GC–MS Analysis of Acylated Derivatives of the Side-Chain Regioisomers of 4-Methoxy-3-Methyl-Phenethylamines Related to Methylenedioxymethamphetamine

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Abstract

The five side-chain regioisomers of 4-methoxy-3-methylphenethylamine constitute a unique set of compounds having an isobaric relationship with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy). These isomeric forms of the 4-methoxy-3-methylphenethylamines have mass spectra essentially equivalent to 3,4-MDMA, and all have a molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. Mass spectral differentiation of 2,3- and 3,4-MDMA from primary and secondary amine regioisomeric side chains of 4-methoxy-3-methylphenethylamines was possible after formation of the perfluoroacyl derivatives, pentafluoropropionamides and heptafluorobutyrylamides. The mass spectra for these derivatives are significantly individualized, and the resulting unique fragment ions allow for specific side-chain identification. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond, which yielded unique hydrocarbon fragments. The heptafluorobutyrylamide derivatives offer more fragment ions for molecular individualization among these regioisomeric substances. Gas chromatographic separation on relatively non-polar stationary phases successfully resolves these derivatives.

Introduction

Previous studies (1–4) in this series have shown that the ten direct regioisomeric substances, 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy) and its nine regioisomeric equivalents, have identical molecular weights and mass spectral fragments of equivalent mass-to-charge ratios. Therefore, direct analysis of these regioisomers by electron ionization mass spectrometry (MS) does not provide data for the specific differentiation and identification of one of these regioisomers (specifically the drug of abuse Ecstasy, 3,4-MDMA) to the exclusion of all the other isomers. All 10 compounds of MW = 193 showed major fragment ions for the imine at *m/z* 58 and the substituted benzyl fragment at *m/z* 135/136. Thus, specific identification must be based on a combination of mass spectral data as well as chromatographic resolution of these regioisomeric substances. Further studies have demonstrated that some of these compounds have very similar gas chromatographic (GC) retention properties; indeed, 3,4-MDMA was found to coelute with one of its non-drug regioisomeric equivalents, *N*-ethyl-2,3-methylenedioxyphenethylamine (1), under common conditions used to identify Ecstasy (3,4-MDMA) in forensic drug samples. Additional studies (2) have shown that all ten compounds can be resolved using the more polar GC stationary phases and specific temperature programming conditions. Additional background information on the structures of these ten regioisomeric substances as well as their individual mass spectra and chromatographic properties can be found in the literature (1,2).

Another report (4) showed that the perfluoroacyl derivatives of the eight primary and secondary amines provide unique mass spectral fragment ions to differentiate among the side-chain substitution patterns for the direct regioisomers of MDMA.

A recent report (5) described the preparation and analytical evaluation of the ring-substituted methoxy methyl methamphetamines, a series of isobaric compounds related to 3,4-MDMA. The ten methoxy methyl methamphetamines were compared to 2,3- and 3,4-MDMA. All 12 of these compounds have the same side chain structure generating the *m/z* 58 ion, the base peak in the electron ionization mass spectrum for these amines. Mass spectral differentiation of 3,4-MDMA from some of the methoxy methyl methamphetamines was possible after formation of the perfluoroacyl derivatives. GC separation on non-polar stationary phases successfully resolved subsets of the methoxy methyl methamphetamines, based on ring position of the methoxy group, from 2,3- and 3,4-MDMA as the perfluoroacyl derivatives.

Differentiation of regioisomers and isobaric substances is a significant issue in forensic drug chemistry that has been addressed in a number of drug categories (5–14).

As early as 1983, forensic scientists recognized the need for

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regioisomeric differentiation in the methylenedioxyphenethylamine series of drugs (9). Soine et al. (9) described the differentiation of 2,3-methylenedioxyamphetamine from 3,4-methylenedioxyamphetamine. Additionally, differentiation between the diethylamide of lysergic acid and its regioisomeric methylpropylamide has been a concern among forensic chemists for many years (14). The present work focuses on preparation and analytical evaluation for a series of side-chain regioisomers of 4-methoxy-3-methyl phenethylamines (Figure 1) having an isobaric relationship to 3,4-MDMA. These compounds have the same nominal mass but with different elemental composition (isobaric substances), yet they are expected to yield major mass spectral fragments of equivalent mass to those observed for 2,3- and 3,4-MDMA.

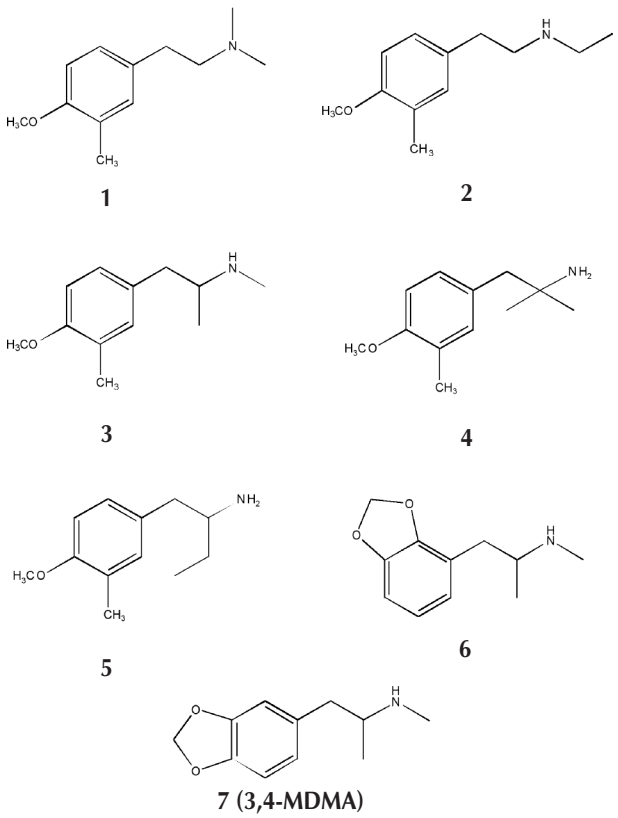


Figure 1. Structures of the regioisomeric 4-methoxy-3-methylphenethylamines and 2,3- and 3,4-MDMA.

Experimental

Analytical

GC–MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto). The MS was operated on the electron impact mode using ionization voltage of 70 eV and a source temperature of 230°C. The mass spectra presented in Figures 2–4 were obtained by background subtraction and are the average of at least five scans. The mass spectral scan rate was 1.2 s per scan. The GC was operated in splitless mode with a flow rate of 1.37 mL/min and a column head pressure of 10 psi using helium grade 5 as carrier gas. Samples were diluted in high-performance liquid chromatography (HPLC) grade acetonitrile (Fischer Scientific, Fairlawn, NJ) and manually introduced (1 µL), individually and in a physical mixture, using a 10-µL Hamilton syringe (Hamilton Co., Reno, Nevada).

The retention data was collected and separations were carried out on a 30 m × 0.25-mm i.d. column coated with 0.25 µm 100% dimethyl polysiloxane (Rtx-1) and a 30 m × 0.25-mm i.d. column coated with 0.25 µm trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek corporation (Bellefonte, PA).

The retention data in Table I were generated using a temperature program consisting of an initial temperature hold at 100°C for 1 min, ramped up to 180°C at a rate of 9°C per min, held at 180°C for 2 min, and then ramped to 200°C at a rate of 10°C per min.

Table I. Relative Retention Time (min) of the Underivatized, HFBA, and PFPA Derivatives of Compounds 1–7*

	Rtx-1†			Rtx-200‡		
		Derivatives			Derivatives	
Compound No	Underivatized	HFBA§	PFPA§	Underivatized	HFBA	PFPA
1	0.948	N/A	N/A	0.966	N/A	N/A
2	1.012	0.988	0.988	1.046	0.912	0.915
3	0.969	0.950	0.949	0.988	0.850	0.850
4	0.935	0.849	0.844	0.930	0.778	0.780
5	1.044	0.910	0.903	1.082	0.884	0.888
6	0.953	0.927	0.918	0.970	0.874	0.878
7	1	1	1	1	1	1
3,4-MDMA	9.701	13.349	12.876	10.770	19.666	18.959

* Results are the average of three experiments.
† Rtx-1 is a 30 m × 0.25-mm i.d. column coated with 0.25 µm 100% dimethyl polysiloxane
‡ Rtx-200 is a 30 m × 0.25- mm i.d. column coated with 0.25 µm trifluoropropyl methyl polysiloxane
§ Abbreviations: HFBA, heptafluorobutyrylamide and PFPA, pentafluoropropionamide.

Drugs and reagents

Samples of 2,3- and 3,4-MDMA and 4-methoxy-3-methylmethamphetamine were synthesized as previously described (1,5). Other side-chain regioisomers of 4-methoxy-3-methylmethamphetamine were synthesized in analogous procedures to side chain regioisomers of 3,4-MDMA as reported in the literature (1) using 4-methoxy-3-methylbenzaldehyde as a starting material. All laboratory reagents and chemicals were obtained from Aldrich Chemical Company (St. Louis, MO) and Fisher Scientific (Atlanta, GA). Pentafluoropropionic anhydride and heptafluorobutyric anhydride were purchased from UCT (Bristol, PA).

Derivatization procedure

Each perfluoroamide was prepared individually from the hydrochloride salts of the regioisomers by dissolving approximately 0.3 mg (1.33×10^{-5} mole) of each amine in 50 µL of ethyl acetate followed by the addition of a large excess (250 µL) of the appropriate derivatizing agent (pentafluoropropionic anhydride or heptafluorobutyric anhydride), and the derivatization reaction mixtures were incubated in capped tubes at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of air at 55°C and reconsti-

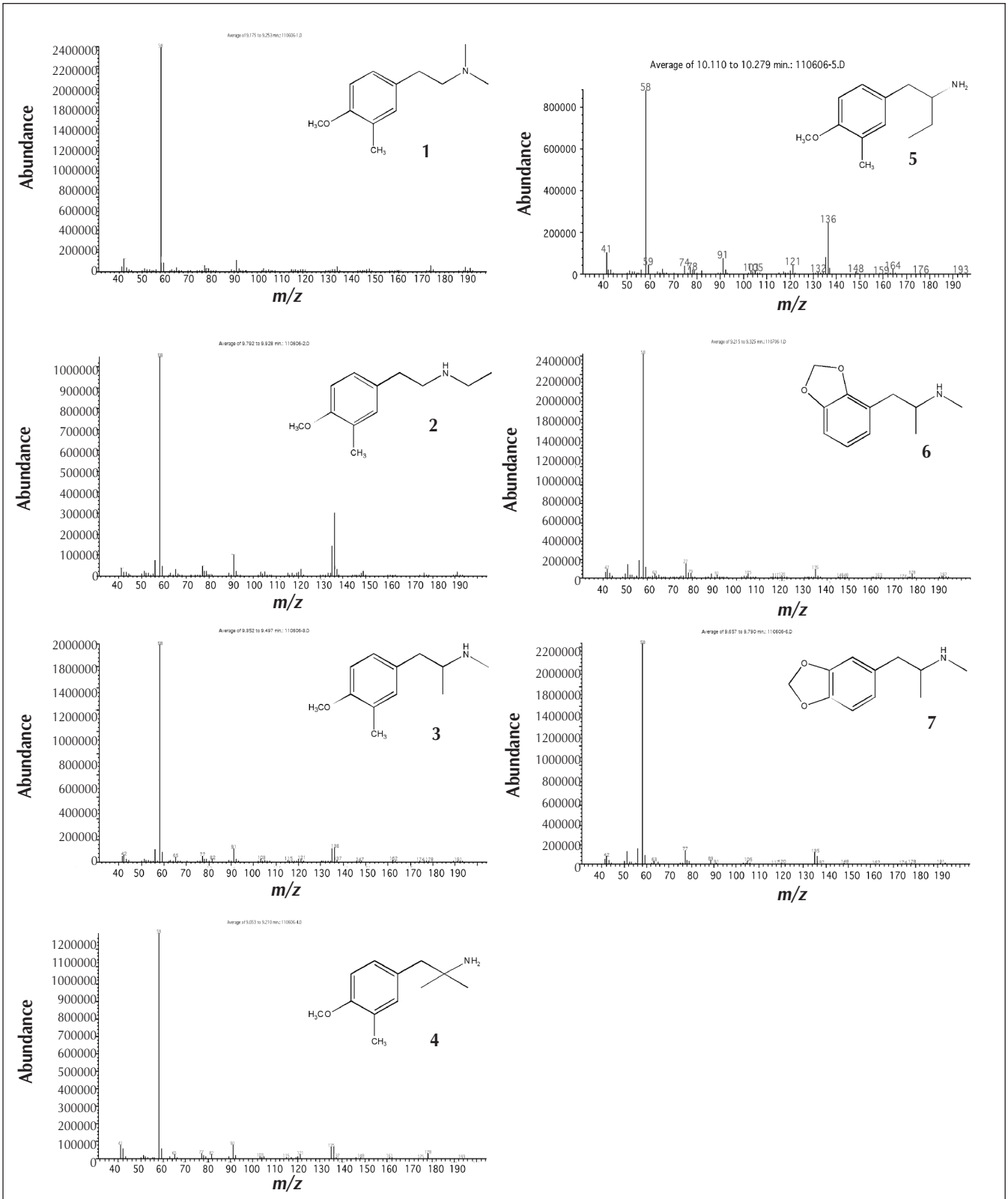


Figure 2. Mass spectra of the underivatized amines.

tuted with 200 µL of ethyl acetate and 50 µL of pyridine.

Results and Discussion

Synthesis

Condensation of the 4-methoxy-3-methylbenzaldehyde with a nitroalkane (nitromethane, nitroethane, or 1-nitropropane) under basic conditions yields the 1-(4-methoxy-3-methylphenyl)-2-nitroalkene, which, upon reduction with lithium aluminum hydride (LAH), yields the primary amines. The *N*-methyl and *N*-ethyl analogues were prepared from the primary amines by acylation followed by LAH reduction. Alternately, the nitroalkenes are hydrolyzed to the corresponding 4-methoxy-3-methylphenylketones and reductively aminated with methyl-, dimethyl-, or ethylamine in the presence of sodium cyanoborohydride. The 1-(4-methoxy-3-methylphenyl)-2,2-dimethylethanamine was prepared from 4-methoxy-3-methylbenzaldehyde following conversion to the corresponding benzylchloride and condensation with isobutyric acid. The resulting 2,2-dimethyl-3-(4-methoxy-3-methylphenyl)-1-propionic acid was treated sequentially with sodium azide, ethyl chloroformate, and benzyl alcohol, followed by catalytic hydrogenation under low pressure to yield the desired 1-(4-methoxy-3methylphenyl)-2,2-dimethylethanamines (1). The methods for the preparation of the 2,3- and 3,4-methylenedioxy-isomers have been described in the literature (1,3,9,10,11). The general procedure for the synthesis of these compounds begins with 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxybenzaldehyde (piperonal) as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde has been reported (1,11).

MS

MS is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The

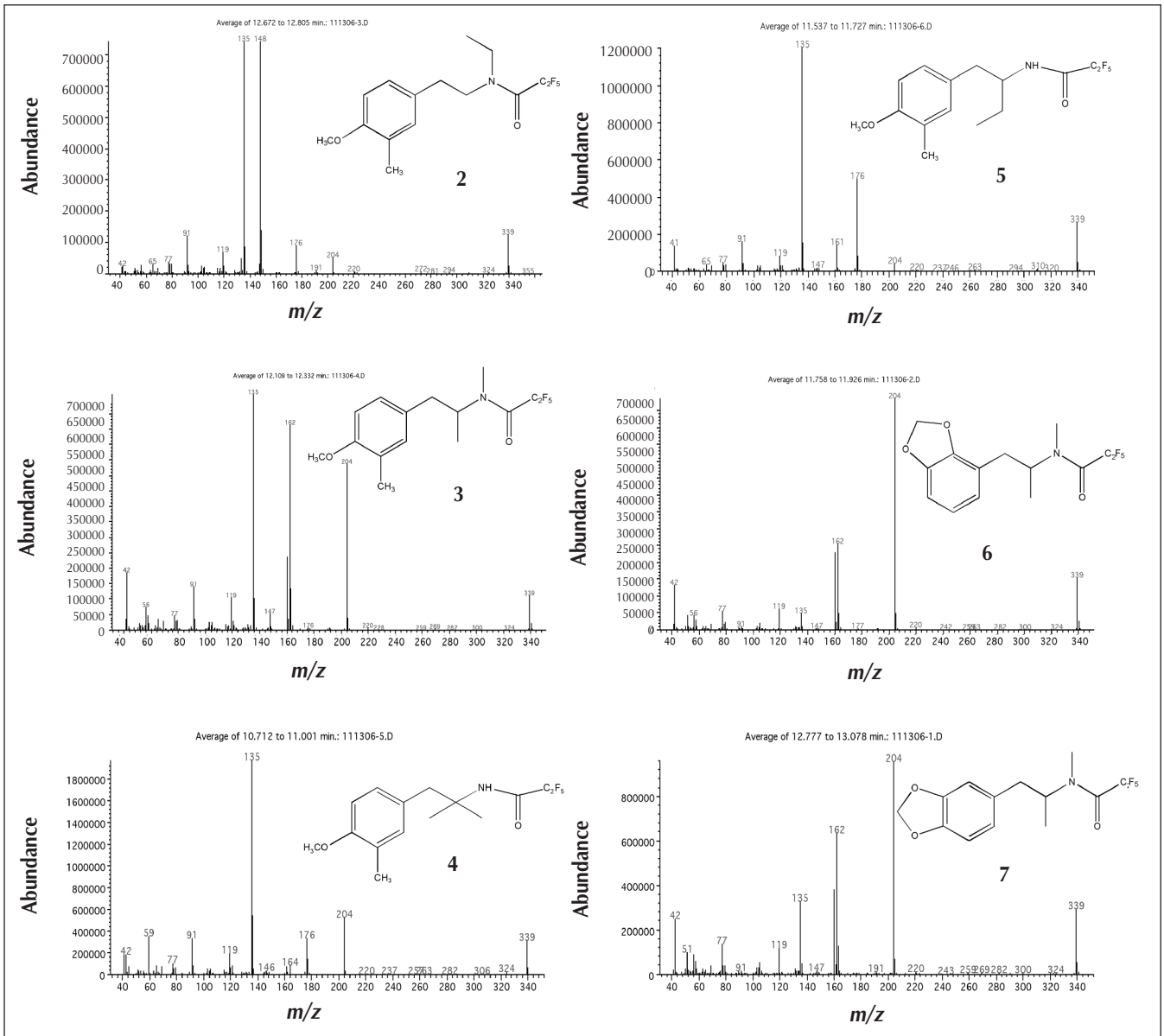


Figure 3. Mass spectra of the PFPA derivatives of compounds 2–7.

mass spectra of phenethylamines are characterized by a base peak formed from an amine-initiated alpha-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-methylenedioxymethamphetamine (MW = 193), the alpha-cleavage reaction yields the substituted imine fragment at *m/z* 58 and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-methylenedioxymethamphetamine contains major ions at *m/z* 58 and 135/136 as well as other ions of low relative abundance (1). There are a total of five regioisomeric forms of the *m/z* 58 imine species.

The five side-chain regioisomers of 4-methoxy-3-methylphenethylamine (Compounds 1–5) have the potential to yield mass spectra essentially equivalent to 3,4-MDMA and 2,3-MDMA. All have a molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136 (Figure 2). The isobaric methoxy methyl benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. Furthermore, the *m/z* 58 ion in the methoxy methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA (Figure 5). The individual mass spectra for 2,3- and 3,4-MDMA are also presented in Figure 2 (Compounds 6 and 7).

This lack of mass spectral specificity, in addition to the possibility of chromatographic coelution with 3,4-MDMA, could result in misidentification of the target drug. Furthermore, the lack of available reference samples could complicate the individual identification of any one of these substances. This constitutes a significant analytical challenge where the specific identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the regioisomeric/isobaric non-drug substance from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for

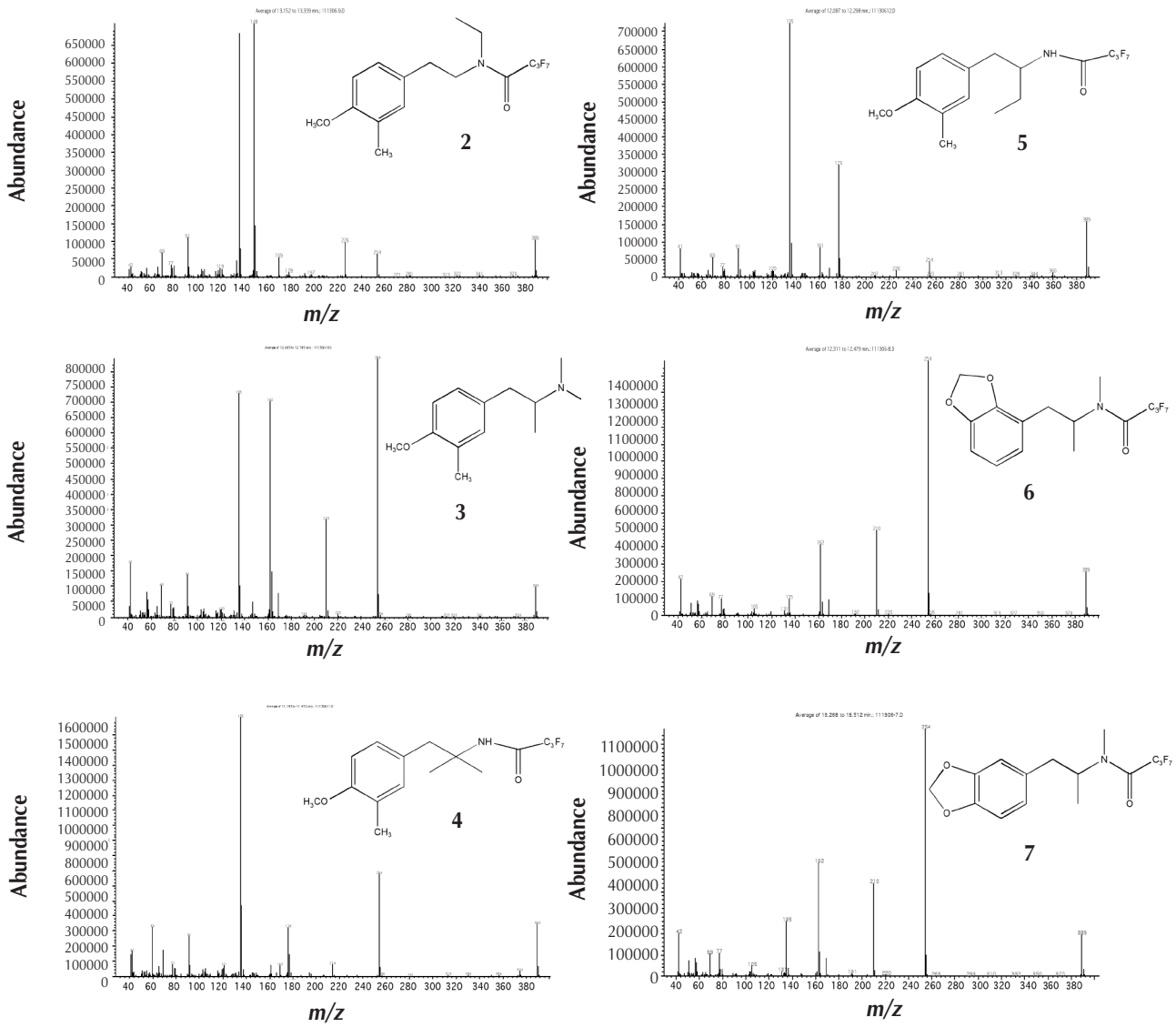


Figure 4. Mass spectra of the HFBA derivatives of compounds 2–7.

the target drugs of interest.

In the following phase of this study, various perfluoroacylated derivatives of the regioisomeric primary and secondary amines were prepared and evaluated in an effort to individualize their mass spectra and provide unique marker ions for specific identification. Acylation of the amines significantly reduces the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum (4).

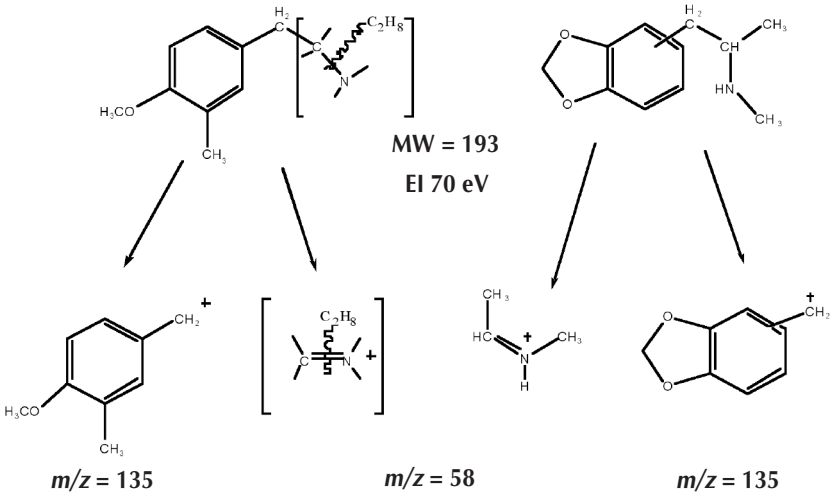


Figure 5. Electron ionization fragmentation pattern for compounds 1–7.

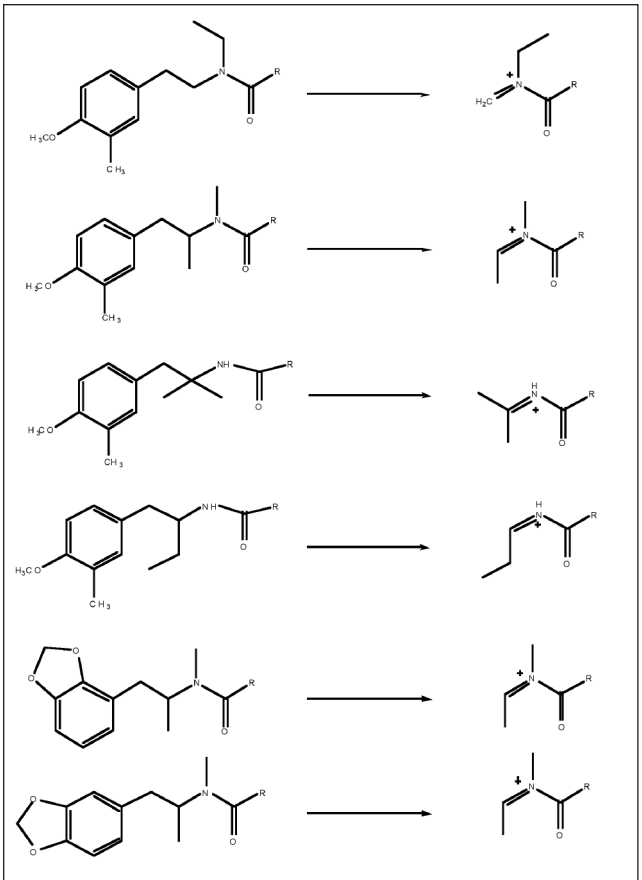


Figure 6. Formation of the (M-135)+ ions in the perfluoroacyl-derivatives of compounds 1–7.

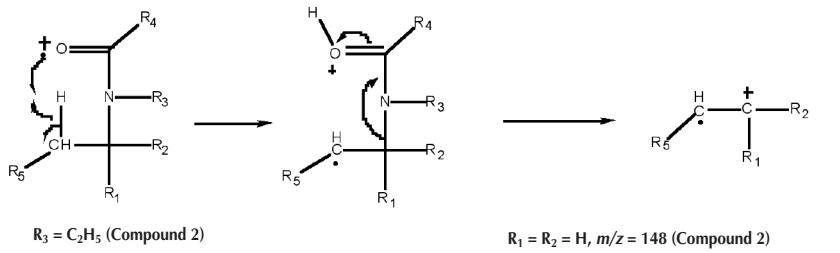
The mass spectra for the 12 pentafluoropropionyl and heptfluorobutyryl amides are shown in Figures 3 and 4, respectively. In these spectra, a common peak occurs at *m/z* 204 and 254, which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for pentafluoropropionamides (PFPA) and heptafluorobutyrylamides (HFBA). This ion at *m/z* 204 and 254 is the PFPA and HFBA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the 4-methoxy-3-methylbenzyl radical as well as the 2,3- and 3,4-methylenedioxybenzyl radical. Thus, the *m/z* 204 and 254 ions in the PFPA and HFBA amides are analogous to *m/z* 58 in the underivatized species because all of these ions represent the $(M-135)^+$ species. The general fragmentation pattern and structures for the *m/z* 204 and 254 ions are shown in Figure 6. The 4-methoxy-3-methylbenzyl cation (*m/z* 135) and the methylenedioxybenzyl cation (*m/z* 135) are fragments common to all spectra in Figures 3 and 4. However, the 4-methoxy-3-methylbenzyl cation at *m/z* 135 in the perfluoroacyl derivatives shows a very high relative abundance. Indeed, the *m/z* 135 ion is the base peak in all the PFPA derivatives of compounds 2–5 and in the HFBA derivatives of compounds 4 and 5. The remaining two HFBA derivatives of compounds 2 and 3 show the *m/z* 135 ions as a major fragment of at least 90% relative abundance. This would suggest that the perfluoroacyl derivatives offer a distinct discrimination between the methylenedioxy and the 4-methoxy-3-methyl substitution patterns based on the difference in relative abundances of the substituted benzyl cation at *m/z* 135.

The decreased role of the alpha cleavage reaction in the fragmentation of these amides allows the formation of ions that are more diagnostic of each individual isomer. Acylation weakens the bond between nitrogen and the alkyl carbon of the phenethyl side chain, allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structure information. The mass spectra in Figures 3 and 4 illustrate the role of hydrocarbon fragments at *m/z* 148, 162, and 176 in the electron impact mass spectral differentiation among these regioisomers.

The spectra for the *N*-ethyl isomer (compound 2) in Figures 3 (Compound 2) and 4 (Compound 2) show a base peak at *m/z* 148 corresponding to the alkene radical cation, which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (see Figure 7). This ion at *m/z* 148 would only occur for the *N*-ethyl regioisomer. The spectra in Figures 3 (Compound 3), 3 (Compound 6), 3 (Compound 7), 4 (Compound 3), 4 (Compound 6), and 4 (Compound 7) show the substituted

phenylpropane hydrocarbon ion at *m/z* 162, identifying these molecules as the PFPA and HFBA derivatives of 4-methoxy-3-methylmethamphetamine and the 2,3- and 3,4-methylenedioxymethamphetamines, respectively. The proposed mechanism for the formation of the hydrocarbon fragment is illustrated in Figure 7. However, the base peak of *m/z* 135 in the spectra of the PFPA derivative of compound 3 offers a significant discrimination of 4-methoxy-3-methylmethamphetamine from its two isobaric methylenedioxymethamphetamines isomers (Compounds 6 and 7). The spectra for the PFPA and HFBA derivatives of the primary amines (Compounds 4 and 5) show ions at *m/z* 176 from the corresponding substituted phenylbutene radical cation. This ion at *m/z* 176 results from hydrogen rearrangement and subsequent fragmentation of alkyl carbon to nitrogen bond. The lower abundance of *m/z* 176 for the 4-methoxy-3-methylphentramine (compound 4) may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

Although the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the *N*-ethylphenethylamines [Figure 3 (Compound 2)] is the appearance of an ion at *m/z* 176 in addition to the base peak at *m/z* 148. The 176 ion suggests a four-carbon chain directly attached to the aromatic ring, which also occurs for the alpha-ethyl- (Compound 5) and alpha, alpha-dimethyl-(Compound 4) phenethylamines [Figures 3 (Compound 4), 3 (Compound 5) and 4 (Compound 4), 4 (Compound 5)]. The *m/z* 176 ion in the spectra for the PFPA derivatives of the *N*-ethyl regioisomers [Figure 3 (Compound 2)] is a rearrangement of the *m/z* 204 ion, resulting in the loss of mass 28 (the *N*-ethyl group) via hydrogen transfer (see Figure 8). This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the *N*-ethylphenethylamines shown in Figure 4 (Compound 2). The loss of 28 mass units from the acylimine fragment at *m/z* 254 yields the equivalent fragment ion at *m/z* 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances.



R_3 = C_2H_5 (Compound 2)
CH_3 (Compounds 3, 6, 7)
H (Compounds 4 and 5)
R_4 = C_2F_5, PFPA Derivatives
C_3F_7, HFBA Derivatives
R_5 = 4-Methoxy-3-methyphenyl (Compounds 2–5)
= 2,3-Methylenedioxyphenyl (Compound 6)
= 3,4-Methylenedioxyphenyl (Compound 7)

R_1 = R_2 = H, *m/z* = 148 (Compound 2)
R_1 = H, R_2 = CH_3, *m/z* = 162 (Compounds 3, 6, 7)
R_1 = R_2 = CH_3, *m/z* = 176 (Compound 4)
R_1 = H, R_2 = C_2H_5, *m/z* = 176 (Compound 5)

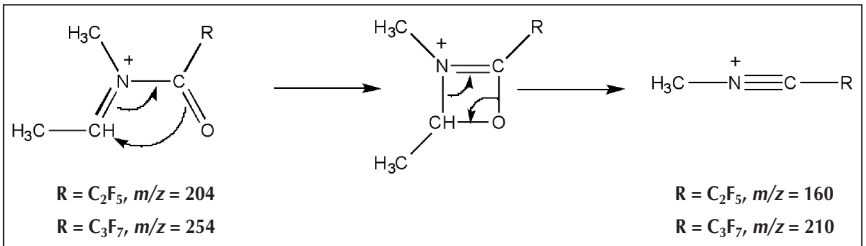
Figure 7. Mechanism for the formation of the alkene radical cation in the perfluoroacyl derivatives of compounds 1–7.

R = C_2F_5, *m/z* = 204
R = C_3F_7, *m/z* = 254

R = C_2F_5, *m/z* = 176
R = C_3F_7, *m/z* = 226

Figure 8. Mechanism for the loss of mass 28 from the perfluoroacyl-*N*-ethyl-imine cation.

A comparison of the mass spectra for the PFPA and HFBA derivatives of all three ring-substituted methamphetamines (Compounds 3, 6, and 7) indicates unique ions at *m/z* 160 and 210 [see Figures 3 (Compound 3), 3 (Compound 6), and 3 (Compound 7); and 4 (Compound 3), 4 (Compound 6), and 4 (Compound 7)]. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively. An analysis of the masses of the components that make up the fragment at *m/z* 160, for example, include C_2F_5 (119 mass units) and CH_3 (15 mass units), leaving only a mass of 26 available for the total of 160. The mass 26 would correspond to CN, and the proposed mechanism for the formation of $(C_2F_5CNCH_3)^+$ is shown in Figure 9. An equivalent fragmentation pathway has been reported (13) for methamphetamine and is further supported by the analysis of the mass spectra of the PFPA and HFBA derivatives of d_3- and d_5-MDMA in a previous study (4).



R = C_2F_5, *m/z* = 204
R = C_3F_7, *m/z* = 254

R = C_2F_5, *m/z* = 160
R = C_3F_7, *m/z* = 210

Figure 9. Mechanism for the formation of the m/z 160 and 210 ions in the mass spectra of the perfluoroacyl derivatives of the ring-substituted methamphetamines (Compounds 3, 6, and 7).

GC

The PFPA and HFBA derivatives of the six primary and secondary amines were compared on two stationary phases, the relatively nonpolar 100% dimethyl polysiloxane (Rtx-1) and the more polar trifluoropropyl methyl polysiloxane (Rtx-200). Several tem-

perature programs were evaluated, and one program showing the best compromise between resolution and analysis time was used to generate the retention data in Table I and the chromatograms in Figure 10. Table I shows the relative retention of these compounds compared to *N*-methyl-3,4-methylenedioxyphenyl-2-propanamine (3,4-MDMA) under identical chromatographic conditions. The chromatograms of the perfluoroacyl compounds show that 2,3-MDMA elutes before the corresponding 3,4-isomer, which elutes last. When the ring-substitution pattern is held constant (4-methoxy-3-methyl), the side-chain elution order is secondary amides before the tertiary amides, and in this limited set of examples, branched isomers elute before the more linear ones. Therefore, the amides of compound 4 elute first, followed by the amide of compound 5 (both secondary amides), then the amides of compound 3 (the methamphetamine side chain), and finally, the amides of compound 2 (the more linear of the tertiary amides). The amides of 2,3-MDMA elute between the secondary and tertiary amides in this group of compounds. Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance 3,4-MDMA and its closest eluting regioisomeric and isobaric equivalents. Both the PFPA and HFBA derivatives of 3,4-MDMA elute last, and the *N*-ethyl-4-methoxy-3-methylphenethylamine PFPAs and HFBAs are the closest eluted compounds in the 4-methoxy-3-methyl phenethylamine series. The isobaric *N*-ethyl amides show very distinct mass spectra with several characteristic ions to differentiate it from the corresponding amides of the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with both chromatographic and mass spectral procedures can allow for the complete differentiation of the side-chain substitution pattern of the 4-methoxy-3-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA.

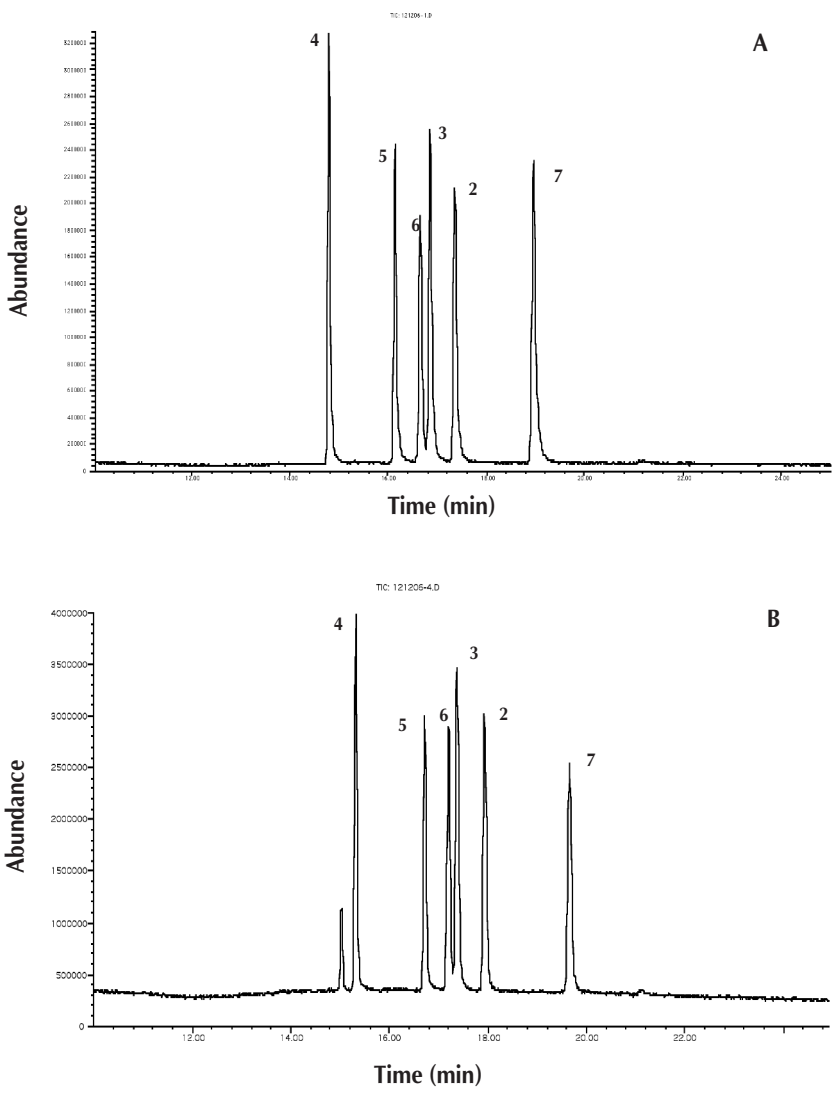


Figure 10. GC separation of the PFPA (A) and HFBA (B) derivatives of compounds 2–7. Rtx-200 column.

Conclusion

3,4-MDMA, 2,3-MDMA, and the five side-chain regioisomers of 4-methoxy-3-methyl phenethylamines are a unique subset of regioisomeric and isobaric molecules; each compound has a molecular weight of 193 and yields a base peak at *m/z* 58 in the mass spectrum from the loss of the corresponding methylenedioxybenzyl and 4-methoxy-3-methylbenzyl groups, respectively. Thus, the traditional electron impact mass spectrum provides little structural information for differentiating among these seven compounds. Because of the unique similarity of these compounds shown by MS, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate other regioisomeric and isobaric substances.

This elimination process could be accomplished on the basis of chromatography alone, but ultimately, it would require the analyst to use reference samples of the other substances. Derivatization of the primary and secondary amines with various acylating agents yields amides with improved resolution compared to the underivatized amines by capillary GC on Rtx-1 and Rtx-200 stationary phases. Additionally, the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for specific identification. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond, which yields characteristic hydrocarbon fragments at *m/z* 148, 162, and 176, as well as other unique fragments.

Acknowledgments

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