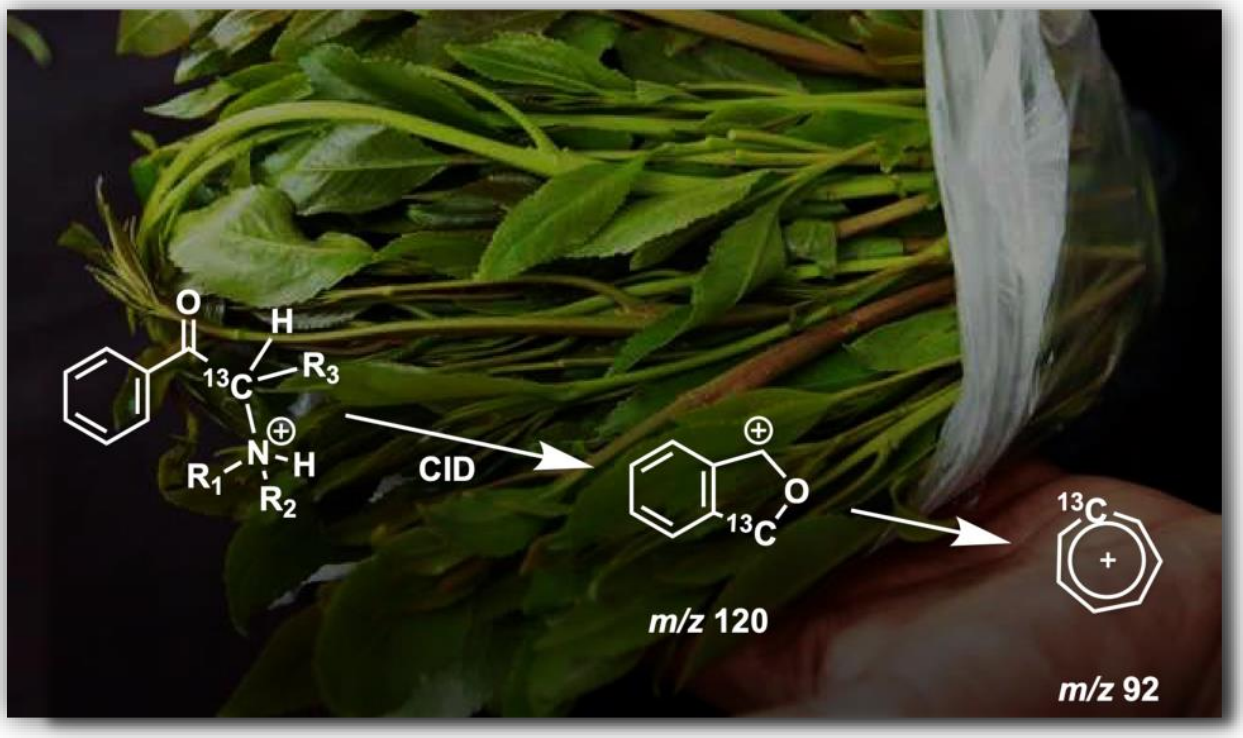
Structural Characterization of Emerging Synthetic Drugs



Final Research Report

Glen P. Jackson, C. Randall Clark

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Project Summary

The major goal of this project was to answer a defined research need of the NIST OSAC subcommittee on Seized Drugs, which was "to develop an interpretation strategy that allows consistent conclusions to be drawn from [spectrometric] data," and to provide guidance to the forensic community about how to extend such knowledge to the identification of future analogs. The identification of well-characterized seized drugs is performed thousands of times a day in the United States; however, the expanding use of emerging synthetic drugs is creating a growing problem for both toxicological and seized drug analyses. In this project, we demonstrate the combination of multi-stage mass spectrometry (MS^n), accurate mass measurements with high-resolution mass spectrometry (HRMS), and isotopic labeling for the structural characterization of synthetic cathinones and fentanyl analogs. The deliverables of this research include the identification of conserved fragmentation pathways for synthetic cathinones and fentanyl analogs, proposed mechanisms for the formation of characteristic ions through both protonated tandem mass spectrometry (MS/MS) and electron ionization mass spectrometry (EI-MS), and a discussion about how to apply the broadened understanding of the fragmentation behavior to the identification of novel synthetic cathinones and fentanyl analogs. This project helped answer the following questions: 1) what fragment ions are characteristic for a chosen subset of analogs of emerging synthetic drugs?; 2) to what degree can quantitative differences in ion abundances between two isomers be differentiated?; 3) by what mechanisms are diagnostic ions formed?; and 4) how do instrument conditions affect the generation of diagnostic ions?

The first major finding about the fragmentation behavior of synthetic cathinones is that the tropylium ion (*m/z* 91), or substituted derivative thereof, forms through different oxygen-containing intermediates that do not contain a formal C=O bond but instead contain a phthalane-like core structure. The phthalane-like intermediates were elucidated through gas-phase ion spectroscopy measurements and density functional theory calculations. Likewise, the use of stable isotope labeling revealed the unprecedented finding that, during collision-induced dissociation (CID) of α-pyrrolidinophenone synthetic cathinones, the α-carbon is retained almost exclusively in the tropylium ion and the carbonyl carbon is not retained in the tropylium ion. Isotope labeling also identified competitive pathways for the loss of CO and ethylene (C_2H_4) from a primary intermediate ion, which provides support for the direct loss of CO from the alkyl side chain.

A second major finding was the identification of characteristic protonated MS/MS fragmentation pathways and proposed mechanistic origins for both protonated MS/MS and EI-MS fragmentation for α-pyrrolidinophenone and *N*-alkylated synthetic cathinones. For MS/MS spectra of protonated α-pyrrolidinophenone synthetic cathinones. the dominant fragmentation pathways are through 6- or 4- center hydrogen rearrangements to produce pyrrolidine ring cleavage, characteristic iminium ions and diagnostic ions at *m/z* 91 and *m/z* 105. For EI mass spectra, radical-directed alpha cleavages result in dominant iminium ions. In contrast to the α-pyrrolidinophenones, MS/MS of protonated *N*-alkylated synthetic

cathinones provided abundant radical losses from both the *N*-alkylated and aliphatic side chains, a dominant loss of H_2O for 2° amines and no loss of H_2O for 3° amines. Instead, 3° amines formed abundant alkyl-phenones. These findings help advance our current understanding of the MS/MS analysis of synthetic cathinones, and they help analysts better understand and defend their observations and interpretations in existing and future casework.

For fentanyl-related compounds (FRCs), a combination of isotope labelling, HRMS and MS^n experiments identified a novel isobaric product ion at nominal *m/z* 188, which is elementally distinct from the previous two known isobars at nominal *m/z* 188 and forms through an intermediate product ion at *m/z* 216 (for fentanyl). These studies also confirmed the pathways through which the three nominal isobars are formed and how substitutions to the aniline ring and amide moieties result in remarkably conserved fragmentation pathways. In contrast, substitutions to the piperidine ring, the *N*-alkyl chain, and the cyclic substituent of FRCs resulted in distinct differences in fragmentation pathways, the abundance of which is related to the identity of the specific substitution. For example, an OH group on the alkyl chain between the piperidine ring and the phenyl group causes the MS/MS spectrum to be dominated by an uninformative neutral loss of water. By understanding the fragmentation behavior of fentanyl and the impact of substitutions to the core fentanyl structure, toxicologists and seized drug analysts will be better prepared to identify emerging FRCs, which are increasingly common and deadly adulterants in the growing opioid epidemic.

The final major contribution from this work was the comparison and contrast between in-source CID and beam-type CID experiments of the same cathinones and FRCs on the same instrument. Whereas the relative abundances of certain fragments were often readily distinguishable between in-source CID and beam-type CID, the fragment m/z values and the overall pattern of fragmentation were sufficiently consistent that the spectra from the two different activation methods could serve as proxies for one another. However, because in-source CID involves fragmentation of all precursors from the source within the source region of the mass spectrometer rather than through isolation and fragmentation in the collision cell, caution should be used when analyzing potential mixtures or complex biological samples where strict control of precursor ions present in the source region may not be possible.

By understanding the origins of mass spectral fragmentation rules of emerging synthetic drugs, and by explaining trends, mechanisms, and interpretation strategies in various presentations, workshops, and publications, we have helped disseminate the results to practitioners. We have therefore helped the criminal justice system to be prepared to respond more quickly to the emergence of new and dangerous drugs. Practitioners will be able to better assist emergency responders by more quickly and more confidently identifying the drugs involved in accidental overdoses in the future.

OUTCOMES

What were the major goals of the project?

The original proposal was separated into two main aims, each with four major goals or objectives.

Aim 1: Understand the mechanisms of fragmentation of odd-electron and even-electron cathinone analogs. One specific deliverable was to understand the mechanism of formation of the tropylium ion and the methylenedioxy analog of synthetic cathinones; these fragments are abundant and important, yet their formation mechanism remained elusive until our study. Aim 1 was accomplished through the following tasks:

1. Synthesized and/or acquired synthetic cathinones with the ^{13}C isotope label at the alpha or carbonyl position.
2. Synthesized and/or acquired deuterated synthetic cathinones with the D isotope labels on the pyrrolidine, benzyl, or alkyl moieties.
3. Synthesized and/or acquired synthetic cathinones with an ^{18}O isotope label in the carbonyl position.
3. Comparison of the fragmentation patterns of each synthetic cathinone using EI-MS, DART-MS/MS and ESI-MS/MS, Infrared ion spectroscopy, molecular modeling, and high-resolution mass spectrometry (HRMS).
4. Proposed fragmentation mechanisms for each compound and confirmed with MSn analyses of additional analogs.

Aim 2: Understand the mechanisms of fragmentation of odd-electron and even-electron fentanyl analogs. Aim 2 was accomplished through the following tasks:

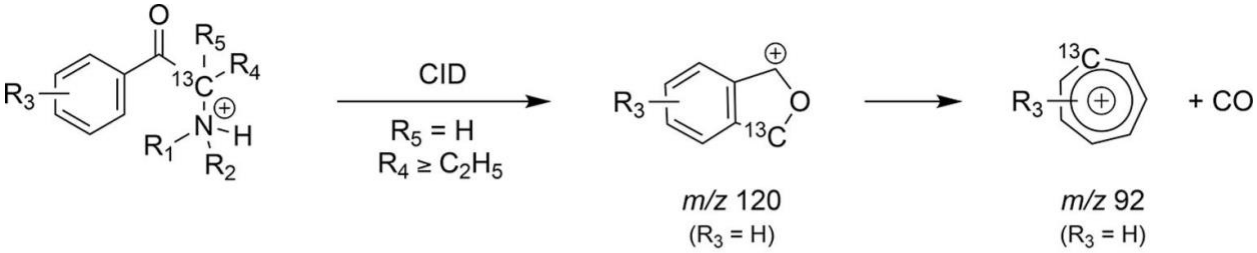
1. Synthesized and/or acquired fentanyl analogs with the ^{13}C isotope label in various positions.
2. Synthesized and/or acquired fentanyl analogs with D or ^{18}O labels in various positions.
3. Comparison of the fragmentation patterns of each synthetic fentanyl analog using EI-MS, DART-MS/MS and ESI-MS/MS, and HRMS.
4. Proposed fragmentation mechanisms for each compound and confirmed with MSn analyses of additional analogs.

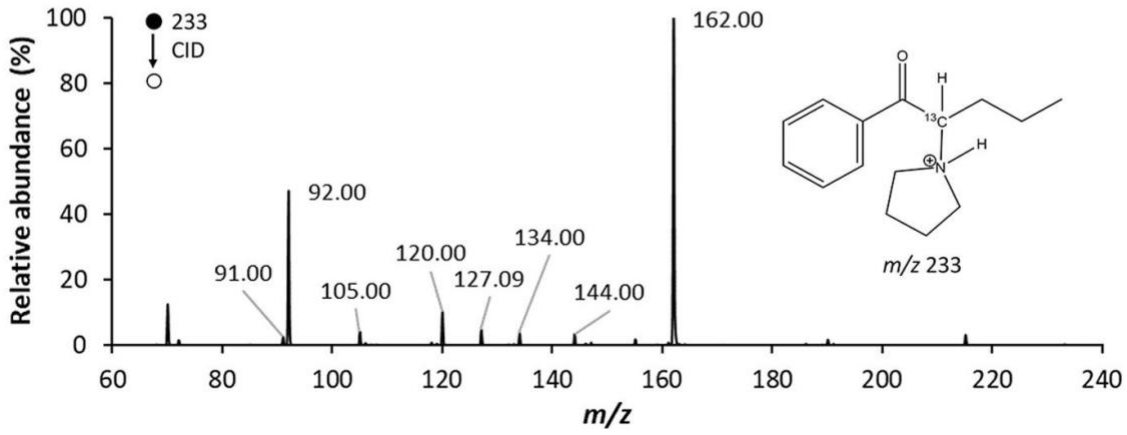
Activities/Accomplishments

The following paragraphs summarize the findings in seven different publications that resulted from this work.

Manuscript #1. Identification of novel fragmentation pathways and fragment ion structures in the tandem mass spectrometric analysis of protonated synthetic cathinones. Published in *Forensic Chem.*, **2020**, 19. 100245 (Open Access).
https://doi.org/10.1016/j.forc.2020.100245:

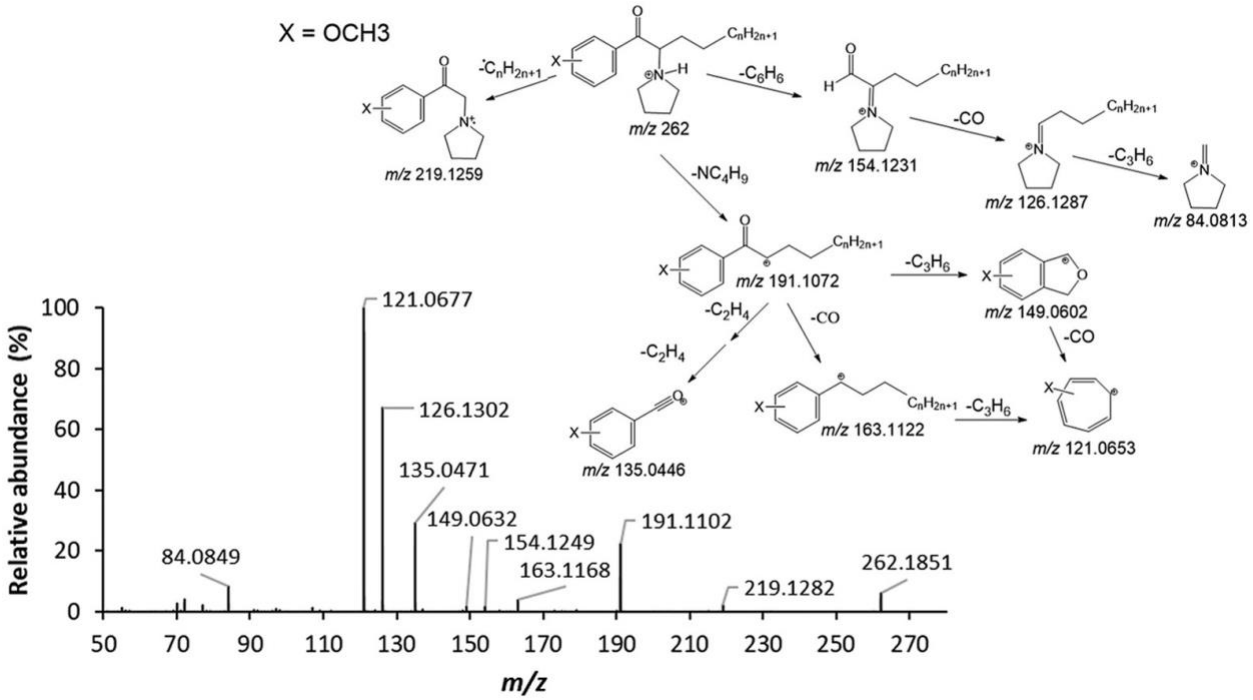
The expanding use of emerging synthetic drugs such as synthetic cathinones, or "bath salts", is a growing public health concern and a continual challenge for drug analysts. In the tandem mass spectra of protonated α-pyrrolidinophenone cathinones, the tropylium ion at *m/z* 91 is often among the most abundant product ions, but its mechanistic origin is currently unexplained. This project combined electrospray ionization multi-stage mass spectrometry (ESI-MS^n), high-resolution mass spectrometry (HRMS), isotopic labeling and ion spectroscopy to enhance our understanding of the fragmentation pathways and mechanisms of a variety of α-pyrrolidinophenone cathinones. The fragmentation trends derived from these ESI-MS/MS studies are: 1) unlike N-alkylated cathinones, abundant radical cations are not observed from even-electron precursors of α-pyrrolidinophenones; 2) the loss of a 71 Da pyrrolidine neutral to form an alkylphenone cation is always observed; 3) a series of neutral alkenes are lost from the alkylphenone cation to form intermediate cations with phthalane-like structures. The phthalane intermediates then eliminate the carbonyl carbon as CO or C_2H_2O to form a tropylium ion at *m/z* 91. The α-carbon of the original cathinone is almost exclusively retained in the tropylium ion. If the original cathinone is substituted on the aromatic ring, the observed tropylium ion will be shifted by the mass of the substitution. These findings explain the characteristic ions in ESI-MS/MS spectra of synthetic cathinones and will help analysts better employ mass spectral observations in future casework.





Manuscript #2. Fragmentation pathways of α-pyrrolidinophenone synthetic cathinones and their application to the identification of emerging synthetic cathinone derivatives. Published in *Int. J. Mass Spectrom.* **2020**, 453, 116343. https://doi.org/10.1016/j.ijms.2020.116343.

The expanding use of emerging synthetic drugs is creating a growing problem for both seized drug analysts and toxicologists because the clandestine suppliers continually tweak the chemical structures to keep one step ahead of the law. Synthetic cathinones, commonly referred to as bath salts, are a specific class of emerging synthetic drugs. These substances are derivatives of cathinone, which is the psychoactive component of the Catha edulis plant, commonly referred to as khat. Of the synthetic cathinone class of compounds, the a-pyrrolidinophenone synthetic cathinone derivatives stand out as one of the most abused designer drugs.

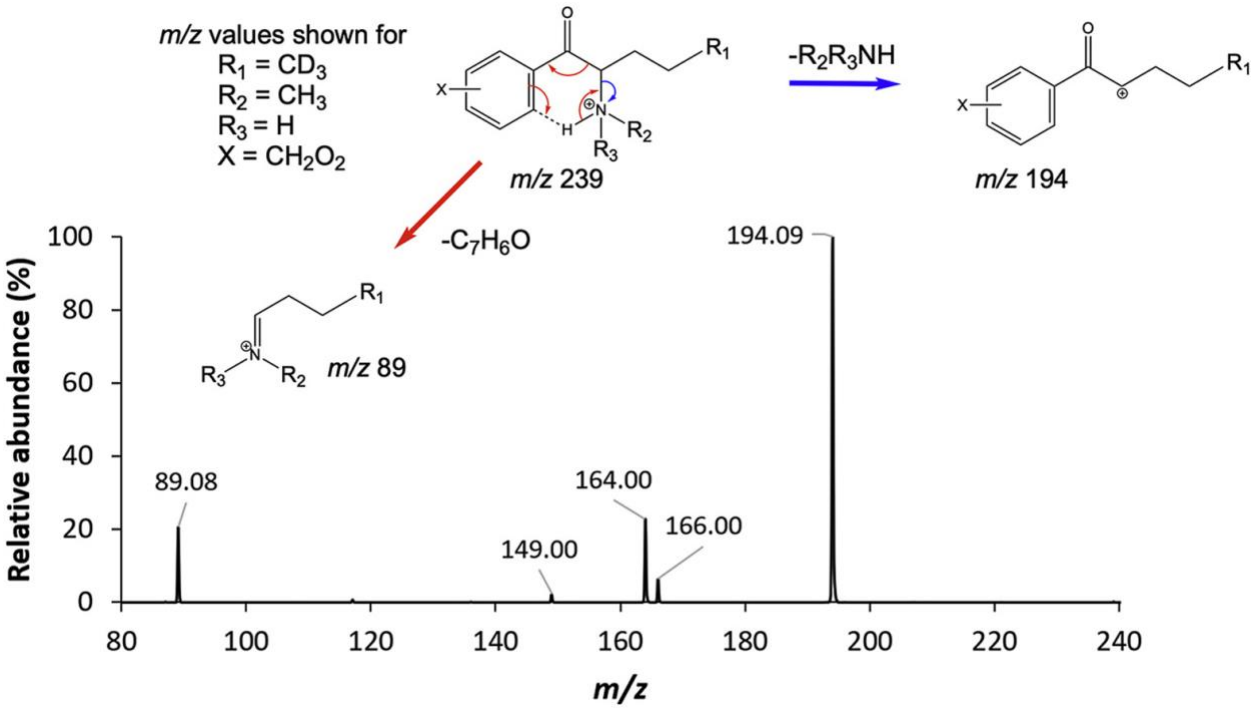


The fragmentation behavior of a series of a-pyrrolidinophenone synthetic cathinones was studied with three different ionization and fragmentation techniques to enhance the current understanding of pyrrolidinophenone synthetic cathinones in mass spectrometers. Gas chromatography-electron ionization- mass spectrometry (GC-EI-MS) fragmentation is commonly used by seized drug analysts, whereas liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) is more commonly used in toxicological analyses. Direct analysis in real time mass spectrometry (DART-MS) is becoming more popular as a screening technique, especially in national laboratories. Each ionization and activation method encourage particular pathways of fragmentation, and whereas some pathways are conserved across all platforms, other pathways are unique to a particular instrument. This study combines isotope-labeling, multi-stage mass spectrometry (MS^n) and accurate mass measurements with high-resolution mass spectrometry (HRMS) to

enhance the current understanding about a-pyrrolidinophenone synthetic cathinones. This manuscript provides characteristic protonated tandem mass spectrometry fragmentation pathways and the mechanistic origins of the EI-MS fragmentation observed for this class of synthetic cathinones and provides examples of how this knowledge can be applied to the identification of novel synthetic cathinones.

Manuscript #3. Fragmentation pathways of odd- and even-electron N-alkylated synthetic cathinones. Published in *Int. J. Mass Spectrom.* **2020,** 453, 116354. https://doi.org/10.1016/j.ijms.2020.116354

Three ionization techniques, isotopic labeling, high-resolution mass spectrometry (HRMS) and multistage mass spectrometry (MS^n) were used to analyze a series of N-alkylated synthetic cathinone derivatives and gain a deeper understanding of their fragmentation behavior during mass spectrometric analysis. The compounds analyzed represent 15 unique structures with common substitutions to the core synthetic cathinone structure, including substitutions to the aromatic ring and the number and types of N-alkyl functionalities.



The analytical techniques employed include gas chromatography-electron ionization-mass spectrometry (GC-EI-MS), electrospray ionization-tandem mass spectrometry (ESI-MS/MS) with HRMS and direct analysis in real time tandem mass spectrometry (DART-MS/MS) with HRMS. These techniques cover a variety of forensic applications, including seized drug analysis, toxicological analysis and screening analysis, in local, state and federal laboratories. For collision-induced dissociation (CID) of protonated precursors, the spectra of 2° and 3° amines showed evidence for charge-remote and charge-irected fragmentation mechanisms.

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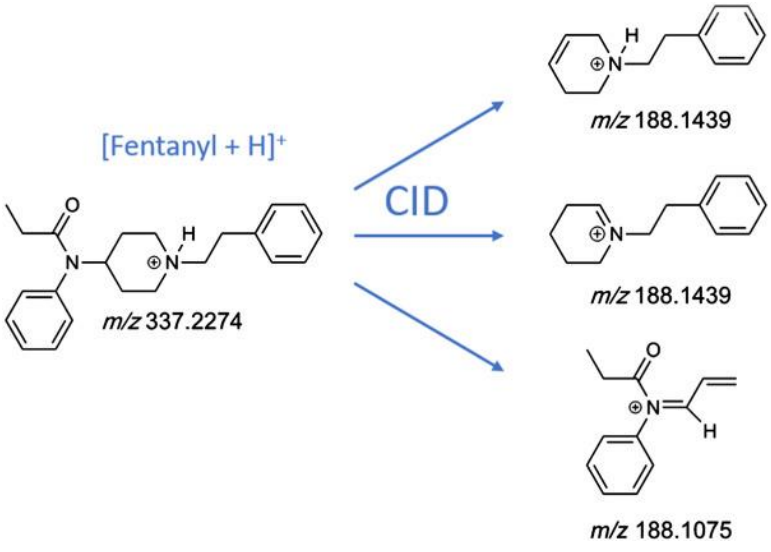
The 2° amines lost H_2O as a dominant pathway whereas the 3° amines favored the formation of alkylphenones. As reported by others, CID of protonated N-alkylated cathinones containing 2° and 3° amines also provided an abundance of odd-electron product ions from the even-electron precursors. In contrast to the rearrangements observed in CID of protonated cathinones, EI fragmentation patterns were dominated by radical-directed cleavages to form iminium ions and charge-directed cleavages to form acylium ions. A comparison between the fragmentation behaviors of N-alkylated synthetic cathinones under all three ionization techniques enables a deeper understanding of N-alkylated synthetic cathinone fragmentation under varying instrumental setups.

Manuscript #4. The characterization of isobaric fentanyl product ions using multi-stage mass spectrometry, high-resolution mass spectrometry and isotopic labeling. Published in *Drug Test. Anal.,* **2020,** 12, 496-503. https://doi.org/10.1002/dta.2758.

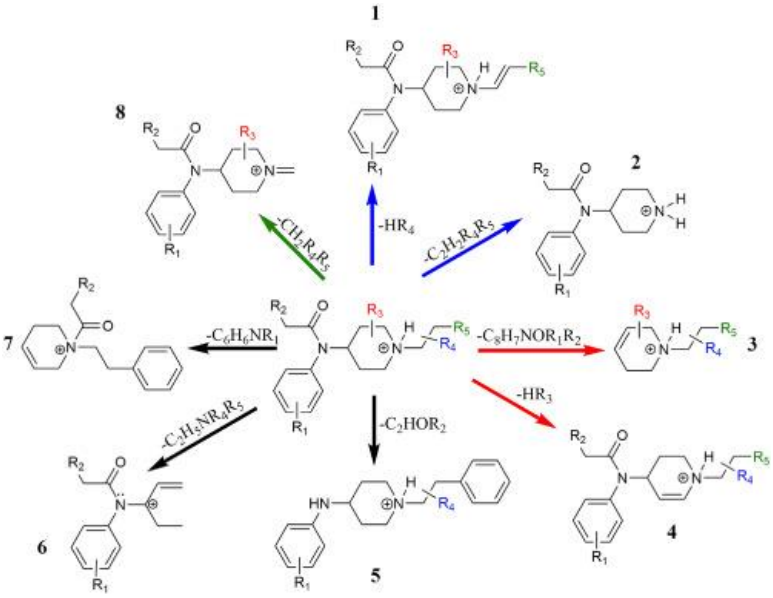
This study uses a combination of multi-stage mass spectrometry (MS^n), accurate mass measurements with high-resolution mass spectrometry (HRMS) and isotopic labeling to characterize the fragmentation behavior of fentanyl and 4-ANPP. By understanding the fragmentation behavior of fentanyl and its analogs in more detail, toxicologists and seized drug analysts will be better poised to identify new and emerging fentalogs, which are increasingly common and deadly adulterants in the growing opioid crisis.

Throughout the literature the product ion at m/z 188 is often the most abundant fragment in the mass spectrometric analysis of fentanyl and fentanyl analogs, and this fragment is used for both qualitative and quantitative determinations. Our work shows there are at least three different structures for the isobaric fentanyl product ions at m/z 188, and they each form and fragment via different pathways. The development of fragmentation mechanisms to explain the observed fragmentation pathways of fentanyl and its main precursor 4-ANPP helps contribute to the advancement of knowledge about fentanyl fragmentation and could provide important information for the identification of future fentanyl analogs.



Manuscript #5. The influence of chemical modifications on the fragmentation behavior of fentanyl and fentanyl-related compounds in electrospray ionization tandem mass spectrometry. Published in *Drug Test. Anal.,* **2020,** 12, 657-967. https://doi.org/10.1002/dta.2794

Fentanyl is a synthetic opioid that has been approved by the FDA as a general anesthetic because of its rapid onset and high potency. However, since 2013 an opioid epidemic involving fentanyl or fentanyl-related compounds (FRCs) has swept the United States and caused numerous deaths in every state. The identification of novel FRCs is complicated by the rapid turnover of modifications to the core fentanyl structure. In this study, a series of 16 FRCs were analyzed using electrospray ionization tandem mass spectrometry (ESI-MS/MS) to gain a deeper understanding of the conserved and unique fragmentation behaviors associated with substitution to the core fentanyl structure.

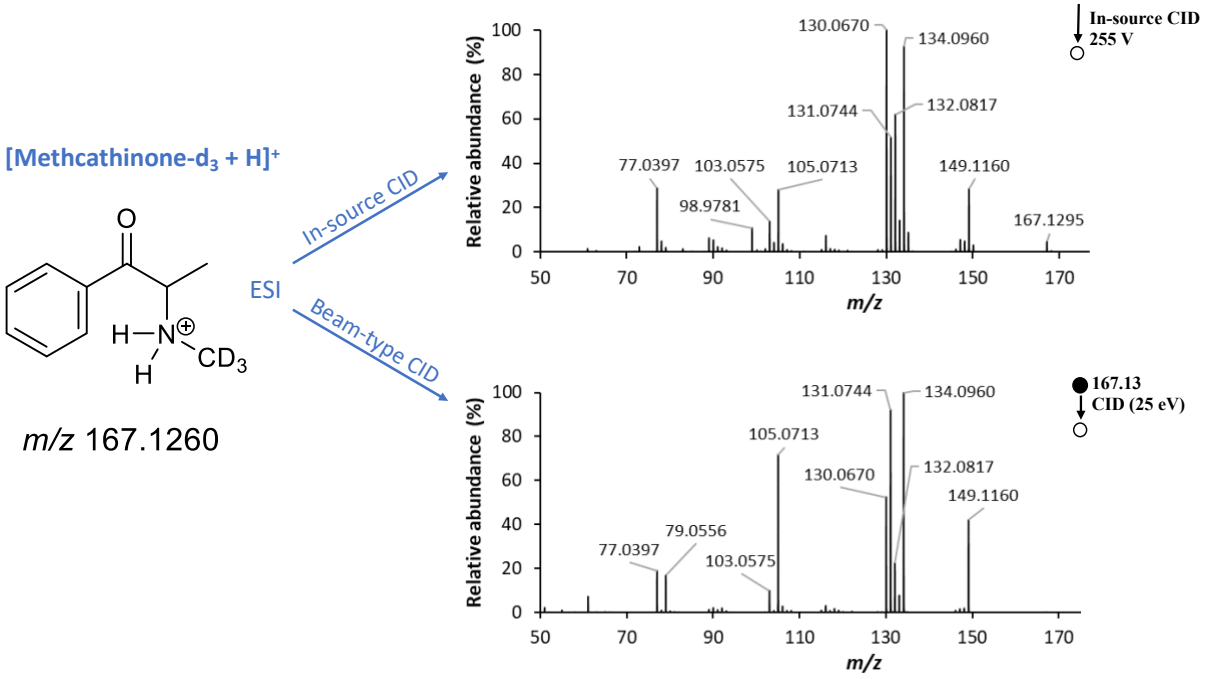


This work provides an approach, based on the product ions from ESI-MS/MS, to identify the modification site(s) on the core fentanyl structure for FRCs. Five common locations of substitution to the core fentanyl structure were used to assess the effect of substitution on the fragmentation behavior of FRCs. The proposed fragmentation pathways are supported through the combination of isotopic labeling, multi-stage mass spectrometry (MS^n), and accurate mass measurements with high-resolution mass spectrometry (HRMS). The identification of primary product ions specific to regions of substitution provides an additional tool for the identification of the location of substitution to the core fentanyl structure, which ultimately will assist toxicologists and seized drug analysts in the identification of emerging FRCs.

Manuscript #6. Comparison of in-source collision-induced dissociation and beam-type collision-induced dissociation of emerging synthetic drugs using a high-resolution quadrupole time-of-flight (Q-TOF) mass spectrometer. Published in *Rapid Commun. Mass Spectrom*. **2020**, 56, e4679. https://doi.org/10.1002/jms.4679.

In-source collision-induced dissociation (CID) is commonly used with single-stage high-resolution mass spectrometers to gather both a molecular formula and structural information through the collisional activation of analytes with residual background gas in the source region of the mass spectrometer. However, unlike tandem mass spectrometry, in-

source CID does not involve an isolation step prior to collisional activation leading to a product ion spectrum composed of fragment ions from any analyte present during the activation event. This work provides the first comparison of in-source CID and beam-type CID spectra of emerging synthetic drugs on the same instrument to understand the fragmentation differences between the two techniques and to contribute to the scientific foundations of in-source CID.

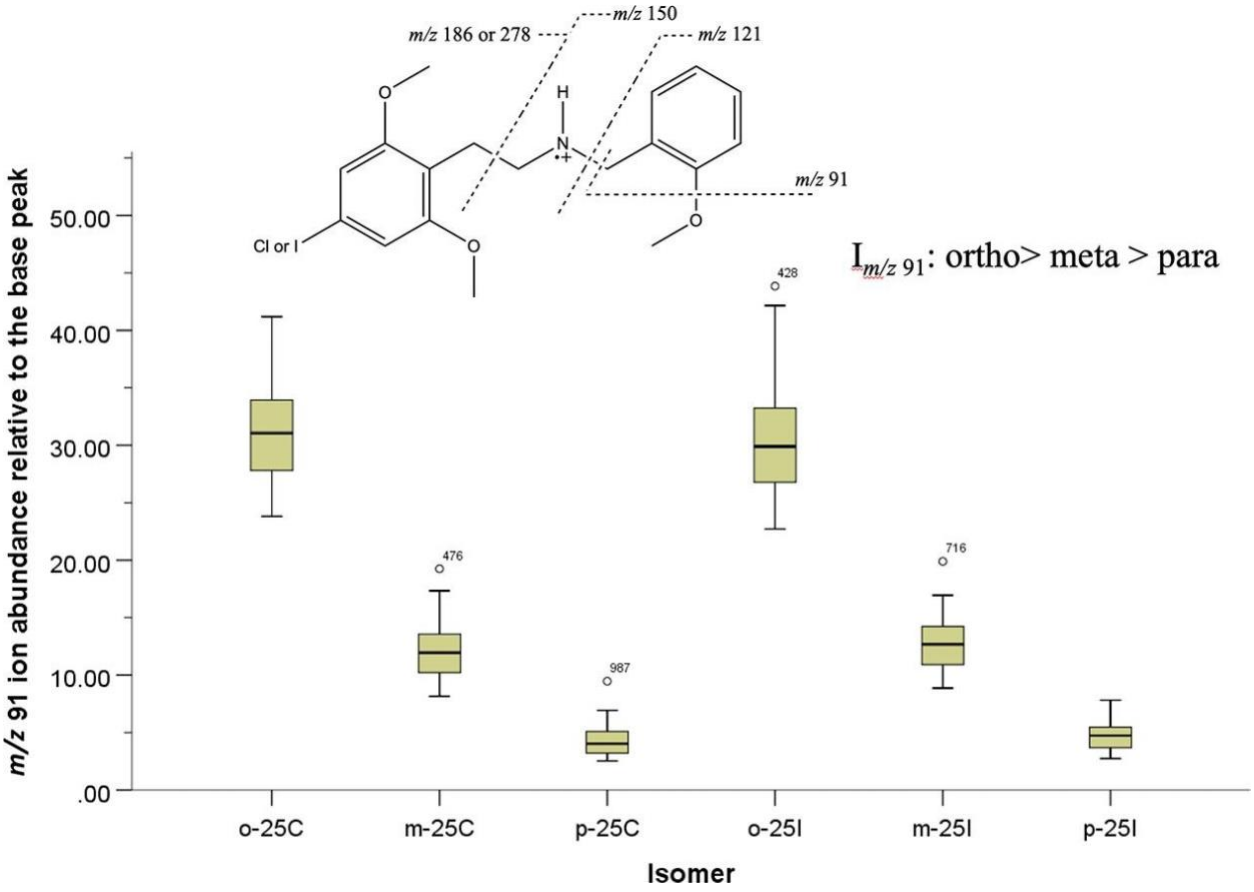


Electrospray ionization–quadrupole time-of-flight (ESI-Q-TOF) mass spectrometry was used to generate product ion spectra from in-source CID and beam-type CID for a series of well-characterized fentanyl analogs and synthetic cathinones. A comparison between the fragmentation patterns and relative ion abundances for each technique was performed over a range of fragmentor offset voltages for in-source CID and a range of collision energies for beam-type CID. The results indicate that large fragmentor potentials for in-source CID tend to favor higher energy fragmentation pathways that result in both kinetically favored pathways and consecutive neutral losses, both of which produce more abundant lower mass product ions relative to beam-type CID. Although conditions can be found in which in-source CID and beam-type CID provide similar overall spectra, the in-source CID spectra tend to contain elevated noise and additional chemical background peaks relative to beam-type CID.

Manuscript #7. The differentiation of 2,5-dimethoxy-N-(N-methoxybenzyl)phenethylamine (NBOMe) isomers using GC retention indices and multivariate analysis of ion abundances in electron ionization mass spectra. Published in *Forensic Chem.* **2019**, 14, 100160. https://doi.org/10.1016/j.forc.2019.100160

Synthetic phenethylamine derivatives known as 2,5-dimethoxy-N-(N-methoxybenzyl)phenethylamines (NBOMes) are a common class of novel psychoactive

substances (NPS) that are causing many accidental deaths across the United States. Many derivatives are now banned at the federal and state levels, but such control requires reliable identification of the different positional isomers. This manuscript helps establish retention indices and characteristic ion ratios that can be used to distinguish between the positional isomers of 25C-NBOMe and 25I-NBOMe. This manuscript also provides additional support for the ortho effect as a reliable, general, fragmentation mechanism to differentiate positional isomers of NBOMes in electron ionization (EI) mass spectra.



The retention indices and fragment ion abundances of the positional isomers of 25C-NBOMe and 25I-NBOMe were measured on two instruments using three different GC columns and parameters. The measured retention indices for the six compounds on three different 5% diphenyl columns are as follows: ortho-25C-NBOMe = 2614 ± 15; meta-25C-NBOMe = 2666 ± 13; para-25C-NBOMe = 2692 ± 13; ortho-25I-NBOMe = 2821 ± 16; meta-25I-NBOMe = 2877 ± 15; and para-25I-NBOMe = 2904 ± 12, where the errors represent the 95% confidence interval of the measurements. Principal component analysis (PCA) and canonical discriminant analysis (CDA) were used, respectively, to assess the variance and classification of NBOMe isomers based on the 15 most abundant ions relative to the base peak. The CDA classification accuracy for the six NBOMe compounds was 99.5% when the data set included spectra from three instrumental setups and the widest range of concentrations. Isomer classification was greater than 99.9% within an instrument and

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excluding low abundance spectra. These results support the use of chemometric approaches for the classification of unknown compounds, even when non-ideal lower-abundance spectra are used for classification.

Mass spectral comparison algorithm

As described in the original proposal, we also made progress on a new algorithm to assist practitioners with identifying substances from their mass spectra. Although there a dozens of commercial algorithms available for the purpose, existing algorithms struggle to cope with the variance in ion abundances cause by different mass spectrometer platforms. The proposed algorithm formed the basis of a new grant proposal, which was recently funded by NIJ (15PNIJ-21-GG-04179-COAP) titled "Expert Algorithm for Substance Identification (EASI)." Work on the algorithm is currently in progress.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

The following researchers contributed to the project:

Dr. Glen Jackson (WVU), Principal Investigator
Contribution to project: Responsible for managing the project at WVU. Dr. Jackson oversaw the instrument analysis and data analysis aspects of the project. He also oversaw the administrative aspects of the project, including the preparation of presentations, manuscripts and reports.

Dr. J. Tyler Davidson (WVU), PhD Graduate Student in FIS
Contribution to project: Tyler helped conceive of the original proposal and experiments, he took the lead on most of the data analysis and spectral interpretation, he helped communicate with the various collaborators, and he also drafted the seven publications that resulted from this work. Six of these manuscripts formed the basis of his dissertation. He has served as the first point of contact for other students on the project.
Funding support: Tyler was primarily funded through this NIJ award.

Dr. Zachary J. Sasiene (WVU), PhD Graduate Student in Chemistry
Contribution to project: Zach helped Tyler with MS/MS data analysis and spectral interpretation, and he helped draft and edit several manuscripts.

Ms. Alexandra Adeoye (WVU), MS Graduate Student in FIS
Contribution to project: Alex contributed through the development of the mass spectral comparison algorithm.

Ms. Alia Hacker (WVU)

MS Graduate Student in FIS
Contribution to project: Alia studied a novel fragmentation pathway that we recently identified for fentanyl analogs. The pathway involves an intramolecular transamidation, such as through the transfer of the propionaldehyde group from the amide group to the pyrrolidine ring (for fentanyl).

Ms. Isabel Galvez (WVU), MS Graduate Student in FIS
Contribution to project: Isabel has been focusing on the instrumental variables that influence the fragment ion abundances in replicate EI spectra. She has used cocaine as a model compound, and through ANOVA.

Ms. Samantha Mehnert (WVU), Undergraduate Student in Chemistry
Contribution to project: Sam collected MS data of various drugs, on various instruments, and she helped develop the algorithm using cocaine as a model compound.

Ms. Emily Ruiz (WVU), Undergraduate Student in Chemistry
Contribution to project: Emily collected MS data in a GC-MS instrument. To help support the development of the mass spectral comparison algorithm.

Mr. Jacob King (WVU), Undergraduate Student in Chemistry
Contribution to project: Jacob collected ESI-MS/MS data for CBD and THC on the Q-TOF instrument. He helped developed the algorithm to discriminate between these two cannabinoids with greater than 95% confidence.

Dr. Randall Clark (Auburn U), Co-Principal Investigator
Contribution to project: Prof. Clark oversaw the organic syntheses of the synthetic cathinones at Auburn University. He also contributed to the syntheses of the fentanyl analogs. He supervised the postdoctoral researcher on the project, and he helped devise synthetic strategies for the isotope-labeled targets.

Dr. Jack DeRuiter (Auburn U), Co-Investigator
Contribution to project: Prof. DeRuiter oversaw the organic syntheses of the fentanyl analogs at Auburn University. He also contributed to the syntheses of the cathinone analogs. He co-supervised the postdoctoral researcher on the project, and he helped devise synthetic strategies for the isotope-labeled targets.

Dr. Younis F. Abiedalla (Auburn U), Postdoctoral researcher
Contribution to project: Dr. Abiedalla conducted the organic syntheses of the cathinone and fentanyl analogs at Auburn University, and he ensured their purification and structural characterization before sending them to WVU for analysis. He also helped edit manuscripts.

Dr. Victor Rhyzov (Northern Illinois U), Professor

Contribution to project: Dr. Rhyzov oversaw the ion spectroscopy measurements and DFT calculations to help identify the structure of the precursor ions at m/z 133 and 119 in the fragmentation of cathinones.

Dr. Elettra L. Piacentino (Northern Illinois U), Graduate Student
Contribution to project: Elettra conducted the DFT calculations to help identify the structure of the precursor ions at m/z 133 and 119 in the fragmentation of cathinones.

Dr. Jos Oomens (FELIX Laboratory, Radboud University), Professor
Contribution to project: Prof. Oomens helped conduct the ion spectroscopy measurements at the FELIX laboratory in the Netherlands to help identify the structure of the precursor ions at *m/z* 133 and 119 in the fragmentation of cathinones.

Dr. Giel Berden (FELIX Laboratory, Radboud University), Researcher
Contribution to project: Dr. Berden helped conduct the ion spectroscopy measurements at the FELIX laboratory in the Netherlands to help identify the structure of the precursor ions at *m/z* 133 and 119 in the fragmentation of cathinones.

Other organizations involved as partners

Broward Sherriff's Crime Laboratory (in Broward County, FL), the Virginia Department of Forensic Science (VDFS), the University of Massachusetts Drugs of Abuse Laboratory, and the Drug Enforcement Agency. Individuals involved include Benny Lum (Broward Sherriff's Crime Laboratory), Dr. Gina Nano (U. Mass. Drugs of Abuse Lab), Ms. Jennifer Bonetti (VDFS) and Dr. Jeanette Perr (DEA special testing, Dulles, VA). All provided spectra for the development of the mass spectral comparison algorithm,

Opportunities for training and professional development

At WVU, at least eight students were involved with the project, including five graduate students and three undergraduates. PhD Student J. Tyler Davidson helped conceive of the original proposal and experiments, he took the lead on most of the data analysis and spectral interpretation, he helped communicate with the various collaborators, and he also drafted the seven publications that resulted from this work. Six of these manuscripts formed the basis of his dissertation. He has served as the first point of contact for other students on the project. Dr. Sasiene assisted with the tandem mass spectrometry experiments and with data analysis. An MS student, Alia Hacker, made significant progress on understanding the factors that influence a particular R-group transfer (an intramolecular trans-amidation) during the fragmentation of fentanyl analogs, and that project is still ongoing. An MS student, Alexandra Adeoye, has been making significant progress on developing the MS algorithm to fentanyl analogs. All the students have been trained in the procedures for sample preparation and dilution, GC-MS analysis, model application, and data interpretation. All students have been actively involved in preparing abstracts, progress reports, conference presentations and manuscripts.

Ms. Mehnert and Ms. Ruiz have both graduated with the BS degrees and are now pursuing PhDs in chemistry. Ms. Adeoye is now pursuing her PhD in forensic science and Dr. Sasiene is a postdoctoral fellow at Los Alamos National Laboratory. Dr. Davidson is now an assistant professor in the forensic science department at Sam Houston State University.

At Auburn, all of the synthetic work was conducted by a postdoctoral researcher, Dr. Younis Abiedalla, and he, Dr. Clark and Dr. DeRuiter all assisted with drafting the collaborative manuscripts. We also collaborated with Prof. Ryzhov and his students at U. Northern Illinois, and he and his student conducted spectroscopic measurements at the synchrotron light source in the Netherlands (with Dr. Oomens et al.).

Artifacts and Dissemination Activities

How have the results been disseminated to communities of interest?

Publications:

1) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, "Comparison of In-Source Collision-Induced Dissociation and Beam-Type Collision-Induced Dissociation of Synthetic Cathinones and Fentanyl Analogs using a High-Resolution Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer," *J. Mass Spectrom.*, **2020**, e4679.
2) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, "Fragmentation pathways of odd- and even-electron *N*-alkylated synthetic cathinones," *Int. J. Mass Spectrom.*, **2020**, 453, 116354.
3) J. T. Davidson, Z. J. Sasiene, Y. Abiedalla, J DeRuiter, C. R. Clark, G. P. Jackson, "Fragmentation pathways of α-pyrrolidinophenone synthetic cathinones and their application to the identification of emerging synthetic cathinone derivatives," *Int. J. Mass Spectrom.*, **2020**, 453, 116343.
4) J. T. Davidson, E. L. Piacentino, Z. J. Sasiene, Y. Abiedalla, J DeRuiter, C. R. Clark, G. Berden, J. Oomens, V. Ryzhov, G. P. Jackson, "Identification of Novel Fragmentation Pathways and Fragment Ion Structures in the Tandem Mass Spectra of Protonated Synthetic Cathinones," *Forens. Chem.*, **2020**, 19, 100245.
5) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, "The Influence of Chemical Modifications on the Fragmentation Behavior of Fentanyl and Fentanyl-Related Compounds in Electrospray Ionization Tandem Mass Spectrometry," *Drug Test. Anal.*, **2020**, 12(7), 957-967.
6) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, "The Characterization of Isobaric Product Ions of Fentanyl using Multi-Stage Mass Spectrometry, High-Resolution Mass Spectrometry and Isotopic Labeling," *Drug Test. Anal.*, **2020**, 12(4), 496-503.
7) M. K. Santos, G. B. Walber, L. J. Danielli, T. Kreutz, K. C. Mariotti, M. Ritter, L. E. Arroyo, G. P. Jackson, R. P. Limberger, "Evaluation of the Presence of 1,3-Dimethylamylamine in Pelargonium Leaves and Essential Oils by Mass Spectrometric and Chromatographic Methods," *Chromatographia,* **2019**, 82(5), 875-883.
8) J. T. Davidson, G. P. Jackson, "The Differentiation of 2,5-dimethoxy-N-(N-methoxy-benzyl)phenethylamine (NBOMe) Isomers using GC Retention Indices and Multivariate

Analysis of Ion Abundances in Electron Ionization Mass Spectra," *Forens. Chem.,* **2019**, 14, 100160.

Presentations and Workshops:

The results of this research have been presented in a variety of venues. These include:

1) 3rd School of Forensic Sciences of the Brazilian Federal Police, (Remote due to Covid Pandemic). ***Structural Characterization of Emerging Synthetic Drugs using Mass Spectrometry Webinar***. Taught a 45-minute webinar with a live question and answer session to ~50 participants.
2) Through RTI Forensic Technology Center of Excellence (FTCOE), Research Triangle Park, NC, I presented a Webinar/Workshop on the results and ongoing work of this grant. The webinar was titled: "Structural Characterization of Emerging Synthetic Drugs using Mass Spectrometry". The webinar lasted one-hour and included a live question and answer session to ~150 participants. The presentation is archived here: https://learning.forensicac.org/course/view.php?id=435
3) G. P. Jackson, S. Mehnert, J. T. Davidson, "New Algorithm for Mass Spectral Identifications," at Pacifichem, Honolulu, HI, (Virtual due to COVID), Dec 2021. (Oral)
4) G. P. Jackson, S. Mehnert, J. T. Davidson, "Expert Algorithm for Substance Identification (EASI): A New Paradigm for Mass Spectral Identifications," presented in the Innovation Award Session at SciX Conference, Providence, RI, Sept 2021. (Oral)
5) G. P. Jackson, A. Adeoye, J. T. Davidson, E. Ruiz, B. Lowe, "A New Mass Spectral Identification Algorithm to Discriminate Between Structurally Similar Fentanyl Analogs," at SciX Conference, Providence, RI, Sept 2021. (Oral)
6) G. P. Jackson, J. T. Davidson, Z. J. Sasiene, B. Lowe, Y. Abiedalla, C. R. Clark, E. L. Piacentino, V. Ryzhov, "Mass Spectrometric Characterization of Emerging Synthetic Drugs and an Algorithm for Confident Identifications," at the 72nd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, (virtual due to COVID), Mar 2021. (Oral)
7) J. T. Davidson, Z. J. Sasiene, G. P. Jackson, "Comparison of In-Source Collision-Induced Dissociation and Beam-Type Collision-Induced Dissociation for Emerging Synthetic Drugs," at the 73rd Meeting of the American Academy of Forensic Sciences (virtual due to COVID), Feb 2021. (Oral)
8) J. T. Davidson, Z. J. Sasiene, Y. F. Abiedalla, C. R. Clark, G. P. Jackson, "Fragmentation Pathways of α-pyrrolidinophenone Derivative Synthetic Cathinones," at the 68th ASMS "Reboot" Conference on Mass Spectrometry and Allied Topics (virtual due to COVID-19), June 2020. (Poster)
9) G. P. Jackson, J. T. Davidson, Z. J. Sasiene, B. Lowe, Y. Abiedalla, C. R. Clark, E. L. Piacentino, V. Ryzhov, "Towards an Improved Understanding of the Mass Spectrometric Identification of Cathinones and Fentalogs," at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Chicago, IL, Mar 2020. (Oral)

10) S. A. Mehnert, B. D. Lowe, E. Ruiz, J. T. Davidson, G. P. Jackson, "A Regression-Based Algorithm to Maximize the Confidence in Mass Spectral Identifications," at the 72nd Meeting of the American Academy of Forensic Sciences, Anaheim, CA, Feb 2020. (Oral)
11) J. T. Davidson, Z. J. Sasiene, Y. F. Abiedalla, R. Clark, G. P. Jackson, "On the Fragmentation Behavior of Fentanyl and Its Analogs in Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS)," at the 72nd Meeting of the American Academy of Forensic Sciences, Anaheim, CA, Feb 2020. (Oral)
12) *J. Tyler Davidson received the Forensic Science Foundation (FSF) Emerging Forensic Scientist Award at the AAFS Meeting.
13) S. A. Mehnert, B. D. Lowe, E. Ruiz, J. T. Davidson, G. P. Jackson, "Development of a Flexible Algorithm for Substance Identification Using Mass Spectrometry," at Eastern Analytical Symposium, Plainsboro NJ, Nov 2019. (Poster)
14) G. P. Jackson, S. A. Mehnert, , B. D. Lowe, E Ruiz, J. T. Davidson, "A Regression-Based Algorithm to Maximize the Confidence in Mass Spectral Identifications," at Eastern Analytical Symposium, Plainsboro NJ, Nov 2019. (Oral)
15) G. P. Jackson, S. A. Mehnert, J. T. Davidson, B. D. Lowe, "A Regression-Based Algorithm to Maximize the Confidence in Mass Spectral Identifications," at SciX Conference, Palm Springs, CA, Oct 2019. (Oral)
16) G. P. Jackson, J. T. Davidson, Z. J. Sasiene, Y. Abiedalla, C. R. Clark, "On the Mass Spectral Interpretation of Cathinones and Fentanyl Analogs," at SciX Conference, Palm Springs, CA, Oct 2019. (Oral)
17) G. P. Jackson, S. A. Mehnert, B. D. Lowe, J. T. Davidson, "The Development of a Flexible Algorithm for Substance Identification Using Mass Spectrometry," at the Pittsburg Conference, Philadelphia, PA, Mar 2019. (NIJ Poster session).
18) G. P. Jackson, J. T. Davidson, Z. J. Sasiene, Y. Abiedalla, C. R. Clark "On the Tandem Mass Spectrometry of Cathinones and Mass Spectrometric Identification of Drugs" at the Spring National Meeting of the American Chemical Society, Orlando, FL, 2019. (Oral)
19) S. A. Mehnert, B. D. Lowe, J. T. Davidson, G. P. Jackson, "The Development of a Flexible Algorithm for Substance Identification Using Mass Spectrometry," at the 71st Meeting of the American Academy of Forensic Sciences, Baltimore, MD, Feb 2019. (Oral)
20) J. T. Davidson, Z. J. Sasiene, Y. F. Abiedalla, R. Clark, G. P. Jackson, "The Identification of a Novel Fragmentation Pathway of Synthetic Cathinones," at the 71st Meeting of the American Academy of Forensic Sciences, Baltimore, MD, Feb 2019. (Oral)
21) S. E. Chaffman, T. Williams; J. T. Miller, J. T. Davidson, G. P. Jackson, "Identification of an Ultraviolet (UV) -Induced Promethazine Dimer," at the 71st Meeting of the American Academy of Forensic Sciences, Baltimore, MD, Feb 2019. (Poster)

The project did not result in any website(s) or other internet site(s), technologies or techniques, inventions, patent applications, and/or licenses. We did produce several

datasets, and these have been made publicly available through supplemental material accompanying each published project.