Final Summary Overview, NIJ award 2013-DN-BX-K022

Bath Salt-type Aminoketone Designer Drugs: Analytical and Synthetic Studies on Substituted Cathinones

Purpose of Project:

This project has focused on issues of resolution and discriminatory capabilities in controlled substance analysis providing data to increase reliability and selectivity for forensic evidence and analytical data on new analytes of the so-called bath salt-type drugs of abuse. The overall goal of these studies is to provide an analytical framework for the identification of individual substituted cathinones to the exclusion of all other possible isomeric and homologous forms of these compounds. A number of aminoketones or beta-keto/benzylketo compounds (bk-amines) have appeared on the illicit drug market in recent years including methcathinone, mephedrone, methylone and MDPV (3,4-methylenedioxypyrovalerone). These substances represent a variety of aromatic ring substituent, hydrocarbon side chain and amino group modifications of the basic cathinone/methcathinone molecular skeleton. The broad objective of this research was to improve the specificity, selectivity and reliability of analytical methods used to identify ring substituted aminoketones and related compounds. This improvement comes from methods which allow the forensic analyst to identify specific regioisomeric forms of substituted aminoketones among many isomers of mass spectral equivalence. Mass spectrometry is the most common method of confirmation in forensic drug analysis. This project provides methodology and analytical data to discriminate between those regioisomeric and isobaric molecules having the same molecular weight and major fragments of equivalent mass (i.e. identical mass spectra). Furthermore, this work has anticipated the future appearance of some designer aminoketones and developed reference data and analytical reference standards for these compounds.

Project Subjects:

This project involves basic chemical sciences and focuses on forensic drug chemistry. This research project did not involve human subjects and no laboratory animals were used in this work. The project has explored issues of analytical specificity in forensic drug analysis involving synthetic designer drugs related to cathinone. Cathinone is a natural product found in the leaves of Catha edulis Forsk, the Khat plant, discovered in Yemen in the eighteenth century. Cathinone's stimulatory effects have been known for centuries, mostly prevalent in Middle Eastern countries ranging from Southern Africa to the Arabian Peninsula. Synthetic cathinones, the active component in "bath salts," have surfaced as a popular alternative to other illicit drugs of abuse, such as cocaine, MDMA (ecstasy), and methamphetamine, due to their potent psychostimulant and empathogenic effects.

The stimulatory effects of synthetic cathinones are the result of elevations in synaptic catecholamine concentrations, primarily via two mechanisms. These molecules bind to and inhibit the monoamine uptake transporters for dopamine, norepinephrine, and serotonin diminishing their clearance from the synaptic cleft. Second, as substrate releasers, they exhibit non-exocytotic neurotransmitter release from intracellular stores by reversal of transporter flux and inhibiting the vesicular monoamine transport receptor. Pyrovalerone and MDPV, the pyrovalerone–cathinones, represent a subset of cathinone derivatives whose actions occur via potent and selective monoamine uptake inhibition. MDPV is a potent monoamine transporter inhibitor with the highest affinity for the dopamine transporter and lowest affinity for the serotonin transporter. The selectivity of MDPV for the dopamine transporter protein (>100 dopamine/serotonin inhibition ratio) is higher than that described for methamphetamine and cocaine ratio, >10 and 3.1, respectively, revealing a high abuse potential for this pyrovalerone-type cathinone derivative.

Project Design:

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions of the molecule: A) the aromatic ring, B) the alkyl side chain; C) the amino group and a fourth, the carbonyl group (not explored in this project). Based on the structures of previously identified designer cathinone derivatives, it is clear that designer modification in regions A, B and C are currently being explored by clandestine chemists. Futhermore, the strong interest and unique pharmacology of MDPV will likely yield future designer compounds based on modification of the MDPV molecule. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules. The commercial availability of many sets of precursor substances further provides additional interest in designer modifications. This project has focused on those designer concepts used in the amphetamine-type molecules applied to the MDPV series.

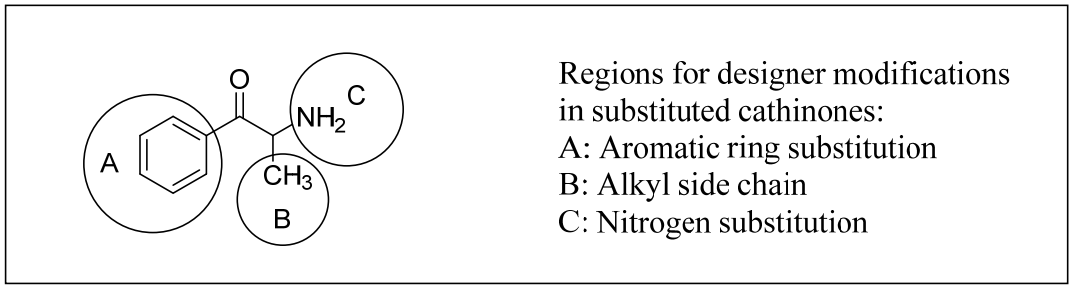


Figure 1. Regions for potential designer modifications in the cathinone derivatives.

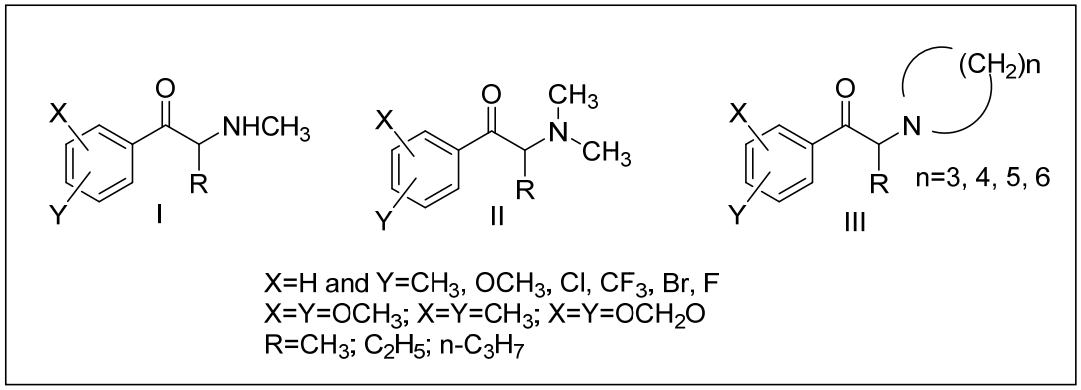


Figure 2. General structures for the Series I-III bath salt aminoketone derivatives in this study.

Project Methods:

The purpose of this project is to develop regioisomer specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). This work included: 1) the chemical synthesis of all regioisomeric forms of selected aromatic ring substituted aminoketones, 2) generation of a complete analytical profile for each compound, 3) chromatographic studies to separate/resolve all regioisomeric aminoketones having overlapping analytical profiles, and 4) design and validation of confirmation level methods to identify each compound to the exclusion of other regioisomeric forms.

The initial phase of this work is the organic synthesis of aminoketones of varying aromatic ring substituents, hydrocarbon side chains and amino groups. In this phase of the work numerous substituted aminoketones of potential forensic interest have been evaluated. The analytical phases included chemical characterization, using tools common to forensic science labs such as MS and IR and these studies were carried out on each of the compounds. The chromatographic retention properties for each series of isomers have been evaluated by gas and liquid chromatographic techniques on a variety of stationary phases. These studies have established a structure-retention relationship for the regioisomers and isobaric aminoketones.

The results of this project will serve to increase the forensic drug chemistry knowledge base for aminoketone-type designer drugs. When compounds exist which produce the same mass spectrum (same MW and fragments of equivalent mass) as the drug of interest, the identification by GC-MS must be based upon the ability of the chromatographic system to resolve these substances. This project included the synthesis and generation of complete analytical profiles as well as methods of differentiation for regioisomeric and isobaric substances related to the substituted aminoketones. This project has generated proactive data for the forensic drug analyst and described a unified approach for specific drug identification based on structure correlated analytical properties.

Figure 3 illustrates the methods used in our laboratory for the synthesis of the isomers needed to complete modifications at Regions A, B, and C shown in Figure 1. The starting point in the synthetic pathway is a substituted benzaldehyde or benzoic acid. Many mono-substituted benzaldehydes or benzoic acids are available from commercial sources and all six regioisomeric dimethoxybenzaldehydes are commercially available. The precursor chemicals for all the possible regioisomeric imposter compounds are commercially available. Isomer issues are not quite so central in forensic analysis for those natural product drugs (THC, cocaine, etc.) synthesized by a plant in an enzymatically controlled (isomeric specific) process.

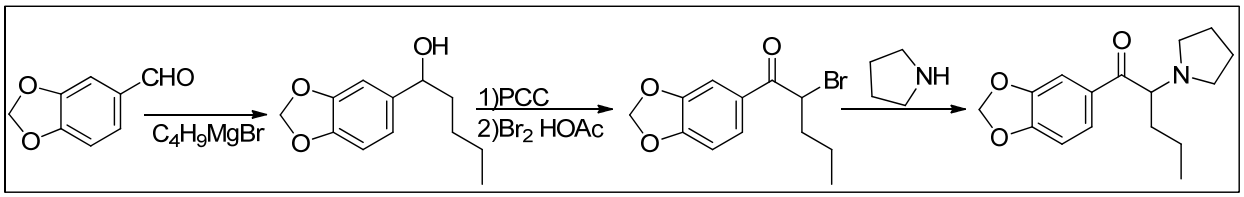


Figure 3. General synthesis for the bath salt aminoketones using the synthesis of MDPV as an example.

The analytical methods have focused on those techniques in routine use in forensic laboratories: GC, GC-MS, IR, GC-IR and related techniques. Additionally some GC-MS/MS product ion studies have been done as well as some preliminary GC-TOF-MS studies. The initial analytical studies consisted of a complete profile on each of the amines and ketone, including GC-MS, IR and GC-IR. The chromatographic elution properties and retention data via capillary GC retention studies have been collected for each compound followed by the chromatographic evaluation of each group of compounds based upon structural types. Spectrophotometric techniques such as infrared spectrophotometry have allowed for the differentiation among some of the aromatic ring substitution patterns and allow for the detection of those isomeric substances containing a carbonyl-group in the side chain. With an ample number of structural types available in this study, the various ring substituents and patterns can be individualized as a result of this work.

Project Data Analysis:

The general approach for the analytical identification of an individual cathinone derivative based on the results of this work would consist of the generation of a classical electron ionization mass spectrum (EI-MS). These data should assist with a molecular weight and major fragmentation between the carbonyl carbon and the alkyl carbon as indicated in Figure 4.

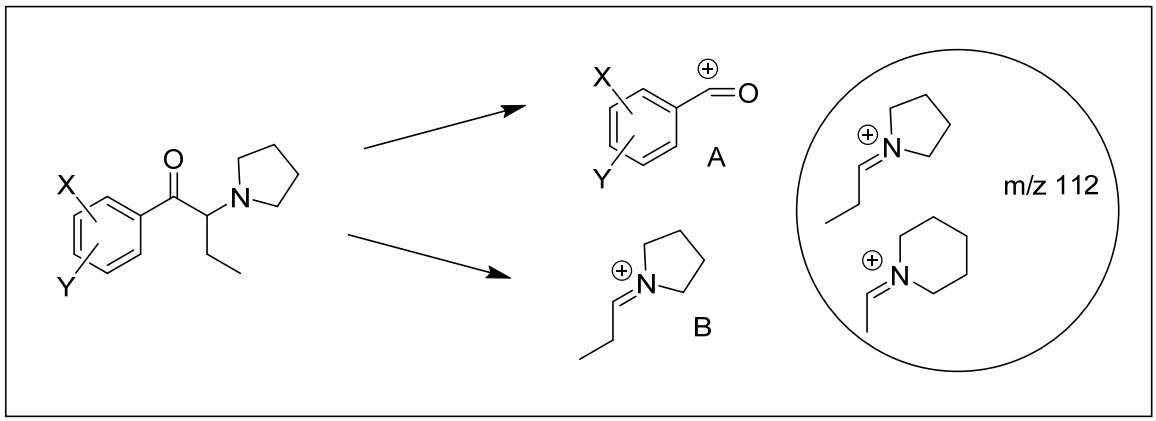


Figure 4. General EI-MS fragmentation for substituted cathinones.

The mass of the molecular ion as well as that for the acyl cation (A in Figure 4) helps to identify the aromatic ring substituents based on mass differentiation from the basic benzoyl fragment mass. The iminium cation is the base peak in the mass spectrum in our examples studied so far and this ion provides information on the number of carbons attached to the amine group and the alkyl side chain in combination. IR and vapor phase GC-IR allow for differentiation of the aromatic ring substitution patterns. For example GC-IR vapor phase spectra clearly differentiate the 2,3-methylenedioxy substitution pattern from the 3,4-pattern across a number of side chain structural categories. Product ion MS-MS studies have provided data on the make-up of the iminium cation species and allow a clear differentiation of the hydrocarbon content of the tertiary amine group verses the alkyl side-chain in regioisomeric examples. See the regioisomeric m/z 112 iminium cation structures inside the circle in Figure 4. The synthesis, GC-MS and MS/MS studies of various deuterium labeled analogues has confirmed these product ion observations.

A series of regioisomeric cyclic tertiary amines were prepared and evaluated in EI-MS and MS/MS product ion experiments. The cyclic amines azetidine, pyrrolidine, piperidine and azepane were incorporated into a series of aminoketones related to the cathinone derivative drug of abuse known as MDPV. Deuterium labeling in both the cyclic amine and alkyl side chain allowed for the confirmation of the structure for the major product ions formed from the EI-MS iminium cation base peaks. Each of the regioisomeric m/z 126 base peaks gave a unique and characteristic major product ion (see Figure 5). An example of the data collected in this work is presented in the Appendix.

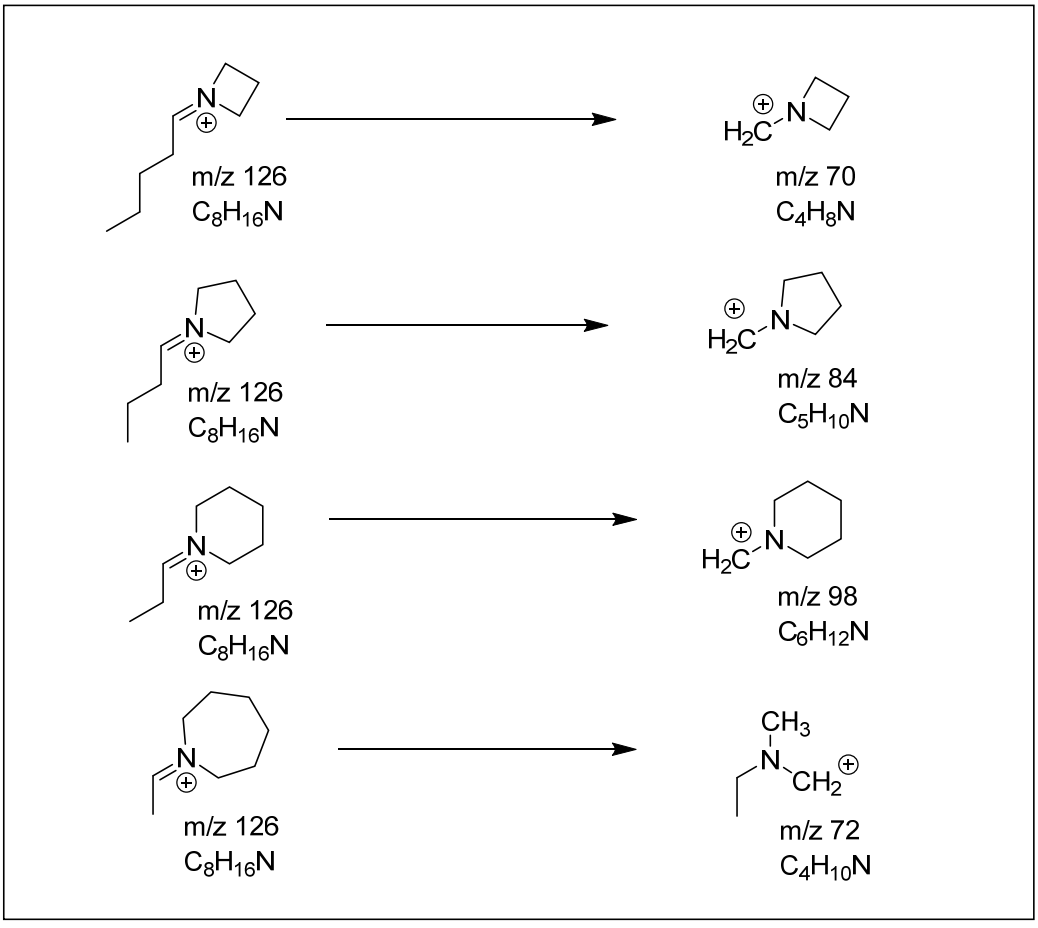


Figure 5. The characteristic product ion MS/MS species from regioisomeric m/z 126 ions.

List of Scholarly Products Produced or in Progress:

The scholarly products for this project will consist of peer reviewed publications in scientific journals in the field of forensic science, forensic drug chemistry, analytical chemistry and related subjects. We expect to submit results of this research for publication in national and international journals such as Journal of Forensic Science, Forensic Science International, Drug Testing and Analysis, Rapid Communications in Mass Spectrometry, and other appropriate scientific outlets. We have two manuscripts submitted for publication and under review at this time.

1-Younis F. Hamad Abiedalla, Karim Abdel-Hay, Jack DeRuiter and C. Randall Clark, "GC-MS and GC-IR Analysis of a Series of Methylenedioxyphenyl-Amino-Ketones: Precursors, Ring Regioisomers and Side-Chain Homologues of 3,4-Methylene-dioxypyrovalerone (MDPV)," Drug Testing and Analysis, submitted for publication.

2-Younis F. Hamad Abiedalla, Karim Abdel-Hay, Jack DeRuiter and C. Randall Clark, "Differentiation of Cyclic Tertiary Amine Cathinone Derivatives by Product Ion Electron Ionization Mass Spectrometry," Rapid Communications in Mass Spectrometry, submitted for publication.

We expect to prepare and submit other manuscripts on additional structural subsets of cathinone derivatives evaluated in this study. We anticipate the submission of at least two more manuscripts from this research project.

Implications for Criminal Justice and Practice in the United States:

The appearance of increasing structurally diverse aminoketone derivatives in clandestine samples highlights several key issues of immediate forensic significance. First, most of these compounds have regioisomeric analogues which would not be readily differentiated and specifically identified using standard forensic analytic methodology. The second key issue of forensic

importance among the aminoketone compounds involves designer drug development. Many of these compounds contain aromatic substituents known to enhance hallucinogenic or entactogenic activity in the phenylalkyl-amine (amphetamine and methylenedioxyamphetamine) drugs of abuse series. As regulatory controls tighten with respect to available aminoketones, designer derivative exploration and synthesis is expected to continue. This is particularly important in these aminoketone compounds since the syntheses are relatively straight-forward and many starting materials are readily accessible. Further designer exploration would likely parallel that observed in the phenylalkylamine series of drugs and involve methoxy, dimethoxy, bromo-dimethoxy, methylenedioxy, trifluoromethyl, chloro, and methyl substituents, as well as regiosiomers of these substitution patterns.

A proactive investigation of the forensic analytical chemistry of these future designer substances was the objective of this project. The resulting analytical data as well as methods for reference standard synthesis represent important advancements in forensic drug chemistry. The goal of this work is to have the data and methods for differentiation among these designer regioisomeric substances available to assist in drug identification.

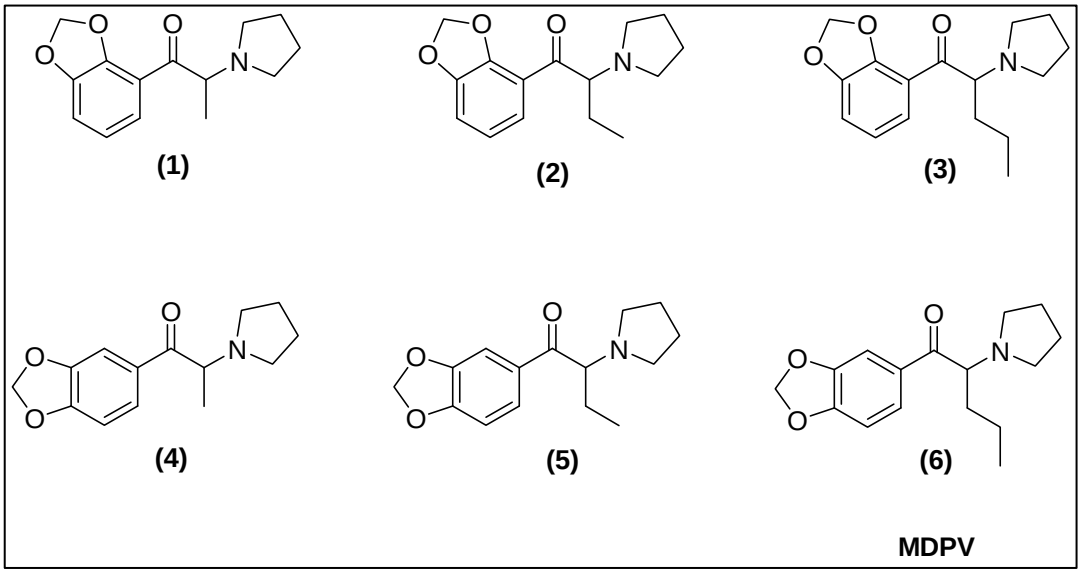
The results of this project should provide a detailed framework of analytical properties and their relationship to drug structure. This project will also provide a scientific framework for understanding the spectroscopic properties (especially mass spectral fragmentation chemistry) and chromatographic retention details. These studies will yield synthetic methods and analytical reference material for rapid production of drug reference standards. More importantly these results will provide for a more capable scientifically prepared forensic expert to interact at the interface of the legal system and the science of forensic drug chemistry.

In July of 2011 the National Drug Intelligence Center (NDIC) issued a "Situation Report" examining the threat that synthetic cathinone abuse poses to the United States and the difficulty that U.S. law enforcement faces in preventing the manufacture and distribution of synthetic cathinones and synthetic cathinone products. The four major concerns in the NDIC report were as follows: 1-the distribution and abuse of synthetic cathinones in the US will increase in the near term; 2-more synthetic cathinones will be abused in the long term; 3-different synthetic cathinones will surface as commercial drug testing companies develop drug screens to detect existing synthetic cathinones; 4-the global nature of Internet chemical sales, particularly of synthetic cathinones, will present increasing challenges to U.S. law enforcement in the long term.

Most of these predictions have now come true and continue to highlight the need to develop specific, selective and reliable analytical methods to identify existing cathinone products in the clandestine market place, and versatile methods that can be used to readily identify new designer analogues as they emerge.

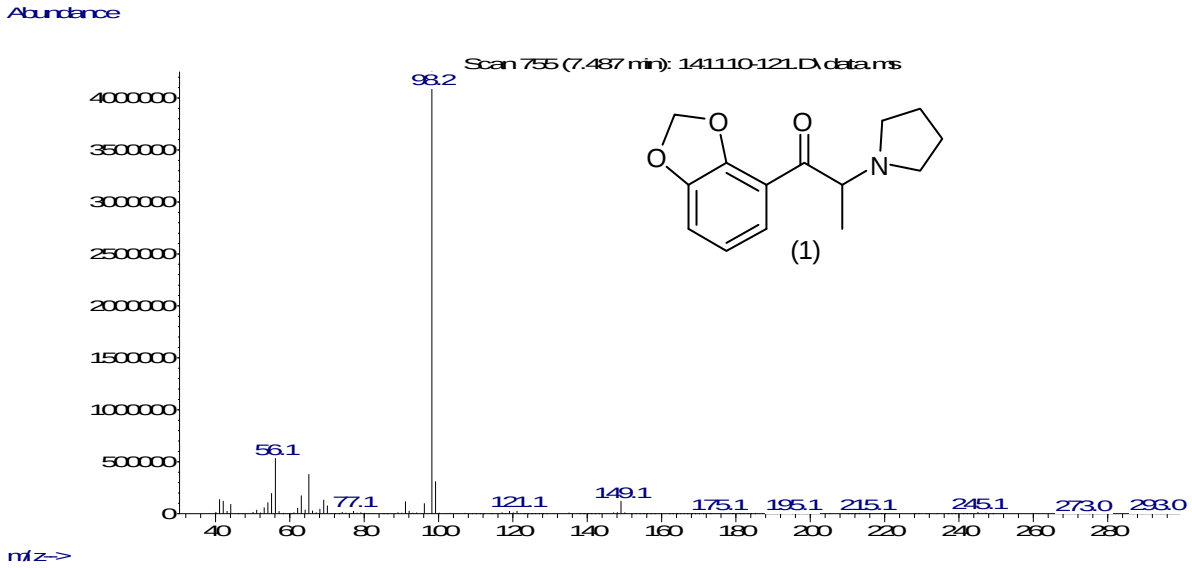
Appendex Material:

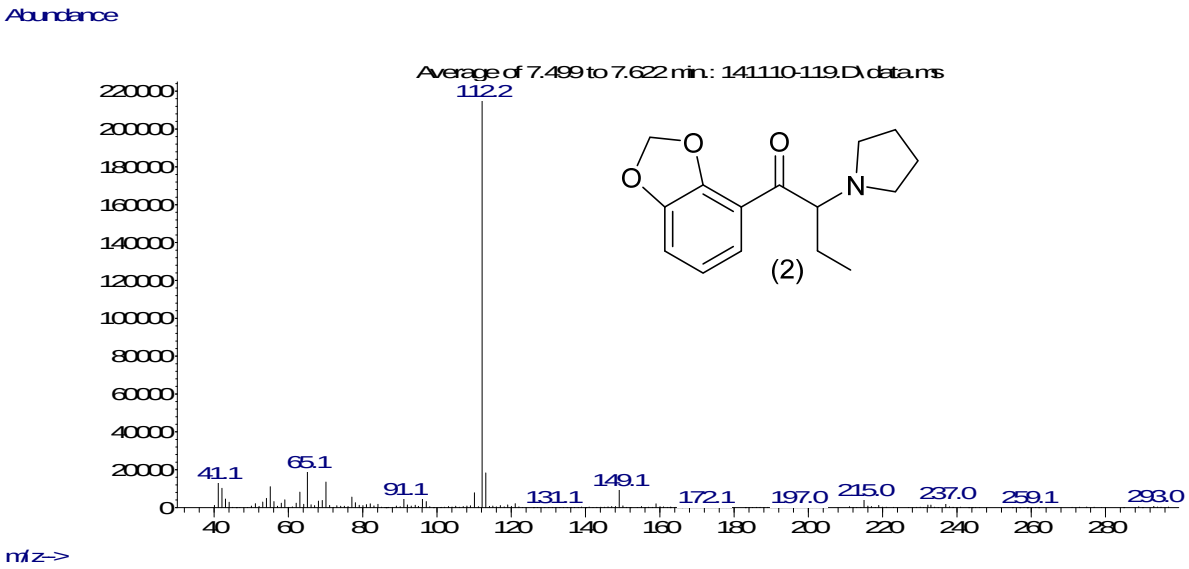
This section contains an example of the data generated in this project using one subset of compounds, the 2,3- and 3,4-methylenedioxyaminoketones. This is a series of homologues and regioisomers directly related to MDPV.

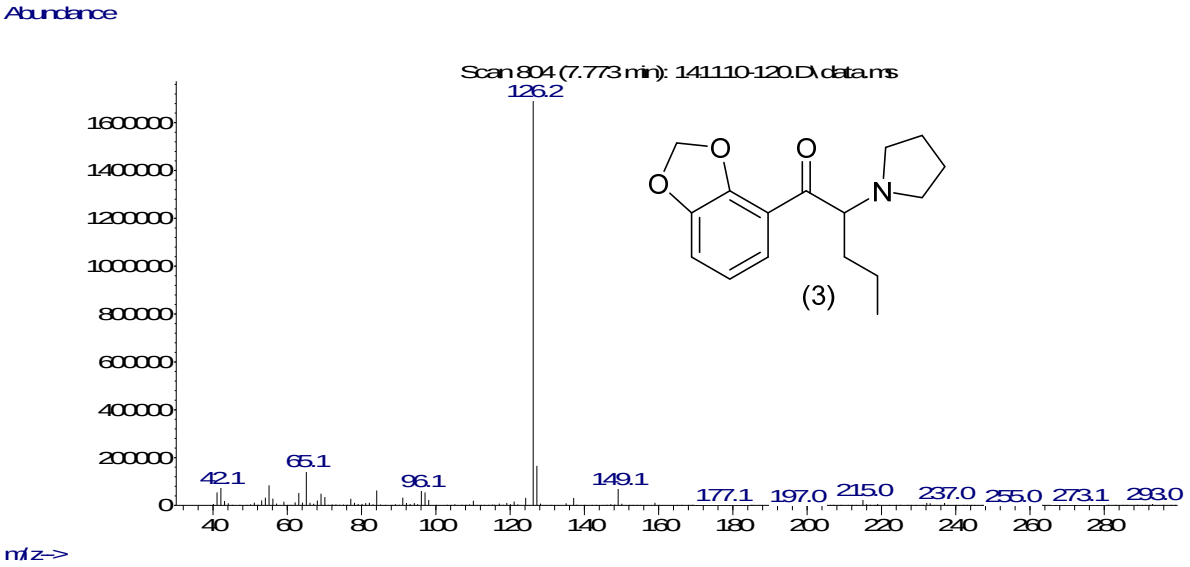


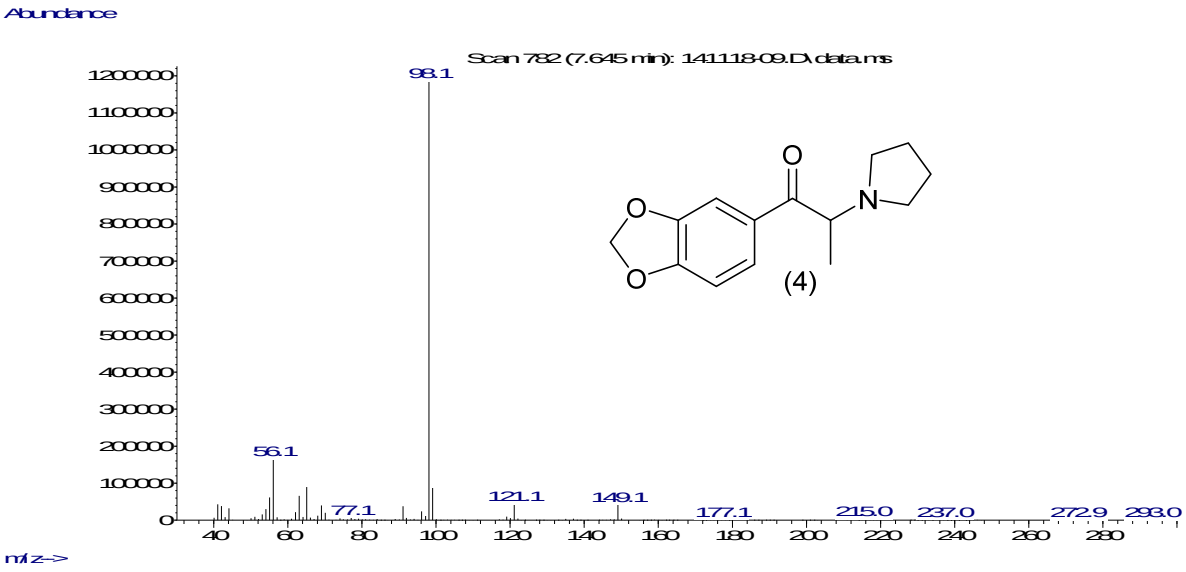
The EI mass spectra of the isomeric and homologous aminoketones (Compounds 1-6) in this study are shown below. The base peak in the mass spectra of all these compounds are the result of the amino-group dominated alpha-cleavage process producing the iminium cation fragments having homologous and regioisomeric relationships. The base peak in all these spectra occurs in a mass range of m/z 98, m/z 112 and m/z 126 and represents a homologous series of iminium cations based on the number of methylenes in the alkyl side-chain. These iminium cations are the result of fragmentation of the bond between the carbonyl carbon and the adjacent side chain carbon bearing the amine nitrogen of the pyrrolidine ring. The m/z 149 ion seen in most of the spectra is the methylenedioxybenzoyl cation ($ArCO^+$) resulting from initial ionization of the carbonyl oxygen and fragmentation of the same carbon-carbon bond to result in charge retention on the carbonyl portion of the structure. Loss of CO from the m/z 149 cation is

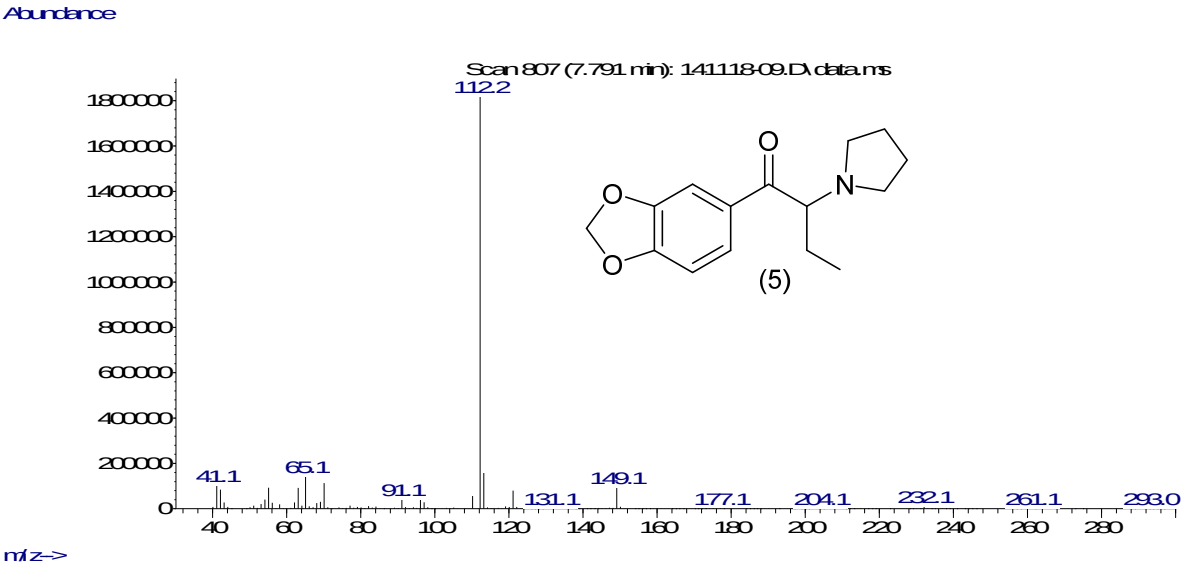
the likely source of the m/z 121 cation observed in most of the spectra. The iminium cation essentially characterizes the nature of the alkylamino-group attached to the carbonyl carbon, however these ions as well as the substituted benzoyl cations and resulting fragments do not provide characterization of the regioisomeric position of the methylenedioxy-group.

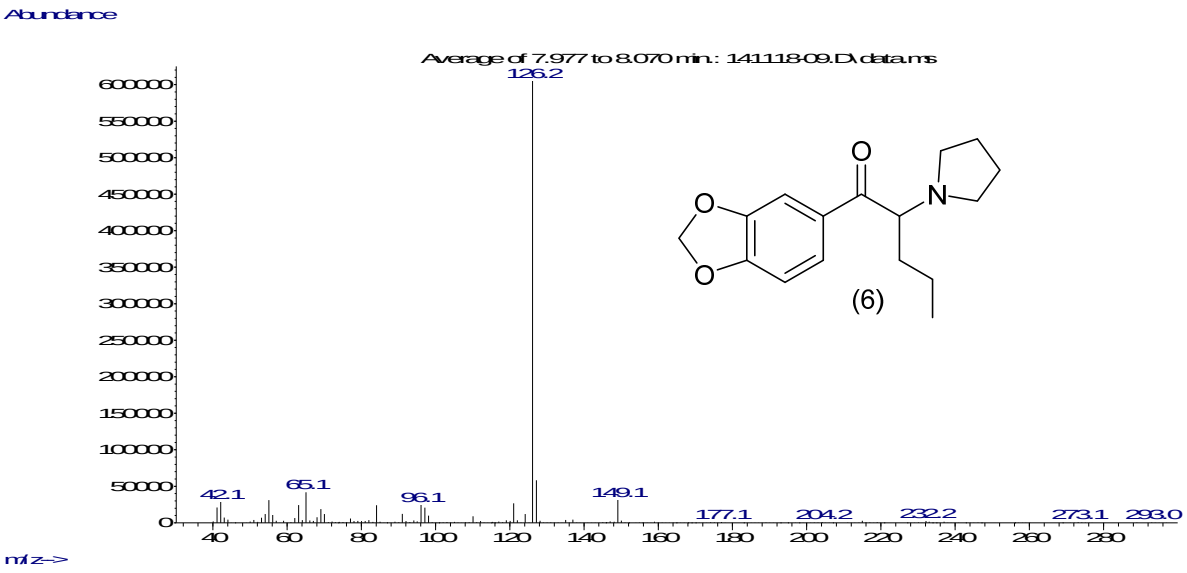




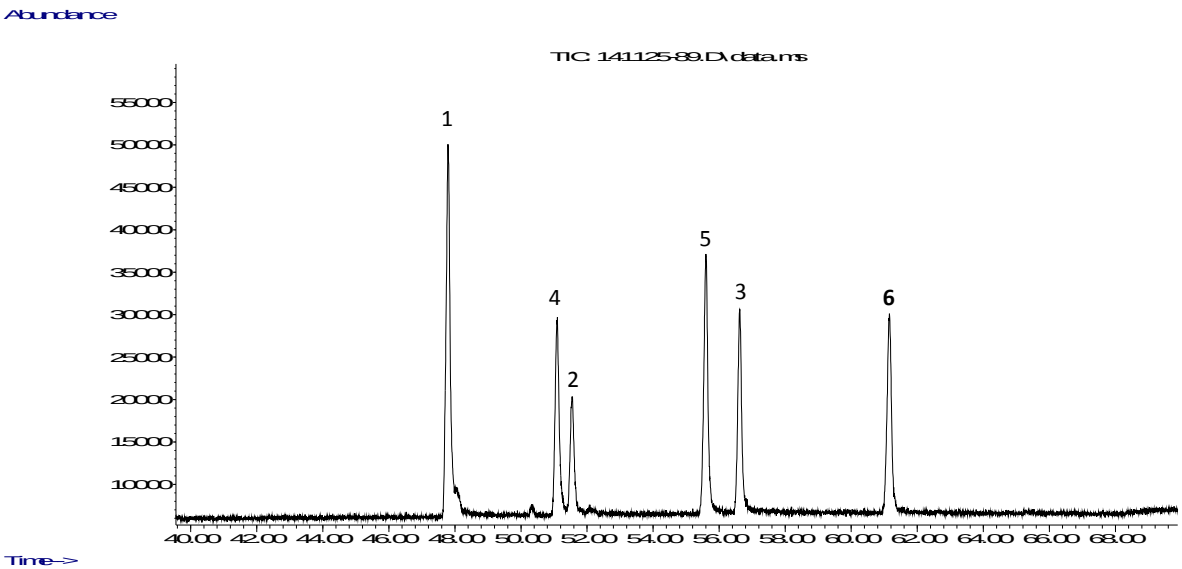








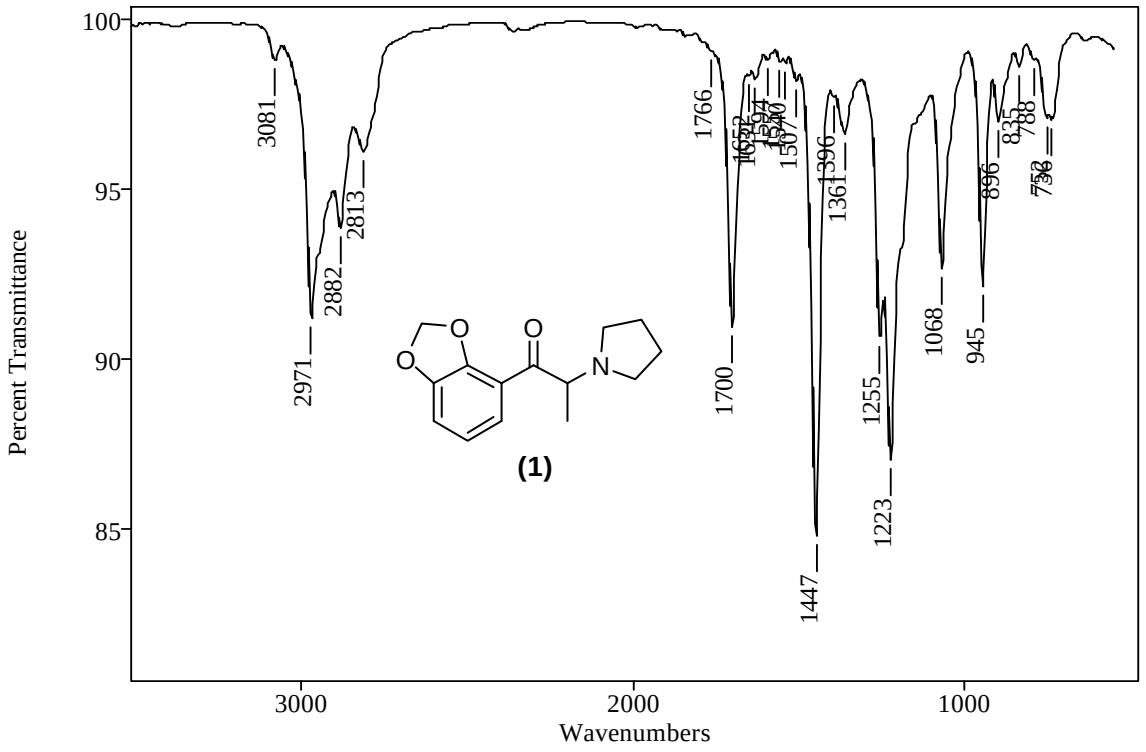
EI mass spectra of the six regioisomeric and homologous methylenedioxy aminoketones.

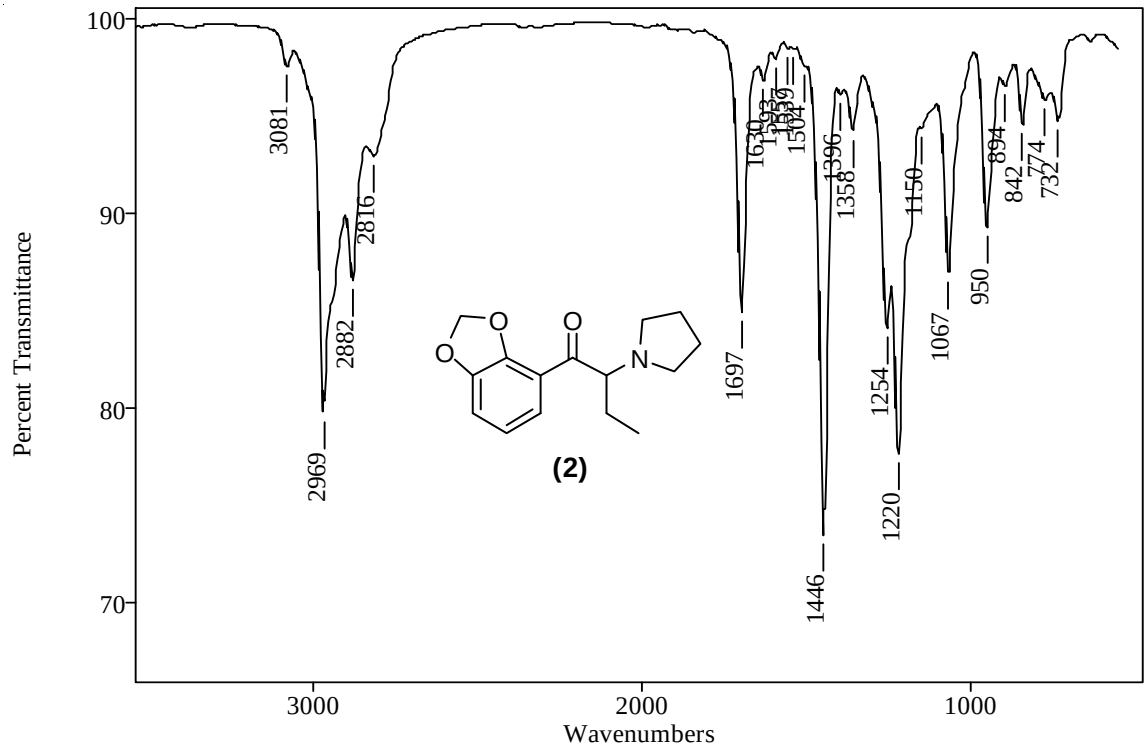


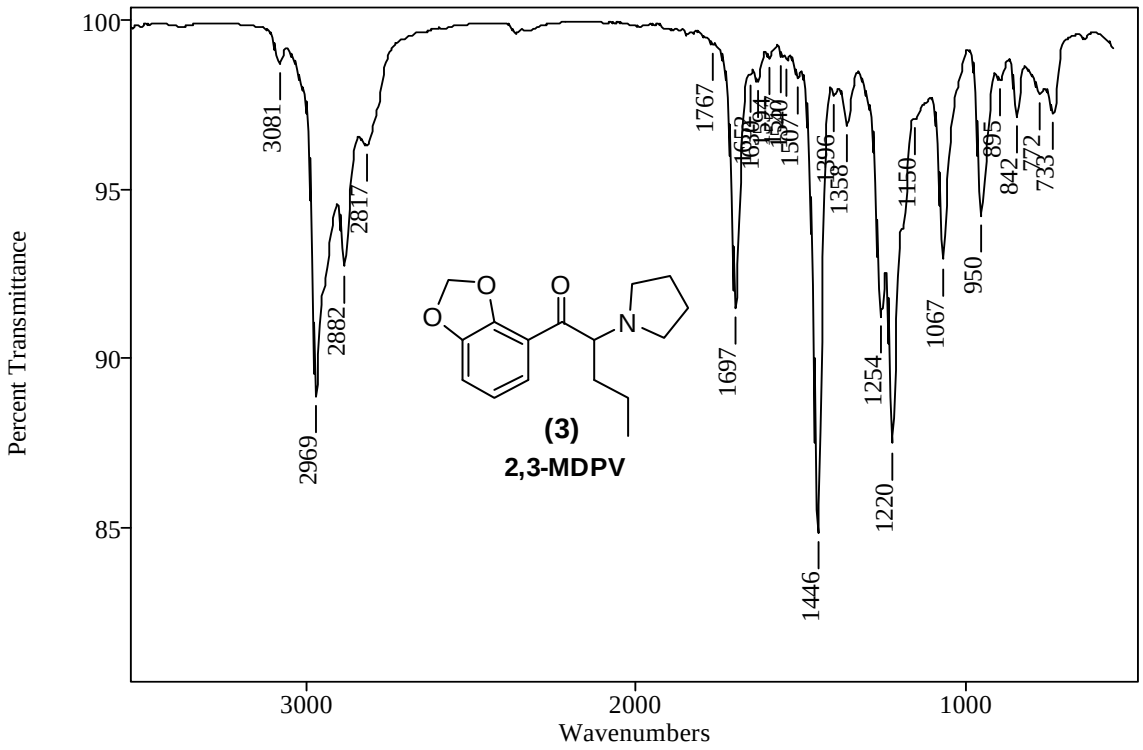
Capillary gas chromatographic separation of the six regioisomeric and homologous methylenedioxy aminoketones using Rtx-5 stationary phase.

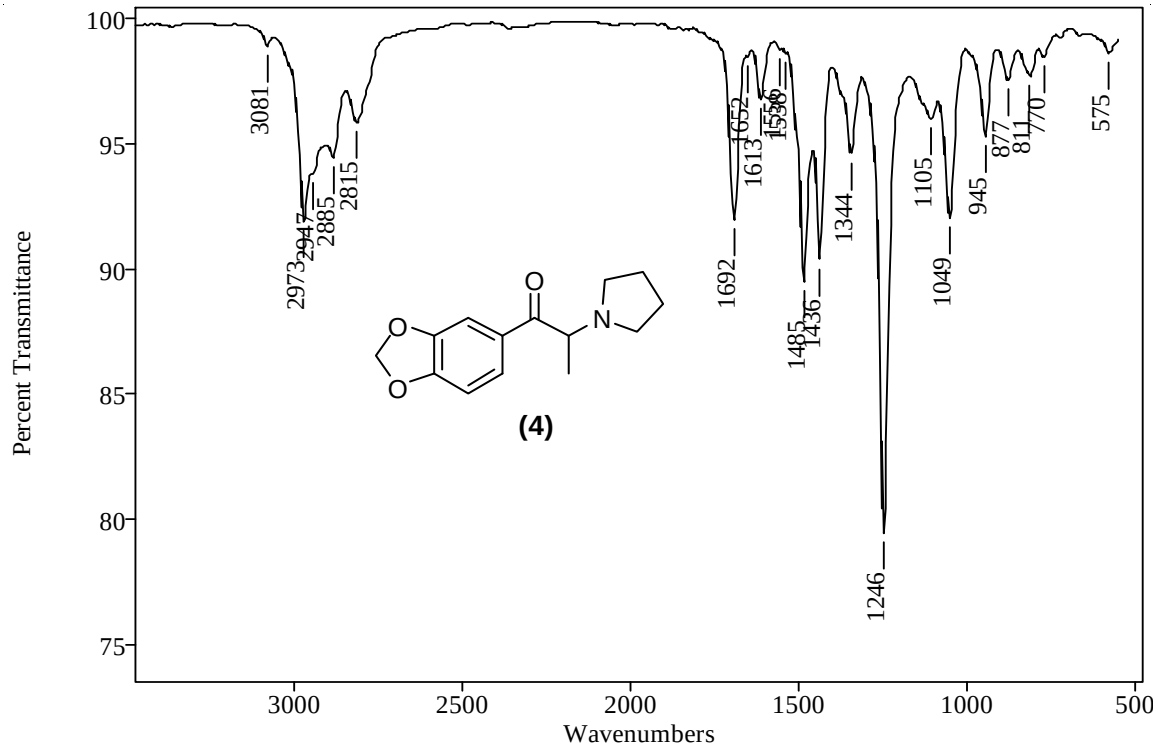
The chromatogram above shows the GC separation of the six regioisomeric and homologous methylenedioxyphenylaminoketones in this study. The separation was obtained on a 30 meter capillary column coated with Rtx-5, 5% diphenyl and 95% dimethylpolysiloxane. The elution order reflects these structure-chromatographic relationships showing increased retention with increasing side chain length for the homologous series. Compounds 1 and 4 show the lowest retention and both represent the methyl side chain, this is followed by the ethyl side chain for compounds 2 and 5, and the highest retention occurs for the n-propyl side chain for compounds 3 and 6. Furthermore, within each pair of equivalent homologues the position of the ring methylenedioxy substitution controls the relative retention of the pair. In every case the 2,3-isomer elutes before the 3,4-isomer, for example MDPV (the 3,4-methylenedioxyphenyl isomer, compound 6) has much higher retention (about 4 minutes) than its' corresponding 2,3-isomer, compound 3.

The vapor phase infrared spectra generated in GC-IR experiments are shown below. These spectra were generated directly from the chromatography peak as each compound eluted from the capillary GC column. Thus these infrared spectra have an added level of reliability based on the purity of the gas chromatographic peak. The GC-IR vapor phase infrared spectra were recorded in the range of 4000 – 550 cm^{-1} with a resolution of 8 cm^{-1}. All these compounds show a carbonyl band in the 1690 cm^{-1} range and characteristic bands in the 1500 cm^{-1} to 1200 cm^{-1} range. The characteristic bands in the 1500 cm^{-1} to 1200 cm^{-1} range provide information concerning the position of the methylenedioxy ring and its relationship to the aminoketone side chain. The 2,3-methylenedioxy substitution pattern in compounds 1, 2 and 3 show a characteristic pattern in the 1500 cm^{-1} to 1200 cm^{-1} range consisting of a strong single band absorption centered in the 1447 cm^{-1} range and a less intense doublet peak in the 1254/1220 cm^{-1} range. However, the 3,4-methylenedioxy substitution pattern in compounds 4, 5 and 6 show a doublet pattern with peaks centered at 1485 cm^{-1} and 1436 cm^{-1} in all three spectra and an intense singlet absorption band at 1446 cm^{-1}.









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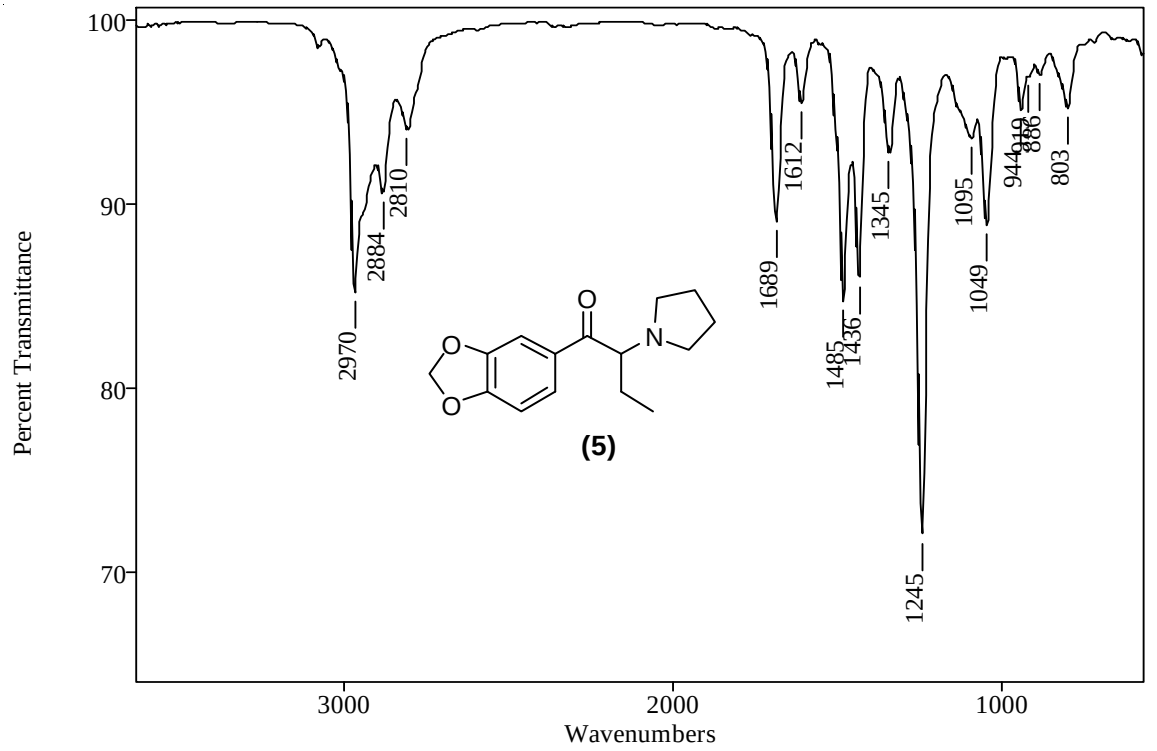

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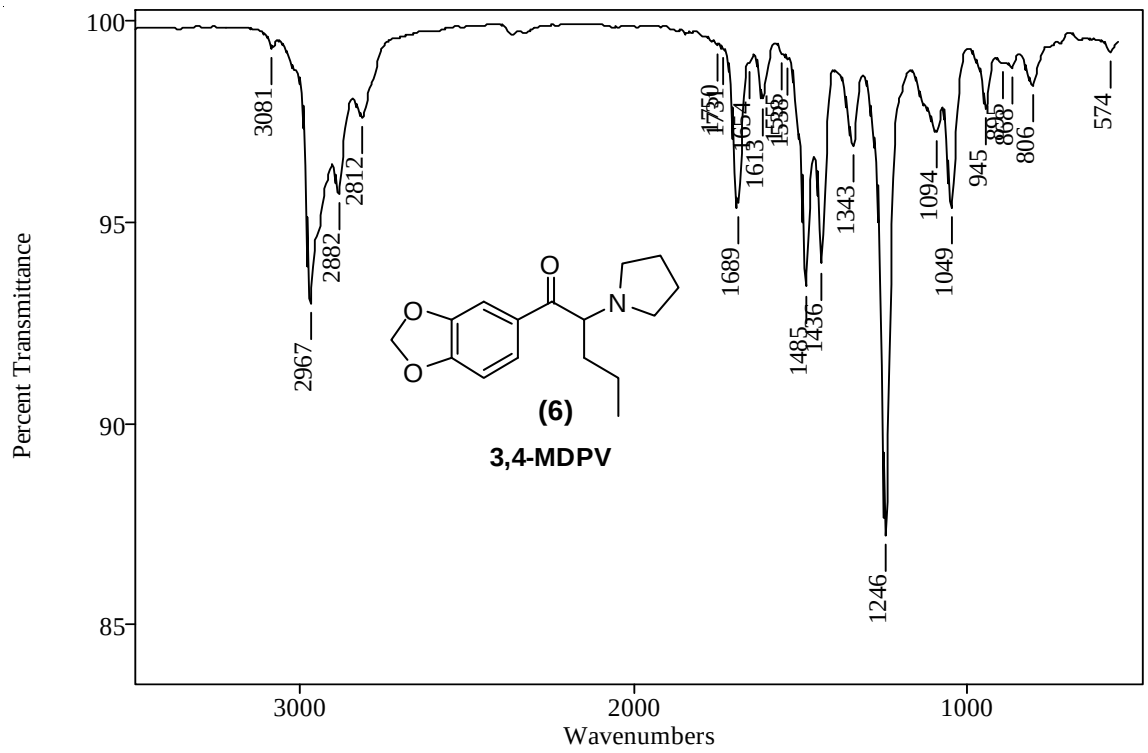
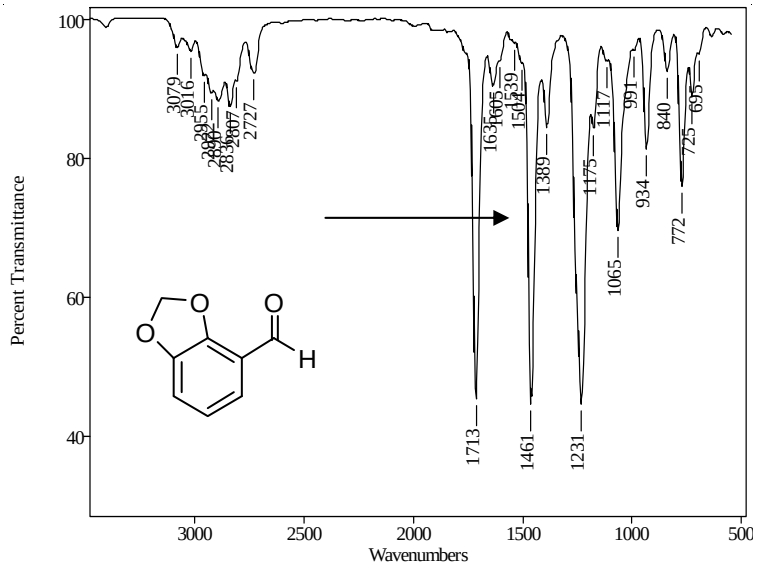
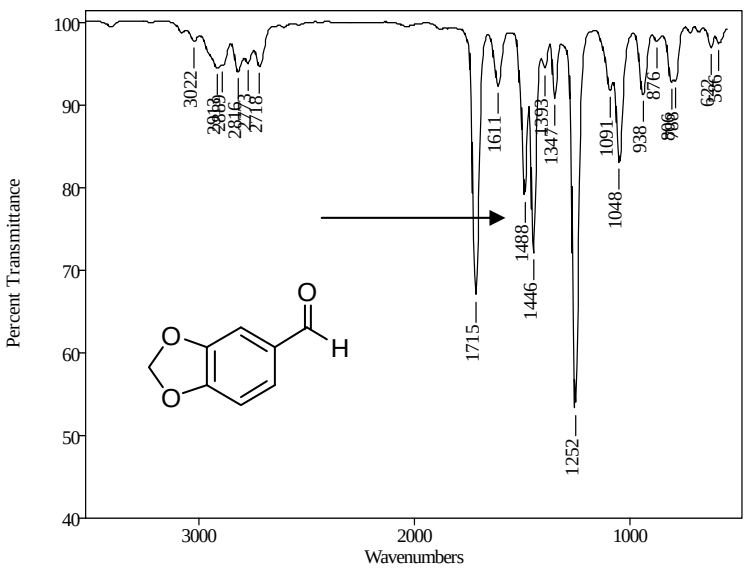
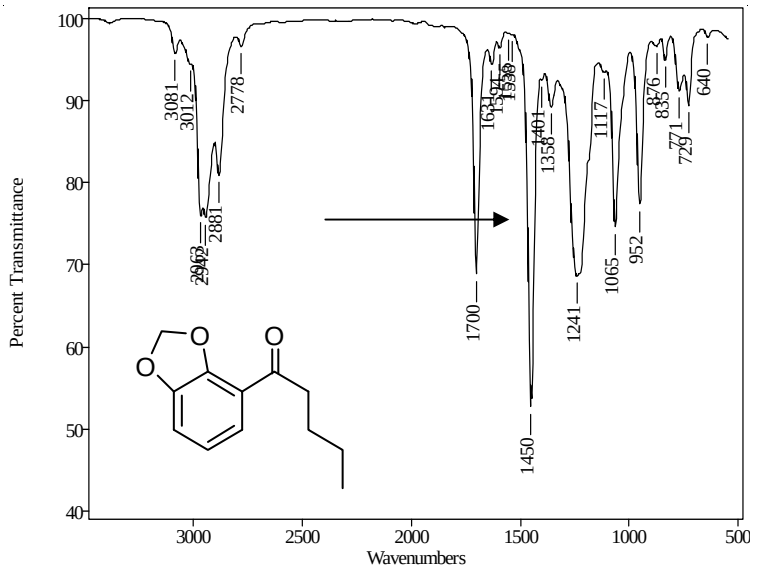
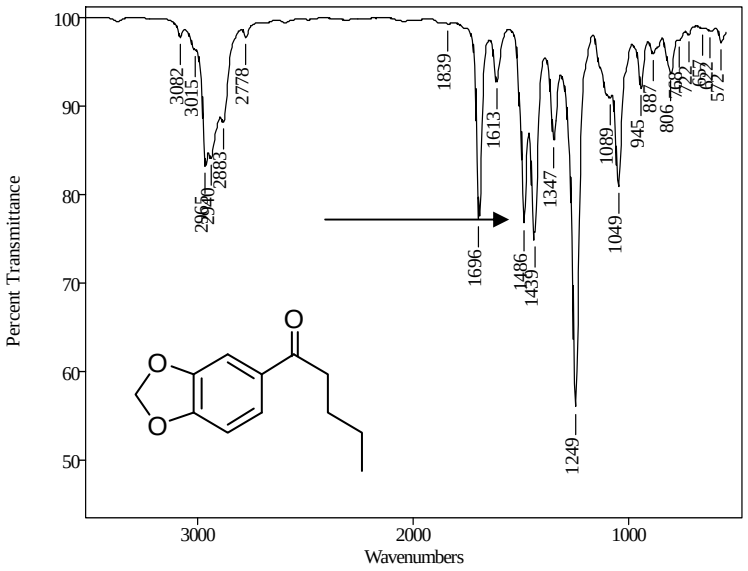
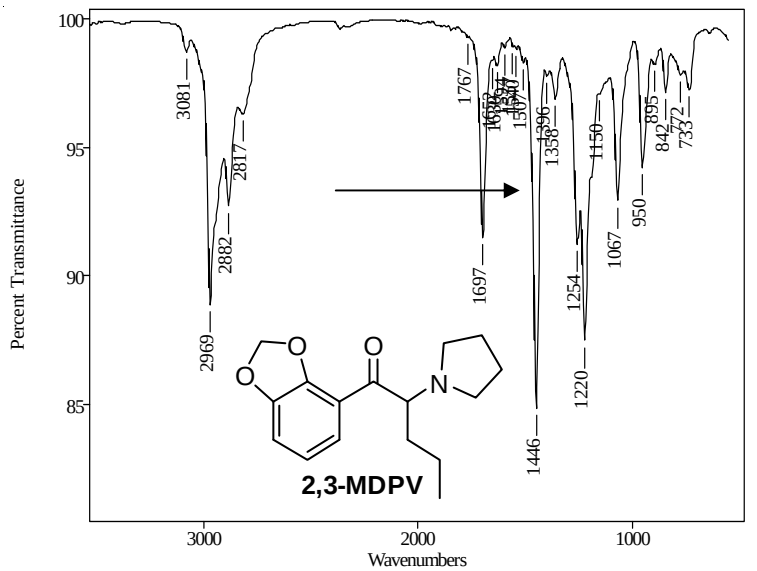


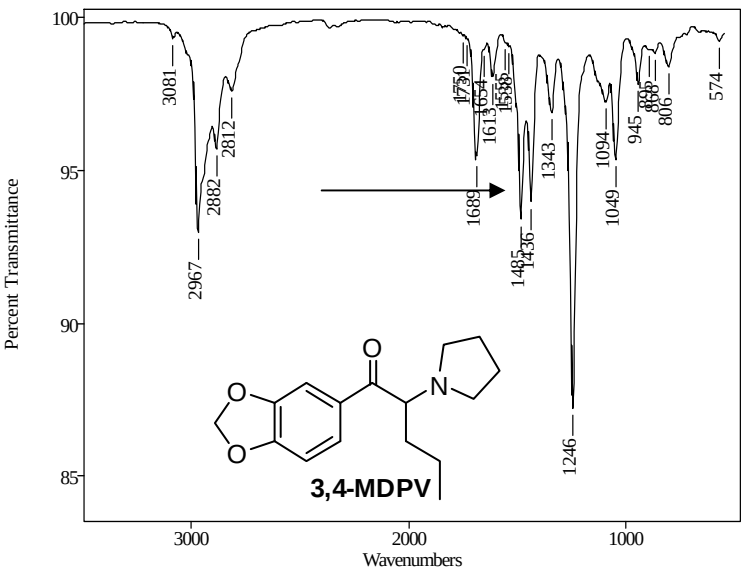


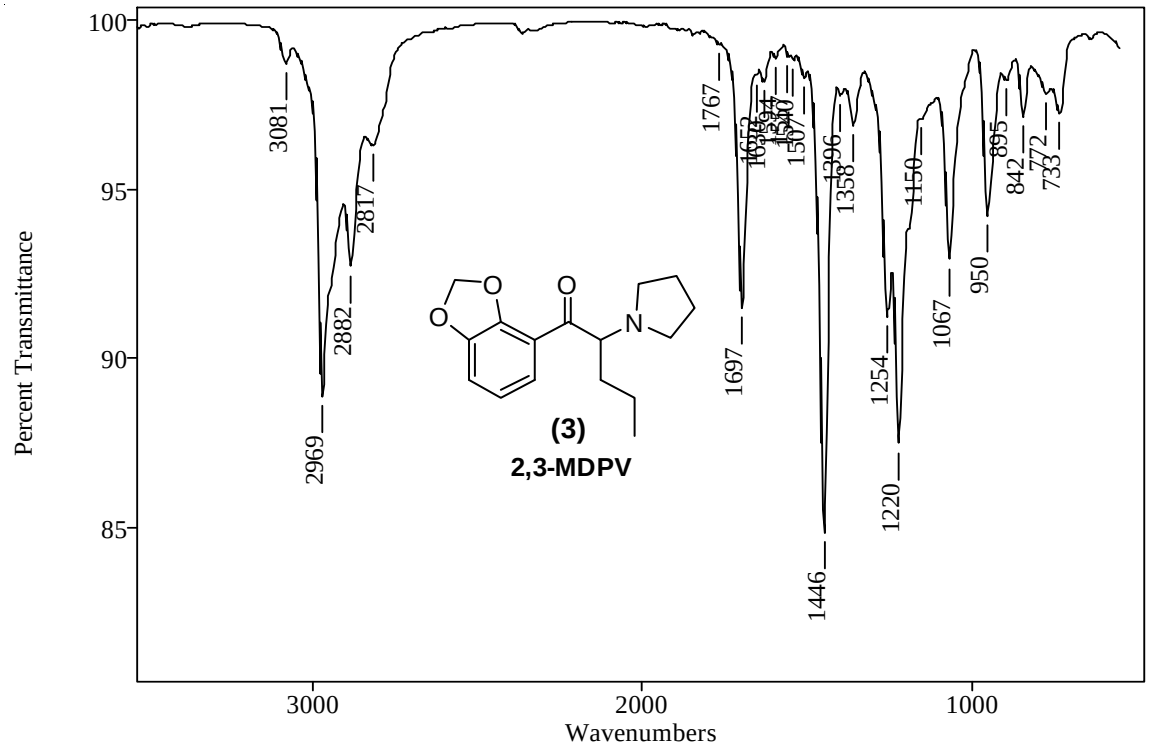


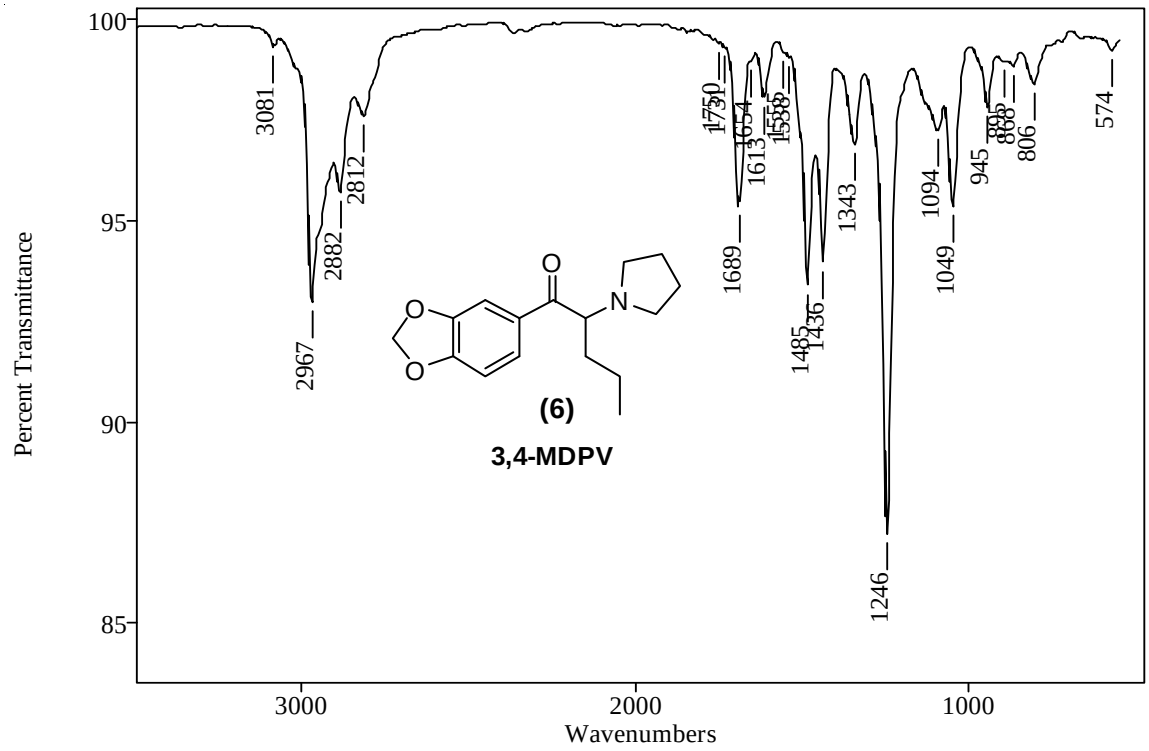






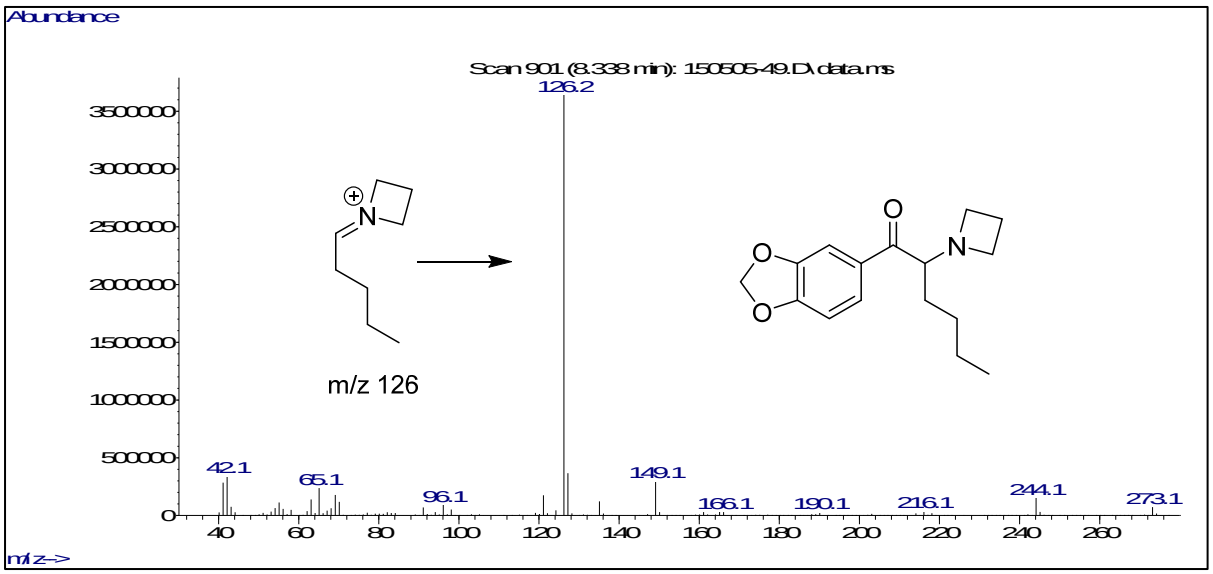


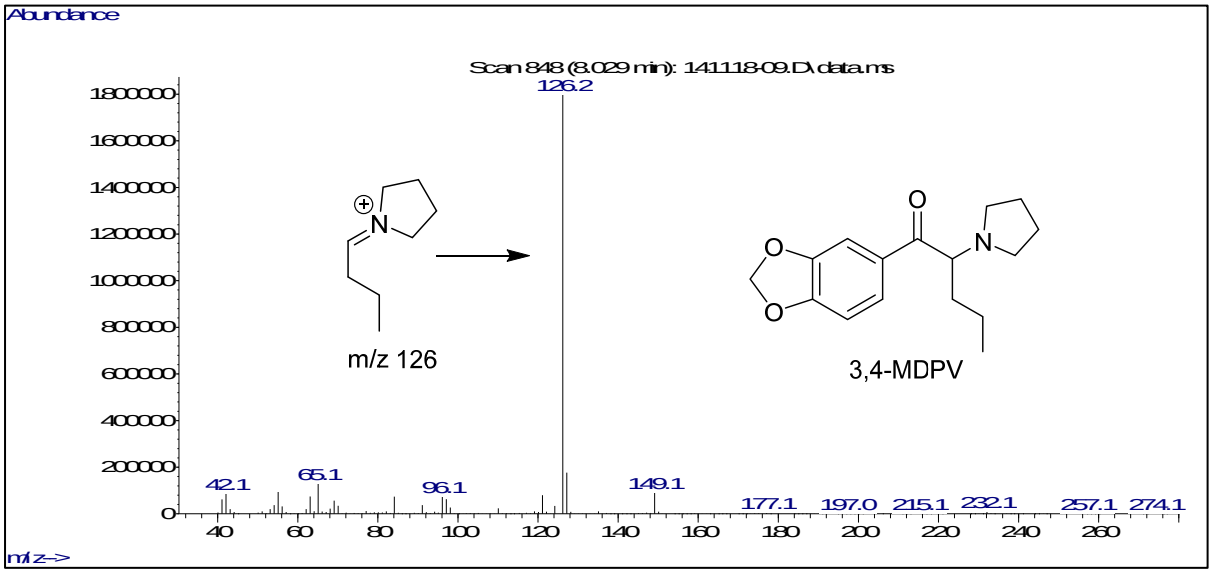


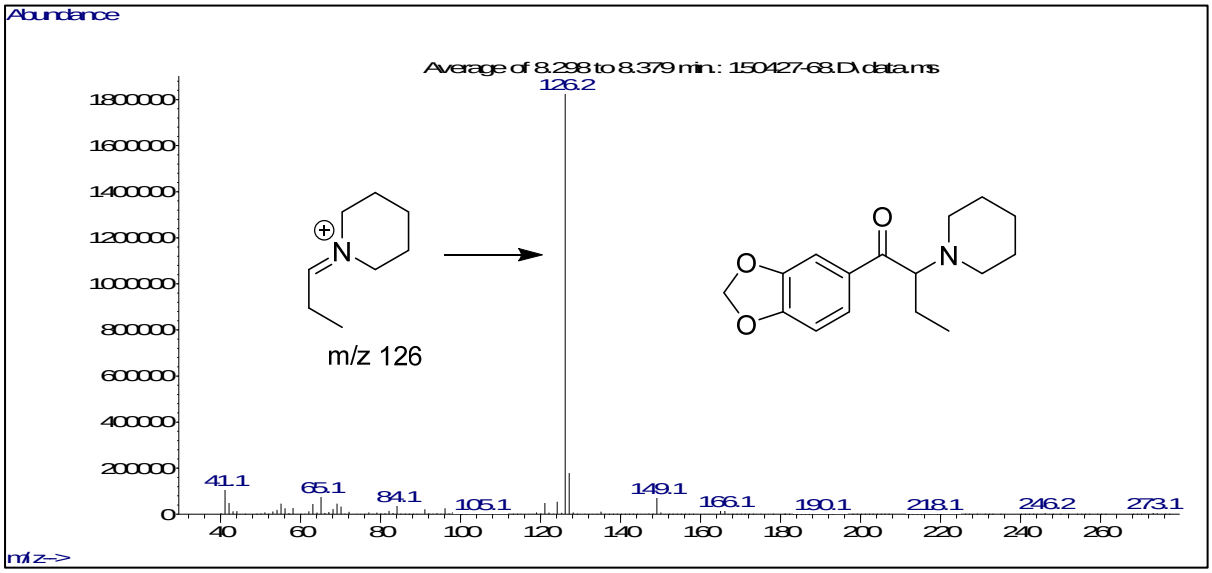


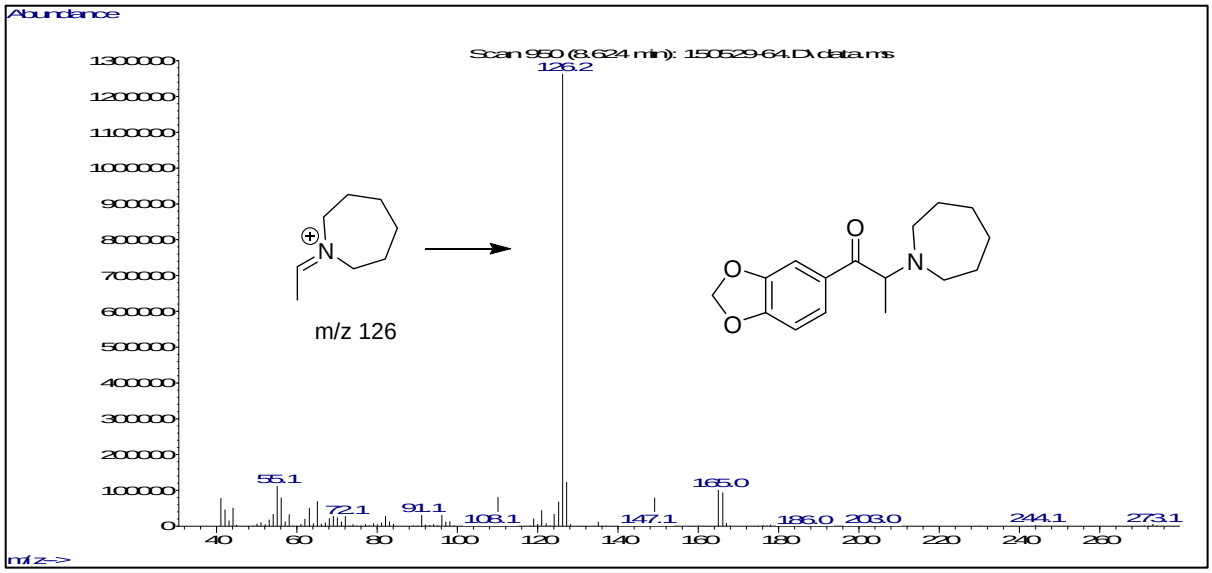
The cyclic amines azetidine, pyrrolidine, piperidine and azepane were incorporated into a series of aminoketones related to the cathinone derivative drug of abuse known as MDPV. Deuterium labeling in both the cyclic amine and alkyl side chain allowed for the confirmation of the structure for the major product ions formed from the EI-MS iminium cation base peaks. These iminium cation base peaks show characteristic product ion spectra which allow differentiation of the ring and side chain portions of the structure. The small alkyl side chains favor ring fragmentation in the formation of the major product ions. The higher side chain homologues appear to promote product ion formation by side chain fragmentation. Both side chain and ring fragmentation yield a mixture of product ions in the piperidine and azepane series.

The following mass spectra and product ion spectra show the results for the regioisomeric cyclic tertiary amines related to MDPV.

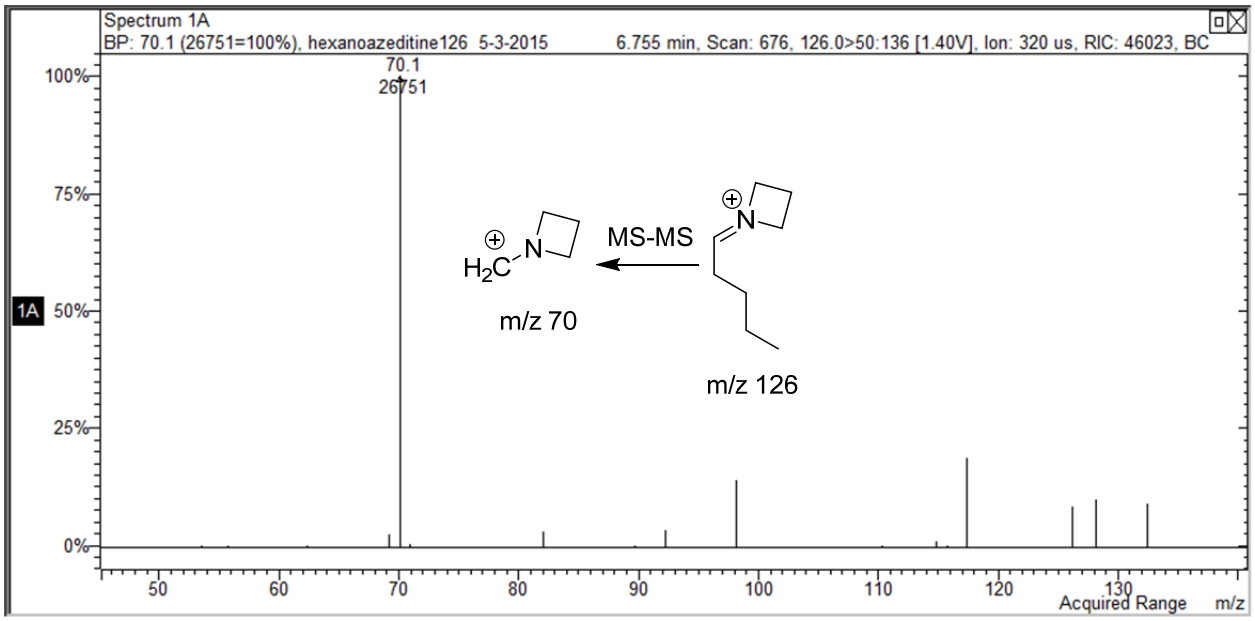


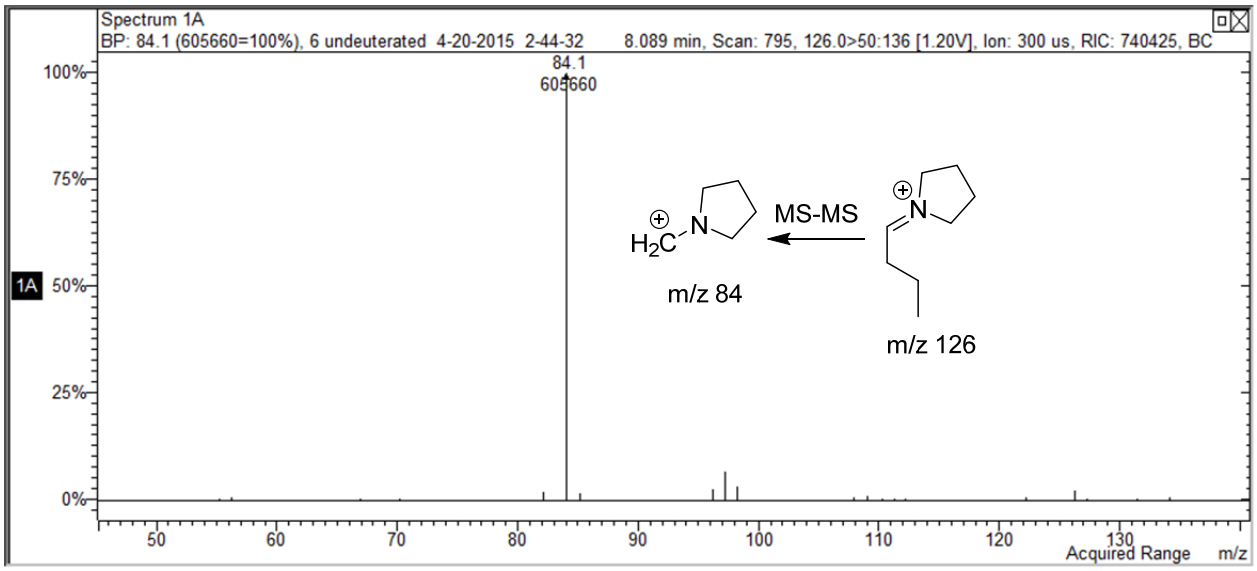


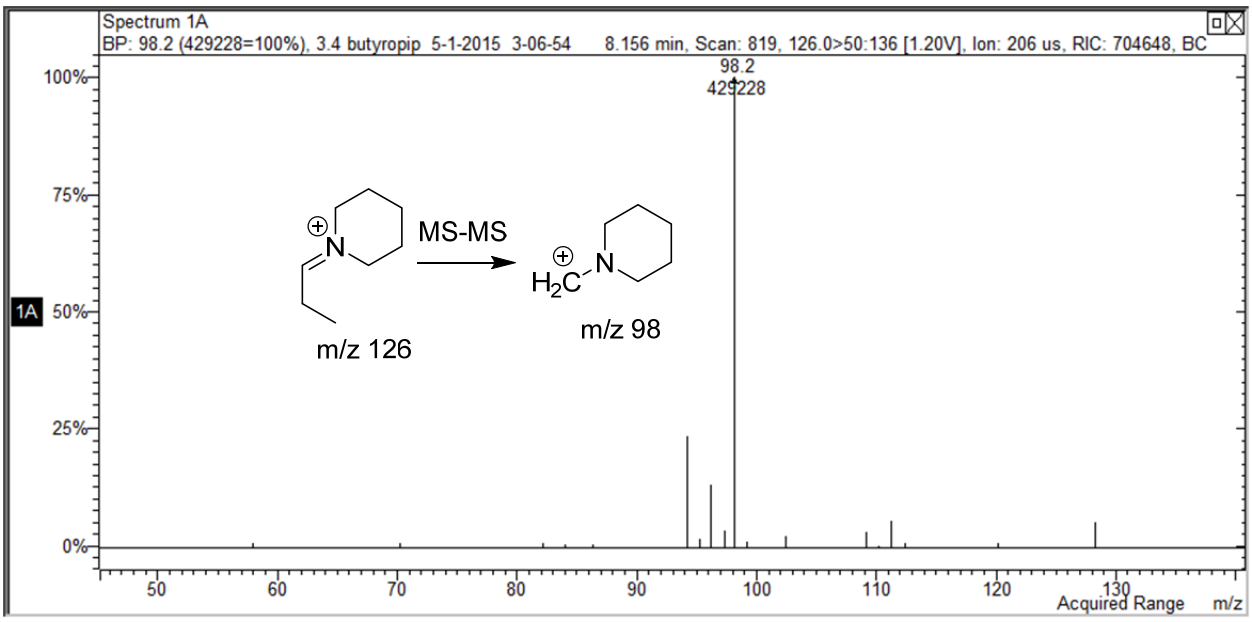


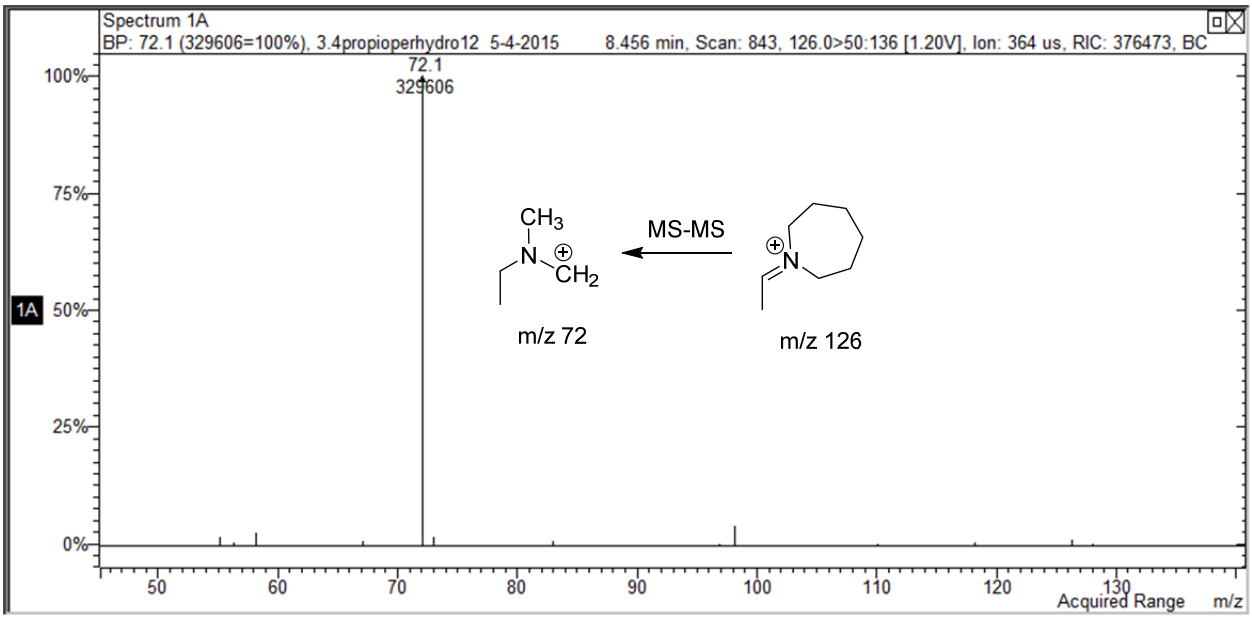


EI-MS for the four regioisomeric cathinone derivatives of MW= 275 and regioisomeric base peak iminium cations at m/z 126.









MS/MS product ion spectra for the four regioisomeric m/z 126 base peak iminium cations.