Chromatographic and Mass Spectral Studies on Isobaric and Isomeric Substances Related to 3,4-Methylenedioxymethamphetamine

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Abstract

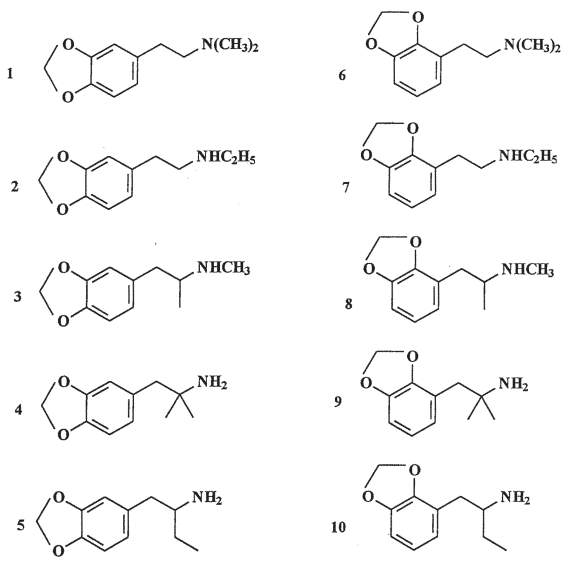
A series of isobaric and isomeric molecules related to 3,4-methylenedioxymethamphetamine (3,4-MDMA) are prepared and evaluated as potential mass spectral equivalents to this controlled substance. These compounds have the potential to produce a mass spectrum equivalent to 3,4-MDMA, thus making mass spectrometry a nonconclusive method for confirming the identity of any one of the substances. The various isomeric forms of the methoxymethylphenethylamines and the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA, but the ethoxy substituted phenethylamines show a unique fragment at *m/z* 107. Gas chromatographic separation on nonpolar stationary phases successfully resolved these compounds from 3,4-MDMA, however only a limited set of side chain regioisomers and ring substitution patterns are evaluated in this initial study.

Introduction

Previous studies (1,2) in this series have shown that the 10 direct regioisomeric substances, 3,4-methylenedioxymethamphetamine (MDMA) and its nine regioisomeric equivalents, have mass spectral fragments of equivalent mass and identical molecular weights (Table I). Therefore, direct analysis 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 Ecstacy (3,4-MDMA)] to the exclusion of all the other isomers. All 10 compounds of molecular weight 193 showed major fragment ions for the imine at *m/z* 58 and the benzyl fragment at *m/z* 135/136. 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, compounds 3 and 7 in Table I—were shown to coelute (1) under common conditions used to identify compound 3 (3,4-MDMA) in forensic drug samples. Recent reports (2) have shown that all 10 compounds can be resolved using the more polar GC stationary phases.

This report describes the initial evaluation of a variety of isobaric substances that have the potential to yield mass spectra equivalent to that of the drug of abuse, 3,4-MDMA. Isobaric substances are defined (3) as compounds of the same nominal mass but of different elemental composition. For example, the methoxy-methyl disubstitution pattern (mass 46, C_2H_6O) is isobaric with methylenedioxy (mass 46, CH_2O_2) disubstitution on the aromatic ring. Therefore, other ring substitution patterns have the potential to produce mass spectra with fragments of equivalent mass to those of 3,4-MDMA. Monosubstitution of the aromatic ring of methamphetamine with mass 45, such as the

Table I. Structures of the 10 Direct Regioisomeric Substances of Molecular Weight 193 and Major Fragments at *m/z* 58 and 135/136*



* The controlled drug substance 3,4-MDMA is compound 3.

ethoxy-group, yields an amine of isobaric equivalence to that of the methylenedioxy-group. The present work focuses on preparation and analytical evaluation of representative molecules from various isobaric structural categories using currently available precursor substances. The goal of this work is to determine whether misidentification as MDMA is possible for any of these types of compounds. Additionally, chromatographic evaluation of these compounds establishes whether any of these example substances have similar retention properties to the target drug of abuse, MDMA. The results of these studies will be used to guide future research in this area.

Experimental

Instrumentation

GC–MS analyses were performed with an HP 6890 GC coupled with an HP 5973 mass selective detector (Hewlett-Packard, Little Falls, DE). The MS was operated in the electron impact mode utilizing an ionization voltage of 70 eV and a source temperature of 230°C. The samples were dissolved in pH 8.9 Trizma base buffer (1 mg/mL), extracted with iso-octane (1 mL), and introduced (1.0 µL) into the MS via the GC, which was equipped with an HP 7673 automatic injector. The separation was carried out on a 25-m × 0.20-mm i.d., coated with 0.11 µm of 5% phenyl methyl silicone (Ultra-2) purchased from Hewlett-Packard. The injection was carried out using splitless mode with an injector temperature at 250°C in which the split purge valve opened after 1 min. The injection volume was 1 µL/column. The temperature program started with 1 min isothermal hold at 60°C, followed by a linear ramp (8°C/min) to 180°C and then to the final temperature of 300°C at a rate of 30°C/min with a hold time of 10 min. The helium carrier gas was adjusted to 25 cm/s at 60°C in the constant flow mode.

GC analyses were performed with an HP 6890 GC equipped with split/splitless inlet, HP 7683 automatic injector, and flame ionization detector (Agilent Technology, Little Falls, DE). ChemStation software (Rev. A.08.03) was used for data acquisition and processing. Carrier gas (hydrogen) was adjusted at 60°C to give the optimum average velocity (50 cm/s). Inlet pressure was converted according to the constant flow mode, and the total flow was 60 mL/min. The injection was in the split mode with an injector temperature at 260°C.

The temperature program optimization was carried out using DryLab 2000 chromatography optimization software, version 3.00.06 (LC Resources, Walnut Creek, CA).

Capillary columns

The conventional columns used were an HP Ultra 1 (25 m × 0.2 mm, 0.33 µm), HP Ultra 2 (25 m × 0.2 mm, 0.33 µm), DB-35MS (25 m × 0.2 mm, 0.33 µm), HP-50+ (25 m × 0.2 mm, 0.33 µm), DB-17MS (30 m × 0.25 mm, 0.25 µm), and HP-1701 (25 m × 0.2 mm, 0.20 µm).

Narrow bore columns

The narrow bore columns used were an HP-1 (10 m × 0.1 mm, 0.1 µm), HP-5 (10 m × 0.1 mm, 0.17 µm), DB-17 (10 m × 0.1 mm, 0.2 µm), and SPB-50 (10 m × 0.1 mm, 0.17 µm).

Synthetic procedures

Compounds 11–14

General procedures for the synthesis of nitroalkenes from available substituted benzaldehydes, lithium aluminum hydride reductions, and reductive aminations with methylamine and sodium borohydride have been reported previously (1,4,5). These methods were used to prepare compounds 11–14.

Compound 15

Procedures for the preparation of 2,2-dimethylphenethylamines, such as compound 15, using base catalyzed addition of isobutyric acid to 3,4-methylenedioxybenzyl chloride have been described previously (4).

Compounds 16–18

Compounds 16–18 were prepared by the same general procedure using the appropriately substituted methoxyphenylacetone. Dry tetrahydrofuran (THF) was added dropwise to 60% sodium hydride, followed by dropwise addition of the methoxyphenylacetone and methyliodine in THF. The reaction mixture was refluxed for 24 h and quenched with aqueous THF. The solvent was evaporated to yield a yellow oil 1-(methoxyphenyl)-1-methyl-2-propanone purified by vacuum distillation. The ketones were converted to the desired amines via sodium cyanoborohydride reductive amination.

Compound 19

4-Methoxypropiophenone was dissolved in carbon tetrachloride along with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide. The mixture was refluxed, filtered, and the solvent evaporated under reduced pressure yielding 4-methoxy-2-bromo-propiophenone.

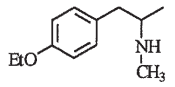
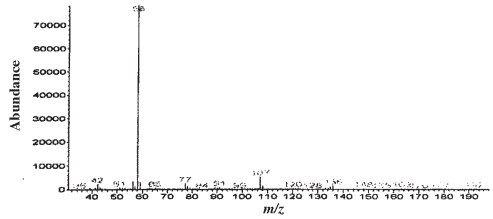
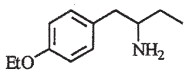
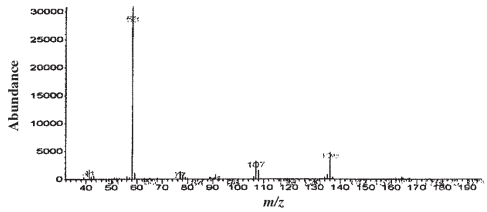
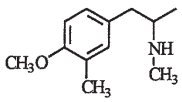
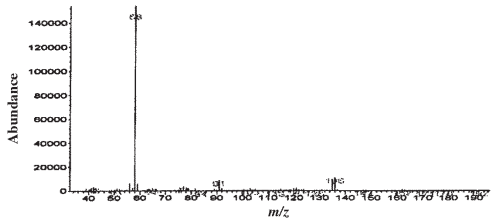
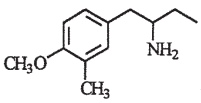
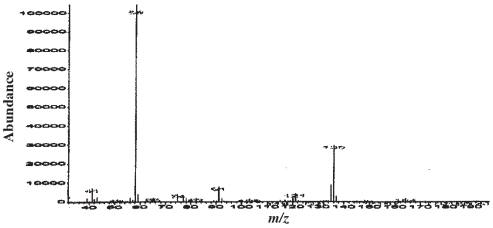
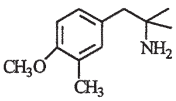
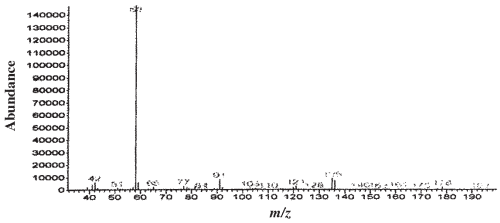
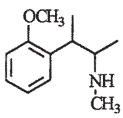
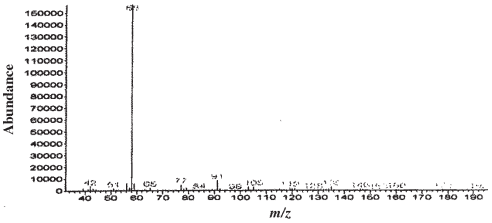
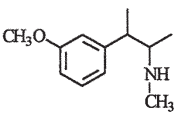
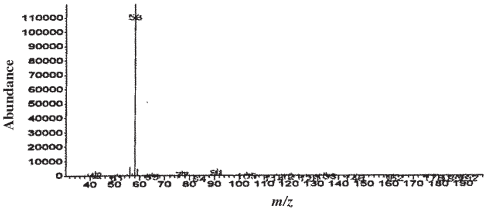
The 4-methoxy-2-bromo-propiophenone in acetonitrile was added dropwise to a mixture of methylamine hydrochloride and sodium bicarbonate in acetonitrile. The mixture was stirred at room temperature, filtered, and the solvent evaporated under reduced pressure. Water was added and acidified with concentrated hydrochloric acid and washed with methylene chloride. The water layer was alkalinized with sodium hydroxide pellets and extracted with methylene chloride. Evaporation of the solvent gave a light yellow oil, which was converted to the hydrochloride salt.

Results and Discussion

Synthesis

There are other substances that have the potential to yield the same mass spectrum as the drug of abuse (3,4-MDMA) but that do not contain the methylenedioxy substitution pattern in the aromatic ring (e.g., methoxy, methyl-substitution in the aromatic ring is isobaric with methylenedioxy and may produce essentially the same mass spectrum). Numerous alternatives of mass spectral equivalence to MDMA are possible, and some of the more likely ones (based on currently available precursor chemicals) are evaluated in this paper. The specific compounds evaluated in this report are shown in Table II along with their mass spectra. The

Table II. Structures and Mass Spectra of Ring and Side-Chain Regioisomers of MDMA

Structure of regioisomer	Mass spectrum
 11	
 12	
 13	
 14	
 15	
 16	
 17	

compounds in Table II are numbered 11–19 for clarity and consistence with previous reports in this series (1,2).

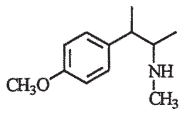
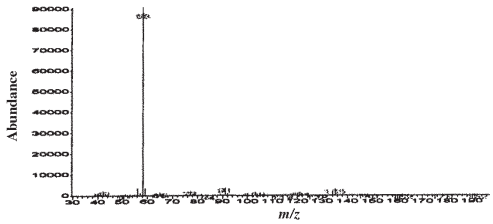
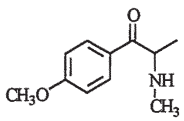
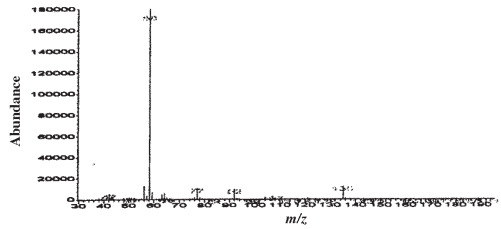
The methoxymethyl-disubstituted aromatic ring and the monosubstituted ethoxy-group have the same mass as the methylenedioxy-group, and therefore the potential to produce a similar mass spectral fragmentation pattern as the drugs of abuse 3,4-MDMA or its side chain regioisomer, 3,4-methylenedioxphenyl-2-butanamine (BDB) (compounds 3 and 5 in Table I). The MDMA- and BDB-type side chain, *N*-methyl-1-aryl-2-propanamine and 1-aryl-2-butanamine, respectively, were prepared from the substituted benzaldehydes according to previously described methods (2,4,5). The *N*-methyl-1-aryl-2-propanamines [compounds 11 and 13 (MDMA side chain)] were prepared from the corresponding ketones by reductive amination with methylamine and sodium cyanoborohydride. The 1-aryl-2-butanamines, compounds 12 and 14 (BDB-type side chain) regioisomers were synthesized using the nitrostyrene method to prepare 1-aryl-2-nitrobutenes, which were then reduced with lithium aluminum hydride to yield the desired primary amines.

The 1-aryl-2,2-dimethylphenethylamine derivative was prepared according to the procedure outlined in Figure 1. A sample of 3-methyl-*p*-anisaldehyde was reduced with sodium borohydride to the corresponding alcohol, which was then converting to the corresponding chloride. Treatment with the dianion of isobutyric acid yielded 2,2-dimethyl-1-(3-methyl-4-methoxyphenyl) propionic acid. Reaction with ethyl chloroformate and sodium azide followed by Curtius rearrangement (6) gave the isocyanate derivative, which was allowed to react with benzyl alcohol to form the benzyloxycarbonyl derivative. The last step in the reaction sequence was hydrogenolysis of the benzyloxycarbonyl derivative to the desired 2,2-dimethyl-1-(3-methyl-4-methoxyphenyl)-2-ethanamine.

A combination of aromatic-ring and side-chain modifications yields regioisomeric methoxymethcathinones such as *p*-methoxymethcathinone, which have the potential to produce the same mass spectrum as MDMA. Although not a direct regioisomer of MDMA, the methoxybenzoyl fragment ($C_8H_7O_2$) is equivalent to the methylenedioxybenzyl fragment ($C_8H_7O_2$). The synthesis of *p*-methoxymethcathinone was carried out using *p*-methoxypropiophenone as a starting material. Bromination of the α-position with *N*-bromosuccinimide followed by bromine displacement with methylamine gave *p*-methoxymethcathinone (Figure 2).

In the case of the methoxy-methyl disubstitu-

Table II. (continued) Structures and Mass Spectra of Ring and Side-Chain Regioisomers of MDMA

Structure of regioisomer	Mass spectrum
 **18**	
 **19**	

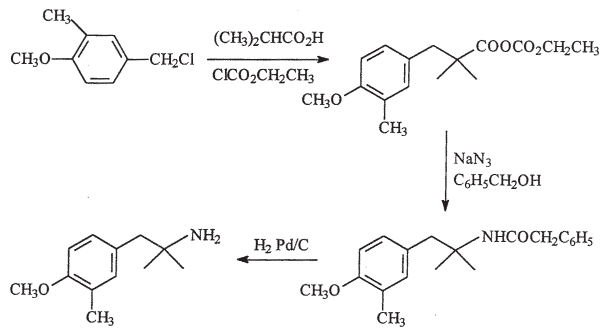


Figure 1. Synthesis of 2,2-dimethyl-1-(3-methyl-4-methoxyphenyl)-2-ethanamine.

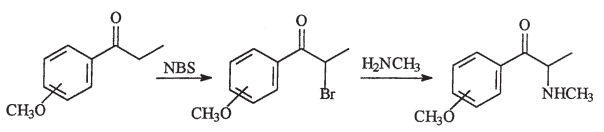


Figure 2. Synthesis of methoxymethcathinones.

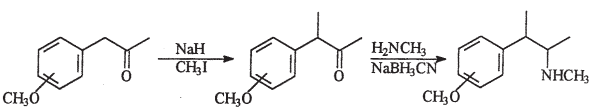


Figure 3. Preparation of *N*-methyl-1-(methoxyphenyl)-1-methyl-2-propanamines.

tion pattern the methyl substituent at the benzylic carbon will likely yield a mass spectrum equivalent to that of 3,4-MDMA. Figure 3 shows commercially available methoxyphenylacetones were used as a starting material, and the methylation of the side chain was achieved with methyliodine in the presence of sodium hydride. The resulting ketones were converted to the desired *N*-methyl amines via reductive amination using sodium cyanoborohydride.

Mass spectra

Table II shows that the ring and side-chain regioisomers produce very similar mass spectra, and these mass spectra are essentially equivalent to those for the methylenedioxy substituted regioisomers (1). The mass spectral fragmentation of the ring and side-chain regioisomers examined in this study (compounds 11–19) showed the same major fragments at mass 58 and 135/136 as the methylenedioxy substituted isomers (compounds 1–10). The molecular ion at *m/z* 193 is either absent or weak, and there are few specific ions of significant relative intensity that could be useful for the specific identification or differentiation of these compounds from their methylenedioxy-substituted substances. Previous studies (1,2) have shown that the mass spectra for compounds 1–10 are essentially equivalent, and differentiation must depend heavily on chromatographic resolution of these regioisomeric substances.

The mass spectra for the *p*-ethoxy regioisomers (compounds 11 and 12) show a specific ion at *m/z* 107 ($HOC_6H_4CH_2^+$), which results from elimination of ethane from the *p*-ethoxy-benzyl cation at *m/z* 135. This characteristic fragment should allow ethoxy substituted phenethylamines to be differentiated from the methylenedioxy substituted amines based solely on mass spectral data.

The mass spectra of 4-methoxy-3-methylphenyl regioisomers (compounds 13–15) do not show any unique fragment ions, and they are therefore not easily differentiated from their methylenedioxyphenyl regioisomers. The major difference among these three 4-methoxy-3-methylphenyl regioisomers is the greater relative abundance of the radical cation at *m/z* 136 for the butanamine (compound 14). However, this is a fairly common observation for primary amines of other regioisomeric phenethylamines (7).

The methoxyphenyl-1-methylpropanamines (compounds 16–18) also show very similar mass spectra compared with the other regioisomers, and the position of methoxy substitution does not yield any characteristic ions useful for differentiation. Additionally, 4-methoxymethcathinone (compound 19) produces a mass spectrum, which is virtually identical to that of 3,4-MDMA and the other direct regioisomers in Table I. These preliminary studies show that only the ethoxy substitution pattern in the phenethylamines gives characteristic fragment ions to allow differentiation from the MDMA-type molecules based on mass spectra alone.

Chromatography

The mass spectral studies showed that most of these representative MDMA isobars (compounds 11–19) have fragmentation equivalence to compounds 1–10. Preliminary GC studies on compound 3 (3,4-MDMA) and compounds 11–19 were performed with various stationary phases, and the obtained data were used in resolution modeling. In this limited set of compounds, complete separation from compound 3 (MDMA) was obtained on nonpolar stationary phases.

The nonpolar columns used in this study (Ultra 1 and 2) showed similar resolution maps and both columns separated MDMA (compound 3) from its ring and side-chain regioisomers (compounds 11–19) with low temperature program rates. Baseline separation can be obtained with a temperature programming rate of 7.3°C/min (the results are shown in Figure 4). The elution properties of 3,4-MDMA differ from regioisomers 11–19 and, thus, misidentification caused by coelution is unlikely in this limited set of isomers as long as these reference materials are available for the analysts. However, it must be emphasized that the goals of this initial work did not require a complete set of side-chain regioisomers for each of the aromatic ring substitution patterns having equivalent mass to the methylenedioxybenzyl fragment of *m/z* 135. A complete set of the ring and side-chain regioisomers of the methoxymethylphenethylamines will likely produce several compounds similar in retention properties to compound 3, MDMA. When more polar columns (DB-35MS and HP50+) were applied for the separation, 3,4-MDMA coeluted with either compound 12 or 14. These polar phases provided maximum chromatographic separation for the direct regioisomeric compounds related to 3,4-MDMA, compounds 1–10 (2).

When the same stationary phase—but narrow-bore column (HP-5)—and segmented temperature ramps were used, the last eluting compound (19) had a retention time of 12.60 min. The analysis time was decreased by optimization of the temperature program. Figure 5 shows the segmented temperature ramp used to generate the chromatogram in Figure 6. All 10 regioisomers (compounds 3, 11–19) in Figure 6 eluted inside of 1.5 min window, and the last compound eluted at 6.07 min. The elution order for side-chain regioisomers having a constant ring substitution seems to follow the same order observed for the methylenedioxy-regioisomers (1,2). The first compounds to elute were the compounds that had the greatest degree of side-chain branching, (2,2-dimethyl), followed by *N*-methyl substituted (MDMA-type) and the butanamines (BDB-type), which displayed the greatest retention. These experimental results and those obtained in previous reports will guide additional future experiments.

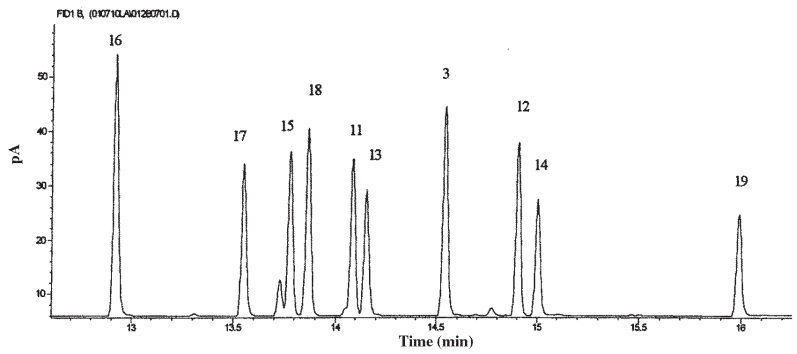


Figure 4. Experimentally obtained separation of compounds 3 and 11–19 on Ultra 2 (25 m × 0.2 mm, 0.33 µm) with the temperature program rate of 7.3°C/min.

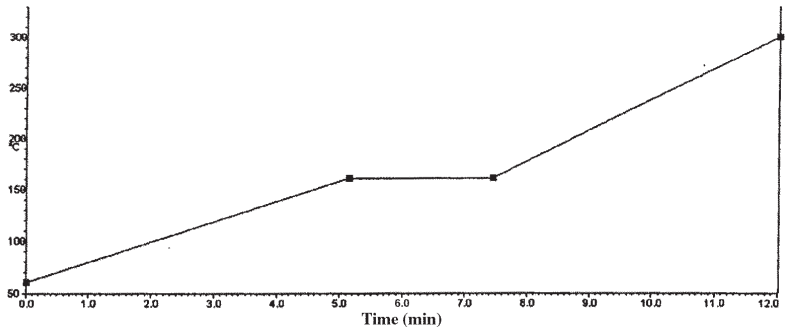


Figure 5. The segmented temperature ramp used in the separation of compounds 3, 11–19 in Figure 6.

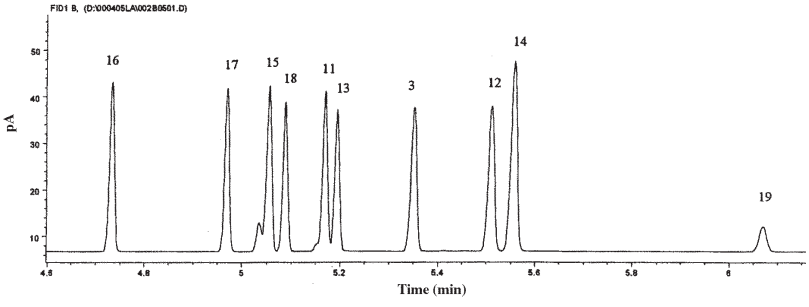


Figure 6. The GC chromatogram obtained with the HP-5 column and the segmented temperature ramp shown in Figure 5.

Conclusion

In summary, the limited set of isobaric and isomeric compounds examined in this study indicates that MS has limited utility in confirming the identity of the drug of abuse 3,4-MDMA to the exclusion of many of its mass spectral equivalents. The ethoxy-substituted phenethylamines show a unique fragment at *m/z* 107; however, the various isomeric forms of the methoxymethylphenethylamines and the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA. GC separation on nonpolar stationary phases successfully resolved the regioisomeric and isobaric compounds from 3,4-MDMA in this limited series.

Acknowledgments

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