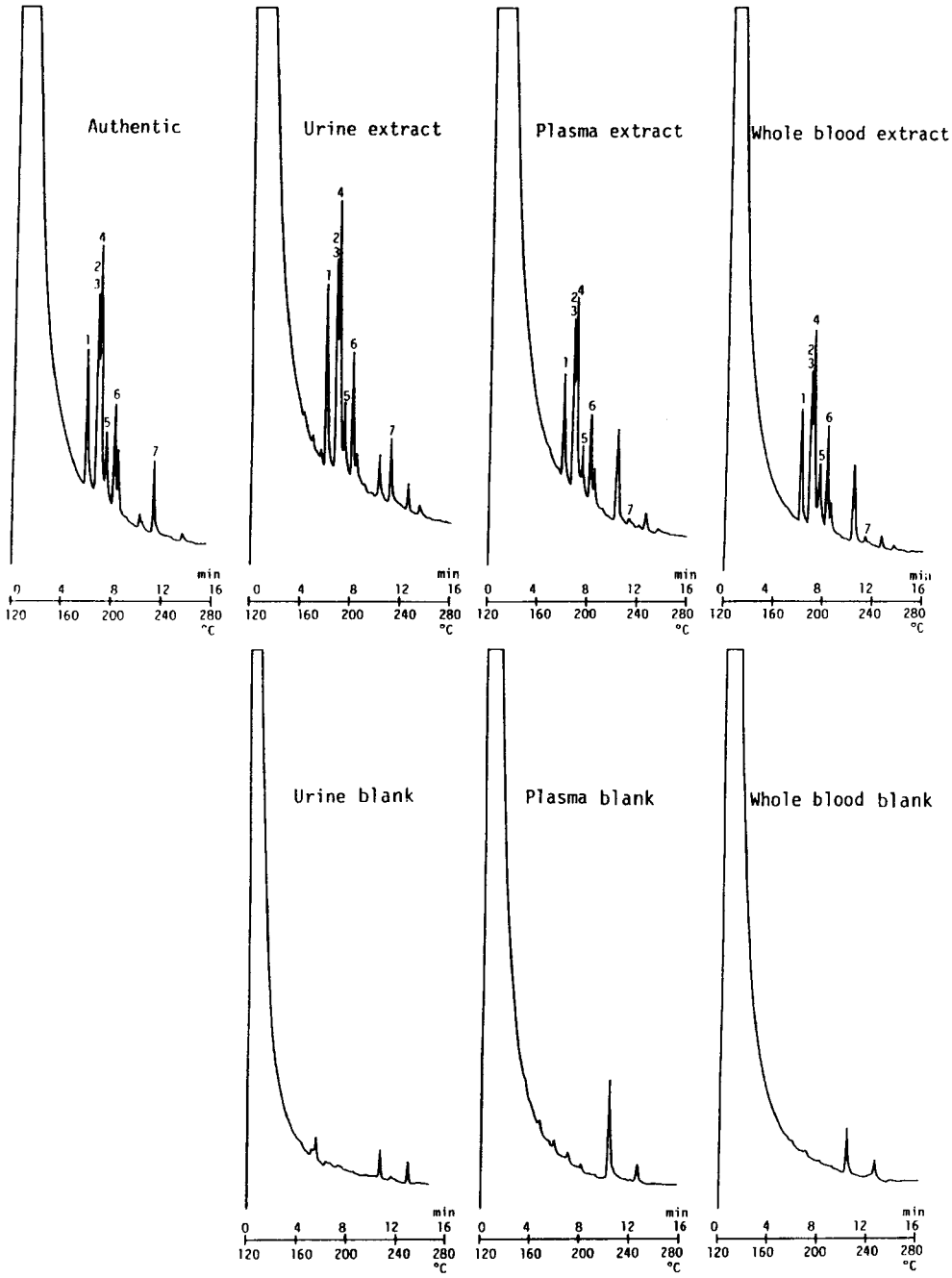

Fig. 11. Wide-bore capillary GC for pyrazolones and pyrazolidines extracted from human urine, plasma and whole blood with use of Sep-Pak C_{18} cartridges. Keys: 1, antipyrine; 2, isopropylantipyrine; 3, aminopyrine; 4, 4-aminoantipyrine; 5, dipyrone; 6, isopyrin; 7, phenylbutazone. GC was carried out with an SPB-1 fused silica wide-bore capillary column (15 m x 0.53 mm i.d., film thickness 1.5 μm). Its conditions were: column temperature 120−180 ℃ (10 ℃/min) and nitrogen flow rate 20 ml/min. The mixture of the drugs (10 μg each) was added to 1 ml of samples.

only for ketophenylbutazone.

Drug screening is important in forensic science practice. Each mode gave common ions at m/z 56 for pyrazolones and m/z 183 for pyrazolidines in the PIEI mode, at m/z 120 for pyrazolidines in the PICI mode, and at m/z 106 for pyrazolones in the NICI mode. These ions are a good indication for the possibility of the presence of pyrazolones or pyrazolidines in a sample.

Figure 11 shows gas chromatograms by wide-bore capillary GC for 10 μg each of the drugs, which had been added to 1 ml of human urine, plasma and whole blood, and extracted with Sep-Pak C_{18} cartridges. It was reported that the drugs were relatively stable during passage through a wide-bore capillary column due to much faster flow inside the column and thus much shorter exposure to heat [5]. However, for pyrazolidines, only phenylbutazone was detectable, in contrast to the fact that all pyrazolones could be detected. Therefore, derivatization should be required for detection of pyrazolidines.

Small interfering peaks overlapped the drug peaks, but they gave almost no problems. The recovery for the pyrazolones were more than 60%; that for phenylbutazone was excellent for urine extracts, but was very poor for plasma and whole blood extracts, suggesting that the drug is bound to blood protein firmly. The efforts were unsuccessful to improve the recovery for phenylbutazone in plasma and whole blood samples by acidifying the sample before loading on Sep-Pak cartridges (unpublished data).

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