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Accessing the Probative Value of Physical Evidence at Crime Scenes with Ambient Mass Spectrometry and Portable Instrumentation

Award No. 2011-DN-BX-K552

**Applied Research and Development in
Forensic Science for Criminal Justice Purposes**

Final Report

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Abstract

The amount and variety of evidence collected at a typical crime scene is extensive. While many significant analytical methods have been established over the years, particularly hyphenated mass spectrometric techniques, forensic laboratories cannot keep up with the demand, and in many cases, significant backlogs of evidence have amassed. While this points to a need for more rapid, streamlined technologies for forensic analysis, a significant reduction in collected evidence, leading to a subsequent reduction in backlogged evidence, would come from the ability to access the probative value of chemical evidence at the crime scene itself, allowing only pertinent samples to be sent to off-site laboratories for confirmation. Screening of physical evidence at the crime scene also has the capability to rapidly determine whether a criminal investigation is needed and provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial. To assist in the reduction of collected samples while increasing the overall quality of said evidence, it would beneficial for forensic science practitioners to have technology at their disposal that is not only portable, allowing the screening of potential evidence before collection, but also flexible in terms of chemical species and sample substrates that can be analyzed. This flexibility, in particular, would allow this technology to be robust towards the ingenuity of criminals and emerging threats.

In an effort to fulfill the current technological needs of forensic science practitioners and associated laboratories, we sought to create a broadly-applicable, portable chemical detector based on a state-of-the-art mass spectrometer (MS) capable of sampling externally-generated ions. This capability allows the use of novel "ambient" ionization methods that allow direct screening of target compounds or "analytes" in their native environment and state without prior preparation. Utilizing the Flir Systems AI-MS 1.2 portable instrument as a testbed, several ambient ionization techniques were created and/or validated for coupling to the system. The suite of ionization sources investigated allowed the sensitive analysis of solid, liquid, and gas-phase chemicals, as well as chemical traces on everyday surfaces, at low concentrations with high chemical specificity. Swab-based physical transfer methods were developed and extended to this instrumentation to allow the flexibility in analyzing geometrically-complex samples and large surface areas, as well. Ambient ionization techniques coupled to this portable system were shown to perform well when analyzing complex samples (*i.e.* bulk powders, chemical residues in latent fingerprints, pharmaceutical tablets, clandestine synthetic reaction products/apparatus etc.), authentic evidentiary seizures, and emerging threats. Base and tandem MS (MS/MS) spectra obtained on the AI-MS 1.2 were marked by high congruency to that collected or reported on lab-scale, commercial MS systems, showing high potential for adoption as an accepted technique in crime scene investigation and forensic analyses. Furthermore, automated library searching via data dependent scanning and chemical identification via MS/MS fragmentation spectra offers the potential for usage by non-technical operators, reducing the need for spectral interpretation by the end-user.

Building upon their extensive characterization and evaluation of this technology and considering interactions with forensic science practitioners, project personnel developed and deliver a Flir Systems AI-MS 1.2 prototype with optimized instrumental methods to NIJ for evaluation and testing, along with appropriate operational documentation and a comprehensive spectral library.

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Executive Summary

The amount and variety of evidence collected a typical crime scene is extensive. While many significant analytical methods have been established over the years, particularly hyphenated mass spectrometric techniques, forensic laboratories cannot keep up with the demand, and in many cases, significant backlogs of evidence have amassed. While this points to a need for more rapid, streamlined technologies for forensic analysis, a significant reduction in collected evidence, leading to a subsequent reduction in backlogged evidence, would come from the ability to access the probative value of chemical evidence at the crime scene itself, allowing only pertinent samples to be sent to off-site laboratories for confirmation. Screening of physical evidence at the crime scene also has the capability to rapidly determine whether a criminal investigation is needed and provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial. To assist in the reduction of collected samples while increasing the overall quality of said evidence, it would beneficial for forensic science practitioners to have technology at their disposal that is not only portable, allowing the screening of potential evidence before collection, but also flexible in terms of chemical species and sample substrates that can be analyzed. This flexibility, in particular, would allow this technology to be robust towards the ingenuity of criminals and emerging threats.

In an effort to fulfill the current technological needs of forensic science practitioners and associated laboratories, we sought to create a broadly-applicable, portable chemical detector based on a state-of-the-art mass spectrometer (MS) capable of sampling externally-generated ions. This capability allows the use of novel "ambient" ionization methods that allow direct screening of target compounds or "analytes" in their native environment and state without prior preparation. Utilizing the Flir Systems AI-MS 1.2 portable instrument as a testbed, several ambient ionization techniques were created and/or validated for coupling to the system. The suite of ionization sources investigated allowed the sensitive analysis of solid, liquid, and gas-phase chemicals, as well as chemical traces on everyday surfaces, at low concentrations with high chemical specificity. Swab-based physical transfer methods were developed and extended to this instrumentation to allow the flexibility in analyzing geometrically-complex samples and large surface areas, as well. Ambient ionization techniques coupled to this portable system were shown to perform well when analyzing complex samples (*i.e.* bulk powders, chemical residues in latent fingerprints, pharmaceutical tablets, clandestine synthetic reaction products/apparatus etc.), authentic evidentiary seizures, and emerging threats. Base and tandem MS (MS/MS) spectra obtained on the AI-MS 1.2 were marked by high congruency to that collected or reported on lab-scale, commercial MS systems, showing high potential for adoption as an accepted technique in crime scene investigation and forensic analyses. Furthermore, automated library searching via data dependent scanning and chemical identification via MS/MS fragmentation spectra offers the potential for usage by non-technical operators, reducing the need for spectral interpretation by the end-user.

Building upon their extensive characterization and evaluation of this technology and considering interactions with forensic science practitioners, project personnel developed and deliver a Flir Systems AI-MS 1.2 prototype with optimized instrumental methods to NIJ for evaluation and testing, along with appropriate operational documentation and a comprehensive spectral library.

ES.1 Project Goals

Project goals for this research project centered around answering five (5) principle scientific questions in order to gauge performance and potential applicability: **(i)** can a portable mass spectrometer be adapted to allow direct analysis of solid, liquid, and gas-phase chemical species? **(ii)** can evidence be effectively screened in a non-destructive nature? **(iii)** is physical transfer of chemical residues more effective that direct surface analysis? **(iv)** is the developed technology on par with current methods in terms of reliability, reproducibility, selectivity, and sensitivity? **(v)** is this technology robust in terms of the current and changing needs of forensic science and law enforcement personnel?

Seven discrete tasks were undertaken to develop and implement the first-ever portable MS system that allows screening of probable evidentiary samples at crime scenes, yet is flexible and broadly-applicable to many different classes of chemicals and surface substrates. We began by evaluating the use of electrospray ionization (ESI), desorption electrospray ionization (DESI), low temperature plasma (LTP), and atmospheric pressure chemical ionization (APCI) on Flir Systems AI-MS 1.2, producing high quality MS and MS/MS spectral data for over 70 analytes of forensic evidence; paper spray ionization (PSI) was examined to a lesser extent. The ability to screen chemicals of interest as trace residues from common surfaces found at crime scenes (*e.g.* glass, plastic, metal) and as bulk powders was successfully demonstrated. Intensive investigation of the analytical performance of the Flir AI-MS 1.2 was then undertaken, including spectral data quality and accuracy, sensitivity and quantitative ability, sample throughput, and reliability in terms of false positive/false negative rates. Swab-based physical transfer methods were developed and extended to this instrumentation to allow the flexibility in analyzing geometrically-complex samples and large surface areas, as well. A custom APCI-MS source was developed and implemented on the system to extend application to high volatility analytes, particularly common accelerants pertinent to arson investigations, solvents used in clandestine drug operations, and chemical warfare agent simulants. Utilizing optimized instrumental methods developed in-house, a comprehensive mass spectral reference library consisting of both full scan and MS/MS fragmentation spectra was constructed to assist possible end users of the instrumentation. Prototypical, simplified user software methods allowing automated library searching and negating the need for spectral interpretation were developed and rigorously tested, showing high potential for use by non-technical operators. Proof of concept experiments were conducted to show application of the system to the daunting task of chemical identification at clandestine laboratory operations; toward this, the Flir AI-MS 1.2 performed well, easily identifying common illicits and their precursors as powders, tablets, and residues from surfaces and storage media. The ability to perform real-time reaction monitoring of clandestine methamphetamine production was also demonstrated; this, in essence, allows the Flir AI-MS 1.2 to conclusively identify a clandestine methamphetamine operation regardless of synthesis stage. Field testing and demonstrations for forensic science and law enforcement personnel was pursued to demonstrate the technology on authentic contraband samples and seized materials. The culmination of the project was the preparation and delivery of a modified Flir AI-MS 1.2 system coupled with the associated ionization sources, as well as associated training documentation, spectral libraries and methods, to the NIJ.

ES.2 Methods Implemented During Project

In all studies, a Flir Systems AI-MS 1.2 cylindrical ion trap (CIT) mass spectrometer (Flir Mass Spectrometry, West Lafayette, IN, USA) ruggedized of field use was implemented. This portable system allows sampling of externally-generated ions via a capillary-based atmospheric pressure inlet, and analyte confirmation is possible via tandem MS (MS/MS). All AC/DC voltages needed for instrument operation and desorption electrospray ionization (DESI) are incorporated, as well as an on-board helium supply for the CIT damping gas needed for MS/MS fragmentation. Nebulizing gas (N_2 or air) necessary for DESI analysis is supplied by a stand-alone tank, allowing the use of small, self-contained breathing apparatus (SCBA) tanks commonly utilized by the first response community for field implementation. The size (24" x 20" x 15", L x W x H), weight (98 lbs.) and ruggedness of this instrument makes it an amenable platform for field-based, CSI applications.

Over the course of the project, several conventional and ambient ionization sources were constructed and tested on the Flir AI-MS 1.2, including ESI (for solutions and extracts), DESI (for solids, powders, residues and transfer swabs), LTP (for residues), PSI (for powders, residues and transfer swabs), and APCI (for gas-phase analytes). For ESI and DESI studies, The AI-MS 1.2 system has a position-stationary ionization assembly that allows implementation of both spray-based methods. The stationary source is mounted to fix both the ESI/DESI spray angle (55°) and DESI emitter tip-to-analysis surface distance. Spray parameters included a 4 kV spray voltage, nebulizing gas pressure (N_2) of 100 PSI, and solvent flow rates of either 10 µL/min for ESI or 3 µL/min for DESI. All spray-based ionization was performed with spray solvent compositions consisting of 1:1 methanol:water with 0.1% formic acid in positive-ion mode and 1:1 methanol:water in negative-ion mode. LTP was investigated extend application of the system to explosives, and was implemented constructing a custom source using a stock nanoESI holder with gas assistance port (Warner Instruments, ESW-M15P), through which helium gas is flowed at a rate of 0.2-0.3 L/min. To provide the necessary dielectric barrier, a quartz capillary (Sutter Instruments) is sheathed over the electrode of the nanoESI probe (grounded), to which a small section of adhesive-backed copper tape is wrapped and connected to the AC power supply (Information Unlimited, PVM500, 3-4 kV, 33.6 kHz) needed for plasma generation. PSI-MS works by combining two mechanisms, paper chromatographic separation and electrospraying. After a chemical is applied to the paper triangle (Whatman chromatography paper) by spotting or swab transfer, the combination of added solvent and 4 kV of high voltage provided by an on-board high voltage "alligator clip" electrode produces a stable electrospray of inherent analyte ions detected by the MS. For gas-phase analytes, a custom-built teflon APCI source featuring an auxiliary pumping system to sample volatile chemicals in proximity to the Flir Systems AI-MS 1.2 was constructed. Inside the source, a corona discharge is generated by application of 4 kV under resistivity (1 MΩ) to a tungsten wire discharge needle held perpendicular to the MS inlet capillary. Before data collection on the portable MS system, instrument calibration and tuning was performed to provide optimal operating conditions. Mass spectral data was collected in either positive or negative-ion mode, depending on the nature of the chemical of interest.

For characterization studies with ESI/DESI/LTP/PSI, analytical standards of illicit chemicals and others of forensic interest were purchased as either standard solutions (Cerilliant Co., Round Rock, TX, USA) or pure solids (Sigma-Aldrich Co., St Louis, MO, USA) in 1000 ppm (1.0 mg/mL) concentrations. Serial dilution of the stock solutions were performed as desired using appropriate solvents, including HPLC-grade methanol and acetonitrile obtained

from Fisher Scientific (Pittsburg PA). Residues of known mass were deposited on substrates of interest for direct analysis experimentation (DESI/PSI/LTP) or swab transfer by spotting and drying small aliquot(s) of known concentration solutions. Powder-based samples and authentic forensic evidence was analyzed by deposited ~1 mg onto an adhesive-based glass slide or via swab transfer. Transfer swabbing procedures for DESI/PSI involved wetting the sampling medium with a predetermined amount of methanol to assist with efficient surface transfer and probing of the surface or sample of interest, followed by direct analysis of said swab without further preparation. Volatile analytes were examined via APCI-MS by sampling ambient air or headspace vapors from sealed flasks containing pure liquid chemicals (Sigma-Aldrich).

ES.3 Results

ES.3.1 *Task 1: Test Proof of Concept Detection of Chemicals of Interest on the Flir Systems AI-MS 1.2*

Ambient ionization coupled with the Flir AI-MS 1.2 system has far exceeded our initial expectations in regards to performance and applicability to analytes and samples of interest to forensic science and crime scene investigation. At the time of writing, our database of detected and confirmed analytes numbers **73** and continues to grow as new and emerging illicits continue to be added. This spectral database contains MS and MS/MS from a wide array of illicit drugs, abused pharmaceuticals, potential cutting agents, explosives and species related to ballistics, and accelerants. For the analytes tested, all non-volatile species were analyzed at a deposited mass of 100 ng via DESI-MS, the milestone amount targeted for this grantwork, unless they were directly analyzed via APCI due to volatility. Of note, a majority of the compounds tested produced very high spectral signal intensity even at 100 ng, meaning that most limits of detection for these species lie in the very low to sub-ng range. Spectral data collected on the AI-MS 1.2 was highly congruent in regards to both MS and MS/MS ion signatures collected on in-house, lab-scale MS instrumentation and also seen in scientific literature; this is seen as a major benefit towards actual implementation in CSI applications and potential acceptance as a validated method. When comparing spectra collected using ESI, DESI and PSI on a similar analyte, they are marked by high similarity in regards to ions seen; however, differences in signal intensities are seen depending the mechanisms employed by the ionization technique (*i.e.* DESI signal intensity is typically lower than that of ESI or PSI for similar mass of compound analyzed due to its required surface desorption mechanism).

Analysis of residues from flat surfaces like glass or plastic was demonstrated with very high throughput and sensitivity, but given the potential complexity of authentic forensic evidence in terms of chemical composition, geometry, and size, experimentation on unconventional substrates and complex samples was thoroughly investigated. The Flir AI-MS 1.2 performed well in this testing, with the suite of ionization methods utilized proving robust to a wide selection of test samples. Over the course of the project, representative surfaces tested included glasses, metals, polymers/plastics, non-stick coatings, common paraphernalia, phone keypads, and many others. Detection of chemical signatures in latent fingerprints and organic components in gunshot residue was also successfully demonstrated.

Spectral quality (in terms of accuracy of m/z determinations and relative abundance in both MS and MS/MS mode) of the Flir Systems AI-MS 1.2 was tested by direct comparison with a commercially-available, spectral reference database, the Wiley Registry® of Tandem Mass

Spectral Data ([MSforID](#)). To compare to the database, MS and MS/MS data using spray and ambient ionization techniques for thirty-two total "blind" analytes, including five negative controls were subjected to library searching, providing the correct identification for all positive controls tested with high sensitivity (*i.e.* true positive rate). Authentic seized evidence was also able to be rapidly identified, providing accurate determinations and demonstrating high potential for use in forensic casework and potential crime scene investigation.

Ambient MS analyses upon the Flir AI-MS 1.2 were marked by very high throughput, usually dictated by the user themselves (*i.e.* how quickly they can document a sample and present it for analysis), and well as little to no carryover. Reliability of the system was assessed by determination of false positive/false negative rates for lab-generated control samples, yielding 100% success rate for positive controls and 98% success rate for negative controls for 400 control samples.

ES.3.2 *Task 2: Investigate Alternative Surface Sampling Methodologies*

Analysis of surface swabs with DESI, following the probing of samples of interest, was successfully demonstrated from several surfaces of interest (*e.g.* latent fingerprints from touchscreen cellphone, prescription pill bottles, etc.) with relevance to drugs of abuse. The effect of surface probing with "wet" swabs vs. dry swabs was also evaluated. For this evaluation methanol, which has high solubility towards drugs of abuse, was utilized with wet swabbing. It was determined that wet swabbing drastically outperformed dry swabbing. Different swabs were also investigated for their applicability to direct DESI analysis, including chromatography paper, knitted polyester, non-woven hydroentangled foam polyester, polyurethane foam, and spun cotton "Q-Tip"-style applicators. Considering all factors tested, Berkshire polyurethane foam swabs were determined to be the best candidate for developing an optimized transfer swabbing protocol for use with DESI-MS.

ES.3.3 *Task 3: Demonstrate Direct Air Analysis via APCI on the Flir Systems AI-MS 1.2*

High volatility analytes have long been examined using GC/MS, but when implementing specialized ionization sources, they can also be analyzed on the Flir AI-MS 1.2 directly from ambient air. To demonstrate the ability to monitor gas-phase analytes directly from the air via APCI-MS, several iterations of source design were undertaken, producing a robust, sensitive ionization source easily coupled to the system without interference with the on-board ESI/DESI assembly. By utilizing a small auxiliary diaphragm pump, non-proximate, ambient air samples can be pulled into the APCI source for analysis via a sampling tube; this provides flexibility in what and where air samples are investigated. Vapors from an array of potential accelerants were investigated to show potential applicability to arson investigation evidence, and limits of detection for several of these were demonstrated to be less than 1 ppm (a milestone threshold for this grantwork). Mock arson evidence was created by combusting wood substrates in the presence of accelerants of interest, enclosing charred materials in sealed flasks, and sampling headspace vapors for detection. From the data collected, accelerants used for combustion are able to be detected, along with other signatures from chemicals inherent to the substrate itself.

ES.3.4 *Task 4: Develop Comprehensive Mass Spectral Libraries and Optimized Methods for Target Chemicals*

Over the course of the project period, high-quality MS and MS/MS spectra and methods for their collection were generated for numerous analytes of forensic interest for development of a comprehensive mass spectral library. Optimization of MS/MS instrumental methods was undertaken by systematically adjusting ion trap parameters during precursor isolation and fragmentation, including frequency of the AC dissociation waveform applied, collision energy (*i.e.* the energy imparted to isolated ions via collision-induced dissociation, CID, with helium damping gas), and dissociation time. Said MS/MS methods not only gave fragmentation data congruent to our own testing on lab-scale MS instruments and that reported by other research groups in literature, but also have proven to be reproducible in both intra- and inter-day testing. User software upgrades released by Flir Mass Spectrometry for the AI-MS 1.2 allowed creation of semi-automated optimization methods of MS/MS experimental conditions and fully-automated library searching protocols based of MS/MS-based data dependent scanning. Of note, automated library searching has the potential to alleviate the need for spectral interpretation by the instrument operator, expanding its use by non-technical operators by incorporating "red light/green light" protocols (*i.e.* red indications on a graphical user interface affirms the presence of a target analyte, green indications that the sample is innocuous).

ES.3.5 *Task 5: Demonstrate Detection Capability of Materials of Interest at Clandestine Methamphetamine Labs*

Clandestine laboratory installations represent the worst-case scenario for field analysis, as the variety of "samples" found are diverse in nature, of high magnitude, rarely marked and stored in proper containers, and most likely located in unsafe conditions. To help provide a robust platform for general sample screening in these situations, proof-of-principle experimentation on the Flir AI-MS 1.2 focused on synthetic routes for methamphetamine and the emerging clandestine drug desomorphine (aka "krokodil"). Testing during this phase of the project involved trace residues and bulk powder of precursors and illicit products, and potential solvents used in clandestine operations were examined via APCI-MS. Active ingredients in pharmaceutical and over-the-counter tablets utilized as a source of precursors can easily be identified as either bulk compositions or residues from production and storage media commonly found in clandestine settings. The AI-MS 1.2 was also shown able to monitor in real-time both the Birch reduction and Nagai synthetic routes for methamphetamine, identifying both precursor and product species at any point during the reactions. This, in essence, allows the Flir AI-MS 1.2 to conclusively identify a clandestine methamphetamine operation regardless of synthesis stage. Preliminary results also point to the ability to confirm fentanyl clandestine synthesis.

ES.3.6 *Task 6: Conduct Field Experiments/Dissemination to Practitioners*

While many of the results obtained on the Flir AI-MS 1.2 during this project involved analytical standards in controlled laboratory settings, special efforts were taken to perform field testing on authentic forensic evidence. Field testing conducted with law enforcement, forensics and criminal justice practitioners served dual purposes, as the effect of environmental variables (*i.e.* transport and calibration, ambient conditions, etc.) can be assessed while gathering integral

feedback from the target user groups. Overall, practitioners who witnessed our demonstrations and field experimentation were impressed by the performance of the Flir AI-MS 1.2, size of the device, and especially by the ease of use. Through discussion with these groups, information regarding needs for true field implementation and use by untrained personnel was acquired that helped craft and improve our developed methods and ionization source design. Furthermore, important troubleshooting experiences were gathered during these exercises.

Several opportunities to analyze authentic forensic evidence stemmed from these interactions, to which ambient MS on the Flir AI-MS 1.2 performed admirably. Evidence tested during this phase of the project included authentic synthetic cathinone "bath salt" powders that were seized as commercially-available products at local retailers, powdered cocaine, and methamphetamine residue on the inside of a plastic bag used to transport the drug. Of interest, experimentation with bulk forensic evidence was able to be accomplished without extensive carryover between analyses by implementing proper hygiene protocols involving cleaning of the ionization source and outer surface of the MS inlet capillary and analysis of pre-sample blanks.

ES.3.7 *Task 7: Preparation of Deliverable Instrument and Associated Documents*

An important final aspect of this project was to prepare and deliver a modified Flir AI-MS 1.2 system coupled with the associated ionization sources investigated to the NIJ for testing purposes. Along with the instrumentation, a comprehensive mass spectral library (including MS and MS/MS data for project analytes) and optimized compound-specific MS/MS methods were delivered. Also, methods allowing automated library searching via data dependent scanning were able to be constructed and delivered. Documentation delivered with the Flir AI-MS 1.2 included extensive training and troubleshooting manuals, with substantial detail to ensure that NIJ personnel and other users will be able to quickly learn the operation of all facets of the system.

ES.4 Conclusions

The Flir Systems AI-MS 1.2 was demonstrated to be broadly-applicable, portable instrument with high potential for use in crime scene investigation and evidentiary analysis. The flexibility to screen and identify solid, liquid and gas-phase analyte when utilizing the suite of ionization methods developed and/or investigated on the system has the potential to provide capabilities that no other fieldable technology currently available can offer. Switching between trace level residues and bulk-level samples was achievable with no carryover when implementing simple hygiene protocols, and coupling with physical transfer swabs extends application of the system to large and geometrically-complex surfaces, meeting the demands that real evidence present. The congruency of spectral data collected on the AI-MS 1.2 in regards to both MS and MS/MS ion signatures collected on lab-scale MS instrumentation and also seen in scientific literature is seen as important milestone towards acceptance as a validated forensic analysis method and actual implementation in CSI applications.

When considering the end product of this project, a portable instrument capable of assessing the probative value of physical evidence typically found at crime scenes, the impact of providing the forensic science community with said technology would have a positive effect on criminal justice practice at the local, state and national level. There are also potential implications in regards to reducing evidence backlogs that hinder publicly-funded forensic

laboratories, as this technology provides both higher throughput and a potentially reduced influx of evidence entering the lab system. When considering the feasibility of implementing the proposed technology in forensic settings, the cost of this instrumentation and maintenance could be off-set by the reduction in evidence sent to forensic laboratories and funds being used for outsourced analyses to private laboratories.

While the effect on current criminal justice practice can be presumed, the implications on current criminal justice policy are not as clear. Field-portable units for accurate contraband detection in the condensed phase and from surfaces would allow greater versatility in routine traffic stops and criminal investigations, but if this technology is used to gather evidence prior to an arrest, there are implications regarding unreasonable search and seizures. Overall, implementation of the proposed research could allow greater flexibility in law enforcement, but its application to legal convictions under current criminal justice policy will need to be considered before common usage.

1. Introduction

1.1 Statement of the Problem

It is hard to envision a more nebulous, demanding discipline than forensic science in terms of chemical analysis. The nature of forensic science suggests than almost any chemical could be potential evidence, given that it was involved in or alludes to criminal activities. To further complicate the issue, this large breadth of chemical species can be found in various states of matter, as residues on many different substrates, or in the presence of complex chemical matrices. Due to this, both the amount and variety of evidence collected is extensive. While several analytical techniques for identification and quantitation of unknown chemical species have been established for use in forensic science, they have had to be adapted over the years to incorporate high-throughput sampling into the instrumental method in an attempt to keep up with the magnitude of submitted evidence while still fulfilling the requirements of the legal system. While many significant analytical methods have been established, forensic laboratories cannot keep up with the demand, and in many cases, significant backlogs of evidence have amassed.

The most notable example of this backlog is DNA evidence. While DNA profiling is regarded as one of the most discriminating and powerful types of evidence, many laboratories struggle to obtain needed results on a timeframe that is consistent with corresponding legal proceedings, requiring considerable outsourcing of evidentiary analyses. In fact, it was recently estimated that nearly half of publicly funded forensic laboratories outsourced at least one type of forensic service to private laboratories, which is disadvantageous from a budgetary standpoint.[1] While this points to a need for more rapid, streamlined technologies for forensic analysis, a significant reduction in collected evidence, leading to a subsequent reduction in backlogged evidence, would come from the ability to access the probative value of chemical evidence at the crime scene itself, allowing only pertinent samples to be sent to off-site laboratories for confirmation. Screening of physical evidence at the crime scene also has the capability to rapidly determine whether a criminal investigation is needed and provide law enforcement personnel with necessary information in a timely manner, which in many cases is crucial. To assist in the reduction of collected samples while increasing the overall quality of said evidence, it would beneficial for forensic science practitioners to have technology at their disposal that is not only portable, allowing the screening of potential evidence before collection, but also flexible in terms of chemical species and sample substrates that can be analyzed. This flexibility, in particular, would allow this technology to be robust towards the ingenuity of criminals and emerging threats.

1.2 Relevant Literature Review

1.2.1 Current Forensic Analysis Technologies

The field of forensic science has matured concurrently with advancements in analytical instrumentation. While several colorimetric assays are still implemented in the field and laboratory setting,[2,3] a majority of forensic analyses have grown to incorporate instrumental methods with specific sample preparation requirements, and have become both routine and accepted as relevant in judicial proceedings. Instrumentation commonly utilized in the forensic laboratory setting for chemical analyses includes gas (GC) and liquid chromatography (LC)

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coupled with mass spectrometry (MS) for illicit drug analyses, toxicology, and arson investigations, infrared spectroscopy (IR) for blood alcohol content, atomic spectroscopy for elemental speciation, and capillary electrophoresis (CE) for DNA profiling, and new methodologies are currently being investigated.[4-6] While each of these technologies offers its own benefits, these techniques often require multiple instrumental methods to cover a broad range of analytes, suffer from long analysis times, and require extensive sample preparation, commonly in the form of liquid-liquid and solid phase extraction.

A significant disadvantage of these techniques is that they are confined to the laboratory (with the exception of a few GC-MS and IR portable systems currently available), requiring all potential evidence to be collected at the crime scene and sent back to laboratory facilities. This is disadvantageous in terms of chain of custody errors and sample degradation, but more importantly, the time between evidence collection and interpretation of chemical data is extensive. To combat this, several portable technologies have been investigated,[7-9] but few have been introduced as commercial products. Manufacturers such as Inficon, Torion Technologies, and Griffin Analytical Technologies have introduced portable GC/MS instruments, which is a significant milestone given the substantial usage of GC/MS in forensic science, but these instruments have comparable, if not more extensive, sample preparation requirements and suffer from low sample throughput.

1.2.2 Necessary Criteria of Field-Portable Detection Technologies

To be considered a viable technology, portable analytical instruments have long been required to fulfill the three main analytical criteria of sensitivity, selectivity, and speed.[10] Sensitivity is required because compounds of interest are often at very low levels—parts per million to parts per billion. Selectivity in detection, or the ability to accurately discriminate the response from the desired target compound against other compounds present in the sample, is vital, given the complexity of samples of interest in their natural states or locations. Because important actions—such as arrests for criminal activities and closing of public venues–could be based on the response of such analytical instrumentation, selective detection is key in avoiding false alarms, or more importantly, false negative responses, where a target analyte is actually present but analysis yields a "negative" response for its presence. An instrument's ability to provide real-time data for first responders at an incident site is also important.

Considering the incidents that often involve forensic and law enforcement personnel and first responders, including arson investigations and domestic terrorism involving explosives and other harmful chemical/biological agents,[11] the current criteria must be expanded for portable technologies to account for these situations. It is critical that the tools at the disposal of forensic science practitioners be versatile so that they are broadly applicable to incident sites and the samples of interest they contain, but also robust in terms of new threats and applications for which they will utilized.

1.2.3 Field-Portable Mass Spectrometry

Several technologies with varying levels of performance and feasibility have emerged as candidates for continued development and evaluation as portable analytical instruments, as recently reviewed.[7-9] Of these technologies, several exhibit significant drawbacks, including long analysis times, high false positive and negative rates, high limits of detection, and narrow applicability. Mass spectrometry (MS) emerges as a technique with the potential of fulfilling

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the major criteria needed for field-portable analytical instruments. Of the general-purpose methods of chemical analysis, mass spectrometry has proven to be one the most sensitive techniques, and detection of ultra-trace quantities of specific compounds has been reported, even from complex mixtures.[10] The high specificity of MS comes from tandem mass spectrometric analysis (two or more coupled stages of mass analysis), which allows for both molecular weight and structural data to be gathered.

Recent advances in vacuum and electronic technologies have led to the miniaturization of MS instruments that make portability feasible. Several research groups have designed and reported portable MS prototypes,[11-13] and on-site analysis with instruments typically requiring as little as 50 ms to acquire a complete mass spectrum leads to rapid collection of data. However, conventional sample preparation typically can take several hours to days, depending upon the sample in question and the desired analyte. Novel sample ionization methods at atmospheric pressure, or "ambient mass spectrometry," have allowed mass spectrometric analysis to take place with little to no sample preparation.[14-16] These flexible ionization methods require that the instrumentation being employed allows for sampling of analyte ions at atmospheric pressure into the instrument's vacuum system. Although this constraint typically requires large, power-hungry vacuum systems that are not amenable to fieldable mass spectrometers, portable[17] and handheld[18] MS prototypes have been developed to allow coupling to ambient mass spectrometry. While these prototype instruments mark an important step towards a fieldable MS instrument, these systems are not constructed or ruggedized to allow constant operation in harsh environments. Recently, Flir Systems, Inc. has developed a field-ready mass spectrometer (AI-MS 1.2) featuring an atmospheric ion inlet, marking the development of the first high performance instrument of this kind.[19] Technologies like Flir Systems AI-MS 1.2 becoming commercial products is important in terms of availability and applicability to practitioners of this technology.

1.2.4 Ambient Mass Spectrometry

Ambient mass spectrometric methods, a topic of much interest in analytical chemistry today, have allowed the analysis of "ordinary" samples in their native environment with little or no sample preparation, different from traditional analytical methods that require extensive sample preparation and/or introduction of sample into an instrumental vacuum system before ionization. Electrospray ionization,[20] for which John Fenn was awarded the Nobel Prize in Chemistry in 2002, readily allows the analysis of chemical species in solution, revolutionizing the analysis of biological macromolecules. Techniques such as atmospheric pressure chemical ionization (APCI) have allowed rapid analysis of volatile and semi-volatile chemical species in complex gas matrices without preconcentration.[21,22] While these techniques allow analysis of liquid and gaseous samples, the ability to directly detect surface-bound analyte from native samples had been lacking until recently.

Desorption electrospray ionization (DESI), the first reported ambient MS method, allows ions to be created directly from surfaces under ambient conditions and then collected and analyzed by MS systems equipped with an atmospheric pressure inlet.[23] DESI has been reported for the analysis of solid and liquid chemical species of forensic interest with little or no sample preparation,[24-27] while exploiting the inherent advantage of sensitivity afforded by MS and the specificity of tandem mass spectrometric analysis. DESI is carried out by directing charged microdroplets generated by an electrospray of appropriate solvent (typically methanol/water) onto a contaminated surface or solid analyte, as seen in Figure 1-1. Neutral analytes present on the surface are then desorbed under ambient conditions as secondary ions and detected almost instantaneously through a capillary inlet. DESI mass spectra are similar to typical ESI mass

3

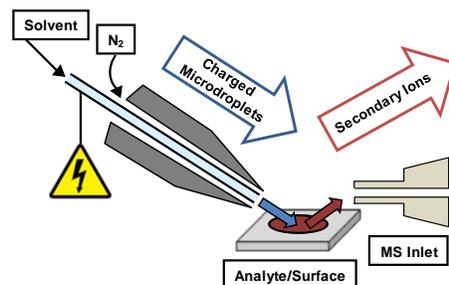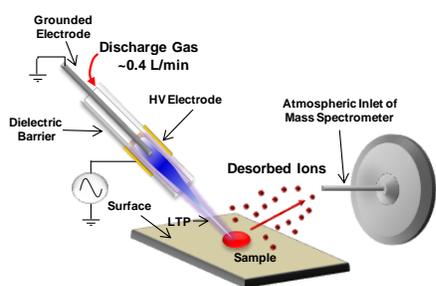**Figure 1-1.** Schematic of the experimental setup for DESI-MS, allowing chemical analysis of untreated surfaces.

spectra in that they are dominated by the protonated molecular ion $[M + H]^+$ of analytes of interest and other simple adducts with little molecular fragmentation.

The versatile nature of DESI-MS has made it an attractive alternative for forensic applications, as recently reviewed.[28] Forensic applications reported in literature include direct analysis of cannabis,[29] determination of counterfeit pharmaceuticals,[30] analysis of latent fingerprints for suspect identification[31] and for chemical residues,[32] direct analysis of clothing for drugs of abuse and explosives residues,[33] and analysis of gunshot residue.[34] Substrates analyzed by DESI-MS include glass, plastics, metals, stone, cloth, skin, and vegetation, and this list continues to grow. The rapid, direct analysis afforded by DESI-MS opens up new avenues of forensic analysis, and many new types of evidence could be potentially screened for relevance.

Other reported ambient MS techniques have high potential in regards to forensic sample analysis, such as low temperature plasma mass spectrometry (LTP-MS).[35] In LTP-MS, depicted in Figure 1.2, an ambient plasma is generated by a dielectric barrier (*i.e.* glass) discharge in the presence of either nitrogen or helium at atmospheric pressure. The generated plasma can desorb/ionize surface-bound chemicals, yielding data very similar to that obtained by DESI-MS, but without the need for solvent. Recently introduced paper spray ionization (PSI) touts the ability to turn physical transfer swabs commonly employed in forensic investigations directly into disposable ionization sources.[36] PSI-MS works by combining two mechanisms, paper chromatographic separation and electrospraying. After a chemical is applied to the paper triangle, the combination of added solvent (a flexible variable) and high voltage allows the now dissolved analyte to migrate through the paper medium, eventually coming to the terminal point of the triangle (as seen in Figure 1.3). The build-up at this point generates a stable electrospray of charged droplets, which contain the added solvent and deposited analyte, that then go on to desolvate and generate dry, gas-phase analyte ions that are detected by the MS. PSI has two distinct advantages towards potential field usage. First, PSI is marked by extreme simplicity in setup and operation. Second, besides depositing a pre-dissolved sample onto the paper triangle for PSI analysis, the paper itself can be used as a surface swab, effectively sampling large areas.

Figure 1-2. Schematic of LTP-MS ionization source, utilizing an ambient plasma to desorb/ionize surface-bound analytes (reproduced from Ref. 35)
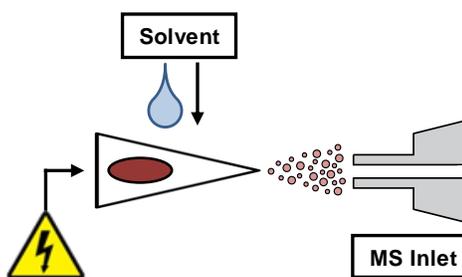

Figure 1-3. Representation of PSI-MS analysis directly from a paper triangle containing analyte.

1.3 Rationale for the Research

In an effort to fulfill the current technological needs of forensic science practitioners and associated laboratories, we sought to create a broadly-applicable, portable chemical detector based on a state-of-the-art mass spectrometer (MS) capable of sampling externally-generated ions. Utilizing the Flir Systems AI-MS 1.2 portable instrument as a testbed, several conventional and ambient ionization techniques were created and/or validated for coupling to the system to allow screening of solid, liquid, and gas-phase chemicals of forensic interest. Application of ambient ionization techniques coupled to this portable system towards complex samples (*i.e.* bulk powders, chemical residues in latent fingerprints, pharmaceutical tablets, clandestine synthetic reaction products/apparatus etc.), authentic evidentiary seizures, and emerging threats was of increased interest to show feasibility in field implementation. Development of swab-based physical transfer methods extended to this instrumentation was intended to provide flexibility in analyzing geometrically-complex samples and large surface areas. Demonstrating congruency of both MS and MS/MS data collected on the Flir AI-MS 1.2 to that obtained on lab-scale, commercial instrumentation was important in showing potential for adoption as an accepted technique in crime scene investigation and forensic analyses.

The principal scientific questions that were addressed in order to gauge performance of the proposed technology included: **(i)** can a portable mass spectrometer be adapted to allow direct analysis of solid, liquid, and gas-phase chemical species? **(ii)** can evidence be effectively screened in a non-destructive nature? **(iii)** is physical transfer of chemical residues more effective than direct surface analysis? **(iv)** is the developed technology on par with current methods in terms of reliability, reproducibility, selectivity, and sensitivity? **(v)** is this technology robust in terms of the current and changing needs of forensic science and law enforcement personnel?

2. Methods

NOTE: This section serves to provide operating conditions and associated variables for project-related methods. As method development was a critical aspect of this project, expanded detail for several topics is provided in *Section 3 – Results*.

2.1 Instrumentation and Ionization Sources

2.1.1 Flir Systems AI-MS 1.2

All investigations into ambient MS utilized a Flir Systems AI-MS 1.2 (Flir Mass Spectrometry, West Lafayette, IN, USA), a portable, cylindrical ion trap (CIT) mass spectrometer that has been ruggedized for field usage. This portable system allows sampling of externally-generated ions via a capillary-based atmospheric pressure inlet, and analyte confirmation is possible via tandem MS (MS/MS). All AC/DC voltages needed for instrument operation and spray-based ionization (*i.e.* ESI, DESI and PSI) are incorporated, as well as an on-board helium supply for the CIT damping gas needed for MS/MS fragmentation. Nebulizing gas (N_2 or air) necessary for DESI analysis is supplied by a stand-alone tank, allowing the use of small, self-contained breathing apparatus (SCBA) tanks commonly utilized by the first response community for field implementation. The size (24" x 20" x 15", L x W x H), weight (98 lbs.) and ruggedness of this instrument makes it an amenable platform for field-based, CSI applications. In selecting appropriate ionization sources to implement on the AI-MS 1.2 testbed, only those that were relatively small in size and could operate without the need of bulky external power supplies were chosen; these selection criteria eliminated other ambient techniques that have shown promise in forensic analysis, but are not quite field-ready (*e.g.* direct analysis is real time, or DART[28]).

2.1.2 MS and MS/MS Analysis

MS analysis on the Flir Systems AI-MS 1.2 was typically performed with a 70 to 450 m/z range for DESI, ESI, and PSI experiments. General data collection utilized an average of 5 scans/spectrum. APCI experiments utilized a 50 to 450 m/z range due to the lower molecular weights of the volatile compounds analyzed. MS/MS analysis utilized the same m/z ranges as in MS analysis. Multiple settings for MS/MS analysis were available for adjustment, including the precursor ion isolation window, frequency of the AC dissociation waveform applied, collision energy (*i.e.* the energy imparted to isolated ions via collision-induced dissociation, CID, with helium damping gas), and dissociation time. These values were initially set to ±10 m/z, 173.5 kHz, an instrument-calculated value based on analyte m/z value, and 15 ms respectively, and were then systematically optimized for each individual analyte.

2.1.3 ESI and DESI

ESI and DESI experiments utilized an on-board ESI/DESI ionization assembly that fixed a 55° spray angle and spray emitter tip-to-analysis surface distance; this source is described in detail in *Section 3.1.1.* Operating conditions for ESI and DESI included a 4 kV spray voltage, 100 PSI nebulizing gas pressure (N_2), and 3 (DESI) or 10 (ESI) μl/minute solvent flow rate. During field use, the N_2 nebulizing gas tank is replaced with a small SCBA tank filled with

OSHA D breathable air. Positive-mode operation utilized a 1:1 methanol:water with 0.1 % formic acid spray solvent, and negative-mode operation generally utilized a 1:1 methanol:water spray solvent. Selected experiments utilized a home-built, positionable DESI source constructed of a fused silica capillary held within a ¼ inch stainless steel Swagelok tee with nebulizing gas supplied axially, pictured in Figure 2-1.

2.1.4 LTP

LTP ionization utilized a home-built source constructed using a stock nanoESI holder with a gas assistance port (Warner Instruments, ESW-M15P) for the LTP probe and operated with helium gas flowed at a rate of 0.2-0.3 L/min. Voltages of 3-4 kV were provided by a commercially-available power supply (Information Unlimited, PVM500), operated with a 33.6 kHz AC signal needed for plasma generation. The electrode of the nanoESI probe was grounded and sheathed within a quartz capillary (Sutter Instruments) that was wrapped with a small section of adhesive-backed copper tape and connected to the LTP power supply to create a dielectric barrier. A schematic of this source is provided in *Section 3.1.1*.
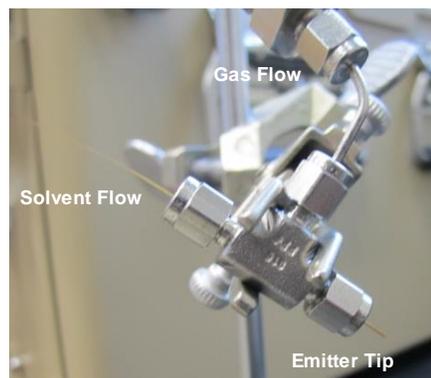
Figure 2-1. Home-built, position-flexible DESI source. A Swagelok tee assembly houses fused silica capillaries for solvent flow and nebulizing gas.

2.1.5 PSI

PSI ionization utilized paper triangles pre-cut into 10 mm by 5 mm isosceles triangles from Whatman chromatography paper (Fisher Scientific, Inc., Hampton NH, USA). After analyte is transferred to the paper, it is allowed to dry and attached to a toothless "alligator clip"-style electrode connected to the instrument's high voltage supply. After application of 1.7 µl of appropriate spray solvent and 4 kV, a stable electrospray can be generated from the paper substrate. Further, the paper triangle can be utilized as a surface transfer swab by pre-wetting with 2.0 µL of methanol prior to probing the substrate of interest.

2.1.6 APCI

APCI ionization utilized tungsten discharge needles under 10 MΩ resistance to generate a corona discharge after application of 4 kV through the high voltage supply of the Flir AI-MS 1.2. The entire discharge needle assembly was housed within a chemically-resistant source body possessing inlet and outlet ports for sample introduction and removal of exhausted vapors that could otherwise lead to a buildup of backpressure in the source, as illustrated in Figure 2-2 with one iteration of our source design. A length of chemically-resistant, perfluoroalkoxy alkanes (PFA) tubing was attached to the inlet port to enable non-proximate sampling, and it possessed a removable Swagelok tee fitted with a rubber septum to serve as in injection port to facilitate introduction of headspace samples through Hamilton Gastight syringes. A small diaphragm pump (KNF Neuberger UNMP015M) powered by an AC adapter and connected to the exhaust port via PFA tubing was utilized to continually draw fresh sample vapors through the sampling tube into the discharge region for ionization, past the inlet capillary for intake of ions into the

instrument, and then out through the exhaust port. The source body attached directly over the inlet capillary of the instrument with a compression fitting.
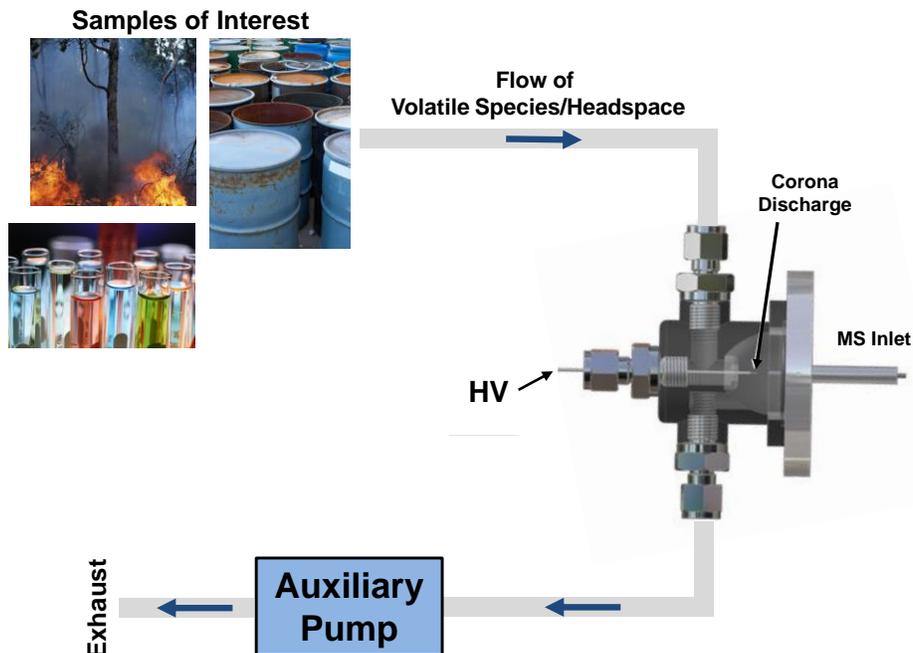
Figure 2-2. Basic design and usage of an APCI source to sample volatile chemical signatures from ambient air. An auxiliary pump continually draws fresh sample vapor through chemically-resistant PFA tubing into the corona discharge region. Resulting ions enter the MS inlet while the pump evacuates used sample through an exhaust port.

2.1.7 GC-MS

GC-MS analysis was performed with a Thermo ITQ 1100, and was utilized only for assessment of transfer and extraction efficiencies of transfer swabs, detailed in *Section 3.2.1*. Prepared and extracted samples were spiked with controlled amounts of internal standard (d_3-methamphetamine) and injected via autosampler for analyses. The temperature profile implemented had at an initial temperature of 50°C held for 0.50 minutes, ramped to 200°C at a 60°C/minute rate, and then raised to the 290°C final temperature at 30°C/minute. Calibration curves were then generated using a minimum of three replicates for standard solutions, and all swabs investigated were analyzed using a minimum of five replicates.

2.2 Sample Preparation

2.2.1 Standards and Liquid-Phase Samples

For characterization studies, stock analytical standards of multiple drugs of abuse, pharmaceuticals, and synthetic precursors were purchased from Cerilliant Co. (Round Rock, TX, USA) in 1000 ppm (1.0 mg/mL) concentrations. Serial dilution of the stock solutions was then performed using analyte appropriate solvents, including methanol and acetonitrile purchased from Fisher Scientific (Pittsburg PA) at HPLC grade. DESI and PSI analysis primarily utilized

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100 ppm dilutions, and ESI utilized dilutions ranging in 0.1 to 10 ppm concentrations depending on the ionization efficiency of individual analytes. Selected unrestricted substances with forensic relevance as potential cutting agents or impurities from clandestine syntheses were purchased as bulk powders from Sigma-Aldrich Co. (St Louis, MO, USA). Appropriate masses of powder were then dissolved in methanol using a volumetric flask to produce a 1000 ppm stock solution used for serial dilutions.

Samples for direct presentation to the DESI source were prepared by using adjustable pipets to spot 1 µL aliquots of a standard solution upon printed Teflon slides (Prosolia Inc., Indianapolis, IN) or upon a surface of interest to deposit a known mass of analyte. The solvent was then allowed to fully evaporate before the substrate itself was positioned in the DESI spray region. Samples for indirect presentation using transfer swabs were prepared in a similar fashion. Samples for PSI-MS analysis were prepared by spotting aliquots of 2.0 µL or less directly upon the paper triangle substrate itself and allowing it to dry before application of spray solvent. For indirect application of the analyte, the paper triangle was wetted with 2.0 µL of methanol and used to swab a surface of interest in a circular motion. The paper triangle was then allowed to dry and analyzed using similar methodology.

2.2.2 Solid-Phase Samples and Powders

Powder samples were prepared from bulk powders purchased from Sigma-Aldrich Co. (St Louis, MO, USA), pharmaceutical tablets purchased from local retailers ground into a powder with a mortar and pestle, or from seized evidentiary materials obtained through collaboration with law enforcement. A small mass of powder, typically ~ 1 mg, was placed upon a surface of interest using a clean spatula. DESI analysis of powder samples used either transfer swabs or adhesive-backed glass slides to hold and present the powder to the spray region. For slide preparation, a gentle stream of canned air was applied to remove any loose powder that may otherwise be transferred into the inlet capillary of the instrument during presentation to the DESI source and cause carryover. For transfer swabs, a spatula was first used to separate the powder into small portions. A swab wetted with a variable amount of methanol (see *Section 3.2.2*) was used to sample the powder and then presented using a positioning guide for direct analysis. For PSI analysis of powder samples, a paper triangle was wetted with 2.0 µL of methanol prior to sampling the powder, followed by direct analysis of the paper substrate.

2.2.3 Gas-Phase Samples

Gas-phase samples for APCI analysis were prepared by enclosing small liquid volumes of volatile chemicals of interest in flasks sealed with rubber septa. The flasks were set aside to allow headspace vapors to build up and reach equilibrium to allow determination of gas-phase concentrations via the analytes partial pressure. An aliquot of this headspace is sampled with a Gastight syringe (Hamilton Co., Reno, NV) and accurately flowed via Harvard Apparatus syringe pump into a calibrated flow of room air (for these studies, 5 L/min). This diluted air sample (now containing a known amount of accelerant vapor) can then be flowed directly into the custom APCI source constructed for the Flir AI-MS 1.2. Analysis of undiluted vapor was performed by positioning the inlet of the sampling tube near bulk liquids or objects releasing gaseous vapors, taking care to avoid direct contact with the object or liquid to prevent coating the sampling tube with the material in question, which could lead to saturating the instrument detector or carryover.

To simulate the direct analysis of evidence that may be present at an actual arson investigation, representative charred samples were prepared by ignition with potential accelerants. Initial testing involved exposing 1 in. x 1 in. x 0.25 in. pinewood blocks with small quantities (~5 mL) of a potential liquid accelerant and igniting it in a glass petri dish. The wood sample was then ignited and allowed to openly burn until all accelerant liquid was exhausted, and the block was allowed to become visibly charred before being capped to extinguish any remaining flames. Charred samples were then placed in Erlenmeyer flasks sealed with rubber septa for transport and to allow generation of headspace vapor within. These headspace vapors were then analyzed by APCI after transporting them via a pump-assisted sampling tube (shown in Figure 2-2) into the ionization source.

2.3 Analytical Characterization

2.3.1 Spectral Quality and Accuracy

MS and MS/MS spectral quality was assessed by comparison of both the intensity and breadth of ions (*i.e.* molecular ion signatures in base MS mode and fragment ions seen in MS/MS with relative abundances) to data collected on lab-scale, commercial MS systems utilizing similar ionization methods and by that reported in chemical literature. While some deviation in the population and abundances of fragment ions were seen, they were overall congruent with all direct comparisons; MS/MS yields are very much effected by both the fragmentation method (*e.g.* CID) and mass analyzer employed.

Accuracy of spectral data obtained with the Flir AI-MS 1.2 was verified by comparison with that obtained in the Wiley Registry® of Tandem Mass Spectral Data (MSforID) through collaboration with Dr. Herbert Oberacher (Associate Professor, Innsbruck Medical University, Austria). Blind MS/MS spectra generated with the AI-MS 1.2 utilizing three separate collision energies for each analyte were submitted to the MSforID spectral database to calculate "relative average match probability," or *ramp* value; the searching algorithm of the MSforID database has been reported in detail in literature.[37] Single *ramp*-values range between 0 and 100, and a high compound-specific *ramp* value (40 or higher) indicates high similarity between the unknown and the reference compound. The database identifies the submitted chemical data as the entry that produces the highest *ramp* value. More detailed information regarding these assessments can be found in *Section 3.1.7*.

2.3.2 Limits of Detection and Quantitation

Limits of detection (LODs) were determined for all examined ionization methods in MS/MS mode by measuring signal responses from purchased analytical standards and utilizing the traditional threshold of a signal-to-noise ratio of 3 (*i.e.* signal intensity corresponding to the analyte being three times higher than the standard deviation of the mass spectral noise level). In instances where there was no appreciable noise level in MS/MS analysis, the LOD was determined as the quantity of analyte that gave lowest fragment ion signal that was both stable and detectable.

To determine detection limit of APCI analysis on the Flir AI-MS 1.2, defined as a signal-to-noise ratio of 3, accurate concentration gas standards were produced and analyzed. To create these known standards, equilibrated headspace was taken from sealed flasks containing liquid accelerant at standard temperature and pressure. As the vapor pressure is a known variable for

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most of the common accelerants (accessed from the Stanford University SRC Physical Properties Database – PHYSPROP), the corresponding gas-phase concentration (in parts per million [ppm]) can be calculated from the headspace. A known volume of this headspace is sampled with a Hamilton Gas-Tight syringe and accurately flowed via Harvard Apparatus syringe pump into a calibrated flow of room air (for these studies, 5 L/min). This diluted air sample (now containing a known amount of accelerant vapor) is then flowed directly into the constructed APCI source of the Flir AI-MS 1.2.

Quantitative ability was assessed by constructing calibration curves for select drugs of abuse using DESI-MS. To construct these curves, standard solutions of each analyte were spotted onto glass substrates to reach the target deposited mass. The average of four replicate measurements of direct DESI analysis intensity was used for plotting purposes. Linear dynamic range and precision could then be assessed.

2.3.3 Reliability (False Positive/False Negative Rates)

Reliability was assessed by calculating false positive/false negative rates for a large quantity of control samples (*i.e.* "positive" samples where a known chemical residue was present and "negative" samples with no residue). Analysis of these controls was completed over a one month period at different times of day in an effort to determine inter- and intraday variability in collected spectral intensity for all positive controls.

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3. Results

The overall project was separated into seven discrete tasks in order to determine performance and breadth of applicability of ambient ionization methods coupled to the Flir Systems AI-MS 1.2 portable mass spectrometer, as delineated below.

3.1 Task 1: Test Proof of Concept Detection of Chemicals of Interest on the Flir Systems AI-MS 1.2

The Flir Systems AI-MS 1.2 is a portable, cylindrical ion trap (CIT) mass spectrometer that has been ruggedized for field usage. This portable system allows sampling of externally-generated ions via a capillary-based atmospheric pressure inlet, and analyte confirmation is possible via tandem MS (MS/MS). All AC/DC voltages needed for instrument operation and desorption electrospray ionization (DESI) are incorporated, as well as an on-board helium supply for the CIT damping gas needed for MS/MS fragmentation. Nebulizing gas (N_2 or air) necessary for DESI analysis is supplied by a stand-alone tank, allowing the use of small, self-contained breathing apparatus (SCBA) tanks commonly utilized by the first response community for field implementation. The size (24" x 20" x 15", L x W x H), weight (98 lbs.) and ruggedness of this instrument makes it an amenable platform for field-based, CSI applications. A photo of the testbed instrument can be seen in Figure 3-1a.

3.1.1 Compatible Ionization Sources for Solid and/or Liquid-Phase Samples

Over the course of the project, several conventional and ambient ionization methods were shown applicable on the Flir Systems AI-MS 1.2 system, including electrospray ionization (ESI), desorption electrospray ionization (DESI), and, to a lesser extent, low temperature plasma (LTP) and paper spray ionization (PSI). The AI-MS 1.2 system (Figure 3-1a) has a position-stationary ionization assembly that allows the use of ESI-MS for solution-based analytes and DESI-MS for solids and surface-bound residues (See Figure 3-1b). The stationary source is mounted to fix both the DESI spray angle (55°) and DESI emitter tip-to-analysis surface distance. The DESI sprayer is held in a hemispherical enclosure, and once relatively-flat samples are placed at the enclosure egress, they undergo desorption/ionization. A depiction of this enclosure is seen in Figure 3-2a, with a photo depicting the direct analysis of a glass side with DESI seen in Figure 3-2b.

Figure 3-1a. Photo of Flir Griffin AI-MS instrument, with aluminum can in place for scale.

Figure 3-1b. Stock ionization assembly that allows both ESI (solution-based analytes) and DESI (surface-based analytes)

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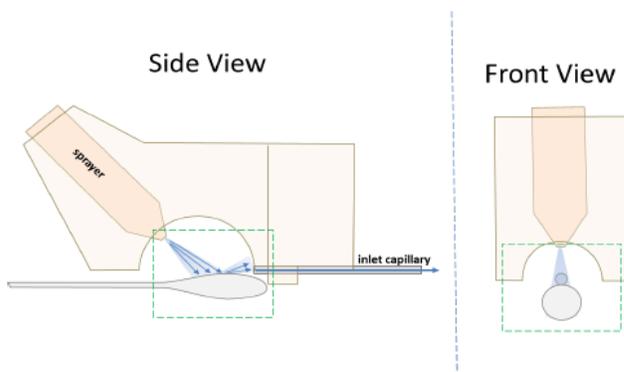
Figure 3-2a. Depiction of swab analysis using DESI. Charged droplets impinge upon the swab surface, desorbing/ionizing analytes present and projecting them toward the MS capillary inlet for mass analysis. Courtesy of Flir Mass Spectrometry.
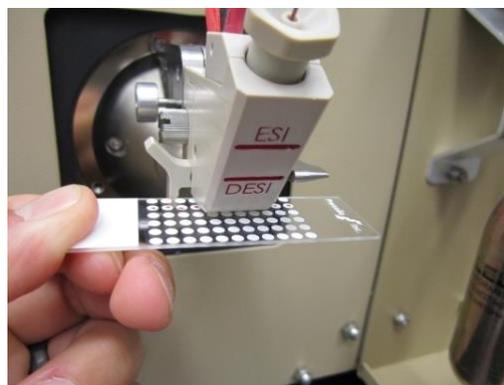
Figure 3-2b. Photo depicting direct analysis of a Teflon-coated glass microscope slide. Upon placement in the DESI spray, surface-bound analytes are instantaneously ionized and detected with minimal carryover

To facilitate the analysis of more geometrically-complex samples (*i.e.* those that require flexibility in positioning), a position-adjustable DESI source was constructed in-house using a ¼" stainless steel Swagelok tee and fused-silica capillaries for solvent flow and axial nebulizing gas (Figure 3-2c). Positioning of this source relative to both the sample surface and capillary inlet of the MS allows direct analysis of a variety of substrates of forensic interest, including pill bottles and plastic baggies (discussed later in this report). While demonstrated as useful to certain samples, the position-adjustable DESI source has significant disadvantages for field usage compared to the stationary ESI/DESI assembly. As the DESI spray angle and DESI emitter tip-to-analysis surface

Figure 3-2c. Photo of the home-built DESI source, composed of a Swagelok tee assembly and fused silica capillaries for solvent flow and nebulizing gas.

distance must be set and maintained by the operator with the custom source, there is a greater potential for misuse compared to the factory spray assembly. Enclosing the spray emitter (as in the factory spray assembly) also protects from breakage and contamination when samples are introduced. The use of surface swabbing as a means to investigate residues from geometrically-complex or large area samples and sub-sample bulk powders was extensively investigated during this project (discussed further in *Section 3.2*). Used swabs can then be presented directly into the ESI/DESI spray assembly to detect and identify transferred compounds, as depicted in Figure 3-2a. DESI-MS was determined to be an ambient ionization method of high flexibility and utility, making it the focus of much of the research and spectral databasing efforts herein.

A custom-built, low temperature plasma (LTP) ionization source (see Figure 3-4) was constructed and implemented for proof-of-principle testing on the Flir AI-MS 1.2, as it has been recently shown able to detect surface-bound explosive residues using metastable helium species produced in a dielectric barrier discharge The LTP probe was produced using a stock nanoESI holder with gas assistance port (Warner Instruments, ESW-M15P), through which helium gas was flowed at a rate of 0.2-0.3 L/min. To provide the necessary dielectric barrier, a quartz capillary (Sutter Instruments) was sheathed over the electrode of the nanoESI probe (grounded),

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to which a small section of adhesive-backed copper tape was wrapped and connected to the LTP power supply. When AC voltage is applied to the copper tape, a plasma discharge region is produced on the inside of the capillary due to the dielectric medium; this discharge produces energetic He species that go on to directly or indirectly (through production of secondary reagent ions) ionize surface-bound analytes. A commercially-available power supply was utilized for this source (Information Unlimited, PVM500), producing the 3-4 kV, 33.6 kHz AC signal needed for plasma generation. LTP-MS was examined toward explosives analysis, but was determined to be a sub-optimal solution when compared to the ruggedness and reliability of the stationary ESI/DESI assembly.

Figure 3-3. Depiction of the home-built LTP source. A discharge is produced between a (A) grounded electrode and (C) high voltage outer electrode, with a (B) quartz glass capillary serving as the dielectric barrier needed and ionization probe. The excited gas species produced can desorb/ionize surface-bound samples for MS analysis.

While not a main focus of the initial project proposal, paper spray ionization (PSI) was also investigated for use with the Flir AI-MS 1.2 system, as it offers a cost-effective analysis method coupled with surface swabbing capability.   Generated mass spectra are very similar to that obtained with both ESI and DESI-MS on this instrument.
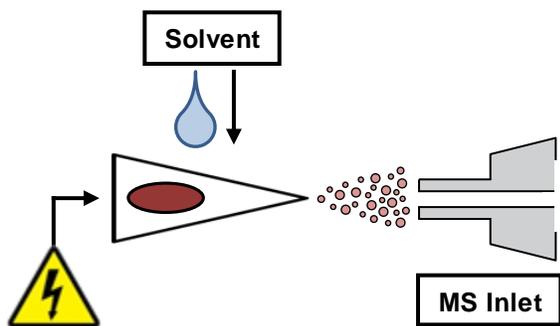
Figure 3-4. Depiction of paper spray ionization directly from a paper triangle containing analyte. By application of appropriate solvent and high voltage, deposited or swabbed analytes on the paper can be directly ionized/detected with the Flir Systems AI-MS.
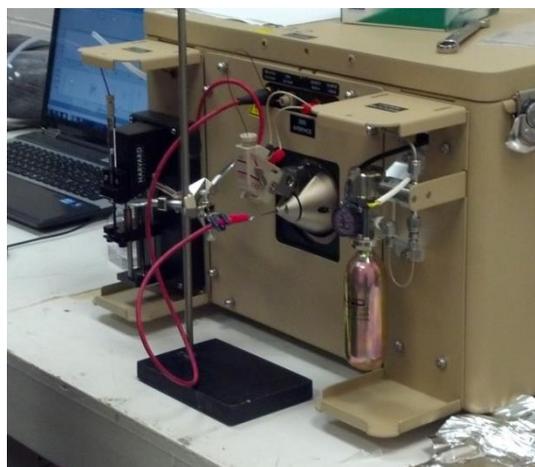
Figure 3-5. Photo of the PSI source in use on the Flir AI-MS system. The red cable serves dual purposes of holding the paper substrate in place and delivering the required high voltage for ionization.

PSI-MS works by combining two mechanisms, paper chromatographic separation and electrospraying. After a chemical is applied to the paper triangle, the combination of added solvent (a flexible variable) and ~ 4 kV of high voltage allows the dissolved analyte to migrate through the paper medium, eventually coming to the terminal point of the triangle (as seen in

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Figure 3.4). The build-up at this point generates a stable electrospray of charged droplets, which contain the added solvent and deposited analyte, that then go on to desolvate and generate dry, gas-phase analyte ions that are detected by the MS. PSI has two distinct advantages towards potential field usage. First, PSI is marked by extreme simplicity in setup and operation. As seen in the photo in Figure 3-4, the only materials needed to perform these analyses that are not supplied by the Flir AI-MS 1.2 is the paper substrate itself and the delivered solvent; the on-board high voltage "alligator clip" cable delivers the needed electrospray voltage and holds the paper substrate in place. Second, besides depositing a pre-dissolved sample onto the paper triangle for PSI analysis, the paper itself can be used as a surface swab, effectively sampling large areas. This ability aligns itself well with goals of *Task 2: Investigate Alternative Surface Sampling Methodologies,* as it has the potential to eliminate the extra steps needed for DESI-MS analysis of utilized sampling swab. For PSI, the surface swab **and** ionization source are effectively the same.

3.1.2 Data Quality and Broad Application to Forensic Chemical Analysis

Ambient ionization coupled with the Flir AI-MS 1.2 system has far exceeded our initial expectations in regards to performance and applicability to analytes and samples of interest to forensic science and crime scene investigation.   At the time of writing, our database of detected and confirmed analytes utilizing the AI-MS 1.2 numbers **73** and continues to grow as new and emerging illicits continue to be added. This spectral database contains MS and MS/MS from a wide array of illicit drugs, abused pharmaceuticals, potential cutting agents, explosives and species related to ballistics, and accelerants, and representative mass spectra for these compounds can be seen in *Appendices A* and *B*. Tabulated MS and MS/MS information, including optimized MS/MS method conditions for each analyte and observed ion fragmentations, can be seen in Table 3-1.

For the analytes listed, all non-volatile species were analyzed at a deposited mass of 100 ng via DESI-MS, the milestone amount targeted for this grantwork, unless they were directly analyzed via atmospheric pressure chemical ionization (APCI, see *Section 3.3*) due to volatility. Analytes specifically confirmed via APCI are known accelerants or chemical warfare agent simulants and are indicated as such. Of note, a majority of the compounds tested produced very high spectral signal intensity even at 100 ng, meaning that most limits of detection for these species lie in the very low to sub-ng range. Target analytes can also be detected and identified in complex matrices and authentic forensic evidence, as discussed in *Sections 3.5 and 3.6*. Furthermore, spectral data collected on the AI-MS 1.2 was highly congruent in regards to both MS and MS/MS ion signatures collected on lab-scale MS instrumentation and also seen in scientific literature; this is seen as a major benefit towards actual implementation in CSI applications and potential acceptance as a validated method.

Table 3-1. MS and MS/MS Characterization of Project Analytes on the Flir Systems AI-MS 1.2

Compound	MW (Da)	Precursor Ion (m/z)	MS/MS Transitions (m/z)**	Frequency (KHz)	Voltage (V)	Time (ms)
Acetaminophen	151.06	152 [M+H]+	152 (39%) 110 (100%)	175.5	0.230	15.0
Acetone†	58.08	59 [M + H]+ 117 [2M + H]+	N/A	N/A	N/A	N/A
Alprazolam	308.77	309 [M + H]+	281 (100%) 274 (62%) 241 (19%) 206 (20%) 165 (6%)	172.5	0.370	22.0
Amobarbital	226.27	225 [M – H]-	182 (100%)	168.5	0.280	30.0
Amphetamine	135.2	136 [M + H]+	119 (100%) 91 (18%)	175.0	0.195	22.5
Benzene†	78.11	79 [M + H]+	N/A	N/A	N/A	N/A
Benzocaine	165.19	166 [M + H]+	138 (100%) 120 (14%) 93 (2%)	174.0	0.235	15.0
Benzoylecogonine	289.33	290 [M + H]+	168 (100%)	171.0	0.275	15.5
Caffeine	194.19	195 [M + H]+	138 (100%)	173.0	0.340	19.5
Chlorodiazepoxide	299.75	300 [M + H]+	283 (100%) 241 (23%)	172.0	0.360	9.0
Cocaethylene	317.38	318 [M + H]+	196 (100%) 150 (11%)	174.0	0.455	15.0
Cocaine	303.35	304 [M + H]+	182 (100%) 150 (7%)	172.5	0.280	13.5
Codeine	299.36	300 [M + H]+	282 (11%) 243 (17%) 225 (22%) 215 (100%) 183 (23%) 161 (14%)	172.5	0.410	16.0
Creatine	131.13	132 [M + H]+	90 (100%)	174.5	0.200	10.5
Desomorphine	271.35	272 [M + H]+	215 (100%) 197 (16%)	172.5	0.335	22.0
Dextromethorphan	271.43	272 [M + H]+	215 (100%) 213 (23%)	171.5	0.405	15.0

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			147 (27%)			
Diazepam	284.74	285 [M + H]$^+$	257 (92%) 228 (25%) 222 (100%) 193 (28%) 182 (28%) 154 (83%) 105 (15%)	173.0	0.390	13.5
Dimethyl Methylphosphonate†	124.08	125 [M + H]$^+$	125 (25%) 111 (100%) 93 (15%)	TBD	TBD	TBD
Dimethyl Phthalate	194.18	195 [M + H]$^+$	163 (100%)	173.0	0.255	14.0
1,3-Dinitrobenzene	168.11	168 [M]$^-$	TBD	N/A	N/A	N/A
2,4-Dinitrotoluene	182.13	181 [M – H]$^-$	TBD	N/A	N/A	N/A
Diphenylamine	169.23	170 [M + H]$^+$	92 (100%)	173.5	0.300	15.0
Ecgonine Methyl Ester	199.25	200 [M + H]$^+$	182 (100%)	173.5	0.255	15.5
Ephedrine	165.23	166 [M + H]$^+$	148 (100%)	TBD	TBD	TBD
Ethanol†	46.07	93 [2M + H]$^+$ 139 [3M + H]$^+$	N/A	N/A	N/A	N/A
Ethyl Centralite	268.35	269 [M + H]$^+$	148 (100%) 120 (76%)	170.0	0.250	16.0
Ethyl Ether†	74.12	75 [M + H]$^+$ 149 [2M + H]$^+$	N/A	N/A	N/A	N/A
Fentanyl	336.47	337 [M + H]$^+$	188 (100%)	171.5	0.375	14.0
Flunitrazepam	313.27	314 [M + H]$^+$	300 (94%) 286 (75%) 268 (100%) 240 (34%)	173.0	0.380	17.5
Guaifenesin	198.09	199 [M+H]$^+$	181 (23%) 163 (81%) 151 (17%) 125 (100%)	TBD	TBD	TBD
Heroin	369.41	370 [M + H]$^+$	328 (70%) 310 (30%) 268 (100%)	172.0	0.485	16.0

			237 (14%)			
			211 (27%)			
Hydrocodone	299.37	300 [M + H]$^+$	243 (22%) 225 (9%) 199 (100%) 183 (16%)	173.5	0.420	17.5
Hydromorphone	285.14	286 [M + H]$^+$	243 (4%) 229 (16%) 211 (21%) 185 (100%)	172.5	0.350	14.5
Hydroxyzine	374.9	375 [M + H]$^+$	201 (100%) 166 (21%)	171.0	0.315	15.0
Isopropanol†	60.1	61 [M + H]$^+$ 121 [2M + H]$^+$	N/A	N/A	N/A	N/A
Kerosene	Various	Various	N/A	N/A	N/A	N/A
Ketamine	237.73	238 [M + H]$^+$	220 (100%) 208 (32%) 152 (21%)	172.5	0.430	16.5
Levamisole	204.29	205 [M + H]$^+$	178 (100%) 145 (45%) 88 (20%)	173.0	0.275	15.0
Lidocaine	234.34	235 [M + H]$^+$	85 (100%)	171.5	0.275	14.5
Lorazepam	320.2	321 [M + H]$^+$	303 (69%) 275 (100%)	172.5	0.385	18.5
LSD	323.43	324 [M + H]$^+$	281 (53%) 223 (100%) 197 (26%)	172.0	0.430	20.0
MDMA	193.25	194 [M + H]$^+$	163 (100%)	171.5	0.160	18.5
MDPV	275.34	276 [M + H]$^+$	205 (95%) 175 (100%) 135 (31%) 126 (21%)	171.5	0.255	14.0
Mephedrone	177.24	178 [M + H]$^+$	160 (100%) 147 (6%)	TBD	TBD	TBD
Mescaline	211.26	212 [M + H]$^+$	195 (195%)	173.5	0.260	14.0
Methadone	309.45	310 [M + H]$^+$	265 (100%)	171.0	0.345	12.5
Methamphetamine	149.23	150 [M + H]$^+$	119 (100%) 91 (23%)	174.5	0.180	15.0
Methanol†	32.04	65[2M + H]$^+$	N/A	N/A	N/A	N/A

		97[3M + H]$^+$				
Methyl Centralite	240.3	241[M + H]$^+$	134 (100%)	172.5	0.315	13.0
Methyl ethyl ketone†	72.11	73 [M + H]$^+$ 145 [2M + H]$^+$	N/A	N/A	N/A	N/A
Methyl salicylate†	152.15	153 [M + H]$^+$ 121 [MH – CH$_3$OH]$^+$	121 (100%)	TBD	TBD	TBD
Methylone	207.23	208 [M + H]$^+$	190 (100%) 160 (96%)	TBD	TBD	TBD
Methylphenidate	233.3	234 [M + H]$^+$	84 (100%)	173.0	0.250	4.0
Morphine	285.34	286 [M + H]$^+$	268 (11%) 229 (28%) 211 (32%) 201 (100%) 183 (37%) 173 (29%) 155 (12%)	172.5	0.375	15.0
Oxycodone	315.36	316 [M + H]$^+$	298 (100%)	172.0	0.315	15.0
4-nitro diphenylamine	214.22	215 [M + H]$^+$	198 (100%)	172.5	0.275	11.0
N-nitroso diphenylamine	198.22	199 [M + H]$^+$	169 (100%)	173.0	0.475	15.0
PCP	243.39	244 [M + H]$^+$	159 (100%) 86 (83%)	173.0	0.290	13.0
Pentedrone	191.27	192 [M + H]$^+$	174 (100%) 161 (10%) 132 (31%)	172.5	0.265	17.0
Pentobarbitol	226.27	225 [M – H]$^-$	182 (100%)	168.5	0.280	30.0
Phenacetin	179.22	180 [M + H]$^+$	138 (100%) 110 (23%)	173.5	0.215	13.0
Phenobarbitol	232.24	231 [M – H]$^-$	188 (100%)	168.5	0.280	30.0
Phenylephrine	167.09	168 [M+H]$^+$	150 (100%) 135 (10%)	172.5	0.290	15.0
Phenylephrine In-Source Fragment (m/z 150)	N/A	150 [MH – H$_2$O]$^+$	150 (9%) 135 (18%) 121 (36%)	TBD	TBD	TBD

			119 (100%)			
			109 (72%)			
			91 (64%)			
Pseudoephedrine	165.23	166 [M + H]$^+$	148 (100%)	172.0	0.205	14.5
Secobarbitol	238.28	237 [M – H]$^-$	194 (100%)	172.5	0.296	20.0
Styphnic acid	245.11	244 [M – H]$^-$	227 (100%)	N/A	N/A	N/A
Δ-9-THC	314.47	315 [M + H]$^+$	259 (79%) 221 (29%) 207 (21%) 193 (100%) 181 (26%) 135 (38%)	172.0	0.360	17.0
Turpentine† (Pinene)	136.24	137 [M + H]$^+$ 155 [MH + H$_2$O]$^+$	137 (10%) 95 (35%) 81 (100%)	N/A	N/A	N/A
Triazolam	343.23	344 [M + H]$^+$	315 (26%) 308 (100%)	172.5	0.390	14.5
Trinitrotoluene (TNT)‡	227.13	227 [M – H]$^-$ 226 [M]$^-$ 210 [M – OH]$^-$ 197 [M – NO]$^-$	TBD	TBD	TBD	TBD
Zaleplon	305.45	306 [M + H]$^+$	288 (18%) 264 (100%) 236 (34%)	173.5	0.390	23.0
Zolpidem	307.4	308 [M + H]$^+$	263 (100%) 235 (56%)	173.0	0.370	15.5
Zopicolone	388.81	389 [M + H]$^+$	345 (100%) 277 (70%) 245 (52%) 217 (35%)	173.0	0.545	15.0

†Headspace of this analyte was analyzed via APCI   N/A = MS/MS confirmation not possible due to low mass cutoff
‡ Analyzed via LTP only   TBD = exact assignments still in progress
* Tentative Assignment   █ = Analyzed in Negative-Ion Mode
** Relative intensities of observed transitions included (0-100%)

3.1.3 Comparison of Spray and Ambient Ionization Methods

Spectra collected utilizing spray-based conventional (ESI) and ambient (DESI, PSI) ionization techniques on target analytes are marked by high intensity signatures for the

protonated molecule, similar to data generated on lab-scale instrumentation. These techniques are typically "soft" ionization methods, meaning little to no fragmentation is seen during initial ionization, but some of these analytes do show in-source fragmentation. This unintentional fragmentation stems from the ion optics used for ion transport from ambient conditions (*i.e.* capillary inlet and subsequent ion focusing electrodes) into the reduced-pressure vacuum system, and they can be partially controlled by manipulating the potentials applied to said optics. The in-source fragments seen are reproducible and coincide directly to common MS/MS transitions for each specific analyte, allowing them to be used for enhanced analyte confirmation.

To assess the efficacy of the implementable ionization methods, comparison studies between ESI, DESI and PSI-MS were undertaken, using methamphetamine as a model system. Characteristic spectral data can be seen in Figure 3-6.
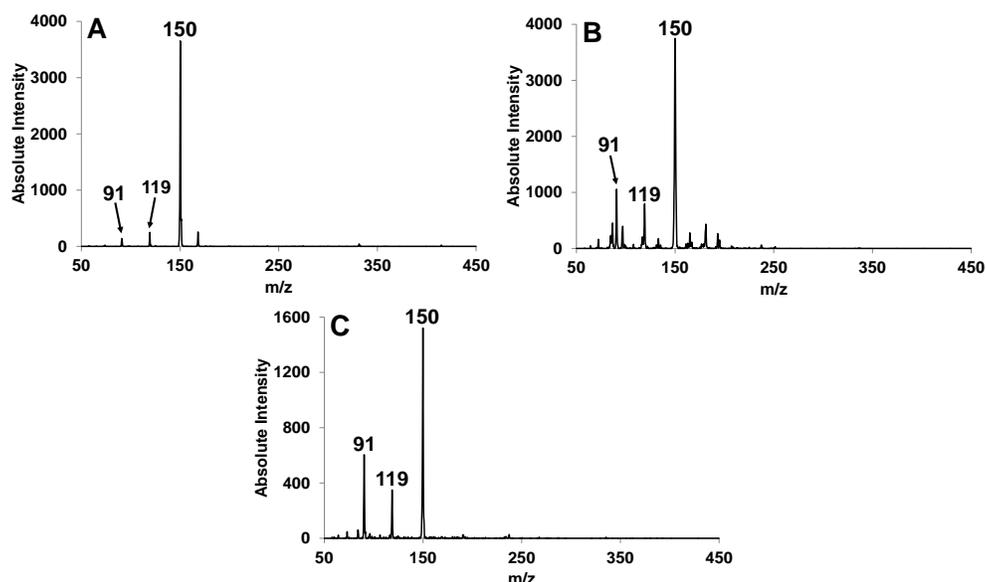
Figure 3-6. (a) ESI, (b) PSI, and (c) DESI mass spectra collected on the Flir AI-MS for 15 ng of methamphetamine. As seen, the obtained spectra are quite similar. ESI data was collected on a 1.5 ppm methamphetamine solution flowed at 5 μL/min for 2 min, resulting in a total analyzed mass of 15 ng.

As seen in Figure 3-6, spectra collected from the differing ionization methods are marked by high similarity in regards to ions seen; however, when the overall signal intensities are compared amongst the data, there are some distinct differences. ESI data seen in Figure 3-6a is marked by high intensity and very low baseline noise, typical of overall ESI-MS data collected on the Flir AI-MS 1.2. When a similar total amount of methamphetamine (15 ng) is spotted as a residue onto a paper substrate, the signal intensity generated from direct PSI analysis is nearly identical to ESI, except for a slightly higher degree of in-source fragmentation that increases the yield of corresponding m/z 119 and m/z 91 fragments and other chemical noise resulting from the substrate itself. The performance of PSI in these studies shows its analytical potential, warranting further investigation.

When analyzing a 15 ng residue of methamphetamine deposited onto porous Teflon, DESI produces the lowest signal intensity at nearly 1500 arb. units. As DESI is a two-stage ionization method (that is, the DESI spray solvent impacts the surface, projecting secondary droplets containing surface-bound analyte towards the MS inlet for detection), it is much more

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sensitive to sample positioning in respect to the ionization assembly and MS inlet. Of note, while the spectral intensity of DESI was less than that of the other tested methods, the 1500 mark is still quite high considering the trace sample analyzed; signals of 25 or below are typically considered at noise level. Similar performance between these ionization methods is seen with all other project analytes investigated. While there are differences in performance between these techniques, they are all viable for use on the Flir AI-MS 1.2 system.

3.1.4 Analysis of Unconventional Surfaces and Complex Samples

Analysis of residues from flat surfaces like glass or plastic was demonstrated with very high throughput and sensitivity, but given the potential complexity of authentic forensic evidence in terms of chemical composition, geometry, and size, experimentation on unconventional substrates and complex samples was thoroughly investigated. The Flir AI-MS 1.2 performed well in this testing, with the suite of ionization method utilized proving robust to a wide selection of test samples. Over the course of the project, representative surfaces tested included glasses, metals, polymers/plastics, non-stick coatings, common paraphernalia, phone keypads, and many others; further detail is provided in *Sections 3.2* (Tasks 2) and *3.6* (Task 6). Chemical residues residing in latent fingerprints were also shown detectable directly and indirectly through swab transfer when utilizing DESI-MS. The ability to rapidly screen and identify chemicals of interest from mock samples and authentic forensic evidence was routinely shown, including from soft drinks containing adulterants, abused pharmaceutical tablets, "white powder" evidence types (see *Section 3.6*), organic signatures attributed to gunshot residue, and even methamphetamine and precursors in real-time during a clandestine synthesis (see *Section 3.5*).

Representative spectra from our investigation into the effect of cutting agents can been found in Figures 3-7 and 3-8. Figure 3.7 shows the direct DESI-MS analysis from a residue containing the synthetic cathinone MDPV (80 ng) in the presence of two common cutting agents, phenacetin (analgesic, 136 ng) and benzocaine (anesthetic, 64 ng) placed onto the lip of a used prescription pill bottle. The protonated molecules of MDPV, phenacetin, and benzocaine are all clearly seen at m/z 276, 180, and 166, respectively, and data is collected instantaneously once the pill bottle is placed below the DESI emitter. Figure 3.8 shows the DESI-MS analysis of 100 ng of cocaine in the presence of three cutting agents, phenacetin (analgesic, 200 ng), hydroxyzine (antihistamine, 200 ng), and benzocaine (anesthetic, 200ng). The protonated molecule of each analyte present on the glass slide is easily seen. Identifying cutting agents present simultaneously with the illicit chemical itself in seized drug evidence has the potential to discriminate drug manufacturers and suppliers

Latent fingerprint analysis via DESI-MS has been shown to not only allow suspect identification like traditional developer-based methods, but also provide insight regarding substances that the individual has come in contact with through other chemical signatures present in collected spectra. To show the ability to screen chemical residues from latent fingerprints with DESI on the Flir AI-MS 1.2, a fingerprint was deposited onto a glass slide after exposure to 1 μg of MPDV spiked via a solvent aliquot. After complete evaporation of the solvent, a latent fingerprint was transferred onto a glass microscope slide. Without further preparation, the fingerprint was directly screened via DESI-MS, yielding the spectrum seen in Figure 3-9. The protonated molecule of MDPV is clearly present. Interestingly, protonated nicotine is also seen at m/z 163, present due to fact that the latent fingerprint analyzed was that of a cigarette smoker.
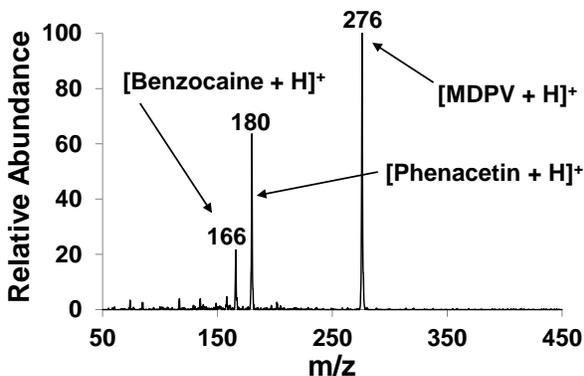
Figure 3-7. Direct DESI-MS analysis of the lip of a prescription pill bottle spiked with the synthetic cathinone MDPV and common cutting agents benzocaine and phenacetin.
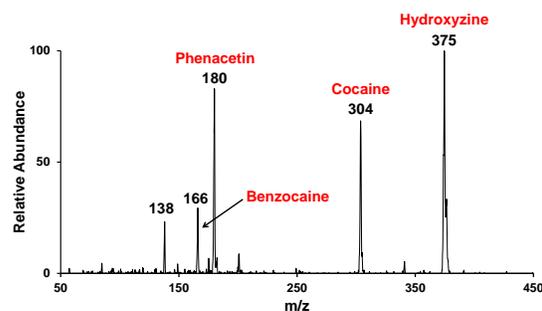
Figure 3-8. Positive-ion DESI mass spectrum of cocaine in the presence of common cutting agents.

Figure 3-10 shows the PSI-MS mass spectrum obtained from the swabbing of gun powder residues deposited onto a glass surface. For this experiment, a small amount of Winchester® smokeless powder was deposited, and a paper triangle pre-wetted with a small aliquot of acetone (acetone assists in the transfer of powder residue to the paper swab and dissolvation) was used to swab the surface. Immediately, the paper swab was placed into the high voltage mount (as seen in Figure 3-4), and a 1 μL aliquot of spray solvent (4:1 methanol:water with 0.1% formic acid) was added to produce ionization. After roughly 2 sec. of delay (as the analyte must migrate through the paper to the triangle point), characteristic spectra of the smokeless powder is collected, yielding ion signatures for the organic constituents *n*-nitrosodiphenylamine (m/z 199), diphenylamine (m/z 170), and ethyl centralite (m/z 149, seen as an in-source fragment). In total, this experiment took less than 1 minute to perform. Utilizing PSI-MS to investigate trace residues attributed to gunshot residue (GSR) is an interesting future application of this portable MS system.
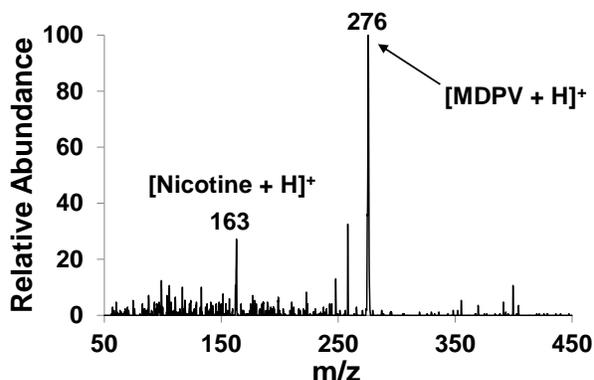
Figure 3-9. Direct DESI analysis of a latent fingerprint deposited onto a glass slide. Before deposition, the pad of the finger was spiked with MPDV (seen at m/z 276). A signature for nicotine is also seen due to the test subject handling a cigarette prior to this experiment.
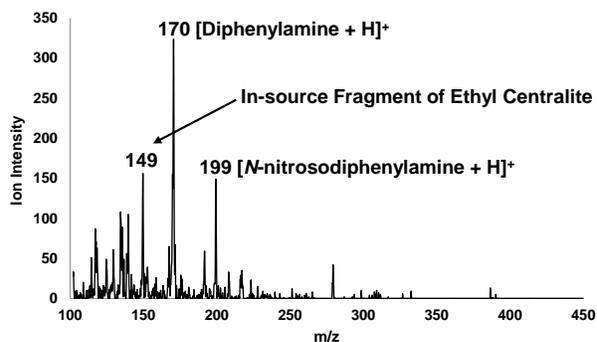
Figure 3-10. PSI mass spectrum collected on the Flir AI-MS 1.2 from the direct swabbing of Winchester® smokeless powder from a solid surface.

3.1.5 Negative-Ion Mode on the Flir AI-MS 1.2

While a majority of analytes of forensic interest shown applicable to the Flir AI-MS 1.2 are optimally detected in positive-ion mode, negative-ion mode capability of the system can be utilized to extend viability towards analytes that exclusively or primarily deprotonate during ionization (*i.e.* barbiturates). To demonstrate negative-ion mode detection of compounds of interest, select barbiturates of high probability of abuse, including amobarbitol, pentobarbital, phenobarbital, and secobarbitol, were successfully investigated. Figure 3-11 shows representative spectra for (A) ESI-MS and (B) ESI-MS/MS analysis for secobarbitol. Overall

Figure 3-11. (A) ESI-MS and (B) MS/MS spectra collected for secobarbitol using the Flir AI-MS 1.2 in negative-ion mode. Deprotonated (*i.e.* [M-H]⁻) secobarbitol can be seen at m/z 237, with its characteristic MS/MS fragment signature at m/z 194.

simplicity of collected spectra in both modes is comparable, while overall signal intensity during negative-ion mode operation was lower compared to that of positive-ion mode. Increasing the ionization time (*i.e.* the time period that externally-generated ions are allowed to fill the CIT mass analyzer prior to generation of a mass spectrum) from the typical 150 ms to 450 ms signal was able to rectify some of this signal loss in negative-ion mode operation.

3.1.6 Explosives Analysis and Implementation of LTP-MS

To test application to common military explosives and ordnance-related species, surface-bound residues were investigate at low levels (100 ng) with the on-board DESI ionization source of the Flir AI-MS 1.2. The most sensitive way to detect common military explosives (RDX, HMX and PETN) via DESI-MS is through doping the spray solvent with the Cl⁻ anion (commonly from small volumes of HCl or NaCl), which creates chloride-bound adducts with the analytes. Interestingly, these chloride-bound adducts are not observed in DESI-MS testing of explosives on the AI-MS 1.2 at any appreciable level, as well as signatures for another common explosive, TNT. As DESI-MS analysis of explosives using the chloride adduction methodology is quite routine on larger-scale mass spectrometers, as was validated in experiments not reported here, this result was not expected. This phenomenon potentially arises from the optics utilized for ion introduction at the high pressure stages of the system energizing the formed adducts and making them unstable; this also can cause the in-source fragmentation observed for other analytes on the Flir AI-MS 1.2.

Interestingly, DESI-MS was shown applicable to low-level explosives related to primer and detonator mixtures and commonly found in the "volatile bouquet," chemicals detected in the headspace surrounding armaments. Negative-mode DESI-MS spectra were obtained from surface residues of 100 ng for the following analytes: *styphnic acid* (*i.e.* trinitroresorcinol, explosive used to produce lead styphnate, detected at m/z 244 [M − H]⁻), *1,3-dinitrobenzene* (volatile explosive, detected at m/z 168 [M]⁻·), and *2,4-dinitrotoluene* (volatile explosive, detected as m/z 181 [M − H]⁻). Positive-mode DESI-MS was also used to investigate ethyl centralite, an organic constituent found in gunshot residue (GSR).

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To accommodate the nonperformance of direct detection of military-grade explosives via DESI-MS, a custom-built, LTP ionization source was constructed and tested (as depicted in Figure 3-3). Initial characterization of the source was performed using TNT as a model explosive analyte. Representative negative-mode LTP-MS spectra were readily obtained for 100 ng surface residues of TNT, as seen in Figure 3-11. Several ion signatures are seen for this

analyte on the Flir AI-MS 1.2, including the molecular (m/z 227) and deprotonated (m/z 226) ion and simple losses of OH (m/z 210) and NO (m/z 197), all of which have been reported in literature using lab-scale mass spectrometers.[35]

These initial results allude to LTP-MS being an effective ionization method when considering explosives analysis on this portable system, and it warrants further investigation. Due to the overall success of the ESI/DESI assembly on the Flir AI-MS 1.2, DESI-MS was chosen as the primary ionization source of emphasis to this project.
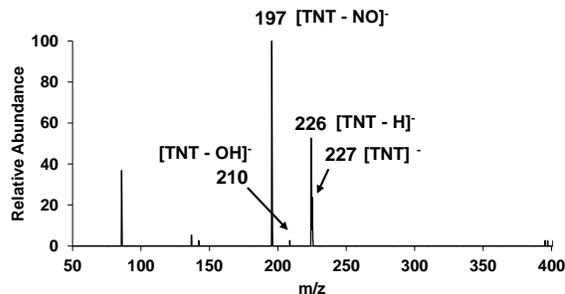
Figure 3-11. Negative-mode LTP-MS mass spectra of 100 ng TNT deposited onto a glass surface. Upon analysis with the plasma probe, several ion signatures corresponding to TNT are seen, adding to the selectivity of the analysis.

3.1.7 Analytical Characterization

Spectral Data Quality and Accuracy

Mass spectral data collected using both conventional and ambient ionization methods on the Flir Systems AI-MS 1.2 are congruent with that obtained on lab-scale MS instrumentation, and important attribute in the pursuit of adopting this technology as an accepted method for crime scene investigation and forensic analyses.

Spectral quality (in terms of accuracy of m/z determinations and relative abundance in both MS and MS/MS mode) was tested by direct comparison with a commercially-available, spectral reference database. Through collaboration with Dr. Herbert Oberacher (Associate Professor, Innsbruck Medical University, Austria), creator of the Wiley Registry® of Tandem Mass Spectral Data (MSforID), data obtained on the Flir AI-MS 1.2 was compared to that of an array of other MS instrumentation and analyzer classes. The MSforID database contains spectral data obtained on commercial, lab-scale QqTOF instrumentation, but does not presently contain any MS data generated on portable MS prototypes, making this a pioneering study. To compare to the database, MS and MS/MS data (processed as m/z assignment and relative intensity) using spray and ambient ionization techniques (ESI, DESI, and PSI) for thirty-two total "blind" analytes, including five negative controls (*i.e.* compounds not currently present in the MSforID database), were compared to the library, providing the correct identification for all positive controls tested with high sensitivity (*i.e.* true positive rate). Authentic seized evidence ranging from bulk powder to trace residue was also able to be rapidly identified, providing accurate determinations and demonstrating high potential for use in forensic casework and potential crime scene investigation. Library searching through the Wiley Registry was shown to provide both rapid and highly accurate chemical identification without the need for spectral interpretation by the end-user, allowing operation by non-technical personnel.

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For this study, correlation between collected data and the reference spectra is calculated as a "probability of match" known as the RAMP variable, ranging from 1-100. This variable does not read as a percentage per-se (where 60 means 60% match); any collected value over 40 is considered a positive match with this database. Figure 3-12 shows the comparison of an ESI-MS/MS "sample" mass spectrum collected on the Flir AI-MS 1.2 from authentic methamphetamine evidence and the ESI-MS/MS "reference" mass spectrum present in the MSforID database.

In-source fragmentation observed on the AI-MS 1.2 was shown to complicate library searching for certain analytes. Phenylephrine (Figure 3-13a) was of particular interest, as it possesses an in-source fragment at m/z 150 that when isolated and fragmented, results in fairly similar MS/MS data to that of methamphetamine; methamphetamine is seen in ESI/DESI/PSI spectra as m/z 150, also, exacerbating the issue. This occurrence of similar data is a potential issue for instrument users, as it not only results in a subsequent misidentification when compared to the MSforID database, but poses a problem for field implementation. The incorrect usage of phenylephrine as a precursor in clandestine methamphetamine production is becoming more prevalent as difficulty in ephedrine/pseudoephedrine acquisition increases.

The misleading identification of the m/z 150 in-source fragment of phenylephrine as methamphetamine can be explained by the functional principle of the MSforID search algorithm. In essence, this algorithm was optimized to give preference to compounds with matching

Figure 3-12. Comparison of the ESI-MS/MS "sample" spectrum of authentic methamphetamine evidence collected on the Flir AI-MS 1.2 to the ESI-MS/MS reference spectrum present in the MSforID database. A corresponding RAMP value of 75 was obtained by comparing the characteristic MS/MS fragments present at m/z 119 and m/z 91.

precursor ions (that is, the ion that is isolated for MS/MS analysis). Therefore, when the in-source fragment of phenylephrine is compared to the molecular ion of methamphetamine library entry, the algorithm detects the nominal precursor and fragment ion m/z assignments as identical, resulting in a misidentification. This phenomenon is represented in ESI-MS and MS/MS mass spectra collected on the FLIR AI-MS 1.2 for phenylephrine and methamphetamine in Figure 3.13. As the fragmentation pattern of phenylephrine does contain ions specific only to this analyte, further refinement of the library identification algorithm to include these signatures could negate false identification of phenylephrine as methamphetamine.

Limit of Detection and Quantitative Ability

Conventional and ambient ionization methods coupled to the Flir AI-MS 1.2 performed quite well in regards trace analysis of target analytes. Sensitivity towards illicit chemical detection has surpassed expectations so far regarding residue analysis, yielding limits of detection (LOD) in the low-to-sub nanogram (ng) range; in fact, a LOD of 500 picograms was recorded for residues of ecstasy (MDMA) via DESI-MS. All of the solid-phase analytes listed in Table 3-1 have been detected at 100 ng, and in most cases, at much lower amounts. The

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instrument also shows high sensitivity towards DESI-MS analysis of swabs utilized to probe surface-bound residues. Table 3-2 shows representative detection limits for surface-bound residues deposited on surface of interest in clandestine settings.   Here, residues of methamphetamine and its pseudoephedrine precursor were sampled via a wetted transfer swab, and the swab was then analyzed directly via DESI-MS. As seen, sub-µg detection limits were obtained, which is notable considering the variables involved in swab transfer and imperfections of the swab surface towards DESI analysis.
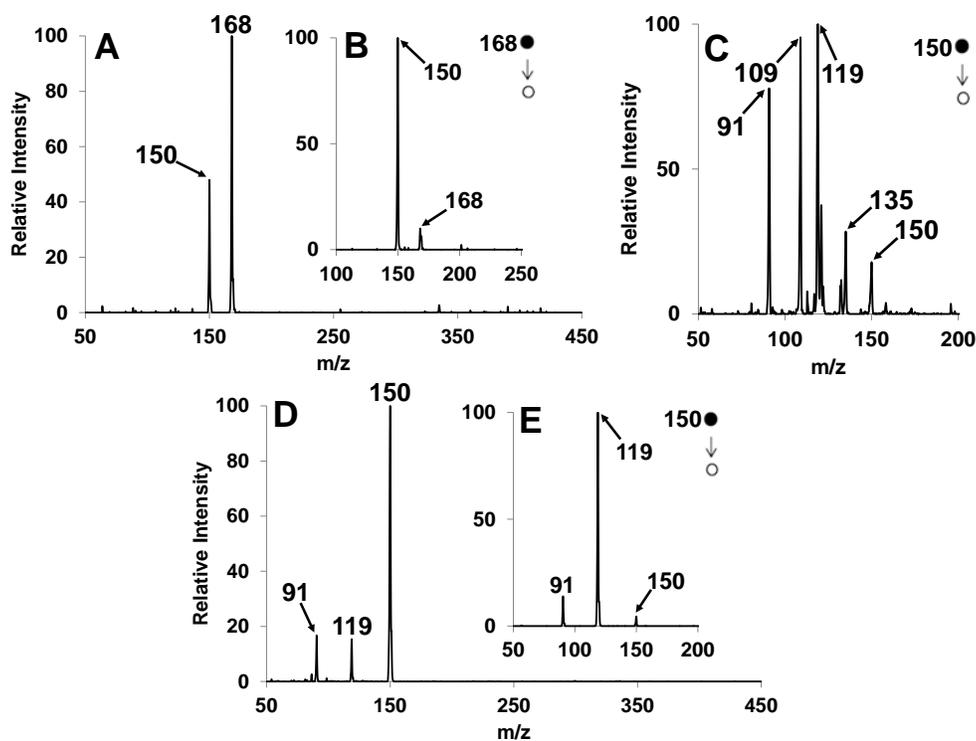
Figure 3-13. ESI-MS and MS/MS mass spectra collected for phenylephrine and methamphetamine. (A) ESI-MS spectrum of phenylephrine, with the protonated molecule present at m/z 168, as well as an in-source fragment (loss of water) at m/z 150. (B) ESI-MS/MS of protonated phenylephrine, yielding m/z 150 through dehydration. (C) ESI-MS/MS of the m/z 150 in-source fragment. Several products are present, with m/z 119 and m/z 91 being similar to MS/MS analysis of methamphetamine. (D) ESI-MS of methamphetamine, showing the protonated molecule at m/z 150 and characteristic in-source fragments at m/z 119 and 91. (E) ESI-MS/MS of protonated methamphetamine.

Table 3-2. Detection limits for methamphetamine and pseudoephedrine residues swabbed from surfaces of interest

Substrate	Methamphetamine	Pseudoephedrine
Pill bottle	350 ng	500 ng
Steel	325 ng	300 ng
Enamel	225 ng	475 ng
Nonstick	200 ng	450 ng
Glass	400 ng	500 ng

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Detection limits obtained in MS/MS mode were shown to be highly sensitive to the instrumental settings that control precursor ion isolation and fragmentation via collision-induced dissociation. As part of our efforts of develop optimized methods for operation and comprehensive spectral reference libraries, MS/MS settings were meticulously optimized to produce favorable levels of fragment ion intensity and diversity (for chemical identification purposes); further detail regarding these efforts can be seen in *Section 3.4*. To demonstrate this effect, detection limits for the emerging illicit chemical desomorphine and its precursor codeine from representative surfaces were assessed via swab transfer DESI-MS/MS using both non-optimized, factory-default settings and our own optimized variables. The use of desomorphine (street name "krokodil") has been seen in Europe, but recent reports of its production and use in the U.S. have made it news-worthy As seen in Table 3-2, detection limits obtained using optimized MS/MS settings (mid to low ng) were more than an order of magnitude lower than those using default settings (low µg).

Table 3-2. DESI detection limits utilizing optimized and non-optimized (default) MS/MS conditions

	Default		Optimized	
Surface	Desomorphine	Codeine	Desomorphine	Codeine
Steel	3 µg	5 µg	90 ng	100 ng
Nonstick cookware	2.5 µg	2 µg	100 ng	150 ng
Enamel cookware	1 µg	4.5 µg	200 ng	200 ng
PET bottle	1.5 µg	3 µg	100 ng	270 ng
Glass	1 µg	5 µg	150 ng	350 ng

While the Flir AI-MS 1.2 has been extensively demonstrated as a rapid, yet sensitive screening method, the ability to quantitatively assess samples and surfaces of interest could be of interest to forensic investigations. To test this capability, calibration curves were generated for surface-bound samples of ketamine, MDMA, and heroin using DESI-MS, as seen in Figure 3-14. Modest linearity was obtained for these studies, with $R^2 = 0.9976$ (heroin) representing the best calibration data obtained. To construct these curves, standard solutions of each analyte were spotted onto glass substrates to reach the target deposited mass. The average of four replicates for each deposited mass was used for plotting purposes. Linear dynamic range varied from 1.5 (heroin) to 2.25 (MDMA) orders of magnitude.

Precision of each data point obtained is represented by the included error bars, which are relatively large in some instances. This loss of reproducibility (and therefore linearity) comes from the numerous variables that must be controlled during DESI-MS analysis, including surface preparation, orientation of the sample surface relative to the DESI analysis point, and positioning relative to the inlet capillary of the Flir AI-MS 1.2 (this variable is particularly crucial given the reduced vacuum system of the instrument). While reproducibility of routine analysis does increase with user experience, it is anticipated that semi-quantitative analysis on the Flir AI-MS 1.2 represents the best-case scenario when utilizing DESI-MS, and even then may be difficult when additional surface variables are introduced (*e.g.* porosity, dirty surfaces, fingerprint oils, etc.). While viable quantitative data could be generated using the conventional ESI ionization source, requiring generation of sample solutions from forensic evidence, it is our opinion that ambient MS performed with the Flir AI-MS 1.2 is best suited for rapid screening purposes, where it has been demonstrated as highly proficient for common forensic analytes.

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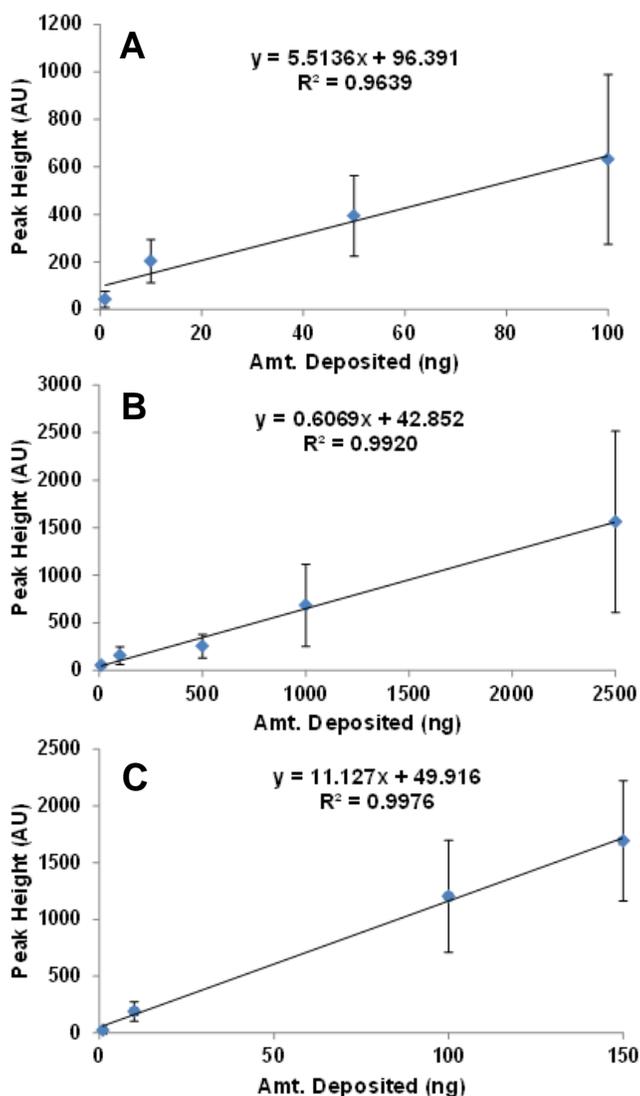
Figure 3-14. Representative calibration curves generated via DESI-MS from surface residues of known mass, depicting linearity for (A) ketamine, (B) MDMA, and (C) heroin. Modest linearity is obtained over at least two orders of magnitude of deposited mass. The average of four replicates for each mass was used for plotting purposes.

Sample Throughput

A major benefit of direct sample analysis via ambient MS is enhanced throughput over other techniques requiring extensive sample preparation. To assess sample throughput on the Flir AI-MS 1.2 with DESI-MS, trace residues (100 ng) of the synthetic cathinones MDMA (3,4-methylenedioxy-methamphetamine), MDPV (3,4-methylenedioxypyrovalerone HCl), methylone (3,4-methylenedioxy-N-methylcathinone HCl), and mephedrone (4-methylmethcathinone HCl) were spotted separately onto a printed teflon slide and analyzed in sequence by rastering the substrate by hand at a rate of ~1 mm/sec, as seen in the selected ion chromatograms depicted in

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Figure 3-15. The entire analysis of all four samples took ~35 sec, including several seconds of blank data collection for background subtraction. The protonated molecule for each analyte is seen in high abundance at the expected time intervals (*i.e.* the timepoint to when that spot underwent desorption/ionization), and no carryover was recorded, as evidenced by each selected ion chromatogram retreating back to noise level once the corresponding sample spot is no longer in the ionization region. The rapidity of sample screening and resistance to chemical carryover seen on the AI-MS 1.2 would be of great benefit if utilized for authentic evidence screening.
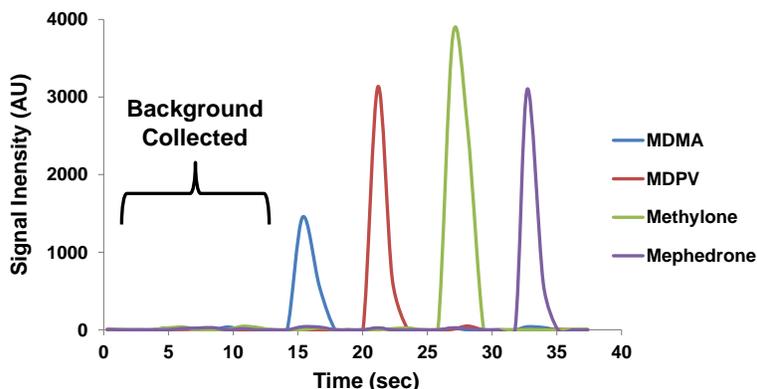
Figure 3-15. Selected, color-coded ion chromatograms for 4 synthetic cathinones spotted in sequence onto a glass slide. By rastering by hand underneath the DESI source assembly, signatures of each analyte are seen at the corresponding time of analysis. No carryover is seen between analytes

Reliability (False Positive/False Negative Rates)

As seen by Table 3-1, numerous analytes of forensic relevance have been successfully detected and characterized using the Flir AI-MS 1.2, but for true application at crime scenes or other sites of interest, reliability of data obtained and accurate analyte identification is just as crucial as broad applicability. To test false positive/false negative rates on the Flir AI-MS 1.2 using DESI-MS, two separate studies were completed.

First, 100 ng residues of cocaine on glass surfaces were used as a model sample for direct surface screening via DESI. Here, a total of 200 samples were analyzed (100 "positive" samples where cocaine residue was present and 100 "negative" blank slides with no cocaine residue) over a time period of several weeks. Acquiring instrumental data at differing date and time helps to show inter- and intraday variability of results. All samples (positive and negative samples) were analyzed, and the generated data were simultaneously compared to predetermined library entries for cocaine DESI-MS (protonated cocaine at m/z 304) and DESI-MS/MS (fragmentation of the m/z 304 precursor to produce the characteristic m/z 182 fragment ion) data. Specifics of this library searching protocol are described in detail in *Section 3.4*. For this comprehensive false positive/false negative rate study, a positive "confirmation" was denoted by the library protocol both warning the presence of cocaine in MS mode and alarming the confirmation of cocaine via MS/MS. A negative response was denoted by a lack of both the warning and alarming of cocaine. For the 200 sample set, the Flir AI-MS 1.2 performed perfectly, that is it recorded a 0% false positive and 0% false negative rate, successfully identifying all true cocaine residues and

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blank samples correctly analyzed. To visually examine inter- and intraday spectral variability for these repetitive analyses, the MS (Figure 3-16) and MS/MS (Figure 3-17) intensities were plotted with time dependence for all 100 "positive" cocaine samples. The average of the MS mode intensity seen was 3058 ± 2648 AU, showing that there was a decent amount of variability in spectral intensity day-to-day, further suggesting that this platform is better suited for screening and identification of forensic evidence, not quantitative assessment. A similar amount of variability is seen in the corresponding plot of the MS/MS results.
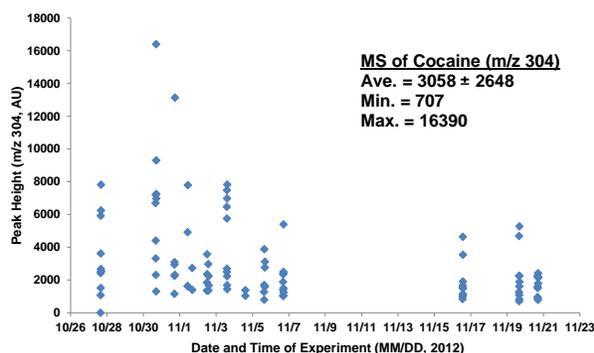
Figure 3-16. Graphical depiction of spectral intensity obtained for all 100 "positive" confirmations of cocaine in MS mode for direct DESI-MS surface analysis. The intensity of the protonated molecule at m/z 304 is tracked over date and time of analysis. Statistical analysis of the dataset is seen in the corner inset.
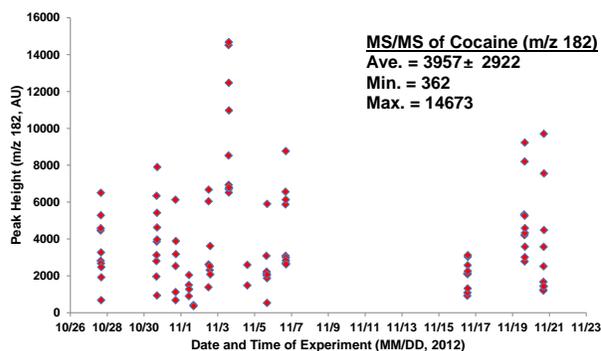
Figure 3-17. Graphical depiction of spectral intensity obtained for all 100 "positive" confirmations of cocaine in MS/MS mode for direct DESI-MS surface analysis. The intensity of the corresponding fragment ion at m/z 182 for the isolated m/z 304 precursor is tracked over date and time of analysis.

In a second study, the variability of utilizing surface swabbing for probing surface residues was investigated. Here, 200 total control samples (100 "positive" glass slides containing 100 ng residues of cocaine and 100 blank "negatives") were analyzed by using a pre-wetted swab to interrogate the glass slide, introducing said swab without further preparation into the DESI source of the Flir AI-MS 1.2. As in the first study, an automated library searching protocol (*See Section 3.4*) was used to confirm the detection and identification of cocaine from each sample. Samples were analyzed over a one month period at differing times for variability testing. This study yielded a <u>total of zero false positives and four false negatives</u>. Signal intensity varied greatly for both MS screening and MS/MS verification, more so than direct analysis of glass slide via DESI-MS, Overall MS/MS intensity tended to be lower, as the cocaine residue was partially exhausted during base MS scanning. The enhanced deviation of this study is a result of inherent variability of the surface swabbing and swab placement in the DESI source. It is anticipated that the extent and location of cocaine transfer to the swab can change. This source of variability, in addition to the sensitivity to positioning inherent to swab analysis, suggests the use of physical transfer swabs is limited to qualitative screening purposes.

Taking into account both reliability studies, the **Flir AI-MS had a 100% success rate for positive controls and 98% success rate for negative controls for 400 control samples**.

3.2 Task 2: Investigate Alternative Surface Sampling Methodologies

For traditional DESI-MS, the actual analysis point (*i.e.* the area on the surface of interest that is undergoing desorption/ionization) is relatively small (~1 cm^2) and is directly in front of

the electrospray emitter of the ionization source (see Figures 3-2A and B). While this is suitable for small substrates of simple geometry, large surfaces pose significant problems for DESI-MS. For larger surface areas or samples with complex geometry, physical transfer of chemical residues via surface swabbing is an effective method that can be rapidly applied to DESI-MS analysis on the Flir AI-MS 1.2. It is expected that many substrates too large in size to allow positioning in the ionization source can be screened by using a swabbing protocol followed by direct analysis of said swab material.

To determine the viability of swab transfer coupled to DESI-MS, known amounts of analyte were deposited onto surfaces by spotting aliquots of standard solutions and allowing complete evaporation of solvent. Several swab candidates were identified and tested to determine the best candidate for adoption, with attributes including transfer efficiency, chemical noise during MS analysis inherent to the swab, ease of positioning in the DESI assembly, and reliability of analysis. Tested swabs included chromatography paper (Whatman 1 Chr, GE Healthcare Life Sciences), knitted polyester (LTP70R, Berkshire Corporation), non-woven hydroentangled foam polyester (LTN70F, Berkshire), polyurethane foam (LTO70R, Berkshire), and spun cotton "Q-Tip"-style applicator (A5005-1, American Scientific Products), as

Figure 3-18. Examined commercial swabs. From left to right: Whatman chromatography paper used for PSI-MS, woven fiber, non-woven foam, polyurethane foam buccal, and spun cotton "Q-Tip"-style. Also seen is a residue-containing glass slide used for physical transfer studies.

shown left to right in Figure 3-18. Note that Whatman chromatography paper that can be utilized for PSI-MS was also included, as the paper substrate itself can be implemented as a physical transfer swab prior to PSI analysis. Extensive testing of wet vs. dry swabbing shows that dampening the swab prior to surface probing (typically with 5-10 µL of methanol or other suitable solvent) results in significantly higher transfer efficiencies and subsequent analyte intensity.

To show the potential of direct transfer swab analysis, mock scenarios of forensic interest were created and tested. For instance, using wetted transfer swabs to probe latent fingerprints for chemical residues from surfaces not amenable to direct DESI analysis was demonstrated. Figure 3-19 depicts using a physical transfer swab to probe latent fingerprints from a cellular phone. To confirm this capability, 500 ng of methylone was deposited onto the glass display of a touchscreen cellular phone. A dampened, sterile swab was then quickly run over the entire glass surface and directly analyzed, with resultant data seen in Figure 3-20. The protonated molecule of methylone is seen at m/z 208 even when present at trace levels. Swabbing of latent fingerprints on large surfaces found at a crime scene (*e.g.* drywall, windows, and countertops) could be done in a similar fashion, keeping in mind that said method would be destructive to the fingerprint topology. It is expected that a similar swabbing protocol could be used extensively in clandestine methamphetamine operations, where residue analysis from unmarked glassware and other paraphernalia could be high utility, and this expounded on in *Section 3.5* of this report.

Figure 3-19. Depiction of using a transfer swab to probe the keyboard of a cellular phone for residues found in latent fingerprints.
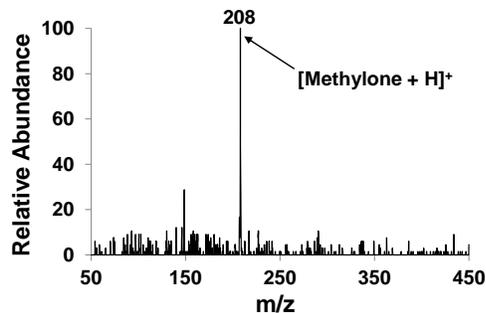
Figure 3-20. DESI mass spectrum of a swab after probing the touchscreen display of a cellular phone. The protonated molecule of methylone (spiked onto phone prior to swabbing) is seen at m/z 208.

3.2.1 Wet vs. Dry Swabbing and Transfer Efficiencies

Characterization of surface probing with "wet" swabs vs. dry swabs was undertaken; swab "wetting" comes from the predisposition of solvent directly to the swab head. It was anticipated that wet swabbing would take advantage of surface-bound analyte solubility, removing more residue from the sample surface and increasing the sensitivity of the overall method. Initial test involved the use of methanol, a common organic solvent with high solubility towards drugs of abuse, and wet swabbing drastically outperformed dry swabbing. It is important, though, not to overly wet the utilized swab, as excess solvent can dissolve the surface residue, wick from the swab during contact, pool, and dry, reducing the transfer efficiency of the desired experiment. Damp, not saturated, swabs are recommended. To this end, each particular swab was determined to have an optimal wetting volume. The success of this study warrants further investigation, particularly with solvents of low human toxicity (*i.e.* isopropanol, water) for instances where direct skin swabbing is of interest. Skin swabbing could provide interesting opportunities in search and seizure and post-mortem/toxicology applications.

The transfer swabbing/DESI-MS protocol has two distinct phases that need characterized in order to obtain full method optimization: assessment of swab transfer efficiency and ionization efficiency of direct DESI analysis from used swabs. Using standard GC/MS procedures, transfer efficiencies (*i.e.* how much analyte can be transferred from surface to the swab) for commonly-available swabs seen in Figure 3-18 were determined for methamphetamine residues deposited onto glass. Utilized swabs were solvent extracted in methanol, and internal standard (d_3-methamphetamine) doped extracts were analyzed via GC/MS and compared to calibration data to determine concentration; transfer efficiencies were then be back calculated after incorporating volume and the pre-determined extraction efficiency (*i.e.* how much analyte can be extracted from the used swab) for each swab type.

Experimentally-determined extraction and transfer efficiency values were found for all swab types and reported in Tables 3-3 and 3-4, respectively. As seen, swab composition not only effects the relative efficiencies investigated, but also the repeatability of the surface transfer process, which is not surprising given the inherent variability of this swabbing action. Interestingly, the PSI paper substrate has very high transfer efficiency for residues below 2 µg, which could be another benefit of investigating this ambient ionization method for physical transfer experimentation. The buccal swab, in contrast, has been shown to recover only around

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58%, but can do so for up to ~16 μg of the target compound. Thusly, usability factors must be taken into account when validating the various swabs and making our final recommendation for swabbing procedures.

Table 3-3. Extraction Efficiencies for Physical Transfer Swabs

Extraction Efficiency For Cocaine Residues	
Type of Swab	**Average**
Polyester Buccal	96.72% ± 6.45%
Woven Fiber	73.28% ± 4.77%
Paper Triangle (PSI)	70.92% ± 0.96%
Non-Woven Foam	80.43% ± 1.77%
Spun Cotton	78.37% ± 3.85%

Table 3-4. Transfer Efficiencies for Physical Transfer Swabs

Transfer Efficiency For Cocaine Residues	
Type of Swab	**Average**
Polyester Buccal	57.49% ± 7.22%
Woven Fiber	67.68% ± 14.99%
Paper Triangle (PSI)	98.20% ± 4.05%
Non-Woven Foam	66.78% ± 11.27%
Spun Cotton	61.90% ± 1.70%

Relative ionization efficiencies of direct DESI analysis from surface swabs (*i.e.* how much analyte can be removed/ionized from the swab surface with DESI) were determined by deposition of known quantities of illicit drugs onto swabs of interest and comparison of corresponding spectral intensity generated. It was found that ionization efficiency, as expected, was effected by the surface composition of each swab. Porous and absorbent materials tended to retain the incoming DESI spray and hinder the secondary release of surface-bound analyte residue. The paper substrate yielded high ionization efficiency via PSI-MS due the combined analyte extraction and ionization process of the technique. Swab geometry also played a role, with flat, smaller surface area swabs proving easier to orient in the DESI assembly, enhancing ionization efficiency.

3.2.2 Recommended Transfer Swabbing Protocol

Considering all factors tested during Task 2, Berkshire polyurethane foam swabs (identified as "non-woven foam" in Tables 3-3 and 3-4) were determined to be the best candidate for developing optimal transfer swabbing protocols for use with DESI-MS. Prior to use, the swab is prewetted with 5 μL of methanol, spread equally by splitting the total volume to both sides. Depositing should be closer to the end of the swab head rather than its center. To sample a surface of interest, the swab should be held at ~30º relative to the surface with light pressure and slowly moved up and down with light pressure across the entire surface with slight rocking to expose all of one face of the swab to the surface. Once finished, the other face of the swab was

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utilized to sample the surface in a similar way in the right to left direction to collect as much of the potential surface residues as possible. The angle of contact determines the swab area that actually contacts the surface of interest. After the surface was sampled, the swab is then presented to the DESI spray region using a swab positioning guide from Flir Mass Spectrometry, seen in Figure 3-21. The swab is placed within the alignment groove of the guide and initially held flush against the right edge of the guide. The swab is then slowly rotated to expose the entire swab surface to the DESI spray, and it was then pulled back along the left-right axis by approximately 2 mm to expose the swab head region to the spray, again with slow rotation to analyze the entire circumference.

When utilizing the automated library searching protocol developed for the Flir AI-MS 1.2 (see *Section 3.4* for more detail), rotation of the swab should be paused and the swab held in place to allow time to collect confirmatory MS/MS scans for that surface region if the software interface warns the user of the presence of a potential analyte of interest. If the software does not alarm in MS/MS mode within ~5 seconds, minor adjustments of the positioning along the left-right axis and circumference via rotation should be made. After MS/MS investigation ceases by the software, examination of the remaining swab surface, as well as the other face of the swab, can continue for other potential analytes present.

Figure 3-21. Photo of DESI swab analysis utilizing a swab positioning guide from Flir Mass Spectrometry.

After completion of swab analysis and in the event of an alarm, a blank swab should be analyzed to ensure no carryover from the preceding experiment. If carryover is seen (determined by the false alarm for the same analyte detected on the preceding analysis), a new swab positioning guide should be placed on the ESI/DESI assembly; subsequent blank swab analyses should be done to confirm hygiene of the ionization source before the next sample is presented.

3.3 Task 3: Demonstrate Direct Air Analysis Ability via APCI on the Flir Systems AI-MS 1.2

The versatility of the direct capillary inlet on the Flir AI-MS 1.2 allows it to couple to most ionization methods that generate analyte ions at ambient conditions. Techniques like DESI allow detection of surface-based or solid chemicals, but are typically not applicable to gas-phase analytes. High volatility analytes have long been examined using GC/MS, but when implementing specialized ionization sources, they can also be analyzed on the Flir AI-MS 1.2 directly from ambient air. To demonstrate the ability to monitor gas-phase analytes directly from the air via APCI, several iterations of source design were undertaken, producing a robust, sensitive ionization source easily coupled to the Flir AI-MS 1.2 platform.

Initial proof-of-principle testing was conducted with a simplified, open air corona discharge source (Figure 3-22), allowing continuous screening of near proximity air contaminants via APCI. The corona discharge is generated via a high voltage applied to a tungsten wire discharge needle. The source itself is quite simple, requiring only 4 kV applied under resistivity (10 MΩ) to a thin wire. Spectra generated with this source were congruent to that obtained on lab-scale instrumentation, showing high promise of APCI as a field-

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implementable technique. On the other hand, safety in regards to inadvertent shock from high voltage and ruggedness was a concern, prompting further revision.

The second revision featured a polyether etherketone (PEEK) enclosure for the corona discharge region, while also allowing air samples to be pulled through the source via sampling tubing by use on a small diaphragm pump (as represented in Figure 3-23; this source was similar in design to one recently reported in literature on portable MS instrumentation.[22] The ruggedized source met all requirements for completion of project tasks, including detection of potential accelerant vapors at or below 1 ppm.

Representative spectra collected using the APCI sampling system seen in Figure 3-23 were obtained on potential accelerants and chemical warfare agent simulants to show field applicability. Figure 3-24A shows a positive-mode APCI mass

Figure 3-22. Simplified corona discharge APCI. High voltage is applied to a thin wire under resistivity. Gas analytes present between the wire and MS inlet are ionized and detected.

spectrum of dimethyl methylphosphonate (DMMP), a common simulant for the agent Sarin, showing the protonated molecule at m/z 125 and an in-source fragment at m/z 111 (the hydrated phosphonium ion). MS/MS confirmation of the m/z 125 precursor can be seen in Figure 3-24B, yielding the characteristic fragments at m/z 93 (phosphonium ion) and m/z 111 (hydrated phosphonium ion). Figures 3-24C and D show MS and MS/MS confirmation of turpentine vapor, yielding spectra indicative of the natural product pinene.

Figure 3-23. Schematic of a ruggedized APCI source built for the Flir AI-MS 1.2 and its utilization to analyze volatile chemicals of interest (*i.e.* accelerants, toxic industrial compounds, etc.). An auxiliary pump pulls ambient air through a sampling tube of defined length into the discharge region of the APCI source. Upon ionization, volatile species are sampled through the inlet system of MS system and detected. Unused portions of the air are exhausted (in a safe location, if need be). Utilization of a sampling tube allows the instrument and operator to be removed from unsafe areas, yet capable of assessing the chemical content of ambient air and samples of interest.

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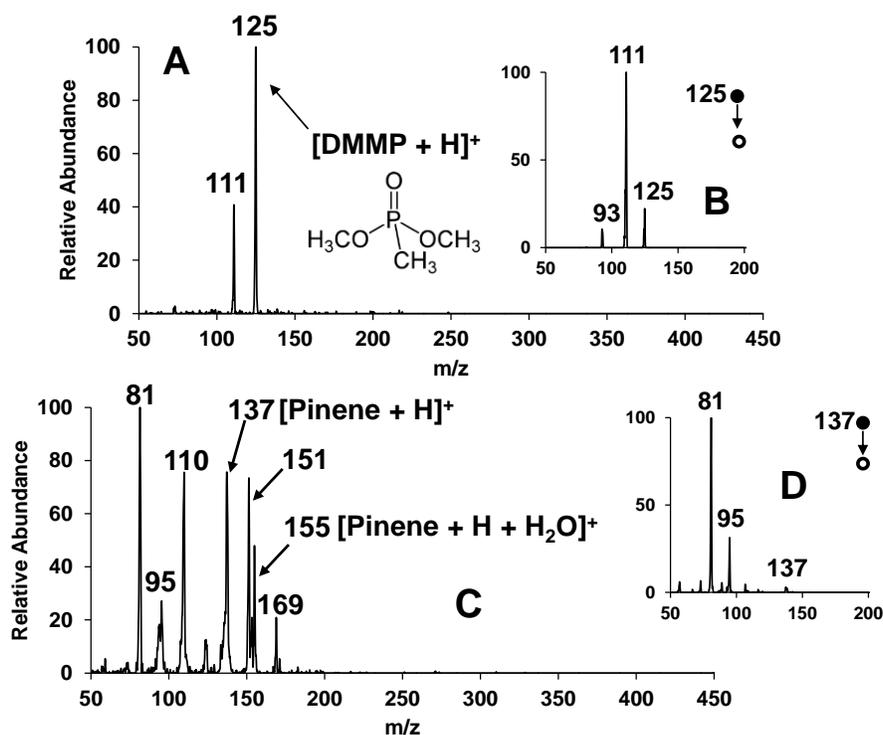
Figure 3-24. Positive-mode APCI mass spectra obtained for volatile headspace of (A) the chemical warfare agent simulant, DMMP and (C) turpentine, with subsequent MS/MS confirmation provided in (B) and (D), respectively. Simplicity of generated spectra depends on the complexity of gaseous headspace analyzed. As turpentine is a mixture of several chemicals, obtained spectra are complex, yet identifiable.

While functional and demonstrating high performance, the PEEK APCI source body possesses potential disadvantages that could complicate its coupling and usage during field usage. Briefly, attachment of the source necessitates the removal of the ESI/DESI spray assembly due to space constraints caused by its large size. This presents the possibility for contamination of the DESI spray body, damage to the emitter tip of the spray head if the source is not stored in a secure location, and misalignment of the DESI source once reattached. Additionally, the weight of the current APCI source represents a complication during its setup due to the possibility of the source placing excess force on the inlet MS capillary.

In order to minimize these concerns, the final design iteration featured a reduced-size APCI source body constructed from lightweight Teflon. As seen in Figure 3-25A, the source is small enough to be attached to the inlet capillary while the DESI source body is raised and secured in its maintenance position. The design possesses a shielded tungsten discharge needle to which the user can quickly attach the voltage lead. The discharge needle is positioned parallel to the flow of sample gas and perpendicular to the inlet capillary, as seen in the top view schematic in Figure 3-25B. The improved machinability of the polymer-based compression fittings used to secure to the MS inlet capillary compared to the stainless steel-based fittings used in the previous source enables fine adjustment, optimizing APCI spectral data intensity and minimizing the

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potential for unwanted arcing between the source and capillary inlet. Overall, this source design was determined as the best option for potential field deployment.
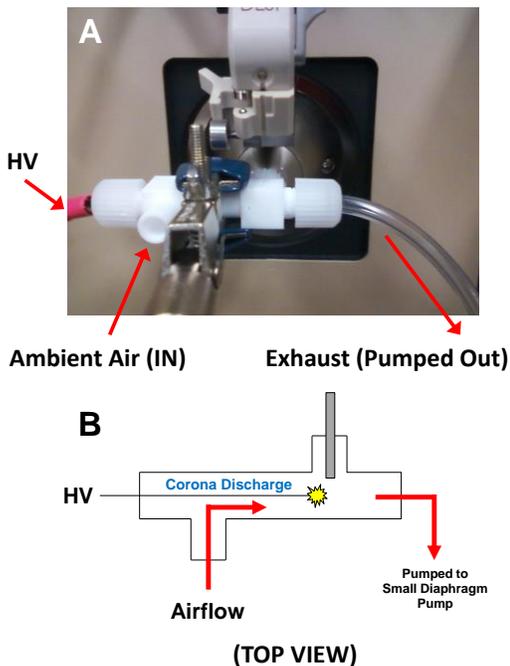
Figure 3-25. (A) Lightweight teflon APCI source attached to the Flir AI-MS 1.2 by simply lifting the DESI/ESI source in the maintenance position and sliding the source over the MS inlet capillary. (B) Top view schematic showing the interworking of the APCI source. The discharge needle is parallel to the flow of sample gas and perpendicular to the inlet capillary.

3.3.1 Assessment of Detection Limits via APCI

To determine detection limit of APCI analysis on the Flir AI-MS 1.2, defined as a signal-to-noise ratio of 3, accurate concentration gas standards were produced and analyzed. Diluted air samples containing a known amount of accelerant vapor were flowed directly into APCI source and analyzed. Detection limits determined for select chemicals and compilations during these studies can be seen below in Table 3-5, along with other volatile chemicals characterized. Overall, several analytes have been detected at gas concentrations **less than 1 ppm**, fulfilling the milestone detection limits for the project. Exact detection limits for complex hydrocarbon mixtures like gasoline and kerosene were difficult to assess, as calculating the gas-phase concentration of each single component depends on its own partial pressure. Detection limits for these samples were represented as a dilution factor (volume of sample/total volume of air analyzed).

3.3.2 Analysis of Mock Arson Evidence via APCI-MS

To simulate the direct analysis of evidence that may be present at an actual arson investigation, representative charred samples were prepared by ignition with potential accelerants. Initial test involved exposing 1 in. x 1 in. x 0.25 in. pinewood blocks with small quantities (~5 mL) of a potential liquid accelerant and igniting it in a glass petri dish. The wood sample was ignited and allowed to openly burn until all accelerant liquid was exhausted, and the block was allowed to become visibly charred before being capped to extinguish any remaining

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flames. Charred samples were then placed in Erlenmeyer flasks sealed with rubber septa for transport and to allow generation of headspace vapor within, as seen in Figure 3-26. These headspace vapors were then analyzed by APCI after transporting them via the pump-assisted sampling tube (shown in Figure 3-23) into the ionization source.

Table 3-5. Potential Accelerants Investigated with APCI and Associated LODs

Compound	MW (Da)	Precursor Ion (*m/z*)	Detection Limit
Acetone	58.08	59 $[M + H]^+$ 117 $[2M + H]^+$	0.5 ppm
Benzene	78.11	79 $[M + H]^+$	Not Tested
Charcoal Lighter	Various	Various	Not Tested
Diesel Fuel	Various	101, 136, various	1/100 Dilution
DMMP (Sarin simulant)	124.08	125 $[M + H]^+$	Not Tested
Ethanol	46.07	93$[2M + H]^+$ 139$[3M + H]^+$	Not Tested
Ethyl Ether	74.12	75 $[M + H]^+$ 149 $[2M + H]^+$	0.5 ppm
Isopropanol	60.10	61 $[M + H]^+$ 121 $[2M + H]^+$	2 ppm
Kerosene	Various	Various	Not Tested
Methanol	32.04	65$[2M + H]^+$ 97$[3M + H]^+$	10 ppm
Methyl Ethyl Ketone	72.11	73 $[M + H]^+$ 145 $[2M + H]^+$	0.5 ppm
Methyl Salicylate (blister agent simulant)	152.15	153 $[M + H]^+$ 121 $[MH – CH_3OH]^+$	Not Tested
Trimethyl benzene	120.19	121 $[M + H]^+$ 105 $[m/z\ 121 – CH_4]^+$ 91 $[m/z\ 121 – C_2H_6]^+$	Not Tested
Turpentine (Pinene)	136.24	137 $[M + H]^+$ 155 $[MH + H_2O]^+$	1/100 Dilution

Figure 3-26. Post-burn wood block visibly charred and cracked after ignition with Coleman Fuel. Generated headspace can then be analyzed directly via APCI-MS

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Charred sample analysis via APCI-MS was shown to produce signatures for residual accelerant vapors and combustion and pyrolysis products released from the charred material, but also natural products inherent to wood substrates. Representative spectra for pinewood burned in the presence of acetone (Figure 3-27A) and Coleman Fuel (Figure 3-27B) show characteristic signatures for each particular accelerant, but data is indicates a strong presence of pinenes originating from the wood itself. Chemical noise related to the burned substrates themselves warrants further investigation to test feasibility of this application of APCI-MS, particularly for polymer-based materials like fabrics and carpet commonly found in housing and commercial structures; these materials are expected to produce strong hydrocarbon-based combustion signatures.
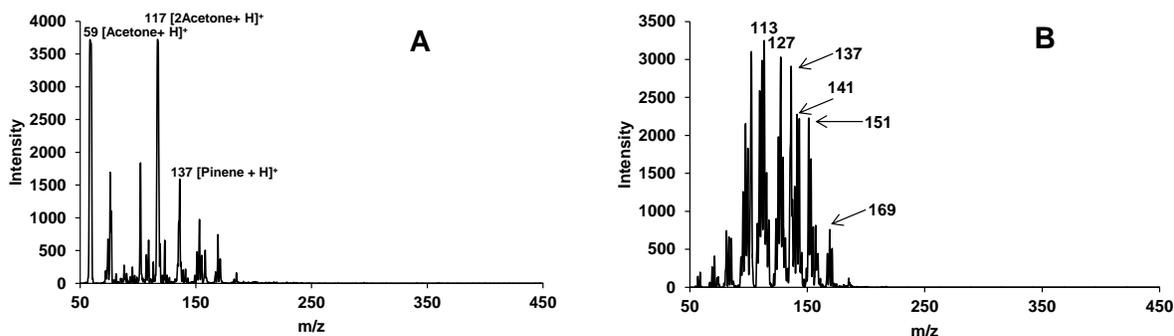
Figure 3-27. (A) APCI-MS spectrum of headspace vapor collected from pinewood charred in the presence of acetone, yielding peaks corresponding to the protonated acetone monomer (m/z 59) and dimer (m/z 117). Ions attributed to the pine substrate include protonated pinene at m/z 137, along with m/z 151 and 169. (B) APCI-MS spectrum of headspace vapor collected from pinewood charred in the presence of Coleman Fuel, featuring distinct peaks at m/z 113, 127, and 141 originating from the accelerant

3.4 Task 4: Develop Comprehensive Mass Spectral Libraries and Optimized Methods for Target Chemicals

Over the course of the project period, high-quality MS and MS/MS spectra and methods for their collection were generated for numerous analytes of forensic interest for development of a comprehensive mass spectral library; analytes residing in the constructed library can be seen in Table 3-1. Optimization of MS/MS instrumental methods was undertaken by systematically adjusting ion trap parameters during precursor isolation and fragmentation, as discussed in *Section 3.4.1.* Said MS/MS methods not only gave fragmentation data congruent to our own testing on lab-scale MS instruments and that reported by other research groups in literature, but also have proven to be reproducible in both inter- and intraday testing; MS/MS comparison studies involving the Wiley Registry® of Tandem Mass Spectral Data (MSforID) are described further in *Section 3.1.7.* User software upgrades released by Flir Mass Spectrometry for the AI-MS 1.2 allowed creation of semi-automated optimization methods of MS/MS experimental conditions and fully-automated library searching protocols based of MS/MS-based data dependent scanning, both of which described in detail below. Of note, automated library searching has the potential to alleviate the need for spectral interpretation by the instrument operator, expanding its use by non-technical operators by incorporating "red light/green light"

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protocols (*i.e.* red indications on a graphical user interface affirms the presence of a target analyte, green indications that the sample is innocuous).

3.4.1 Semi-Automated Optimization of MS/MS Method Conditions

Initial pursuits in optimizing compound-specific MS/MS instrumental methods required an intensive, systematic study of all pertinent variables, requiring the user to alter and test all combinations of settings for each analyte of interest. Updates to the instrument control software (Griffin System Software, GSS) during the project has allowed the streamlining of this method development, as fine optimization of compound-specific, MS/MS method conditions can now be done in a semi-automated fashion, a process termed "macroing." The macro function of the new version of the software allows the user to automatically scan ranges of critical MS/MS method variables, including the frequency of the AC dissociation waveform applied, collision energy (*i.e.* the energy imparted to isolated ions via collision-induced dissociation, CID, with helium damping gas), and dissociation time, in order to maximize MS/MS spectral intensity, fragmentation efficiency and/or fragment diversity. Purposely introducing a higher diversity in generated MS/MS fragments can be beneficial in regards to increasing accuracy of identification of isobaric compounds and structural isomers (*i.e.* codeine and hydrocodone). This macro function was used to produce all of the optimized MS/MS parameters reported in Table 3-1.

In order to macro a particular MS/MS condition for a specific compound, ESI-MS was primarily utilized due to the need for constancy in spectral intensity. First, fragmentation of the compound is examined over a pre-determined range of excitation frequencies, typically between 170 and 178 kHz using 0.5 kHz increments. By looking at the resulting total ion chromatogram, the user can determine the frequency value that results in the highest fragment ion signal intensity, as seen in Figure 3-28 for the optimization of MDMA fragmentation. By observing the maxima of signal intensity (as denoted with the green arrows) for the primary fragment at m/z 163 in MS/MS mode, the most effective frequency value can be rapidly obtained compared to the slower alternative (*i.e.* systematic changing of the variable by the user, collection of spectral data, and determination of spectral intensity maxima by direct observation). The CID voltage variable is then optimized in a similar manner for the compound using the frequency value previously determined. Lastly, the optimal values determined for the frequency and voltage are used to macro for the dissociation time variable, resulting in idealized values for all three variables for generation of MS/MS method files for the specific analyte. Once the main variables are optimized, the overall MS/MS method is then used to analyze the same compound using other ionization methods (*e.g.* DESI, PSI) in order to confirm its ruggedness to broad usage. As anticipated, utilization of non-optimized MS/MS variables results in substandard spectral intensity, as is depicted in Figure 3-29. In the case of MDMA, optimized MS/MS variables via macroing produce a 3x higher spectral intensity for the target m/z 163 fragment, decreasing the limit of detection for the compound in the process.

It should be noted that in the instance of a true unknown analyte observed in an examined sample (*i.e.* compound not found in the developed spectral library), the default MS/MS parameters incorporated in the user software should be utilized until rigorously optimized settings can be determined on appropriate analytical standards.

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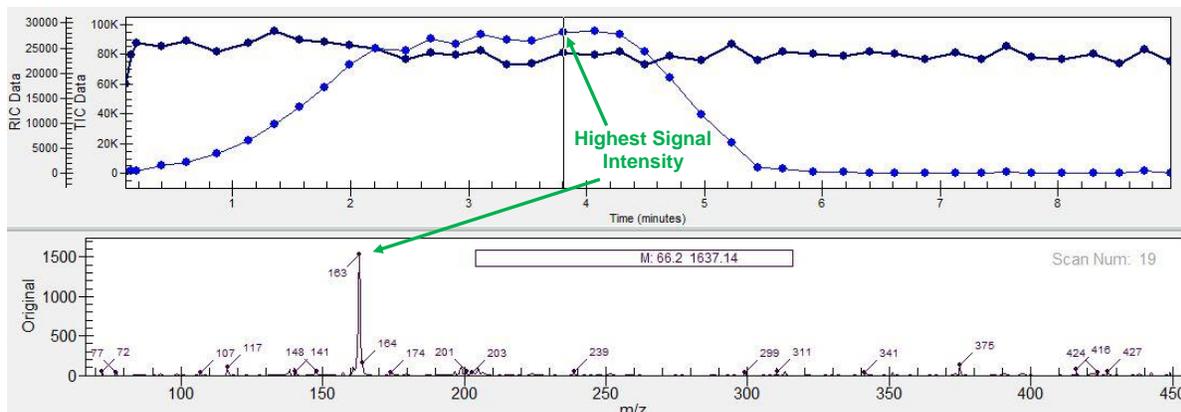
Figure 3-28. Total ion chromatogram depicting of macroed frequency variable for MDMA. Numerous frequency values can be automatically screened (denoted by the lighter blue trace) to determine the value that produces the highest fragment ion signal intensity in the resultant mass spectrum seen in the bottom window.
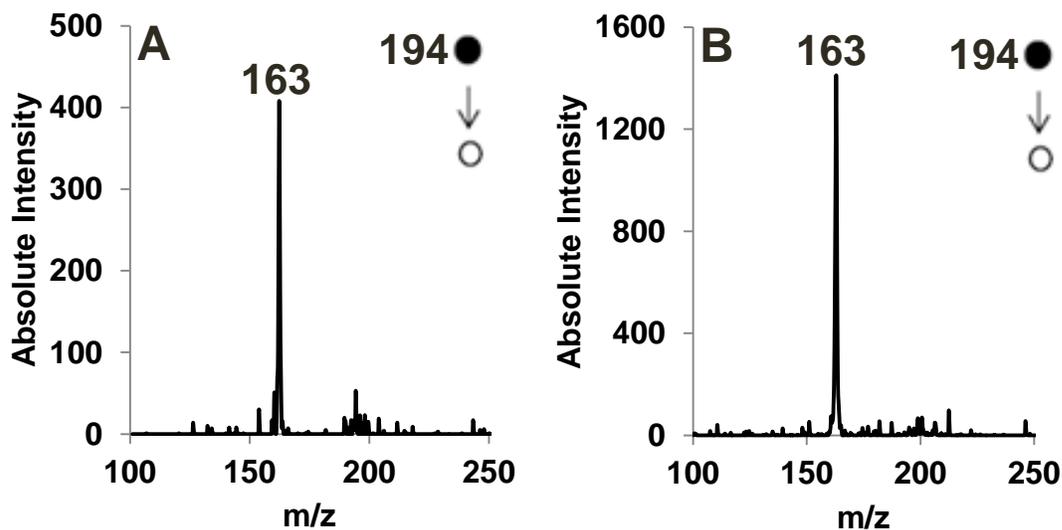
Figure 3-29. ESI-MS/MS of MDMA collected with (A) non-optimized and (B) optimized variables collected via macroing. As seen, optimized variables produces 3x higher signal intensity for the target fragment of MDMA at m/z 163.

3.4.2 Automated Library Searching via Data Dependent Scanning

Early success in project-related research has allowed us to expand and broaden certain proposed tasks, particularly in terms of developing simplified software intended for non-technical users. Updates to the control software for the Flir AI-MS 1.2 during the project allowed development of automated library searching methods that identify target analytes found in investigated samples by comparison to both base MS and MS/MS reference spectra; this, in turn, alleviates the need for user interpretation of collected spectra, as well as guides the user

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through the analysis procedure (*e.g.* documentation, presentation of the sample, saving of spectral data, etc.). This operation is referred to "Level 1" methods, herein.

With Level 1 operation, MS/MS confirmation for target analytes inherent to the presented sample has been integrated into the screening method itself rather than requiring the subsequent loading and implementation of a standalone MS/MS method specific to each controlled substance. The Level 1 protocol is configured to automatically select and run appropriate MS/MS confirmation scans as soon as a target generates a warning (*i.e.* protonated molecule m/z collected in base MS mode matches an existing compound in the on-board spectral library) in real time, removing the need for operators to manually select the appropriate confirmation method. This capability is referred to as data dependent scanning (DDS), as the detection of potential MS precursors prompts the automatic loading and running of optimized MS/MS settings saved within in the method; these optimized MS/MS conditions were determined via macroing, as discussed the previous section. Overall, the user can now screen and identify via MS/MS fragmentation up to 28 different analytes found in a single sample, all within a 4 min. total analysis time. A detailed description of the Level 1 screening process is provided below, with software screenshots for illustrative purposes.

Operation of the Flir AI-MS 1.2 Using the Level 1 Protocol

1. The operator is first prompted to load a Level 1 screening method corresponding to the type of evidence in question (*e.g.* Drugs of Abuse, etc.) and enter a sample name for data recording and chain of custody purposes. After the instrument performs a quick self-calibration to ensure proper operation, the user is prompted to introduce their sample utilizing their desired ionization method. For the purposes of this description, we will focus on our devised Drugs of Abuse method, which performs real-time searches for a variety of illicit drugs, abused pharmaceuticals, potential synthetic precursors, and common cutting agents.
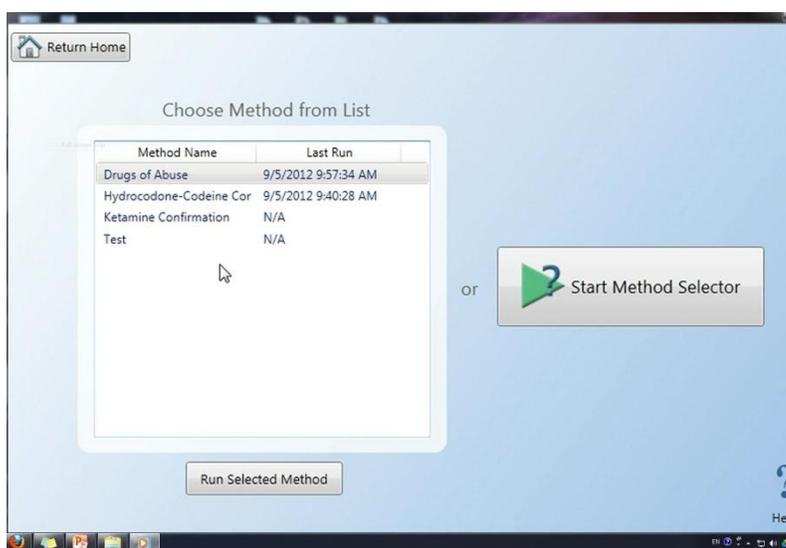
2. Once the analysis prompt appears, the Level 1 protocol enters Monitor View and begins scanning for base MS signatures, particularly parent molecule m/z signatures of analytes

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included in the mass spectral library for the method being used. After the operator introduces the sample, (*i.e.* a physical transfer swab into DESI spray region, as is depicted in this description), MS scanning continues for a predetermined amount of time, currently set to 4 min. total; of note, if no "warnings" occur within 2 min., the method will end to minimize unneeded analysis time.

3. Real-time spectra obtained during this base MS scanning are compared to a pre-determined set of rules for the "warning" thresholds, which include intensity and observed duration of protonated molecule signatures matching the spectral database. Should an observed spectral peak meet these rules for a certain drug of abuse or other illicit substance, the graphical user interface will turn yellow, warning the operator of its possible presence; the compound is also identified by name.

4. After the software registers a "warning," presence of the suspected controlled substance is then verified with compound-specific MS/MS analysis. As stated, the updated instrumental software allows confirmatory MS/MS scans to be included in the general method itself, no longer requiring separate MS/MS methods to be run by the user for each analyte. Any activated MS/MS scans then run sequentially with the base MS scan to allow warning of other potential analytes of interest in the sample. The software itself has a limit of allowing 28 separate MS/MS scans per Level 1 analysis, which allows molecular identification from even the most complicated sample mixtures.

5. Once initiated, each compound-specific MS/MS analysis will continue for two minutes after its starting point and will continue past the two minute cutoff for the MS warning scan if necessary. Rules that govern "alarming" with a red-light designation, that is, confirmed presence of a compound via MS/MS spectral matching, are contained in separate files stored on the AI-MS 1.2 control computer (.MACOI files). "Alarming" for the presence of a target analyte via MS/MS spectra will turn the interface red, alarming the operator of a confirmed target. The MS/MS scan for that specific analyte will then cease, and the software will continue to scan for other target analytes with the time remaining.

In the example shown below, a swab used to collect hydrocodone residue spotted upon a glass surface generated yellow "warnings" for both hydrocodone and its isomer codeine, as both produce a protonated molecular ion at m/z 300. The software then automatically initiates confirmatory MS/MS scans for both hydrocodone and codeine to verify which pharmaceutical triggered the alarm, examining differences in the fragmentation spectra collected. The codeine MS/MS scan did not detect the presence of its unique fragment peak at m/z 215, while the hydrocodone MS/MS scan did detect the presence of its unique fragment at m/z 199. As a result, codeine remained at yellow "warning" status, while the positive MS/MS result prompted a red "alarm" status for hydrocodone.
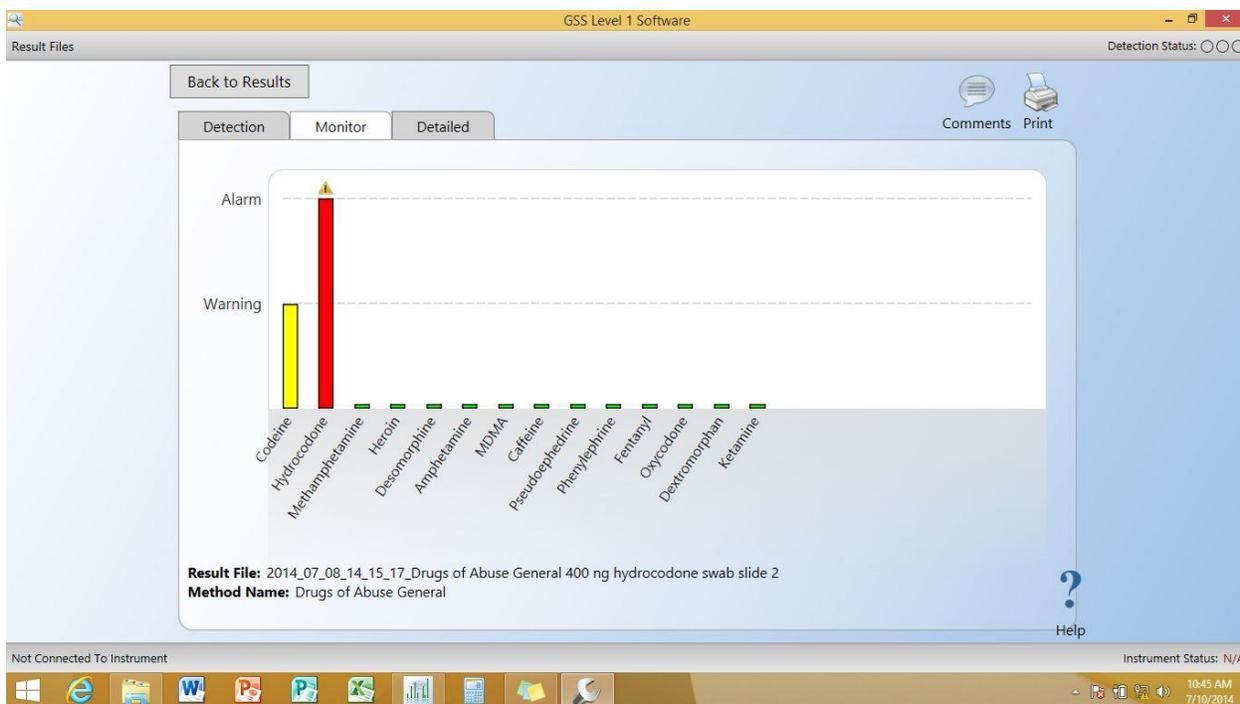
Screenshot of the Monitor View during the hydrocodone analysis discussed in Step 5

6. After any Level 1 analysis that warns or alarms for target analytes, advanced users can inspect the collected MS and MS/MS spectra by clicking the "Detailed" tab to view the raw data; screenshots of this view are seen below. Collected mass spectra are seen in the upper left corner, while a list of chemicals that trigged warnings and full alarms is located in the upper right with the time at which the trigger was recognized. A chromatogram is shown in the lower portion of the screen, depicting specific ion signal over the entire experiment. The total ion chromatogram is shown with the green trace, and each additional MS/MS scan triggered is given a unique color trace (blue and red).

Figure 3-30 shows the tentative flow of a typical DESI-MS screening of evidence with the Flir AI-MS 1.2 and the developed Level 1 software protocol. The flowchart depicts how the method "warns" for potential analytes present and then automatically performs associated MS/MS confirmation; when both MS and MS/MS spectra match the on-board spectral library, the software "alarms" for a confirmed illicit chemical present. This iterative process continues for a pre-set limit (4 min.), after which analysis ends and all warnings/alarms are indicated to the user. Confirmed and non-essential evidence can then be documented at the scene and transported to off-site labs for further confirmation or storage. After cleaning the ionization source to limit cross-contamination, including but not limited to the surface contacting the presented sample (see Figure 3-2), the spray head assembly, and MS inlet capillary, a new sample can be presented for analysis.
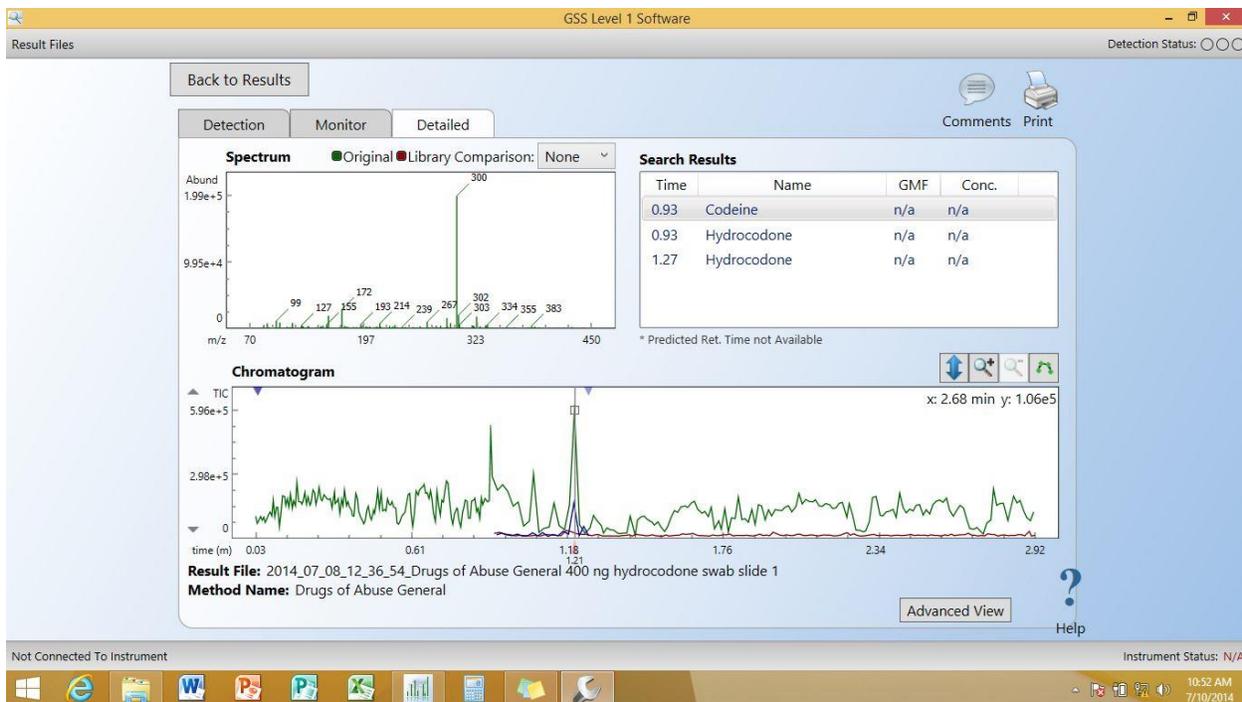
Screenshot of the representative base MS data collected in the Detailed View after alarming for hydrocodone
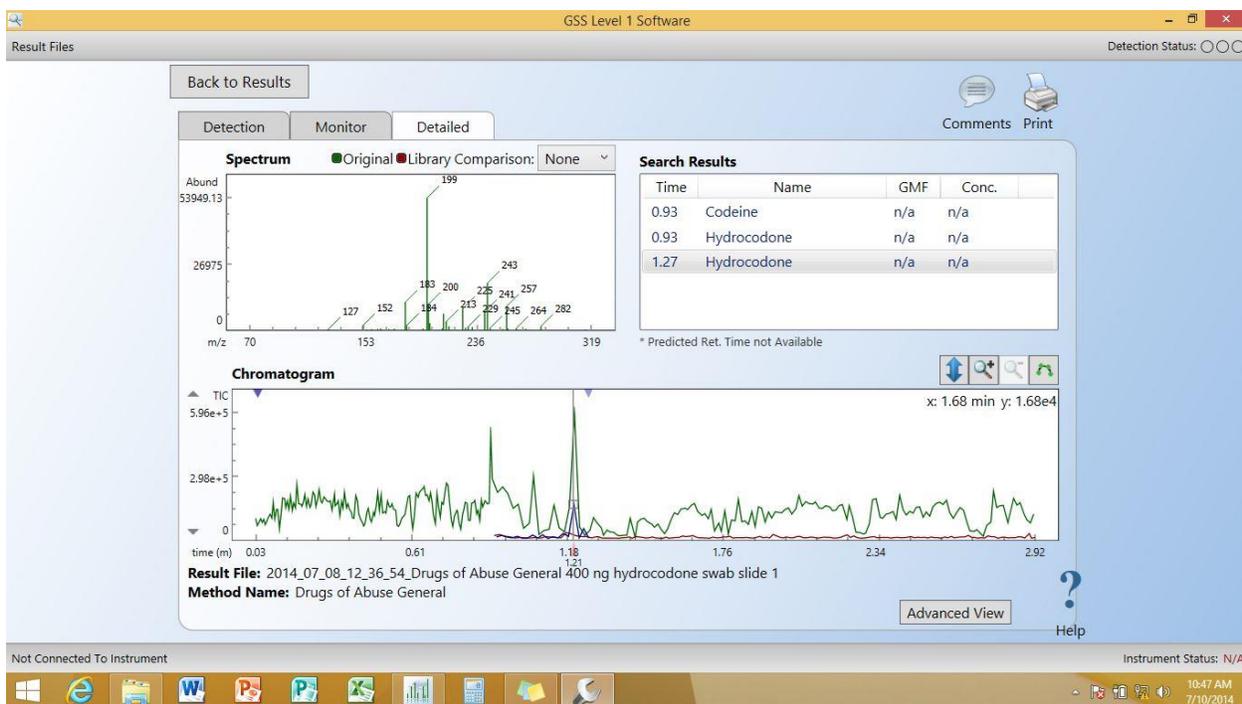
Screenshot of the MS/MS data collected in the Detailed View after alarming for hydrocodone analysis
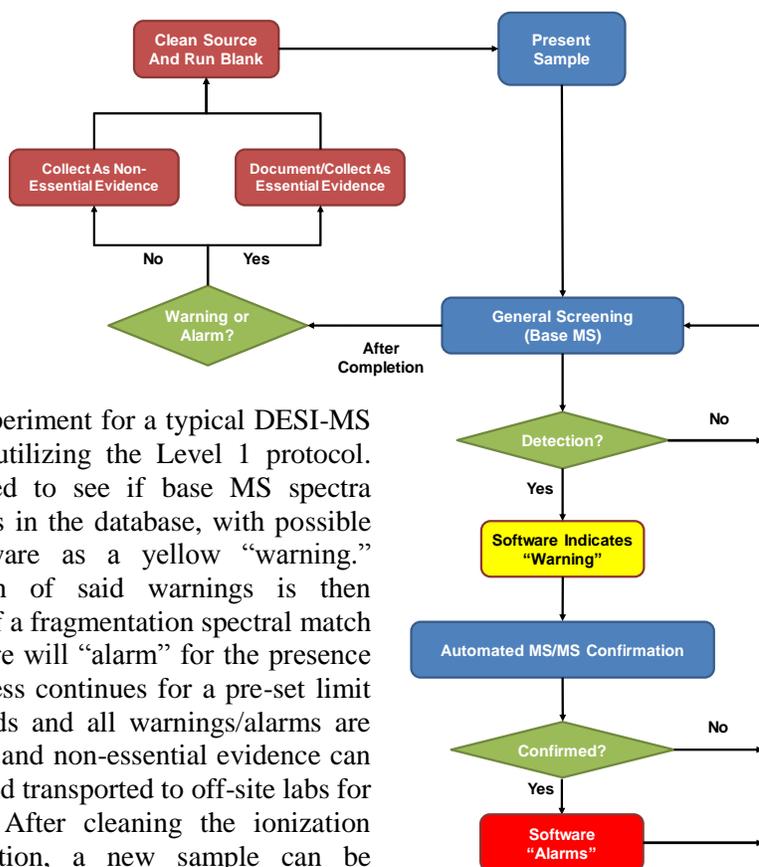
Figure 3-30. Proposed flow of experiment for a typical DESI-MS analysis on the Flir AI-MS 1.2 utilizing the Level 1 protocol. Samples will be initially screened to see if base MS spectra obtained match any target analytes in the database, with possible matches indicated by the software as a yellow "warning." Automated MS/MS confirmation of said warnings is then completed by the instrument, and if a fragmentation spectral match is obtained, the instrument software will "alarm" for the presence of the analyte. This iterative process continues for a pre-set limit (4 min.), after which analysis ends and all warnings/alarms are indicated to the user.   Confirmed and non-essential evidence can then be documented at the scene and transported to off-site labs for further confirmation or storage. After cleaning the ionization source to limit cross-contamination, a new sample can be presented for analysis.

3.5 Task 5: Demonstrate Detection Capability of Materials of Interest at Clandestine Methamphetamine Labs and Investigate Other Forensic Applications

Clandestine laboratory installations represent the worst-case scenario for field analysis, as the variety of "samples" found are diverse in nature, can be large in quantity, rarely marked and stored in proper containers, and most likely located in unsafe conditions. To help provide a robust platform for general sample screening in these situations, proof-of-principle experimentation on the Flir AI-MS 1.2 focused on synthetic routes for methamphetamine and the emerging clandestine drug desomorphine (aka "krokodil"). Testing during this phase of the project involved trace residues and bulk powder of precursors and illicit products, and potential solvents used in clandestine operations were examined via APCI-MS (see *Section 3.3* for discussion of this ionization source). The AI-MS 1.2 was also shown able to monitor both the Birch reduction and Nagai synthetic routes for methamphetamine, identifying both precursor and product species at any point during the reactions.

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3.5.1 Identification of Common Tablets and Powdered Precursors for Methamphetamine Production

In regards to synthetic methamphetamine production, two of the most common precursors, ephedrine and pseudoephedrine (isomeric compounds), can be purchased in limited supply as active ingredients in over-the-counter pharmaceuticals, meaning that corresponding forensic evidence could be found unlabeled as loose tablets, pulverized powder, or extracts (i.e. solution-phase). DESI-MS was successfully used to identify both ephedrine and pseudoephedrine as residues (by direct analysis of the substrate), as a solution (by spotting and drying onto a substrate), and also as a component of crushed pharmaceutical tablets.

For the analysis of pulverized tablets, the user can proceed in two ways: deposition of the powder onto an adhesive-backed glass slide or sampling with a surface swab, both subsequently analyzed directly via DESI-MS. Figure 3-31 shows the DESI-MS spectrum collected from a swab used to sample a pulverized Advil Allergy and Sinus tablet, readily showing the expected spectral profiles for pseudoephedrine and ibuprofen, two active ingredients found in the tablet. Figure 3-32 shows a photo depicting the swabbing aspects of this experiment. For these studies, the surface swab protocol discussed in *Section 3.2.2* was used.
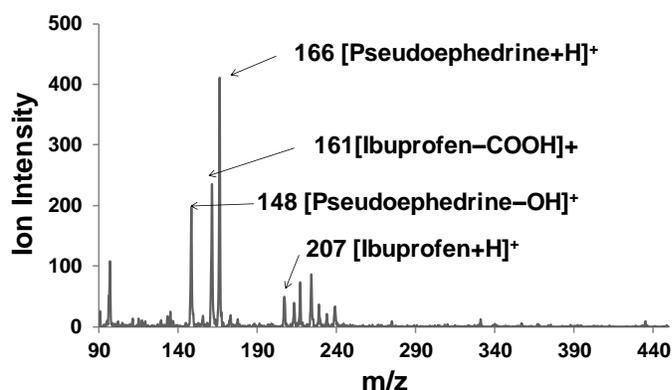
Figure 3-31. DESI-MS from a swab used to sample a pulverized Advil Allergy and Sinus tablet. Spectral signatures for both pseudoephedrine and ibuprofen (both active ingredients) are readily seen.

Figure 3-32. Photo showing the swab sampling of pulverized pharmaceutical tablets. The swab can be subsequently analyzed via DESI-MS.

As manufacturers begin and continue to substitute phenylephrine in place of pseudoephedrine in allergy medications, pharmacies have recently begun selling allergy medications without restrictions while further restricting or discontinuing the sale of the original formulations commonly acquired for the purpose of clandestine methamphetamine synthesis. Due to the ease of purchase and general misinformation, clandestine laboratory operators may accidentally purchase and utilize these newer formulations, causing forensic evidence from a suspected site to contain phenylephrine. To demonstrate the ability to perform both trace and bulk-level detection of this analyte with the Flir AI-MS 1.2, bulk phenylephrine hydrochloride powder was purchased from Sigma-Aldrich and used to make standard solutions for characterization or analyzed as-is. As seen in Figure 3-33, DESI-MS spectra for phenylephrine exhibit a base peak for the protonated molecule at m/z 168 and an in-source fragment at m/z 150,

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corresponding to the loss of water. MS/MS of the m/z 150 fragment (seen in Figure 3-33B) yields signatures at m/z 91, 109, 119, and 135 through a series of complex, gas-phase rearrangements. The similarity of this fragmentation data to that of methamphetamine is of concern, and was a focus of our databasing efforts discussed in *Section 3.1.7 and 3.4.2*.
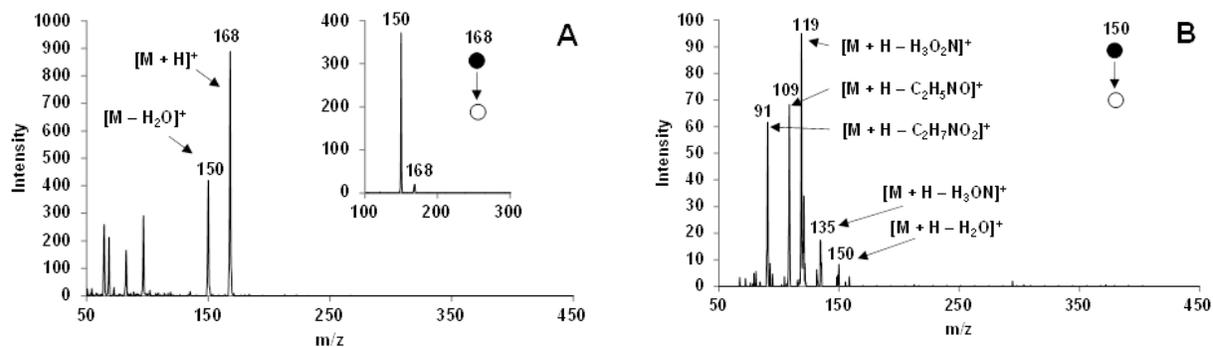
Figure 3-33. (A) DESI-MS data collected for phenylephrine, with MS/MS data for m/z 168 protonated molecule inset. (B) MS/MS data for m/z 150 in-source fragment with molecular assignment.

Additionally, mass spectra were obtained for two different formulations of over-the-counter allergy medications, CVS Sinus Headache PE Non-Drowsy caplets (containing acetaminophen and 5 mg phenylephrine per capsule) and CVS Non-Drowsy Non-Drying Sinus PE Maximum Strength (containing guaifenesin and 5 mg phenylephrine), to simulate medications with potential of being misued for clandestine methamphetamine production. A small section of each pill was ground into a fine powder and sampled via a sampling swab prior to DESI-MS anlaysis. Figure 3-34 shows data for the guaifenesin-containing (34A) and acetaminophen-containing (34B) tablets, yielding intense spectral signatures for all active ingredients, including the inherent phenylephine.
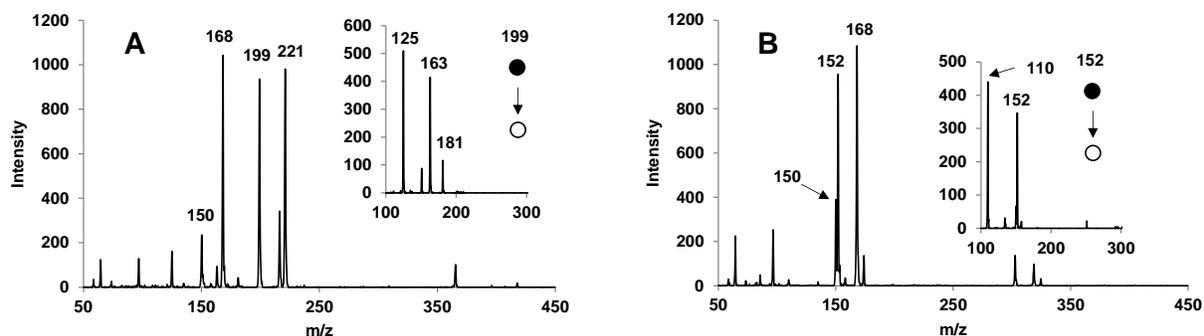
Figure 3-34. (A) DESI-MS spectrum of a pulverized CVS Non-Drowsy Non-Drying Sinus PE Maximum Strength tablet, showing phenylephrine signatures at m/z 168 and 150, as well as two distinct guaifenesin species, $[M+H]^+$ and $[M+Na]^+$, at m/z 199 and 221, respectively. Corresponding MS/MS of the guaifenesin precursor is inset. (B) DESI-MS spectrum of a pulverized CVS Sinus Headache PE Non-Drowsy tablet, showing phenylephrine and the acetaminophen parent at m/z 152. Corresponding MS/MS of the acetaminophen precursor is inset.

3.5.2 Detection of Methamphetamine Residue from Production and Storage Media

To determine if a clandestine laboratory is truly being used for meth production (as opposed to crack cocaine conversion or fentanyl synthesis), detection of powdered

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methamphetamine and its residues is necessary. To show application to this need, the ability to swab various substrates found in clandestine lab scenarios for meth residues and verify their presence with the Flir AI-MS 1.2 was demonstrated. For this testing, various materials were spotted with a known mass of methamphetamine standard, probed via sampling swab following the protocol discussed in *Section 3.2.2*, and analyzed by direct DESI-MS analysis of the swab surface. Table 3-6 shows the detection limit from these methamphetamine residue studies, proving that trace residues can be successfully swabbed, transferred and identified. While there are a multitude of other potential surfaces of interest that can be found in these locations, they are expected to be applicable to our swab transfer protocol, producing variable detection limits depending on the characteristics of the substrates themselves. Residues of the emerging drug desomorphine and its codeine precursor were also shown applicable to our swab transfer protocol, discussed further in *Section 3.5.4*.

Table 3-6. Detection Limit for Methamphetamine
Residues from Surfaces of Interest

Surface	Detection Limit
Aluminum Foil	500 ng
Carbon Steel	500 ng
Glass	2 µg
Polypropylene Bottle	5 µg

3.5.3 Monitoring the Clandestine Synthesis of Methamphetamine via Ambient MS

The Flir AI-MS 1.2 was successful in proof-of-principle screening of the most common methamphetamine precursors, ephedrine and pseudoephedrine, as residues, solutions, and components of crushed pharmaceutical tablets, as well as methamphetamine itself. To demonstrate this capability in a real setting, we participated in the Clandestine Lab Safety Certification course offered through the Midwest Counterdrug Training Center (MCTC) in July 2013 (Camp Dodge, IA). Under the observation of U.S. Drug Enforcement Agency representatives, we had the opportunity to sample, analyze and monitor authentic clandestine methamphetamine syntheses, as well as demonstrate the capabilities of the Flir AI-MS 1.2 instrumentation to practitioners in law enforcement and forensics. Representatives from the Army/National Guard, Iowa State Police, Drug Enforcement Agency, and state and local law enforcement from across the U.S. were in attendance at this course.

During this course, clandestine production of methamphetamine was demonstrated using two common synthetic pathways, the Birch and Nagai (red phosphorous) methods, which both utilized pseudoephedrine as a precursor. After successful instrument setup, calibration and tuning, DESI and PSI-MS was utilized to analyze each significant step in the synthetic pathways. A schematic representation of each synthesis and the success of either DESI or PSI-MS analysis at each procedural step in seen in Figure 3-35.

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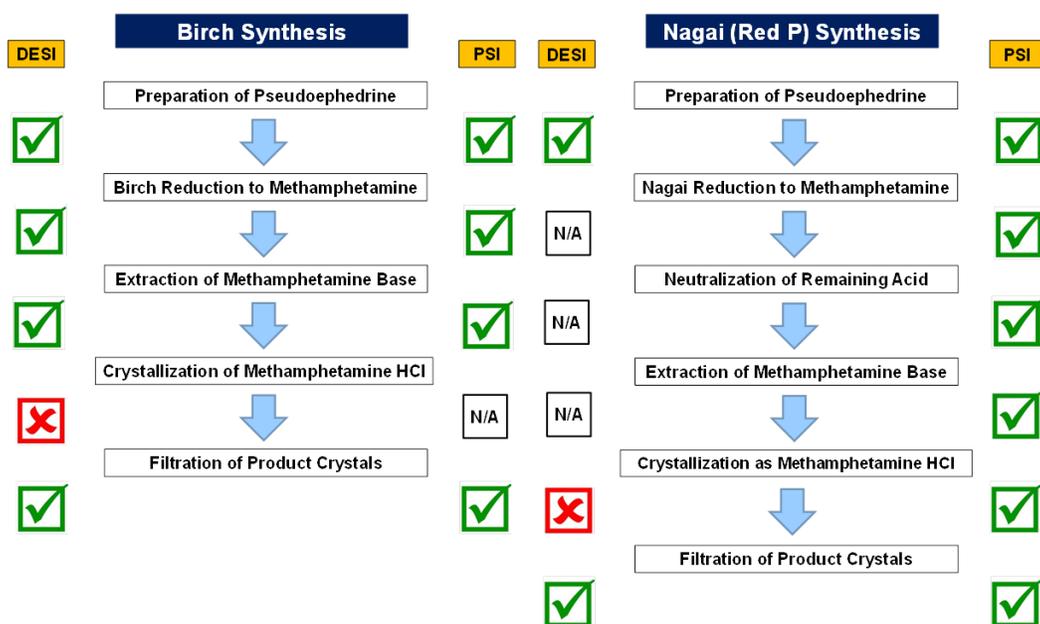
Figure 3-35. Schematic representation of the Birch and Nagai syntheses for clandestine methamphetamine production that were monitored by the Flir AI-MS 1.2 system via DESI or PSI-MS. Green checkmarks correspond to a library match for target compounds. Red crosses correspond to inconclusive data. N/A indicates that analysis at this step was not completed or not possible.

These data represent a crucial achievement for the instrument, as each synthesis can be sufficiently monitored in real-time, showing the consumption of the pseudoephedrine precursor, production and extraction of free-base methamphetamine, and filtration of methamphetamine HCl crystals, all while being fairly robust to the harsh nature of the reaction vessel. This, in essence, allows the Flir AI-MS 1.2 to conclusively identify a clandestine methamphetamine operation regardless of synthesis stage. As seen, the Birch synthesis is thoroughly examined, while testing was a bit more selective with the Nagai synthesis. Since reaction sampling was taking place in conjunction with the regular course instruction, access to the Nagai synthesis was more constrained.

The spectral data that follows represents that obtained during the entire monitoring process. Exact detail of each step of the synthesis is not given in an effort to deter the publication of detailed procedure of methamphetamine production to the general public.

Monitoring of the Birch Synthesis

1) Preparation of Pseudoephedrine

The Birch synthesis utilized reagent-grade pseudoephedrine that required no purification. PSI detection of pseudoephedrine was achieved by swabbing loose powder with a paper triangle. DESI detection of pseudoephedrine was achieved by using a spatula to adhere a small quantity of loose powder to a glass slide with double-sided tape. The PSI-MS and DESI-MS data obtained for pseudoephedrine are represented in Figure 3-36.

51
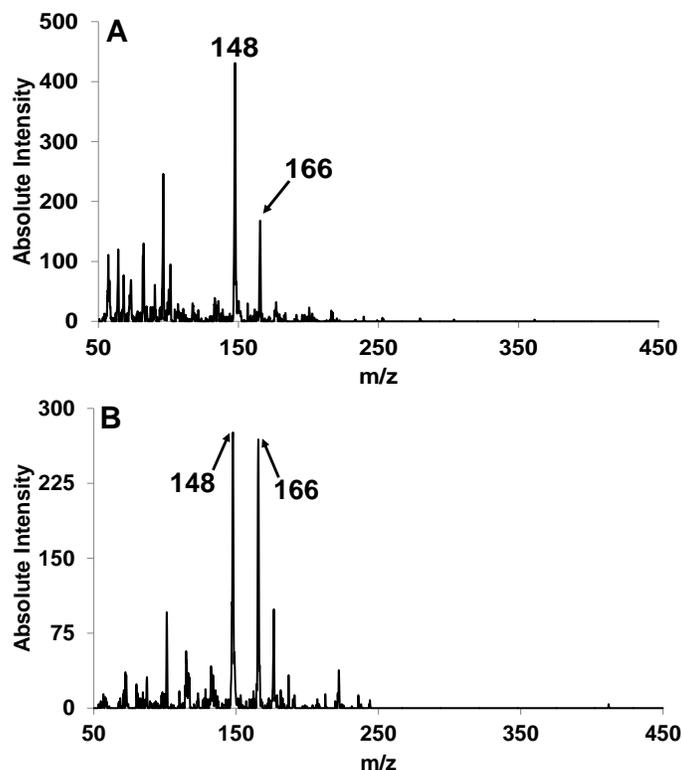
Figure 3-36. PSI-MS and DESI-MS spectra obtained for the pseudoephedrine precursor. (A) PSI-MS spectrum of pseudoephedrine powder collected with a triangular piece of chromatography paper. (B) DESI-MS spectrum of pseudoephedrine powder deposited on a glass slide covered with double-sided tape. The protonated ion of pseudoephedrine and its in-source fragment via loss of water can be seen in both spectra at m/z 166 and 148, respectively.

2) Birch Reduction to Methamphetamine

PSI detection of methamphetamine and residual pseudoephedrine was achieved through use of a disposable glass pipet to sample the aqueous layer and spot a small amount upon a paper triangle. DESI detection of both methamphetamine and residual pseudoephedrine was achieved by spotting a portion of the aqueous layer upon a printed Teflon slide.

3) Extraction of Methamphetamine Base

Addition of Coleman Fuel to the round bottom flask forms an immiscible organic layer to which methamphetamine migrates via liquid-liquid extraction. PSI and DESI detection of methamphetamine was achieved through pipetting and spotting a portion of the organic layer upon the same substrates used in the previous step. Pseudoephedrine was detected at low intensity for both ionization methods.

To demonstrate the detection of potential solvents utilized in clandestine methamphetamine production, vapor from the Coleman Fuel container was transferred and analyzed via APCI with our supplemental pumping system described in *Section 3.3.* Direct analysis of the vapor produced fairly complex, yet characteristic, APCI mass spectra, seen in Figure 3-37. The data is marked by broad hydrocarbon peaks with noticeable species at m/z 85,

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113, 127, and 129, with 127 and 129 tentatively assigned to [Naphthalene - H]$^+$ and [Naphthalene + H]$^+$.
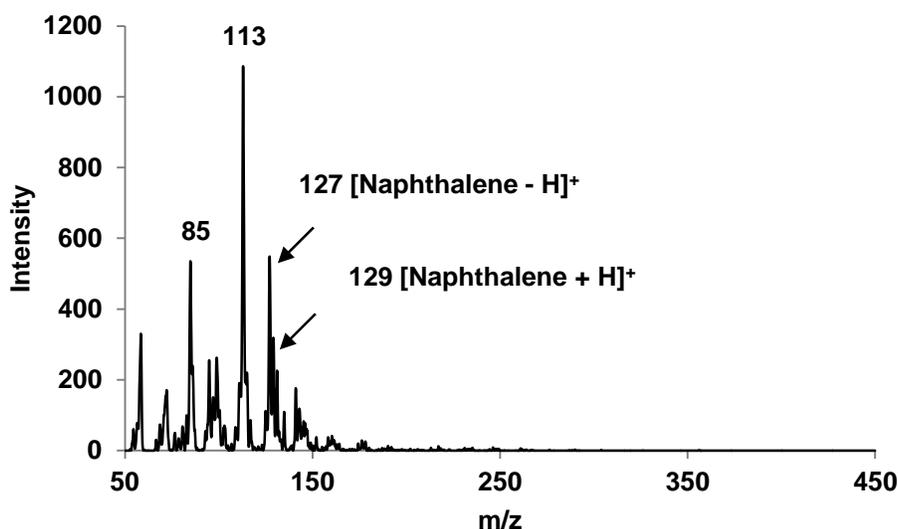
Figure 3-37. APCI mass spectrum of Coleman Fuel vapor, displaying a characteristic hydrocarbon envelope and peaks suggestive of naphthalene.

4) Crystallization as Methamphetamine HCl

5) Filtration of Product Crystals

A standard coffee filter was used to filter the methamphetamine HCl crystals from the organic layer. After filtration, the crystals were washed with acetone to remove any remaining impurities and to aid in drying; acetone vapor was also detected and confirmed via APCI (data not shown). Detection of methamphetamine via DESI was achieved by transferring powderized crystals to double-sided tape adhered to a glass slide, shown in Figure 3-38A. Methamphetamine was also detected with DESI by sampling the product crystals using a nonwoven swab, with pseudoephedrine visible at noise level. Methamphetamine was also detected via PSI by directly swabbing the dried product crystals, with low intensity pseudoephedrine visible, as represented in Figure 3-38B. PSI swabbing of damp crystals produced inconclusive spectra due to low analyte transfer.
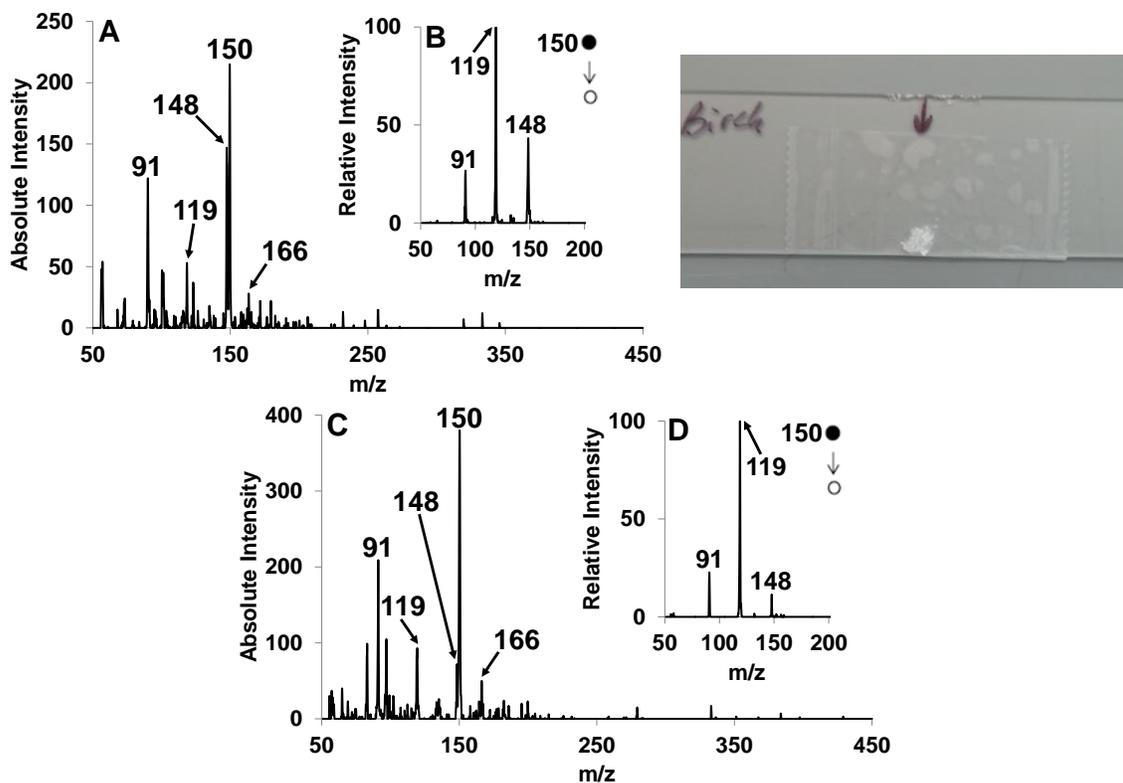
Figure 3-38. DESI-MS and PSI-MS spectra of filtered methamphetamine crystals. (A) DESI-MS spectrum of methamphetamine crystal powder deposited on a glass slide equipped with double-sided tape; this is depicted in the enclosed photograph. Methamphetamine is seen at m/z 150, 119 and 91, while pseudoephedrine is seen at m/z 166 and 148. (B) DESI-MS/MS spectrum resulting from the m/z 150 precursor. (C) PSI-MS spectrum of swabbed methamphetamine crystals. (D) PSI-MS/MS spectrum resulting from the isolation and fragmentation of m/z 150.

Monitoring of the Nagai (Red Phosphorous) Method

1) Preparation of Pseudoephedrine

This synthesis utilized the same reagent-grade pseudoephedrine. DESI and PSI-MS analysis was conducted in the same manner as with the Birch synthesis.

2) Nagai Reduction to Methamphetamine

To support the ability of the Flir Systems AI-MS 1.2 to analyze highly acidic solutions while protecting its critical components, a filter paper swab was used to collect a sample of the contents present in the round bottom flask during the Nagai reduction. Figure 3-39 shows the PSI mass spectrum resulting from the direct analysis of the swab, which also acted as the substrate for ionization. PSI has been shown more apt at minimizing exposure of the Flir AI-MS 1.2 inlet system to harsh samples; DESI-MS of surface-bound samples has a tendency of transferring a majority of the sample matrix along with the analyte of interest, while the PSI

substrate has a tendency to retain it. To this end, PSI was used more extensively to monitor the overall Nagai synthesis.
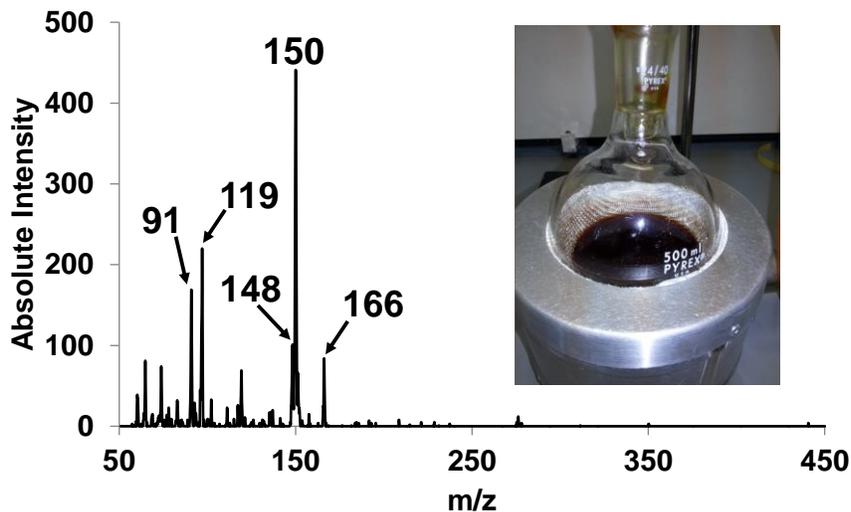
Figure 3-39. PSI-MS spectrum of the contents present in the round bottom flask (photo inset) during Nagai reduction. The molecular ion of methamphetamine is seen at m/z 150, with characteristic in-source fragments present at m/z 119 and m/z 91. Likewise, the molecular ion of pseudoephedrine can be seen at m/z 166, with a characteristic in-source fragment at m/z 148.

3) Neutralization of Remaining Acid

4) Extraction of Methamphetamine Base

PSI detection of freebase forms of methamphetamine and pseudoephedrine was achieved by spotting a portion of reaction mixture to filter paper with a disposable pipet.

5) Crystallization as Methamphetamine HCl

6) Filtration of Product Crystals

A coffee filter was used to filter the methamphetamine as in the Birch method. PSI spectra obtained from swabbing dried crystals exhibited methamphetamine, with pseudoephedrine near noise level. Interestingly, PSI was able to be performed by directly spraying from a triangular piece of the coffee filter that was used in the filtration process. For this, a triangular piece was cut from the coffee filter and directly placed in our PSI ionization source. The PSI-MS spectrum directly from the coffee filter is shown in Figure 3-40, showing methamphetamine and pseudoephedrine signatures.

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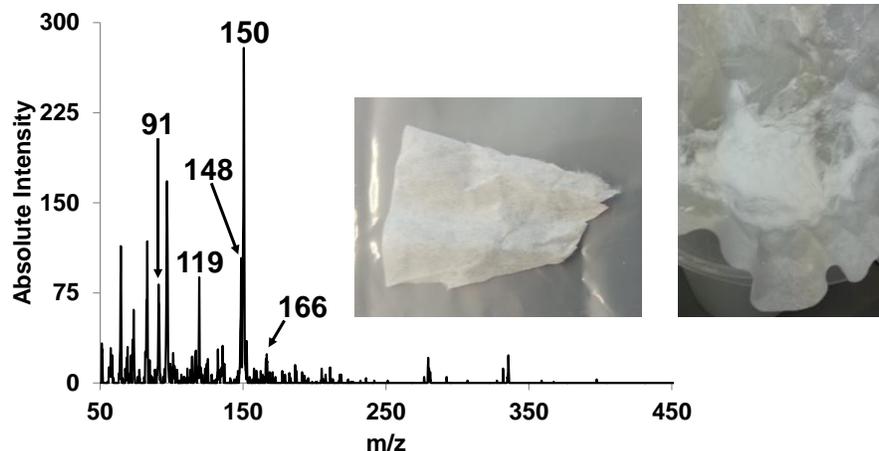
Figure 3-40. PSI-MS spectrum obtained from directly spraying from a triangular section of coffee filter utilized in the filtration process (representative photo inset). Spectral signatures confirm the presence of both methamphetamine product and unreacted pseudoephedrine precursor.

3.5.4 Analysis of Desomorphine, Codeine and Fentanyl

The use of the drug desomorphine (street name "krokodil") has been seen in Europe, but recent reports of its production and use in the U.S. have made it news-worthy. To demonstrate desomorphine analysis on the Flir AI-MS 1.2 platform, an analytical standard of this codeine derivative was purchased and analyzed via DESI-MS. As seen in Figure 3-41, the generated spectra show an intense m/z 272 ion corresponding to protonated desomorphine, while the MS/MS of the m/z 272 precursor yields fragments at m/z 215 and 197, corresponding to a loss of C_4H_9 and $C_4H_{10}O$, respectively.

To demonstrate trace-level detection, residues of desomorphine and its codeine precursor were swabbed and subsequently analyzed via DESI-MS from various surfaces of potential use in clandestine synthesis, including nonstick coated cookware, enamel cookware, glass, steel, and polyethylene terephthalate (PET) pill bottles. The detection limits from these surfaces can be seen in Table 3-7, with LODs typically residing in the low microgram range for most surfaces. Teflon printed slides produced the lowest detection limit, which is typical from our experience with this substrate; the hydrophobicity of Teflon enhances the removal of analyte via the solvent-mediated DESI desorption mechanism.

Clandestine production of desomorphine involves the derivatization of codeine, and given the relative ease of acquiring codeine via prescription pills and cough syrups compared to the availability of ephedrine/pseudoephedrine needed for clandestine methamphetamine production, there is legitimate concern of an uptick of desomorphine use and production. To this end, experimentation on codeine-containing prescription pills was undertaken. Figure 3-42 shows the DESI-MS spectrum collected from a swab brought into contact with a pulverized tablet of Tylenol 3 (active ingredients: acetaminophen (300 mg) and codeine phosphate (30mg)). As seen, the spectra feature strong peaks for the active ingredients of the pill with minimal background. MS/MS of the codeine precursor (inset) produces similar fragments reported in literature and also seen in past Flir AI-MS 1.2 characterization.
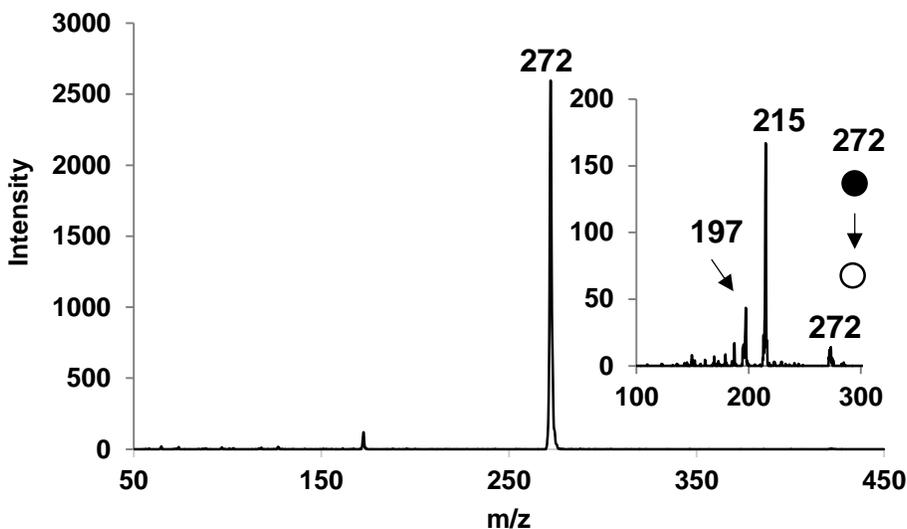
Figure 3-41. DESI-MS and MS/MS (inset) spectra of desomorphine, showing the protonated molecule, [M+H]$^+$, at m/z 272 and fragments at m/z 215 and 197.

Table 3-7. LODs of desomorphine and codeine from surfaces of interest

Surface	LOD	
	Desomorphine	Codeine
Steel	3.0 μg	5.0 μg
Nonstick cookware	2.5 μg	2.0 μg
Enamel cookware	1.0 μg	4.5 μg
PET bottle	1.5 μg	3.0 μg
Glass	1.0 μg	5.0 μg
Teflon Slide	0.50 ng	0.90 ng
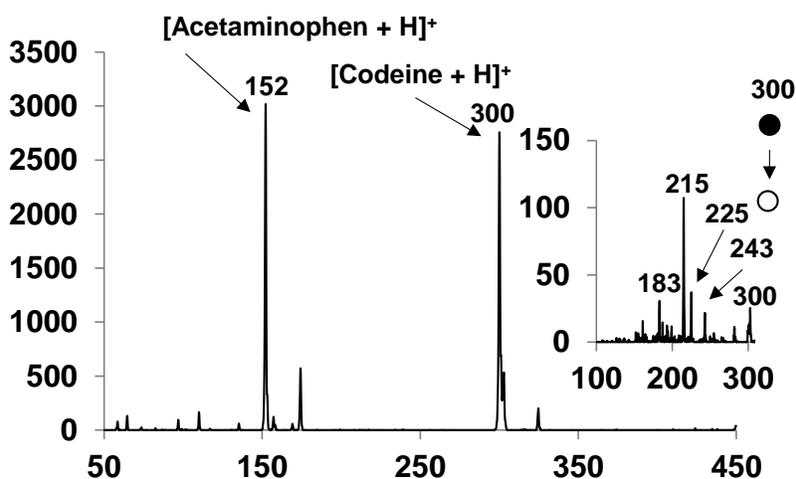
Figure 3-42. DESI-MS spectrum of Tylenol 3 powder from a foam swab. MS/MS of the codeine precursor (inset) matches past data in our spectral database.

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It is expected that, given the broad applicability demonstrated in regards to both drug classes and states they reside in, ambient ionization methods coupled to the Flir AI-MS 1.2 will be capable to detect target species in other known and emerging clandestine syntheses. For instance, initial studies involving fentanyl shows high sensitivity analysis of its residues via DESI-MS. Representative fentanyl data can be seen in Figure 3-43.
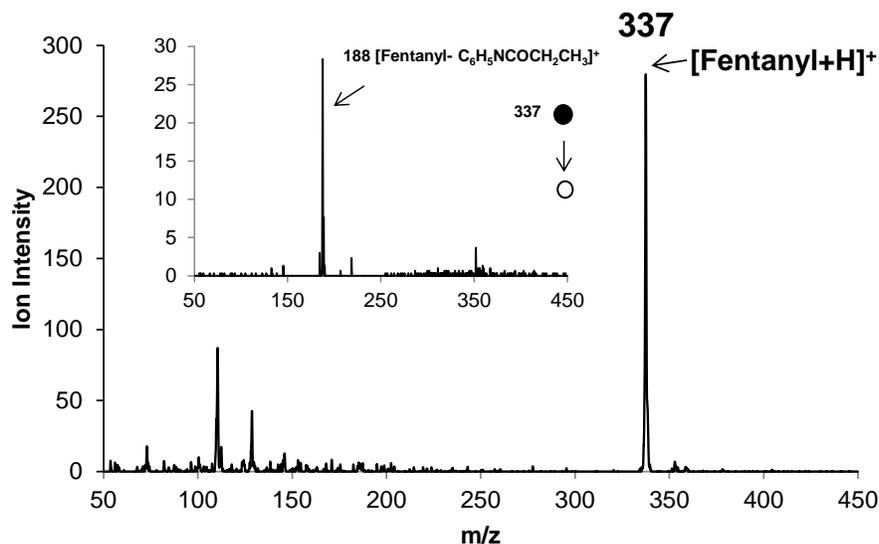
Figure 3-43. DESI-MS and MS/MS (inset) data collected for fentanyl residue from a glass slide collected on the Flir AI-MS. Aspects of this data were sent for comparison to the MSforID database.

3.6 Task 6: Conduct Field Experiments and Disseminate Findings to Practitioners

While many of the results obtained on the Flir AI-MS 1.2 during this project involved analytical standards in controlled laboratory settings, special efforts were taken to perform field testing on authentic forensic evidence. Field testing with law enforcement, forensics and criminal justice practitioners serves dual purposes, as the effect of environmental variables (*i.e.* transport and calibration, ambient conditions, etc.) can be assessed while gathering integral feedback from the target user groups. Overall, practitioners who witnessed our demonstrations and field experimentation were impressed by the performance of the Flir AI-MS 1.2, size of the device, and especially by the ease of use. Through discussion with these groups, information regarding needs for true field implementation and use by untrained personnel was acquired that helped craft and improve our developed methods and ionization source design. Furthermore, important troubleshooting experiences can arise in the field.

3.6.1 Synthetic Cathinone "Bath Salt" Evidence

Trace analysis of drug of abuse residues is an important ability and has been shown repeatedly on the AI-MS 1.2, but the capability to look at condensed phases like powders and tablets from drug seizures is just as much of a necessity. Through collaboration with the Bloomington Police Department Vice Squad (Bloomington, IL), access to authentic synthetic

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cathinone "bath salt" powders that were seized as commercially-available products at local retailers was provided to test screening capability via DESI-MS. These seizures were confirmed positive for their associated illicit drugs via GC-MS analysis through the Illinois State Police forensic laboratory system prior to our analyses. Packaging for these evidentiary samples can be in seen in Figure 3-44, advertised under names such as White Lace, White Horse, and Global bath salts, Global plant food, and Disco all-purpose solution. Figure 3-45 shows representative consistency of select compositions.
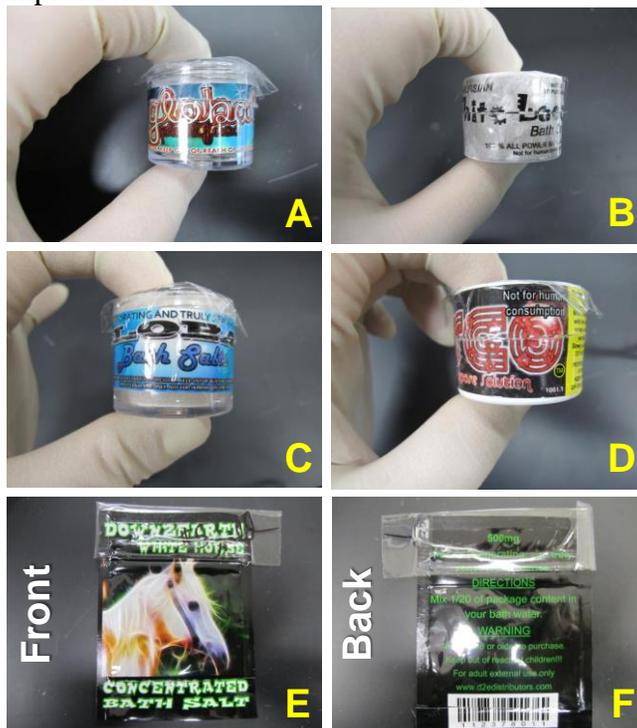
Figure 3-44. Packaging of the seized bath salt products provided through the Bloomington (IL) Police Department. These commercial products were seized or voluntarily forfeited from local retailers in the Bloomington-Normal, IL area. (A) Global Plant Food. (B) White Lace Bath Salt. (C) Global Bath Salts. (D) DISCO All-Purpose Solution. (E) Down2Earth White Horse Concentrated Bath Salt – front of packaging. (F) Down2Earth White Horse Concentrated Bath Salt – back of packaging.
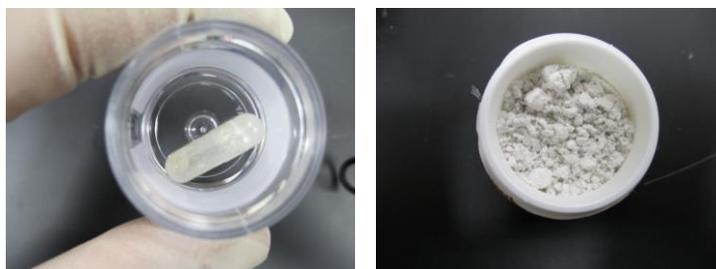
Figure 3-45. Photos showing the consistency of select bath salts that were analyzed. LEFT: Pill capsule containing illicit bath salts. Capsule was opened and powder substance was deposited onto double-sided adhesive tape and directly analyzed via DESI-MS. RIGHT: Most of the seizures were packaged as loose powders. These powders were deposited onto double-sided adhesive tape and directly analyzed with DESI-MS.

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To allow direct detection of these powders with minimal sample preparation, a piece of Scotch® Double-Sided tape adhered to a glass microscope slide was used as the analysis surface. A small amount (~1 mg) of the powder evidence was then deposited onto the tape, using a spatula to break up larger clumps and force contact between the powder and adhesive (as depicted in Figure 3-46C). To prevent sample carryover, loose powder was removed from the surface of the tape using a light flow of compressed air prior to DESI analysis. Of note, no carryover was seen sample-to-sample for this powder analysis method.

Figure 3-46A shows the positive ion DESI mass spectrum of a seizure determined positive for the presence of MDPV, yielding an intense spectral peak for the protonated molecule at m/z 276. MS and MS/MS analyses of this seizure directly matched data collected with analytical standards. Besides the more common cathinones like MDPV, pentedrone, an analogue of methcathinone, was also present in select samples. Figure 3-46B shows the analysis of powdered pentedrone, yielding the protonated molecule at m/z 192 and an in-source fragment at m/z 174, corresponding to loss of water. The corresponding MS/MS confirmation of the protonated molecule precursor can be seen in the inset, showing both the in-source fragment seen in MS mode and other transitions consistent to those reported in literature. The simple preparation utilized for the powdered bath salts can be extended to other powdered drugs of abuse that have been previously investigated as tablets with DESI-MS.
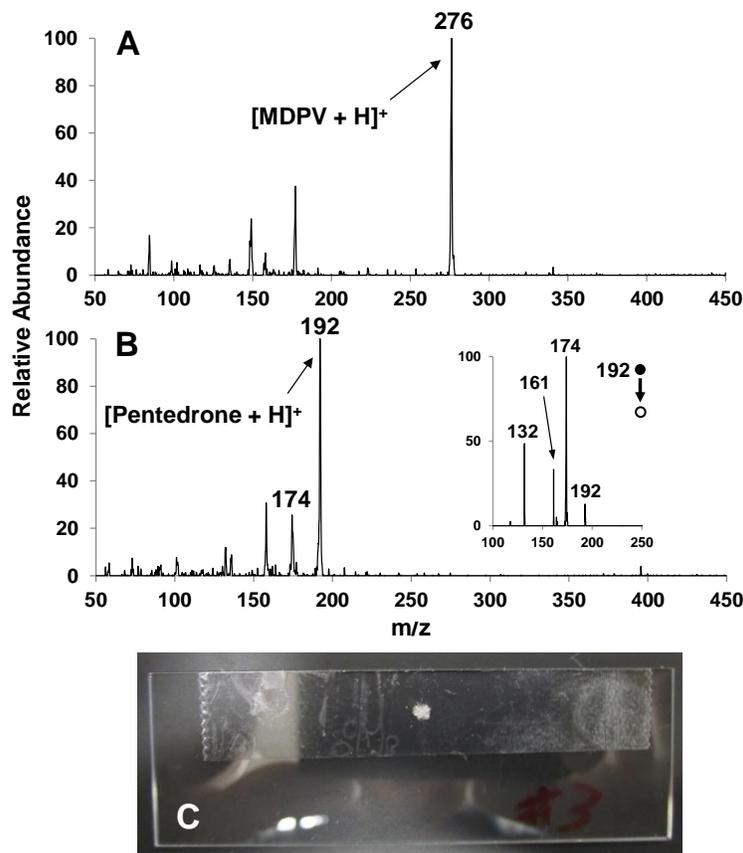
Figure 3-46. DESI-MS data collected on the Flir AI-MS 1.2 from authentic powdered bath salt evidence. (a) Evidence positive for MDPV shows the protonated molecule at m/z 276. (b) Evidence positive for pentedrone shows the protonated molecule and in-source fragment at m/z 192 and 174, respectively. (c) By simple deposition onto double-sided adhesive tape, powdered evidence can be rapidly analyzed with no significant carryover.[38]

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While at Camp Dodge, IA completing the Clandestine Lab Safety Certification course, there was significant opportunity to discuss facets of our NIJ-funded research and the instrumentation being developed. Helpful discussions regarding needs for true field implementation and use by untrained personnel conducted during this opportunity will help guide our continued work on the Flir AI-MS 1.2 system. Through law enforcement contacts, we were also able to further demonstrate and conduct field experiments on authentic evidence provided by the Iowa State Police.

3.6.2 Powder and Residue-Level Cocaine and Methamphetamine Evidence

In cooperation with the Iowa State Police, experimentation on authentic drug evidence, specifically bulk cocaine powder (Figure 3-47) and trace residues of methamphetamine, was conducted. In fact, the methamphetamine evidence that was provided was actually just a plastic bag used to transport the drug, containing an extremely small crystal that could barely be seen with the naked eye. To analyze this sample via DESI-MS, the remaining crystal was dissolved in a small aliquot of methanol and subsequently spotted and analyzed.
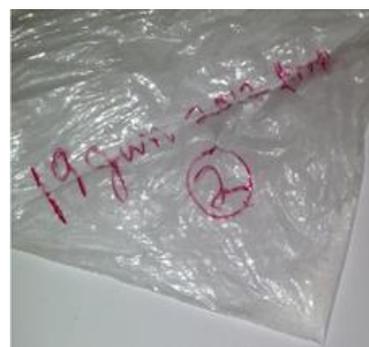
Figure 3-47. Photo showing powdered cocaine evidence provided by the Iowa State Police.

Characteristic data for both PSI and DESI-MS were able to be obtained from each evidence type. Figure 3-48 shows the PSI-MS and MS/MS mass spectra collected from swabbing the inside of the bag known to previously transport crystal methamphetamine. The PSI-MS mass spectrum, collected from the transfer swab itself, not only displays the molecular ion of methamphetamine at m/z 150, but also the characteristic in-source fragments at m/z 119 and 91. Of significance is the high intensity obtained, suggesting that an even smaller amount of methamphetamine could have been successfully detected. This is an interesting finding, as it supports PSI as a probable method for the detection of extremely trace samples. To analyze the bulk cocaine powder via PSI-MS, a paper substrate was first wetted with 2 µL of methanol to assist in the transfer of the analyte to the swab. The paper itself was dipped into the bulk powder, followed by direct PSI analysis. Figure 3-49 shows the PSI-MS and MS/MS mass spectrum obtained for the bulk authentic cocaine powder swabbed from the inside of a plastic bag. The molecular ion of cocaine is seen at m/z 304 with characteristic MS/MS fragments present at m/z 182 and 150.

Of interest, experimentation with bulk forensic evidence was able to be accomplished without extensive carryover between analyses by implementing proper hygiene protocols involving cleaning of the ionization source and outer surface of the MS inlet capillary with methanol-wetted swabs and analysis of pre-sample blanks.
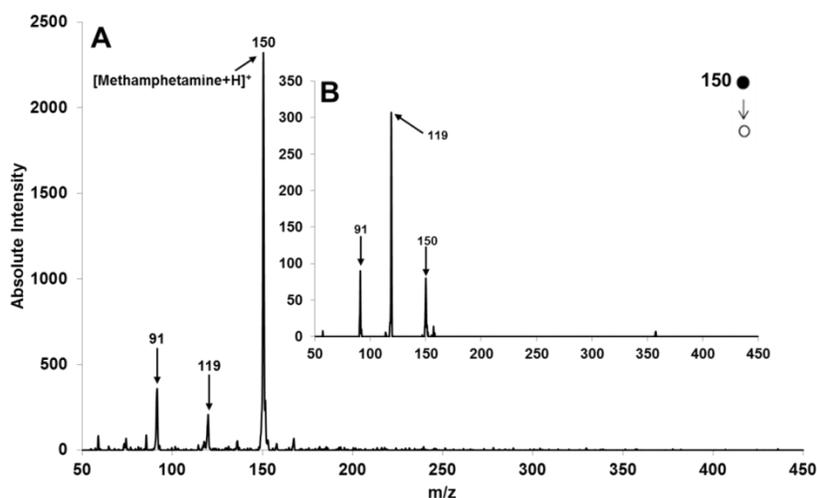
Figure 3-48. (A) PSI-MS and (B) MS/MS data collected for authentic methamphetamine residue present in a plastic bag using the Flir AI-MS. The sample was dissolved in methanol prior to analysis due to the minute amount of sample available for analysis.
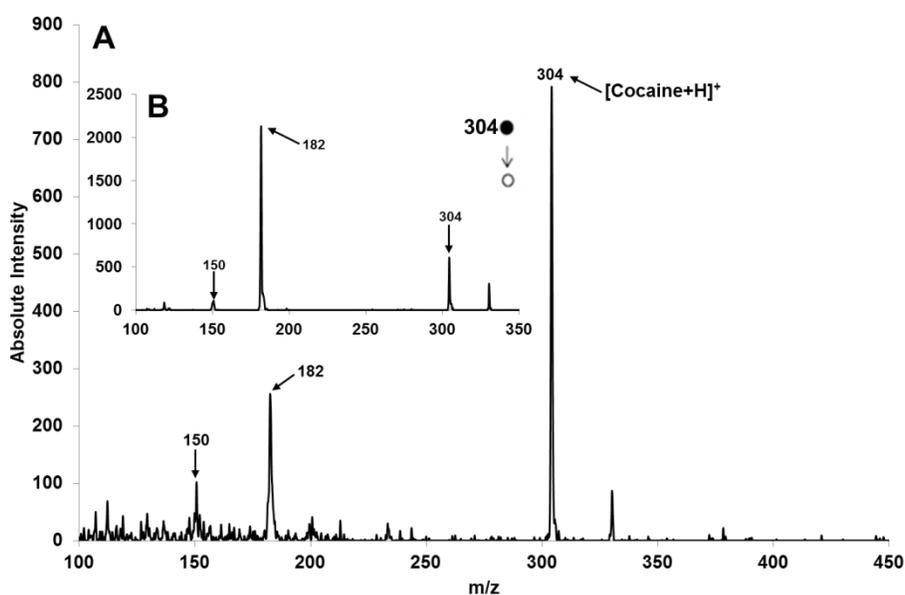
Figure 4-49. (A) PSI-MS and (B) MS/MS data collected from authentic bulk cocaine powder using the Flir AI-MS. The sample was collected using a prewetted paper substrate as a surface swab.

3.7 Task 7: Preparation of Deliverable Instrument and Associated Documentation

An important final aspect of this project was to prepare and deliver a modified Flir AI-MS 1.2 system coupled with the associated ionization sources investigated. Along with the instrumentation, a comprehensive mass spectral library (including MS and MS/MS data for project analytes) and optimized compound-specific MS/MS methods were delivered. Also,

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methods allowing automated library searching via data dependent scanning (Level 1 methods, see *Section 3.4.2*) were able to be constructed and delivered. Documentation delivered with the Flir AI-MS 1.2 included extensive training and troubleshooting manuals, with substantial detail to ensure that NIJ personnel and other users will be able to quickly learn the operation of all facets of the system.

4. Conclusions

4.1 Discussion of Findings

The Flir Systems AI-MS 1.2 was demonstrated to be a broadly-applicable, portable instrument with high potential for use in crime scene investigation and evidentiary analysis. The flexibility to screen and identify solid, liquid and gas-phase analyte when utilizing the suite of ionization methods developed and/or investigated on the system has the potential to provide capabilities that no other fieldable technology currently available can offer. Switching between trace level residues and bulk-level samples was achievable with no carryover when implementing simple hygiene protocols, and coupling with physical transfer swabs extends application of the system to large and geometrically-complex surfaces, meeting the demands that real evidence present. The congruency of spectral data collected on the AI-MS 1.2 in regards to both MS and MS/MS ion signatures collected on lab-scale MS instrumentation and also seen in scientific literature is seen as important milestone towards acceptance as a validated forensic analysis method and actual implementation in CSI applications.

The only class of forensic evidence where the Flir AI-MS 1.2 failed to meet expectations was military grade explosives (*e.g.* TNT, RDX, HMX and PETN), for which DESI-MS analysis is routinely accomplished with lab-scale instrumentation through doping the spray solvent with the Cl^- anion (commonly from small volumes of HCl or NaCl); these dopants create chloride-bound adducts with the analytes. Interestingly, chloride-bound adducts are not observed in DESI-MS testing of explosives on the Flir AI-MS 1.2 at any appreciable level. It is expected that the optics utilized for ion introduction at the high pressure stages of the system energizes the formed adducts and makes them unstable; this also can explain the in-source fragmentation observed for other analytes investigated over the project duration.

4.2 Implications for Policy and Practice

When considering the end product of this project, a portable instrument capable of assessing the probative value of physical evidence typically found at crime scenes, the impact of providing the forensic science community with said technology would have a positive effect on criminal justice practice at the local, state and national level. There are also potential implications in regards to reducing evidence backlogs that hinder publicly-funded forensic laboratories, as this technology provides both higher throughput and a potentially reduced influx of evidence entering the lab system. When considering the feasibility of implementing the proposed technology in forensic settings, the cost of this instrumentation and maintenance could be off-set by the reduction in evidence sent to forensic laboratories and funds being used for outsourced analyses to private laboratories.

While the effect on current criminal justice practice can be presumed, the implications on current criminal justice policy are not as clear. Field-portable units for accurate contraband

detection in the condensed phase and from surfaces would allow greater versatility in routine traffic stops and criminal investigations, but if this technology is used to gather evidence prior to an arrest, there are implications regarding unreasonable search and seizures. It is easy to envision using the capabilities of the Flir Systems AI-MS 1.2 to establish "probable cause" in innovative ways, especially for residues of contraband in latent fingerprints. Overall, implementation of the proposed research could allow greater flexibility in law enforcement, but its application to legal convictions under current criminal justice policy will need to be considered before common usage.

When a new technology is proposed for evidence analysis in the criminal justice system, there is a level of scrutiny to which the method is held. The Daubert Standard, also known as *Rule 702 of the Federal Rules of Evidence*, provides guidelines for admissible expert testimony stemming from a data obtained with new technologies or methods. When this type of expert testimony is being dissected, more effort than not is placed into refuting the method in which the data was obtained. Our characterization studies on the Flir AI-MS 1.2 allude that this system has promise meeting the expectations established by Daubert, particularly in regards to falsifiability error rate, but further validation will be needed to satisfy all provisions.

4.3 Implications for Further Research

There are several areas of interest in regards to future research on the Flir AI-MS 1.2 platform. Particularly, ambient MS ionization methods represent a highly active research area, with new methods reported frequently in the chemical literature. Examination of alternate ionization sources for use on this platform could lead to broader application and flexibility in forensic applications; this is especially true for PSI-MS, which performed well and, in some instances, proved superior to DESI-MS in our proof-of-principle testing. The robust nature of the developed instrumental methods could also prove useful in field-based toxicological assessments and threat detection applications. The Flir AI-MS 1.2 would also benefit from extensive validation at actual crime scenes and harsh environments, allowing the ruggedness of the system to be tested and effect of long-term field use to be assessed. From the research presented herein, the potential impact on the criminal justice system in terms of scientific capabilities can be anticipated, but financial implications (both positive and negative) will not be known until thorough economic assessment and cost-benefit analyses.

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6. Dissemination of Research Findings

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6) Mulligan, C. C.; Hall, S. E.; O'Leary, A. E.; Vircks, K. E.; Wieland, J. R. Towards a Versatile Mass Spectrometric Platform for Comprehensive Crime Scene Analytics. *61st ASMS Conference on Mass Spectrometry and Applied Topics*, *Minneapolis, MN,* **June 9th – 13th, 2013**.

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8) Mulligan, C. C.; O'Leary, A. E.; Hall, S. E.; Wieland, J. R.; Gizzi, M. C. Portable MS Systems for Rapid Screening of Forensic Evidence at Clandestine Drug Operations. *62nd ASMS Conference on Mass Spectrometry and Applied Topics*, *Baltimore, MD.* **2014**.

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9) O'Leary, A. E.; Hall, S. E.; Oberacher, H.; Mulligan, C. C. Comparison of Forensic Tandem Mass Spectral Data Obtained on Portable Instrumentation to an Established Reference Library. *62nd ASMS Conference on Mass Spectrometry and Applied Topics*, *Baltimore, MD.* **2014**.

10) Swiontek, A. I.; Hall, S. E.; O'Leary, A. E.; Mulligan, C. C. Establishing a Surface Swabbing Protocol Compatible with Ambient Sampling Mass Spectrometers for On-Site Forensic Evidence Screening. *62nd ASMS Conference on Mass Spectrometry and Applied Topics*, *Baltimore, MD.* **2014**.

11) Hall, S. E.; O'Leary, A. E.; Traub, A.; Mulligan, C. C. Using an Ambient Sampling, Portable Mass Spectrometer for the Direct Analysis of Species Related to Desomorphine ("Krokodil") Synthesis. *62nd ASMS Conference on Mass Spectrometry and Applied Topics*, *Baltimore, MD.* **2014**.

Appendix A: Positive-Mode MS and MS/MS Spectral Database

*Only compounds for which optimized MS/MS parameters were determined have been included in this appendix.

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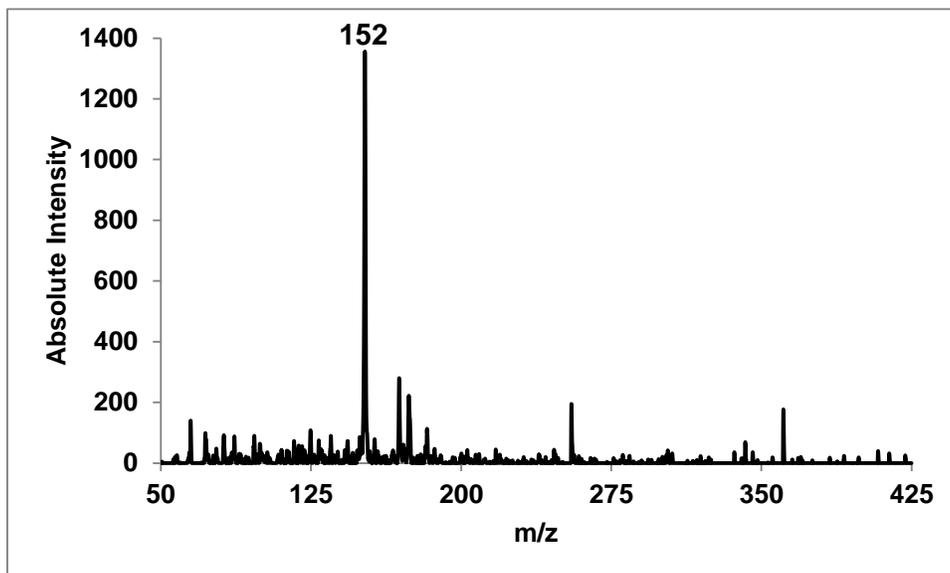
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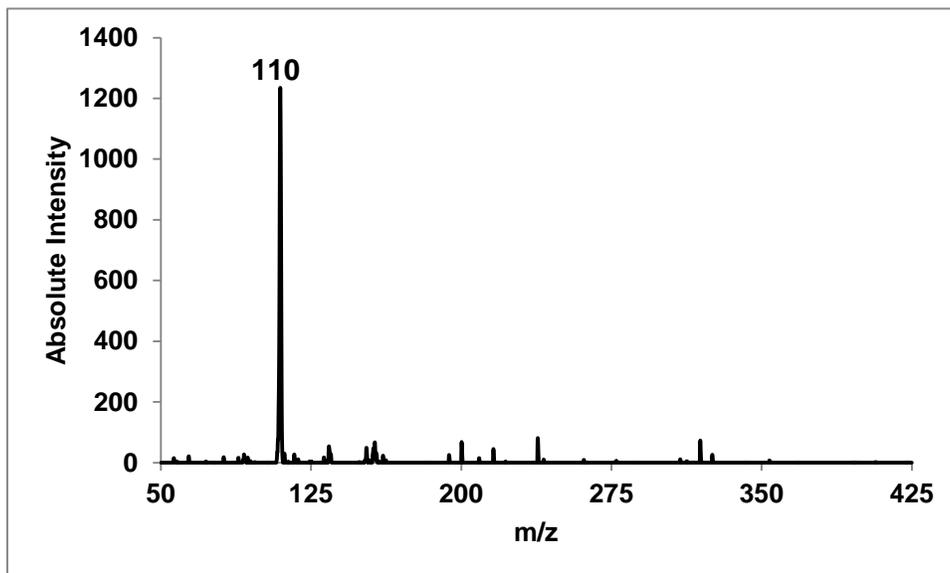
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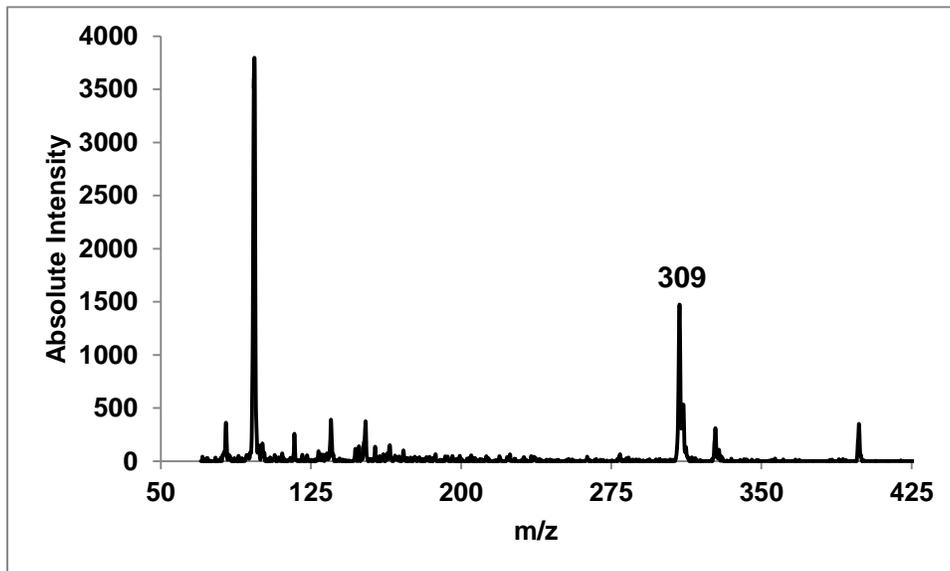
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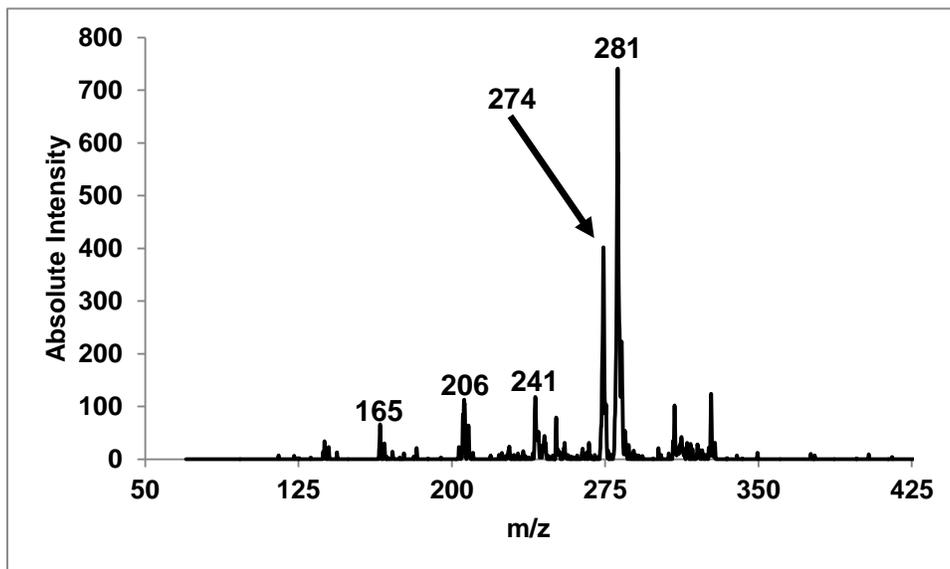
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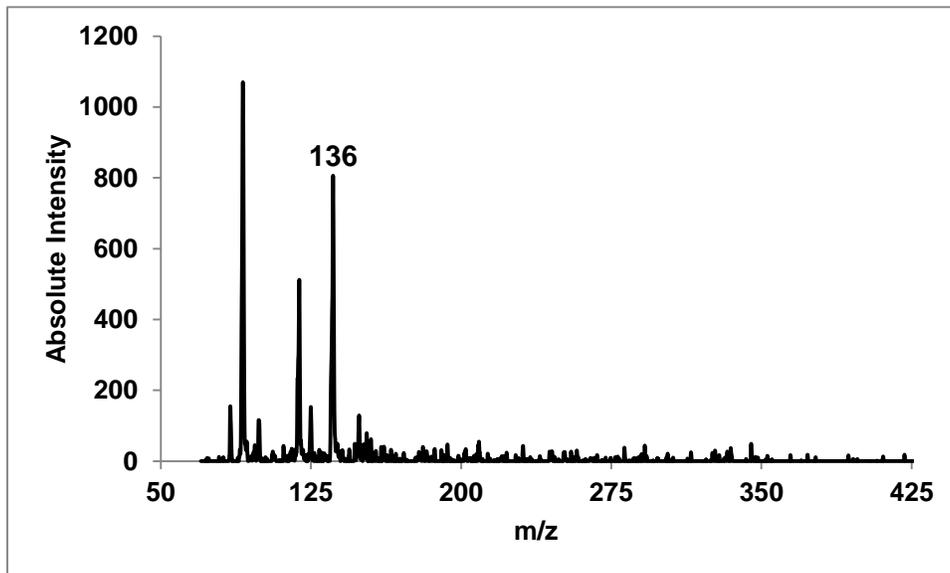
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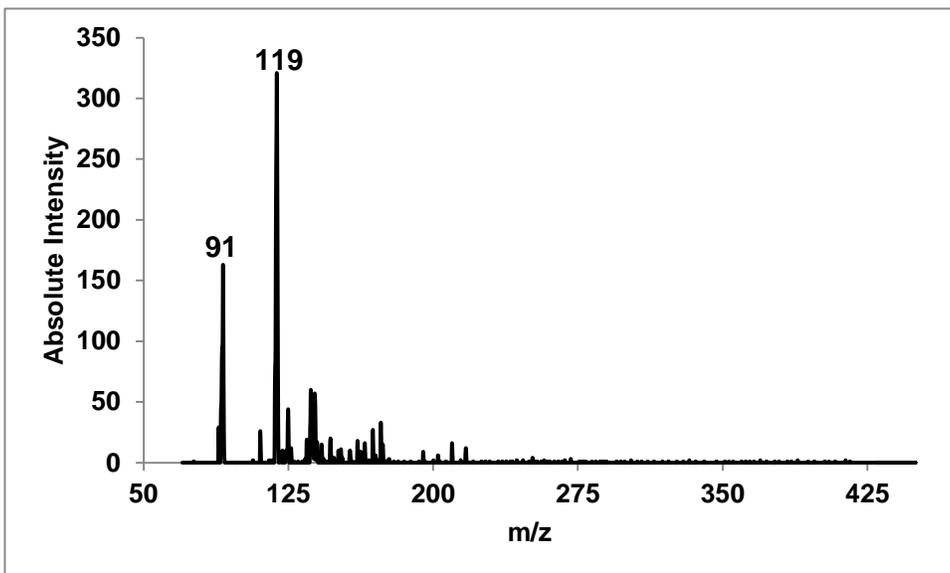
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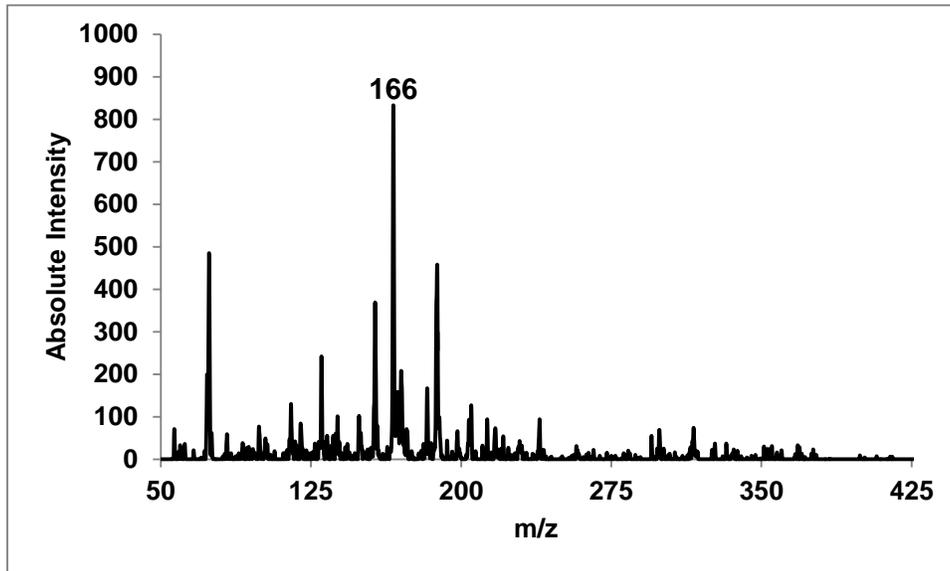
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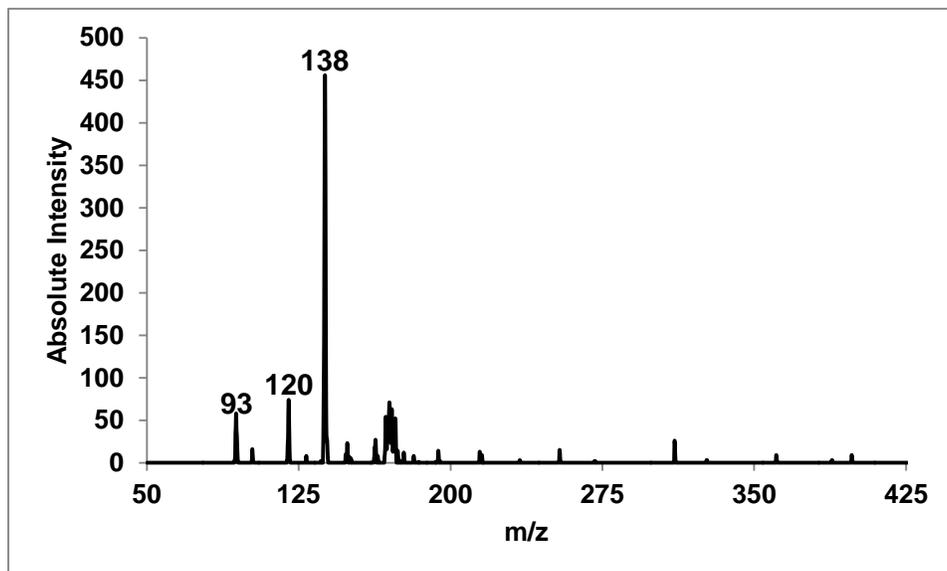
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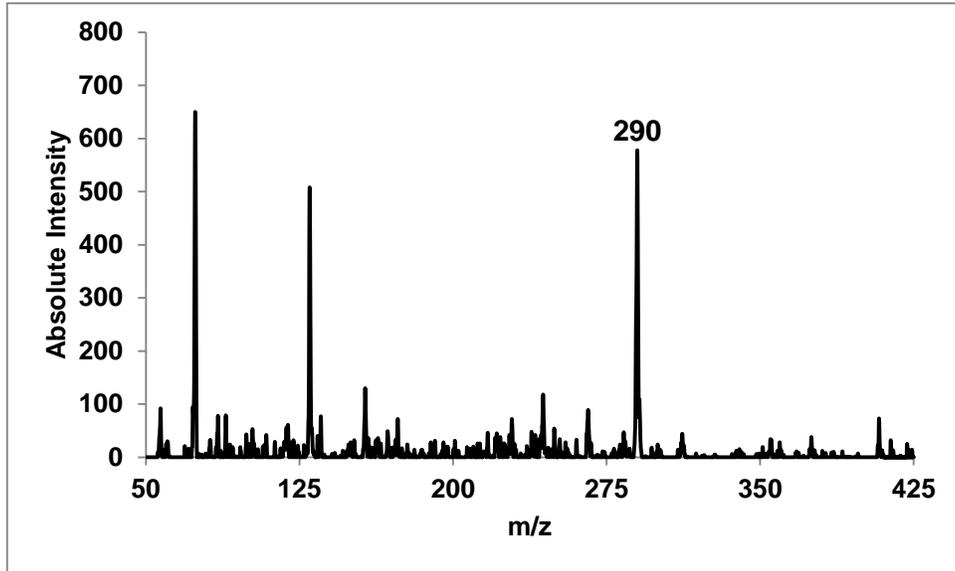
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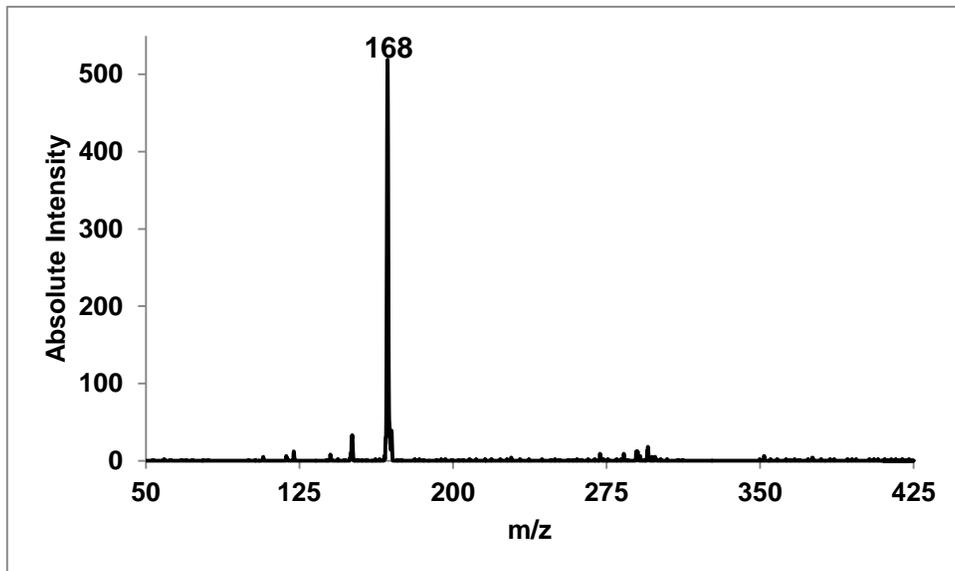
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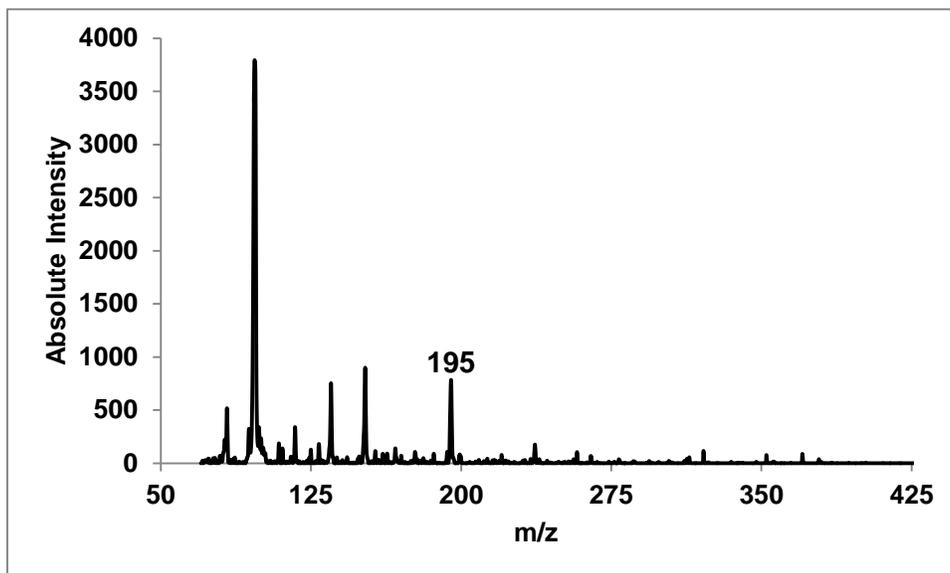
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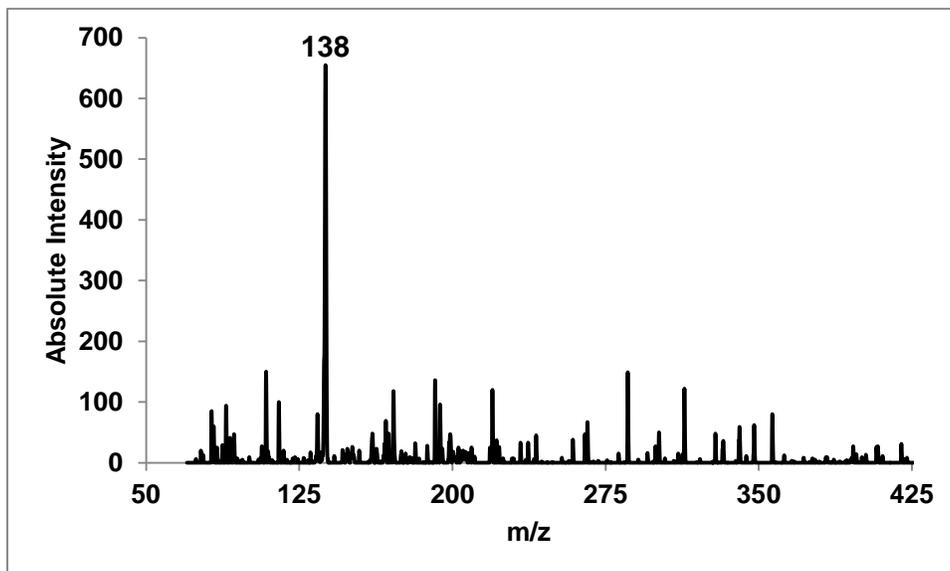
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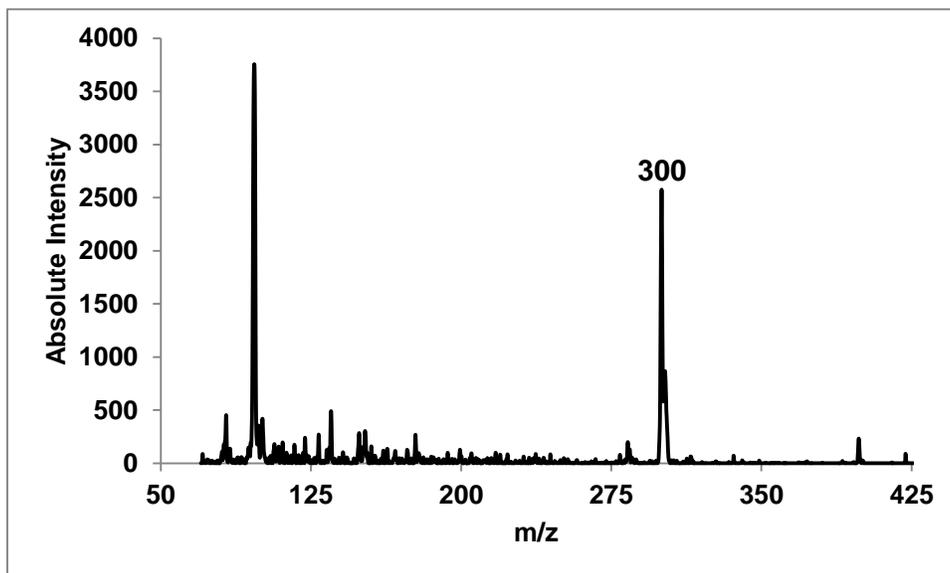
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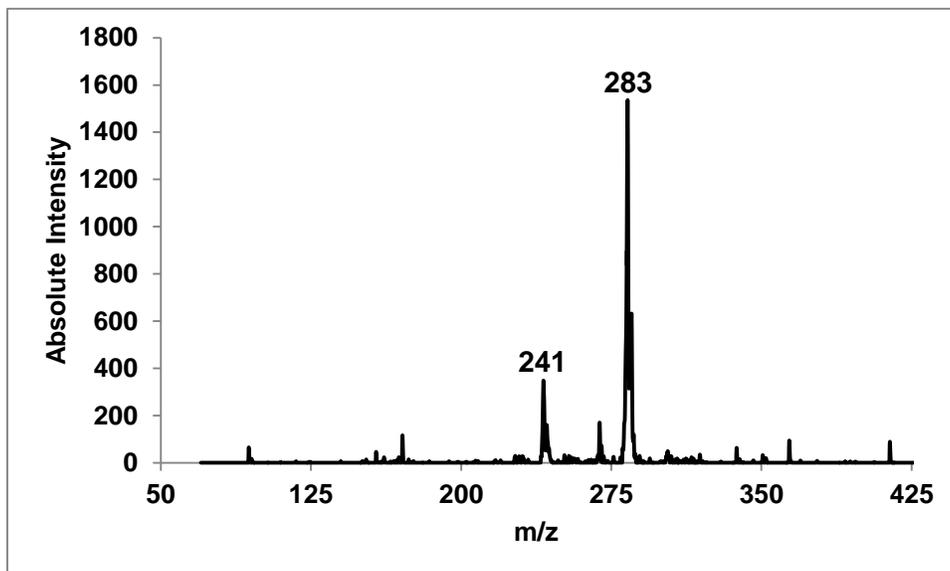
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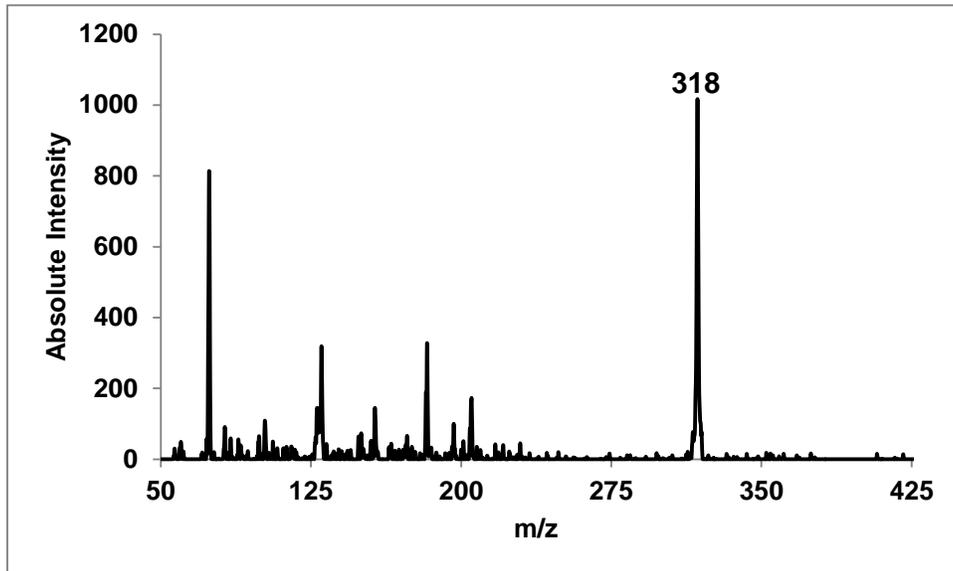
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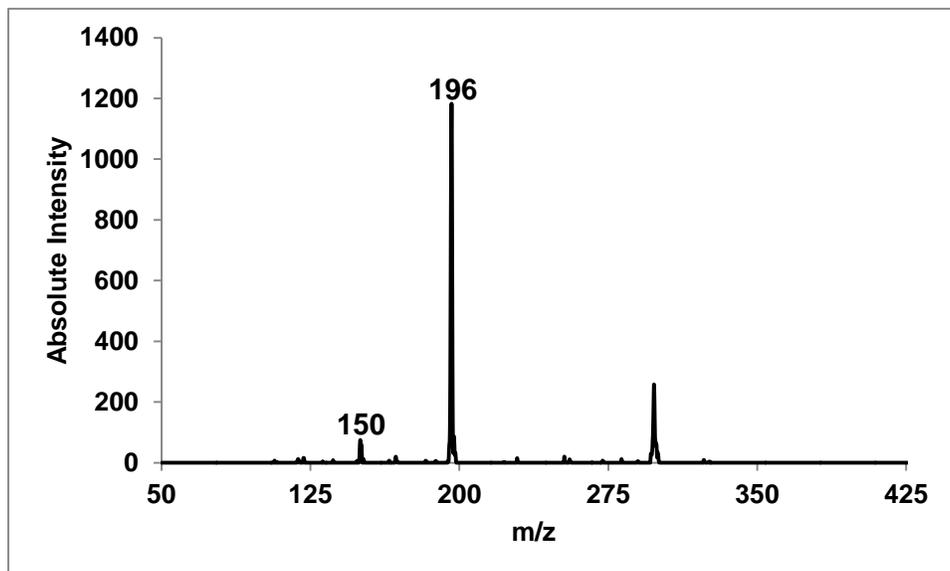
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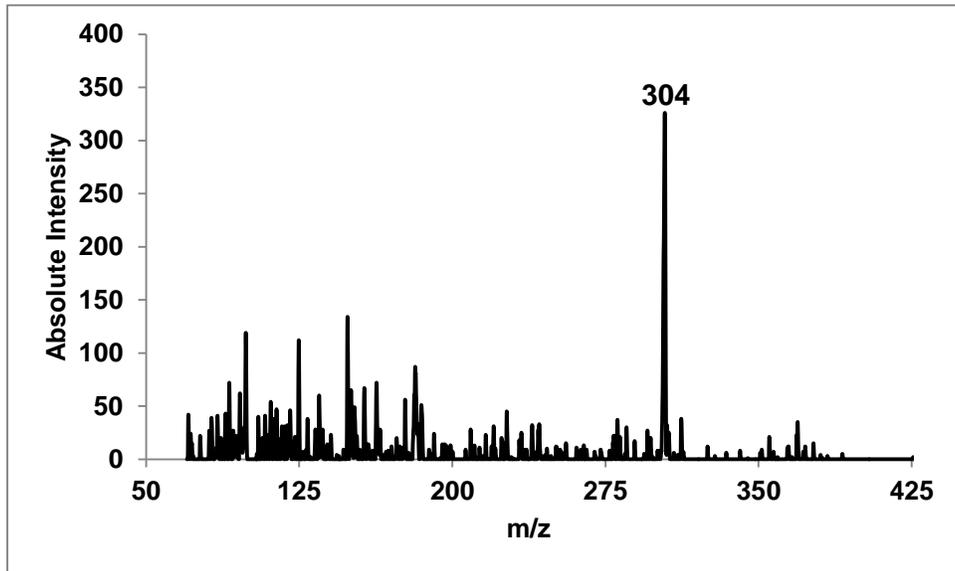
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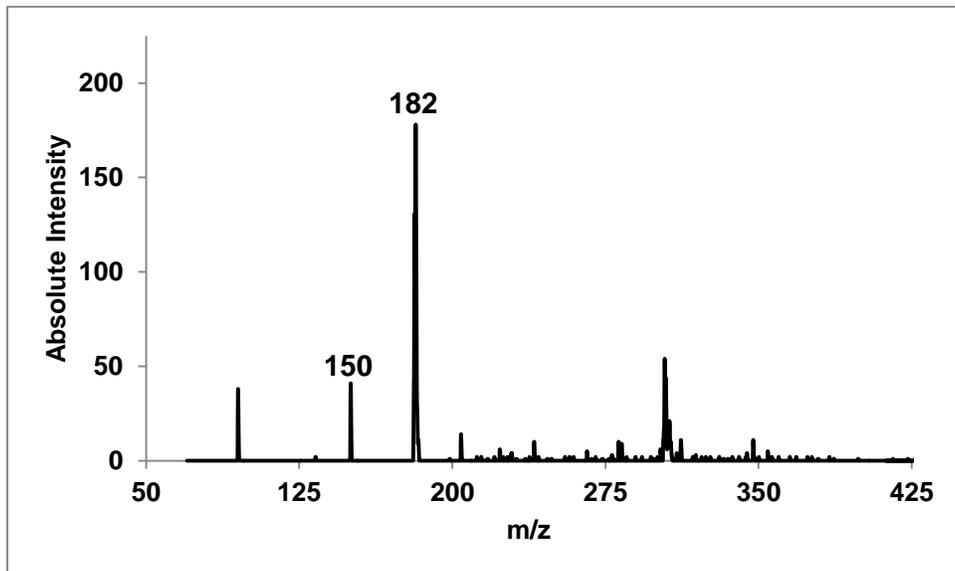
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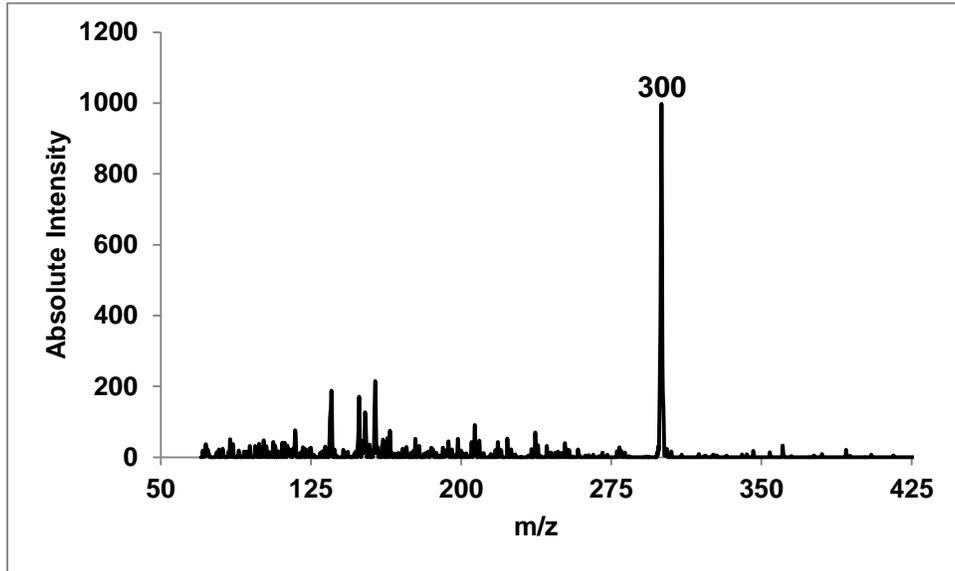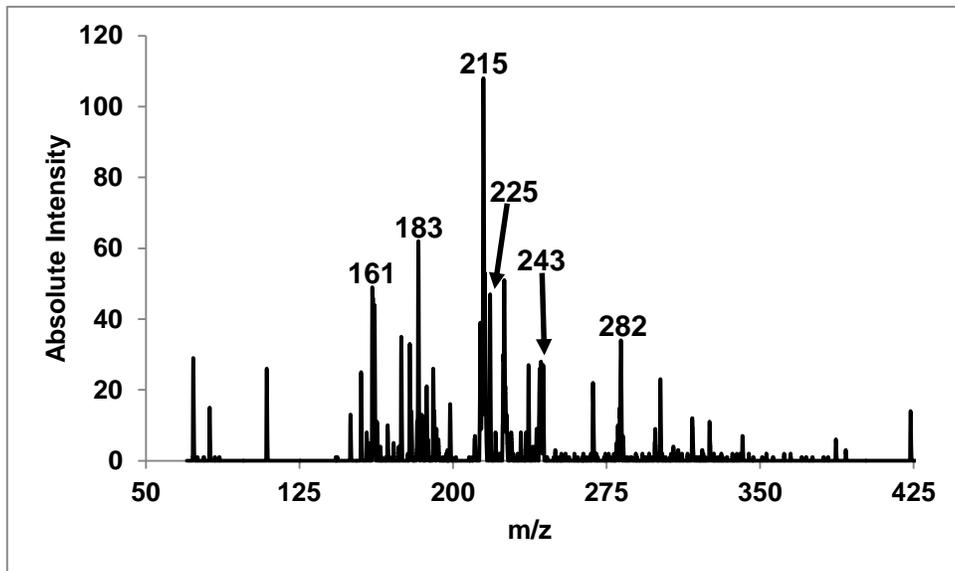
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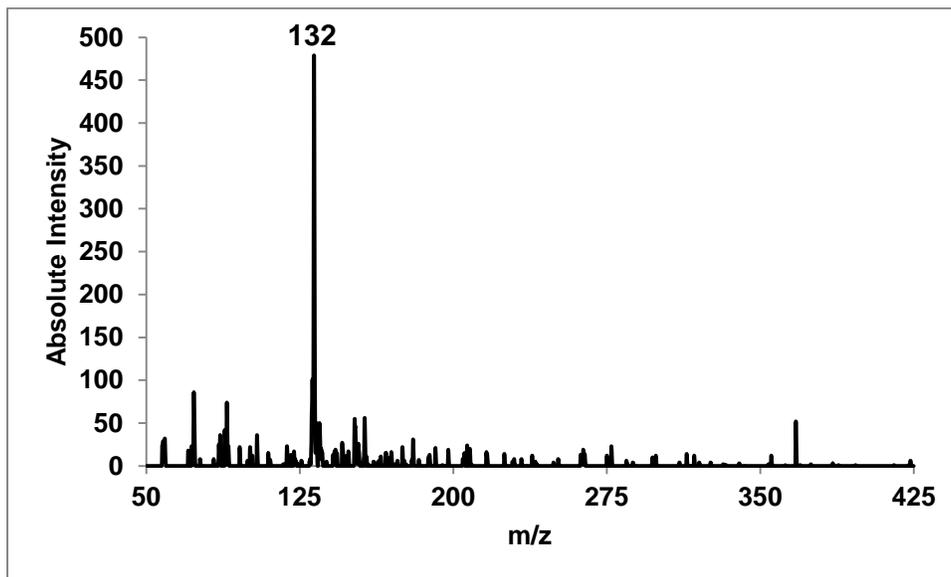
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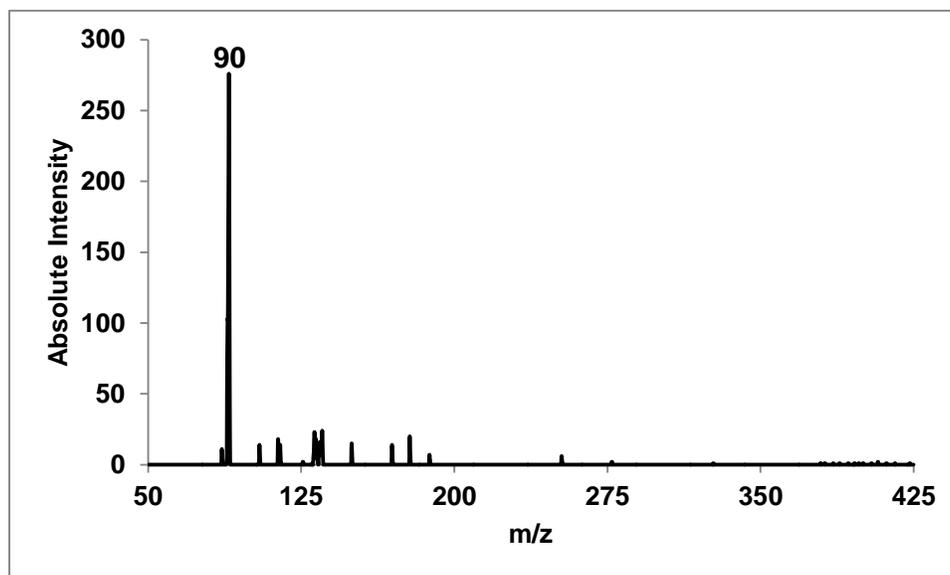
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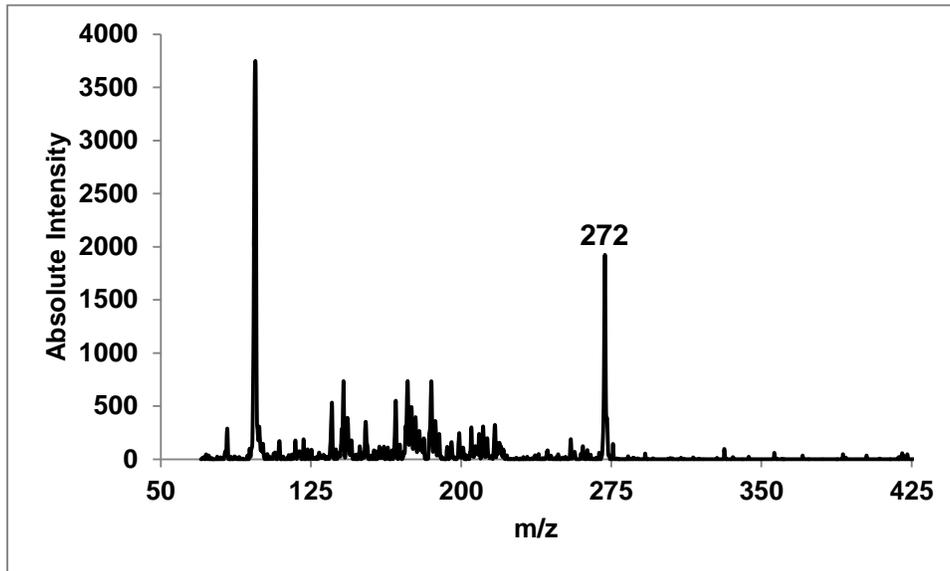
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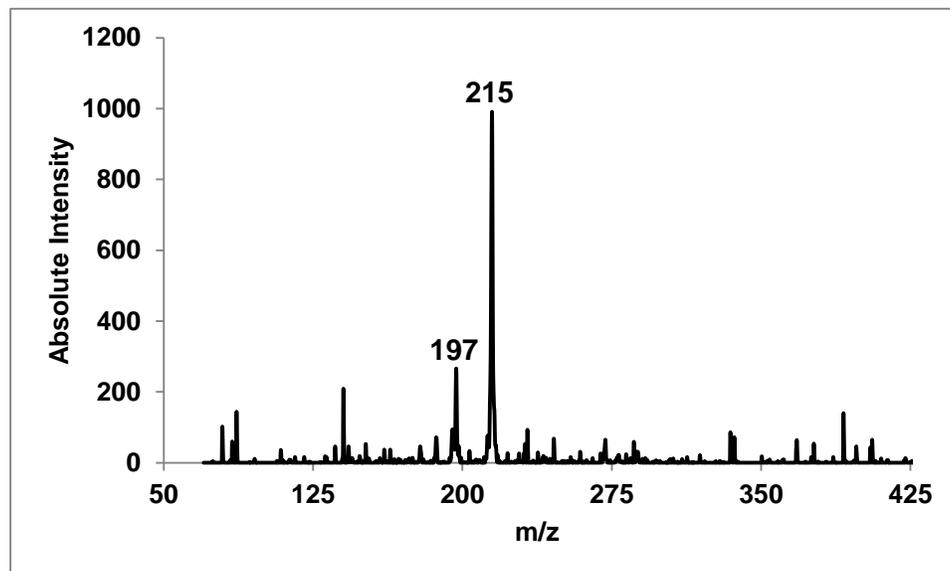
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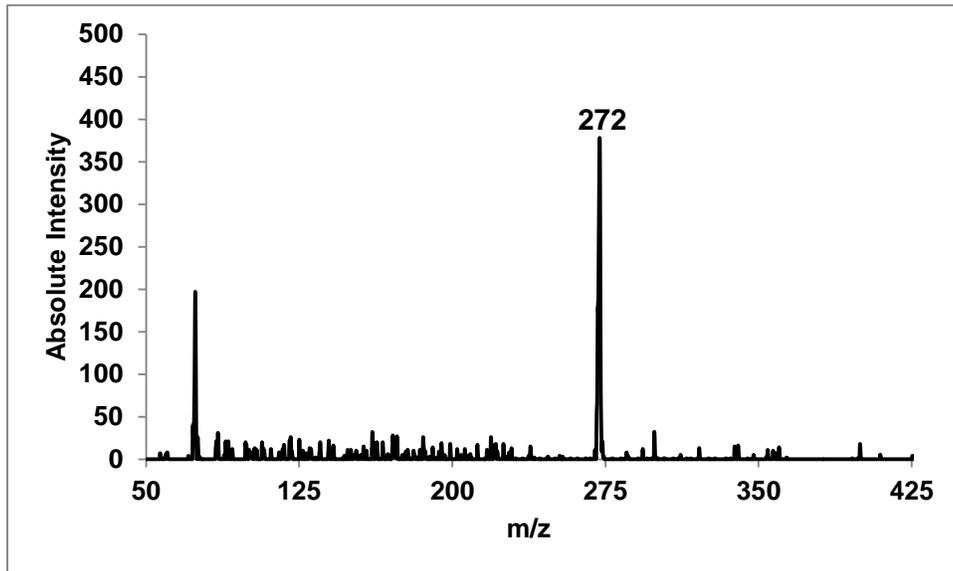
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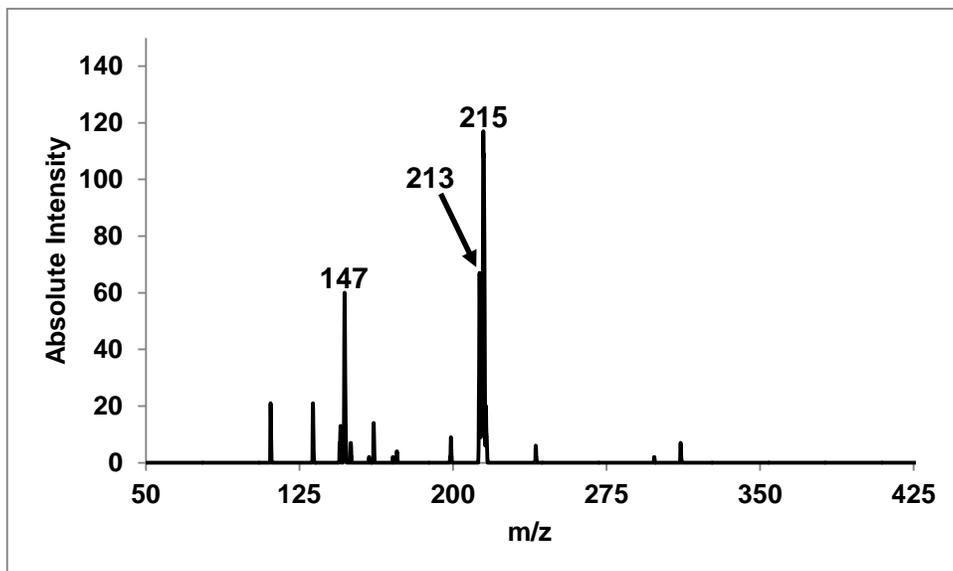
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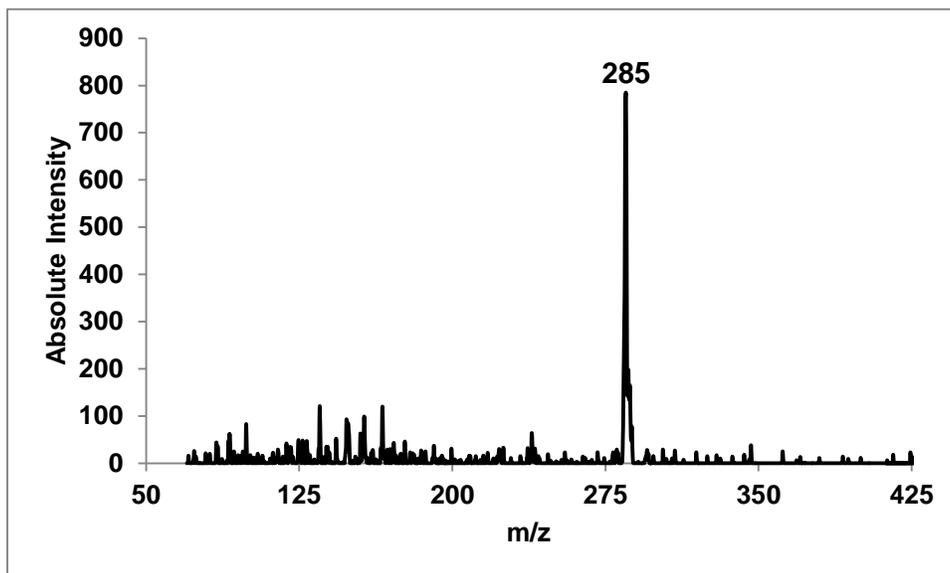
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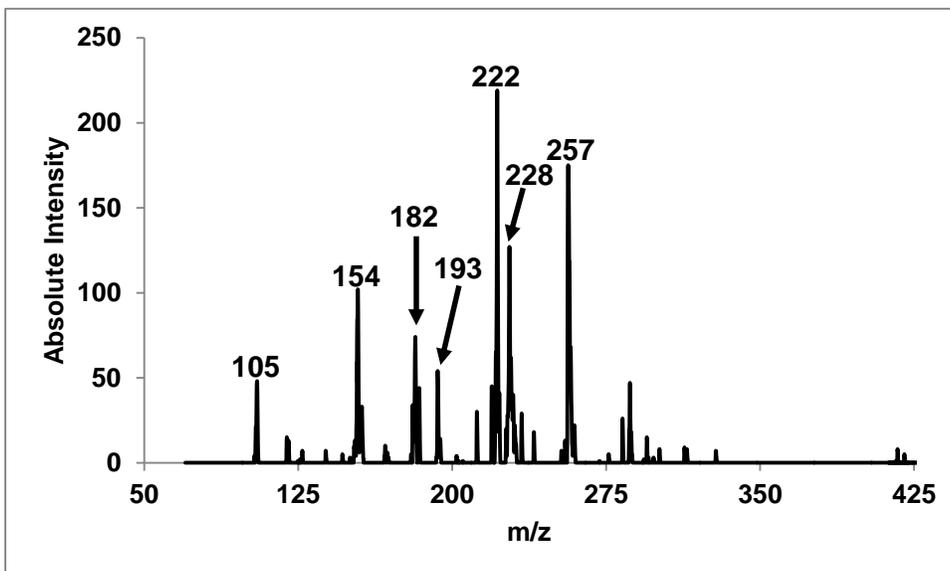
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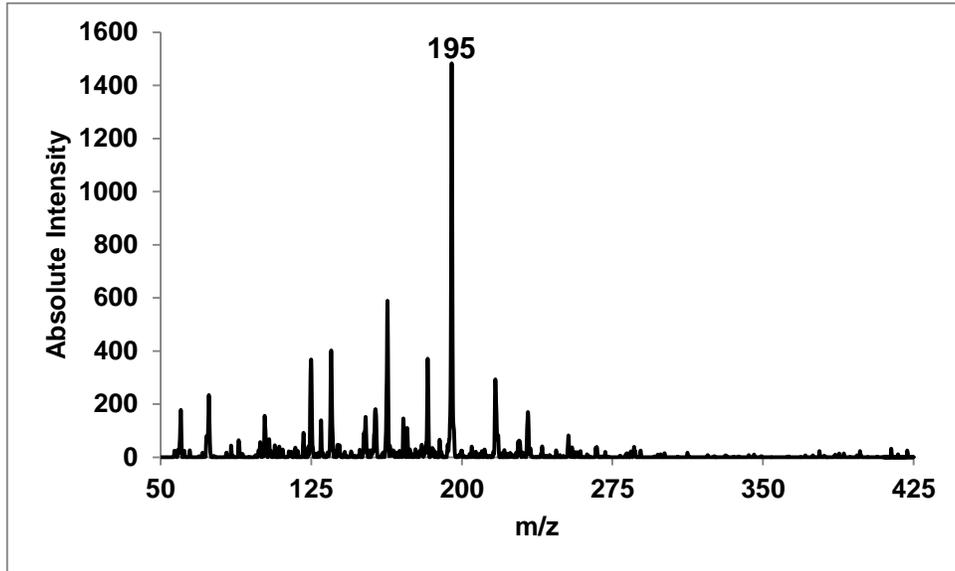
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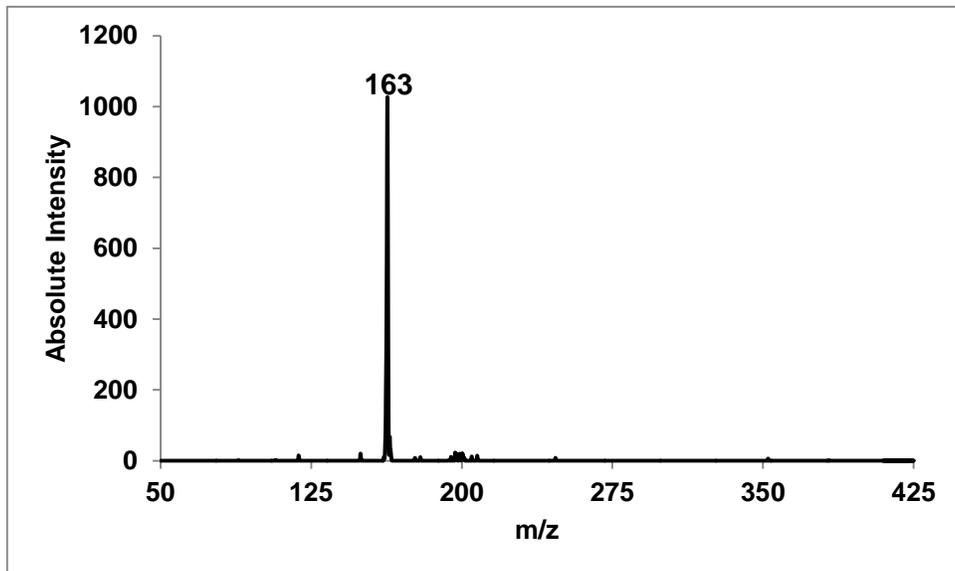
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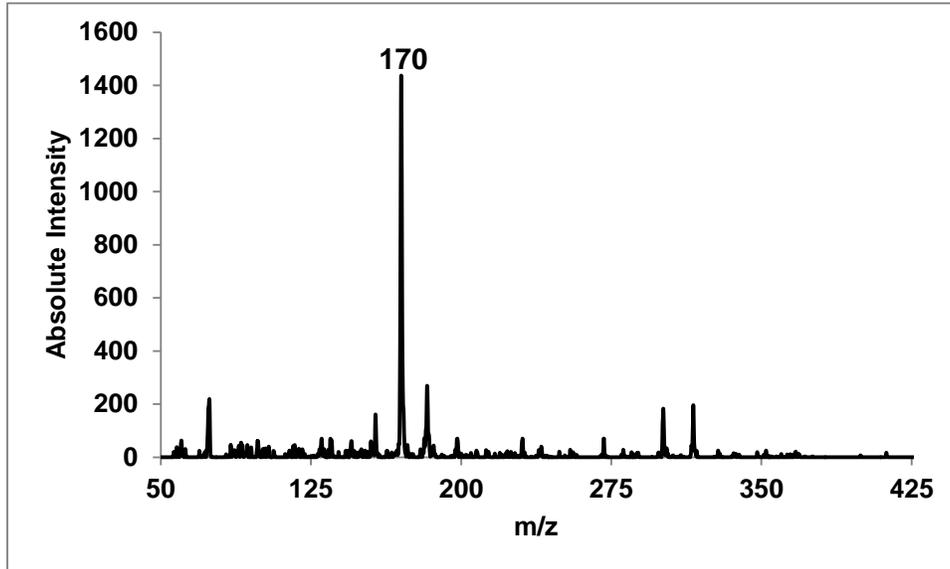
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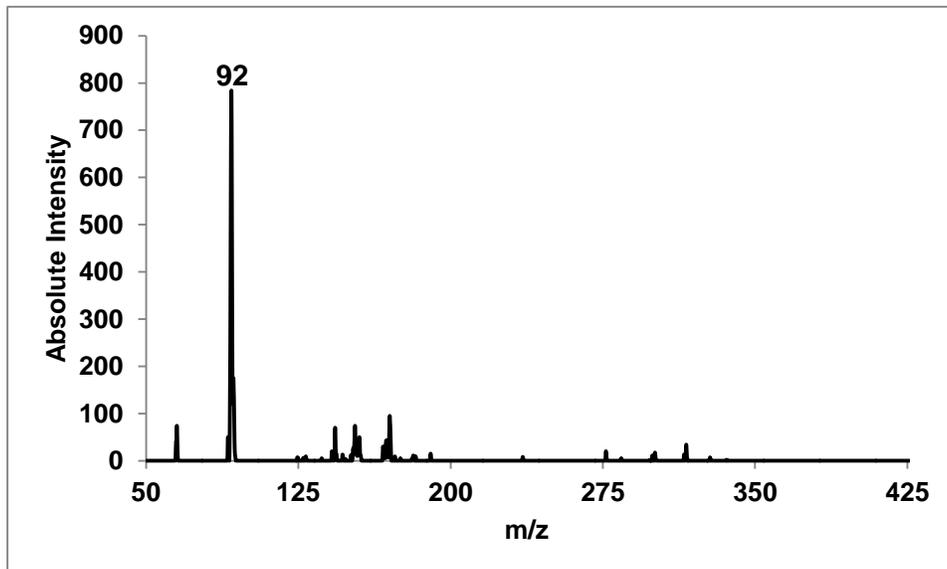
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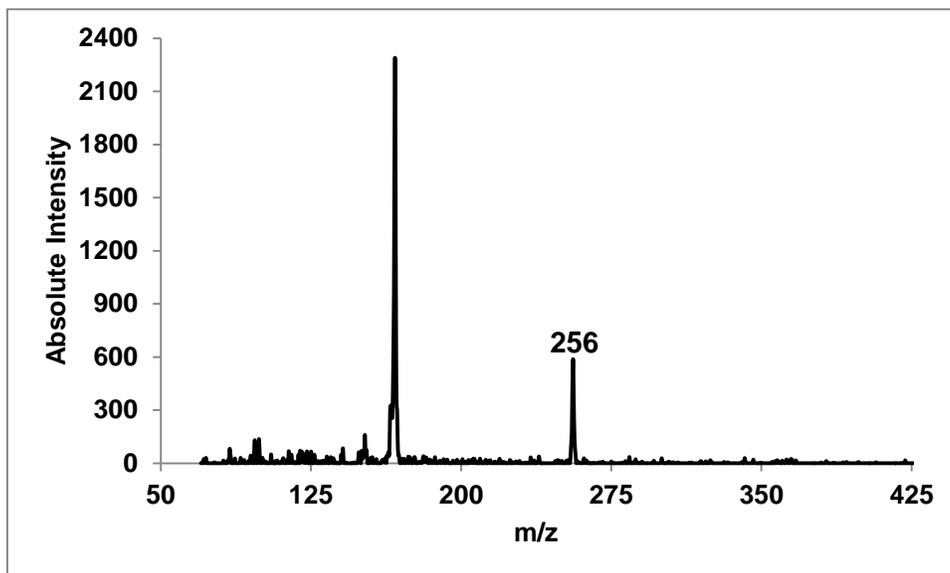
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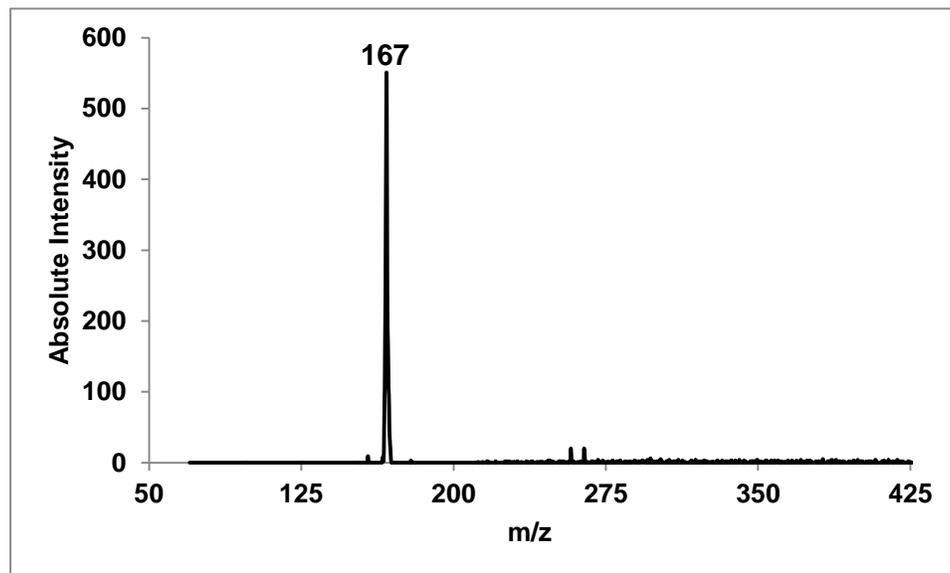
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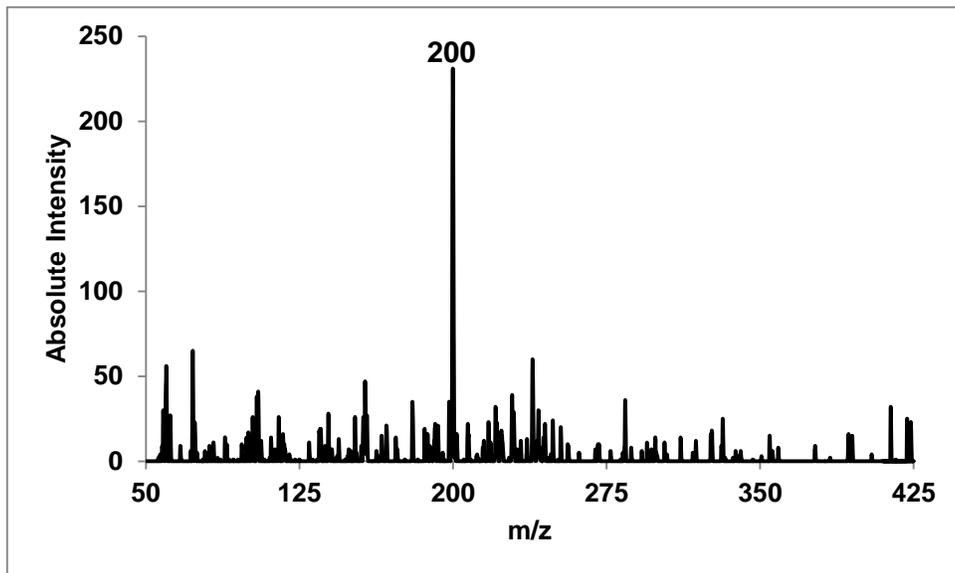
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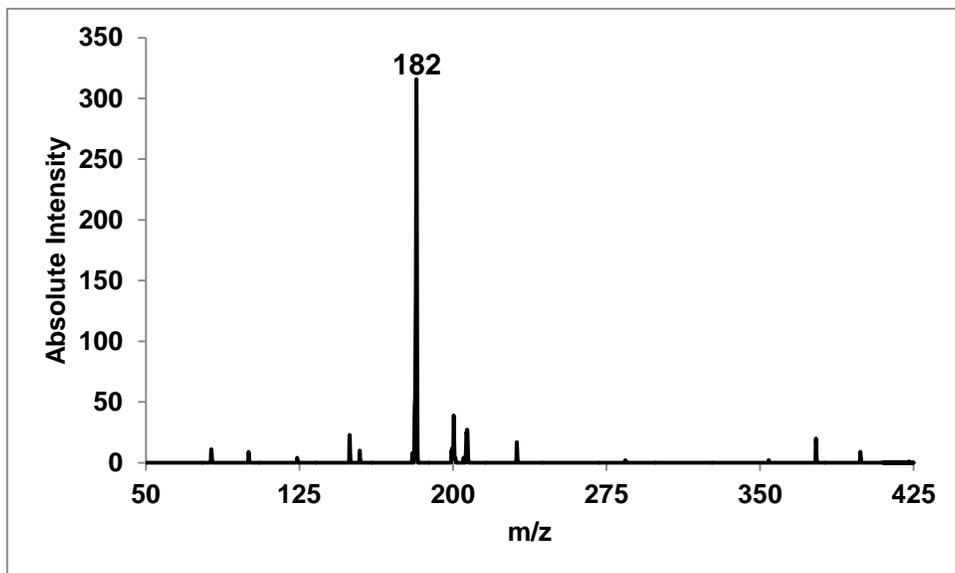
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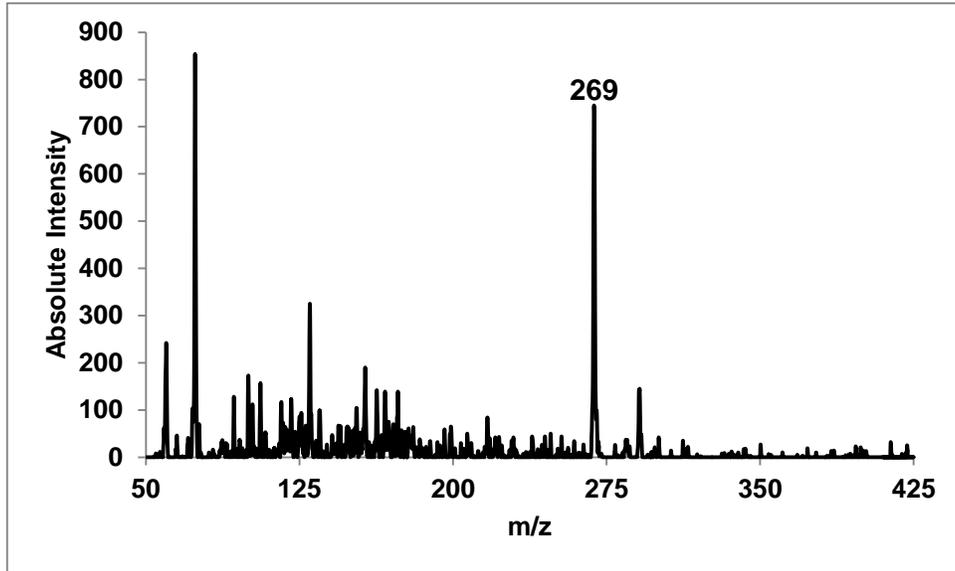
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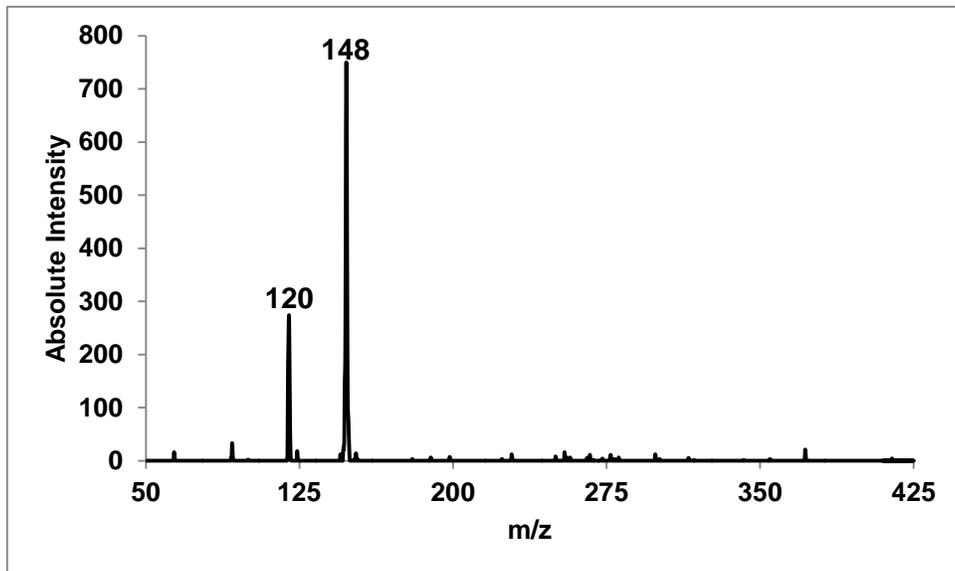
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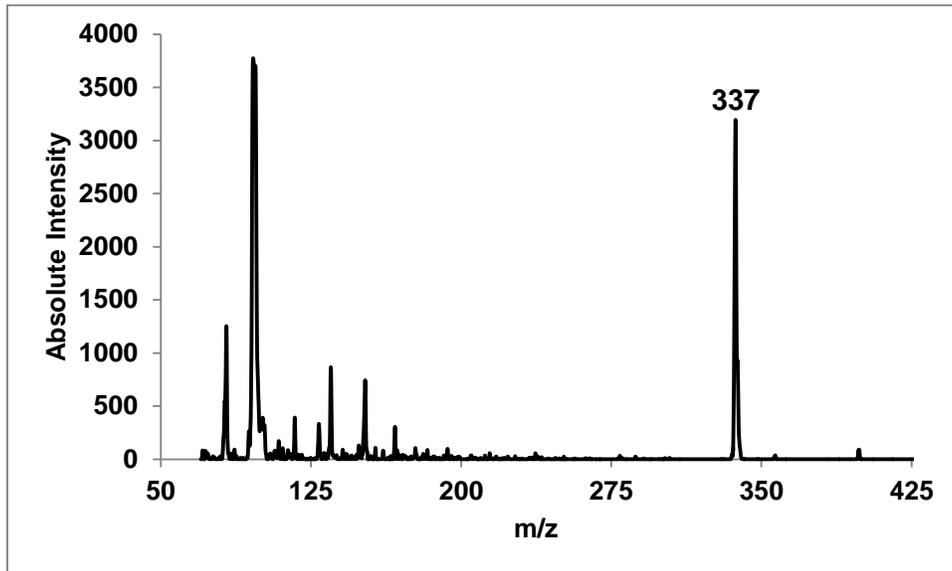
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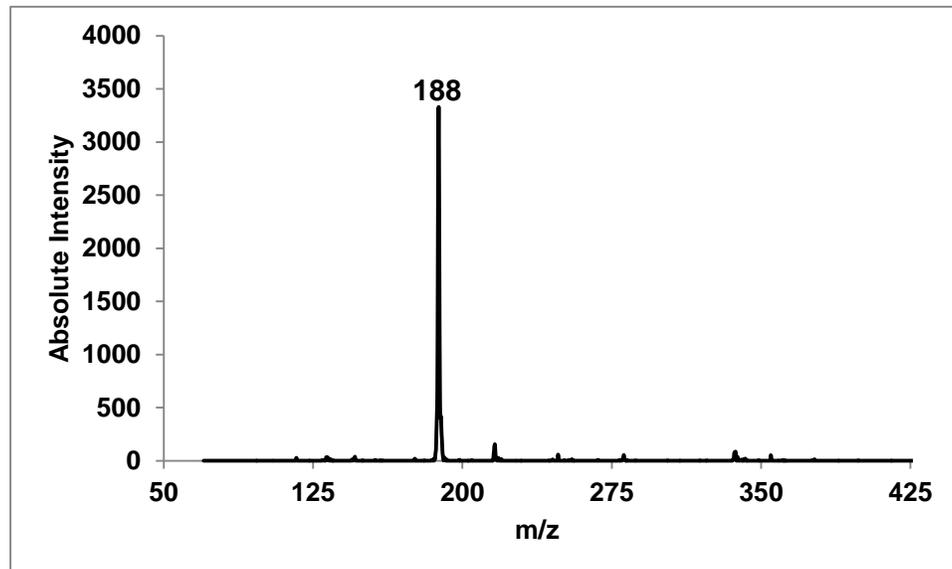
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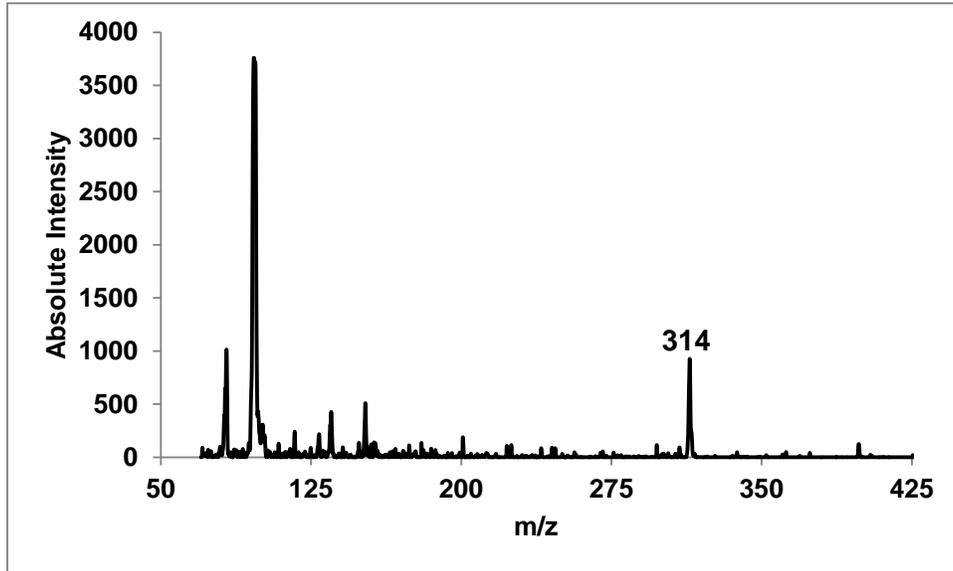
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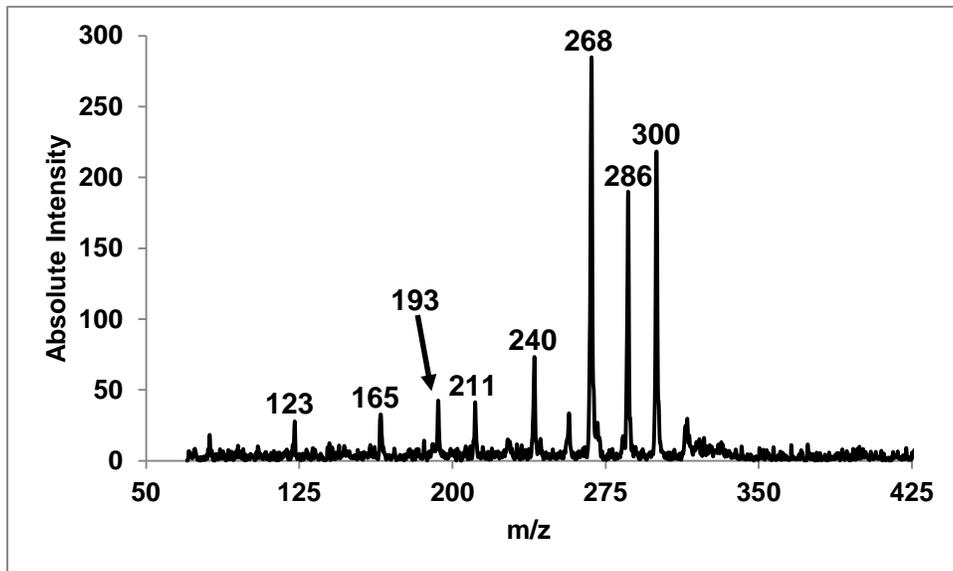
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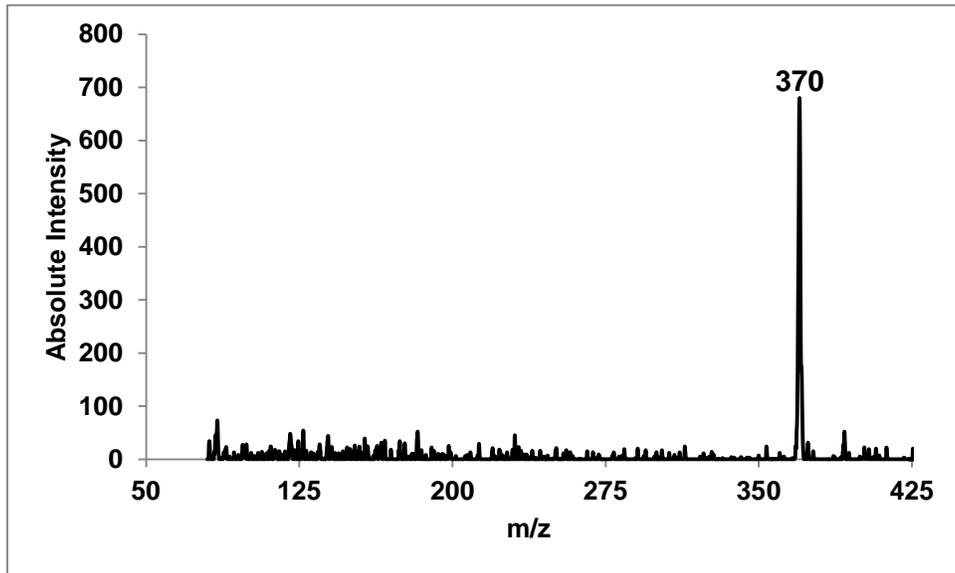
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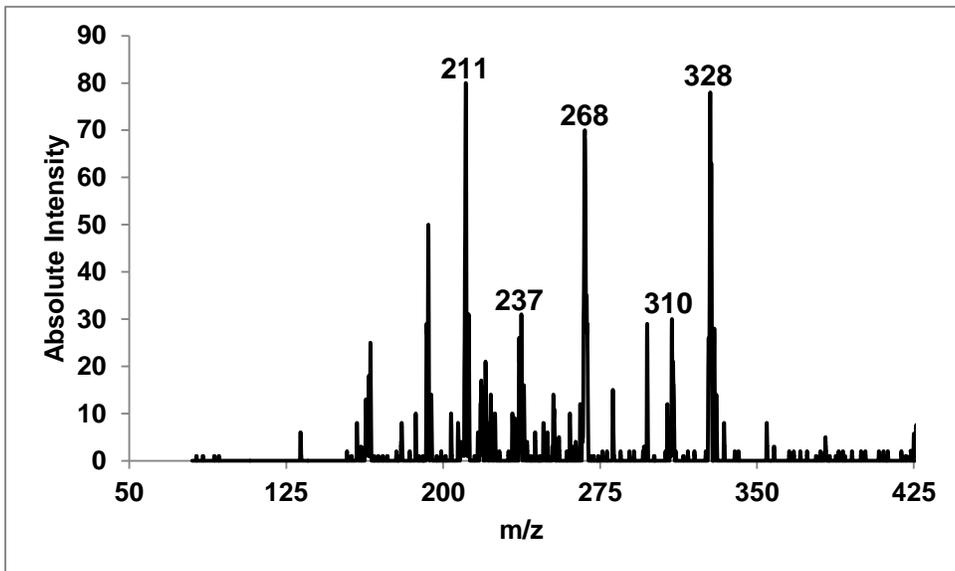
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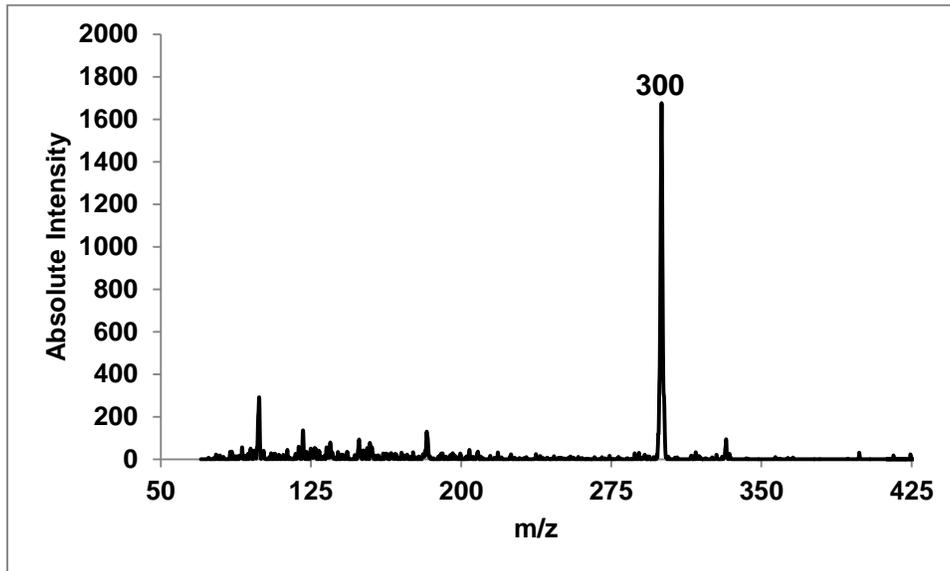
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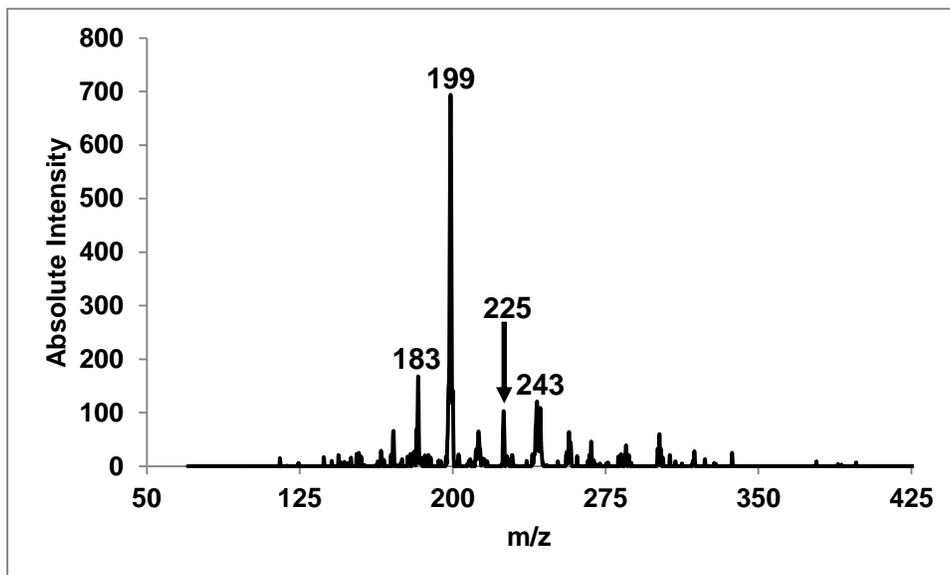
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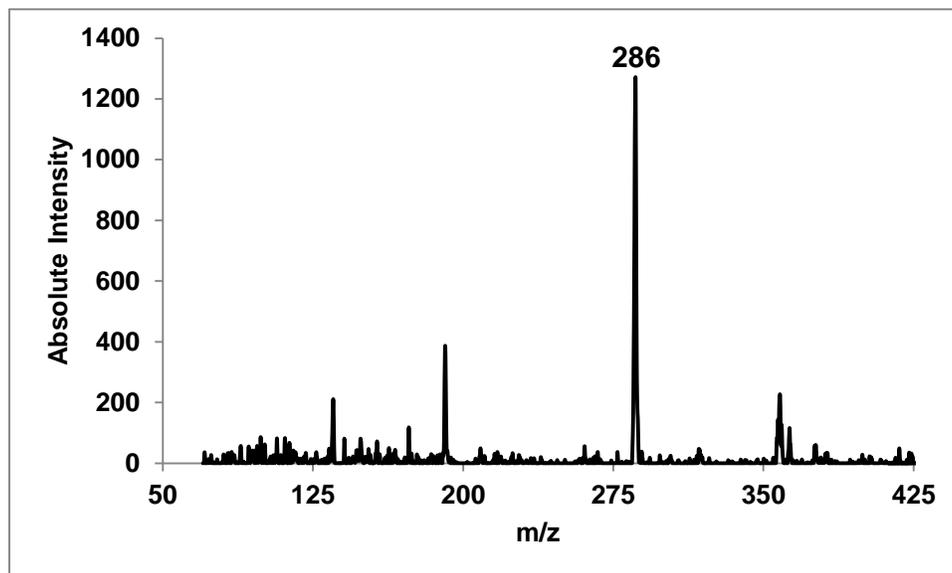
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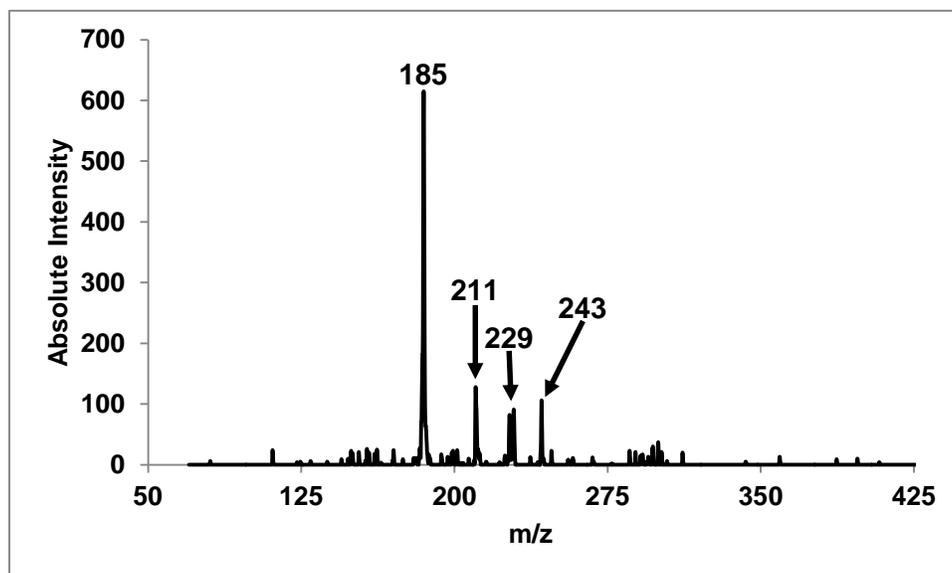
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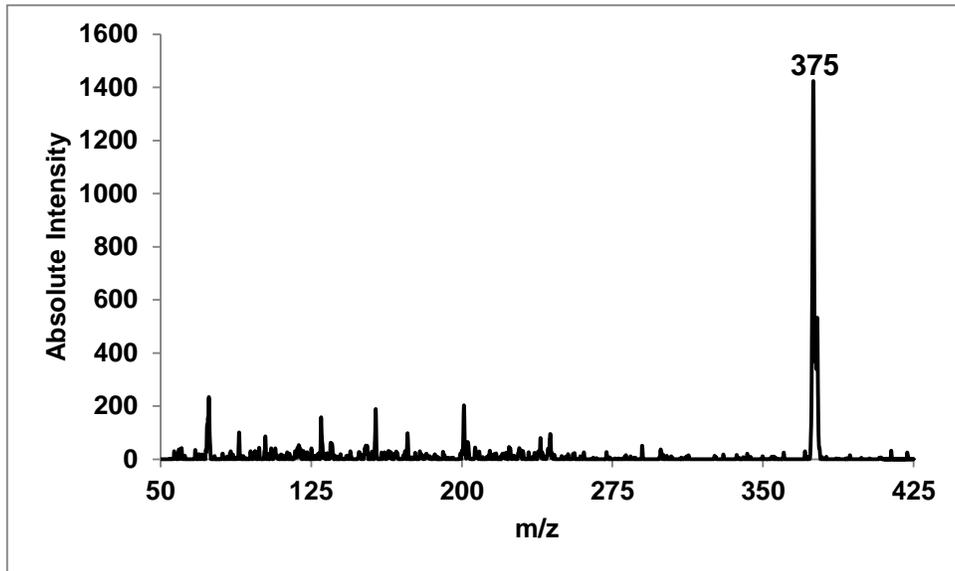
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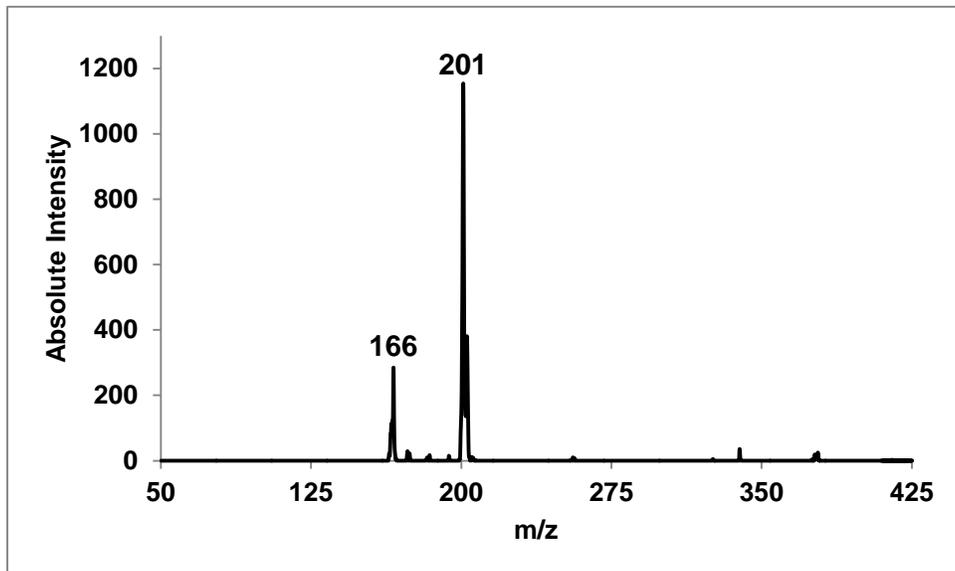
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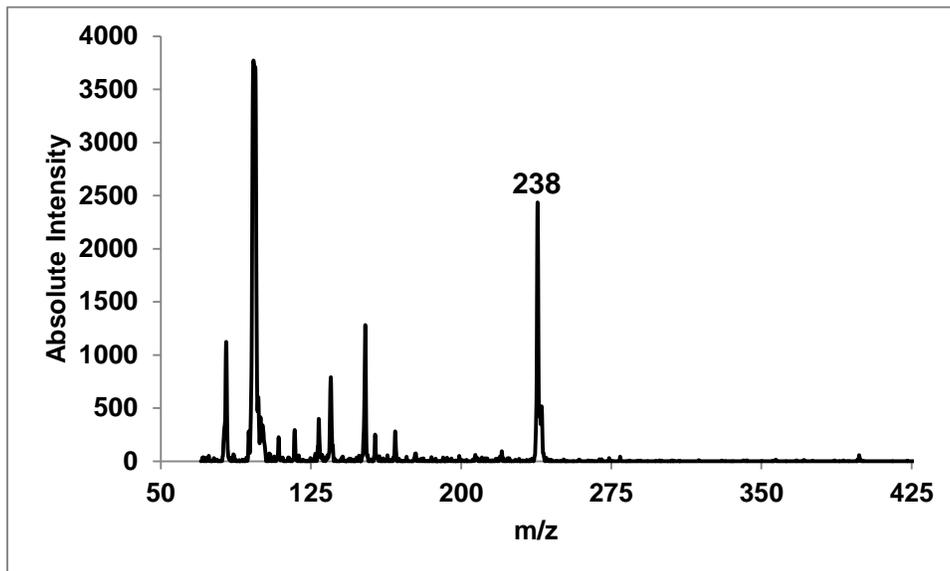
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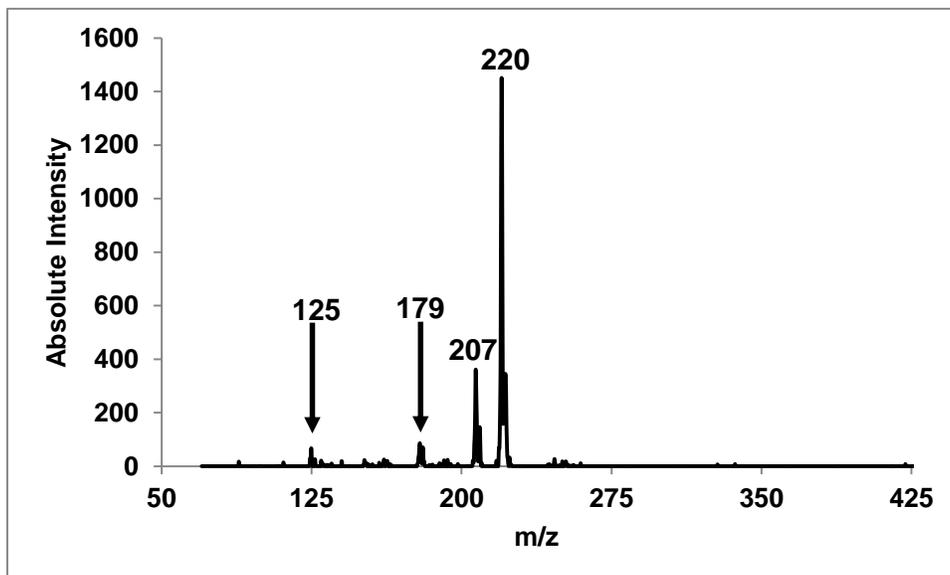
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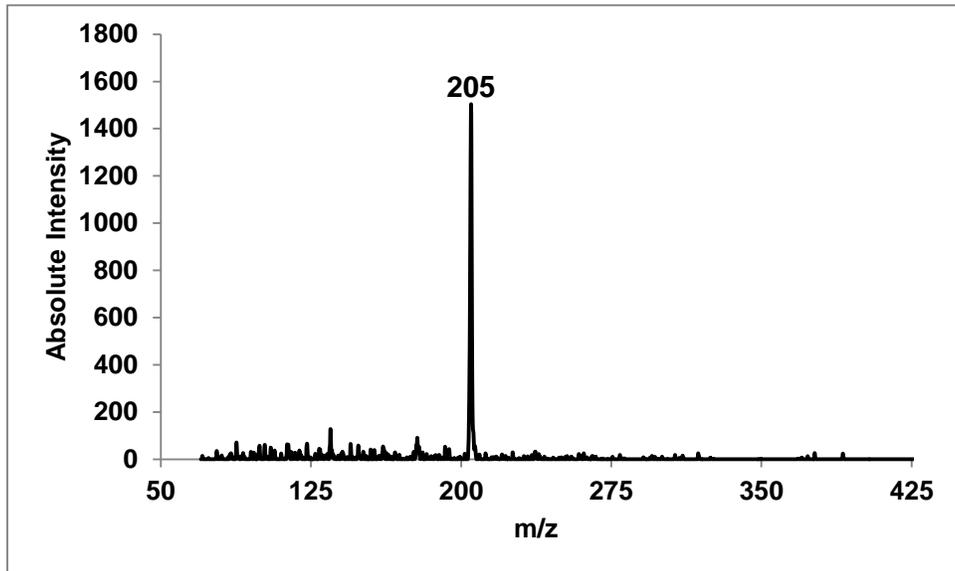
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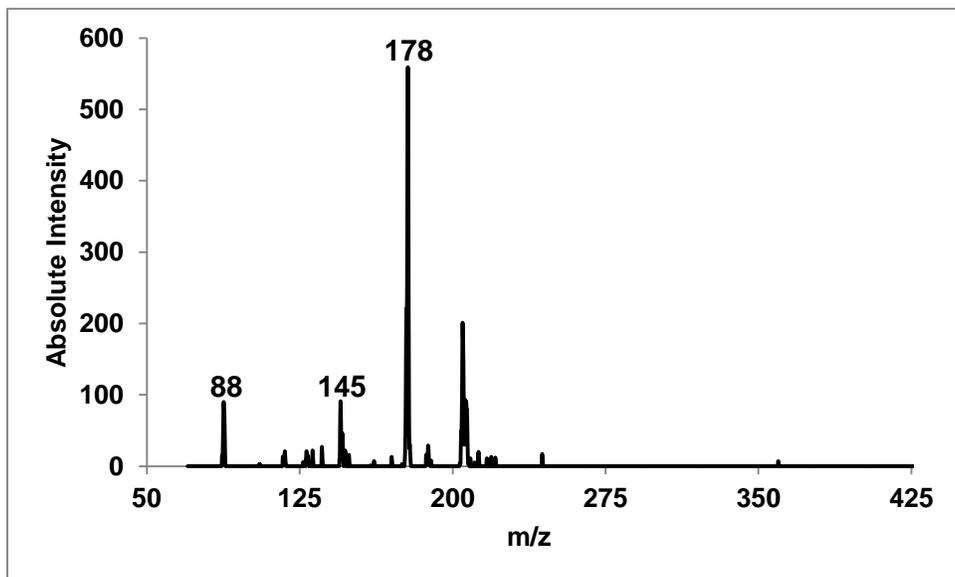
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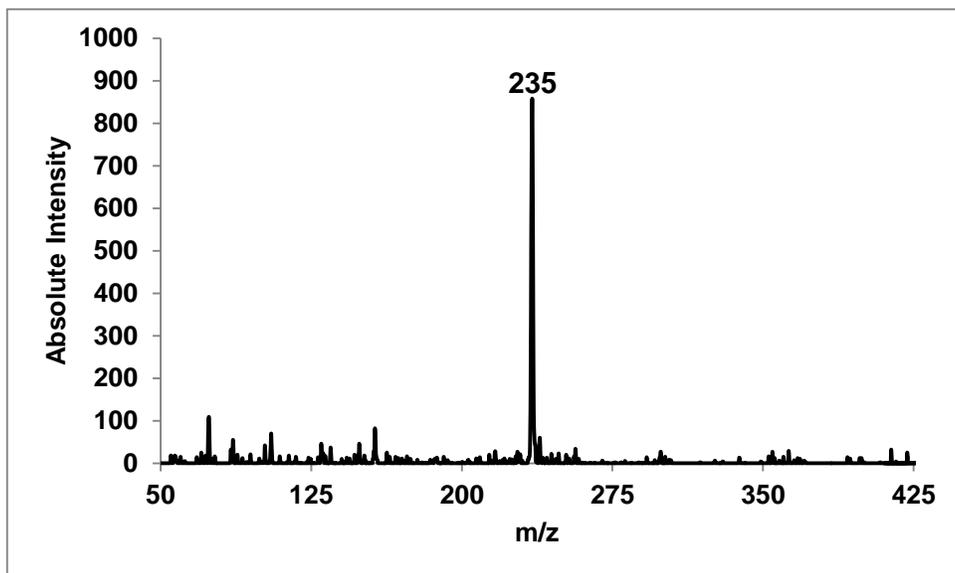
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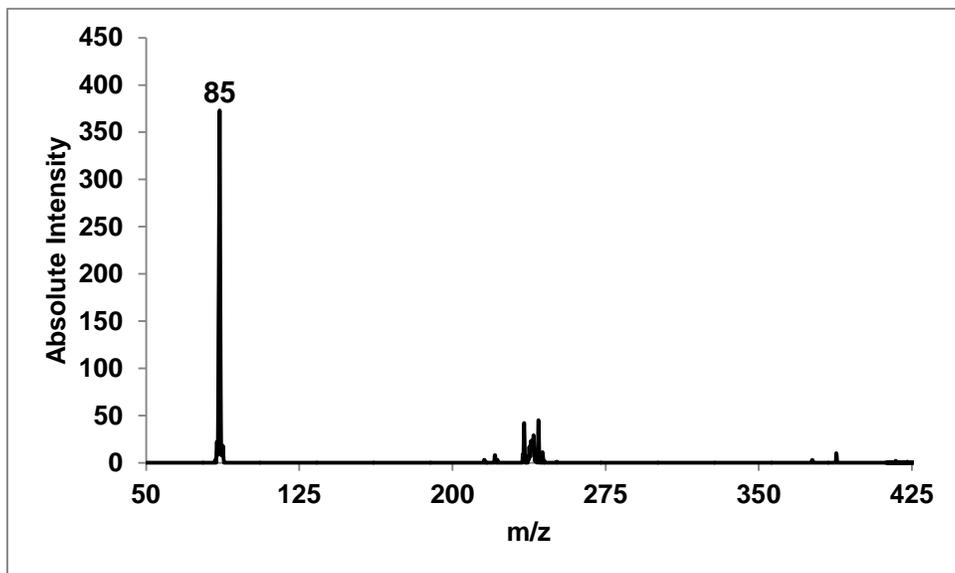
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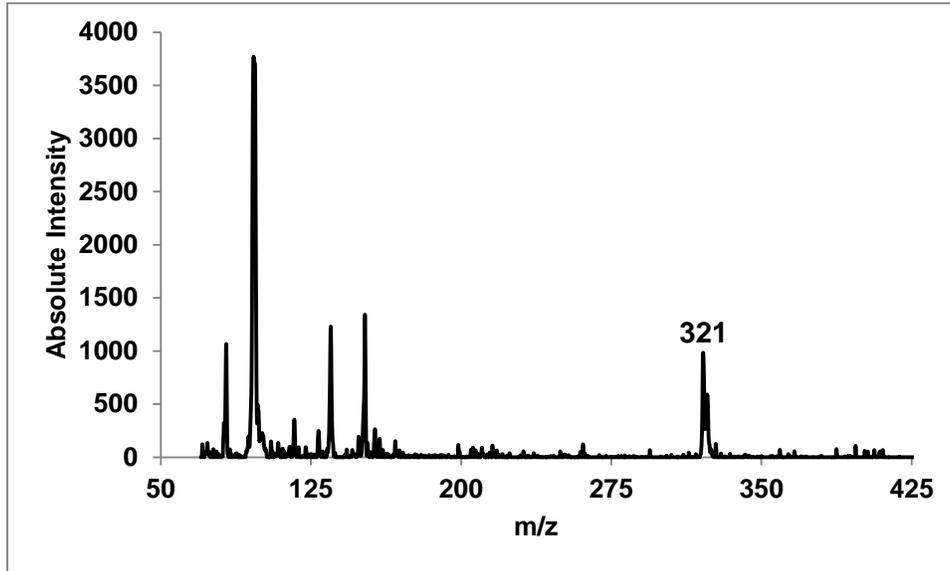
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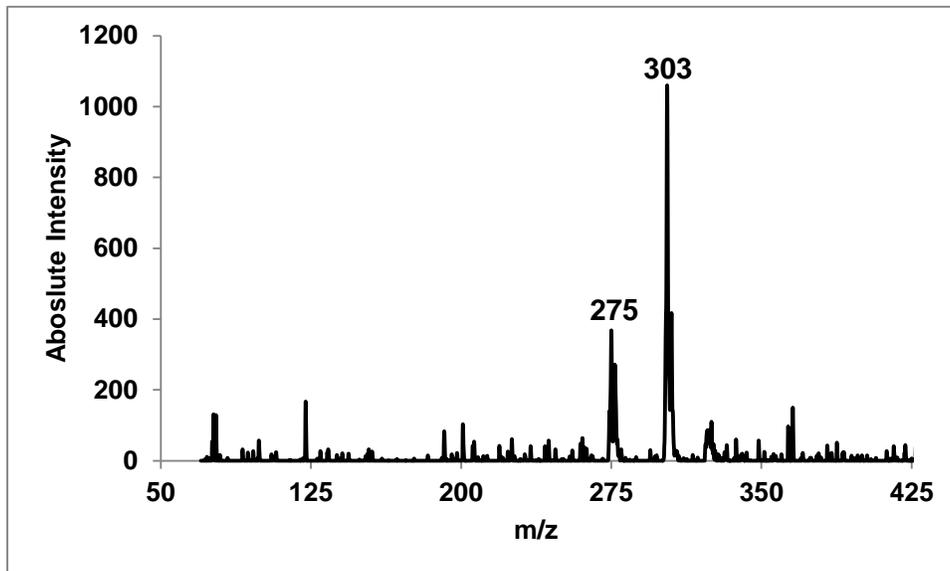
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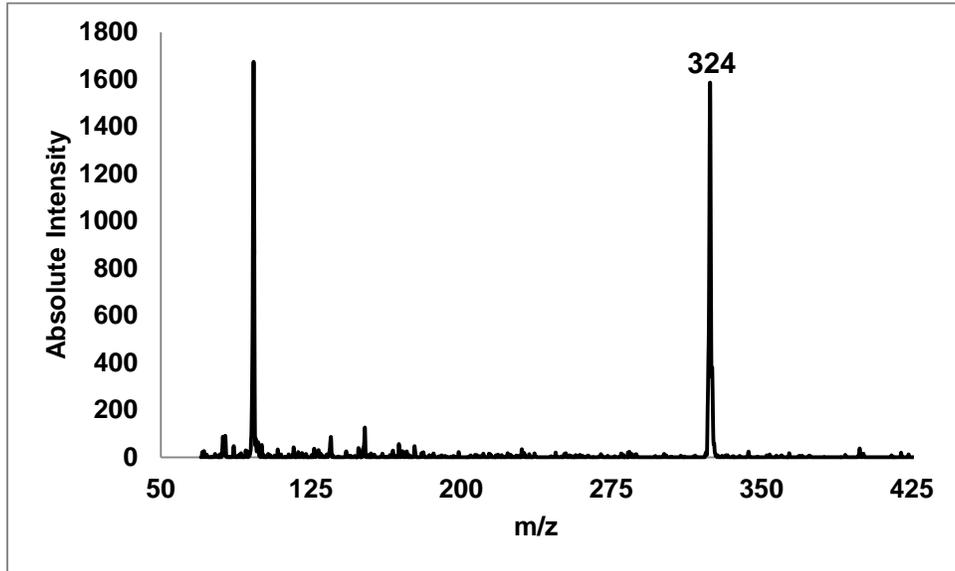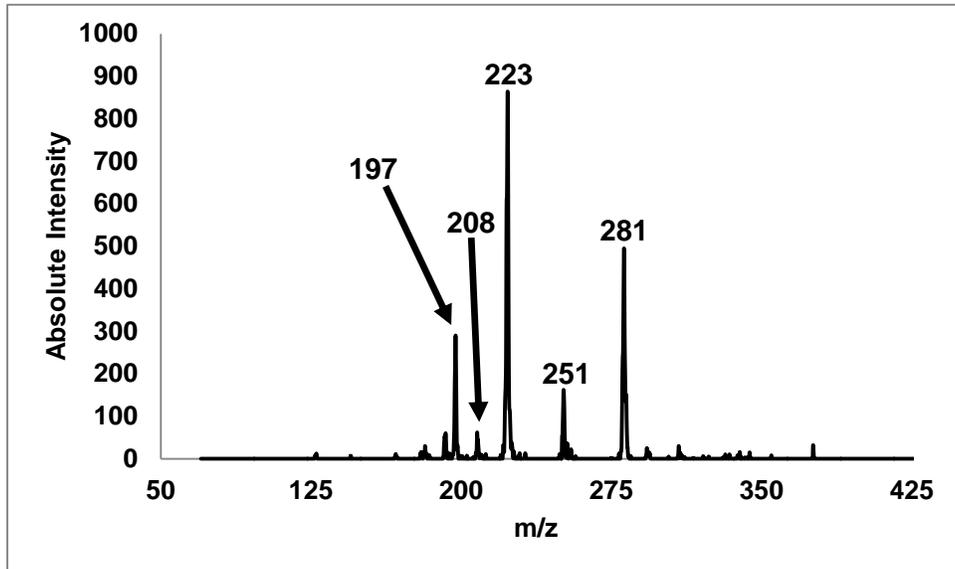
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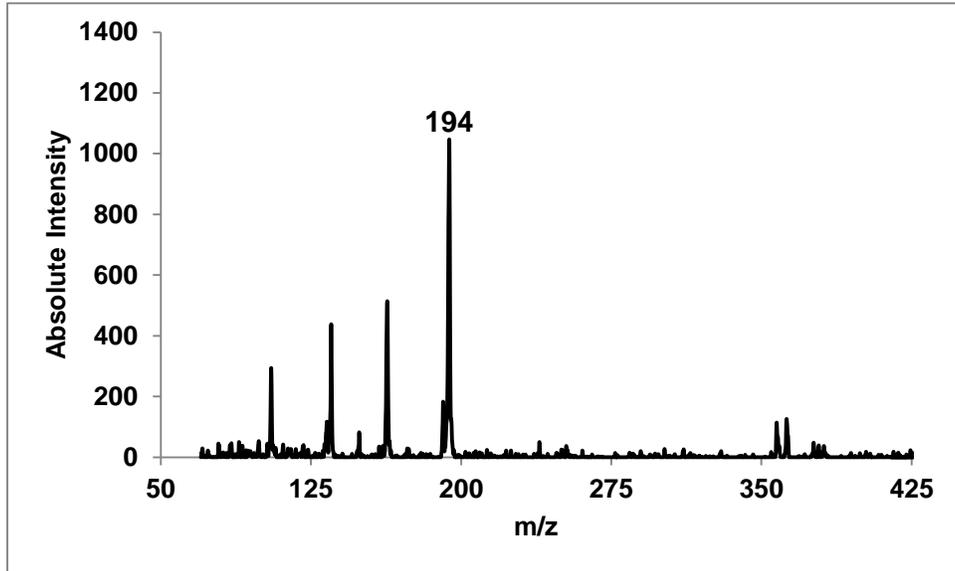
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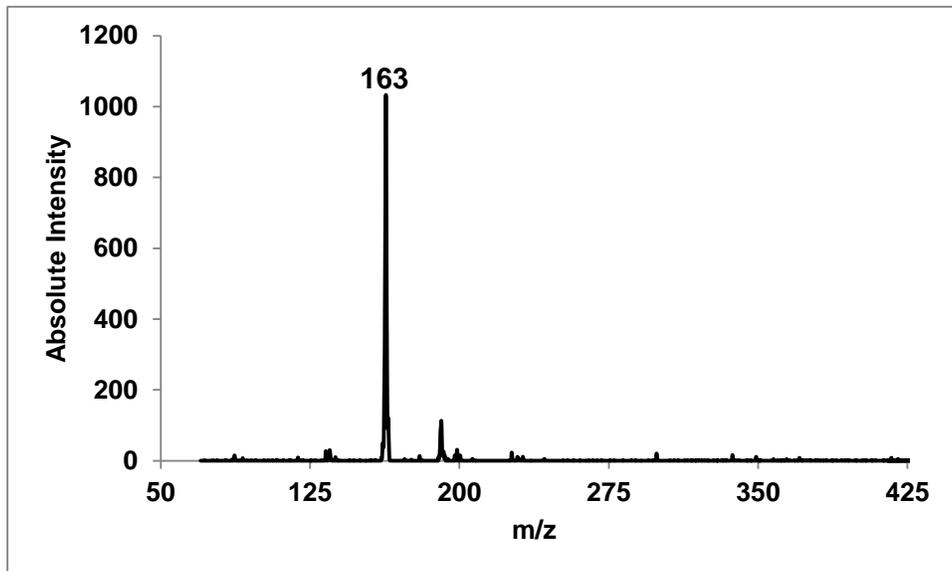
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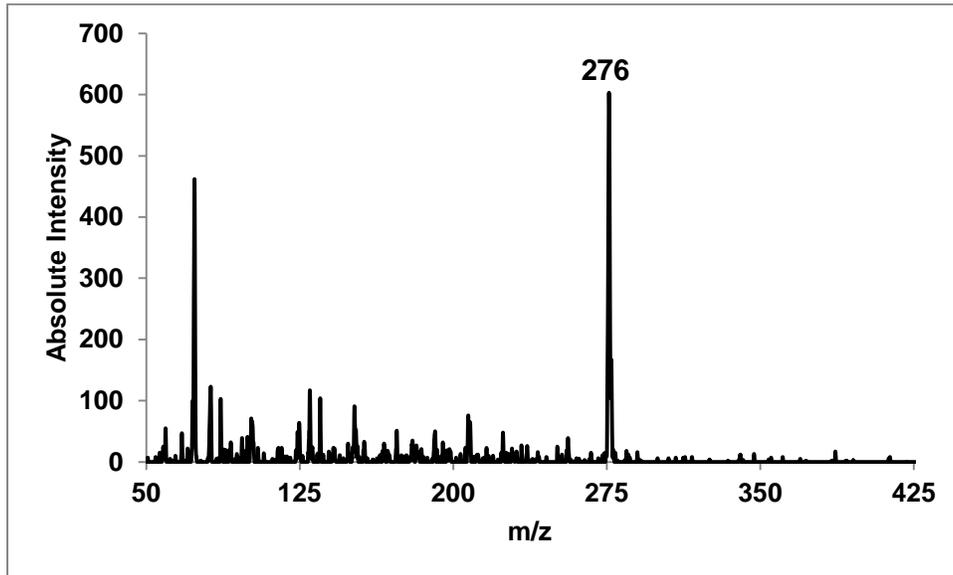
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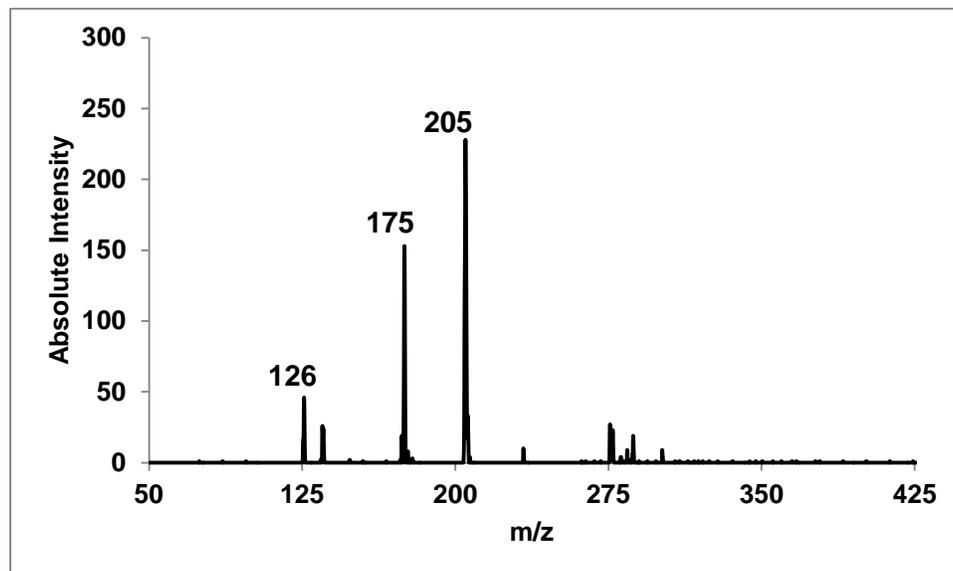
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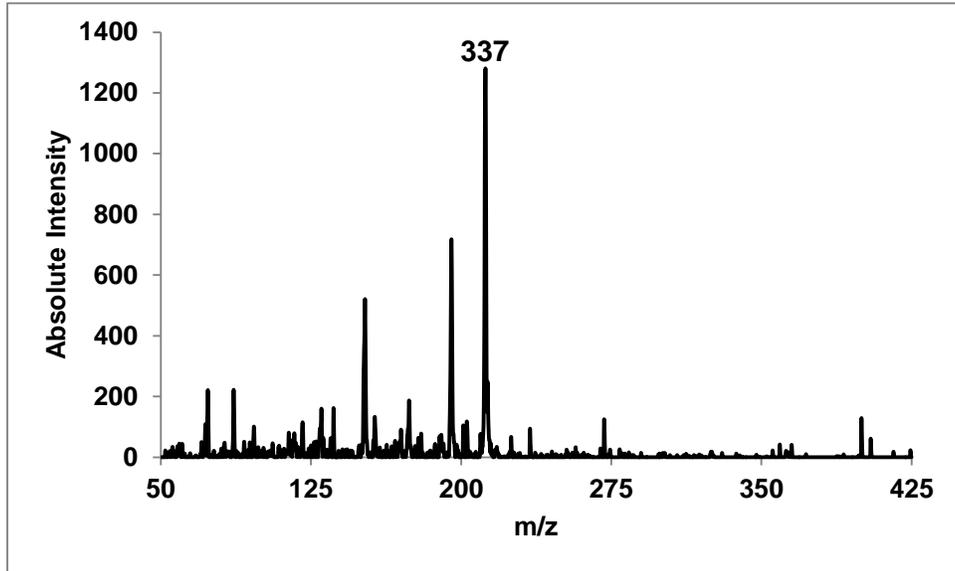
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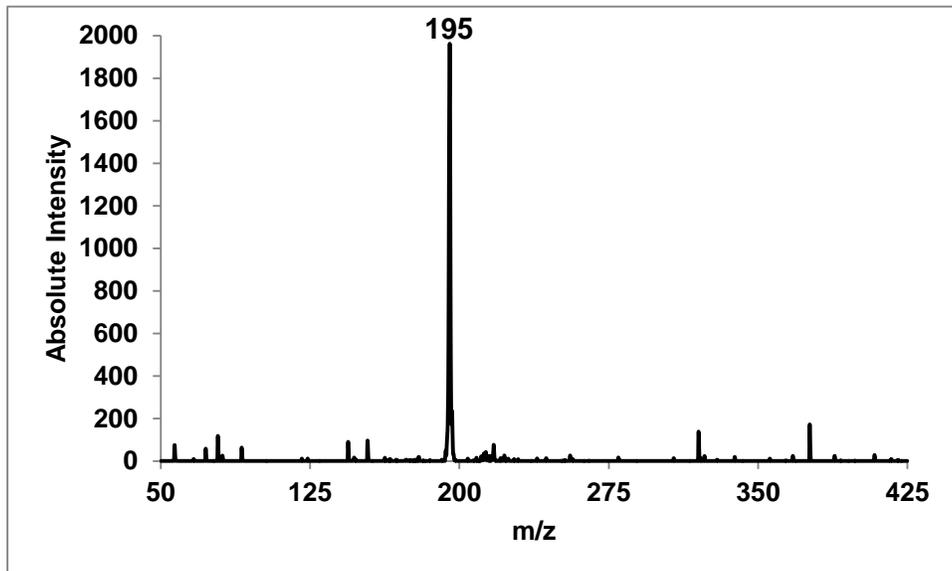
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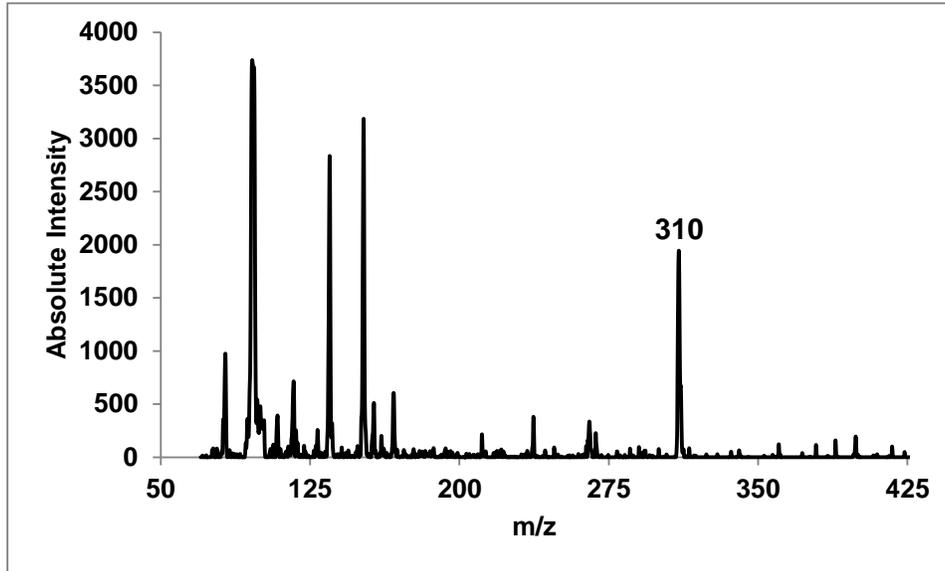
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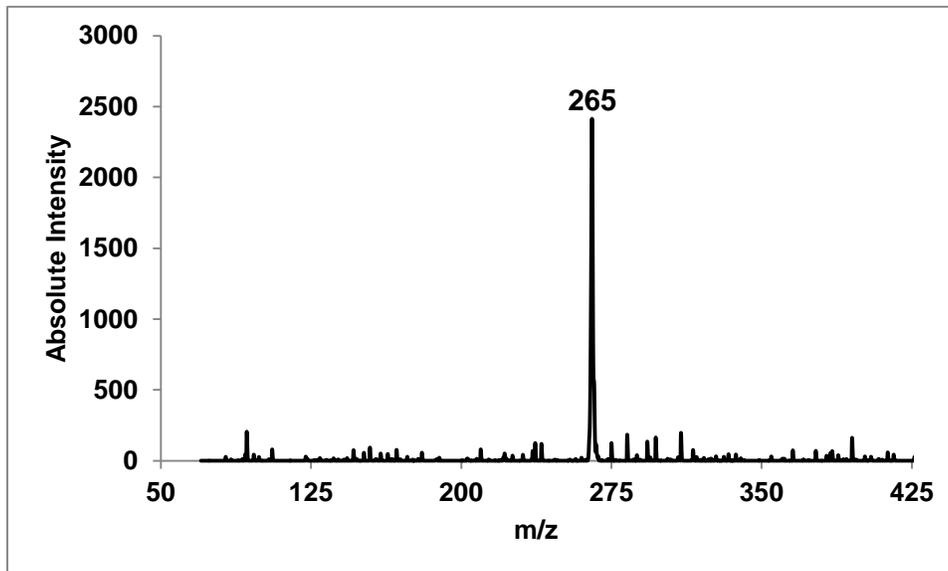
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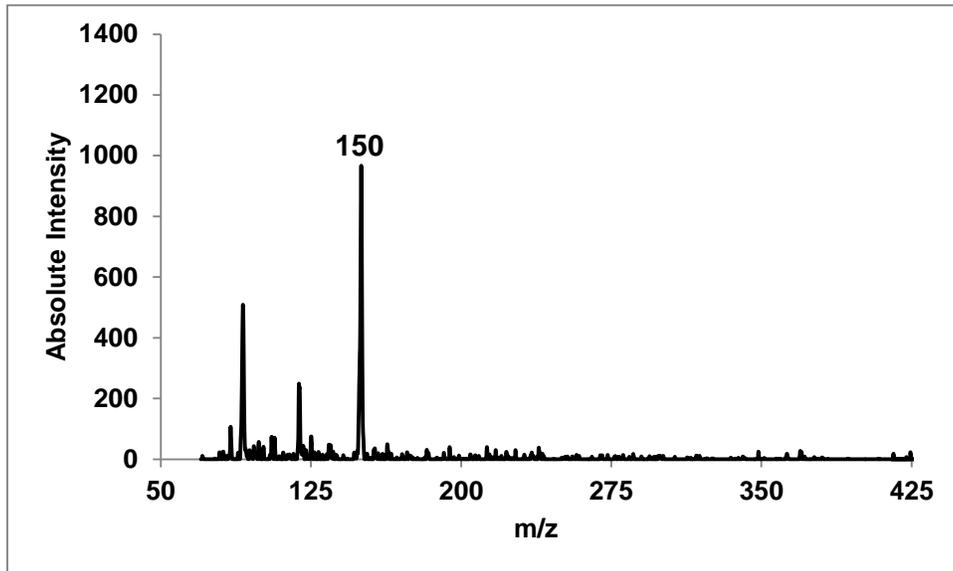
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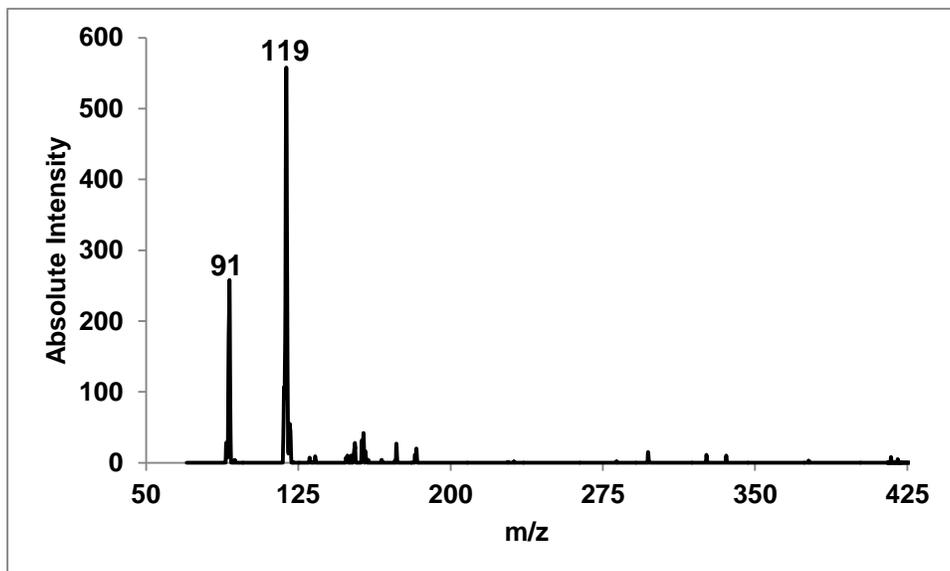
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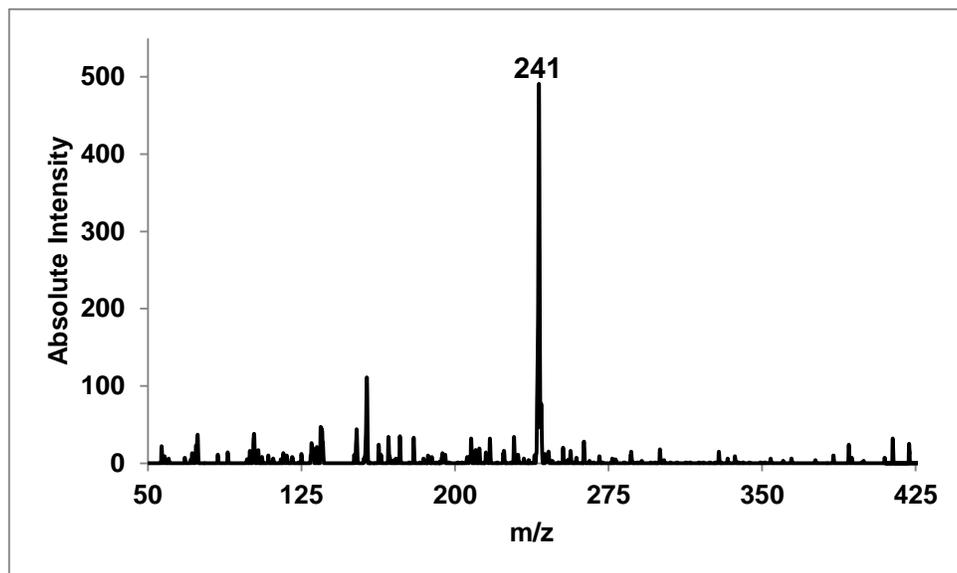
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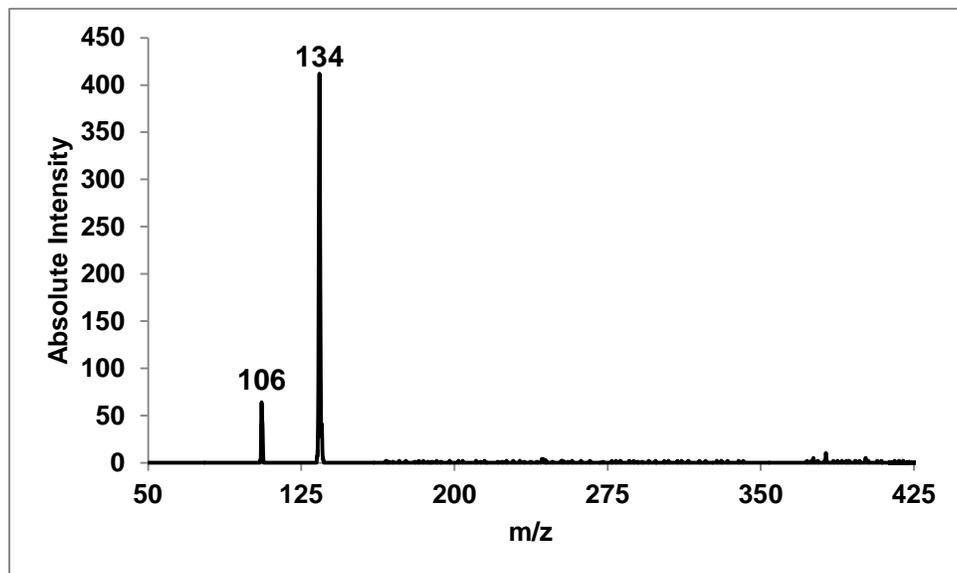
A-37. Methylphenidate

A-37.1. Methylphenidate MS Scan
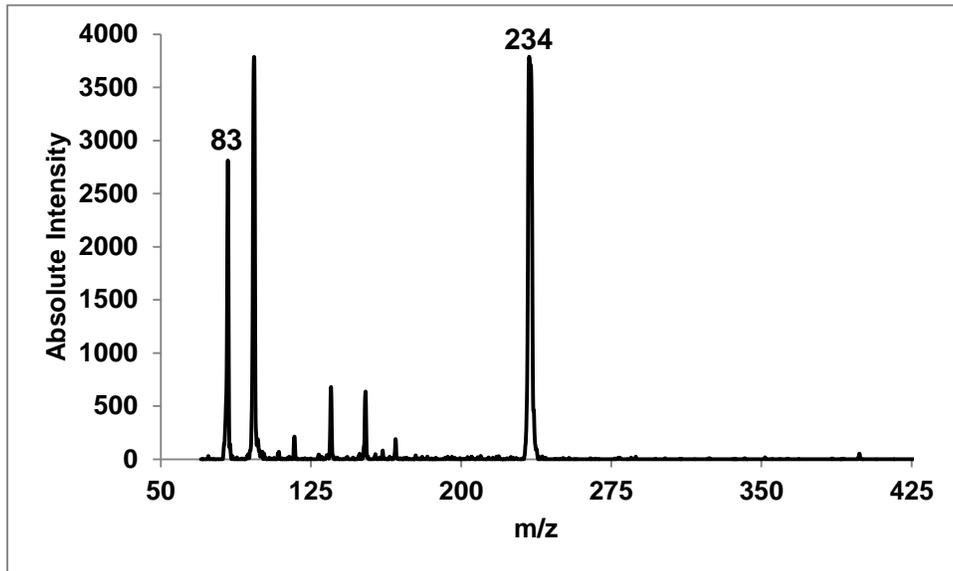
A-37.2. Methylphenidate MS/MS Scan
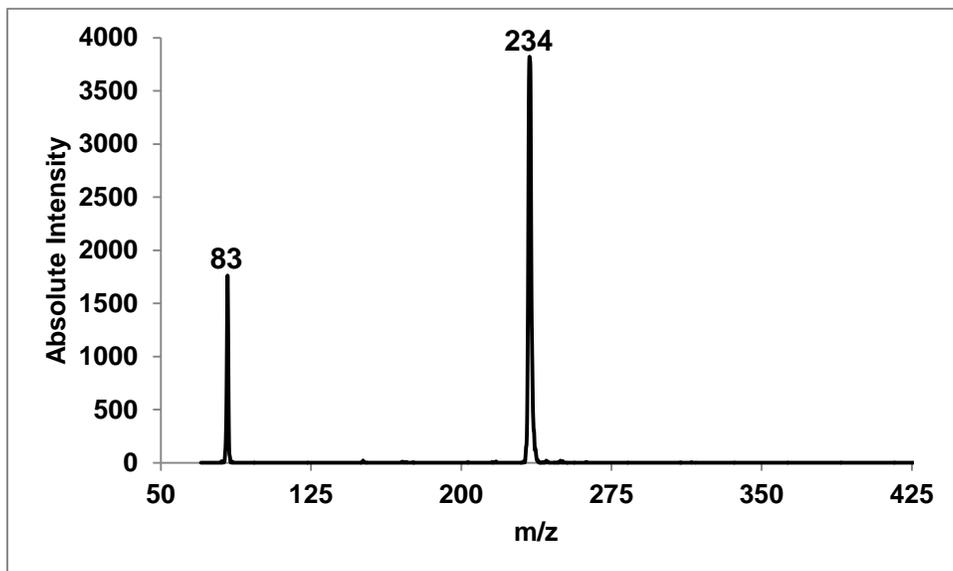
A-38. Morphine

A-38.1. Morphine MS Scan
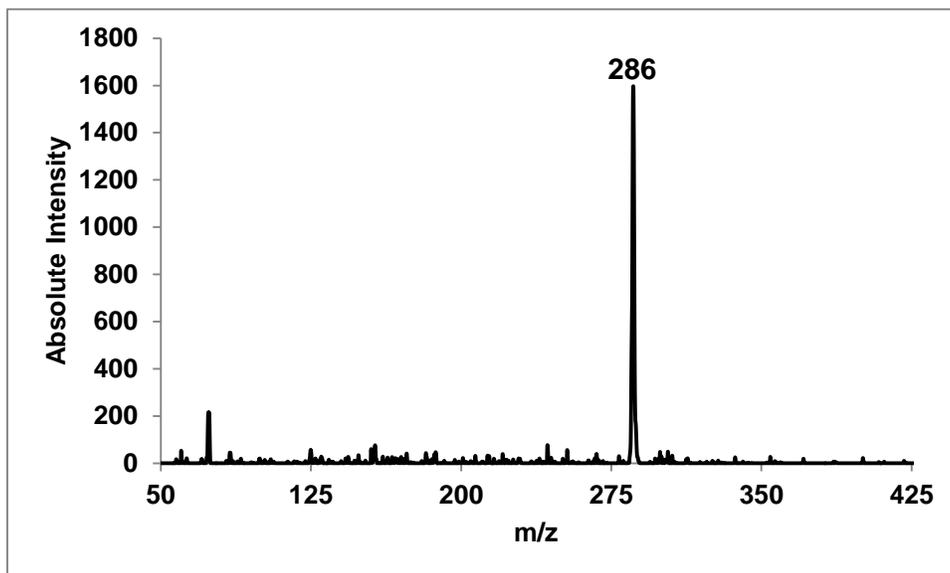
A-38.2. Morphine MS/MS Scan

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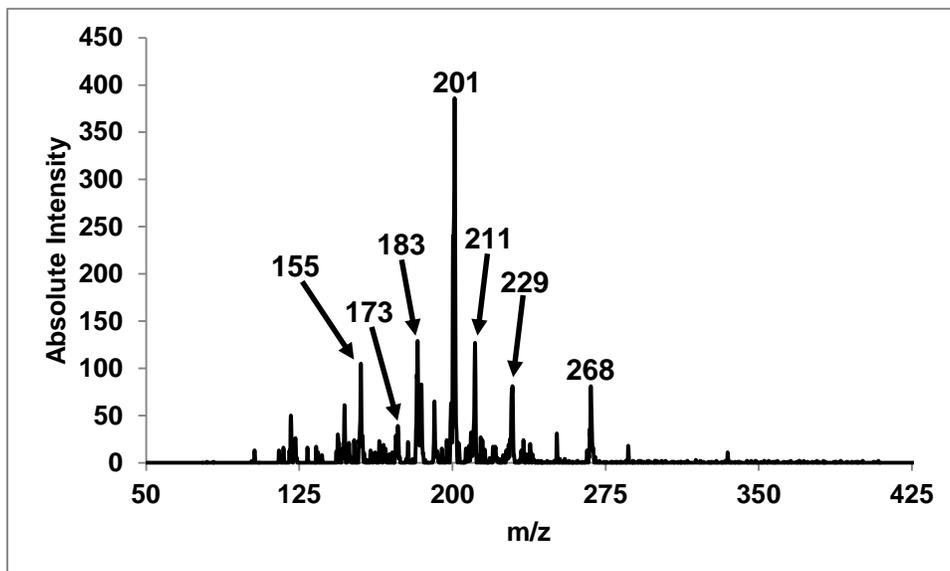
A-39. Oxycodone

A-39.1. Oxycodone MS Scan
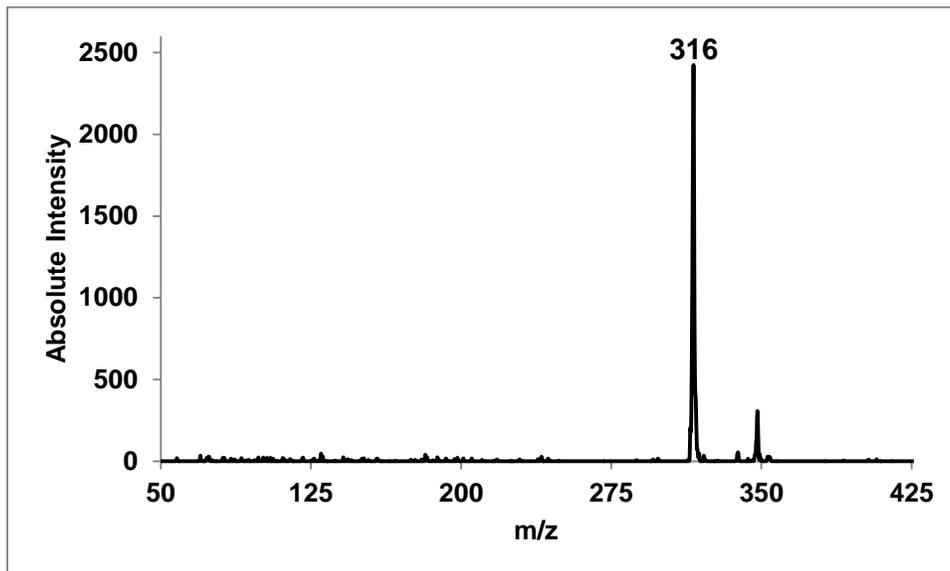
A-39.2. Oxycodone MS/MS Scan
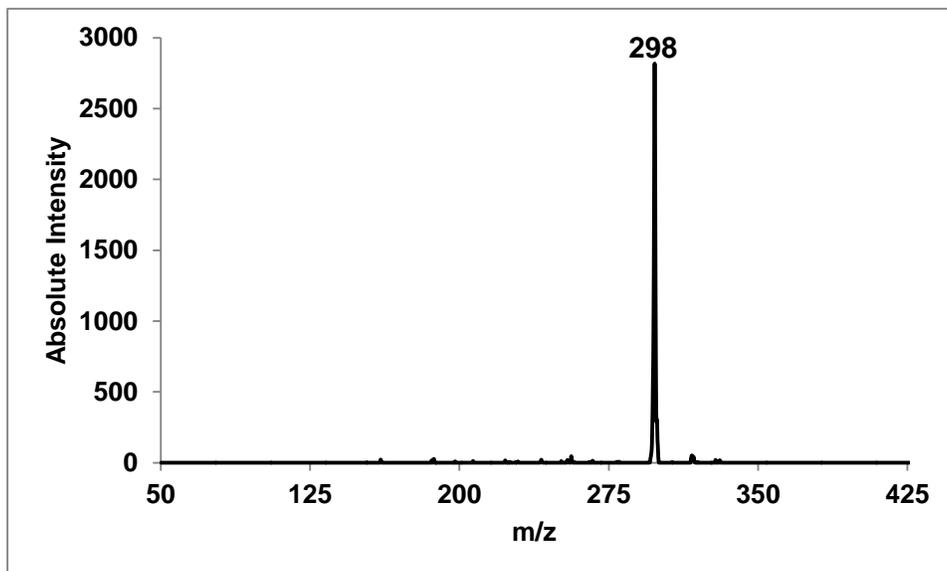
A-40. 4-Nitrodiphenylamine

A-40.1. 4-Nitrodiphenylamine MS Scan
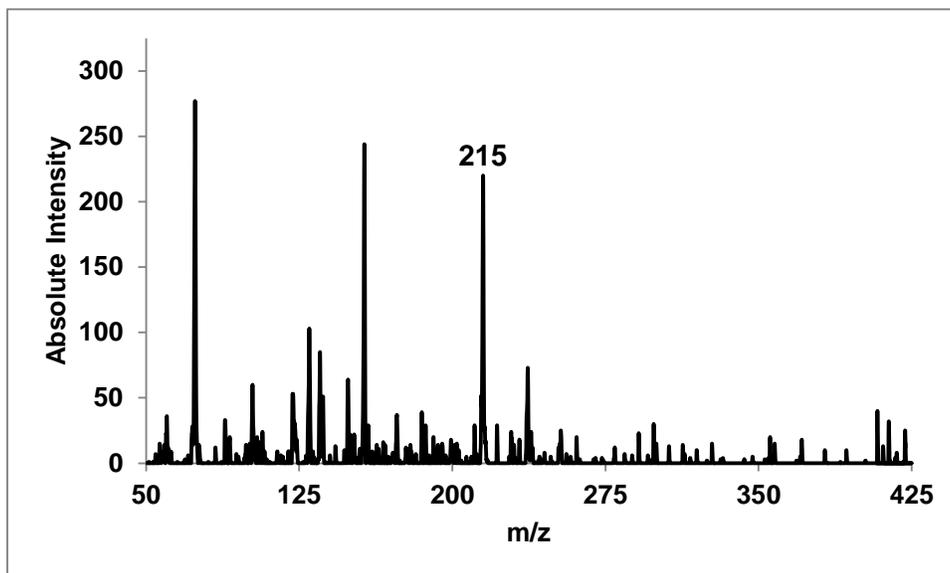
A-40.2. 4-Nitrodiphenylamine MS/MS Scan

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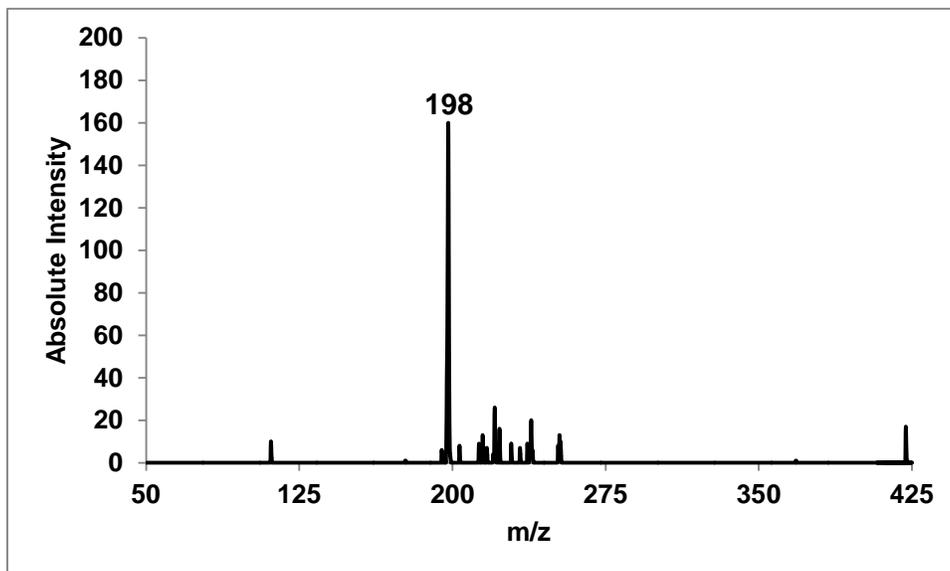
A-41. 4-Nitrosodiphenylamine

A-41.1. 4-Nitrosodiphenylamine MS Scan
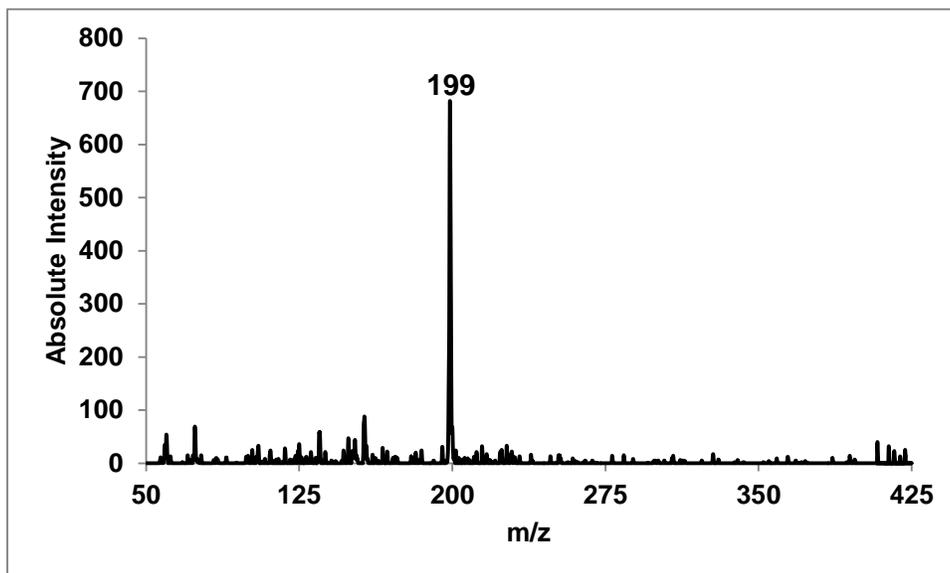
A-41.2. 4-Nitrosodiphenylamine MS/MS Scan

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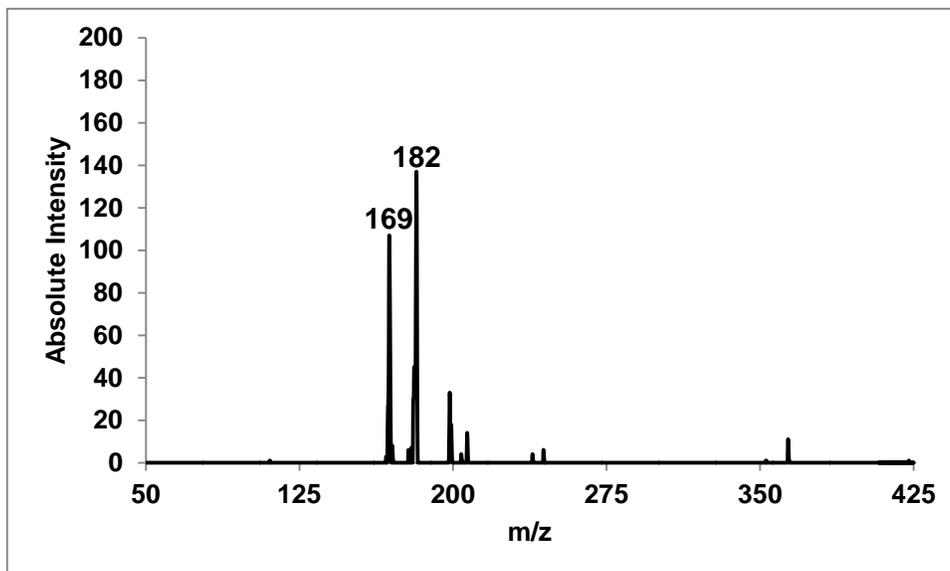
A-42. PCP

A-42.1. PCP MS Scan

A-42.2. PCP MS/MS Scan

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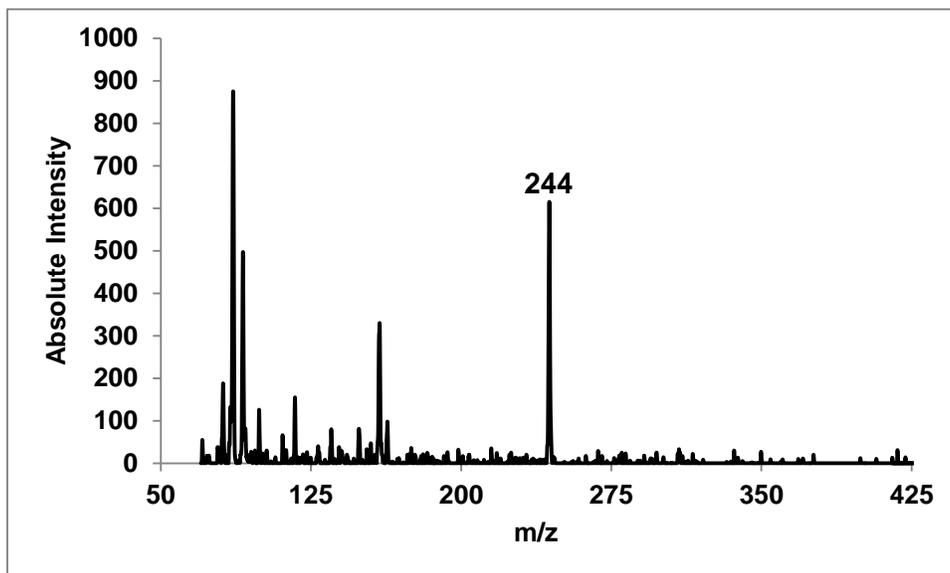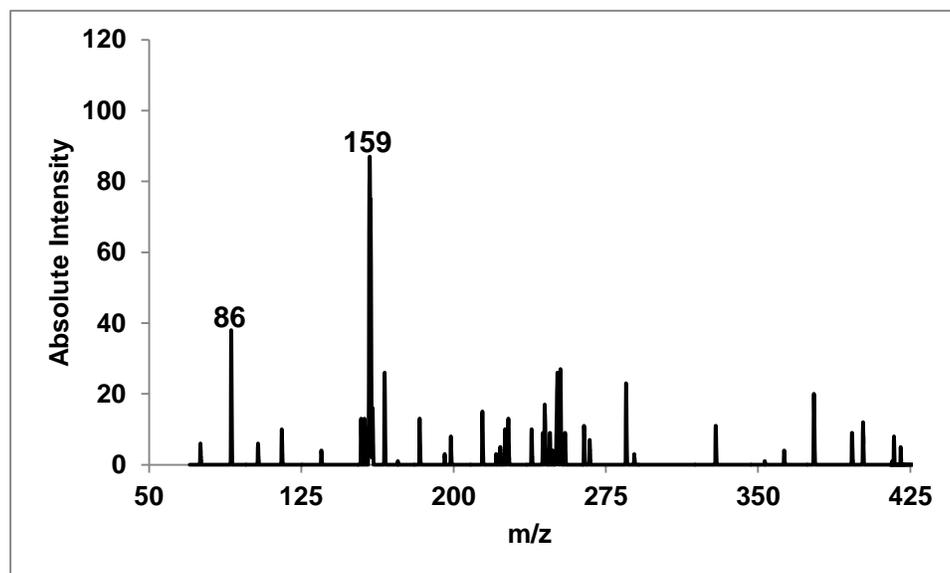
A-43. Pentedrone

A-43.1. Pentedrone MS Scan
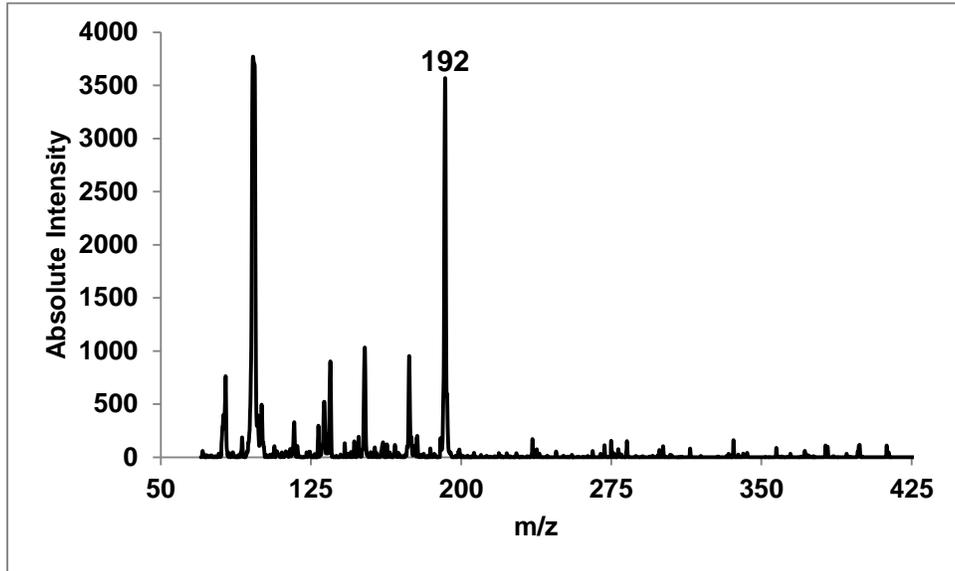
A-43.2. Pentedrone MS/MS Scan
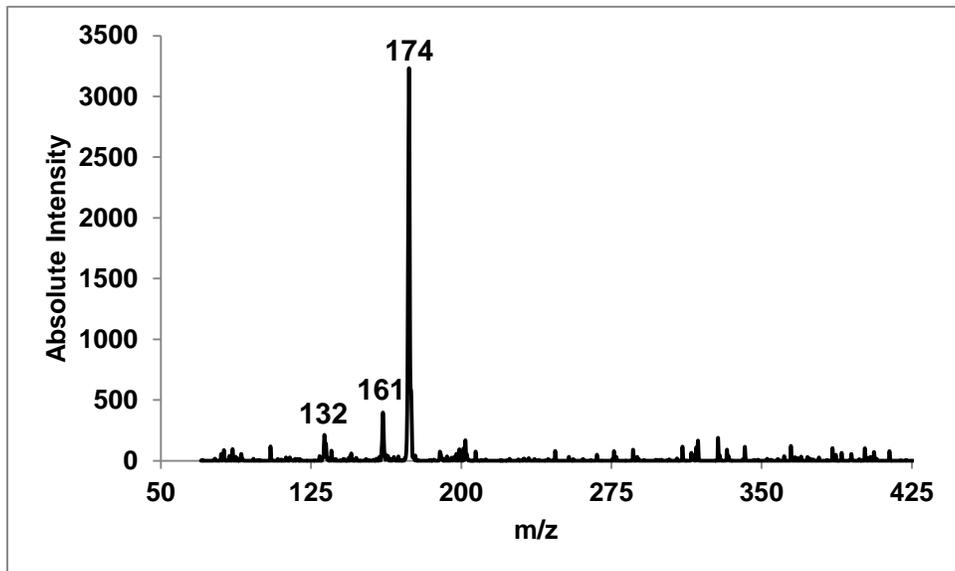
A-44. Phenacetin

A-44.1. Phenacetin MS Scan
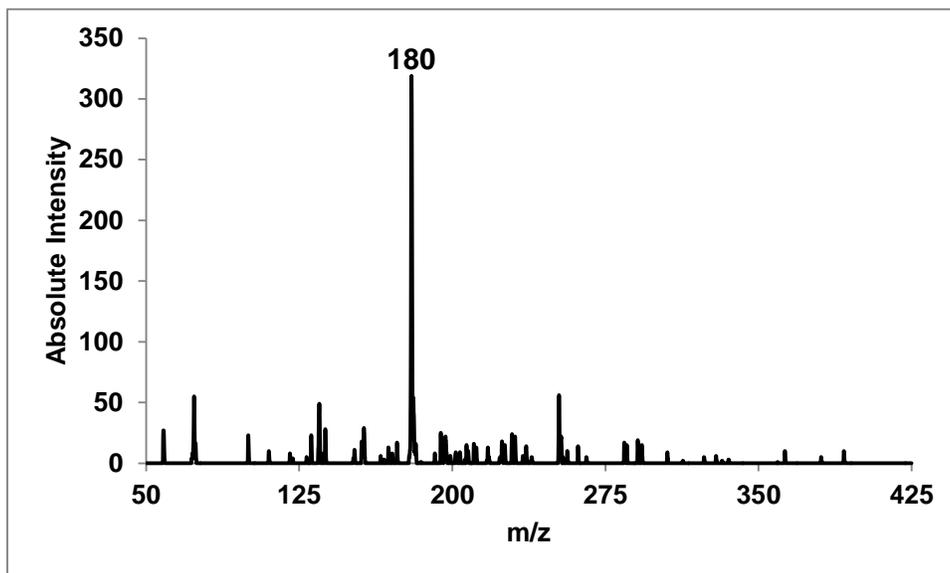
A-44.2. Phenacetin MS/MS Scan
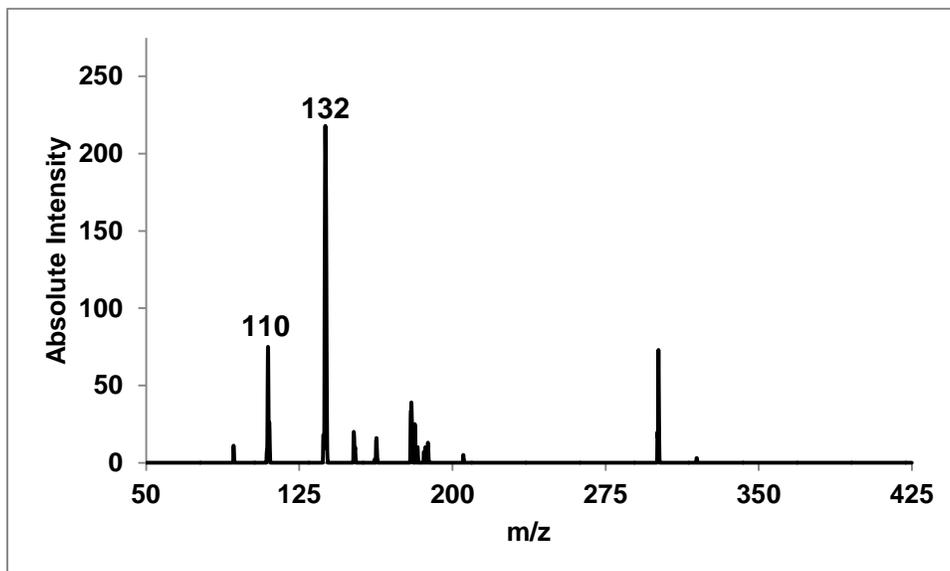
A-45. Phenylephrine

A-45.1. Phenylephrine MS Scan
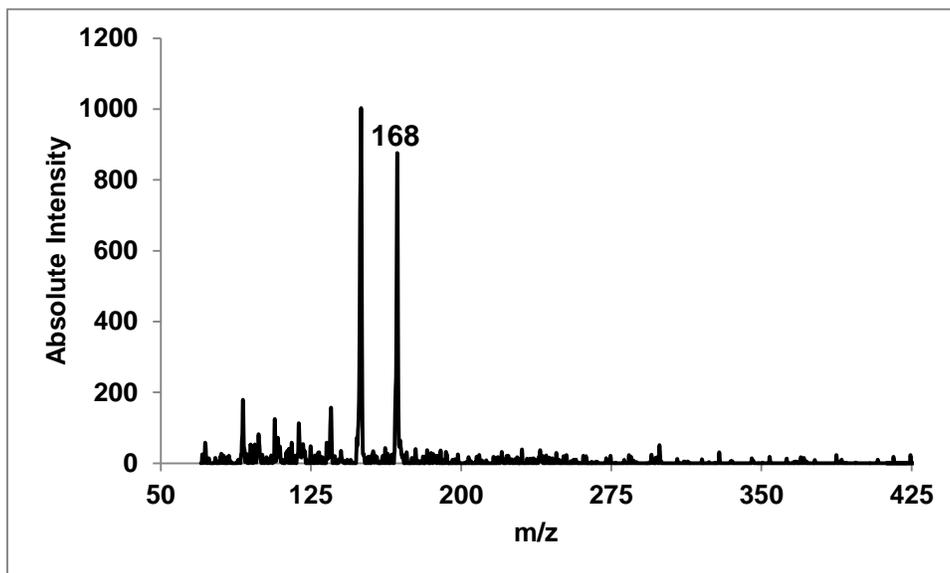
A-45.2. Phenylephrine MS/MS Scan

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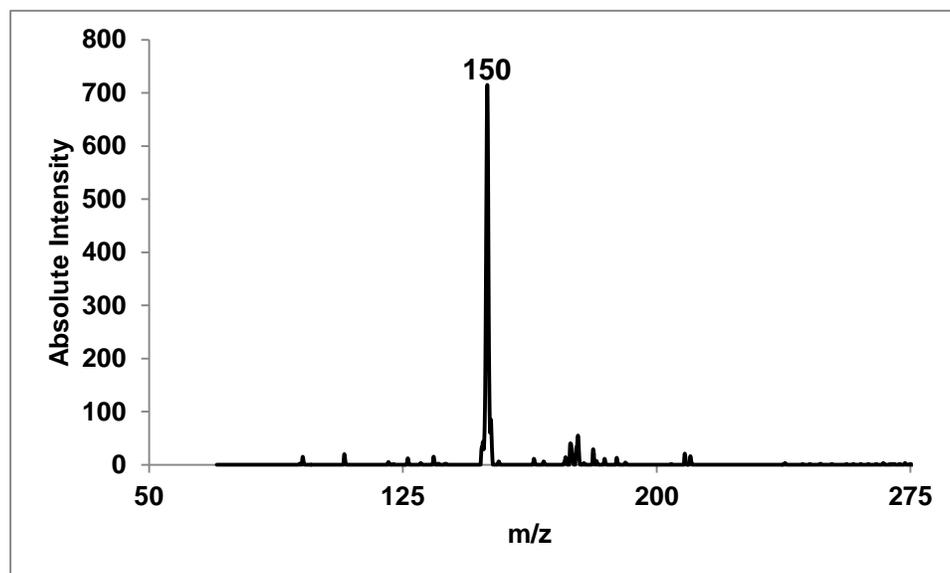
A-46. Phenylpropanolamine

A-46. Phenylpropanolamine MS Scan
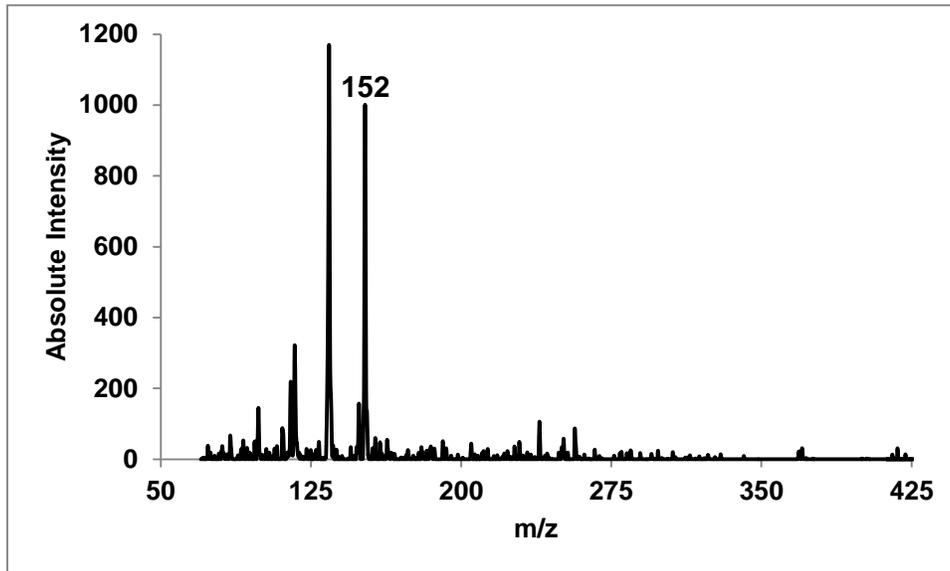
A-46.2. Phenylpropanolamine MS/MS Scan

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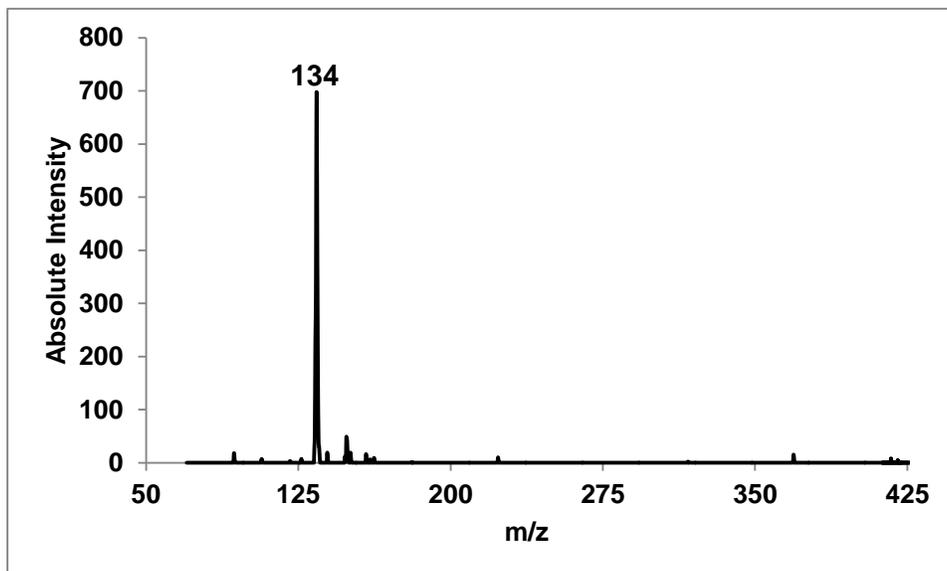
A-47. Pseudoephedrine

A-47.1. Pseudoephedrine MS Scan
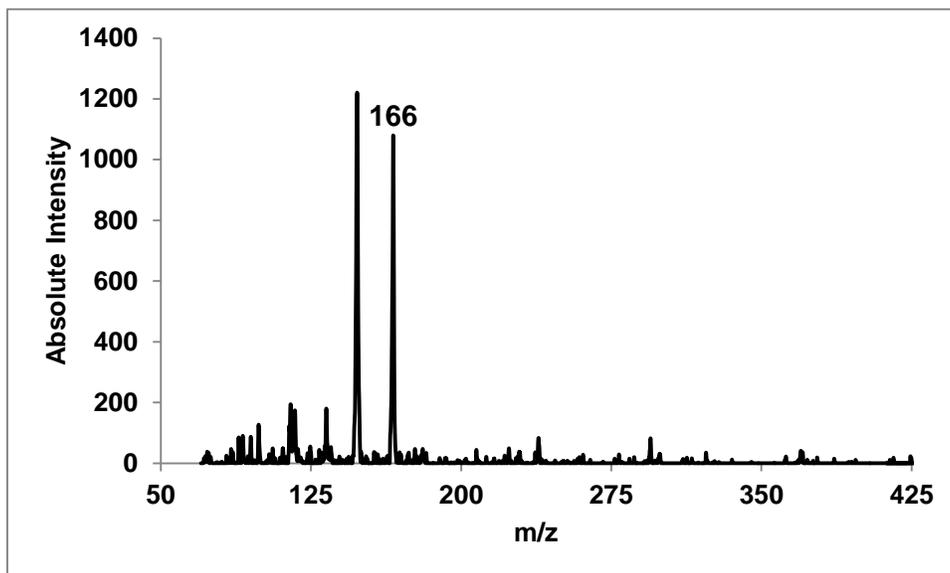
A-47.2. Pseudoephedrine MS/MS Scan

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A-48. Δ-9-THC

A-48.1. Δ-9-THC MS Scan

A-48.2. Δ-9-THC MS/MS Scan

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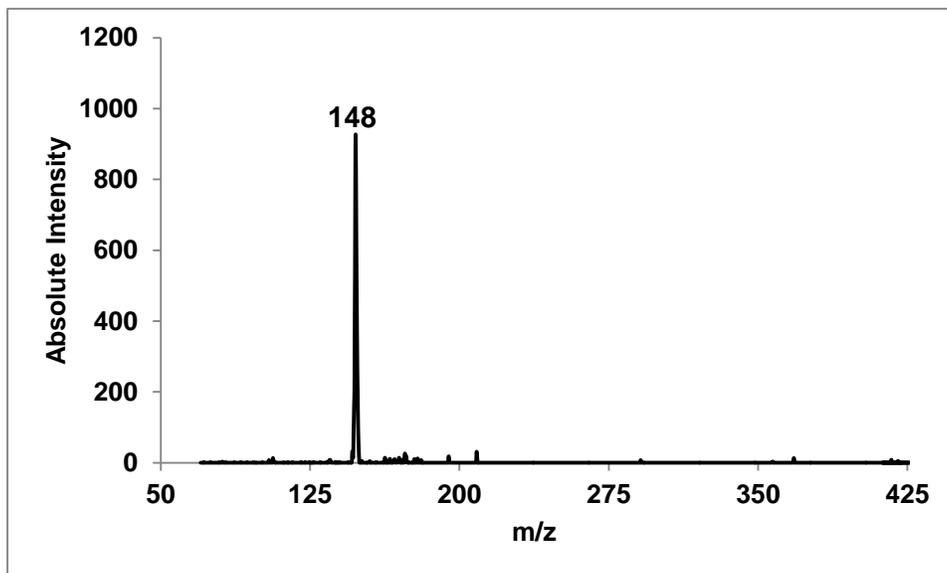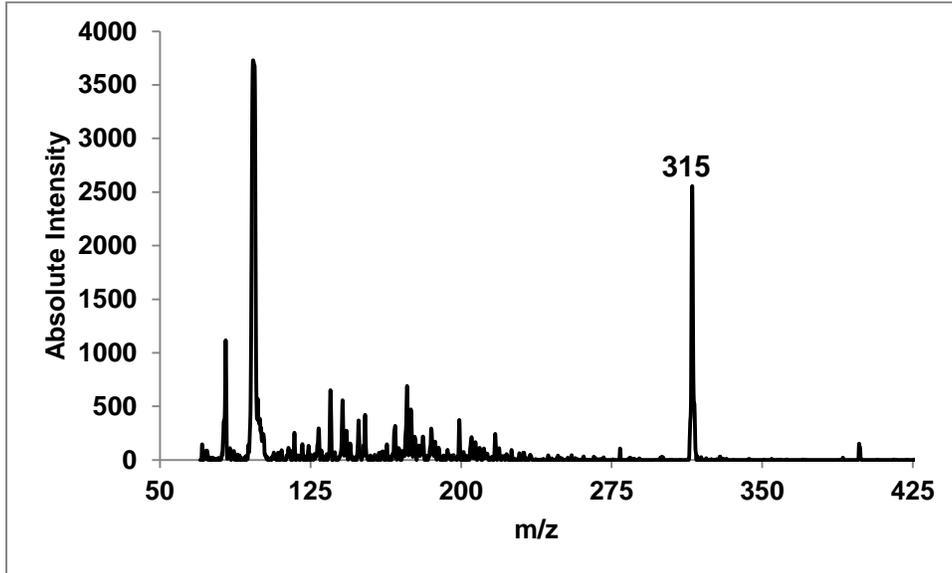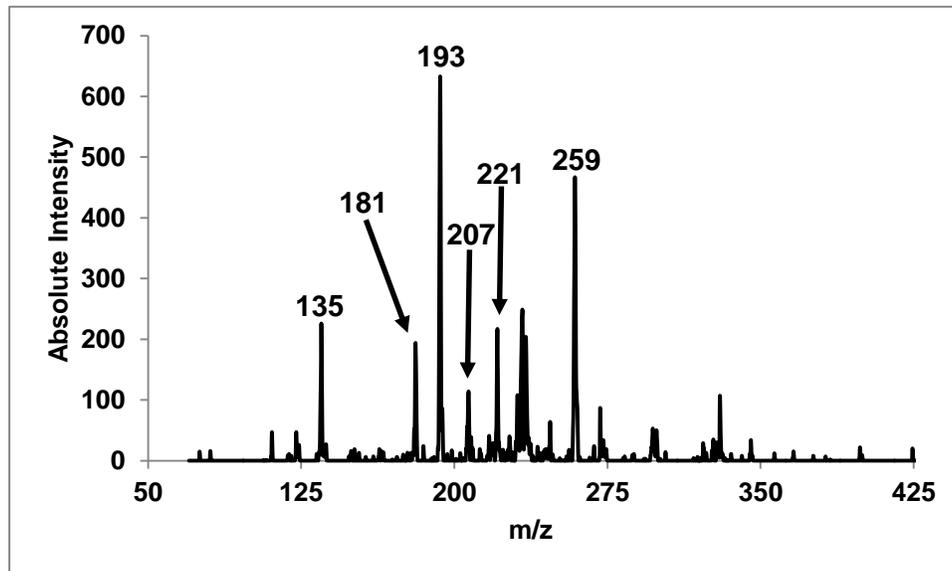
A-49. Triazolam

A-49.1. Triazolam MS Scan
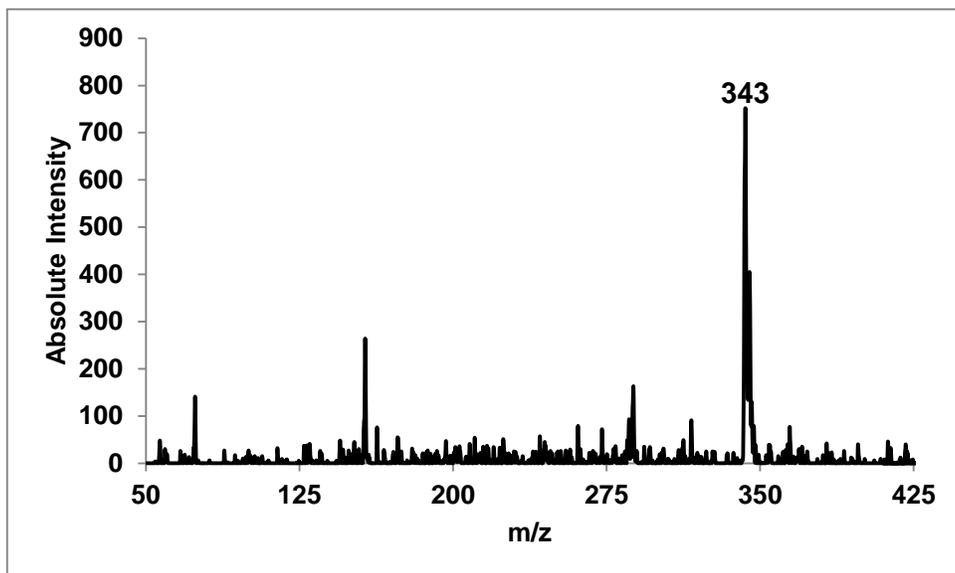
A-49.2. Triazolam MS/MS Scan
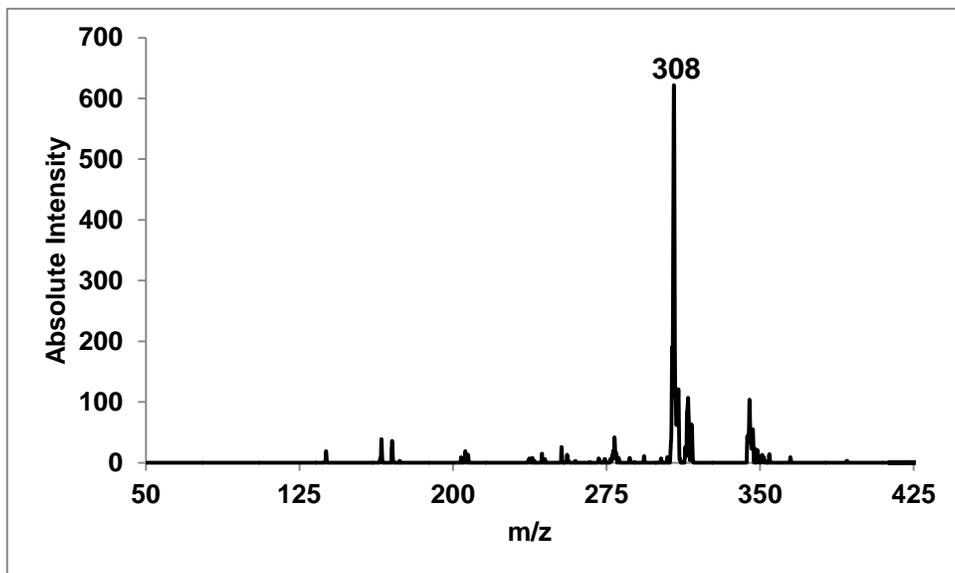
A-50. Zaleplon

A-50.1. Zaleplon MS Scan
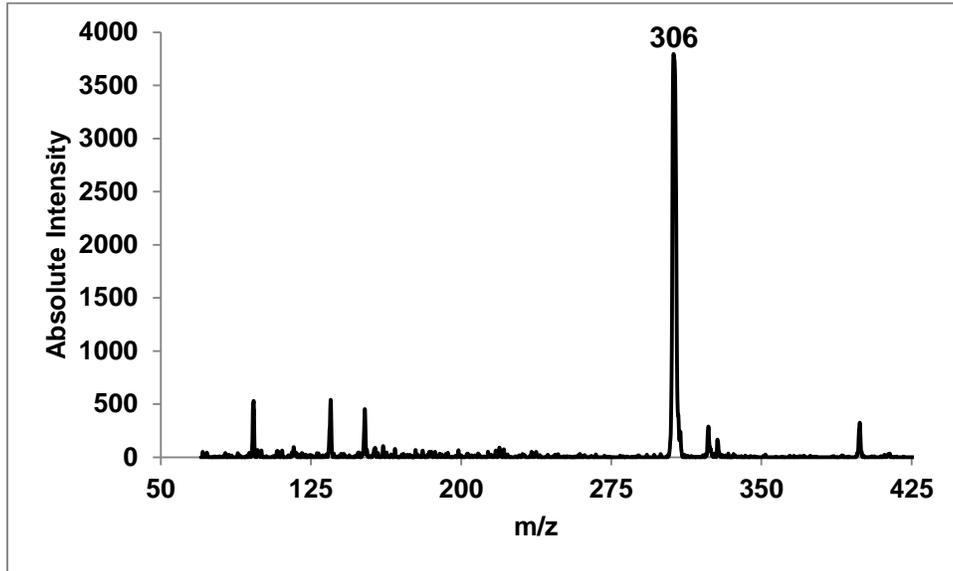
A-50.2. Zaleplon MS/MS Scan
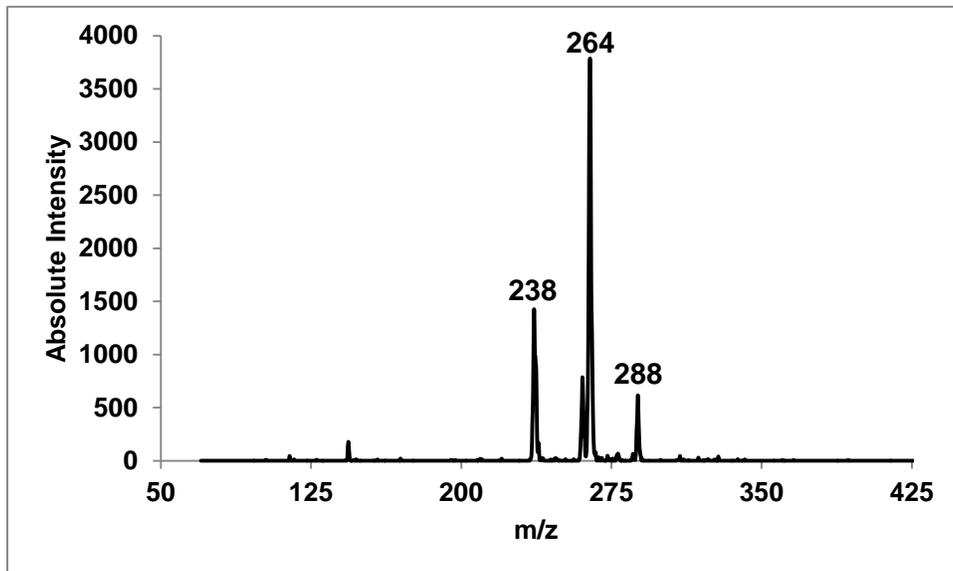
A-51. Zolpidem

A-51.1. Zolpidem MS Scan
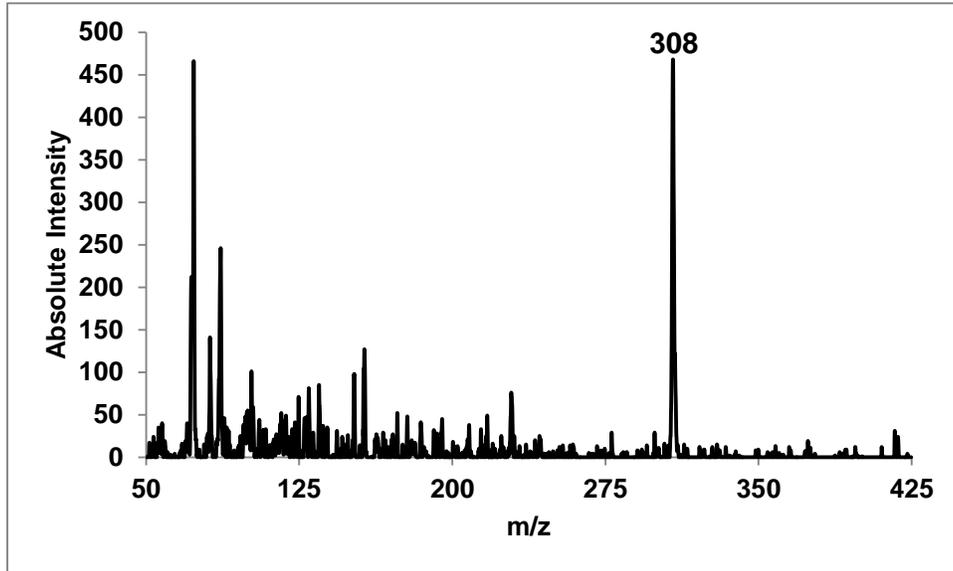
A-51.2. Zolpidem MS/MS Scan
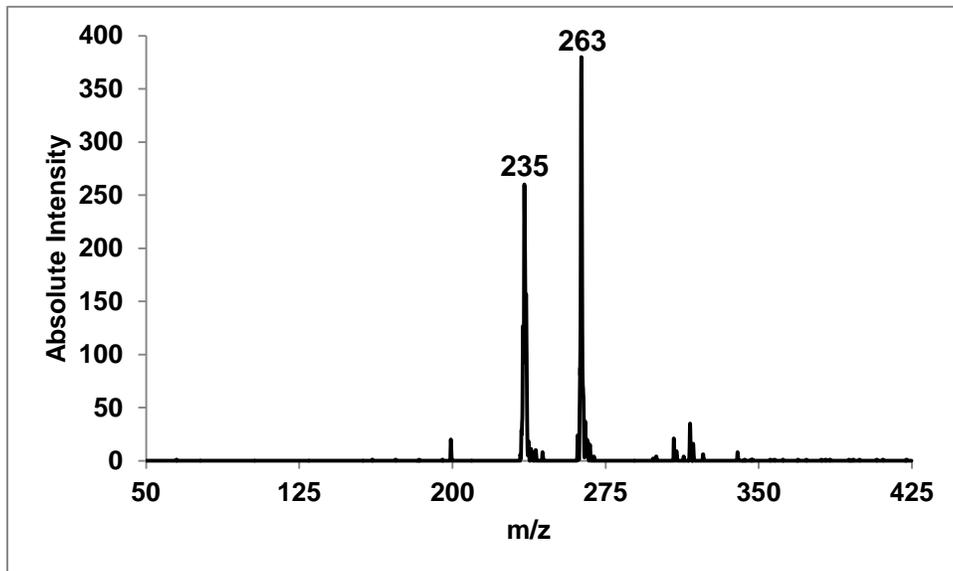
A-52. Zopicolone

A-52.1. Zopicolone MS Scan
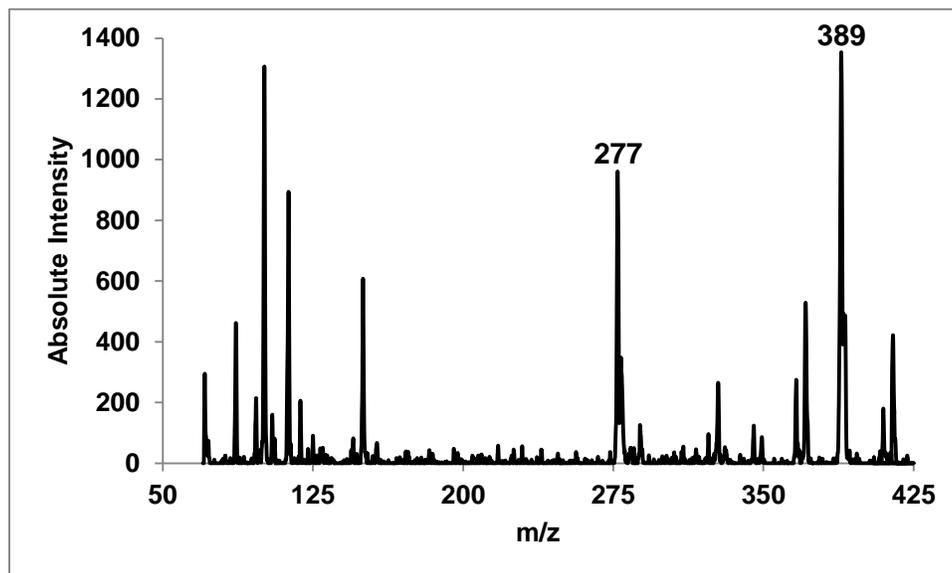
A-52.2. Zopicolone MS/MS Scan

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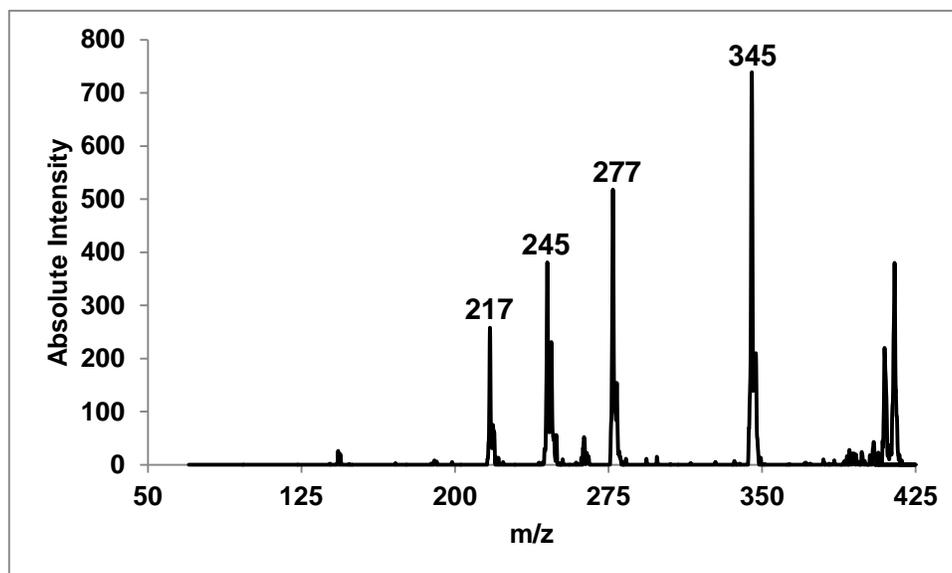
Appendix B: Negative-Mode MS and MS/MS Spectral Database

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B-1. Amobarbital

B-1.1. Amobarbital MS Scan
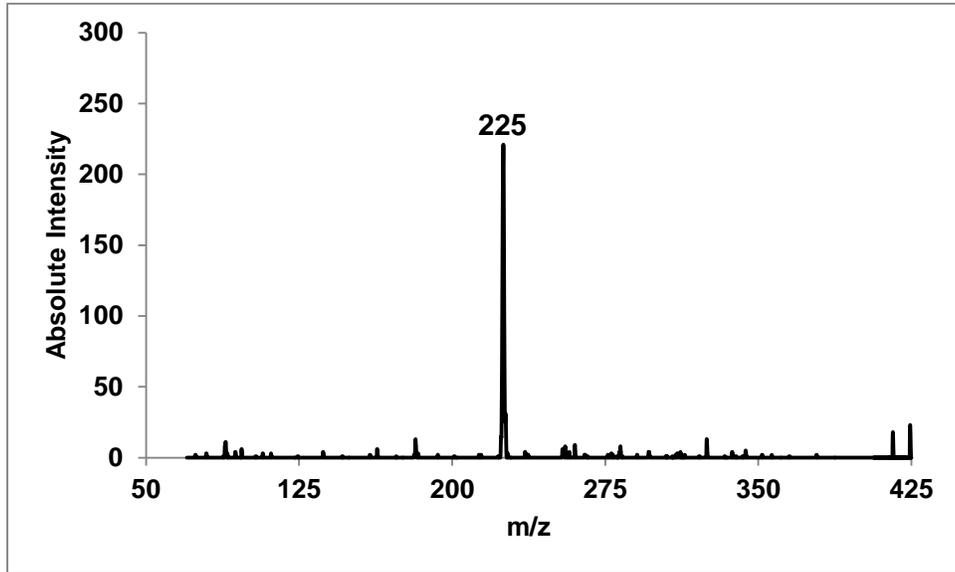
B-1.2. Amobarbital MS/MS Scan

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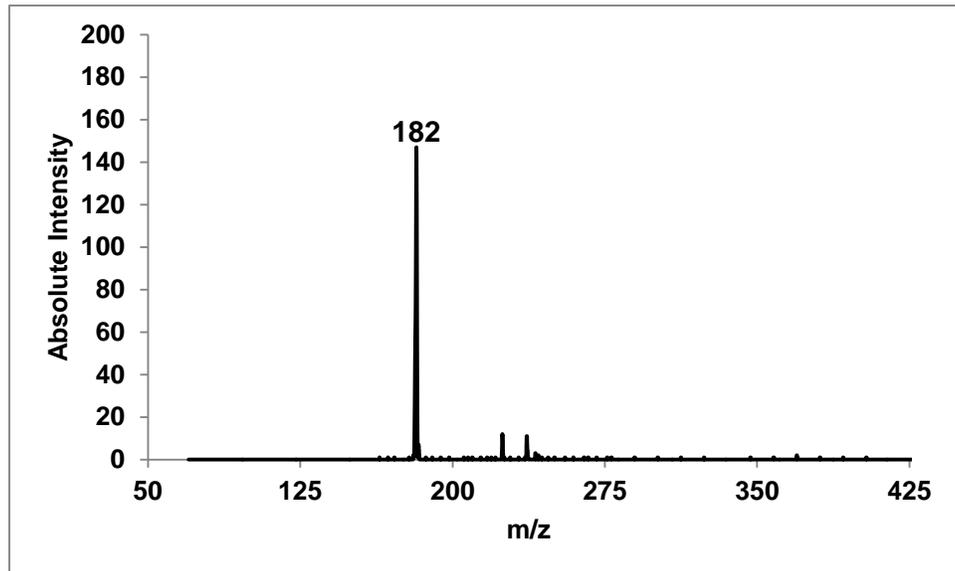
B-2. Pentobarbitol

B-2.1. Pentobarbitol MS Scan
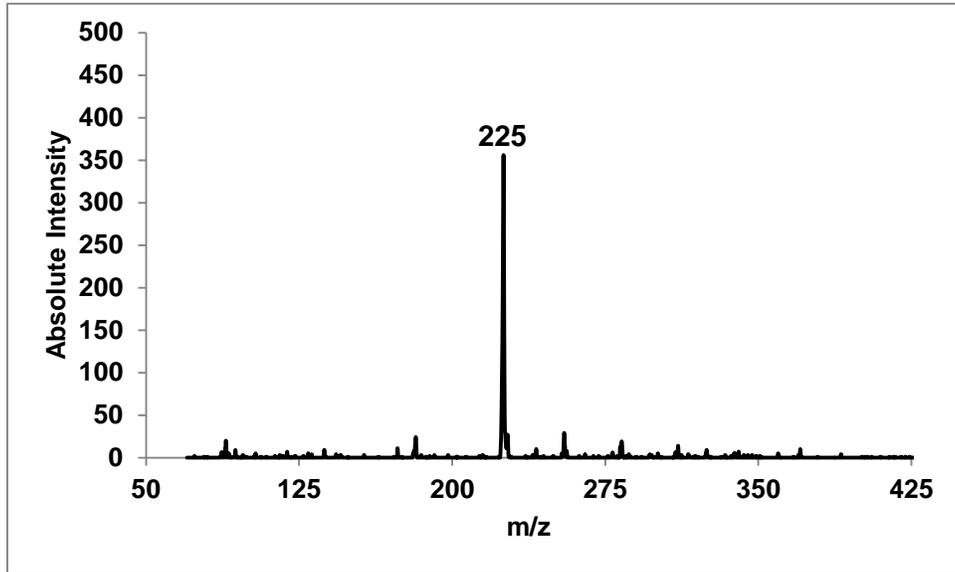
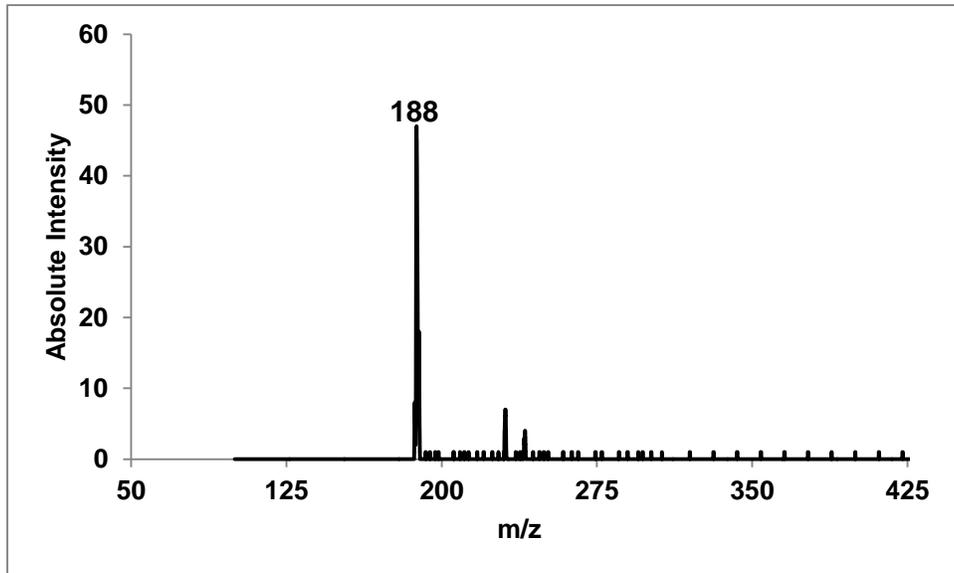### B-2.2. Pentobarbitol MS/MS Scan
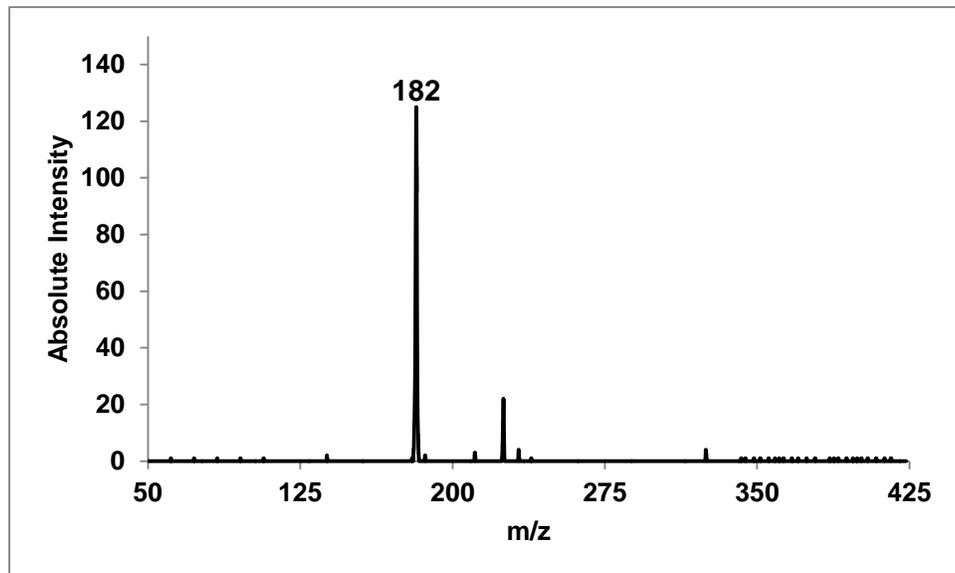
B-3 Phenobarbitol

B-3.1. Phenobarbitol MS Scan
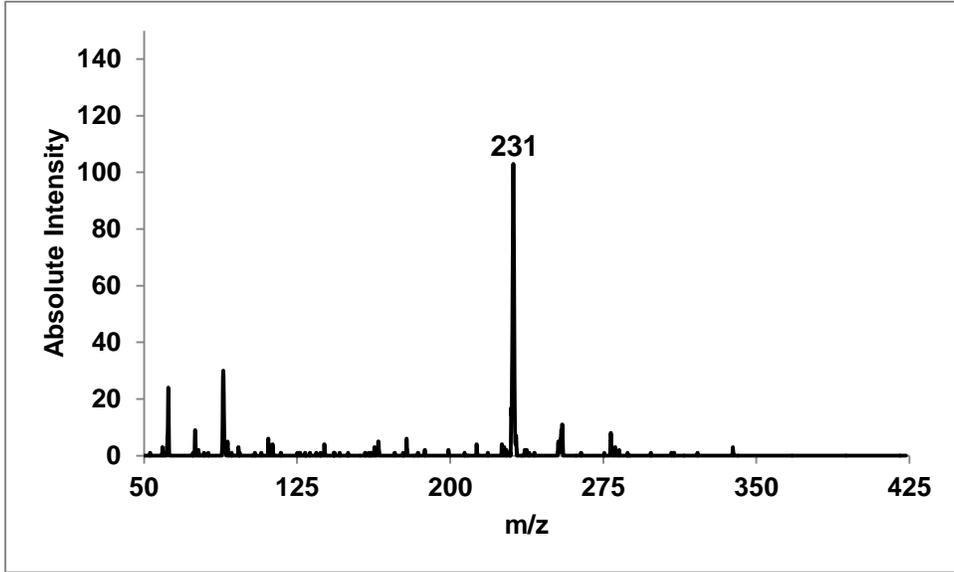
B-3.2. Phenobarbitol MS/MS Scan
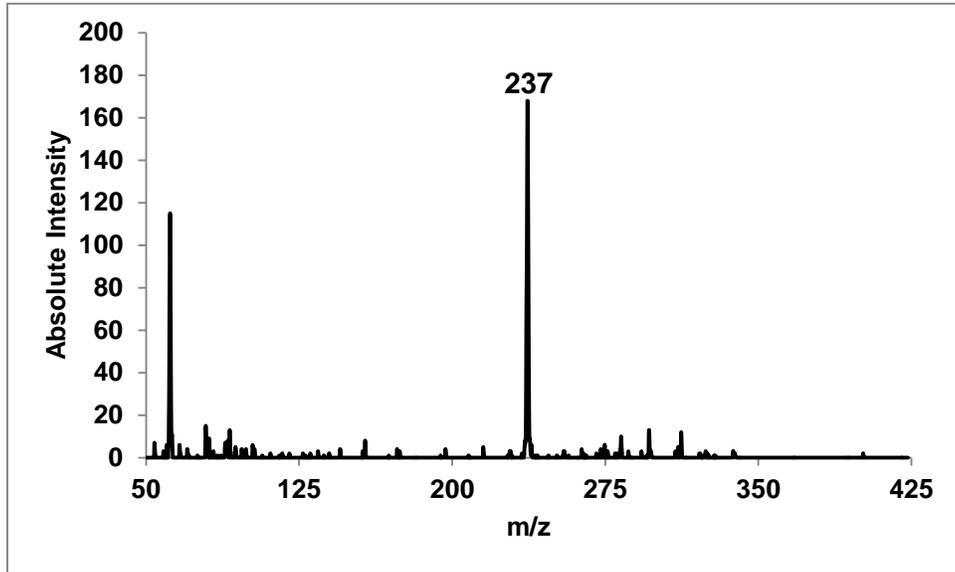
B-4. Secobarbitol

B-4.1. Secobarbitol MS Scan

B-4.2. Secobarbitol MS/MS Scan