PAPER SPRAY-MASS SPECTROMETRY COUPLED WITH PRESSURE-SENSITIVE ADHESIVE-BASED COLLECTION FOR THE RECOVERY AND DETECTION OF DRUGS OF ABUSE

by

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To my parents for their relentless support, Julia for the laughs and encouragement, Drew for always being my biggest fan, and to the grace and glory of God, for without him none of this would be possible.

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AUC	Area under the curve
CBD	Cannabidiol
CI	Chemical ionization
CID	Collision-induced dissociation
DART	Direct analysis in real time
DC	Direct current
DEA	Drug Enforcement Administration
DESI	Desorption electrospray ionization
EI	Electron ionization
ESI	Electrospray ionization
FA	Formic acid
FTIR	Fourier transform infrared spectrometer
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
ISTD	Internal standard
LC-MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection
MALDI	Matrix assisted laser desorption ionization
MAMP	Methamphetamine
MeOH	Methanol
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass to charge ratio
NFLIS	National Forensic Laboratory Identification System
ppm	Parts-per-million, mass to volume
PSA	Pressure-sensitive adhesive
PSI	Paper spray ionization
PS-MS	Paper spray – mass spectrometry

RF	Radiofrequency
RSD	Relative standard deviation
S:B	Signal to blank ratio
S_b	Standard deviation of the blanks
SERS	Surface-enhanced Raman spectroscopy
SPME	Solid phase microextraction
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
THC	∆-9-tetrahydrocannabinol
TIC	Total ion chromatogram
TOF	Time-of-flight
TOM	Traceable Opioid Material
4-ANPP	4-anilino-N-phenethylpiperidine
4-AP	4-aminophenol
4-FIBF	4-fluoroisobutyrylfentanyl

ABSTRACT

Illicit drug abuse is a widespread issue in the United States and worldwide. Many methods seek to ease the analytical workload required to collect, analyze, and identify these drugs. Paper spray-mass spectrometry (PS-MS) is one response to this analytical workload as it offers a rapid, affordable, and simple means for drug identification by mass spectrometry. This work centers on the use of pressure-sensitive adhesive (PSA) lined paper as a PS-MS substrate for drug recovery and detection. The use of PSA paper as a sampling and analysis substrate has been previously established but is expanded herein with new capabilities and applications. Chapter 2 introduces the combination of color tests followed by PS-MS for presumptive and confirmatory drug identification. Three color tests (cobalt thiocyanate, Simon, or Marquis) were performed on the PSA paper with subsequent drug confirmation occurring by PS-MS. Chapter 3 examines the use of PSA paper and PS-MS for the recovery and detection of fentanyl, fentanyl precursors, and analogs from shipping-related surfaces and in the presence of high amounts of cutting agents. The use of a cartridge that accommodates a full-sized PSA paper ticket was also explored for drug detection. Chapter 4 assesses PS-MS with PSA paper on portable MS instrumentation. Analyte recovery and carryover as well as instrument robustness were evaluated. The color test and PS-MS protocol examined in Chapter 2 was also successfully applied to a portable MS instrument. Application of PS-MS to the portable system highlights the potential fieldability of the technique.

CHAPTER 1. INTRODUCTION

1.1 Mass Spectrometry

As is the case with many instruments used by the analytical chemist, the development of the mass spectrometer occurred entirely by accident.[1] During the search for the electron in the early 1900s, much of the preliminary technology used in the first mass spectrometers was developed.[1] Since initial use, mass spectrometry (MS) has been used across the sciences for a myriad of discoveries and advancements ranging from isotope identification to transforming the field of protein and DNA sequencing.[2-4] Even over a century later, the mass spectrometer proves to be an invaluable tool in analytical laboratories.

The mass spectrometer is an instrument that separates and measures ions in a sample based on their mass-to-charge ratios, or *m/z*.[5] This generally is conducted in three simple steps: sample ionization, ion separation, and ion detection.[5] The basic elements of a mass spectrometer are illustrated in Figure 1.1. The most variable of these components is sample ionization, which occurs in the ion source. Ionization can be achieved in a variety of ways based on sample composition and its ionization susceptibility. Ions separation is achieved by the mass analyzer, the heart of any mass spectrometer.[5] Following ionization and separation, the ions strike a detector, generating a signal used in producing a mass spectrum.

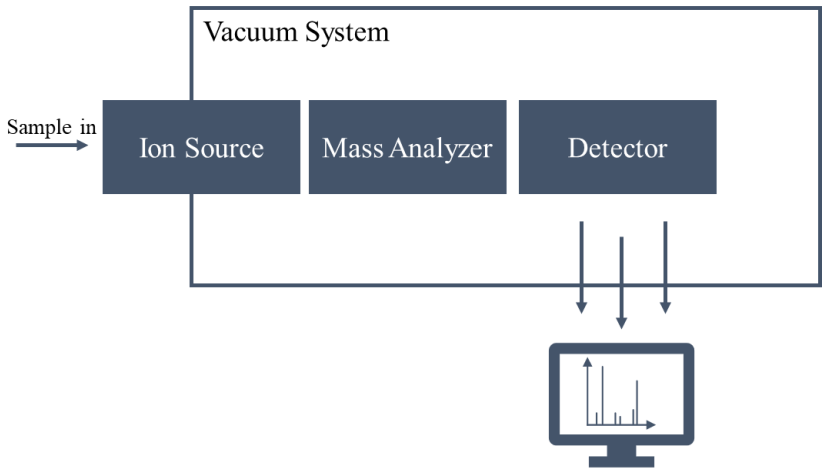

Figure 1.1 Block diagram of a basic mass spectrometer.

Mass spectra are the graphical representations of the detected ions and contain two crucial pieces of data: the mass-to-charge ratio of an ion and its relative abundance. In a spectrum the x-axis is attributed to the mass-to-charge ratio and the y-axis to the relative abundance of ions. Despite the varied applications of MS and the versatility of instrumentation, the net goal is to produce distinct and interpretable mass spectra. Mass spectrometers have been tailored to analyze diverse analytes, with many requiring unique ionization mechanisms, more effective mass analyzers, or more sensitive detectors.

1.1.1 Ionization

The first step in analysis by MS is ionization of the sample. Ionization is the process of generating a charged particle or ion. Ions can have either a positive or negative charge based on how they are altered from the ground state. Though not an exhaustive list, ionization is generally achieved through the loss or gain of an electron(s), protonation or deprotonation, or adduct formation. For protonation and adduct formation, the precursor ion bonds with an atom (or atoms) of hydrogen or other charged species to form a charged molecular ion. Notation for these molecular ions is $[M+H]^+$ indicating a protonated molecular ion or $[M+X]^+$ where X is an adduct ion such as Na^+ or K^+.[6, 7] When deprotonation occurs during ionization, an $[M-H]^-$ molecular ion is produced.

Ionization techniques are generally grouped into two categories: "hard" and "soft."[8] The main distinction between these categories is the extent to which an analyte's molecular ion is retained throughout the ionization process.[9, 10] Hard ionization techniques typically bombard samples with a large amount of energy to induce fragmentation and ionization.[9, 11] Because large amounts of energy are expelled to achieve ionization, the molecular ion is either minimally retained, or not retained at all. Hard ionization techniques are associated with heavy fragmentation. The resulting fragments are often considered a molecular "fingerprint" as they provide important structural information about the intact molecule.[5, 11] Contrary to hard ionization techniques, "soft" ionization techniques result in limited to no fragmentation and a preservation of the molecular ion.[12] Soft ionization techniques are favored when preservation of the molecular ion is preferred, or with analytes that are not stable enough to be ionized by harder techniques.

Electron ionization (EI) is the most common hard ionization technique for MS and is often coupled with separatory techniques such as gas chromatography or liquid chromatography (GC or LC-MS).[11] EI-MS has been applied to the analysis of archaeological, environmental, and forensic

samples.[13-17] Soft ionization techniques are more diverse and include methods such as chemical ionization (CI), electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) among others.[8, 12] "Soft" ionization techniques have been applied to complex biological molecules like lipids and proteins, tissue imaging, inorganic systems and more.[18-22]

The diversity in ionization techniques allows for the analysis of a continuum of analytes, from individual atoms to large and exceedingly complex biological molecules. By utilizing a suitable ionization technique, MS can be used to examine molecular ions or "fingerprints" to learn about a sample and identify its components.

1.1.2 Mass Analyzers

Following ionization, ions are separated based on their mass and charge by the mass analyzer. Much like ionization techniques, there are a variety of mass analyzers. Some of the most common analyzers include quadrupoles, time-of-flight instruments, and ion traps.[23]

Quadrupole mass analyzers separate ions through the use of four parallel metal rods with alternating charges and oscillating frequencies.[23, 24] As ions travel through the quadrupoles the oscillation frequencies are varied with each frequency being specific to a *m/z*. This allows only a specific *m/z* to pass through the quadrupoles and reach the detector, effectively filtering the ions. Quadrupole mass analyzers are affordable, stable, and offer rapid scan speeds.[25] Quadrupoles are often combined with additional analyzers resulting in triple quadrupole or quadrupole time-of-flight mass analyzers.[25] While quadrupoles have a narrower operational mass range than other analyzers and poor mass resolution, they are relatively inexpensive and robust with modest vacuum requirements.[25]

Time-of-flight (TOF) mass analyzers separate ions by applying a constant force that accelerates the ions through a tube. Because the ions have varied masses, smaller ions with reach the detector faster than larger ions, effectively separating ions by mass and charge.[25] TOF mass analyzers offer virtually unlimited mass ranges but often require a correction to achieve mass accuracy.[25]

Another common type of mass analyzer is the ion trap. Much like their name, ion traps function by trapping the ions between hyperbolic electrodes.[25, 26] Ions are then ejected based on their *m/z*, similar to the quadrupole mass analyzer but with the added capability of trapping ions. There are multiple types of ion traps including 3D, 2D (linear), and electrostatic traps. Benefits of

the ion trap include increased sensitivity and ion capacity; however, the resolving power is limited.[25, 26]

Since there are various mass analyzers, each with their own capabilities, understanding the limitations of an instrument's mass analyzer is necessary. An important and widely used feature of modern mass spectrometers is the ability to conduct tandem MS (MS/MS) analysis. MS/MS conducts sequential mass analyses, where the ions generated from the initial ionization event can be isolated and subsequently fragmented.[27] This capability allows for specific ions in a complex sample to be isolated and examined further.[27] Tandem MS analysis is possible for mass analyzers that filter or trap ions of specific masses such as triple quadrupole or ion trap mass analyzers, respectively. The work presented in future chapters was conducted on linear ion trap mass spectrometers with use of both full MS and MS/MS capabilities.

1.1.3 Detectors

There are three main types of detectors common to mass spectrometers: electron multipliers, photomultiplier dynodes, and the Faraday cup.[28] All detectors serve the common goal of amplifying the sample signal into signal great enough to generate electrical current. The generated current can then be interpreted, producing the spectral data that is typically reported.

The simplest of ion detectors is the electron multiplier. In this detector, an electrical current is produced through the generation of secondary electrons from a series of dynodes.[5, 29] When an ion comes in contact with a dynode, secondary electrons are dislodged, magnifying the signal produced by the ion.[5, 28, 29] Since there are a series of dynodes, the signal is magnified repeatedly, until it reaches a readable signal.[5] Electron multipliers offer fast scan rates and are considered robust and sensitive, with ion amplification as much as 10^6.[5, 29]

The photomultiplier detector works in a similar manner to the electron multiplier. Ions strike a dynode, emitting electrons; however, instead of electrons continuing to be amplified by additional dynodes, the electrons strike a phosphorescent screen.[28] The screen emits photons which strike a scintillating surface and release electrons, after which electrons are amplified in the same manner as the electron multiplier.[28, 29] While procedurally similar to the electron multiplier, photomultipliers typically have longer lifetimes. One drawback of the photomultiplier detector is that it has to be sealed in a vacuum and kept away from light to function properly.[5]

The Faraday cup works by the release of secondary electrons when an ion strikes the cup. However, unlike the electron and photomultipliers, the electrons are not continually amplified. Instead, the release of electrons results in the temporary accumulation of a positive charge on the cup surface. In response to the positive charge, there is an induced electrical current that flows to the cup which is measured as the detector's signal.[5, 28] The Faraday cup only offers minimal signal amplification but is considered a relatively flexible detector as it can operate at higher pressures. Additionally, Faraday cups are well-suited to measurements requiring high precision such as isotope analysis.[5, 28]

While additional detectors have been developed for more niche applications of mass spectrometry, all are based on the principle of amplifying the signal of an ion to and electrical signal that can be interpreted. Understanding the capabilities and limitations of the detectors in a mass spectrometer is important to understanding the limitations of the instrument. For all the work detailed in upcoming chapters, an electron multiplier-type detector was employed.

1.1.4 Electrospray Ionization

Electrospray ionization (ESI) generates ions through use of electricity.[30] Generation of ions by ESI is considered a three-step process involving the generation of charged solvent droplets, solvent evaporation, and the ejection of analyte ions from charged droplets.[30, 31] Nebulization, or the generation of charged solvent droplets, occurs when the analyte, in solution, passes through a capillary tube with a high voltage differential between it and the MS inlet.[30, 31] The result is a mist of charged solvent droplets that spray from the tip of the capillary tube and are directed into the mass spectrometer.[30, 31] Charged droplets travel in to the MS inlet, which is kept at an elevated temperature, resulting in the evaporation most of the solvent droplet. The loss of solvent from the charged droplets results in droplet instability due to high surface charge density.[30, 31] This results in the ejection of analyte ions into the gas phase, where they are analyzed.[30, 31]

Though only developed recently, ESI has found a home in analytical chemistry, especially as an ionization technique paired with mass spectrometry. Applications of ESI span across fields including mechanism studies, clinical applications, drug discovery, environmental studies, and forensic science.[32-36] The widespread application of ESI to many areas of research led to the development of further ionization techniques based on the general electrospray ionization process.

1.2 Ambient Ionization

Ambient ionization refers to the ionization technique, typically used with MS, where samples undergo limited to no sample preparation prior to ionization.[37, 38] In limiting or, in some cases, eliminating the sample preparation process, ambient ionization techniques are generally faster and simpler than LC/GC separation techniques coupled with MS. Though ambient ionization techniques only date back to the early 2000s, their use in chemical research has become prolific, with applications including clinical research, environmental chemistry, food safety, forensics, and more.[37-39] Ambient ionization techniques generally fall under one of the following classifications: liquid extraction-based or plasma-based desorption.[37] Like the name suggests, liquid extraction ambient ionization techniques use a liquid to desorb the analyte from a solid or liquid matrix.[37, 40] Liquid extraction ambient ionization techniques include methods like desorption electrospray ionization (DESI) and paper spray ionization (PSI).[37] Plasma-based ionization techniques utilized plasma, generated through electrical discharge, to ionize analyte molecules.[37, 41] The best-known plasma-based ionization technique is direct analysis in real time (DART) ionization. Plasma-based techniques are often applied to the analysis of volatile and semi-volatile compounds.[41]

1.2.1 Paper Spray Ionization

One of the ionization methods developed using an electrospray-like process is paper spray ionization (PSI). With paper spray ionization, a liquid sample is spotted on a paper that is cut to a sharp tip. The paper is then saturated with a solvent, commonly referred to as a spray solvent.[42] Through the application of a spray solvent, analyte molecules are solubilized and able to travel to the paper tip for ionization.[43] Similar to the electrospray process, ions are generated through the application of a high voltage to the saturated paper.[44] Most papers utilized for paper spray are cellulose-based and conduct an electric current when wetted.[44] If a sufficiently high voltage is applied between the paper and the MS inlet, an electrospray is formed at the tip of the paper near the inlet.[44] Subsequent steps of solvent evaporation and the expulsion of charged ions are identical to the electrospray process. This relatively simple process is depicted in Figure 1.2.

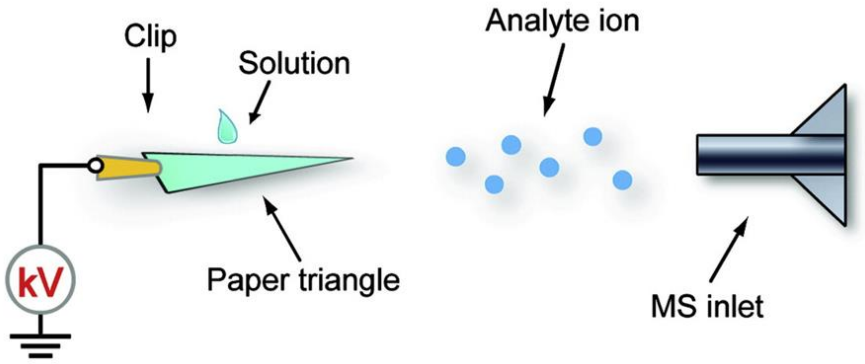

Figure 1.2 Schematic for paper spray ionization coupled with mass spectrometry.[44]

While the ionization process mirrors that of ESI, PSI does offer some unique advantages. First, by using a paper substrate, PSI can be used for the analysis of complex matrices, such as blood, without undergoing the extraction steps frequently required for ESI. Instead, the analyte is extracted from the paper by way of the spray solvent, leaving behind unwanted matrix components.[45] PSI is also able to reduce contamination and carryover concerns through the use of disposable cartridges.[44-47] PSI allows for samples to be prepared and stored on paper prior to analysis. Because of the simplicity of paper spray, the different aspects of the setup including the paper type, distance from the inlet, angle of the paper tip, and spray solvent can be tailored to the specific analyte(s) of interest. Aspects of the paper such as pore size have been demonstrated to affect ionization efficiency and recovery with smaller pore sizes resulting in increased ionization efficiency.[48] The position of the paper tip relative to the inlet impacts analyte signal with the appropriate tip distance being dependent on ionization polarity, voltage, and paper substrate.[44, 49] The angle by which the paper tip is cut plays a role in PSI with larger angles requiring high voltages to achieve ionization.[50] Selection of an appropriate spray solvent is also crucial as the spray solvent serves to both extract the sample from the paper and to ionize, so the solubility of analyte(s) along with the solvents ability to contribute to ionization should be considered.[51, 52] Overall, by modifying various parameters, PSI displays great versatility in the compounds it has been used to analyzed.

Paper spray-mass spectrometry (PS-MS) was originally developed for the analysis of crude biofluids but has since branched into a variety of fields.[44, 53] In the clinical field, PS-MS has been used to analyze complex biological matrices including blood and tissue biopsies.[50, 54] Because of

the simplicity of sample preparations and rapid data collection, PS-MS has been studied for therapeutic drug monitoring as well as point-of-care applications.[55, 56] PS-MS has also been applied to environmental chemistry for the detection of herbicides and chemical warfare agents.[49, 57] Paper spray has also been combined with other techniques to improve or expand capabilities. To increase sensitivity, solid phase microextraction (SPME) preconcentration steps have been introduced.[58] Paper spray-mass spectrometry has also been combined with surface-enhanced Raman spectroscopy (SERS) for illicit drug identification.[59]

While much of the work with PS-MS remains focused on clinical applications, it has also made a name in forensic chemistry. PS-MS has been used for the detection of food and drink adulterants including illegal food dyes and contaminants.[60, 61] In the realm of questioned documents, PS-MS has been used to discriminate between inks in authentic and fraudulent signatures and between genuine and counterfeit bank notes.[62, 63] The analysis of trace explosives by PS-MS with benchtop and portable mass spectrometers has also been demonstrated.[64, 65]

The most expansive applications of PS-MS to forensic science are in seized drugs. The detection of traditional drugs of abuse, such as cocaine and heroin, along with their adulterants, by PS-MS has been demonstrated.[66-69] PS-MS has be used for the detection and semi-quantitation of marijuana components, Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[70] Many recent PS-MS studies have focused on newer drug classes, specifically synthetic opioids, like fentanyl and fentanyl analogs, and cannabinoids.[68, 71-74]

1.3 Pressure-Sensitive Adhesives

As previously indicated, a variety of different paper types can be used as a substrate for PS-MS. The most commonly used paper for PS-MS is chromatography paper.[45] One of the substrates used throughout this work is pressure-sensitive adhesive (PSA) lined papers. Pressure-sensitive adhesives are a class of adhesives that form a bond with a surface without the need for heating or waiting for solvent evaporation.[75] By adhering to a surface through pressure instead of through chemical reactions, these adhesives are removable and repositionable.[75, 76] PSAs date to the 1800s; however, they didn't enter the market as commercial products until the mid-1900s.[77] Commercialization of PSAs led to their integration into a variety of markets where they are commonly found linings tape, stickers, and stationery.[78]

In application to PS-MS, PSA lined papers were chosen as substrate for sample collection and PSI. Because PSAs do not form chemical bonds to a surface, there are no alterations to the surface being sampled. This allows for PSAs to be used to recover trace materials from surfaces without altering the trace particulates. The PSA lined paper used throughout this work is a water-based acrylate polymer with microspheres.[76, 79]

Previous work with PSA paper and PS-MS demonstrated the use of PSA paper for the recovery of drugs of abuse when present on a variety of different surfaces before detection by PS-MS.[69] This work established that the PSA paper was capable of generating ions, could be used to detect drugs of multiple classes, and was capable of recovering drugs from different surfaces such as aluminum, cloth, and asphalt.[69] This work also examined detection limits for the compounds off different surfaces and demonstrated that it could be used with portable instrumentation.[69] Work outlined in subsequent chapters focuses on additional ways in which this PSA paper can be utilized for the recovery and detection of drugs of abuse.

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CHAPTER 2. COMBINING PRESSUMPTIVE COLOR TESTS, PRESSURE-SENSITIVE ADHESIVE-BASED COLLECTION, AND PAPER SPRAY-MASS SPECTROMETRY FOR ILLICIT DRUG DETECTION

2.1 Abstract

Illicit drug trafficking and abuse is a significant public safety and health concern. Color tests are commonly used for drug screening, but their poor specificity can result in false positives. This study demonstrates the combination of drug residue collection using pressure-sensitive adhesive paper, on-paper color testing, and post-reaction analysis by paper spray mass spectrometry on a benchtop ion trap mass spectrometer. All steps, including residue collection, color testing, and paper spray analysis, were performed on the same piece of paper. Three common color tests were investigated: the cobalt thiocyanate test for cocaine, the Simon test for methamphetamine, and the Marquis test for phenethylamine stimulants and opiates. The detection threshold for color tests ranged from 1.25 to 10 µg on paper. The stability of the color test products was assessed through a time study. Drug residues could be detected by MS at least 24 hours after reaction. A series of realistic samples, including false positives, were analyzed to demonstrate the technique's utility in real-world scenarios. Overall, combining color tests with PS-MS offers a rapid, low-cost method for the collection and analysis of illicit drugs.

2.2 Introduction

In 2022, the United States spent $39.3 billion on drug control and enforcement.[1] Of these funds, many were used to combat the transport of illicit drugs into the U.S. via land, sea, and air borders.[1] During the 2022 fiscal year, the U.S. Customs and Border Protection reported seizures of illicit drugs totaling over a half a million pounds.[2] Cocaine, methamphetamine, and heroin, three widely abused illicit drugs, accounted for the majority of non-plant material-containing drug seizures in 2022.[2] Worldwide, the trafficking and seizures of cocaine, methamphetamine, and heroin remain second only to cannabis.[3] As the prevalence of illicit drugs worldwide remains high, there is a need for affordable and simple drug detection methods.

One of the simplest methods for rapid, fieldable drug detection is presumptive color testing. Color tests are conducted by adding a few drops of reagent(s) to an unknown sample to produce a

distinctive color change. These color tests, also referred to as spot tests, are commonly used for presumptive identification of illicit substances by both forensic laboratories and law enforcement personnel.[4] The simplicity of these tests alongside their low cost per test make them desirable options for in-field drug identification.[5] A variety of microchemical tests have been developed over the years targeting specific drugs or drug classes including cocaine, methamphetamine, and heroin.[4] In this work, three color tests will be examined, an acidified cobalt thiocyanate test for cocaine, the Simon test for methamphetamine, and the Marquis test for phenethylamines and opiates.[6] The cobalt thiocyanate test produces a blue precipitate in the presence of cocaine from the formation of a colored cobalt complex (Figure 2.1A).[7, 8] When in contact with methamphetamine, the two-step Simon test produces a dark blue precipitate known as the Simon-Awe complex, with the first step producing an enamine that is hydrolyzed to reach the final product (Figure 2.1B).[6, 9] The Marquis reagent, which is one of most commonly used reagents, reacts with numerous classes of drugs to produce various colored products (Figure 2.1C-D).[4] A dark purple product is produced when reacting with most opiates while a red-to-orange color is obtained when the Marquis reagent reacts with amphetamine and methamphetamine.[4] Formation of a colored product is thought to be the result of the dimerization of the drug molecule in the presence of formaldehyde and a strong acid.[6, 10]

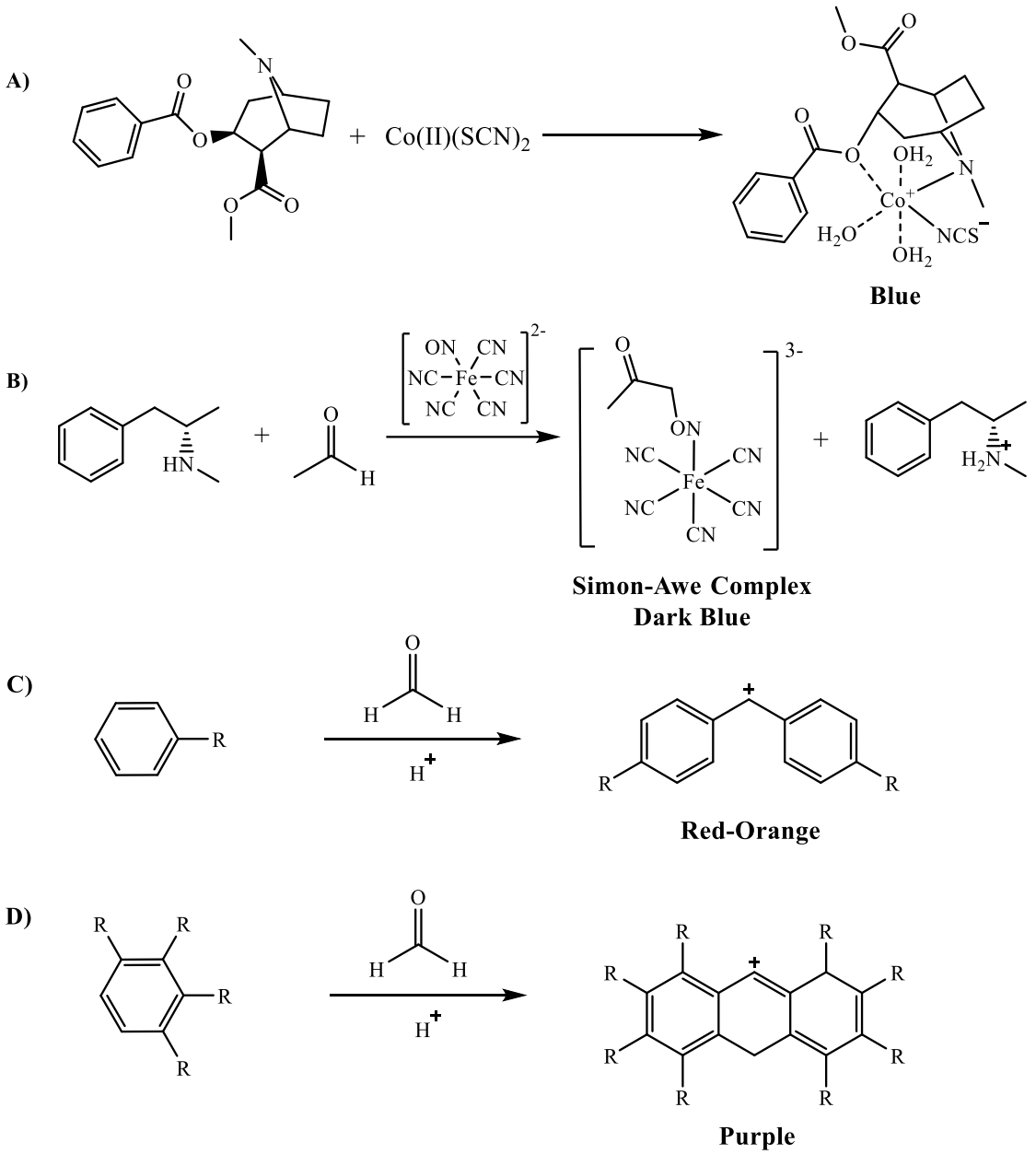

Figure 2.1. Generally accepted reaction mechanisms for color tests, A) Cobalt thiocyanate test with cocaine, B) Simon test with methamphetamine, C) Marquis test with phenethylamines, and D) Marquis test with opiates.

While color tests are rapid, sensitive, and affordable, there remain concerns for their use in drug identification.[11] The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) classifies color tests as a Category C presumptive analytical technique.[12] The SWGDRUG Category A-C classification system is based on the "maximum potential discriminating power" of each techniques, with Category A techniques being the most

discriminating and Category C being the least.[12] Because of the lack of specificity exhibited by color tests, scientists and the public alike are concerned about false positives that occur.[13] Falsely positive color tests have led to arrests and prison time on numerous occasions.[14-17] Steps have been taken to improve the specificity of color tests; however, these improvements often involve increasing the number of steps in a test or increasing the number of different tests that should be conducted.[18, 19] While these improvements do help address color test issues, a confirmatory technique is still required for true drug identification.

In this work, we propose the use of color tests on PSA paper followed by PS-MS analysis for a low-cost drug residue collection and identification technique. In contrast to color tests, mass spectrometry is a SWGDRUG Category A confirmatory technique because of its superior discriminating power.[12] In combining color tests with PS-MS, both presumptive and confirmatory data can be collected from the same sample.

The simplicity and use of affordable paper keeps costs of PS-MS analysis low. The type of paper substrate used for PS-MS can also vary. Broad classes of drugs including cocaine, phenethylamines, synthetic cannabinoids, and opiates (including fentanyl and its analogs) have been analyzed by PS-MS.[20-24] Applications of PS-MS to drug identification have led to its use in harm reduction and drug checking programs.[25, 26]

The use of PSA paper offers an affordable means for sample collection as well as a substrate for performing color tests and PS-MS. The cobalt thiocyanate, Simon, and Marquis tests were examined because of their common use in forensic chemistry. Color changes on PSA paper verses traditional spot well dishes were compared. Characteristics such as sensitivity and stability of color tests were assessed. This technique was also applied to realistic drug consumption scenarios to demonstrate the versatility of the method.

2.3 Materials and Methods

2.3.1 Materials

Solid forms of cocaine hydrochloride and (+)-methamphetamine hydrochloride (MAMP) were purchased from Sigma Aldrich (St. Louis, MO, USA) through the University's DEA license. Lidocaine, methamphetamine-d11, cocaine-d3, and heroin-d9 standards were purchased from Cerilliant at 1 mg/mL concentrations (Round Rock, TX, USA). Street purity samples of cocaine,

methamphetamine, and heroin were obtained from Indiana University-Purdue University Indianapolis's training materials. Ammonium thiocyanate, sodium carbonate, and LC-MS grade methanol (MeOH), water, acetonitrile (ACN), hydrochloric acid, formic acid (FA), formaldehyde, and acetaldehyde were purchased from Thermo Fischer Scientific (Rochester, NY, USA). Cobalt chloride was acquired from MP Biomedicals (Solon, OH, USA). Sodium nitroferricyanide dihydrate was purchased from Acros Organics (New Jersey, USA). White PSA paper (Super Sticky Post-It Notes, 3M) and table sugar were purchased from commercial retailers. Freebase cocaine was obtained from base extraction of the cocaine hydrochloride standard.

2.3.2 Preparation of Standards

Cocaine hydrochloride, MAMP, street heroin, and lidocaine, and were weighed and dissolved in water to a final concentration of 10 mg/mL except for lidocaine at 5 mg/mL. To promote solubility the street heroin solution was acidified with 0.1M HCl. Freebase cocaine was prepared at 5 mg/mL in methanol. Solutions of cocaine hydrochloride, MAMP, and street heroin were serially diluted in water to reach desired concentrations for sensitivity experiments. The freebase cocaine standard was diluted in methanol to reach desired concentrations. When spotting solutions on PSA paper and glass, a single 1 µL aliquot of standard solution was used to minimize dispersal throughout the PSA paper.

2.3.3 Color Test Reagents

All color reagents were prepared according to the 2021 Indiana State Police Drug Unit test methods.[27] An aqueous solution of 2% cobalt thiocyanate (6.8% cobalt chloride and 4.3% ammonium thiocyanate) was acidified with 0.1M HCl to produce the acidified cobalt thiocyanate reagent for use on salt and freebase cocaine.[28] An approximate 2% solution of formaldehyde in concentrated sulfuric acid was prepared for the Marquis reagent. The two-step Simon test was prepared with an aqueous solution of 2% sodium nitroferricyanide and acetaldehyde for Solution A. Solution B was comprised of a 2% aqueous solution of sodium carbonate. The two reagents of the Simon test were stored separately, and Solution A refrigerated until use. All color test reagents were verified monthly according to the Indiana State Police Laboratory Drug Unit Test Methods.

2.3.4 Paper Spray-Mass Spectrometry with PSA Paper

Commercial PSA paper tips were prepared by cutting five layers of PSA paper into 0.5 cm by 1 cm rectangles. Pressure-sensitive adhesive was present on only one face of the tip, covering approximately three quarters of the rectangular paper. A triangular tip with an angle of approximately 45° was cut into the portion of the paper lacking adhesive (Figure 2.2A). Immediately prior to residue collection, the top and bottom layer of paper were discarded leaving a 3-layer tip for collection and analysis. Discarding the outermost layers reduced the potential for contamination that could result from storage or handling. Three layers were used for analysis to provide additional support when dabbing a surface for sampling and to reduce curling of the tip during analysis.

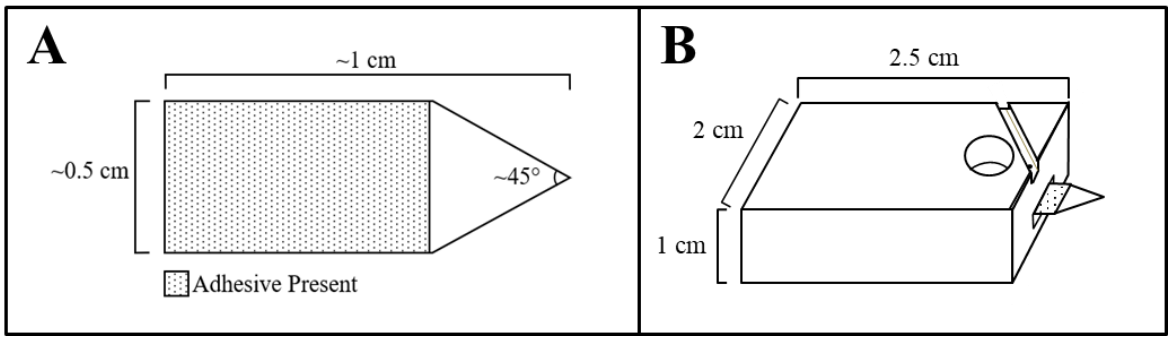

Figure 2.2 Pressure-sensitive adhesive PS-MS requirements. (A) Diagram and dimensions of PSA tip. (B) Diagram of 3-D printed cartridge for PS-MS.

When PSA tips were used for surface sampling, the adhesive-coated portion of the tip was firmly pressed against the surface before lifting for surface recovery. For sampling larger areas, a PSA tip was used to sample the surface three times in different locations. When the cobalt thiocyanate or Simon tests were conducted on paper, 5 µL of each reagent were added to the paper. Reagent volume was reduced to 3 µL for the Marquis test.

Following the addition of color test reagents, the PSA tip was placed in a 3D-printed polypropylene cartridge, adhesive side up. The cartridge (Figure 2.2B) was printed with a channel for connecting high voltage via a wire and a well above the paper slot for spray solvent application. Three different spray solvents, 90:10 MeOH:H2O with 0.1% FA, 0.1% FA in ACD, and 70:30 ACN:MeOH with 0.1% FA were used for PS-MS analysis depending on analyte. For experiments

on the benchtop mass spectrometer, 70 µL of spray solvent were applied slowly for 5-10 seconds. Data collection was carried out for 0.7 minutes with voltage application of +4.5 kV for 30 seconds. Both full MS and MS/MS spectra were collected. The workflow for the use of PSA paper for drug recovery, followed by color test screening, and PS-MS confirmation is illustrated in Figure 2.3.

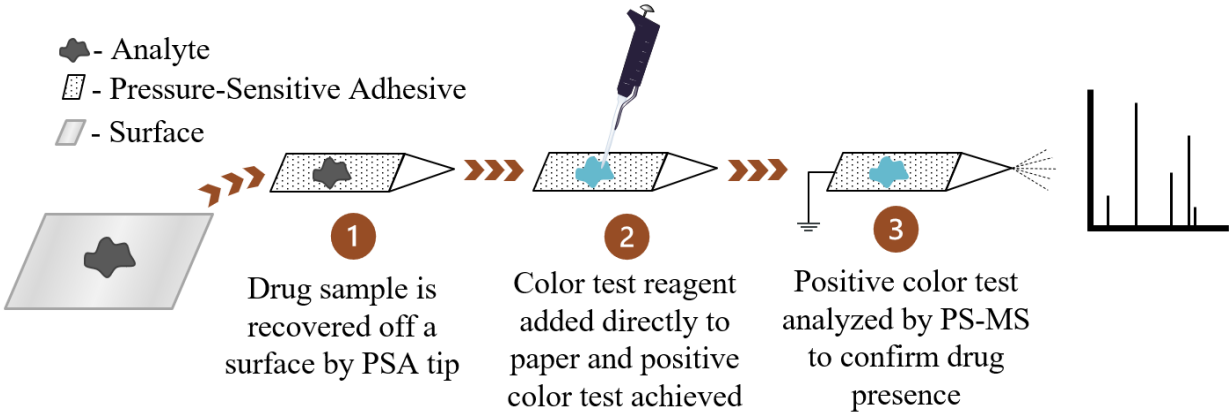

Figure 2.3 Sampling and analysis workflow for use of PSA paper for sample recovery, color testing, and PS-MS for drug identification

All spectral data were collected on a Thermo LTQ-XL linear ion trap benchtop mass spectrometer (Thermo Scientific Inc., San Jose, CA). Instrument parameters are detailed in Table 2.1.

Table 2.1 Benchtop mass spectrometer parameters for full MS and MS/MS analyses

Analyte	**Molecular Weight (g/mol)**	**Precursor Ion (*m/z*)**	**Product Ion (*m/z*)**	**Collision Energy (eV)**	**Deuterated Standard**
Cocaine	303.25	304.25	182	35	D3
Methamphetamine	149.23	150.08	119	50	D11
Heroin	369.42	370.25	268	30	D9

2.3.5 Data Analysis

The threshold for color tests was determined based on visual color changes. The threshold was defined as the lowest amount of analyte by which a positive color test was achieved for five replicate samples. MS signal-to-blank (S:B) ratios for color tests and controls were calculated by

dividing the area under the curve (AUC) for drug-containing samples by the AUC for blank samples. For color test stability experiments, the reported analyte signal was calculated by dividing the AUC of the analyte by that of its internal standard. The internal standards were dissolved in the spray solvent at a concentration of 10 parts-per-million (ppm, mass to volume) for all color test stability experiments. Five trials were conducted for most experiments unless otherwise noted.

2.3.6 Color Test Stability

To examine the impact of time on analyte signal, color tests were performed directly on PSA paper. Excess reagent was removed from the tip immediately following color formation, by using a Kimwipe to absorb the surplus. Samples were aged for up to 24 hours. PS-MS analysis was conducted immediately following the reaction (t = 0 hour) and at 1, 6, and 24 hours after reagent addition. For these experiments, 10 µg/mL deuterated internal standards were included in the spray solvent to improve comparison across time points.

2.3.7 Realistic Drug Sampling Scenarios

Three potential encounters with drugs of abuse were simulated with street grade samples of cocaine, heroin, and methamphetamine. A vacuum setup and a pre-screened dollar bill were used to simulate nasal insufflation of a line of cocaine (~40 mg) off aluminum. PSA tips were used to dab the dollar bill, aluminum surface, and the fingertips of a glove that was swiped across both sides of the bill. A metal spoon, syringe, cotton ball, and flame were used to simulate the preparation of ~1-2 mg samples of heroin and methamphetamine for injection. The spoon (or cooker), needle, and syringe stopper were sampled by PSA paper.

2.4 Results and Discussion

2.4.1 Method Development

The acidified cobalt thiocyanate, Simon, and Marquis tests were selected for analysis because of their prevalence in presumptive drug testing. Both methamphetamine and heroin were utilized for the Marquis test because it is frequently used to screen for methamphetamine and opiates, turning orange-red with amphetamines and purple with opiates.[11] Color test reagents

spotted directly on PSA paper without the drug residues did not result in a color change. Reagent blanks on PSA paper together with positive control PSA papers spiked with 10 µg of drug are shown in Figure 2.4.

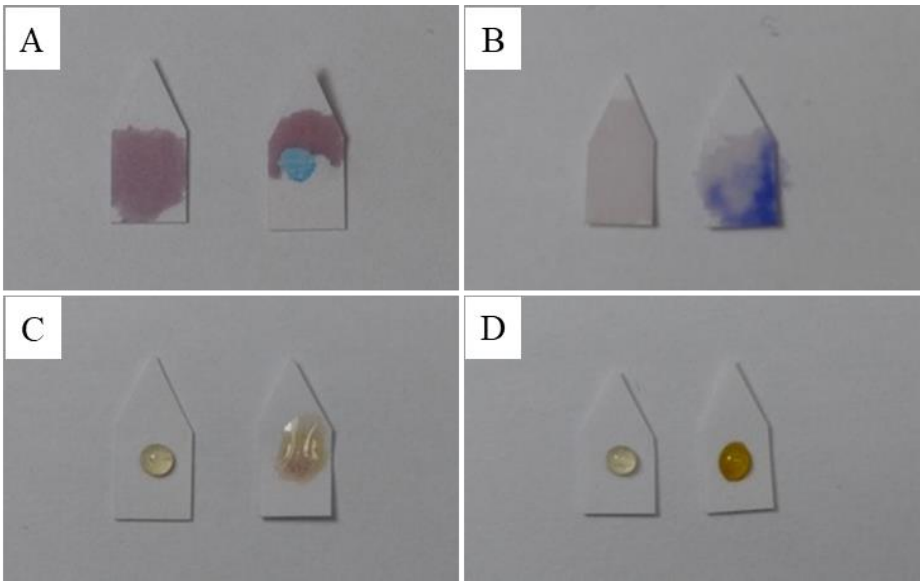

Figure 2.4 Comparison of reagent blanks (left) and positive color tests (right) on PSA-lined paper, A) Cobalt thiocyanate test with and without 10 µg of cocaine HCl, B) Simon test with and without 10 µg of methamphetamine, C) Marquis test with and without10 µg of street purity heroin, and D) Marquis test with and without 10 µg of methamphetamine.

A side-by-side comparison of a positive color tests on PSA paper and in a traditional spot well dish was conducted to determine if performing the reaction on PSA paper altered the color produced by the test (Figure 2.5). All color changes performed on the PSA paper were consistent in color with the spot dish. Notably, the color change for the cobalt thiocyanate and Marquis tests was mostly localized to where the analyte solution was deposited (Figure 2.5A, C, and D). The color change for the Simon test was less localized (Figure 2.5B), likely due to the Simon test reagents absorbing more readily into the PSA paper than other reagents.

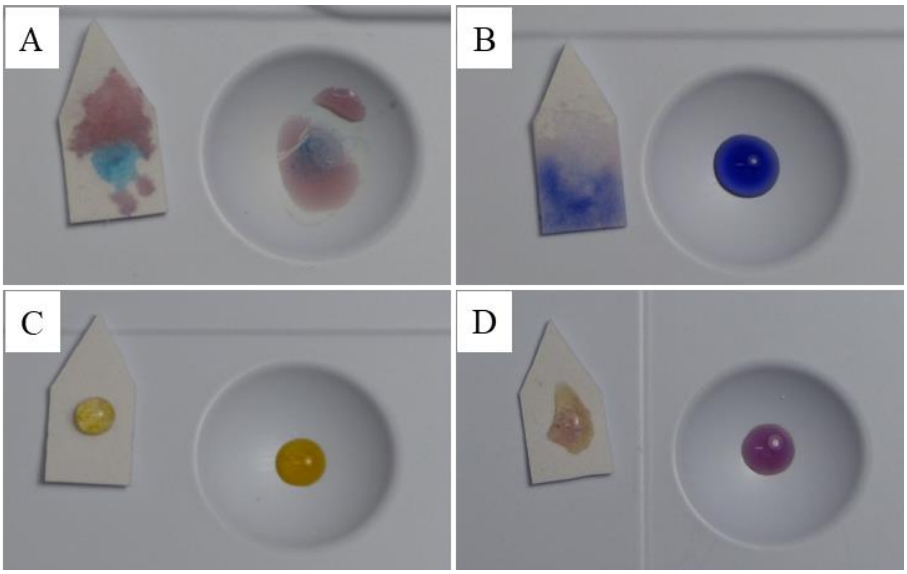

Figure 2.5 Comparison of color tests on PSA-lined paper (left) and traditional spot well dish (right). (A) 10 µg of cocaine HCl reacted with acidified cobalt thiocyanate reagent, (B) 10 µg of methamphetamine reacted with Simon reagents, (C) 10 µg of methamphetamine reacted with Marquis reagent, and (D) 10 µg of street heroin reacted with Marquis reagent.

Paper spray MS and MS/MS spectra were collected from the PSA tips following a positive color test (Figure 2.6). Peaks at 257, 428, and 456 *m/z* were noted as background peaks from the PSA paper. These peaks were visible on both unreacted PSA paper as well as following reactions with cobalt thiocyanate and Simon reagents, but not after treatment with the Marquis reagent. Peaks from cocaine were detected in both full MS and MS/MS following a positive acidified cobalt thiocyanate test on PSA paper (Figure 2.6A-B). Similarly, methamphetamine was clearly identified by full MS and MS/MS following a positive color test with both the Simon and Marquis tests (Figure 2.6C-F). When the Marquis test was applied to street purity heroin (370 *m/z*), the precursor ion was obscured in full MS analysis (Figure 2.6G). The interference likely resulted from the concentrated sulfuric acid and formaldehyde found in the Marquis reagent. Despite interference in the full MS spectra, the MS/MS spectrum at 370 *m/z* yielded peaks at 268, 310, and 328 *m/z* all of which are indicative of heroin (Figure 2.6H). Additional peaks were also present in the MS/MS spectrum that do not correspond to heroin.

For each color test and analyte, three common spray solvents to PS-MS were assessed for test optimization, 90:10 MeOH:H_2O with 0.1% FA, 0.1% FA in ACN, and 70:30 ACN:MeOH with 0.1% FA. Overall analyte signal and stability were considered. For the cobalt thiocyanate test,

90:10 MeOH:H_2O with 0.1% FA was used. The other solvents were excluded from consideration because the cobalt thiocyanate reagent turns blue in the presence of ACN.[29] For the detection of methamphetamine following the Simon or Marquis tests, 0.1% FA in ACN was used. The Marquis test for heroin utilized 70:30 ACN:MeOH with 0.1% FA. For scenarios where simplicity is valued over optimal MS performance, viable detection could be achieved for all cases with 90:10 MeOH:H_2O with 0.1% FA.

No reaction products were identified by PS-MS for any of the color tests. The blue cobalt complex formed with cocaine for the cobalt thiocyanate test was not detected. Likewise, the Simon-Awe complex, which produces the dark blue color for the Simon test, was not detected. No reaction products, including drug dimers, were detected for either of the Marquis test variations.

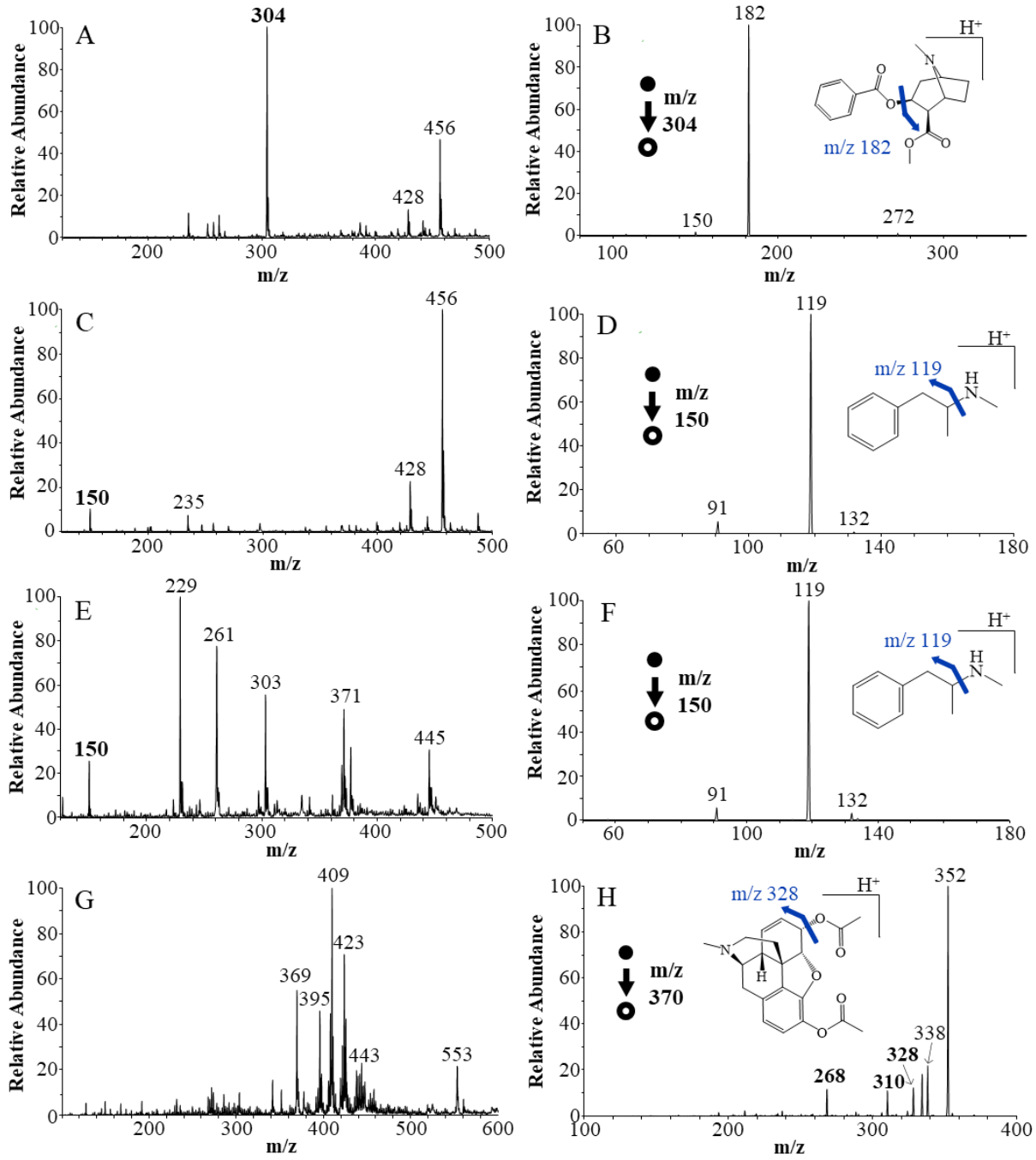

Figure 2.6 Mass spectra for the PSA PS-MS following color tests on benchtop mass spectrometer. A) Full MS of 1.25 µg of cocaine reacted with acidified Co(SCN)2, B) MS/MS of 1.25 µg of cocaine reacted with acidified Co(SCN)2, C) Full MS of 1.25 µg of MAMP reacted with Simon reagents, D) MS/MS of 1.25 µg of MAMP reacted with Simon reagents, E) Full MS of 10 µg of MAMP reacted with Marquis reagent, F) MS/MS of 10 µg of MAMP reacted with Marquis reagent, G) Full MS of 5 µg of street heroin reacted with Marquis reagent, and H) MS/MS of 5 µg of street heroin reacted with Marquis reagent.

2.4.2 Detection Thresholds

The sensitivity of color tests and their impact on MS analysis of the method was assessed. The sensitivity of the color tests, reported as threshold amounts, on PSA paper are shown in Table 2.2. Threshold amounts were defined as the lowest amount of analyte in which five consecutive color tests elicited a positive result. The threshold amounts required for a positive color test ranged from 1.25 to 10 µg when analytes were deposited directly on PSA paper. The threshold amount for positive color tests for recovery of analytes off a glass pane using the PSA paper was also determined (Table 2.2).

Table 2.2 Threshold analyte amount required for a positive color test following direct addition of the analyte to the paper or recovery from a glass pane using the PSA paper

Drug	Color Test	Direct Addition	Recovery off Glass
Cocaine HCl	Cobalt thiocyanate Test	1.25 µg	5 µg
Freebase Cocaine	Cobalt thiocyanate Test	5 µg	30 µg
Methamphetamine	Simon Test	1.25 µg	2.5 µg
Methamphetamine	Marquis Test	10 µg	5 µg
Street Heroin	Marquis Test	5 µg	10 µg

Generally, the color test threshold increased when recovering drug residue from glass, likely due to incomplete recovery. An exception to this trend was the Marquis test with methamphetamine, where the threshold amount decreased when recovering the residue off glass pane. This decrease may be attributed to the analyte remaining localized to the paper surface, allowing for better interaction between the reagent and analyte, though this is not reflected in the street heroin variation of the Marquis test.

The signal-to-blank ratio for MS analysis after use of color test reagents was also determined (

Figure 2.7). The signal for the analyte signal was ratioed to its signal in blank sample for standardization. The addition of color test reagents to PSA paper caused a decrease in the signal-to-blank ratio for all color tests. There was both an increase in the blank signal as well as a decrease in analyte signal for the acidified cobalt thiocyanate and Marquis tests. For the Simon test, the analyte signal decreased while the change in the blank signal was negligible. Despite the decreased S:B, PS-MS was still capable of detecting drugs at the color test threshold.

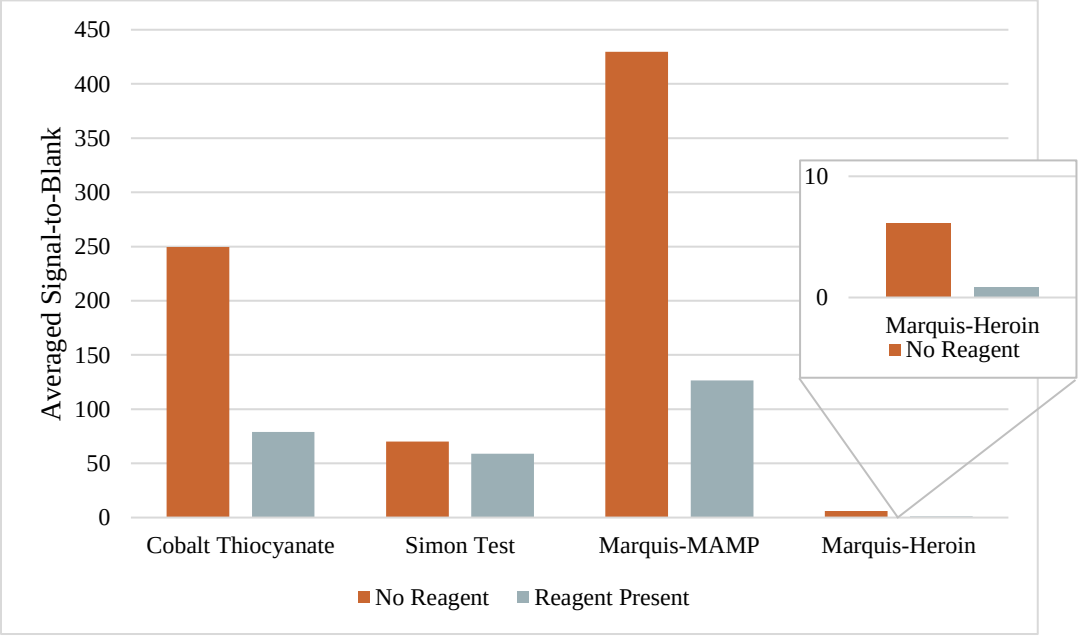

Figure 2.7 Averaged signal-to-blank ratio at threshold amount of analyte with and without color test reagent.

2.4.3 Time Interval between Color Test and MS Analysis

Color test results are usually only considered accurate within a short time period, typically a few minutes.[4] MS analysis, on the other hand, may need to be delayed for practical reasons. We assessed the MS signal at different time points after addition of the color test reagents. Over a 24-hour period after addition of the acidified cobalt thiocyanate test, there was little to no signal depreciation for cocaine. At 24-hour post reagent addition, over 90% of initial cocaine signal was retained (Figure 2.8). The original blue color generated by the test was lost within the first hour,

with the whole PSA tip turning blue due to dried reagent. For both methamphetamine and heroin, approximately half of the analyte signal was lost 24 hours after reaction with the Marquis reagent (Figure 2.8). Similar to the cobalt thiocyanate test, the color was not maintained at 1 hour. The Simon test with methamphetamine experienced the greatest lost in analyte signal, with only 8% of the methamphetamine MS signal intensity remaining after 24 hours (Figure 2.8). Most of the signal loss occurred within the first hour after solvent addition. Much like the other color tests, the dark blue color produced by the Simon test did not persist at 1 hour following reagent addition. The greater decrease in methamphetamine signal for the Simon test in comparison to the Marquis test may result from the reagent interactions with the PSA paper. The Simon reagents wicked readily into the paper, limiting the amount of excess reagent that could be removed after a positive color test. In contrast, the Marquis reagent did not elute through the paper, allowing for the removal of more excess reagent after a positive color test.

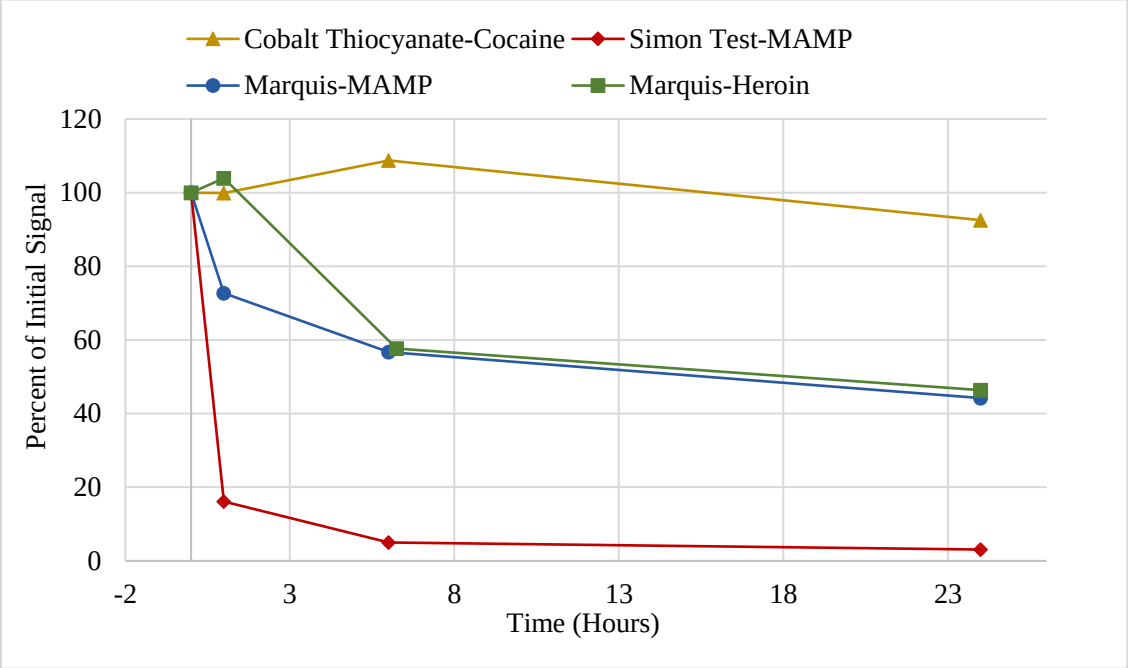

Figure 2.8 Percent of original analyte signal over 24 hours from addition of reagent to PSA paper.

Despite decreases in analyte signal for the Marquis and Simon tests, peaks in both full MS and MS/MS spectra remained detectable over the 24-hour period. For all tests, the appropriate color was no longer present at the one-hour mark. This is consistent with the accepted practice of

noting the initial color change within minutes of adding the reagent. Combined with MS data, this indicates that the color test can be used to prescreen a sample, noting the initial color change, and MS analysis can be conducted later, albeit with some loss of sensitivity.

2.4.4 Color Test False Positives

A main concern of color tests is their noted false positives.[14-16] The cobalt thiocyanate test has numerous false positives reported including lidocaine, diphenhydramine, procaine, and dibucaine.[18] Table sugar and aspirin are known false positives for the Marquis test.[11] PS-MS with the PSA paper after reaction affords much greater selectivity. The improved selectivity was demonstrated by using the reactions of lidocaine and table sugar (sucrose) with the cobalt thiocyanate and Marquis tests, respectively.

For the cobalt thiocyanate test, the lidocaine and cocaine color changes were visually indistinguishable (Figure 2.9A and C). Despite visual similarities, the two reactions are easily distinguishable by their mass spectra. Cocaine (304 *m/z*) was dominant in the true positive sample spectra but absent in the false positive sample (Figure 2.9A and C, respectively). Likewise, lidocaine (235 *m/z*) was prevalent in the false positive sample and absent in the true positive sample.

For the Marquis test, methamphetamine and sugar were compared (Figure 2.9B and D). An indistinguishable orange color was produced for both samples. In the true positive sample, methamphetamine's molecular ion (150 *m/z*) was present, and notably absent in the sugar sample. Possible ions for sucrose, such as $[M+H]^+$ and $[M+Na]^+$, were not visible in the full mass spectrum.

Analysis of false positives for the cobalt thiocyanate and Marquis test demonstrates the superior discriminating power of mass spectrometry. While PS-MS does not utilize a separation step for analysis, the MS/MS capabilities of the instrument allow for discrimination between compounds beyond the precursor ion. In combining color tests with PS-MS, concerns about color test specificity can be addressed.

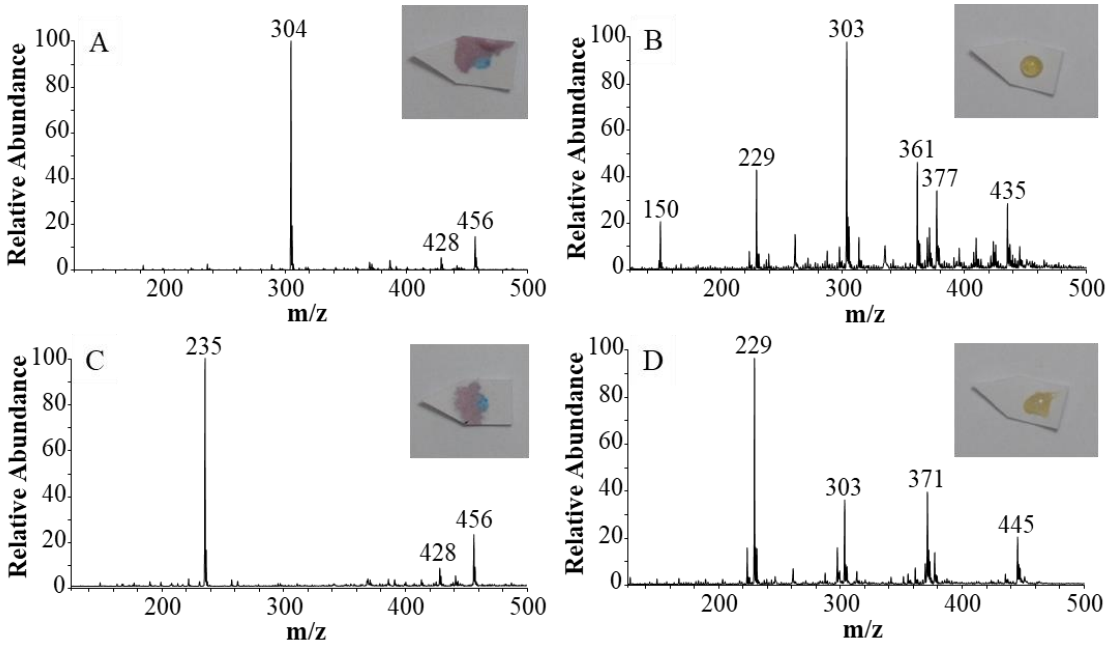

Figure 2.9 Comparison of true positive and false positive color tests by benchtop mass spectrometer, A) Cobalt thiocyanate test true positive with 5 µg cocaine HCl, B) Marquis test true positive with 5 µg methamphetamine, C) Cobalt thiocyanate test false positive with 5 µg lidocaine, and D) Marquis test false positive with 5 µg table sugar.

2.4.5 Realistic Samples

Several realistic drug residue samples were made to further assess the performance of color testing combined with PSA sample collection and PS-MS confirmation. Separate samples of street methamphetamine and heroin were heated with water in a metal spoon, drawn into a syringe, and then discarded. After drying, PSA paper tips were used to dab the spoon, needle, and syringe stopper, and the Marquis test was performed on recovered samples. For the methamphetamine samples, a positive color test was observed with all areas sampled, each of which were successfully confirmed by PS-MS (Table 2.3). Samples of street heroin recovered from the spoon, needle, and syringe underwent the same analysis as methamphetamine. Heroin was detected in samples from the spoon, but not from the syringe or needle (Table 2.3). Detection off the needle and syringe stopper is of little practical significance because many forensic labs do not accept syringes due to the risk of bloodborne pathogens. Street methamphetamine is typically significantly purer than heroin, which may explain the difficultly with heroin detection from two of the surfaces.[30] Also, realistic dosages for methamphetamine and heroin are greater than the 1-2 mg tested here.

Table 2.3. Summary of color test and MS results for the three realistic sampling scenarios

Drug	Location	Color Test Result	MS Result
Cocaine	Aluminum	Positive	Positive
	Dollar Bill	Positive (3/4)*	Positive
	Glove	Positive	Positive
Methamphetamine	Cooker	Positive	Positive
	Needle	Positive	Positive
	Syringe Stopper	Positive	Positive
Heroin	Cooker	Positive	Positive
	Needle	Negative	Negative
	Syringe Stopper	Negative	Negative

*- 1 out of 4 samples was negative

Detection of cocaine residues after simulated nasal insufflation of cocaine off an aluminum surface through a rolled dollar bill was also assessed. The aluminum surface, the dollar bill, and the fingers of a glove ran over the surface of the bill were sampled. The aluminum surface and glove samples indicated the presence of cocaine by color test and PS-MS. The prescreened dollar bill was sampled four times, twice on the front and twice on the back. Three of the four samples indicated the presence of cocaine by color test and PS-MS. One of the samples only indicated the presence of cocaine by PS-MS with a negative color test (Table 2.3). The single negative color test likely resulted from a non-uniform distribution of cocaine on the bill. Representative spectra for all realistic sampling conditions are presented in Figure 2.10.

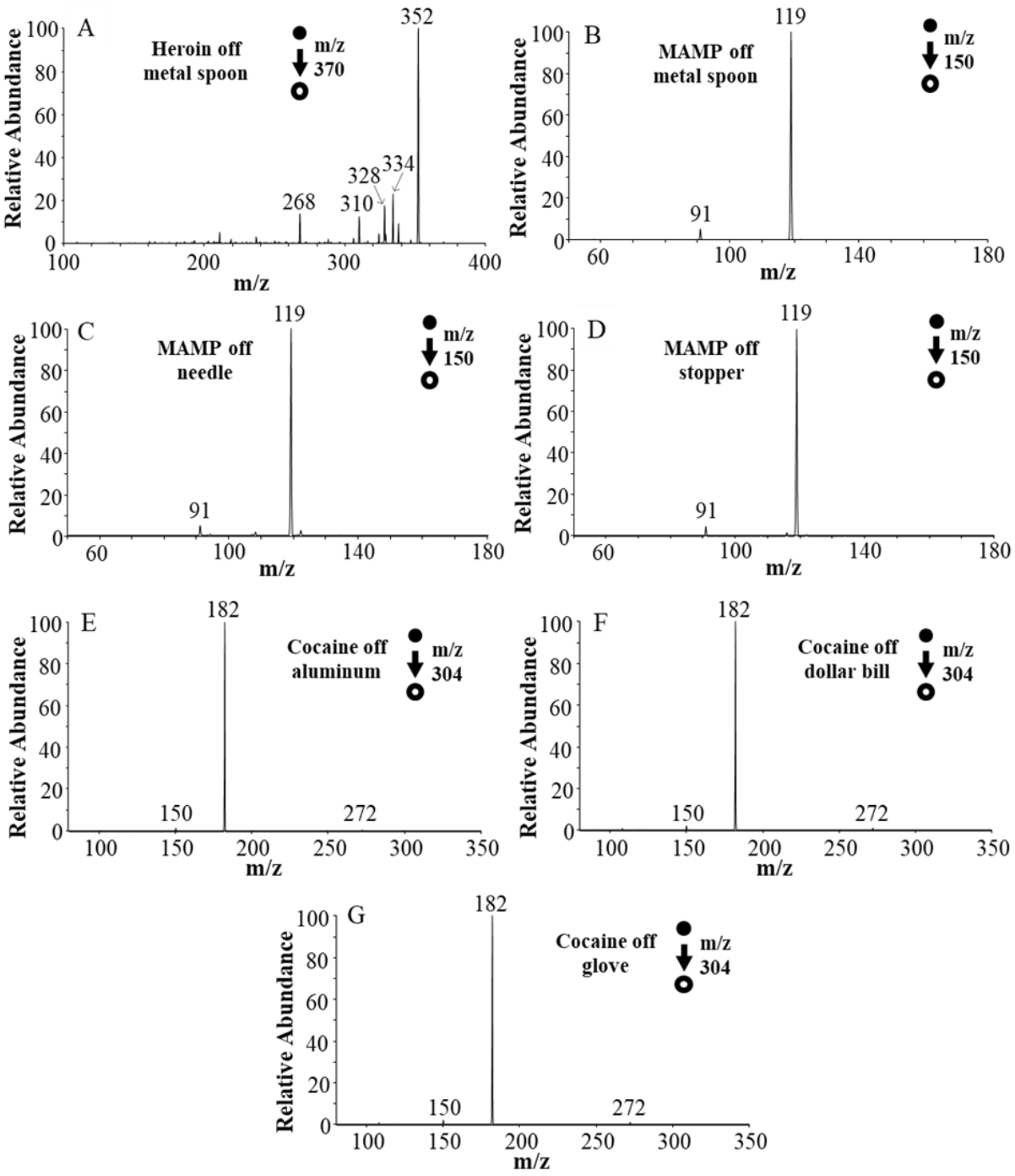

Figure 2.10 Representative MS/MS spectra of realistic sampling procedures, (A) Heroin recovered off metal spoon, (B) Methamphetamine recovered off metal spoon, (C) Methamphetamine recovered off needle, (D) Methamphetamine recovered off syringe stopper, (E) Cocaine recovered off aluminum surface, (F) Cocaine recovered off dollar bill, and (G) Cocaine recovered off glove wiped across the surface of the bill.

2.5 Conclusion

This research describes the combination of color tests with PS-MS for the detection of illicit drugs to gather both presumptive and confirmatory data from a single sample. The color change, sensitivity and stability of the cobalt thiocyanate, Simon, and Marquis tests on PSA paper were examined. Following color testing, cocaine, methamphetamine, and heroin were detectable by PS-MS for all tests. The amount of analyte required to achieve a positive color test on PSA paper for neat addition or recovery from a glass pane were reported to range from 1.25-10 µg and 2.5-30 µg respectively. At all color changes, the analyte of interest was detectable on the benchtop mass spectrometer. The impact of time on color test and PS-MS analysis was studied, and up to 24 hours after reagent addition the analyte was still detectable for all tests. This work was also applied to false positives and realistic drug consumption scenarios, which demonstrated the capabilities of the method. Overall, this work demonstrates a new technique for acquiring the color test data while alleviating specificity concerns through confirmatory analysis by PS-MS. In summary, by combining the affordability of color tests with the simplicity of PS-MS, this method allows for a quick, effective, and cheap method of drug identification that can be conducted on crude samples with the potential for in-field analysis.

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CHAPTER 3. PRESSURE-SENSITIVE ADHESIVES AND PAPER SPRAY-MASS SPECTROMETRY FOR THE COLLECTION AND ANALYSIS OF FENTANYL-RELATED COMPOUNDS FROM SHIPPING MATERIALS

3.1 Abstract

The rise of fentanyl and fentanyl analogs in the drug supply pose serious threats to public health. Much of these compounds enter the U.S. through shipping routes. Here we provide a method for fentanyl screening and analysis that utilizes pressure-sensitive adhesive (PSA) lined paper to recover drug residues from parcel-related surfaces. Mass spectral data were collected directly from the PSA paper by paper spray-mass spectrometry (PS-MS), where PSA paper served as both a sampling and analysis substrate. Seven fentanyl-related compounds were selected for analysis: fentanyl, 4-anilino-N-phenethylpiperidine (4-ANPP), N,1-diphenethyl-N-phenylpiperidin-4-amine (phenethyl-4-ANPP), valerylfentanyl, 4-fluoroisobutyrylfentanyl (4-FIBF), carfentanil, and *p*-fluorofentanyl. These compounds were recovered and identified by PSA paper combined with PS-MS from packaging tape and plastic at 100 ng and from cardboard and shipping labels at 50 ng. The impact of cutting agents on PS-MS analysis of fentanyl analogs was explored. No trends of analyte suppression were found in the presence of high concentrations of the cutting agents caffeine, diphenhydramine, and lidocaine when compounds were recovered from parcel-related surfaces. A cartridge that required no precise cutting of PSA paper prior to sampling or analysis was evaluated for its use in PS-MS for fentanyl screening. Recovery and detection of fentanyl from plastic sheeting was determined using this cut-free cartridge. The cut-free cartridge was somewhat less consistent with lower analyte signal than the standard cartridge, but performance was suitable for potential screening applications. In combining PSA surface sampling with PS-MS for drug screening, both sampling and detection of fentanyl-related compounds is simple, rapid, and low-cost.

3.2 Introduction

The rise of synthetic opioids since the early 2010s have led many to call these compounds the “third wave” of the U.S. opioid epidemic.[1] Of these synthetic opioids, fentanyl and its analogs have garnered widespread attention because of their potency and significant contribution to

overdose deaths.[2] While fentanyl has long been a Schedule II controlled substance under to the Controlled Substances Act, the emergence of its analogs has posed many challenges including the regulation and screening of these compounds.[3, 4] The Drug Enforcement Administration (DEA) has classified many of the emerging fentanyl analogs as Schedule I restricted substances; however, the frequency of occurrence and variety of new analogs have resulted in the emergency scheduling of fentanyl analogs as a broad class of "fentanyl-related substances."[4, 5] Combatting the continued rise of overdoses as the result of fentanyl and its analogs remains dependent on reducing their prevalence in the U.S. drug supply. The development of methods for early detection and supply prevention are crucial to reducing overdoses due to fentanyl and its analogs.

While fentanyl and a few of its analogs are legally manufactured and diverted for abuse, clandestinely manufactured fentanyl is responsible for most of the illicit fentanyl supply.[3, 6] The DEA has identified two major routes by which fentanyl enters the U.S., both of which rely on shipping.[7] The primary route for fully synthesized fentanyl and fentanyl analogs is through direct shipment of fentanyl compounds in small quantities from China to the U.S..[7-9] Synthetic precursors to fentanyl are also shipped to Mexico, as well as Canada and the U.S., for synthesis in clandestine laboratories.[7] These synthesized fentanyl analogs are trafficked into the U.S. across land borders.[7] In addition to these main routes, the shipping channels for fentanyl are expected to diversify in coming years, with fentanyl now originating in India and other nations.[7, 8, 10] Attempts to stem the flow of illicit fentanyl in the U.S. have resulted in increases in international shipping regulations; however, they have not resulted in a decrease in fentanyl in the U.S. drug market.[11, 12]

While fentanyl and its analogs remain pervasive in the U.S., developing methods to sample and detect these compounds, especially before they reach the consumer, is of utmost importance. Due to their high potency and the perceived risk for accidental exposure by first responders, the safe sampling of these compounds is imperative.[13-15] One demonstrated method for low-cost collection of drugs is the PSA lined papers.[16] These adhesives have been demonstrated to recover drug residues from a variety of surfaces and serve a substrate for analysis by PS-MS.[16] In addition to offering an affordable, all-in-one collection and analysis substrate, PSA paper also allows for sampling without direct human contact with the surface in question. In this work, the use of PSA papers combined with PS-MS was expanded to fentanyl-related compounds for their recovery from parcel-associated surfaces. The application of PSA paper to the recovery of fentanyl

compounds from parcel-related surfaces PSA was developed as a potential screening mechanism for fentanyl entering the U.S. by mail.

Paper spray-mass spectrometry has been previously applied to the detection of fentanyl and fentanyl analogs including detection in biofluids and bulk samples.[17-26] The combination of PS-MS with surface enhanced Raman spectroscopy (SERS) paper for fentanyl detection has been demonstrated, including on portable instrumentation.[21, 22, 27] Paper spray-mass spectrometry has also been applied to the detection of fentanyl analogs in casework-type samples including suspected illicit pharmaceutical tablets.[23, 24] Paper spray has also been used in a pilot drug checking program, specifically for the detection of fentanyl compounds, with the goal of harm reduction.[25, 26] Applications of PS-MS have largely been focused on the limited sample prep and quick analysis times offered by the technique. While sample preparation for PS-MS is simplified compared to techniques such as LC-MS, most published methods require the spotting of a prepared solution onto paper for analysis. With the use of PSA paper, these preparations steps are eliminated, streamlining the screening process.

While PS-MS has been utilized for fentanyl detection, many of these methods require additional sample preparation steps, in utilizing PSA paper for sampling these steps are eliminated, simplifying the sample workflow, and minimizing risks for accidental exposure. In this work, the recovery of a subset of common fentanyl-related compounds from four different shipping materials was determined. In addition to screening for fentanyl analogs, the impact of common cutting agents on fentanyl analog signal was evaluated. The use of a cartridge that did not require cutting of the paper tip for analysis by PS-MS was assessed and the performance compared to that of a previously developed cartridge that requires cutting the paper to a particular shape.[16]

3.3 Materials and Methods

3.3.1 Materials

Standards of *para*-fluorofentanyl, 4-anilino-N-phenethylpiperidine (4-ANPP), valerylfentanyl, and 4-fluoroisobutyrylfentanyl (4-FIBF) were obtained from the Traceable Opioid Material (TOM) Kits distributed by Cayman Chemicals through the University's DEA license (Ann Arbor, Michigan, USA). Also purchased from Cayman Chemicals was a standard of N,1-diphenethyl-N-phenylpiperidin-4-amine (phenethyl-4-ANPP). Fentanyl, carfentanil, lidocaine,

and diphenhydramine standards were purchased from Cerilliant (Round Rock, TX, USA). The various structures of fentanyl analogs are shown in Figure 3.1 with variations in structure indicated in green. Powdered caffeine standard was purchased from Sigma Aldrich (St. Louis, MO, USA). LC-MS grade solutions of MeOH, water, and FA were purchased from Thermo Fischer Scientific (Rochester, NY, USA). PSA paper (Super Sticky Post-It Notes, 3M) was purchased from a commercial retailer. Plastic packaging, packaging tape, cardboard, and shipping labels were recovered from shipped items. Both packaging tape and shipping labels were layered on cardboard to simulate actual use in shipping.

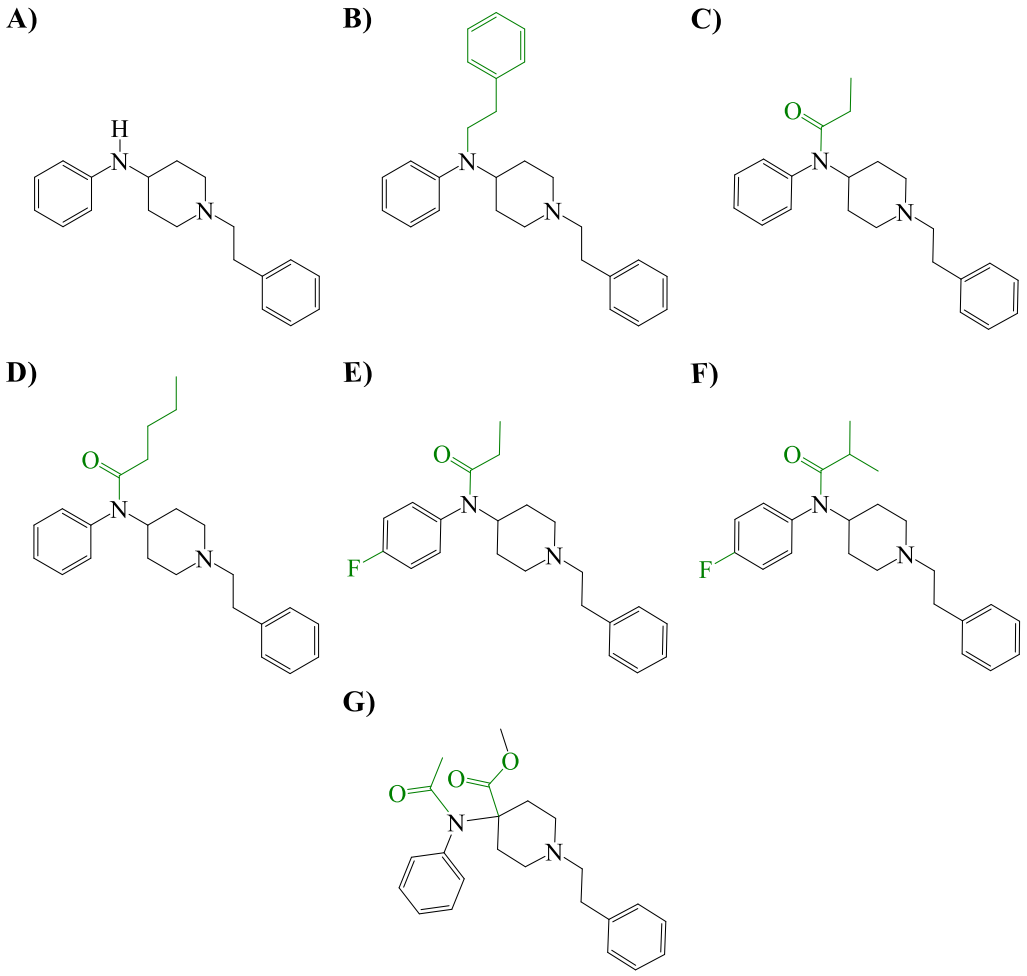

Figure 3.1 Molecular structures of fentanyl-related compounds with structural differences indicated in green. A) 4-ANPP, B) phenethyl-4-ANPP, C) fentanyl, D) valerylfentanyl, E) p-fluorofentanyl, F) 4-FIBF, G) carfentanil.

3.3.2 Standard Preparation

A 10 ppm working solution of *p*-fluorofentanyl, 4-ANPP, valerylfentanyl, 4-FIBF, fentanyl, carfentanil, and phenethyl-4-ANPP was utilized for all experiments. Individual 10 ppm solutions of each fentanyl precursor and analog were prepared for method development. Three cutting agents, caffeine, lidocaine, and diphenhydramine were prepared at 10 ppm, 30 ppm, and 30 ppm respectively. When appropriate, 50 to 100 ng of standard solution was spotted on a surface in 5 µL aliquots. All standard solutions were prepared in a 75:25 H_2O:MeOH solvent ratio to reduce dispersal of the analytes when deposited on a surface.

3.3.3 Pressure-Sensitive Adhesive and Paper Spray-Mass Spectrometry

Commercially available PSA paper was prepared in two ways. The standard PSA tips were prepared as previously described by cutting five-layers of PSA paper into a sharpened tip (Figure 2.2). Photographs of the standard tip and cartridge are shown in Figure 3.2A and B. The top and bottom of the layers were discarded prior to sampling to reduce contamination. The remaining three layers were used as a single unit to provide more support during surface dabbing and paper spray. No changes were made to the standard sampling protocol or cartridge for this application to fentanyl analogs.

A comparison between the standard cartridge used for all previous PSA and PS-MS experiments and a cartridge requiring no paper cutting (referred to as cut-free cartridge) was conducted. For experiments involving the cut-free cartridge a single, full-sized, PSA ticket was used for sampling. Sampling with a full-sized PSA ticket was conducted in the same manner as the cut tickets. The adhesive portion of the ticket was placed in the cut-free cartridge and excess paper torn from the ticket and discarded (Figure 3.2C). The cartridge was comprised of 3-D printed polypropylene containing three solvent wells, an opening for high voltage application via a ball bearing, and an open corner to achieve paper spray ionization (Figure 3.2D).

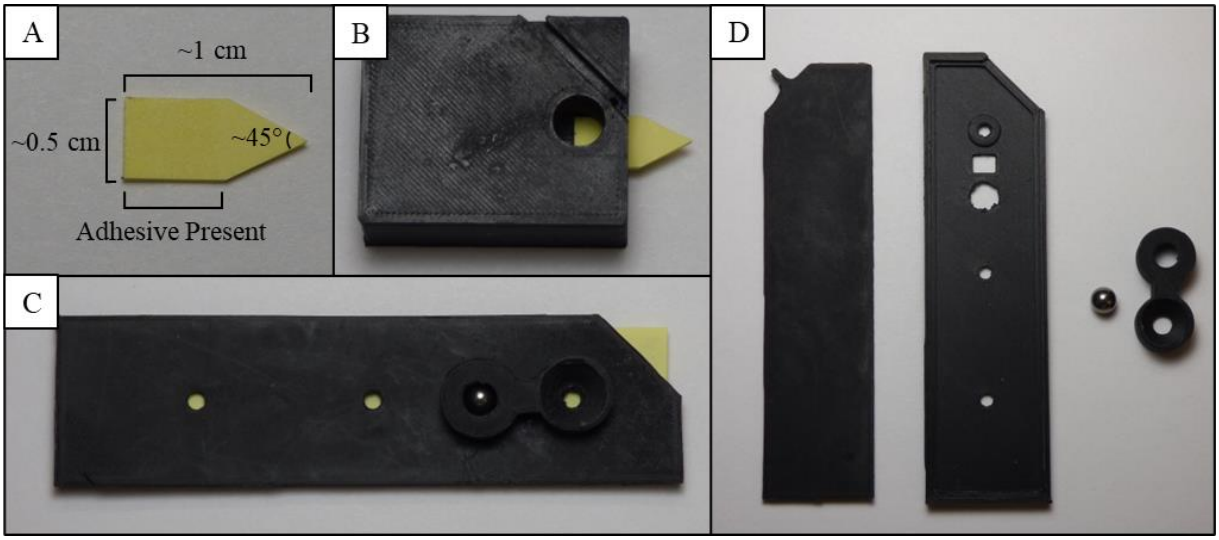

Figure 3.2 Cartridges designed for use of PSA paper for PS-MS. A) Dimensions of cut PSA ticket used with the standard cartridge. B) Standard cartridge used for all experiments. C) Cut-free cartridge with full-sized PSA ticket. D) Parts of cut-free cartridge left to right: base, top piece with solvent wells, ball bearing, and attachment for solvent well and ball bearing.

3.3.4 Data Acquisition

Prior to data acquisition, a spray solvent was applied to the PSA tips before analysis. A spray solvent of 90:10 MeOH:H_2O with 0.1% FA was used for all experiments. The spray solvent was selected based on its common use in PS-MS and the solubilities of fentanyl and fentanyl analogs.[28] For the standard cartridge, a spray solvent volume of 70 µL was applied to the solvent well over 5-10 seconds. For the cut-free cartridge a total of 200 µL of spray solvent was used, with 150 µL applied in the 3 solvent wells prior to analysis and the final 50 µL applied to the tip during data collection to sustain the spray.

All spectral data were collected on a Thermo LTQ-XL linear ion trap mass spectrometer (Thermo Scientific Inc., San Jose, CA). For all experiments, data acquisition was carried out for 0.7 minutes with voltage application of +4.5 kV (45° paper angle) or +5.0 kV (90° paper angle) for 30 seconds. A higher voltage was required as the angle of the paper spray tip increases.[29] Both full MS and MS/MS spectra were collected during analysis. Instrument parameters for MS/MS analysis are detailed in Table 3.1.

Table 3.1 Analyte details and instrument parameters for MS/MS analysis utilized for all experiments.

Analyte	Molecular Weight (g/mol)	Precursor Ion (m/z)	Fragment Ion (m/z)	Collision Energy (eV)
4-ANPP	280.4	281.4	188	53
phenethyl-4-ANPP	384.6	385.4	188	44
fentanyl	336.5	337.4	188	48
***p*-fluorofentanyl**	354.5	355.4	188	55
valerylfentanyl	364.5	365.4	188	46
4-FiBF	368.5	369.4	188	43
carfentanil	394.5	395.6	335	25

3.3.5 Data Analysis

The impact of cutting agents on analyte signal was examined. Reported analyte signal was calculated by dividing the averaged AUC for each analyte's precursor ion by the average AUC of the total ion chromatogram (TIC) for each cutting agent. Analyte signal in the presence of different cutting agents was normalized to positive control (no cutting agents) for comparison. The two cartridges were compared by averaging the AUC:TIC for fentanyl. The relative standard deviations (RSDs), which is a percentage of the sample standard deviation divided by the sample mean, (RSD = s / $\bar{x}$*100%) were calculated. Relative standard deviation is defined as the A student *t*-test (*p*-value = 0.05) was used determination of statistical significance between data sets when applicable. A total of 5 replicates were collected for each experiment unless otherwise noted.

3.3.6 Surface Recovery Experiments

To demonstrate the ability of PSA paper to recover fentanyl-related compounds from packaging materials, 100 ng of the fentanyl analogs both individually, and in a mixture, were deposited on packaging tape, cardboard, shipping labels, and a plastic sheet. Due to absorption into the shipping label and cardboard surfaces, 100 ng was first dried on the plastic sheet and transferred to the porous surfaces (cardboard and shipping label) to ensure the fentanyl analogs were present on their surfaces. Because of effective recovery from the packaging tape and plastic sheet, the analyte amount was decreased to 50 ng. For all additional experiments, 100 ng of transferred fentanyl analogs were utilized for the shipping label and cardboard surfaces and 50 ng of directly deposited analogs were used on packaging tape and the plastic bag.

3.3.7 Cutting Agent Experiments

To assess differences in analyte signal caused by cutting agents, three unregulated cutting agents were assessed (caffeine, lidocaine, and diphenhydramine). Cutting agents and fentanyl analogs were combined by mixing a 10 ppm fentanyl analog solution 1:1 with solutions of 10 ppm lidocaine, 30 ppm caffeine, or 30 ppm diphenhydramine. The cutting agents and fentanyl analogs were recovered from each of the four shipping-related surfaces.

3.3.8 Cartridge Comparison

The performance of the standard and cut-free cartridges were assessed. A total of 50 ng of fentanyl was added directly to the different PSA tips and recovered from a plastic sheet. For the full-sized PSA tip, recovery of the fentanyl residue was focused on the corner of the tip used for paper spray ionization. A total of 8 replicates were carried out for each condition and average fentanyl signal, failure rate, and RSD were compared. Differences in cartridge performance were evaluated for statistical significance.

3.4 Results and Discussion

3.4.1 Method Development

The subset of fentanyl-related compounds (fentanyl, 4-ANPP, phenethyl-4-ANPP, valerylfentanyl, 4-FIBF, carfentanil, and *p*-fluorofentanyl) used in this work were selected because they were reported by the National Forensic Laboratory Information System (NFLIS) as the most commonly encountered fentanyl-related compounds in 2021.[30] In addition to regulated compounds, phenethyl-4-ANPP, a synthesis byproduct, was selected because it can serve as indicator of fentanyl synthetic route.[31] The four surfaces tested, cardboard, packaging tape, shipping labels, and plastic, were selected as they are commonly used in shipping. Collision energies for MS/MS analysis of the compounds were optimized using MS tune function.

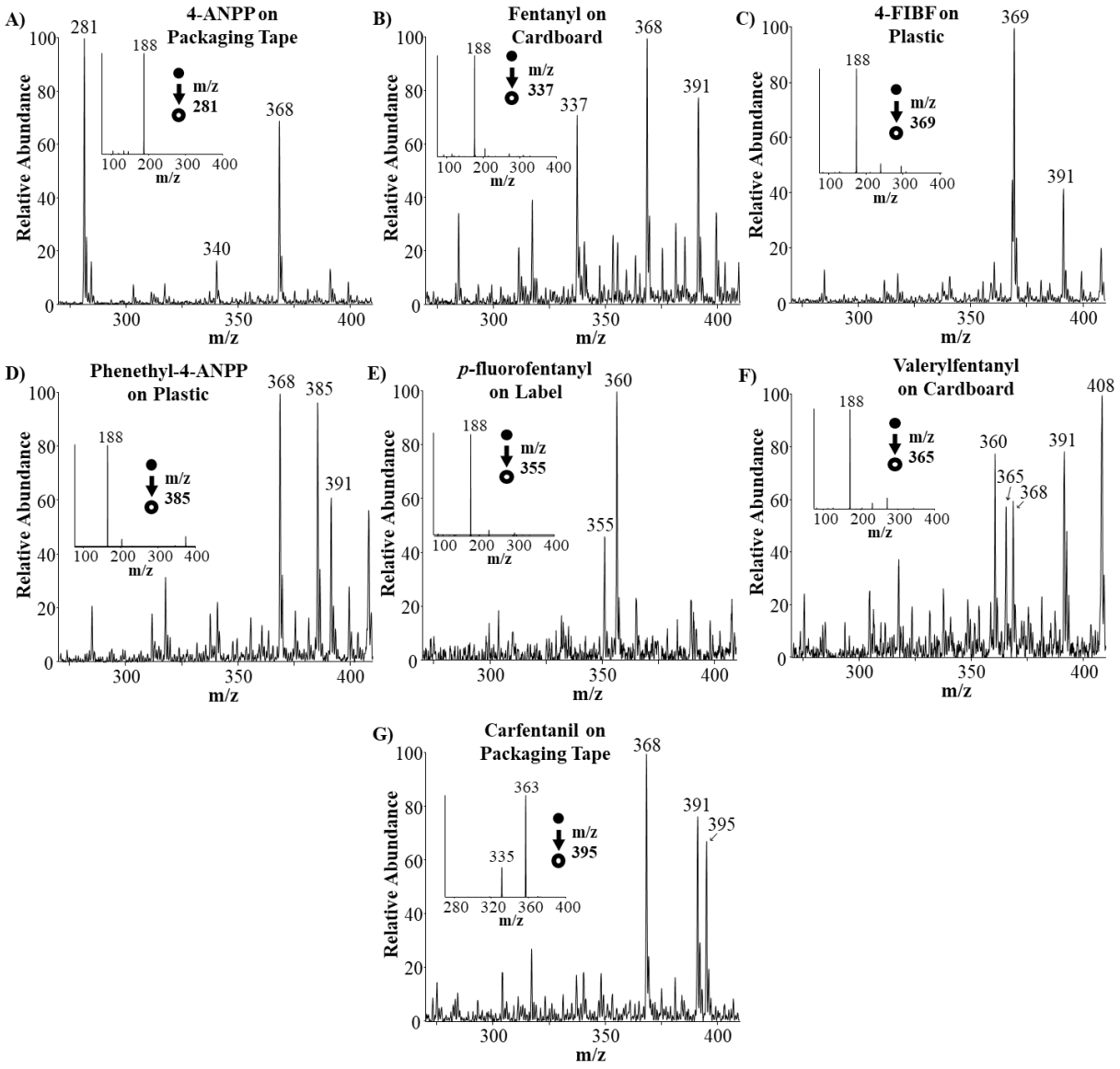

Figure 3.3 Full MS and MS/MS spectra for the recovery of 100 ng of individual fentanyl-related compound residues from packaging materials by PSA paper. A) 4-ANPP recovered from packaging tape, B) fentanyl recovered from cardboard, C) 4-FIBF recovered from plastic, D) phenethyl-4-ANPP recovered from plastic, E) *p*-fluorofentanyl recovered from shipping labels, F) valerylfentanyl recovered from cardboard, and G) carfentanil recovered from packaging tape.

To establish the use of PSA paper for the recovery of fentanyl analogs, full MS and MS/MS spectra were collected for individual compounds spotted and recovered from each of the four surfaces (Figure 3.3). At the 100 ng level, each fentanyl compound was recovered without issue. For all compounds except carfentanil, MS/MS spectra showed the presence of the indicative *N*-phenethylpiperidine moiety (188 *m/z*) found in many fentanyl analogs.[18, 32] Unlike the other

analogs, the dominant fragment ions for carfentanil were 335 and 363 *m/z*. The lack of a peak at 188 *m/z* is consistent with fentanyl analogs that have an additional, stabilizing, substituent group at position 4 of the piperidine ring.[32]

The recovery of a mixture of all fentanyl-related compounds from each surface was also demonstrated (Figure 3.4 and Figure 3.5). For the plastic sheet and packaging tape, 50 ng on the surface was a sufficient amount for identification. For shipping labels and cardboard, 100 ng was required for identification, perhaps owing to the greater surface roughness of the materials.

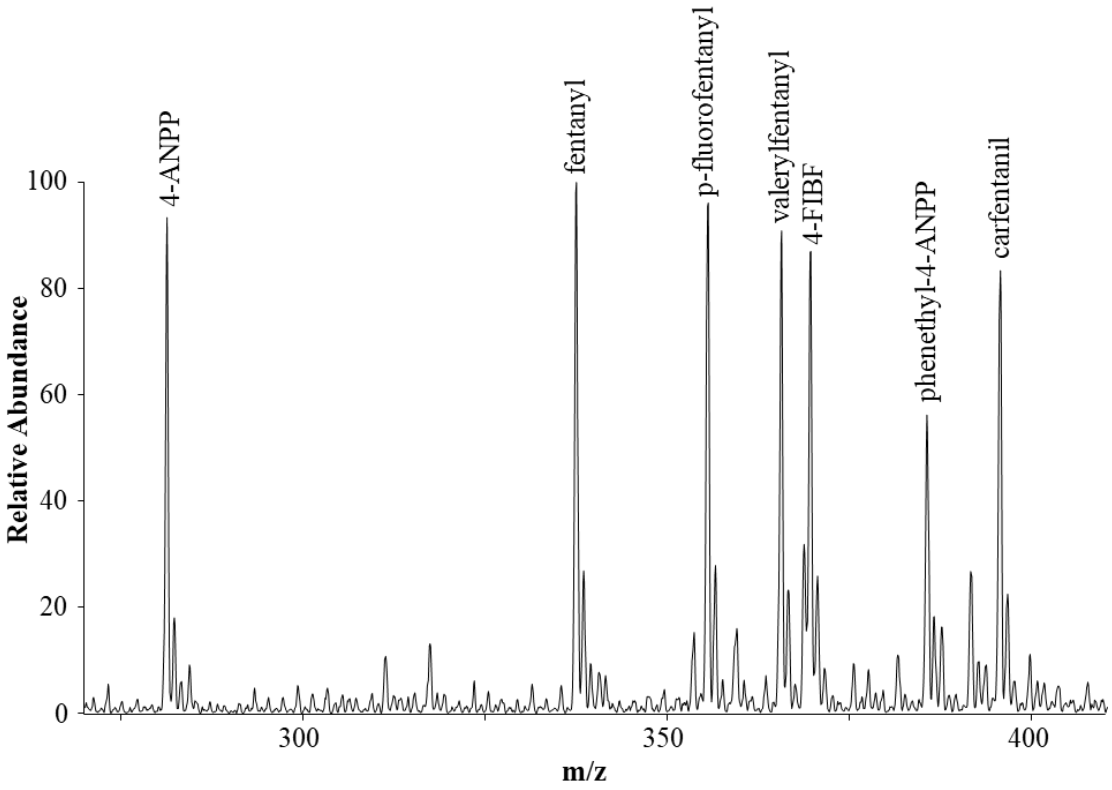

Figure 3.4 Full MS spectrum for 50 ng of fentanyl mix (4-ANPP, fentanyl, p-fluorofentanyl, valerylfentanyl, 4-FIBF, phenethyl-4-ANPP, and carfentanil) recovered from packaging tape by PSA paper.

The examined compounds spanned a mass range of 281-395 *m/z*. Within that mass range, a few peaks, notably at 368 and 391 *m/z*, result from the PSA paper. These peaks are labelled throughout Figure 3.3 and did not interfere with the detection of the fentanyl-related compounds analyzed as they were mass-resolved.

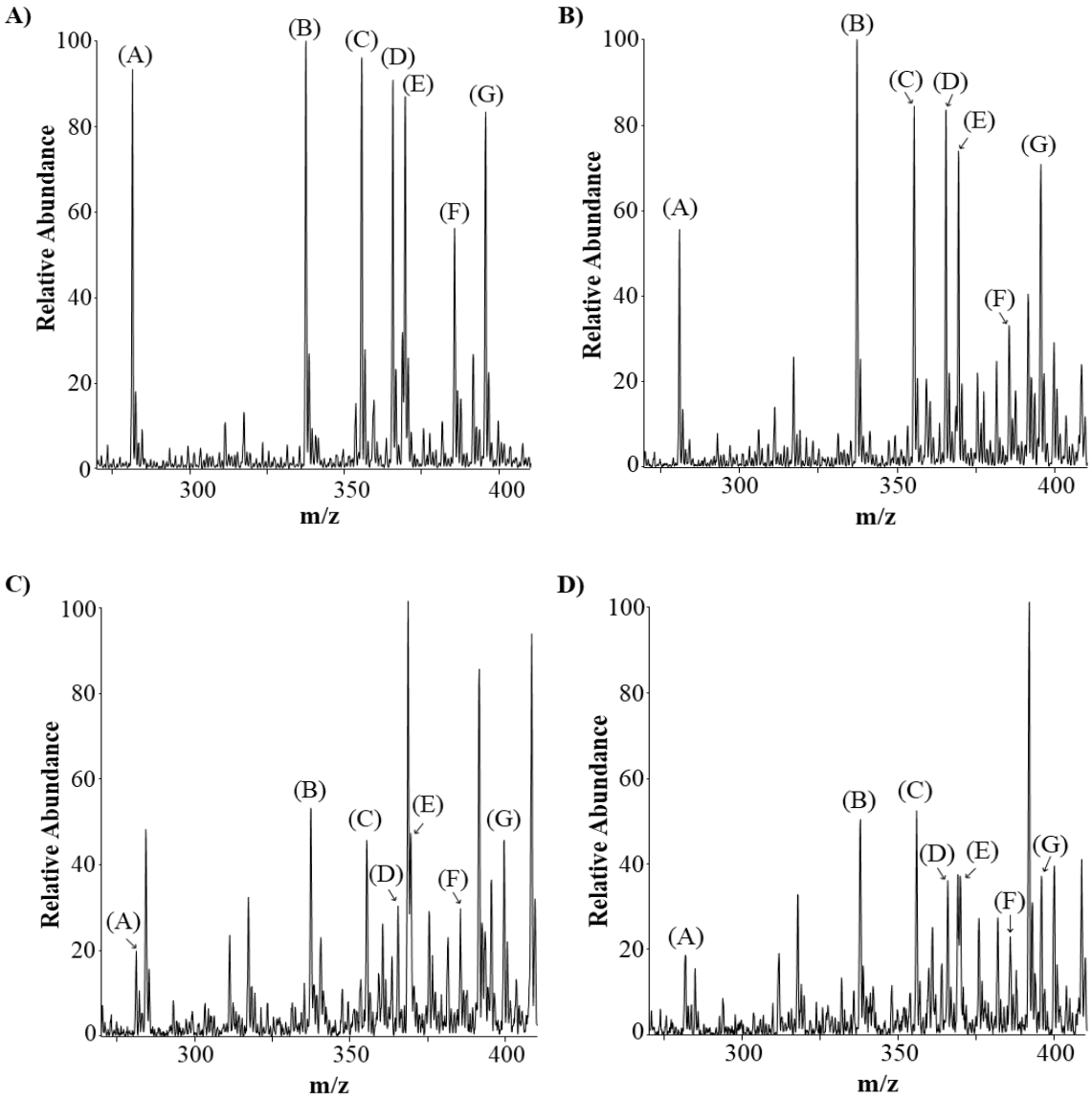

Figure 3.5 Full MS spectra for the recovery of fentanyl mixture (4-ANPP (A), Fentanyl (B), p-fluorofentanyl (C), valerylfentanyl (D), 4-FIBF (E), phenethyl-4-ANPP (F), carfentanil (G)) (A) 50 ng recovered off packaging tape. (B) 50 ng recovered off a plastic bag. (C) 100 ng recovered off cardboard. (D) 100 ng recovered off a label.

3.4.2 Cutting Agents

Three cutting agents, caffeine, lidocaine, and diphenhydramine were examined for their impact on fentanyl-related analyte signal. Fentanyl analog purity was 50% for lidocaine and 25% for caffeine and diphenhydramine. These purities were selected based on casework samples where

fentanyl or fentanyl analogs were the primary controlled substance.[33] Each cutting agent was examined for impacts on analyte signal when recovered from each surface. The cutting agent data for fentanyl are represented in Figure 3.6. As indicated by the figure, the average fentanyl signal for each surface fell within the error range of the control for all circumstances except in the presence of diphenhydramine on tape. In this case, there was a modest decrease in fentanyl signal of about 30%. The lower fentanyl purity of 25% when mixed with caffeine and diphenhydramine was not accompanied by lower fentanyl signal, additionally indicating that the tested cutting agents did not significantly decrease analyte signal at the purities tested here.

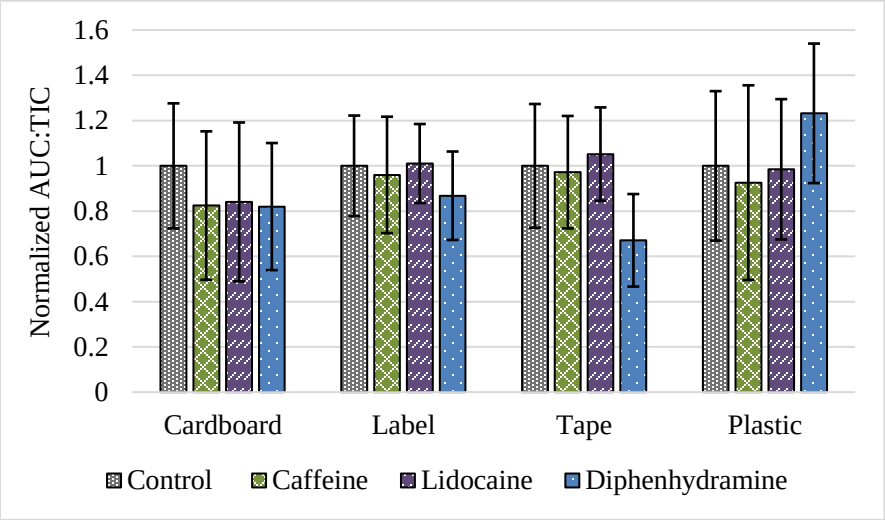

Figure 3.6 Normalized fentanyl signal in the presence of caffeine, lidocaine, and diphenhydramine for recovery from cardboard, shipping label, packaging tape, and plastic by PSA paper.

Results for all compounds on all surfaces in the presence of the cutting agents can be found in Figure 3.7. There was not a notable reduction in analyte signal for any of the fentanyl analog-surface combinations in the presence of any of the cutting agents. The variability in analyte signal was high at times, especially for tape where the median %RSD was 44%. The relatively high variability arises from variation in analyte recovery from the surface as well as variation in ionization by paper spray (Figure 3.7). The variability indicates the method is appropriate for screening and qualitative identification, but not quantitation.

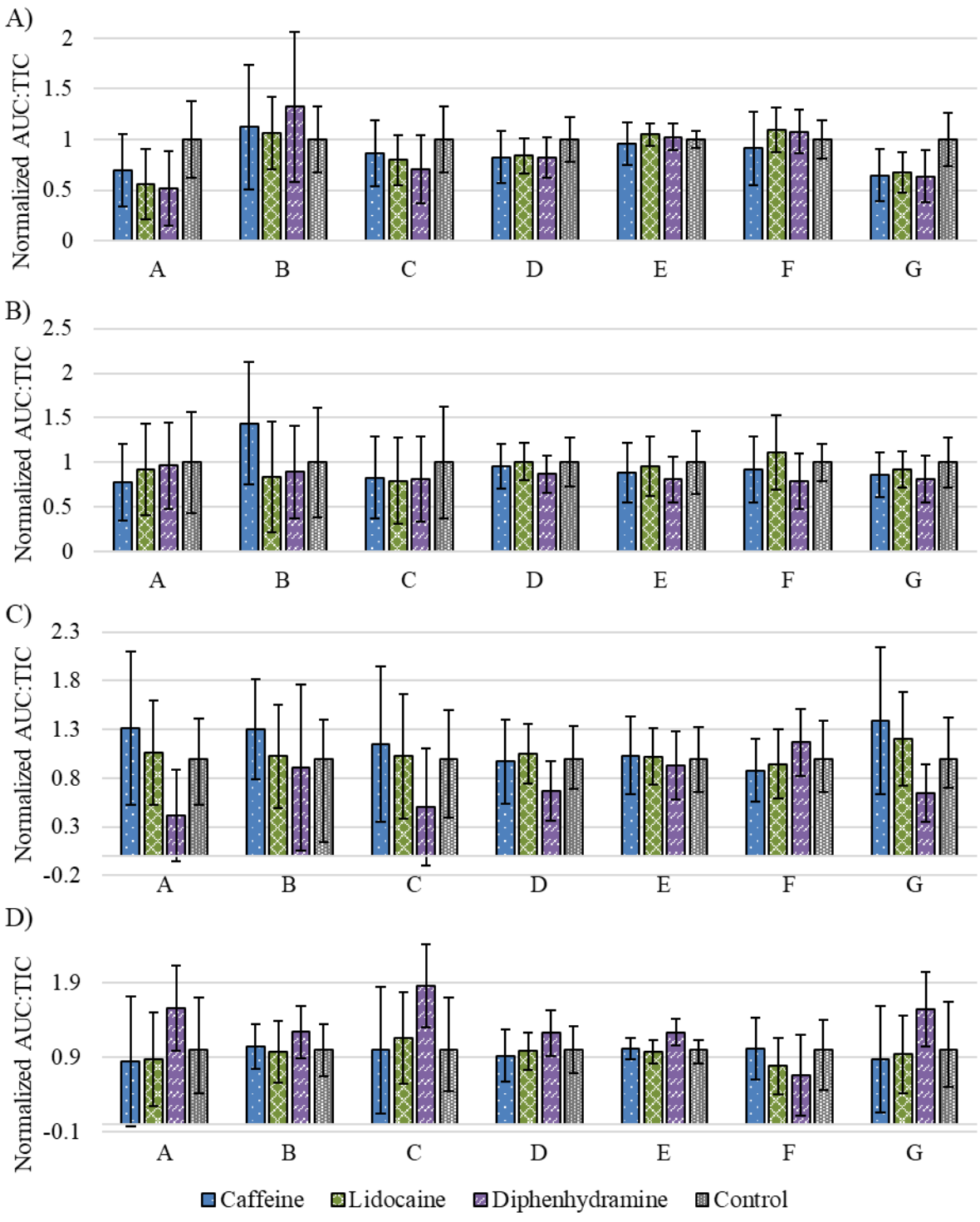

Figure 3.7 Normalized analyte signal for fentanyl-related compounds (A) 4-ANPP, B) fentanyl, C) p-fluorofentanyl, D) valerylfentanyl, E) 4-FIBF, F) phenethyl-4-ANPP, G)carfentanil) in the presence of cutting agents. Recovery from (A) cardboard, (B) shipping label, (C) packaging tape, and (D) plastic.

The largest variations in the analytes' signal were in the presence of diphenhydramine. Despite large variations, average analyte signal did not indicate ion suppression in the presence of diphenhydramine. Large uncertainty values limited the ability to detect subtle changes in fentanyl recovery, but any reduction would be relatively modest and not likely to impact qualitative identifications for most cases.

3.4.3 Cartridge Comparison

A cartridge was designed to eliminate the need to cut PSA paper to a sharp tip prior to sampling. Instead, the existing corner of the PSA paper (~90° angle) was used for ionization. This is an increase in the angle of the tip by a factor of approximately 2. The cut-free cartridge required only a single sheet of PSA paper as opposed to the three layers used in the standard tip. A reduction in layers was possible because of the support built into the cartridge, which increased the stability of the tip during analysis (Figure 3.2D). While no cutting of tips was required prior to sampling, excess paper not lined with PSA was torn off and discarded to reduce solvent dispersal away from the tip. With the increase in surface area between the standard tip and the full-sized PSA ticket, solvent volume was increased to 200 µL. Comparison between the standard cartridge focused on failure rate and fentanyl signal when fentanyl was added directly to paper and recovered from plastic. Failure rate was defined as a sample that did not ionize or produce consistent ionization. Paper spray chronograms and mass spectra obtained for both cartridges are shown in Figure 3.8.

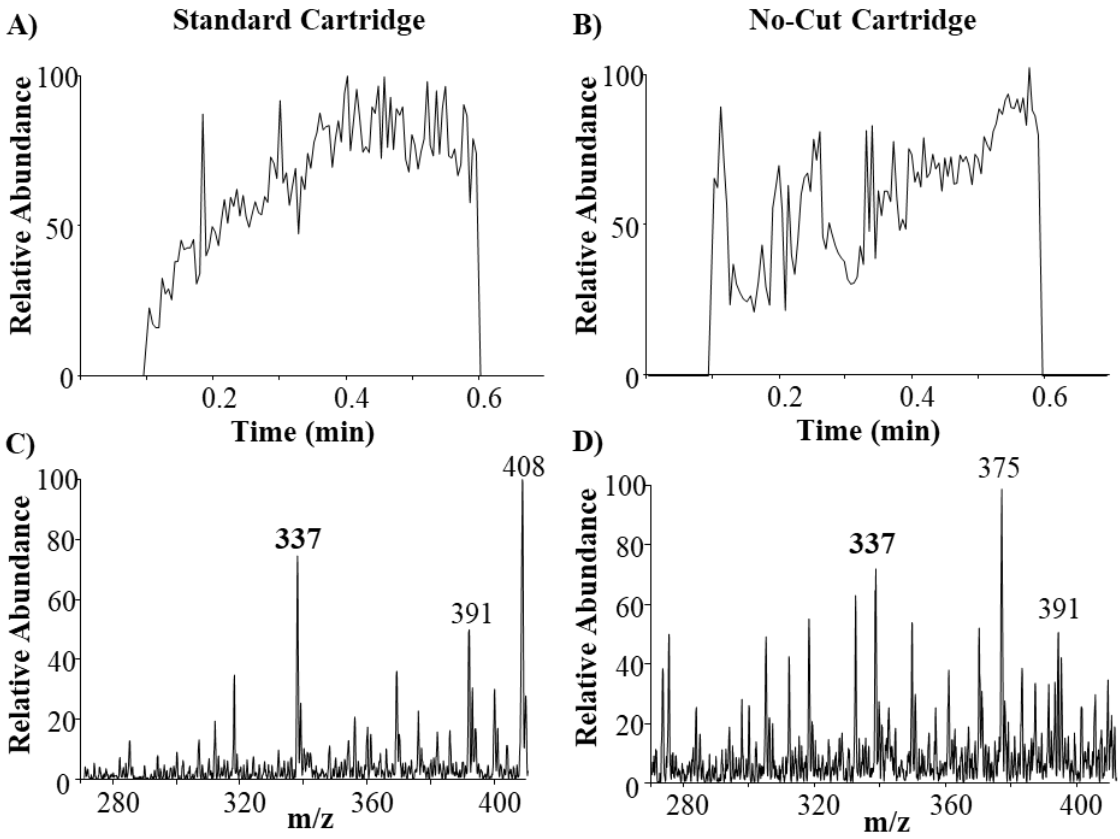

Figure 3.8 Spectral comparison between standard cartridge and cut-free cartridge for the recovery of 100 ng fentanyl from plastic surface. A) chronogram (TIC) for standard cartridge B) chronogram (TIC) for cut-free cartridge, C) Full MS spectrum for standard cartridge, D) Full MS spectrum for cut-free cartridge.

The standard cartridge had a lower failure rate than that of the cut-free cartridge with only 1 of the 16 samples failing to produce consistent ionization. The cut-free cartridge has a failure rate three times greater with 3 out of 16 samples producing inconsistent ionization. Overall, ionization with the cut-free cartridge had more variability than the standard cartridge. The spray instability of the cut-free cartridge as it compared to the standard cartridge is illustrated in Figure 3.8A and B. Reduced spray stability in the cut-free cartridge was attributed to the much larger paper angle. A previous study showed that larger paper angles required higher spray voltages and more precise positioning of the paper tip relative to the inlet.[29]

There was a statistically significant difference in the fentanyl signal between the standard and cut-free cartridges for the neat addition of fentanyl and recovery from plastic (p = 0.0100 and 0.0131 respectively). The average fentanyl signal for the standard cartridge was approximately 2

times greater with the standard cartridge than the new cut-free cartridge for the neat addition of fentanyl directly to the PSA paper (Figure 3.9). For the recovery of fentanyl from plastic sheeting, fentanyl signal remained significantly greater for the standard cartridge but only by a factor of approximately 1.4 times (Figure 3.9). These results, taken together, suggest that the paper spray MS step is somewhat less effective when performed from the 90° corner, but that there may be improved recovery for the larger paper that partially offsets this loss. Despite reduced spray stability and fentanyl signal, the molecular ion for fentanyl was still identifiable with the cut-free cartridge (Figure 3.8D-C).

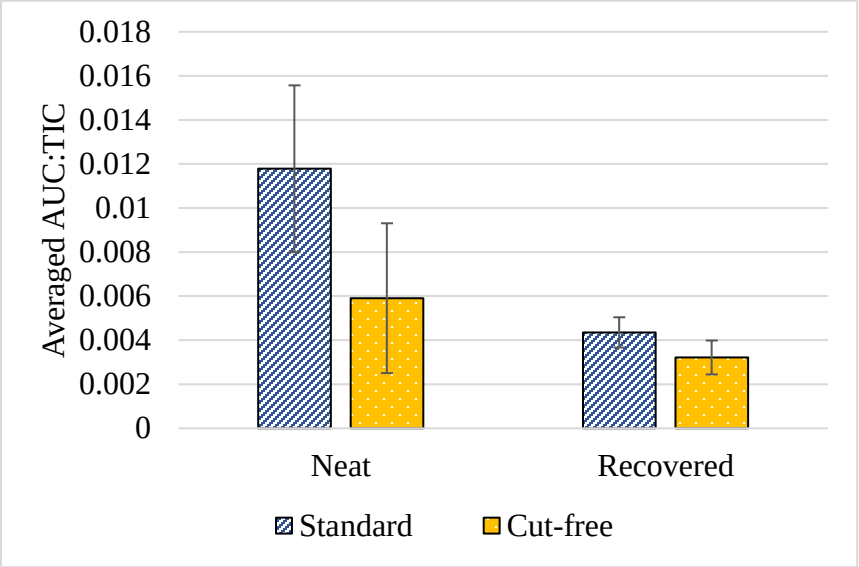

Figure 3.9 Average signal (AUC:TIC) for 50 ng of fentanyl neat and recovered from a plastic surface using the standard and cut-free cartridges.

While the cut-free cartridge generally had poorer performance than that of the standard cartridge, it may still be suitable for qualitative analyses and screening. The cut-free cartridge with a full-sized PSA ticket was able to recover and identify fentanyl at the 50 ng level. Increasing analysis time to accommodate the larger paper may allow for more of the analyte to ionize, making the cartridge more comparable to the standard cartridge. The design of a device that ensures precise placement of the paper tip in front of the MS inlet should aid in reducing the failure rate of the cut-free cartridge.

3.5 Conclusion

This work expands the use of paper spray-mass spectrometry as a screening tool for fentanyl-related compounds by applying pressure-sensitive adhesive paper for drug recovery. In combining the sampling mechanism with the paper spray substrate, this method offers a rapid and highly affordable method for drug identification that does not require sample preparation steps. The recovery of seven fentanyl-related compounds (fentanyl, 4-ANPP, phenethyl-4-ANPP, valerylfentanyl, 4-FIBF, carfentanil, and p-fluorofentanyl) was demonstrated from 4 shipping related surfaces. The impact of three noncontrolled cutting agents (caffeine, lidocaine, and diphenhydramine) on PS-MS analysis of fentanyl analogs was examined. No significant loss of analyte signal was found. This work also evaluated the use of a PS-MS cartridge that utilized a full-sized PSA paper. The detection of fentanyl recovered from plastic sheeting by a full-sized PSA paper and using the cut-free cartridge was reported. In conclusion, by using pressure-sensitive adhesive paper as a substrate for PS-MS, this method allowed for a quick, effective, and selective method of fentanyl screening of mail parcels.

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CHAPTER 4. PORTABLE MASS SPECTROMETRY AND PAPER SPRAY FOR ILLICIT DRUG DETECTION

4.1 Abstract

Portable mass spectrometers bring the analytical power of mass spectrometers out of the laboratory and into the field. Miniaturized mass spectrometers can be applied to forensic chemistry for in-field analysis of drugs, explosives, chemical pollutants, and more. Paper spray with a PSA-lined paper substrate was utilized on a portable mass spectrometer for drug residue collection and detection for a variety of drugs and from several surfaces. Recovery of were demonstrated from aluminum and polyester-cotton blend cloth surfaces with 2 µg of illicit drugs. Drug recovery was not successful at 2 µg from concrete and asphalt surfaces using the method. True blinded trials were conducted to assess false positive and false negative rates of the method. No false positives or false negatives occurred during analysis of cocaine from cloth and aluminum or methadone from glass. The combination of color tests with PS-MS was also investigated on portable instrumentation. Drug residues were successfully confirmed by paper spray MS at the color test threshold in all cases, except for heroin after reaction with the Marquis reagent. In this case, the portable MS detection threshold was 4-fold higher than the color test threshold.

4.2 Introduction

Most mass spectrometry research focuses on the use of benchtop instruments. These instruments offer many benefits such as their sensitivity, resolution, and mass precision.[1] A caveat to these capabilities is that benchtop mass spectrometers are generally heavy and bulky instruments and are often accompanied by one or more external vacuum pumps. Benchtop mass spectrometers are not conducive to use outside of a laboratory environment. In forensic chemistry, benchtop mass spectrometers are frequently coupled with gas chromatography, where it is considered the "gold standard" for drug identification.[2] While GC-MS is a highly effective tool, it requires samples be transported to offsite laboratories for appropriate drug identification. This process is time-consuming and leads to a delay in compound identification, while also contributing to growing evidentiary backlogs in many states.[3, 4] A potential resolution to this issue is the use of portable instrumentation to quickly identify unknown substance in the field. Field identification capabilities

are sought after in a variety of fields including forensic science and environmental chemistry. Applications in these fields have focused on toxin identification and onsite toxicological analyses.[1]

In recent years, handheld Fourier-transform infrared (FTIR) and Raman spectrometers have been developed and commercialized for in-field use by minimally trained personnel.[3] Mass spectrometers have also been miniaturized, though to a lesser extent, for in-field analysis.[5] An example of this miniaturization is illustrated in Figure 4.1. Portable MS systems range in size, but are generally transportable by an individual and have no external vacuum pumps.

Figure 4.1 Side-by-side of ion trap mass spectrometers, (A) benchtop instrument with two external vacuum pumps, (B) portable instrument with no external equipment.

Miniaturization of mass spectrometers has been driven by advances in the miniaturization of the mass analyzer.[6, 7] Mass analyzer miniaturization poses multiple challenges including achieving ion separation with limited space and maintaining a suitable pressure, as mass analyzers require low pressure conditions under vacuum.[1, 5] The most popular mass analyzer for portable mass spectrometers is the ion trap.[6, 7] The ion trap's popularity is due to its ability to operate at

slightly higher pressures and offer MS/MS capabilities.[1, 5] While miniaturization of MS systems has been achieved, there are tradeoffs in terms of instrument performance as the result of reduced mass analyzer size. Portable MS systems are less sensitive with lower resolution and mass precision.[1]

Despite limitations of portable mass spectrometers, their value has been demonstrated in a variety of applications. Forensically relevant applications include the trace detection of explosives, identification of fentanyl analogs, toxic gas monitoring, drug seizures and more.[8-11] Many of these studies use ambient ionization techniques coupled with the portable instrumentation. Use of ambient ionization offers many advantages including minimal to no sample preparation and ability for analysis to be conducted by non-specialized personnel. Paper spray ionization is one such ambient ionization technique that has been demonstrated with portable instrumentation including specific applications to drug detection.[9, 12]

Given the variety of applications of MS to forensic chemistry, portable MS systems have a lot to offer for in-field analysis. Some initial work has highlighted the use of PSA-lined paper with PS-MS on a portable ion trap mass spectrometer for drug detection.[12] Building from this initial work, this chapter seeks to expand the use of PSA with PS-MS on portable instrumentation. This was accomplished by examining PSA paper for the surface recovery of drugs of abuse, the tendency of the instrument to experience analyte carryover at high analyte amounts through blinded sampling, and the ability of color tests to be combined with PS-MS on portable instrumentation.

4.3 Materials and Methods

4.3.1 Materials

Standards of cocaine, fentanyl, and methadone were purchased from Cerilliant (Round Rock, TX, USA). Solid XLR-11 was purchased from Cayman Chemical (Ann Arbor, MI, USA). Solid forms of cocaine hydrochloride and MAMP were purchased from Sigma Aldrich (St. Louis, MO, USA) through the University's DEA license. Street purity heroin was obtained from the University's forensic training materials. Liquid chromatography-MS (HPLC) grade solutions of MeOH, water, ACN, and FA were purchased from Thermo Fischer Scientific (Rochester, NY, USA). All materials for color test reagents were obtained from the same manufacturers outlined in

Chapter 2. PSA paper (Super Sticky Post-It® Notes, 3M) in white and canary yellow were purchased from commercial retailers. Five different surfaces including aluminum sheeting, polyester-cotton blend cloth, glass, asphalt, and concrete were obtained from the U.S. Army Combat Capabilities Development Command (DEVCOM) Chemical Biological Center for surface recovery experiments.

4.3.2 Standard and Reagent Preparation

A stock solution of 1 mg/mL XLR-11 was prepared in methanol. Equal parts cocaine, fentanyl, and XLR-11 standards were combined for a standard concentration of 333 ppm for each compound. Additional individual component solutions of cocaine and methadone were prepared at 333 ppm with a 2:1 water to methanol solvent ratio. Color test reagents were prepared according to the 2021 Indiana State Police Laboratory Drug Unit Test Methods as outlined in Chapter 2.[13]

4.3.3 PSA Tip Preparation and Sampling

For experiment on the portable MS system, PSA tickets were cut to the dimensions outlined in preceding chapters (Figure 2.2A). For general recovery and blinded sampling experiments, PSA paper tickets were cut from canary yellow Super Sticky Post-It Notes. The canary yellow tickets were cut seven layers thick. For all color testing and PS-MS experiments, white PSA paper cut five layers thick was used. The ticket color was changed for better discernment of color changes due to color testing. For all experiments, the top and bottom layers of PSA ticket were discarded prior to sampling. The remaining, intact 5-layer ticket (or 3-layer ticket for color testing experiments),were firmly pressed ("dabbed") against surfaces three times to recover potential drug residues. Following sampling, PSA tickets were placed in the standard polypropylene cartridge (Figure 2.2B). For recovery and blinded sampling experiments, the cartridge was milled Delrin plastic. All color test experiments were conducted on a 3-D printed polypropylene cartridge of the same dimensions.

4.3.4 Data Acquisition and Analysis

Prior to data collection, 45 µL of spray solvent were applied to the PSA tip. For all recovery and blinded trial experiments, acetonitrile with 0.1% FA was used. For all color test experiments,

spray solvents of 90:10 MeOH:H_2O with 0.1% FA, 0.1% FA in ACN, and 70:30 ACN:MeOH with 0.1% formic acid were used for the cobalt thiocyanate, Simon, and Marquis tests, respectively. The experimental setup with the portable instrumentation is depicted in Figure 4.2.

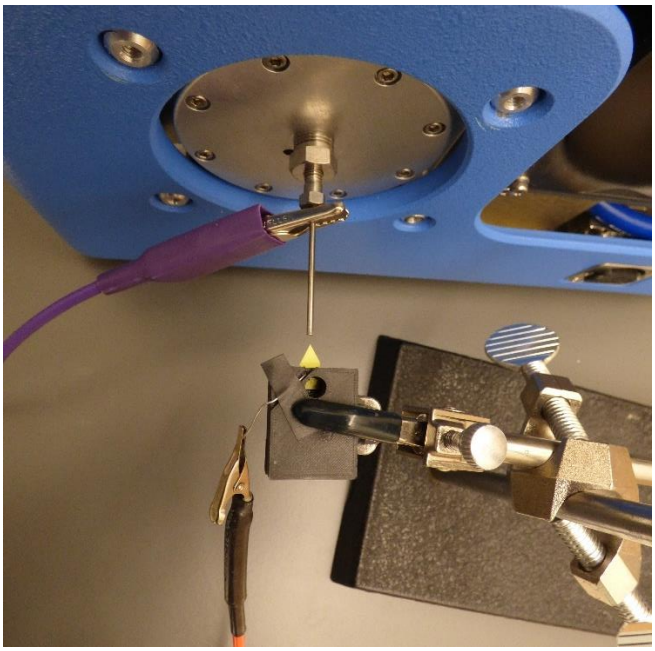

Figure 4.2 Experimental setup for PS-MS on portable mass spectrometer with PSA paper

Data collection was carried out for 0.7 minutes with a voltage of +4.5 kV for 30 seconds. Full MS data was collected for all experiments with a scanning range of 100 to 650 *m/z*. MS/MS data was collected for color test experiments with the parameters detailed in Table 4.1. All data was collected on a BaySpec Continuity Transportable High-Sensitivity Mass Spectrometer (BaySpec, Inc., San Jose, CA).

Table 4.1 Instrument parameters for MS/MS analysis of illicit drugs one a portable mass spectrometer

Analyte	**Precursor Ion (*m/z*)**	**Detector Anode (V)**	**RF Level (V)**	**ISO Freq. Center (kHz)**	**ISO Freq. Width (kHz)**	**CID Level (V)**
Cocaine	304	-1450	150	76	4	0.65
Methamphetamine	150	-1450	150	160	4	0.75
Heroin	370	-1600	250	73.3	2	0.75

These parameters vary from the benchtop MS system. Both detector anode and radiofrequency (RF) level parameters apply to full MS and MS/MS data collection. These parameters adjust the direct current (DC) and RF level of the instrument's ion funnel.[14] The ISO frequency center and width are adjusted to best isolate ions for MS/MS analysis. Along with ISO frequencies voltage for collision-induced dissociation (CID) is also adjusted for fragmentation in MS/MS.

Data analysis was conducted with the use of Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and MATLAB version R2021B (MathWorks, Natick, MA, USA). MATLAB was used for data averaging and spectra plotting.

4.3.5 Surface Sampling and Blinded Sampling

A total of 2 µg of cocaine, fentanyl and XLR-11 was spotted on aluminum, polyester-cotton blend cloth, concrete, and asphalt in three aliquots of 2 µL. After drying completely, the surfaces were sampled by PSA paper.

False positive and false negative rates as well as carryover were assessed through blinded sampling. Positive samples were prepared by spotting 2 µg of cocaine or methadone in three, 2 µL aliquots on a variety of surfaces. Negative samples were prepared by spotting 6 µL of water on the surfaces. Samples were prepared and numbered by an undergraduate student to ensure blinded analysis. An equal number of true positive and blank samples were prepared for each scenario. Three sampling scenarios including cocaine recovered from aluminum, cocaine recovered from polyester-cotton blend cloth, and methadone recovered from glass were assessed. After drying, all samples were collected by pressing the PSA paper firmly against the surface before lifting to recover potential drug residues.

Samples were analyzed on the portable mass spectrometer and determined to be positive or negative based on a visual assignment of the spectra before comparison to expected results. Samples with the molecular ion ($[M+H]^+$ for all drugs studied) present were assigned a "positive" result and samples lacking the molecular ion were assigned a "negative" result. A false positive was defined as the determination of a drug-positive sample when drug residue was absent, and a false negative was defined as the identification of a blank sample when drug residue was present.

A total of 10 samples were prepared each for cocaine recovery from cloth and methadone recovery from glass. The sample size was increased to 20 for the recovery of cocaine from aluminum.

4.3.6 Color Tests

To assess the use of PSA paper, color tests, and PS-MS for in-field drug detection, the technique outlined in Chapter 2 was adapted for use on a portable mass spectrometer. Cobalt thiocyanate and Simon tests were performed by adding 5 µL of reagent(s) to 1.25 µg of cocaine and methamphetamine, respectively. Cocaine and methamphetamine were spotted and dried directly on PSA paper prior to reagent addition. The Marquis test was performed by adding 3 µL of reagent to 10 µg of methamphetamine or 20 µg of street purity heroin. Illicit drug amounts were selected based on the color test thresholds on PSA paper and are reflected in Table 2.2.

4.4 Results and Discussion

4.4.1 PSA Paper and PS-MS on Portable Instrumentation

Earlier work tested PSA and PS-MS on the portable mass spectrometer by pipetting drug standard solutions directly on to the paper. Limits of detection (LODs) for the 10 drugs studied (acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11) were in the low nanogram range, with limits generally falling below 10 ng.[12] The PSA and PS-MS method has also been applied to simulated "real-world" sampling scenarios including the recovery of illicit drugs from a variety of surfaces using portable instrumentation.[12] This work extends the previous study by testing the recovery of known quantities of illicit compounds from surfaces, rather than simply adding standard solutions directly to the paper.

Three illicit compounds–cocaine, XLR-11, and fentanyl–were selected for further study. They were selected as they encompass three commonly abused drug classes: stimulants, synthetic cannabinoids, and opioids. The four surfaces selected for recovery experiments, aluminum, polyester-cotton blend cloth, concrete, and asphalt, were selected because of their varied porosity and topography. These surfaces have also been previously examined for drug recovery on a benchtop mass spectrometer.[12] In order to reduce dispersal of drug standards into surfaces with higher porosities, a 2:1 water:methanol solvent ratio was used.

Recovery and detection of 2 µg of cocaine, XLR-11, and fentanyl were achieved from the aluminum and polyester-cotton blend cloth surfaces. A sample spectrum for the recovery of the compounds from aluminum is provided in Figure 4.3. Peaks at 304 *m/z*, 330 *m/z*, and 337 *m/z* were present in spectra obtained from aluminum and cotton-polyester blend cloth for cocaine, XLR-11, and fentanyl respectively. These surfaces were the smoothest and least porous tested for drug recovery. During sample preparation, the spotted standards remained visible on the surface until dry.

Recovery and detection of cocaine, XLR-11, and fentanyl at 2 µg by portable mass spectrometer was not successful from the concrete and asphalt surfaces. Reduction in aliquot volume to further reduce absorption into surfaces did not improve recovery. Unlike the less porous surfaces, standard aliquots visibly dispersed when deposited on concrete or asphalt. Though drug residues could not be detected for cocaine, XLR-11, and fentanyl for either surface with the use of liquid standards, recovery may be possible with the use of solid drug standards.

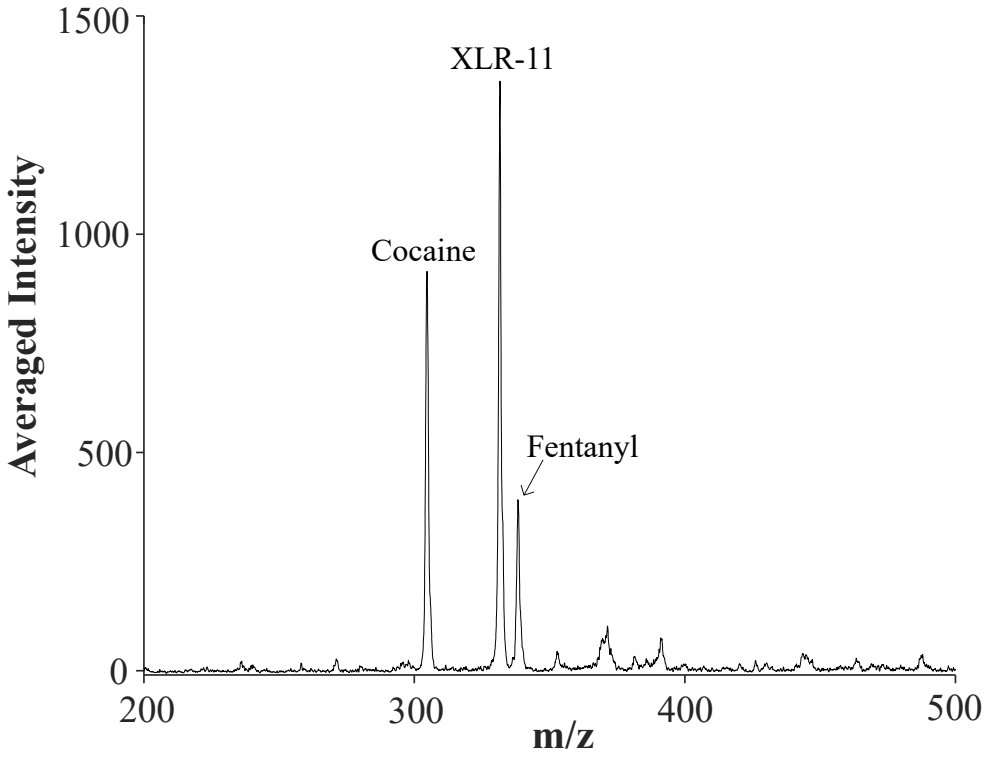

Figure 4.3 Full MS spectrum for 2 µg of cocaine, fentanyl, and XLR-11 recovered by PSA paper from aluminum.

4.4.2 Blinded Sampling

Blinded sampling protocols were conducted to examine false positive and false negative rates as well as carryover for the PSA and PS-MS method. Cocaine and methadone were selected to assess false positive and false negative rates for blinded analysis. Cocaine (304 *m/z*) was selected because it is a commonly used illicit drug, whereas methadone (310 *m/z*) was selected because of previous reports of analyte carryover.[15] Glass was introduced as an additional surface as it is smooth and nonporous and has comparable LODs to aluminum.[12] Following PS-MS analysis, samples were interpreted and categorized as positive or negative before comparison to expected results.

Sample spectra of true negative and true positive samples for cocaine recovered from polyester-cotton blend cloth are provided in Figure 4.4. As shown in Figure 4.4, a clear distinction between the true blank and true positive samples is possible based on the presence or absence of a peak at 304 *m/z*. Similar spectra were obtained for the recovery of cocaine from aluminum and the recovery of methadone (310 *m/z*) from glass.

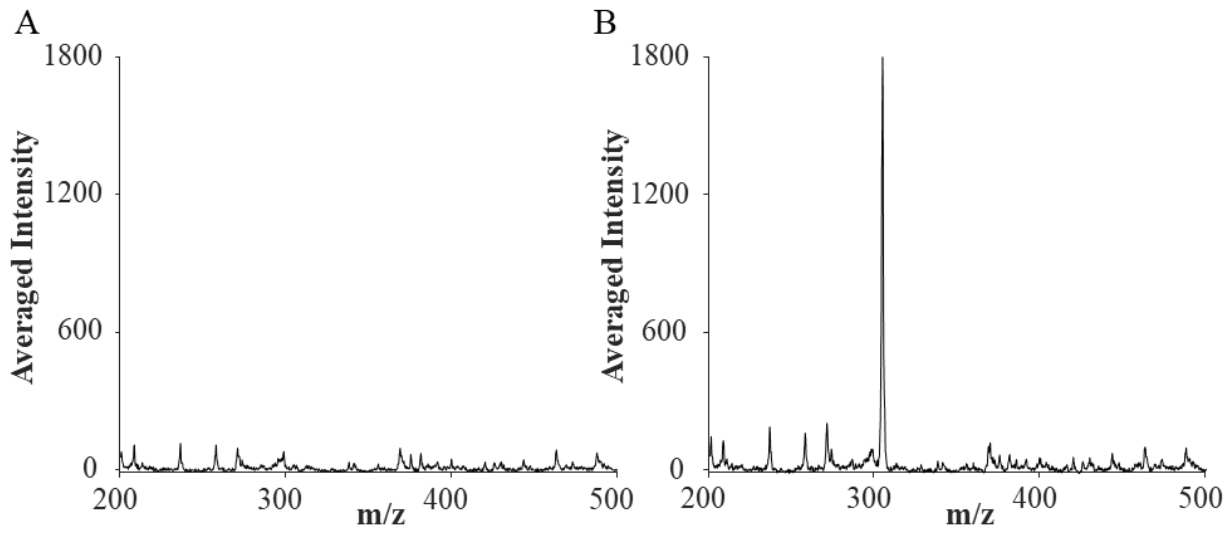

Figure 4.4 Sample spectra for blinded sampling of cocaine from polyester-cotton cloth A) true blank sample, B) true positive sample for cocaine.

The clear distinction offered between true positive and true negative sample spectra allowed for straightforward categorization of spectra as positive or negative. Blinded sampling results for all these scenarios (cocaine on cloth, methadone on glass, and cocaine on aluminum) are summarized in Table 4.2. Notably, the experimental determinations for all surfaces and all compounds were consistent with the true results. All true blank samples and drug positive samples were identified correctly (Table 4.2). This indicates that false positive and false negative rates are low when 2 µg of drug residue are sampled. Despite carryover concerns for methadone, qualitative identification of positive and negative samples was conducted without issue (Table 4.2). This indicates that any methadone carryover was negligible for collection and analysis at 2 µg when recovered from glass.

Table 4.2 Summary of blinded sampling results for cocaine on cloth, methadone on glass, and cocaine on aluminum

Cocaine on Cloth		**Methadone on Glass**		**Cocaine on Aluminum**			
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
1	⭘	1	⭘	1	☐	11	⭘
2	⭘	2	⭘	2	☐	12	⭘
3	☐	3	☐	3	⭘	13	☐
4	⭘	4	☐	4	⭘	14	⭘
5	☐	5	⭘	5	☐	15	⭘
6	⭘	6	☐	6	⭘	16	⭘
7	☐	7	☐	7	☐	17	☐
8	☐	8	⭘	8	⭘	18	⭘
9	⭘	9	☐	9	☐	19	☐
10	☐	10	⭘	10	☐	20	☐
		⭘ − True Positive ● − False Positive		■ − False Negative ☐ − True Negative			

The number of samples of cocaine on aluminum was doubled to assess the instrument's ability to analyze a higher number of samples continually. Expansion of blinded trials to a 20-sample set also did not affect the accurate assignment of negative and positive samples. This indicates increased sample analysis time does not lead to decreases in instrument performance on the portable mass spectrometer for this kind of analysis.

Overall, blinded sampling analysis demonstrated that the PSA and PS-MS method produces distinct spectra for the assignment of blank and positive samples for the recovery of

cocaine and methadone at 2 µg. Analyte carryover and longer instrument operation times did not impact this analysis.

4.4.3 Color Testing

To assess the feasibility of conducting color tests with PS-MS in the field, positive color tests were analyzed on a portable mass spectrometer. Cocaine and methamphetamine at the color test threshold (Table 2.2) were analyzed by PS-MS after reaction with cobalt thiocyanate, Simon, or Marquis reagents. Full MS and MS/MS spectra obtained on the portable mass spectrometer are shown in Figure 4.5. Molecular ion peaks in full MS and characteristic fragment ions in MS/MS mode were detected for all drug-color test combinations except for the Marquis test with heroin. The heroin-Marquis test, in which the precursor ion was obscured on the benchtop mass spectrometer (Figure 2.6G), did not produce characteristic ions in either full MS or MS/MS at the color test threshold of 5 µg. The amount of heroin was increased, and excess reagent was removed from the PSA paper after color formation to decrease interference in the MS/MS data. At a fourfold increase in heroin from the threshold amount (20 µg), three fragment ions for heroin at 268, 310, and 328 *m/z* were identifiable (Figure 4.5H). While an increase in heroin to 20 µg was required for detection, this increase remains a reasonable amount to encounter in field sampling situations as the typical street dose of heroin is in the low milligram.[16] The general consistency between analysis on portable and benchtop mass spectrometer data demonstrates the feasibility of combining color tests and PS-MS for in-field analysis with a portable mass spectrometer.

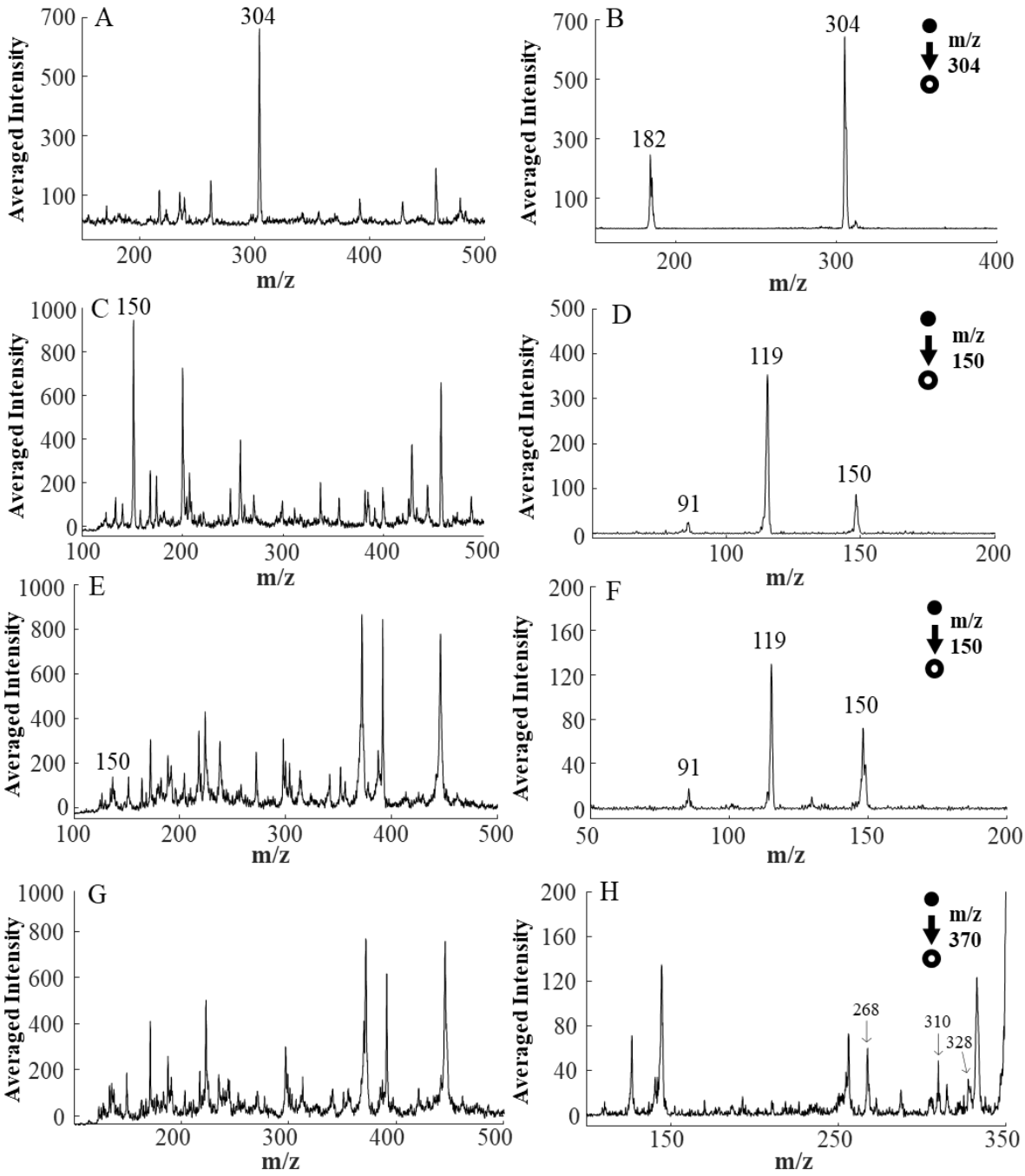

Figure 4.5 Mass spectra for the PSA PS-MS following color tests on portable mass spectrometer, A) Full MS of 1.25 µg of cocaine reacted with acidified $Co(SCN)_2$, B) MS/MS of 1.25 µg of cocaine reacted with acidified $Co(SCN)_2$, C) Full MS of 1.25 µg of MAMP reacted with Simon reagents, D) MS/MS of 1.25 µg of MAMP reacted with Simon reagents, E) Full MS of 10 µg of MAMP reacted with Marquis reagent, F) MS/MS of 10 µg of MAMP reacted with Marquis reagent, G) Full MS of 20 µg of street heroin reacted with Marquis reagent, and. H) MS/MS of 20 µg of street heroin reacted with Marquis reagent.

4.5 Conclusion

This work highlights the power of portable mass spectrometers for in-field drug identification. With the use of PSA paper, the successful recovery of cocaine, XLR-11, and fentanyl was demonstrated from aluminum and polyester-cotton blend cloth. Recovery from concrete and asphalt surfaces was not successful. True blinded sampling boasted zero false positive and zero false negative rates, with all samples being appropriately identified. Increased instrument analysis times did not interfere with analysis, indicating the portable mass spectrometer is robust enough to support extended sample collection periods. The combination of color tests with PS-MS was demonstrated on portable instrumentation with analyte levels comparable to a benchtop mass spectrometer, suggesting the potential adaptation of the technique to the field. The work outlined in this chapter showcased the feasibility and fieldability of PS-MS with PSA paper substrate for in-field detection of illicit drugs with the use of portable instrumentation.

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CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

The work presented in this thesis centers around the idea of using commercially available pressure-sensitive adhesive lined paper for the collection of drug residues and paper spray-mass spectrometry for drug identification. The established use of PSAs with PS-MS for drug detection focused on the recovery of drug residues from various surfaces, constructing limits of detection, and showing feasibility of the technique on portable instrumentation.[1] Building on this foundation, the use of PSAs with PS-MS was expanded for additional drug detection applications.

The combination of presumptive color tests with PS-MS as an all-in-one screening and identification method for illicit drugs is introduced. Color tests are among the simplest in-field drug testing methods but have low specificity. Three color tests, the cobalt thiocyanate test for cocaine, the Simon test for methamphetamine, and the Marquis test for phenethylamines and opiates, were conducted on PSA paper for preliminary drug screening. Following color testing, the post-reacted samples were analyzed by PS-MS to confirm drug identity. This analysis was successful for both color testing and PS-MS for all analytes considered. Parameters of this analysis including color test sensitivity, color test stability, and MS sensitivity were assessed. Finally, the method was applied to simulated "realistic" sampling scenarios where illicit drugs or false positives were successfully identified. This method provides a simple and rapid method for garnering presumptive and confirmatory data on a single sample using an affordable paper substrate.

The PSA and PS-MS method was expanded for the recovery and detection of fentanyl-related compounds including precursors, analogs, and byproducts of synthesis. Since large amounts of fentanyl and its analogs enter the country by mail service, the recovery and identification of these substances by PSA and PS-MS was demonstrated on four parcel-related surfaces: cardboard, shipping labels, packaging tape, and plastic wrapping. Beyond recovery of these compounds, they were examined by PS-MS with high concentrations of cutting agents. In the presence of cutting agents, there was no significant trends indicating a decrease in analyte signal for any of the tested analytes or cutting agents. Additionally, a cartridge was examined that did not require modification of the PSA paper prior to sampling. The cartridge accommodated a

full-sized PSA ticket with ionization occurring at the existing 90° corner of the paper. While performance of the no-cut cartridge was poorer than the traditional cartridge, the cartridge showed potential for fentanyl screening as fentanyl was able to be recovered and detected with the cartridge. Overall, examination of the recovery and analysis of fentanyl analogs by the PSA and PS-MS method demonstrates the versatility of the technique for use in sampling and screening.

This work concludes by examining PSA and PS-MS on a portable ion trap mass spectrometer. The recovery of 2 µg cocaine, XLR-11, and fentanyl was demonstrated from aluminum and polyester-cotton blend cloth by PSA paper. The compounds were not successfully recovered from concrete and asphalt surfaces. A blind study examining false positives and false negatives for the recovery of cocaine and methadone resulted in no false positives or negatives from various surfaces. Additionally, a lack of false positives indicated any potential analyte carryover did not hinder identification of true blank and drug positive samples. Finally, the established color test and PS-MS method was successfully translated to portable instrumentation, suggesting it is a viable technique for in-field analysis.

5.2 Future Directions

5.2.1 Color Testing and PS-MS

Additional studies focusing on the combination of color testing with PS-MS for drug identification could explore color tests targeting different drug classes. Further work on identifying opiates could examine the Mecke reagent (selenious and sulfuric acids).[2] With continual changes on the legality of marijuana by state, investigation of the 3-step Duquenois-Levine reagent for cannabinoids may prove to be valuable for in-field testing. With the recent rescheduling of hemp under the Controlled Substances Act, exploration of the 4-aminophenol (4-AP) or the Cannabis Typification test for distinguishing between marijuana-type and hemp-type cannabis could be valuable to forensic laboratories.[3] With the growing numbers of synthetic cathinones, application of the Zimmerman reagent may also prove valuable.[4] The Dille Koppanyi reagent, which is used to screen for barbiturates, could provide an interesting application as barbiturates have a tendency to ionize negatively with PSI.[2, 5] Expansion of the existing color test and PS-MS method could focus on screening additional surfaces or sampling scenarios. An assessment of drug mixtures or high concentrations of cutting agents could also further the technique.

5.2.2 Fentanyl-Related Compound Screening by PSA and PS-MS

Logical next steps for the screening of fentanyl by PSA paper and PS-MS would focus on the limit of detection determination for the tested compounds on the tested surfaces. With the wide variety of fentanyl analogs, expansion of the method to encompass a larger subset of these analogs would be beneficial. Fentanyl analogs should also be assessed in the presence of additional controlled substances, as they are often used to cut heroin.[6] Examination of more cutting agents, especially substances like xylazine which further contribute to deaths by reducing the efficacy of naloxone, by PS-MS could provide better insight into potential applications for sampling.[7] Testing the method with realistic sampling scenarios, such as packaging a box with fentanyl and then testing the gloves worn, would offer better context to the effectiveness of the method for parcel screening. Finally, further work should be conducted to improve the performance of the no-cut cartridge. This could entail modification of the design for better solvent and high-voltage application, studies to examine the best distance or angle from MS inlet to suspend the cartridge, or improvements to the cartridge materials and machining.

5.2.3 PSA, PS-MS, and Portable Instrumentation

Additional work focused on integrating PSA paper and PS-MS with portable instrumentation could address the determination of limits of detection for drugs recovered from different surfaces. The number and variety of drugs examined could be increased to focus on more compounds. Expansion into fentanyl analogs beyond the fentanyl and its analog acetyl fentanyl, could prove valuable with the opioid epidemic. Blinded sampling and analysis could be expanded to look at additional sampling scenarios and compounds including the examination of false positive and false negative rates at lower analyte amounts. These rates could also be examined in the presence of cutting agents. For the simplicity and efficiency of blinded sampling, improvements to the data analysis process should be pursued. It would be advantageous to develop the data collection software to produce simple “positive” or “negative” responses based on whether the spectrum meets detection requirements. The robustness of the portable instrument could also be assessed through increased sample analysis times. Future work with color tests and PS-MS on portable MS instrumentation could focus on analysis of realistic samples as demonstrated in Figure 2.10 or explore the use of additional color tests or analytes. Finally, as the portable MS is developed for

in-field applications, true use of the instrument in the field would be beneficial in further assessing the ruggedness and robustness of the instrument.

5.3 References

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VITA

Sarah Prunty was raised in Central Virginia. In 2017 she began her collegiate career at the University of Mary Washington in Fredericksburg, Virginia. In 2018 she transferred to the University of Virginia to pursue a degree in chemistry. During her time at UVa, Sarah worked as an undergraduate teaching instructor for general chemistry lab courses. Sarah graduated in the spring of 2021 with a Bachelor of Science in Chemistry and a minor in Environmental Sciences. In fall of the same year, she began graduate coursework and research at Indiana University-Purdue University Indianapolis. Her Master's research was conducted in Dr. Nicholas Manicke's laboratory where she focused on the use of adhesive-lined stationery for the recovery illicit drugs from various environmental surfaces and as a substrate for drug identification.

PUBLICATIONS

Pending Publication

Prunty, S.; Carmany, D.; Dhummakupt, E.; Manicke, N. Combining Presumptive Color Tests, Pressure-Sensitive Adhesive-Based Collection, and Paper Spray-Mass Spectrometry for Illicit Drug Detection. Analyst 2023, 10.1039/D3AN00559C.

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