GC-IRD Analysis of Regioisomeric Substituted Phenethylamines of Mass Spectral Equivalence

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The value of gas chromatography with vapor phase infrared spectrophotometric detection is described for a series of isomeric phenethylamines. The major mass spectral fragments for these unique isomers occur at equivalent mass and members of each series have equal molecular weights. The infrared spectra for these compounds allow for identification of any one of these amines to the exclusion of all other isomers of the series. This differentiation is accomplished without the need for chemical derivatization. All the studied regioisomers could be differentiated from the controlled drug substance via their vapor phase IR spectra. Capillary gas chromatography successfully resolved the side chain and ring regioisomers for those isomers included in this study.

Introduction:

Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar organic molecules. Gas chromatography with infrared detection (GC-IRD) is characterized by scanning quickly enough to obtain IR spectra of peaks eluting directly from capillary GC columns and the technique is not affected by polymorphism since the infrared spectra are obtained in the vapor phase. Thus this analytical method combines the separation power of GC with the identification power of IR. In this brief overview discussion we will focus on some example applications in the analysis of closely related compounds (regioisomers) often encountered in forensic drug chemistry.

Regioisomeric relationships are the result of different positions of functional group attachments in compounds that process the same molecular formula (elemental composition). This situation has special significance when some of these molecules are legally controlled drugs or controlled precursor substances. Indeed, differentiation among regioisomeric substances is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories [1-7]. Mass spectrometry is perhaps the most common confirmatory method of identification in forensic analysis. When the regioisomeric relationships occur within the major mass spectral fragments, there is an increased possibility of misidentification. This is especially the case when reference standards are not available for all impostor substances and the possibility of chromatographic coelution cannot be eliminated.

Experimental:

GC-IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 5965B Infrared detector obtained from Analytical Solutions and Providers, Covington, Kentucky. The vapor phase infrared detector (IRD) recorded spectra in the range of 4000 – 550 cm^{-1} with a resolution of 8 cm^{-1} using a scan rate of 1.5 scans per second and a flow cell temperature was 280°C. 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. Representative mass spectra were collected using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans per second and the GC carrier gas (helium grade 5) flow rate of 0.7 ml/min. The mass spectrometer was operated on the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230°C. The GC injector was maintained at 250°C and the transfer line at 280°C. GC columns used in both instruments were 30 m x 0.25 mm i.d. coated with 0.50 µm 50% phenyl – 50% methyl polysiloxane (Rxi-50) purchased from Restek Corporation (Bellefonte PA, USA). The temperature program consisted of an initial temperature of 100°C for 1 minute, ramped up to 180°C at a rate of 9°C per minute followed by a hold at 180°C for 2 minutes then ramped to 200°C at a rate of 10°C per minute and held at 200°C. Those drug samples not commercially available were synthesized in our laboratory.

Results and Discussion:

Substituted phenethylamines are a diverse group of compounds representing a number of pharmacological categories including stimulants, hallucinogens, entactogens, anorectics, bronchodilators, and antidepressants. Methamphetamine is one example from the substituted

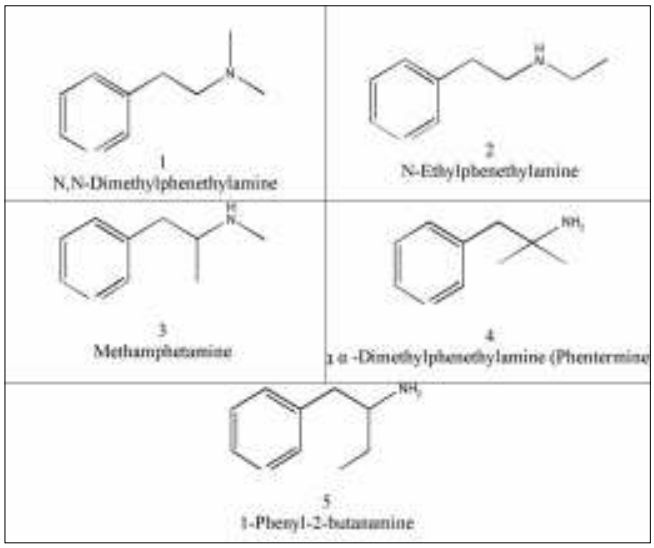

Figure 1. Structures of the five side chain regioisomer of phenethylamines

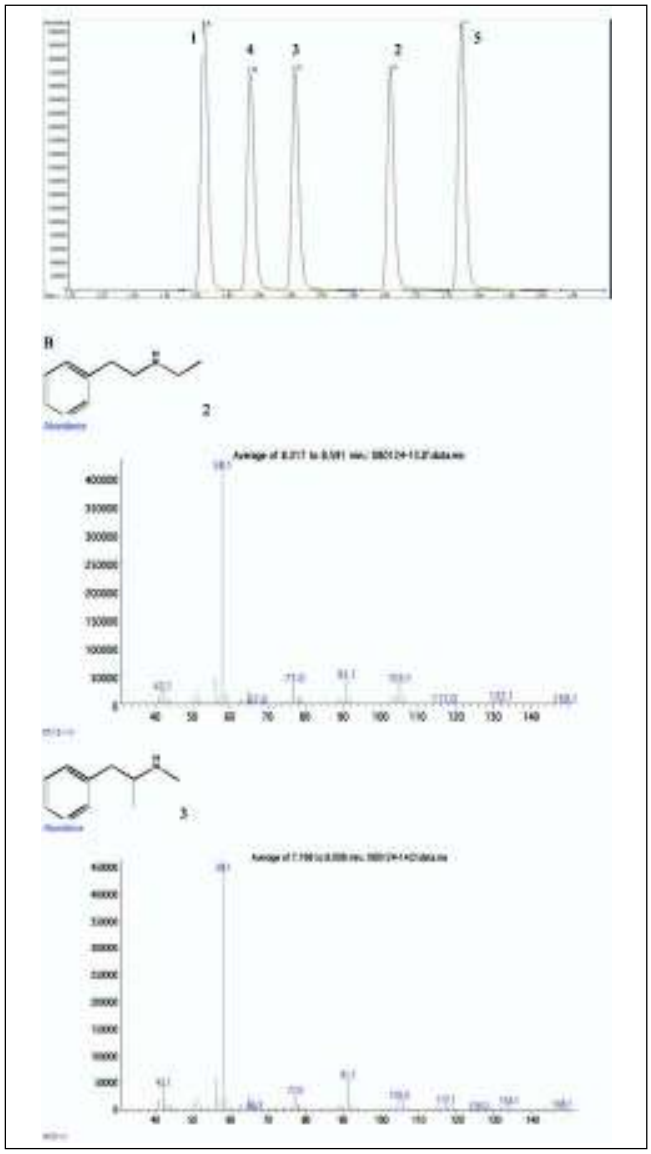

Figure 2. A:Gas Chromatographic separation of compounds 1-5; B: mass spectra of N-ethylphenethylamine (Compound 2) and methamphetamine (Compound 3).

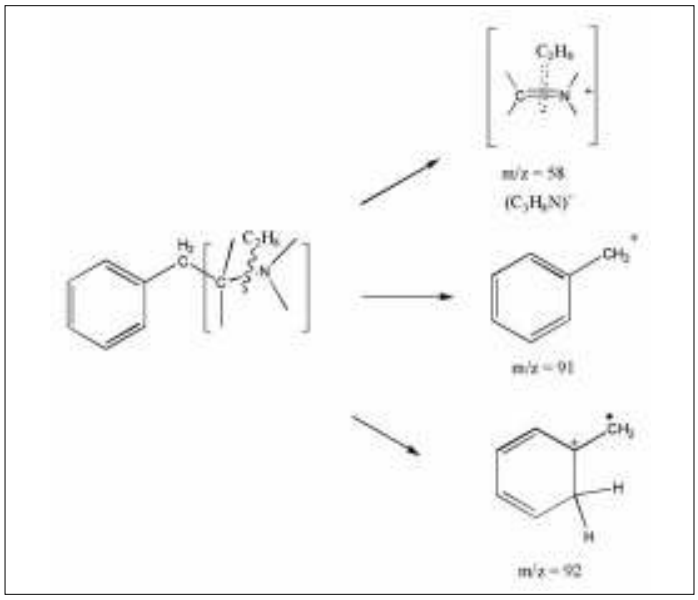

Figure 3. Mass spectral fragmentation pattern of compounds 1-5.

phenethylamines and it is a common drug of abuse [8]. Street samples of methamphetamine have been reported to contain other phenethylamine analogues including N,N-dimethylamphetamine and N-ethylamphetamine [8].

Methamphetamine is one of a total of five regioisomeric phenethylamines that have the same molecular weight (MW=149) and major mass spectral fragment ions at m/z 58 and m/z 91/92 (Compounds 1-5 in Figure 1). The gas chromatographic separation of these five amines is shown in Figure 2 along with example mass spectra for N-ethylphenethylamine and methamphetamine. Figure 3 shows the general fragmentation pattern for these amines. The GC separation was obtained on a nonpolar stationary phase and the elution order appears related to degree of molecular branching. The mass spectra for all five of these amines are very similar and illustrate the potential for misidentification. Indeed phentermine (Compound 4 in Figure 1) has a number of therapeutic applications and is often encountered in forensic drug analysis. It is easily differentiated from methamphetamine since reference standards are available to match chromatographic retention properties. However, a recent report [9] documented the significant challenge of direct mass spectral differentiation among these five regioisomers without the aid of chromatographic reference materials.

The vapor phase infrared spectra provide additional evidence for the confirmation of the exact regioisomer structure. The infrared spectra for N-ethylphenethylamine, methamphetamine and phentermine are shown in Figure 4. These example spectra show many variations in absorption wavelength and relative intensities for differentiation among these similar structures. Indeed, all five regioisomers show distinct vapor phase infrared spectra for differentiation among these amines of mass spectral equivalence. The spectra were obtained as the peaks eluted from a 30 meter column containing a mixture of phenyl and methyl polysiloxane.

The phenethylamines continue to be a common chemical area of interest for designer drug exploration. Some countries have dealt with designer drugs by placing controls on individual molecular species while others

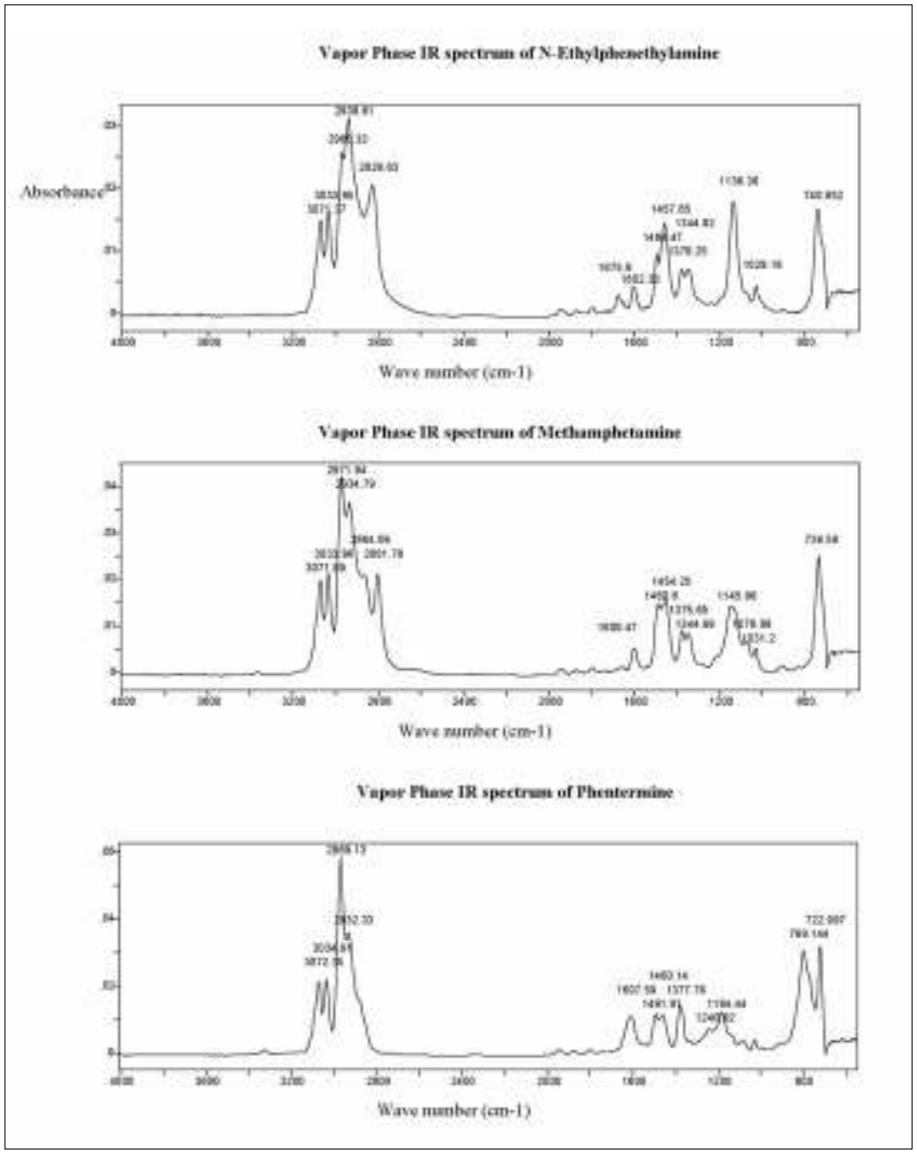

Figure 4. Vapor pase IR spectra of N-ethylphenethylamine, methamphetamine and phentermine.

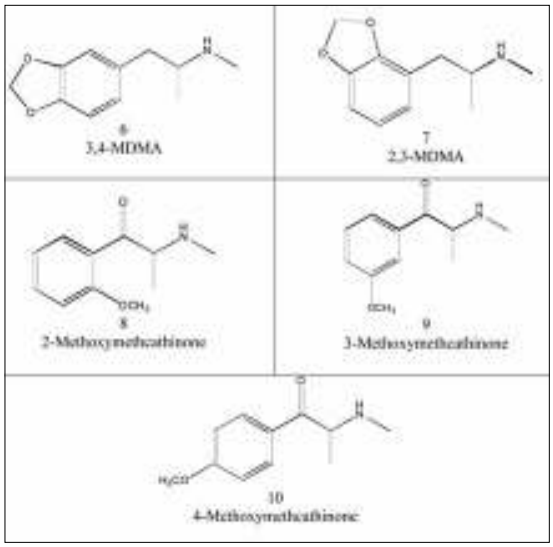

Figure 5. Structures 3,4-MDMA, 2,3-MDMA and the methoxymethcathinones.

have proactively placed controls on drug analogues. In either case the forensic chemist is left with the need to specifically identify any new substances encountered in a drug sample and eliminate regioisomeric impostor molecules.

Substitution on the aromatic ring in methamphetamine-type molecules can expand the number of substances of regioisomeric equivalence. The drug of abuse 3,4-methylenedioxymethamphetamine is often encountered in forensic drug analysis. The other four side chain regioisomers (as described for methamphetamine) would be expected to yield similar mass spectra of less than satisfactory structural confirmation value. Additionally the methylenedioxy ring can be fused to the aromatic ring in the 2,3-substitution pattern doubling the number of possible regioisomers to ten. Furthermore, the methoxymethcathinones represent a nontraditional isomeric arrangement of equivalent elemental composition and mass and would provide the possibility of another fifteen compounds of mass spectral equivalence to 3,4-MDMA. The structures of the methamphetamine-like side chains for each of these methylenedioxy substituents and the equivalent methoxymethcathinone species are shown in Figure 5.

For the purposes of this limited scope overview, we will focus on the five compounds in Figure 5, those having an imine fragment in the mass spectrum equivalent to the methamphetamine side chain. The GC separation of the five amines is shown in Figure 6 along with the IRD generated spectra for the two methylenedioxy substituted methamphetamines and an example 3-methoxymethcathinone. The carbonyl absorption at about 1700 cm-1 for the methoxymethcathinones clearly allows differentiation of this series of compounds from the methylenedioxyphenyl ring system. Additionally the spectra for the two methylenedioxymethamphetamines show a number of variations allowing differentiation between the 2,3- and 3,4-substitution pattern. While this brief discussion has focused on regioisomeric equivalents, substances with an isobaric relationship (equal mass but different elemental composition) can add another level of challenge to the identification of an individual compound from this category [10].

Conclusion:

The vapor phase infrared spectra can provide unique information to assist in the differentiation of regioisomeric substances. The GC-IRD method provides complimentary data to that obtained by GC-MS. In the analysis of substances having the possibility of many regioisomeric equivalents it is necessary to apply multiple methods of structural confirmation. A searchable library of vapor phase generated infrared spectra could offer direct access to an additional level of structural confirmation having

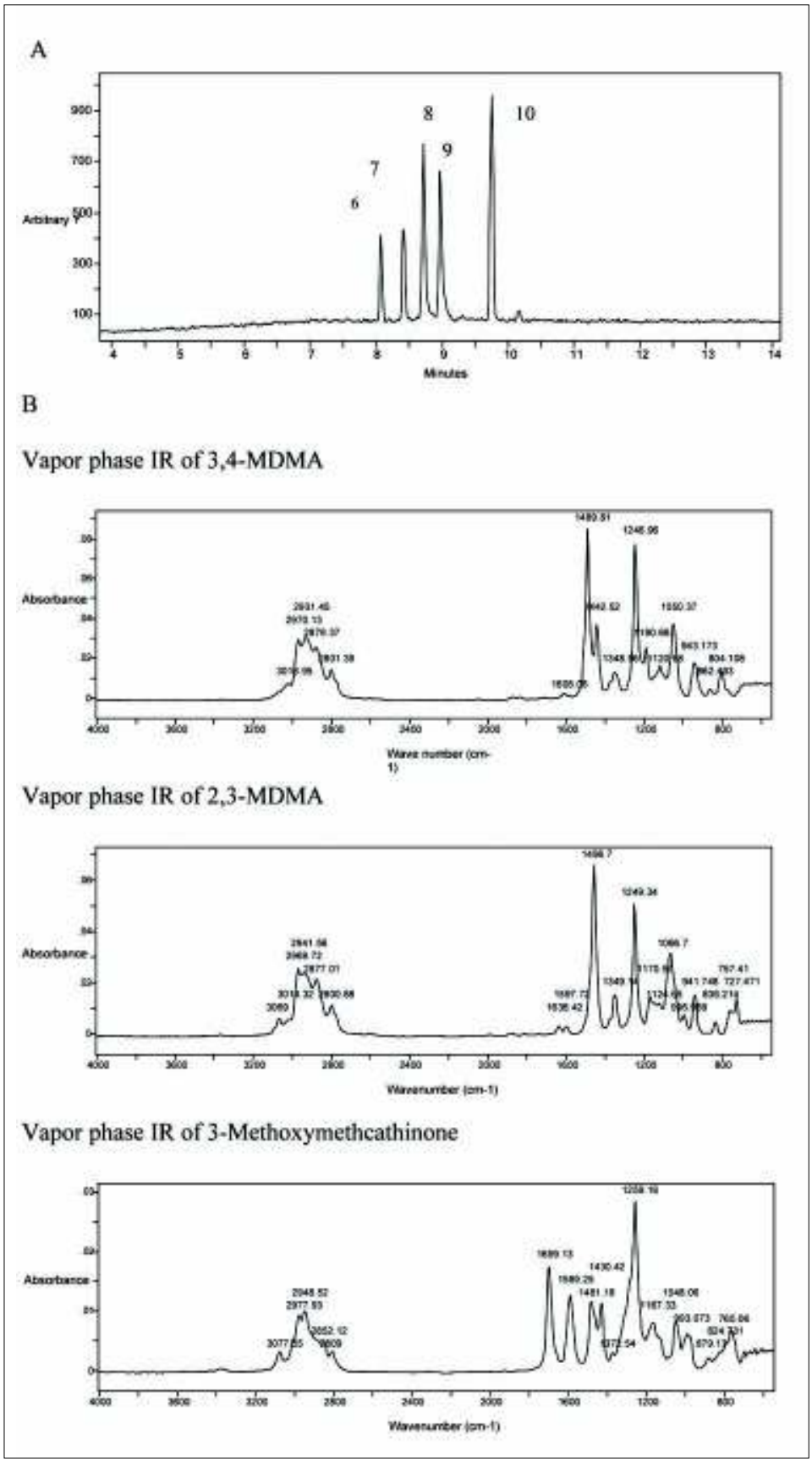

Figure 6. A: Gas chromatographic separation of compounds 6-10; B: Vapor phase IR of 3,4-MDMA, 2,3-MDMA and 3-methoxymethcathinones.

advantages of chromatographic purity as well as the elimination of possible polymorphism often observed in solid phase infrared analysis.

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References:

1. *J. DeRuiter, P.L. Holsten, C.R. Clark, F.T. Noggle, Liquid chromatographic and mass spectral methods of identification for the regioisomeric 2,3- and 3,4-methylenedioxyphenalkylamines, J. Chromatogr. Sci. 36 (1998) 131-138*
2. *C.R. Clark, F.T. Noggle, J. DeRuiter, Chromatographic and mass spectrometric methods for the differentiation of N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine from regioisomeric derivatives, J. Chromatogr. Sci. 34 (1996) 230-237.*
3. *W.H. Soine, R.E. Shark, D.T. Agee, Differentiation of 2,3-methylene-dioxyamphetamine from 3,4-methylenedioxyamphetamine, J. Forensic Sci. 28 (1983) 386-390.*
4. *D.E. Nichols, A.J. Hoffman, R.A. Oberlender, P. Jacob, III, A. T. Shulgin, "Derivatives of 1-(1,3-benzodioxolyl)-2-butanamine: Representatives of a Novel Therapeutic Class," J. Med. Chem., 1986, 29, 2009-2015.*
5. *J.F. Casale, P.A. Hays, R.F.X. Klein, Synthesis and characterization of the 2,3-methylenedioxyamphetamines, J. Forensic Sci. 40 (1995) 391-400.*
6. *C.R. Clark, A.K. Valaer, F.T. Noggle, J. DeRuiter, GC-MS analysis of acylated derivatives of methamphetamine and regioisomeric phenethylamines, J. Chromatogr. Sci. 33 (1995) 485.*
7. *T. Awad, J. DeRuiter, C.R. Clark, GC-MS Analysis of Acylated Derivatives of the Side Chain and Ring Regioisomers of Methylenedioxymethamphetamine, J. Chromatogr. Sci. 43 (2005) 296-303.*
8. *D.A. Cooper, Future synthetics drugs of abuse. Proceeding of the International Symposium on the Forensic Aspects of Controlled Substances. US Government Printing Office, Washington, DC, 1990, 79-103, Stock Number PB90-199472.*
9. *S.B. Sachs, F. Woo, A detailed mechanistic fragmentation analysis of methamphetamine and select regioisomers by GC/MS, J. Forensic Sci. 52 (2007) 308-319.*
10. *T. Awad, C.R. Clark, J. DeRuiter, Chromatographic and Mass Spectral Studies on Methoxy Methyl Methamphetamines Related to 3,4-Methylenedioxy-methamphetamine, J. Chromatogr. Sci. 45 (2007) 468-476.*