GC–MS Evaluation of a Series of Acylated Derivatives of 3,4-Methylenedioxymethamphetamine

Tarek Belal[1], Tamer Awad[2], C. Randall Clark[2,*], and **Jack DeRuiter[2]**

[1]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt; [2]Department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849

Abstract

A series of acylated derivatives of 3,4-methylenedioxymethamphetamine (3,4-MDMA) are prepared and evaluated in gas chromatography–mass spectrometry (GC–MS) studies. The perfluoroalkyl amides of 3,4-MDMA show the lowest GC retention, while the aromatic amides such as the benzamide show the greatest retention on the dimethylpolysiloxane stationary phase (Rtx-1). The mass spectral properties of the acetyl, propionyl, and butyryl derivatives all show a base peak at *m/z* 58 which is the base peak for the underivatized 3,4-MDMA. All acylated derivatives provide mass spectral information (*m/z* 162) to identify the three-carbon side chain for 3,4-MDMA. The perfluoroalkyl amides yield several unique mass spectral fragments for specific identification of 3,4-MDMA. MS fragmentation pathways are illustrated and validated using analogous deuterated derivatives. A combination of excellent chromatographic properties and unique mass spectral fragments allows the perfluoroalkyl amides to provide maximum specific structural information in the GC–MS analysis of 3,4-MDMA.

Introduction

The controlled substance 3,4-methylenedioxymethamphetamine (3,4-MDMA), also known as Ecstasy, belongs to the phenethylamine group of frequently abused drugs. 3,4-MDMA appears to act through the enhancement of serotonin-mediated neurotransmission, producing feelings of euphoria, energy, and desire to socialize (1). However, its abuse is not without risk and severe, even fatal, intoxications have been reported (2–4).

Determination and characterization of 3,4-MDMA in biological and forensic samples has been the focus of many studies over the past years (5–7). Gas chromatography–mass spectrometry (GC–MS) is the most widely used technique in the analysis of controlled substances in forensic laboratories (8–12). One problem encountered in these analyses is that there are a number of regioisomeric and isobaric phenethylamines related to 3,4-MDMA (8,10,11) that yield essentially identical mass spectral fragments in addition to equivalent molecular weights. Chemical derivatization methods (primarily acylation) have been used to address these analytical challenges. Derivatization can alter major fragmentation pathways, often providing additional structural information about an individual phenethlamine as well as altered chromatographic properties (9,12,13). Derivatization agents used for the acylation of 3,4-MDMA and related amphetamines include fluorinated agents such as trifluoroacetic anhydride (TFAA) (14), pentafluoropropionic anhydride (PFPA) (9–12,15), heptafluorobutyric anhydride (HFBA) (9–12,16), pentafluorobenzoyl chloride (17), and perfluorooctanoyl chloride (18); as well as unfluorinated ones like acetic anhydride (19) and propionic anhydride (20).

The aim of this work is to study and compare the mass spectral properties of 3,4-MDMA after derivatization with different reagents commonly used for amine acylation. The results of this study should allow for selection of those derivatives, providing maximum structural information for differentiation between 3,4-MDMA and regioisomeric or isobaric amines. The second task is to evaluate the retention properties of each acyl derivative under chromatographic conditions regularly used by forensic chemists. The derivatizing agents used in this study included TFAA, PFPA, HFBA, pentafluorobenzoyl chloride, acetic anhydride, propionic anhydride, butyric anhydride, and benzoyl chloride. Additionally, the formyl, d_3-acetyl, and d_5-benzoyl derivatives were prepared to confirm mass spectral fragmentation pathways.

Experimental

Instrumentation

Analytical studies were conducted using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans per second. The GC was operated in splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi.

The mass spectrometer was operated on the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230ºC. The GC injector was maintained at 250ºC and the transfer line at 280ºC. The temperature program used consisted of an initial temperature of 100ºC for 1 min, ramped up to 180ºC at a rate of 9ºC per min, followed by a hold at 180ºC for 2 min, then ramped to 200ºC at a rate of 10ºC per min and held at 200ºC.

* Author to whom correspondence should be addressed: email clarkcr@auburn.edu.

The mass spectra reported were obtained by background subtraction and are the average of at least five scans. Samples were diluted in HPLC-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and introduced via the auto injector as individual solutions and in physical mixture.

The chromatographic separations (and collection of retention data) were carried out on a 30 m × 0.25 mm-i.d. column coated with 0.25 µm 100% dimethyl polysiloxane (Rtx-1) purchased from Restek Corporation (Bellefonte, PA).

Drugs and reagents

All reagents and chemicals were purchased either from Aldrich Chemical Company, TCI, or Fisher Scientific. A sample of 3,4-MDMA was synthesized as described in previous publications from this laboratory (1,3) using piperonal as starting material. The N-formyl derivative was prepared by treating the free base of 3,4-MDMA with a 1:1 mixture of formic acid and acetic anhydride. The reaction mixture was quenched with ice water, acidified, and extracted to isolate the N-formyl product.

Derivatization procedure

Each 3,4-MDMA amide was prepared individually from the hydrochloride salt of 3,4-MDMA by dissolving approximately 0.3 mg (1.33×10^{-5} mole) of 3,4-MDMA hydrochloride in 50 µL of ethyl acetate followed by addition of a large excess (250 µL) of the appropriate derivatizing agent, and the derivatization reaction mixtures were incubated in capped tubes at 70ºC for 20 min. Following incubation, each sample was evaporated to dryness under a stream of air at 55°C and reconstituted with 200 µL of ethyl acetate and 50 µL of pyridine. A portion of each final solution (50 µL) was diluted with HPLC-grade acetonitrile (200 µL) to give the working solutions.

Results and Discussion

Mass spectrometry

Mass spectrometry is the principal method used for confirming the identity of drugs and related substances in forensic samples. Figure 1 shows the structure of 3,4-MDMA and the mass spectrum for the underivatized amine. The mass spectra for 3,4-MDMA and numerous regioisomeric molecules have been reported previously (8–12). The mass spectrum for 3,4-MDMA is presented here primarily for comparison with the various acylated derivatives reported in this study. The fragmentation of phenethylamines is generally characterized by a base peak from an amine initiated alpha-cleavage reaction breaking the carbon-carbon bond of the ethyl linkage between the aromatic ring and the nitrogen atom of the amine group. With regards to 3,4-MDMA, initial ionization of nitrogen followed by cleavage of the alpha bond yields the *m/z* 58 imine cation, the base peak in the 3,4-MDMA mass spectrum. Formation of the molecular ion via ionization of the aromatic pi-electrons, followed directly by alpha-cleavage, yields the benzylic cation at *m/z* 135 and hydrogen rearrangement from the equivalent molecular ion followed by alpha-cleavage initiated at the distant radical site yields the radical cation at *m/z* 136 in Figure 1. (8,9). The structures for these fragment ions are shown in Figure 2.

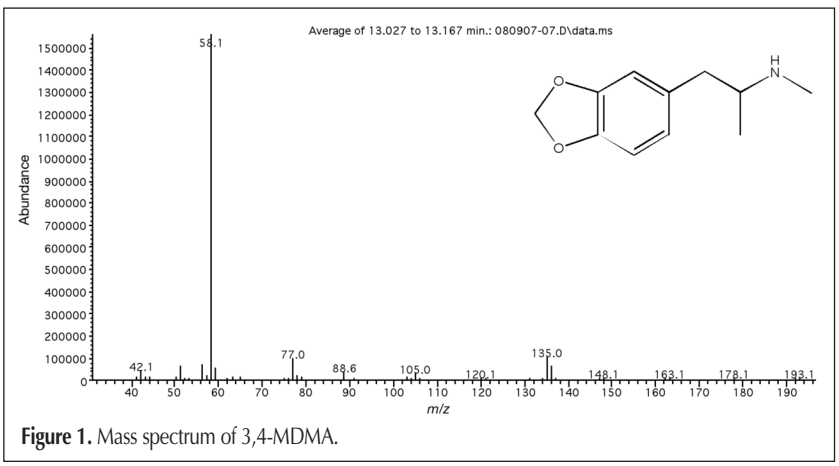

Figure 1. Mass spectrum of 3,4-MDMA.

The initial group of derivatives evaluated in this study were the simple alkyl amides of 3,4-MDMA obtained by derivatization with acetic, propionic, and butyric anhydrides. Acylation of the amine results in significantly lower nitrogen basicity, and this often results in the formation of unique and characteristic fragment ions (9,11,12). Figure 3 (A, B, and C) shows the mass spectra for the homologous acetyl, propionyl, and butyryl amides. These three spectra show molecular ions of low relative abundance at *m/z* 235, 249, and 263, respectively. Additionally, a second homologous series of fragment ions $(M-135)^+$ occurs at *m/z* 100, 114, and 128, suggestive of the acylated imine fragment for each of the amides. The mass spectrum in Figure 3D provides evidence to support these structural assignments and shows the equivalent ion at *m/z* 103 for the d_3-acetamide. The structures for the acylated imine fragments included in this study are shown in Figure 4.

The spectra in Figures 3A, 3B, and 3C show two prominent ions occurring at the same mass for all three derivatives. The base peak for these derivatives is the *m/z* 58 ion, and this ion is equivalent to the base peak in the underivatized amine whose mass spectrum is shown in Figure 1. The *m/z* 58 ion in the spectrum of these derivatives is likely the result of hydrogen rearrangement from the alkyl group of the acyl imine fragments at *m/z* 100, 114, and 128 to yield the common imine fragment at *m/z* 58. This fragmentation pathway is illustrated in Figure 5. Furthermore, the mass spectrum in Figure 3D for the d_3-acetamide shows the deuterated imine species at *m/z* 59 providing support for the structural assignment and fragmentation pathway.

The formamide (N-formyl) and benzamide (N-benzoyl) derivatives were prepared and evaluated to determine the scope of the hydrogen rearrangement in the further decomposition of the acyl imine species. As shown in Figure 6A, the hydrogen of the formyl group does transfer in significant abundance; however, the resulting *m/z* 58 fragment is not the base peak for this compound. The decreased likelihood of hydrogen transfer through the 3-membered ring transition leaves the formyl imine species (*m/z* 86) as the more prominent ion in its mass spectrum. The benzamide in Figure 6B does not show any indication of a *m/z* 58 ion, demonstrating that the aromatic hydrogens do not migrate in the same

manner as the hydrogen of the alkyl groups in the previously described spectra.

The other common fragment seen in all the mass spectra in Figure 3 and those in Figures 6A and 6B is the *m/z* 162 ion. This fragment ion is the 3,4-methylenedioxyphenylpropene radical cation occurring through hydrogen transfer from the propyl group to the carbonyl oxygen followed by loss of the neutral amide species. This mechanism for formation of the *m/z* 162 radical cation is shown in Figure 7. An interesting uniqueness was observed in the mass spectrum of the benzamide derivative in Figure 6B. Two separate fragmentation pathways can yield ions at *m/z* 162, the 3,4-methylenedioxyphenylpropene radical cation and the benzoylimine cation. Figure 6C shows confirmation of this point with the mass spectrum for the d_5-benzamide. The d_5-benzoylimine is shifted to *m/z* 167 as expected, while the 3,4-methylenedioxyphenylpropene fragment remains at *m/z* 162. Additionally, the benzoyl fragment ($C_6H_5CO^+$) at *m/z* 105 in Figure 6B is shifted to *m/z* 110 for the d_5-benzoyl derivative.

The mass spectra for all the derivatives described to this point have some disadvantages for the identification of amines such as 3,4-MDMA. The derivatives formed from alkanoic carboxylic acid anhydrides (acetic, propionic, and butyric) fragment to yield base peaks at *m/z* 58 equivalent to the underivatized amines. Thus without the aid of chromatographic separation, the completeness of the derivatization process itself could be questioned. The loss of significant ion current as *m/z* 58 is a drawback to the use of these derivatives for forensic analysis and the only unique major ion for identification of 3,4-MDMA is the *m/z* 162 ion and this ion appears regardless of the acylating species. The benzamide derivative yields significant ion current at unique masses such as *m/z* 105 and 162. However, the benzamide derivative of 3,4-MDMA was observed to have quite high GC retention properties, as will be described later in this study. These relatively high retention properties may limit the utility of this derivative in some applications.

The mass spectra for a series of perfluoroacyl derivatives of 3,4-MDMA are shown in Figure 8. One of the major features of these spectra is the absence of *m/z* 58 ion. This ion does not occur because no hydrogen is available for migration from the

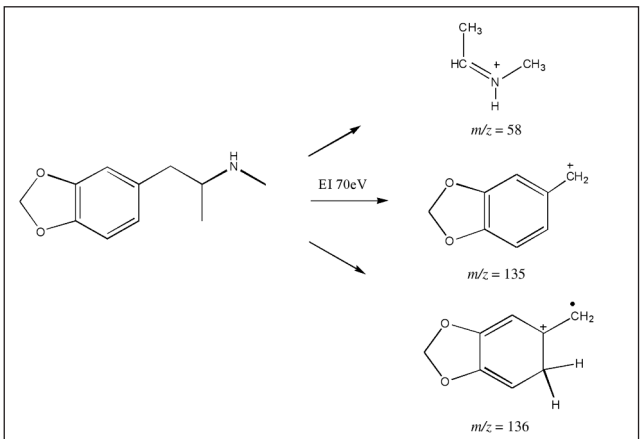

Figure 2. EI fragmentation pattern for 3,4-MDMA.

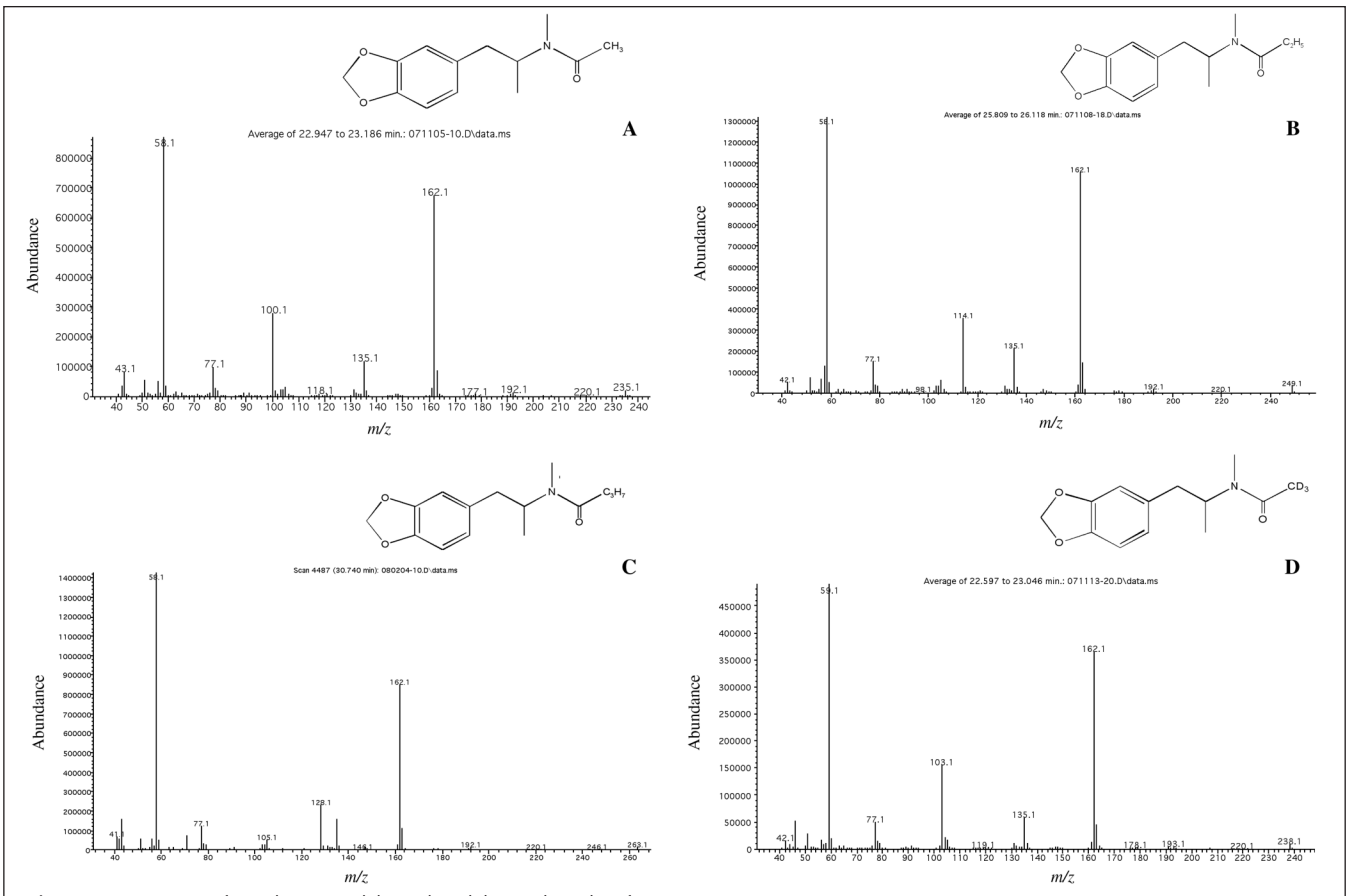

Figure 3. Mass spectra of acetyl, propionyl, butyryl, and d_3-acetyl amides of 3,4-MDMA.

acyl portion of these derivatives. The spectra in Figures 8A, 8B, 8C, and 8D further show common ions at *m/z* 135 and *m/z* 162. These ions are the 3,4-methylenedioxybenzyl cation and the 3,4-methylenedioxyphenylpropene radical cation, respectively. The formation of these ions occurs in an analogous manner to that described earlier in this report. The PFPA and HFBA derivatives of deuterated 3,4-MDMA have been used to confirm the proposed structures (9). The three perfluoroalkyl derivatives, trifluoroacetyl, pentafluoropropionyl, and heptafluorobutyryl amides show several ions in a homologous series separated by 50 mass units (CF_2). The *m/z* 154, *m/z* 204, and *m/z* 254 ions in these three spectra represent the perfluoroacylimine species (see Figure 4). These ions are the base peaks in the three spectra and without hydrogen atom to migrate from the acyl group, no *m/z* 58 decomposition ion is formed. Additionally, these spectra do not show any evidence of analogous fluorine transfer decomposition products.

The second homologous series of ions in Figures 8A, 8B, and 8C occur at *m/z* 110, 160, and 210, respectively. This series of ions differing by 50 mass ions (CF_2) indicates that the perfluoro fragment is a component of these ions. Previous studies (9) using d_3-3,4-MDMA in which the three deuterium atoms are bonded to the N-methyl group (N-CD_3) have shown that all three deuterium labels are contained in the *m/z* 160 ion for the PFPA derivative. This leaves only 26 mass units (CN) to account for all the mass of the *m/z* 160 ion. The mechanism to account for these ions is shown in Figure 9. Based on our studies (9,11) of numerous phenethylamines, this mechanism appears to be specific for the perfluoroacyl derivatives of N-methyl substituted compounds. While this project did not examine any perfluoroacyl derivatives in this homologous series beyond the heptafluorobutyramide, the perfluorooctanoyl (PFO) derivatives of 3,4-MDMA and methamphetamine have been reported (18). These derivatives show a major fragment ion at *m/z* 410 which extends this series of rearrangement ions (see Figure 9) to higher chain homologues. The additional four CF_2 groups in the PFO derivative beyond the HFBA derivative account for the 200 additional mass units. The work of Westphal et al. (18) further described the PFO derivative of d_5-MDMA and clearly showed that only three deuterium labels were incorporated into the analogous fragment ion at *m/z* 413. The base peak in the mass spectrum of the PFO derivative of 3,4-MDMA occurs at *m/z* 454

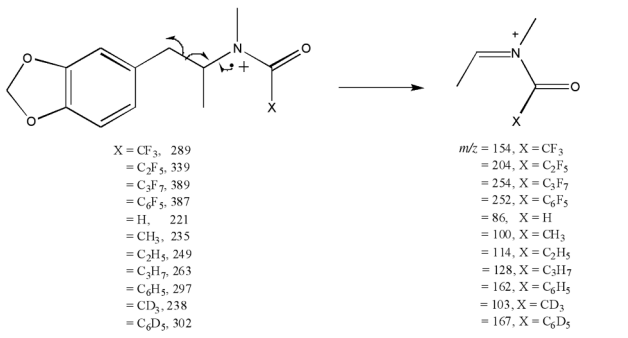

Figure 4. Imine fragment pattern of acylated derivatives of 3,4-MDMA.

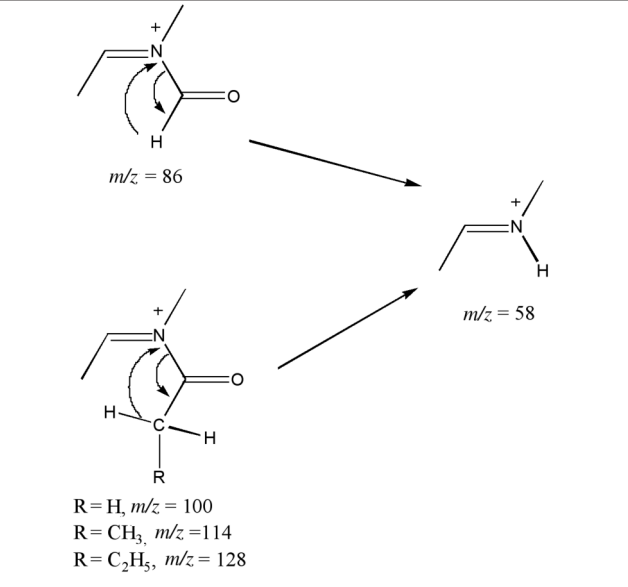

Figure 5. Formation of *m/z* 58 from the N-formyl, acetyl, propionyl, and butyryl imine fragments.

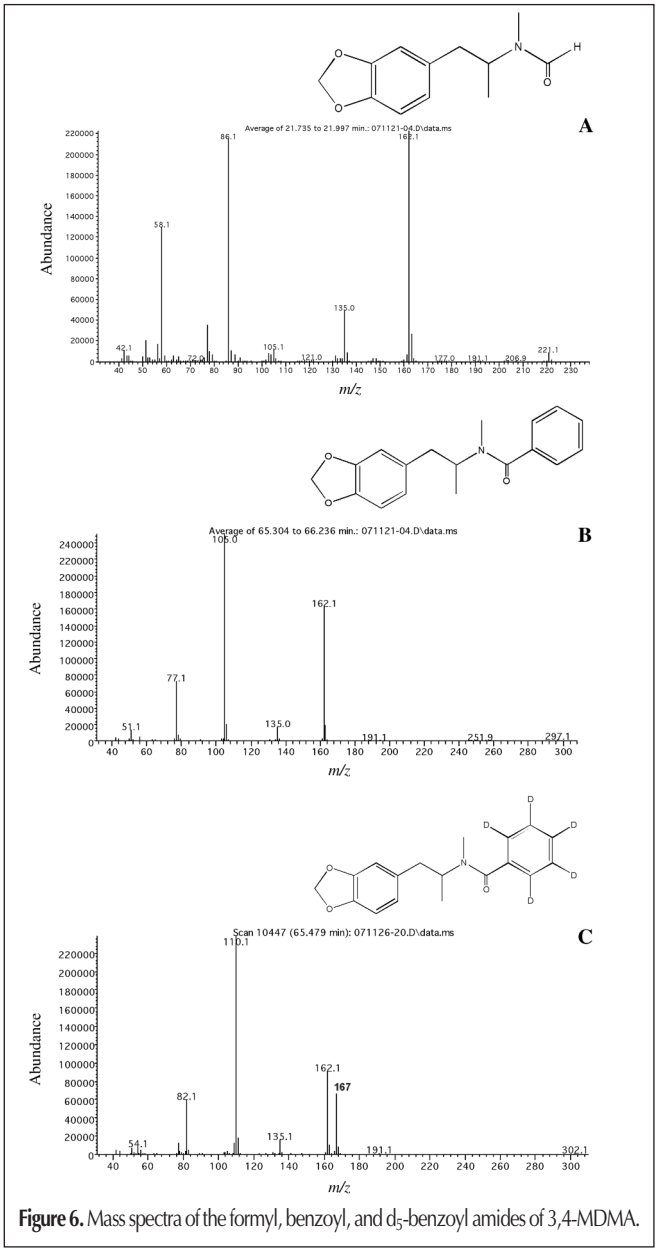

Figure 6. Mass spectra of the formyl, benzoyl, and d_5-benzoyl amides of 3,4-MDMA.

which is the perfluoroacylimine species and analogous to the base peaks in Figures 8A, 8B, and 8C. Thus these data are consistent with the experimental observations reported in this study.

The remaining spectrum in Figure 8D is for the pentafluorobenzamide and the base peak at *m/z* 195 is the pentafluorobenzoyl cation. This ion is analogous to the *m/z* 105 and *m/z* 110 ions for the benzoyl and d_5-benzoyl cations, respectively (see Figure 10). These ions would be major fragments in the benzamide of most amines and thus do not provide ions characteristic of 3,4-MDMA.

The data generated in this study should allow the analyst to select a derivatization reagent to provide maximum structural information for 3,4-MDMA and related amines. However, other issues of product stability and reagent reactivity not addressed in this study may be important criteria for derivative selection in other applications.

Gas chromatography

The chromatogram in Figure 11 provides a comparison of the acyl group structure and relative GC retention properties on a nonpolar stationary phase, 100% dimethylpolysiloxane, Rtx-1. Several temperature programs were evaluated in this project, and one program showing the best compromise between resolution and analysis time was used to generate the chromatogram in Figure 11.

The perfluoroalkylamides are the first group of derivatives to elute on the dimethylpolysiloxane stationary phase. These amides elute much earlier than their corresponding non-fluorinated hydrocarbon counterparts. Both the fluorinated and non-fluorinated alkyl derivatives elute before the aromatic amides. In every case of direct comparison, the perfluoro species eluted much earlier than its non-fluorinated hydrocarbon counterpart. The most obvious example of this effect can be seen for the benzamides in the last two peaks in the chromatogram (Figure 11). The high relative retention for the benzamides coupled with the lack of major fragment ions could limit their value in 3,4-MDMA analysis and identification. The alkyl hydrocarbon derivatives provide significantly increased chromatographic retention yet show the *m/z* 58 ion as the base peak as does the underivatized amine, 3,4-MDMA. Thus, the perfluoroalkyl derivatives offer excellent chromatographic properties as well as a significant number of characteristic fragment ions for 3,4-MDMA identification.

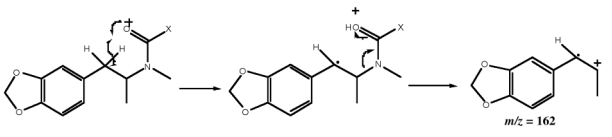

Figure 7. Formation of *m/z* 162 in all amide derivatives of 3,4-MDMA.

Conclusions

The perfluoroacyl derivatives of 3,4-MDMA show excellent chromatographic properties and unique mass spectral fragment ions. The perfluoroalkyl amides of TFAA, PFPA, and HFBA yield a unique series of mass spectral fragments at *m/z* 110, 160, and

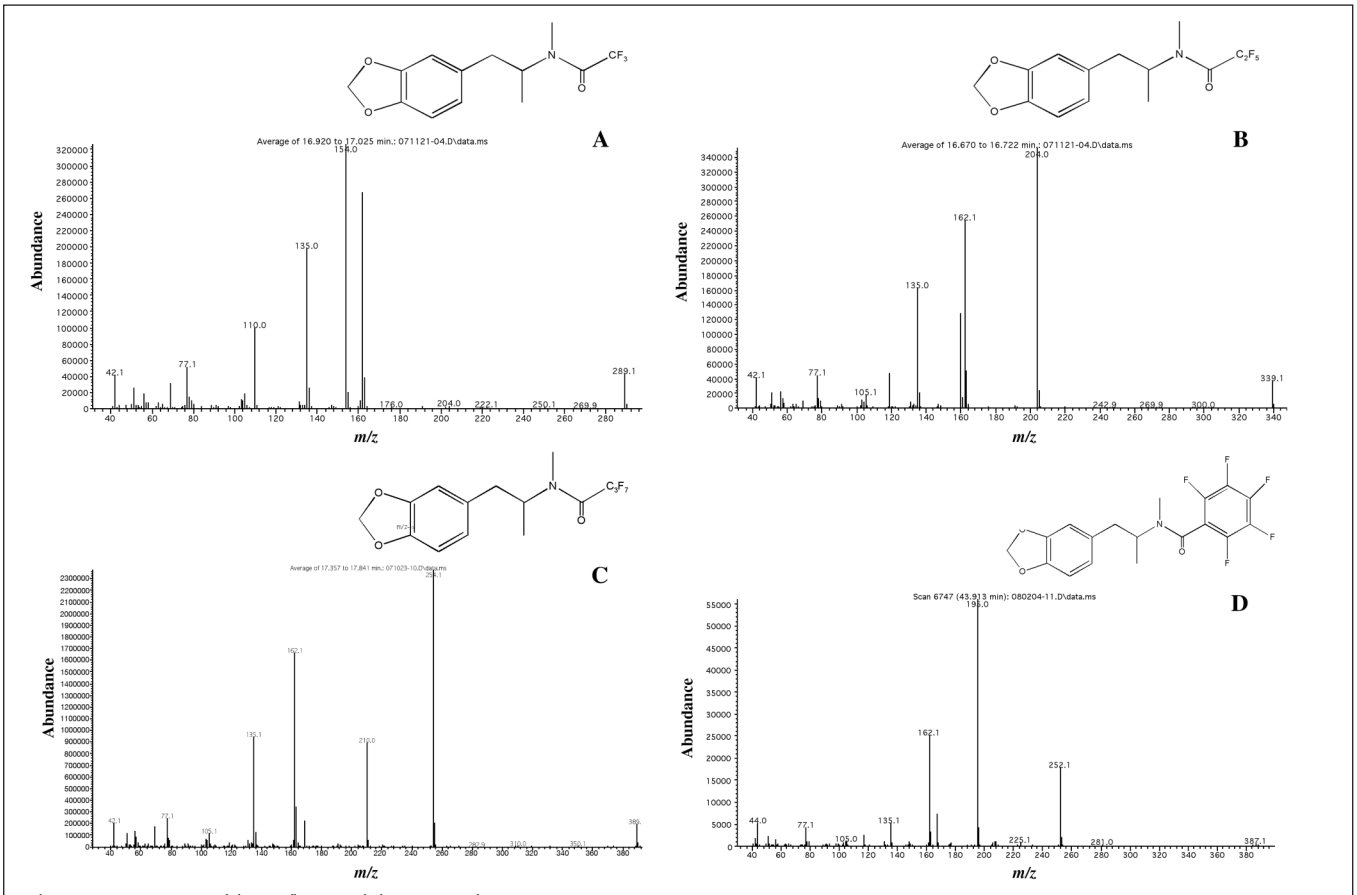

Figure 8. Mass spectra of the perfluoroacyl derivatives of 3,4-MDMA.

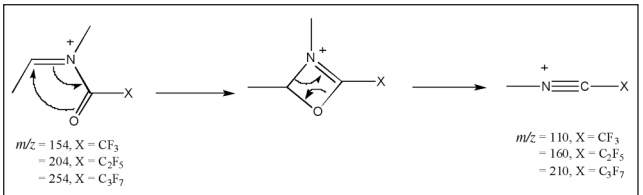

Figure 9. Formation of *m/z* 110, 160, and 210 in the spectra of the TFAA, PFPA and HFBA derivatives of 3,4-MDMA.

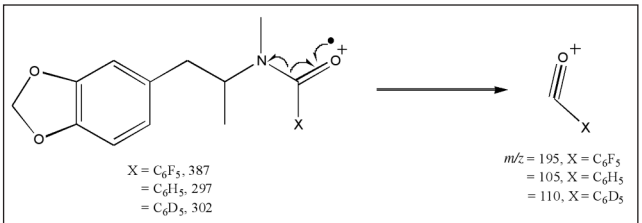

Figure 10. Formation of *m/z* 195, 105, and 110 from the pentafluorobenzoyl, benzoyl, and d_5-benzoyl derivatives of 3,4-MDMA.

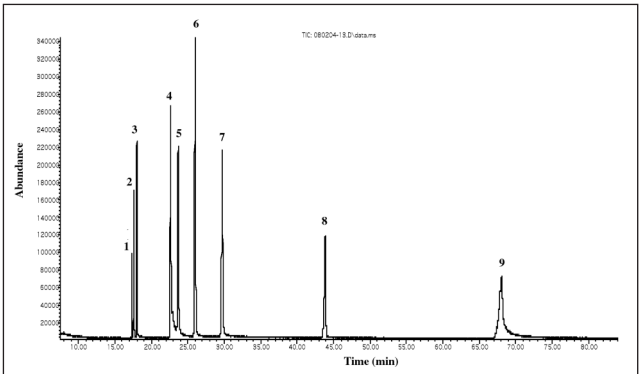

Figure 11. Gas chromatographic separation of 3,4-MDMA derivatives. Peaks: 1, PFPA; 2, TFAA; 3, HFBA; 4, N-formyl; 5, acetyl; 6, propionyl; 7, butyryl; 8, pentaflurobenzoyl; and 9, benzoyl amides.

210, respectively. Additionally, the base peaks in these mass spectra occur at relatively higher masses at *m/z* 154, 204, and 254, respectively. These ions are the perfluoroacylimines resulting from loss of 135 mass units (3,4-methylenedioxybenzyl) from the molecular ion. This $(M-135)^+$ species also occurs for the acetyl, propionyl, and butyryl amides however these ions rearrange through loss of the acyl group to yield a common ion at *m/z* 58. Thus, the base peak for the non-fluorinated hydrocarbon derivatives is the same as that observed for the underivatized 3,4-MDMA, *m/z* 58. The formyl, d_3-acetyl, d_5-benzoyl, and pentafluorobenzoyl derivatives provided chromatographic and confirmatory mass spectral evidence for fragmentation pathways.

Acknowledgment

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