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Regioisomeric differentiation of mono-methoxy ring-substituted amphetamine and methamphetamine by GC-MS

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Abstract

The 2-, 3-, and 4-methoxyamphetamines and methoxymethamphetamines were prepared and analyzed by GC-MS. Regioisomerism at the aromatic ring in these compounds possess similar analytical properties. They show similar gas chromatographic retention properties on a column with phenylmethylsilicone (HP-5) stationary phase. The mass spectra for the underivatized amines are similar and fail to provide sufficient information to differentiate the ring regioisomers. Preparation of the pentafluoropropionylamide derivatives provides adequate GC resolution and distinct mass spectra that can be used to differentiate these regioisomeric amines.

Keywords:GC-MS, Methoxyamphetamines, Methoxymethamphetamines, Chemical derivatization, Regioisomerism

Introduction

Compounds with amphetamine carbon-skeleton are frequently seen on the illicit market and have a high potential for abuse. Amphetamine is a sympathomimetic stimulant, and the substitution of aromatic methoxy group leads to compounds with intense psychotomimetic activity〔1〕. The position of the methoxy group on the aromatic ring strongly influences the degree of psychotomimetic activity. If only one methoxy group is present, it must be in the para position for the compound to have psychotomimetic properties〔2〕. Thus, neither 2- nor 3-methoxyamphetamines has psychotomimetic properties. 2-Methoxymethamphetamine (methoxyphenamine) was not expected to have psychotomimetic properties. It is a sympathomimetic drug for legitimate use in the treatment of bronchial asthma〔3, 4〕but exhibits little〔5〕or no [6] stimulant activity. Clearly there is a need for methods that could differentiate related substances for legitimate use and those having possible psychotomimetic properties.

Although the use NMR and IR as analytical tools for distinguishing mono-methoxy ring-substituted of amphetamine and methamphetamine regioisomers has been reported by many authors [3,7,8,9]; however, they are not practical techniques for direct application to all areas of forensic drug chemistry. The analysis of street drug samples for analytical toxicology heavily depends on chromatography as well as mass spectrometry. The mass spectrum is mandated as a confirmatory information in the identification of drugs of abuse. Although the mass spectrum is often considered a specific fingerprint for an individual compound, there are many substances that produce very similar or almost identical mass spectra. Especially the amphetamine analogues, the aromatic ring-substituted regioisomers frequently yield similar mass spectra [9, 10].

When other compounds have the potential to produce mass spectra similar to the drugs under controlled, the identification by GC-MS must rely mainly on the ability of the chromatographic system to separate the interfering substances from the target compounds. The

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coeluting of interfering substances with the target compounds will yield wrong results. For forensic purpose, the unequivocal identification of the ring position of the methoxy substitution on amphetamine and methamphetamine is needed. The interest in 4-methoxyamphetamine, and 4-methoxymethamphetamine, a series of monomethoxy substituted on aromatic ring of amphetamines and methamphetamines were synthesized, and analyzed by GC- MS. The method for their differentiation are described.

Experimental

Drugs and Reagents

2-Methoxyphenylacetone, 3-methoxyphenylacetone, pentafluoropropionic anhydride (PFPA), and sodium cyanoborohydride ($NaBH_3CN$) were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). 4-Methoxyphenylacetone, ammonium acetate, and methylamine hydrochloride were obtained from Fluka Chemie AG (Switzerland). All solvents and chemicals were of reagent grade or better and were used without further purification.

Synthesis of the methoxyamphetamines and methoxymethamphetamines hydrochloride

A solution of methoxyphenylacetone (10 mmole), ammonium acetate or methylamine (100 mmole) and sodium cyanoborohydride (25 mmole) in 25 mL methanol was stirred at room temperature for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the remaining residue was suspended in dichloromethane (50 mL). The dichloromethane suspension was extracted with 3 N HCl (2 x 50 mL) and the combined acid extracts were made basic (pH 12) with sodium hydroxide solution. The basic aqueous suspension was then extracted with dichloromethane (2 x 75 mL) and the combined organic extracts were dried over anhydrous sodium sulfate. Filtration and evaporation of the filtrate solvent gave the amine product as the free base. Treatment of the amine bases with etheral HCl (50 mL) formed the amine hydrochlorides, which were isolate by filteration and recrystallized from mixtures of anhydrous ether and absolute ethanol.

GC-MS Conditions

The gas chromatography (GC) and electron impact ionization (EI) mass spectrometry (MS) analyses were carried out using a Hewlett- Packard 5890/ 5970B GC-MSD. The ionization voltage was 70 eV. The GC was equipped with a 30-m 0.32-mm i.d. fused-silica column with a 0.25 μm 5% cross-linked phenylmethyl silica gum phase (HP-5). The column temperature was hold at 70℃ for 2.5 min and programmed to 150℃ at a rate of 10℃/min, and from 150℃ to 280℃ at a rate of 15℃/min with a hold time of 6 min. The carrier gas was ultra pure helium at flow rate of 1.0 c.c./min. The injection port and source temperature were 250℃ and 280℃, respectively.

Pentafluoropropionic Anhydride Derivatization

The PFPA-derivatives were prepared by mixing 1.0 mg/mL solution of each amine in 0.5N NaOH (1.0 mL) with 5.0 mL hexane, adding 100 μL pentafluoro-propionic anhydride to the extracted hexane solutions, and heating extracts at 90℃ for 20 min. The hexane solution was evaporated to dryness under nitrogen, the residue was reconstructed with hexane (200 μL), and 1.0 μL was injected into the GC-MS.

Results and Discussion

Standards of mthoxyamphetamines and mthoxymethamphetamines were synthesized from the commercially available 2-, 3-, and 4-methoxyphenylacetones and the appropriate amines under reductive amination conditions (Figure 1). Using the synthetic method followed Borch et al. [11] . The free bases were converted to the hydrochloride salts.

The six compounds synthesized in this study were subdivided into two groups of three regioisomeric amines: 2-, 3-, and 4-methoxyamphetamine; and 2-, 3-, and 4-methoxymethamphetamine. Regioisomerism on the aromatic ring in these two group amines possess similar analytical properties. The major fragmentation process in EI mass spectrometry of phenethylamines is the homolytic cleavage between the alpha and beta carbons on the side chain, yielding benzyl and imine fragments. Thus, regioisomerism on the aromatic ring will yield significantly similar mass spectra.

The three methoxyamphetamine isomers each yielded major fragments of similar mass in the EI mass spectra, m/z 121 for methoxybenzyl fragment, m/z 122

is presumably arise from amino proton transfer to the methoxybenzyl position〔3〕, and base peak m/z 44 for the ethylimine (see Figure 2B-D). Figure 3B-D shows the EI mass spectra for the methoxymethamphetamines yielding the propylimine fragment (m/z 58) as base peak.

These spectra indicated that little structural information is available for the specific differentiation among regioisomeric amines. Because mass spectrometry is often the method of choice or the mandated method for confirmation of drug identity, these two groups compounds represent a unique challenge for the specificity of analytical methods in forensic drug analysis.

The gas chromatographic resolution of these two-group isomers was studied on a column with phenylmethylsilicon (HP-5) stationary phase. The chromatograms in Figures 2A and 3A represent the effective separation achieved in these studies. The methoxyamphetamine isomers eluted in a window of less 1-min, with 2-methoxyamphetamine eluted first, followed by 3-, and 4-methoxyamphetamines. The methoxymethamphetamines eluted in a window also less 1-min and showed the same elution order.

Although these underivatized amines can be separated by capillary gas chromatography using the HP-5 stationary phase, their retention properties remain similar. The compounds eluted in less 1-min window, and detection by mass spectrometry offered little additional structural information for differentiation among these unique positional isomers. Thus, chromatography condition under which some or all these compounds coelute could yield a mass spectrum similar to each other and could have impact on the results of drug testing.

The pentafluoroacylated derivatives of these isomeric amines were studied in an effort to individualize their mass spectra and improve chromatographic resolution. Figures 4B, 4C, and 4D show the mass spectra for the PFPA derivatives of 2-, 3-, and 4- methoxyamphetamines, respectively. These spectra provide unique individualized, each shows a significant molecular ion at m/z 311, and base peak at m/z 121, 148, and 121 for the 2-, 3-, and 4-methoxyamphetamines, respectively. The m/z 190 is the PFPA-derivatized imine fragment that appears at m/z 44 for the underivatized amines. The methoxyamphetamines PFPA derivatives showed a strong m/z 148 ion for the methoxyphenylpropene fragment, and m/z 121 for the methoxybenzyl cation.

Figures 5B, 5C, and 5D show the mass spectra for the PFPA derivatives of 2-, 3-, and 4-methoxymethamphetamines, respectively. These spectra are essentially unique individualized; each shows a significant molecular ion at m/z 325 and a base peak at m/z 204 for the PFPA derivatives of 2-, and 3-methoxymethamphetamines, which resulting from the loss of the methoxybenzyl radical from the molecular ion; 4-methoxymethamphetamine-PFPA derivative show the m/z 121 as base peak. These ions of major abundance allow for the differentiation of 4-methoxy-methamphetamine from the aromatic ring isomers as PFPA derivatives. The m/z 160 ion is a four-centered rearrangement product common to the PFPA derivatives of N-methylphenethylamines〔12〕. The fragmentation for the PFPA derivatives of the regioisomers in this study are summarized in Fig. 6. The GC separation of PFPA derivatives of methoxy-amphetamines and methoxymthamphetamines on a HP-5 capillary column is shown in Figs. 4A and 5A, respectively. The elution order is the same in both chromatograms, with 2- eluting first following by 3-, and 4-, and shows baseline resolution.

Conclusion

In all cases, the PFPA-derivatized amines yielded mass spectra that readily identified the mono-methoxy substituted on aromatic ring of amphetamine and methamphetamine. The pentafluoropropionylamide derivatives have similar resolution to the underivatized amines by capillary gas chromatography on HP-5 stationary phase. Nevertheless, the PFPA-derivatives mass fragmentation were by the affected electron-donated property of methoxy group .The mass spectra of these amides therefore allow specific identification.

The advantages of PFPA-derivatives not only created unique mass spectra for these compounds, the advantage of increasing sensitivity is also useful〔13〕.

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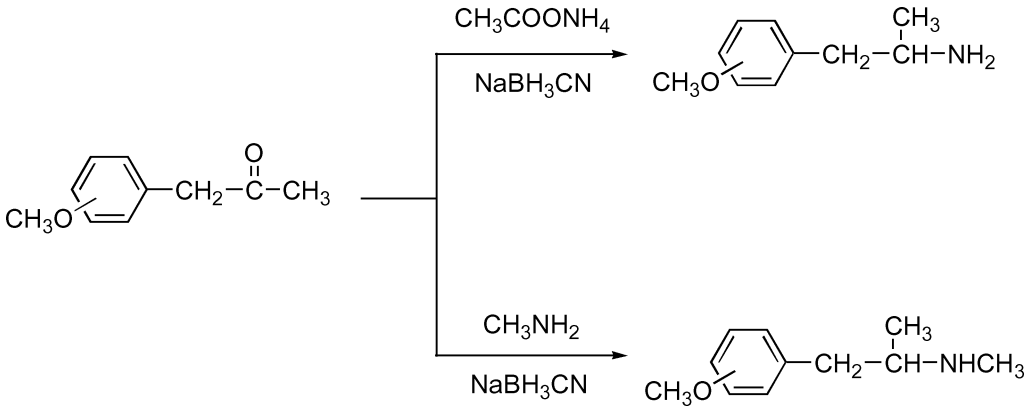

Fig.1 Preparation of the methoxyamphetamines and methoxymethamphetamines

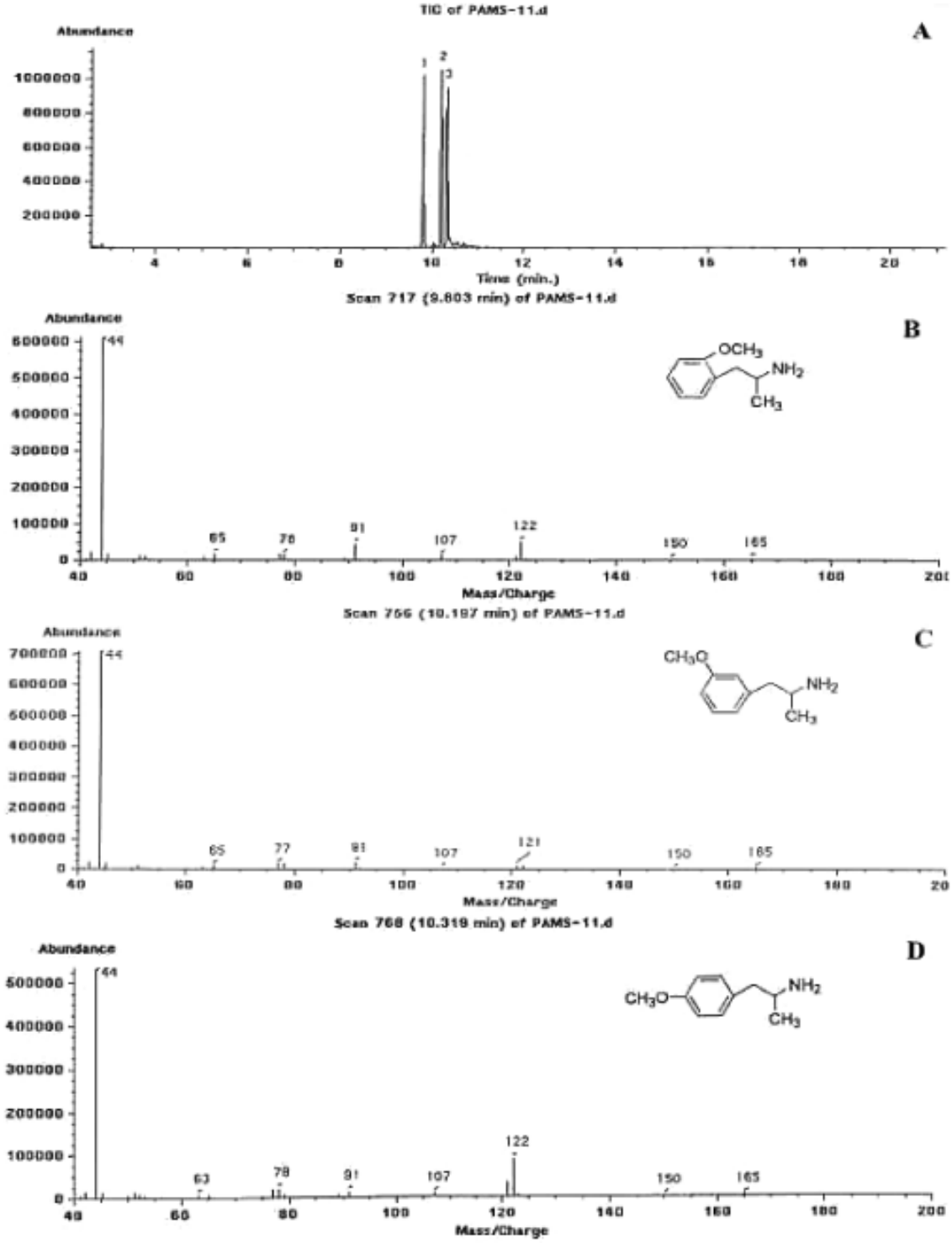

Fig.2 GC-MS analysis of the methoxyamphetamine isomers. (A) chromatogram, Peak 1, 2-methoxyamphetamine; Peak 2, 3-methoxyamphetamine; Peak 3, 4-methoxyamphetamine. (B) mass spectrum of 2-methoxyamphetamine. (C) mass spectrum of 3-methoxyamphetamine.(D) mass spectrum of 4-methoxyamphetamine.

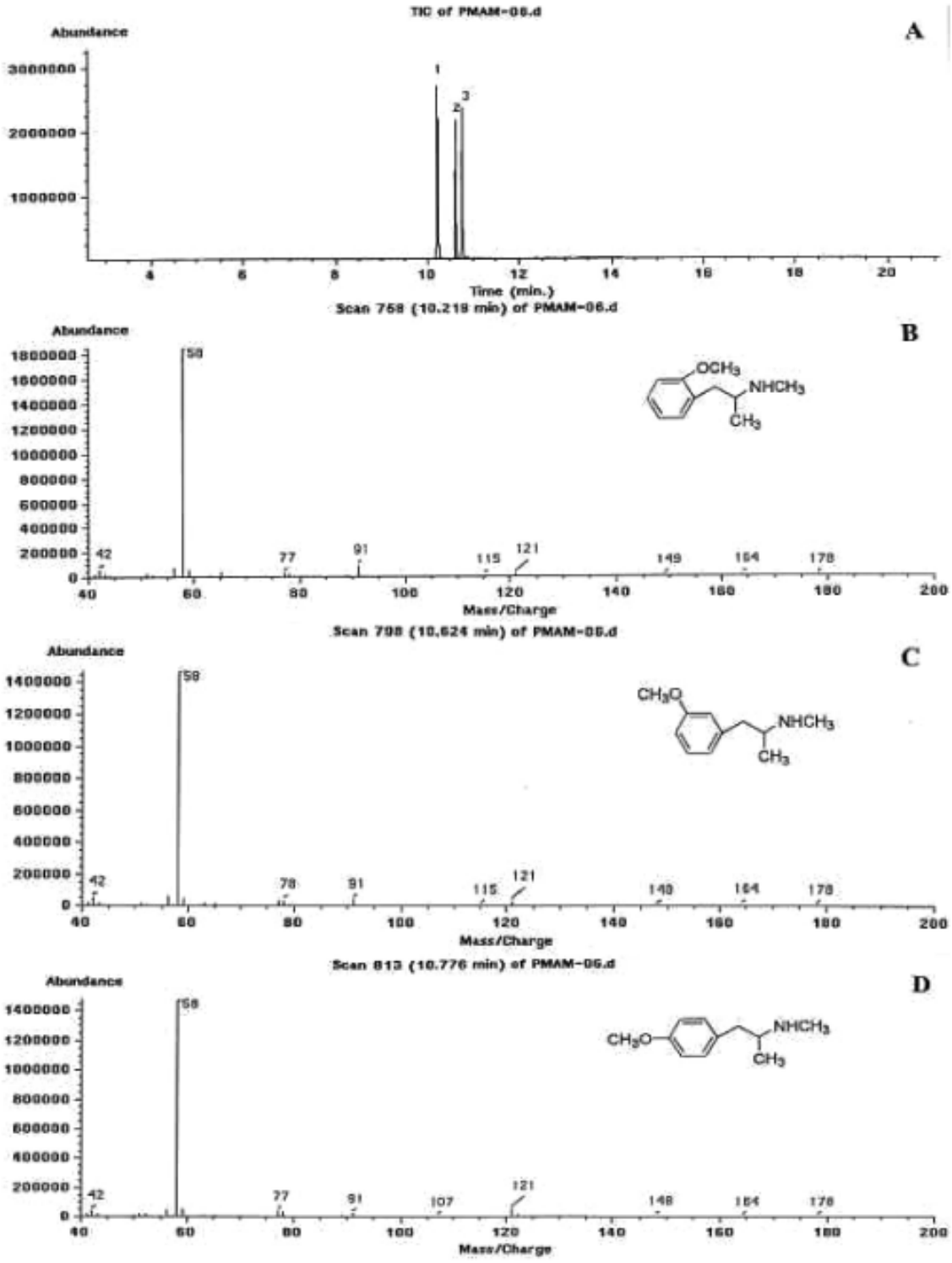

Fig.3 GC-MS analysis of the methoxymethamphetamine isomers. (A) chromatogram, Peak 1, 2-methoxymethamphetamine; Peak 2, 3-methoxymethamphetamine; Peak 3, 4-methoxymethamphetamine. (B) mass spectrum of 2-methoxymethamphetamine. (C) mass spectrum of 3-methoxymethamphetamine. (D) mass spectrum of 4-methoxymethamphetamine.

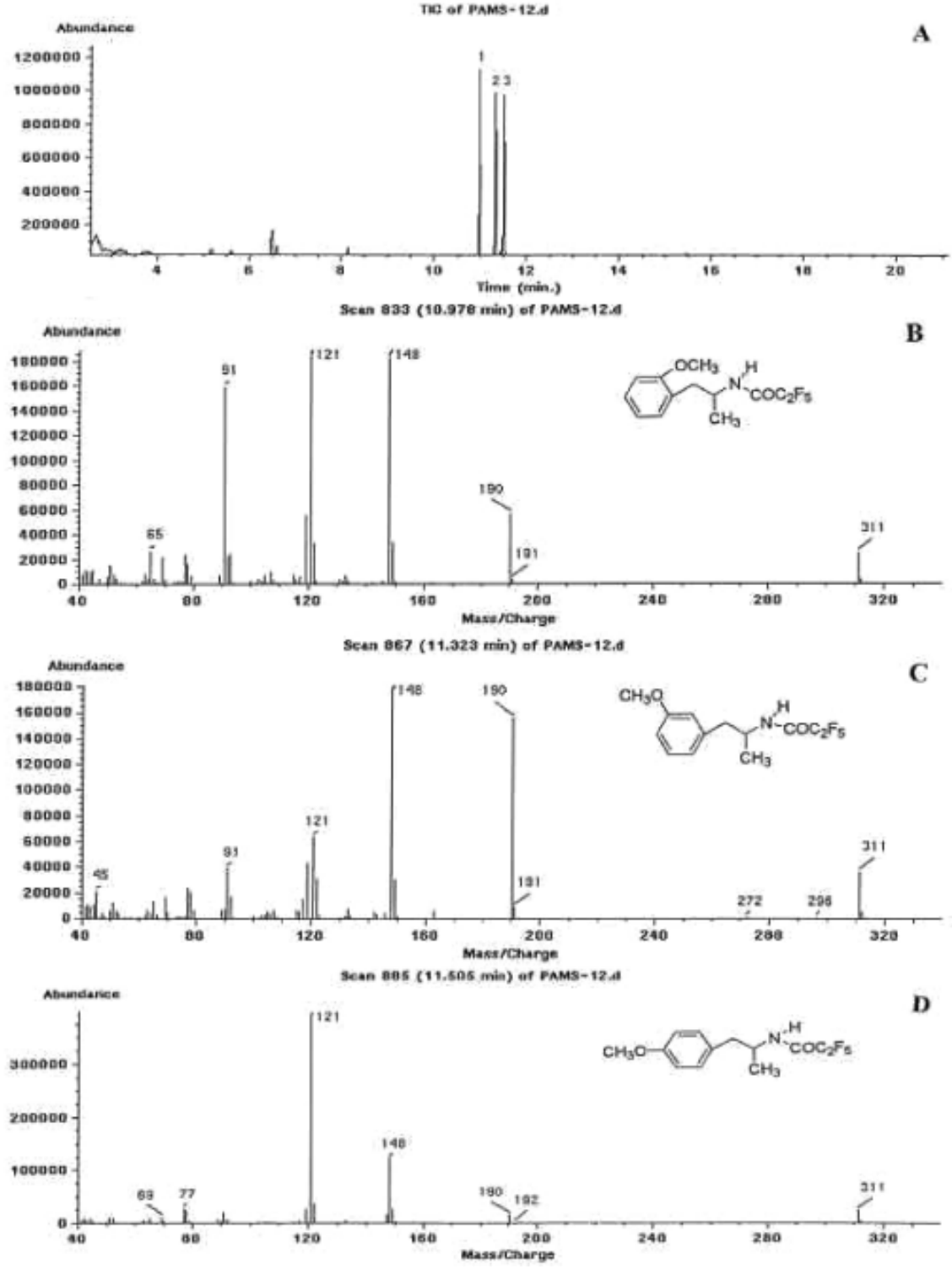

Fig.4 GC-MS analysis of the PFPA-derivatives of methoxyamphetamine isomers. (A) chromatogram, Peak 1, 2-methoxyamphetamine; Peak 2, 3-methoxyamphetamine; Peak 3, 4-methoxyamphetamine. (B) mass spectrum of 2-methoxyamphetamine-PFPA. (C) mass spectrum of 3-methoxyamphetamine-PFPA. (D) mass spectrum of 4-methoxyamphetamine-PFPA.

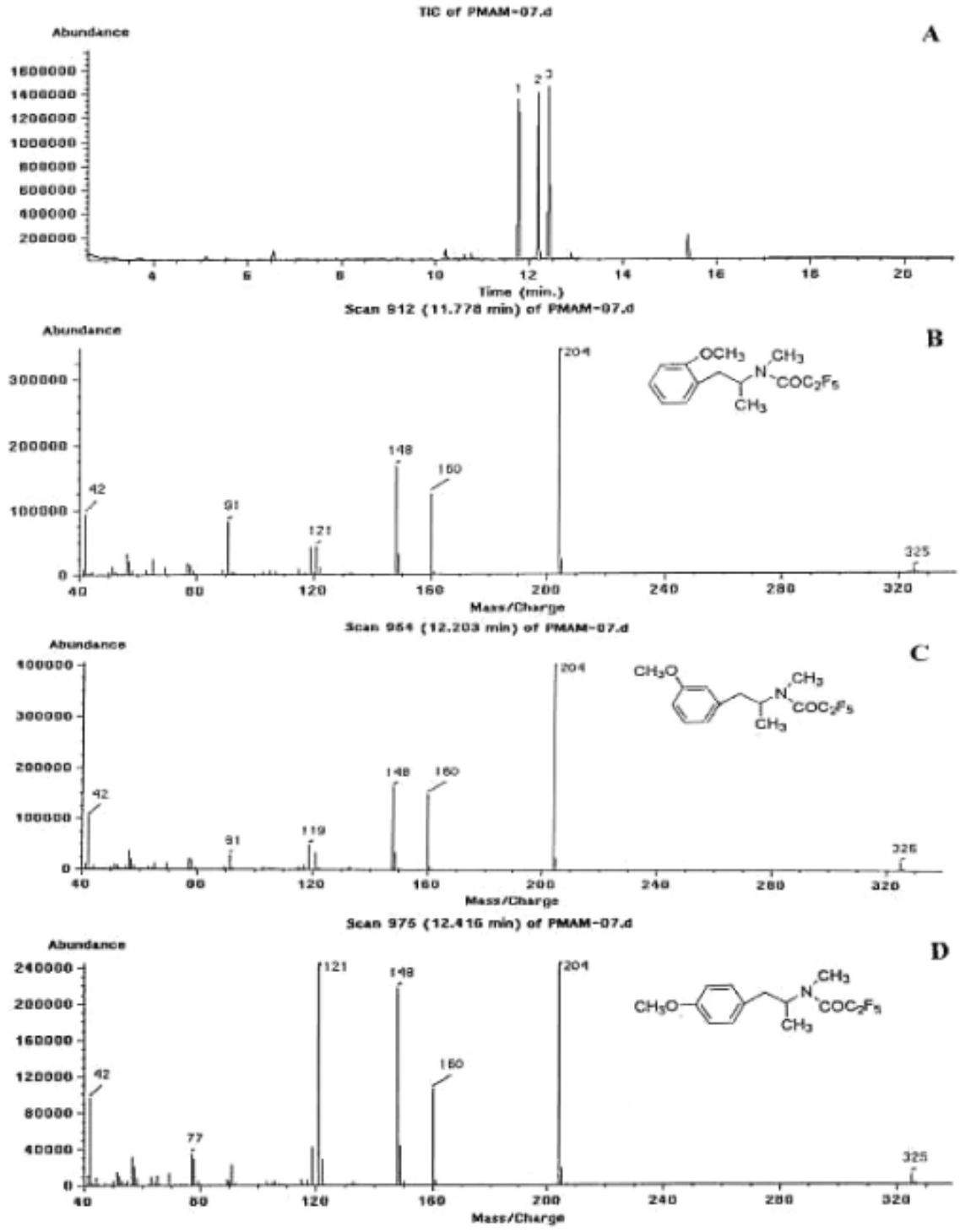

Fig.5 GC-MS analysis of the PFPA-derivatives of methoxymethamphetamine isomers. (A) chromatogram, Peak 1, 2-methoxymethamphetamine; Peak 2, 3-methoxymethamphetamine; Peak 3, 4-methoxymethamphetamine. (B) mass spectrum of 2-methoxymethamphetamine-PFPA. (C) mass spectrum of 3-methoxy methamphetamine-PFPA. (D) mass spectrum of 4-methoxymethamphetamine-PFPA.

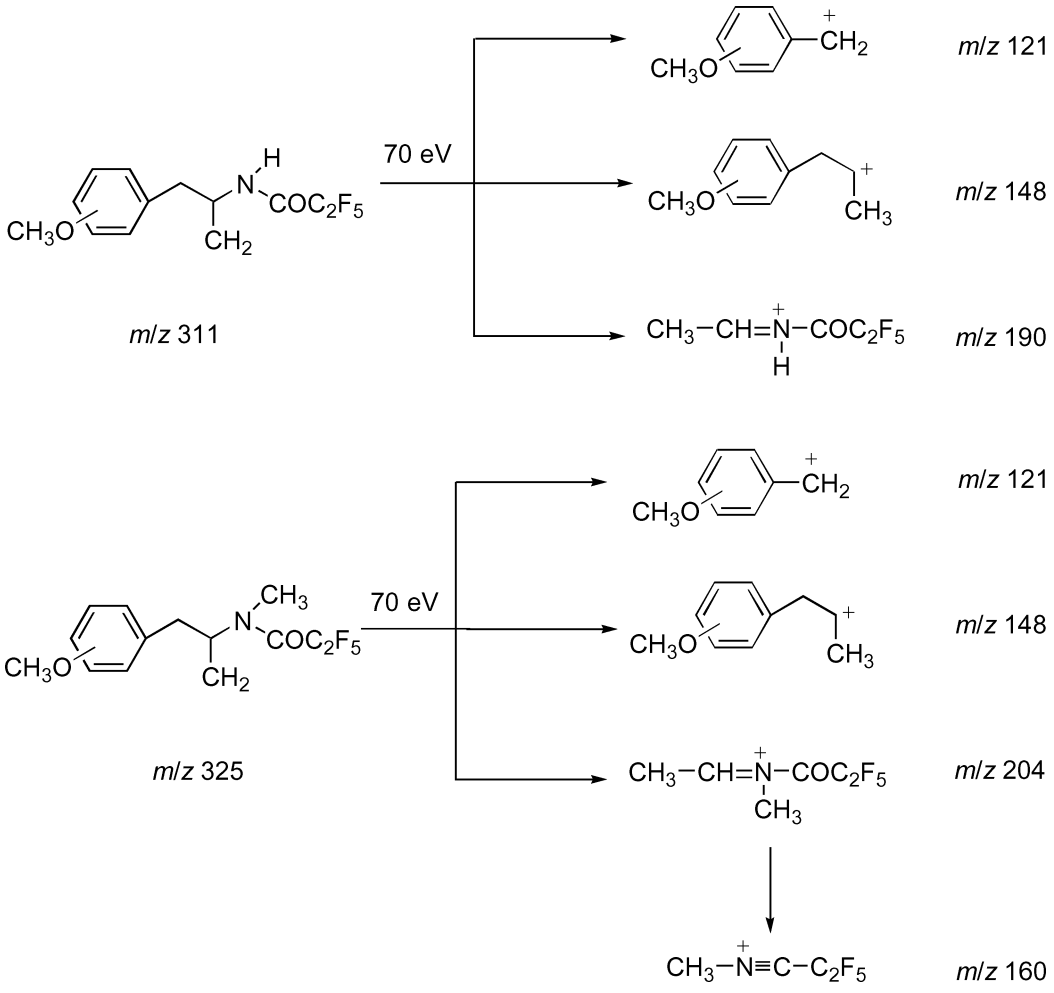

Fig.6 EI fragmentation for the pentafluoropropionylamides of methoxyamphetamine and methoxymethamphetamine.