PAPER SPRAY - MASS SPECTROMETRY: INVESTIGATION OF SAMPLING DEVICES FOR ILLICIT DRUG DETECTION AND QUANTIFICATION

by

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This thesis is dedicated to my beloved parents and my sister, who continually provide their moral, emotional, and financial support, and to my partner, who is always there for me.

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LIST OF ABBREVIATIONS

AUC	Area under the curve
CE	Collision Energies
CID	Collision Induced Dissociation
DAPI	Discontinuous atmospheric pressure interface
DART	Direct analysis in real time
DC	Direct current
DESI	Desorption electrospray ionization
EASI	Easy ambient sonic-spray ionization
ESI	Electrospray ionization
EESI	Extractive electrospray ionization
FN	False negative
FP	False positive
GC	Gas chromatography
IMS	Ion mobility spectrometry
ISO	Isolating
ISTD	Internal standard
LC	Liquid chromatography
LIT	Linear ion trap
LOD	Limit of detection
LTP	Low temperature plasma ionization
MALDI	Matrix assisted laser desorption ionization
MIMS	Membrane introduction mass spectrometry
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass to Charge
NPS	New psychoactive substances
NPV	Negative predictive value
PPV	Positive predictive value

PSI	Paper spray ionization
PS-MS	Paper spray – Mass spectrometry
PSA	Pressure-sensitive adhesive
R^2	Coefficient of Determination
RF	Radio frequency
RSD	Relative standard deviation
S:B	Signal to Blank ratio
SPE	Solid-phase extraction
TN	True negative
TP	True positive
TOF	Time-of-flight

ABSTRACT

Paper spray - mass spectrometry (PS-MS) has been developed as a rapid and direct ionization method for qualitative and quantitative analysis of complex samples at trace levels. In this work, different sampling devices for PS-MS were investigated to improve the assay's simplicity and sensitivity over traditional approaches. In particular, chapter two characterizes an alternate paper substrate to enhance the drug detection on surfaces like asphalt, cloth, concrete, aluminum, and glass. Analysis occurs on a single spray ticket coated with pressure-sensitive adhesive (PSA), also known as Post-it® notes to detect and quantify drug residues. A PS-MS method utilizing PSA paper was developed to detect a mixture of ten drugs off of various surfaces to evaluate the qualitative and quantitative capabilities of the aforementioned substrate. After the method development on a conventional linear ion trap mass spectrometer, the assay was translated for use on a portable mass spectrometer to evaluate the suitability of the pressure-sensitive adhesive paper substrate in the field in chapter three. Chapter four introduces a sampling device combined with a snap-in solid-phase extraction (SPE) column. The new cartridge design not only inherits the functions from the first iteration SPE cartridge, including extraction and preconcentration from complex samples, but also exhibits greater flexibility in volume control and ease of use for on-site sample collection.

1.1 Mass Spectrometry

Mass spectrometry is a commonly used analysis technique for the separation and detection of gaseous, charged species based on their mass-to-charge ratios. A mass spectrometer has five basic components: a sample inlet, through which molecules of interest are introduced, a high vacuum system, an ion source, where the molecules are ionized and transformed into gas phase ions, a mass analyzer that helps separate the ions based on their mass-to-charge ratios, and a detector that converts the separated ion beams into measurable signals[1].

Modern mass spectrometers have different types of mass analyzers utilized to separate and detect ions. The mass analyzer, the heart of the mass spectrometer, can be classified into sector, ion trap, quadrupole, and time of flight. Many analyzers use electric or magnetic fields to apply a force on the charged species[2]. For example, a quadrupole analyzer separates ions then oscillates them from the quadrupole to a detector using a combination of radio frequency and direct current potential[2]. On the other hand, orbitrap analyzers, a subset of ion traps, use a central spindle electrode surrounded by a barrel-like electrode to apply an electrical field on the trapped ions and keep them in oscillating movement[3]. The frequency of axial oscillation of the ions along the central spindle is used to measure their mass-to-charge ratios with induced current[3]. Alternatively, time-of-flight analyzer does not separate ions directly based on mass-to-charge ratios, but rather measures the time it takes for the ions to travel through the flight tube to the detector based on the kinetic and velocity of the analyte. Indeed, these key differences allow modern mass spectrometers to identify a multitude of molecules based solely on the mass-to-charge ratios, making them useful for a variety of applications including forensic toxicology, metabolomics, proteomics, and clinical research[4,5,6].

Apart from the mass analyzer, the ion source also plays an important role as sample introduction was a major challenge in mass spectrometry. In mass spectrometry, molecules of interest are introduced to the ionization source, converted into a gas phase, and charged. The way this mechanism occurs depends on the ionization technique used[7]. For instance, electron impact (EI) directly volatilizes a sample within a vacuum system and bombards the neutral molecules with a beam of electrons, resulting in the generation of positive radicals[8]. Due to the ionization's high internal energy, the molecular ions are broken apart into smaller neutral atoms and fragmented

prior to mass analysis. EI is usually paired with other separation techniques such as gas chromatography. One of the major disadvantages of EI is that molecules are heavily fragmented. While this fragmentation can give excellent selectivity, fragmentation compliances mixture analysis and sometimes eliminates the molecular ion. EI is not an ideal method for large molecule analysis such as peptides and proteins.

The application of MS in detecting and quantifying biological samples has been advanced due to the development of soft ionization techniques such as electrospray (ESI) and matrix assisted laser desorption ionization (MALDI)[9]. MALDI is a type of ionization, in which the molecules are mixed with a matrix that plays a role in laser radiation absorption and charged ions generation when molecules are bombarded with a laser beam instead of electrons. Besides MALDI, the other method that has become more popular overtime due to its massive contribution to quantitative analysis is electrospray ionization (ESI). In ESI, a high voltage is applied to a liquid or solid sample to create an aerosol sprayed into the mass spectrometer's inlet. The charged droplets are then desolvated further to become smaller in size and eventually turned into gas phase. With the ability to transfer ions from solution into gas phase using electrical energy with very little fragmentation, ESI is considered one of the softest ionization methods[10]. As a result, it is often paired with liquid chromatography to analyze biological samples with large masses. Without a doubt, although different methods of ionization can be used and optimized for different purposes, MALDI and ESI have become the most popular ionization technique since they have greatly extended the functionalities of mass spectrometers in analyzing a wide range of compounds, from polymer to drug discovery[11,12].

1.2 Ambient Ionization

To reduce analysis time and labor, there was a need for ionization techniques operate under atmospheric conditions, do not require sample preparation and chromatography separations. Desorption electrospray ionization (DESI) was first introduced by Cook's lab in 2004, showing a technique capable of producing gas-phase ions by desorbing ions from samples to the mass spectrometer using electrosprayed droplets with little or no sample pretreatment[13]. Needless to say, DESI drew attention toward the concept of open-air surface analysis under atmospheric condition, leading to numerous other ambient mass spectrometry techniques.

Ambient ionization can be categorized into three main techniques: spray desorption/ionization, laser ablation/desorption-based ionization, and plasma-based ionization[14,15]. Among spray desorption/ionization techniques, DESI is the most widely practiced. Most of the spray desorption techniques share five analysis steps in their mechanism: 1) charged solvent spraying directly at the intact sample, 2) collision of charged droplets with the sample surface, 3) analytes pick-up during brief contact between charged droplets with sample surface, 4) release of analyte in the form of secondary charged droplets from the liquid layer on sample surface, and 5) desorption of the analyte ions generated from the secondary droplets into the mass spectrometer inlet[15-17]. The applications of spray desorption ionization can be found in various works due to its versatility in sample types. For example, DESI and other desorption methods such as easy ambient sonic-spray ionization (EASI) are only applicable to solid samples, while liquid-DESI and extractive electrospray ionization (EESI) provide a means to analyze liquid samples by infuse the samples through a silica capillary[14,15]. Unlike spray desorption/ionization, laser-assisted ambient ionization uses lasers to ionize molecules of interest. Short bursts of well-defined high energy, ablate or desorb the analytes from the sample, followed by the formation of matrix/analyte clusters after the irradiation, and finally, the generation of highly charged analyte ions via electrospray ion plume[14,18]. Last but not least, direct analysis in real time (DART) and low temperature plasma ionization (LTP) are two well-known examples of plasma-based ionization techniques. In general, plasma-based ionization involves the charge-transfer reactions between the plasma and an ion to form electronic excited gas molecules, usually nitrogen or helium, which will then ionize volatile organic compounds originating from the samples[14,18]. Since the introduction of these ambient ionization techniques, various applications for the analysis or detection of illicit drugs, explosives, pesticides, as well as polymer materials have been presented in literature[19-21].

1.3 Paper Spray – Mass Spectrometry

Along with the techniques mentioned above, paper spray is another ambient ionization method that has garnered significant interest due to the simplicity in set up with little to no sample preparation. This technique involves spotting a sample on a triangular-shaped paper substrate, followed by the addition of a spray solvent and voltage to generate a plume of charged droplets. This induces the formation of a Taylor Cone, in front of the mass spectrometer (MS) inlet (Figure 1.1).

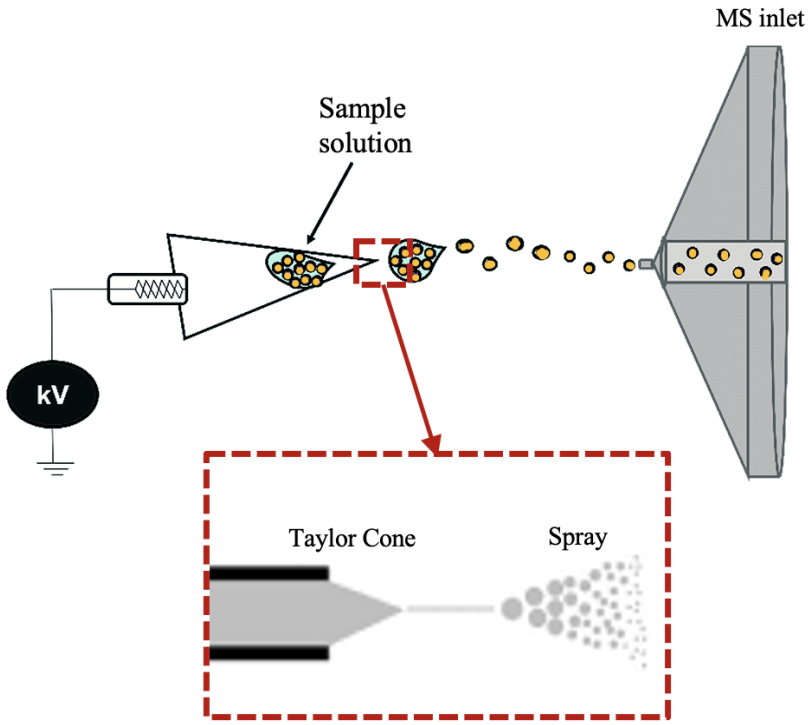

Figure 2.1 Paper spray mass spectrometry spray mechanism. Solvent and voltage applied to the dried sample on paper substrate in front of MS inlet, resulting in ionization occurs under ambient conditions.

Prior to mass analysis, a spray solvent is added from the rear to wet the paper substrate and the dried sample spot, as well as to prevent backward elution. Next, analysis is initiated by applying a high voltage to the paper after analytes of interest have been extracted by the spray solvent and traveled to the tip of the paper. Based on the purpose of the analysis, the applied voltage can be negative or positive, usually ranging from 3.0 to 5.0 kV[4,22-24]. Indeed, several factors can

remarkably impact ions formation and signal stability, including paper spray tip, solvents used, as well as the positioning of the paper tip and its distance from the MS inlet.

Numerous studies have been performed to further enhance signal stability and detection limits. First and foremost, as paper is one of the main components playing a crucial role in paper spray ionization, the usage of a number of different papers and paper pre-treatments have been investigated. The two paper types mostly used are Whatman 1 and Whatman 31ET[22]. Whatman 1 is suitable for a rapid analysis with a minute amount of sample since the thinness of this type of paper not only allows fluid samples to dry faster, but also requires a smaller volume of sample and spray solvent. In contrast, Whatman 31ET, taking advantage of thick and fast chromatography characteristics to accommodate larger sample volumes for longer analysis, can achieve lower detection limits and higher reproducibility of signal[22]. On the other hand, the high content of hydroxyl groups in cellulose and semi-cellulose structures of paper substrate has a tendency to create hydrogen bonds and Van der Waals interactions between the paper surface and the polar analytes[25]. These interactions can hinder the extraction and elution of the targeted analytes from the dried sample to the paper tip and decrease the signal and sensitivity of the assay in overall. As a result, paper pre-treatments such as spray-deposition, dip-coating with silica, metal nanoparticles, and silanization have been studied to improve the performance of paper spray analysis due to different interactions between surface properties and analytes of interest[25].

Other than modifying the paper substrate, solvent selection also plays a crucial role in obtaining stable signals as it is in direct contact with the compounds during the analysis. Organic solvents are usually used as the spray solvents to extract hydrophobic molecules such as drug compounds or biofluids like peptides[26]. Some mixtures commonly used for paper spray assays are 90:10 methanol:water, 90:10:0.01 methanol:water:acetic acid, and 90:10 acetonitrile:water [22,27,28]. Usually, the addition of acetic acid or formic acid helps initiate analyte protonation and also stabilize the spray during the mass analysis[22]. However, several factors should be taken into consideration while choosing spray solvents. Firstly, solvents need to effectively wick through the dried sample and extract the analytes from the matrix because drug molecules and biochemical compounds have different elution behaviors depending on the solvents used. At the same time, the spray solvents should be able to generate a stable electrospray for ionization, ensuring stable signals during the analysis. Moreover, paper pre-treatments and modification can greatly affect the interactions among the paper substrate, the analytes in the matrix, and the solvent; therefore,

choosing the appropriate solvents for extraction and for paper spray as a whole is necessary.

PS - MS exhibits unique advantages over traditional mass spectrometry methods and even other ambient techniques for its simplicity, versatility, rapidity, and affordability. As discussed above, the setup for paper spray ionization cannot get any simpler, with just a paper substrate, spray solvent, and a high voltage power supply. Furthermore, paper spray has been utilized in diverse applications, mentioning clinicals, pharmaceuticals, forensic and other metabolites from blood, plasma, urine, and oral fluids with no sample preparation[22,23,27,29]. In addition, while the sample volume consumed in a paper spray assay is low, this ambient ionization method still shows high sensitivity, with detection limits in single digit ng/ml and sometimes even sub-ng/ml range despite the presence of the matrices[30,31]. Being a cost-effective technique is another advantage of paper spray as it helps reduce cost in numerous ways: the paper substrate is inexpensive and easy to obtain, the amount of spray solvent required for paper spray is low, leading to no solvent waste and no removal cost, and little to no instrument downtime for maintenance and maintenance services needed for paper spray.

PS - MS methods have been developed for drug screening in different research; however, paper spray mass spectrometry has not been fully and widely utilized, especially in clinical applications and forensic[10,32,33]. Gas chromatography (GC) or liquid chromatography (LC), usually coupled with mass spectrometry, has been considered the main methods when it comes to toxicological analysis. They are capable of efficiently separating analytes of interest, gaining high selectivity and specificity, as well as generating data and results that can be compared to databases and libraries. However, while GC - MS and LC - MS are effective analytical methods, they still pose several disadvantages that can be overcome when using PS - MS. For example, the long sample preparation procedure before the actual LC - MS analysis can be replaced with no preparation at all during PS - MS. The fifteen-minute to half an hour-long analysis can be performed within minutes in the case of paper spray ionization. Finally, the sample's properties will not likely be a problem since paper can analyze a wide range of molecules, while mass spectrometry can simultaneously differentiate them based on their unique spectrum. These points make paper spray mass spectrometry a good candidate for initial screening of a wide range of molecules.

1.4 Paper Spray – Mass Spectrometry in Drug Screening

Various research has shown paper spray – mass spectrometry as a potential alternative to GC-MS and LC-MS[34-36]. The detection and quantification of drugs at low or sub-ng/mL levels with high quantitative accuracy has been reported to be in good agreement with the results obtained from LC-MS[22,23,34-36]. In these research, illicit substances could be screened directly from blood, plasma, urine, and oral fluid with low ng/mL detection limits. Explicitly, in a work by McKenna *et al*, over 130 dugs and drug metabolites were screened and semi-quantified in postmortem specimens using a 2.5-min long PS-MS/MS assay[23]. From the results obtained, the drugs were detectable at significantly low concentrations, with the true positive rate being 92.1% while the true negative rate of 99.8%. In another research where PS-MS was adapted for direct, quantitative analysis of tobacco alkaloids from biofluid samples to assess second-hand smoke, limits of quantification of nicotine, cotinine, trans-3'-hydroxycotinine, and anabasine were as low as several nanograms per milliliter[34]. The quantification results of cotinine in blood samples obtained from PS-MA analysis were compared to a traditional analysis protocol using LC-MS, showing he precision of the two methods was similar[34]. These works demonstrated the rapid, sensitive, and simple PS-MS/MS analysis for drug screening[23].

Other than bioanalysis, PS-MS can also be used for drug screening on surfaces. A work of Wichert *et al.* demonstrated the detection of protein toxin simulants from contaminated surfaces with wipe sampling[37]. Porous nylon membrane was used to wipe different proteins and biological toxin simulant on surfaces such as laboratory bench, notebook cover, glass, and vinyl flooring, and then placed onto the paper substrate for PS-MS analysis[37]. Results showed that the tested protein toxin simulants were successfully detected at low microgram quantities using the porous wipe[37]. This application indicated that PS-MS can potentially be used for rapid, sample preparation-free detection of chemicals and biological molecules on a variety of surfaces, which also opened the door for trace detection of different compounds for national security at customs and border checkpoints.

Ion mobility spectrometry (IMS) has been widely employed by military, customs, and border controls for on-site detection of vapor phase species including chemical weapons, explosives, and drugs[38-40]. However, this analytical technique has several weaknesses. First of all, IMS instruments are often coupled with mass spectrometry[41], GC or LC[42] in order to fully achieve a multi-dimensional separation, making the set-up much more complicated in the field. Secondly, while

IMS is considered an ambient analytical technique, matrix effects such as the composition of the sample, high humidity, and fluctuating temperature can have significant impact on the detector's response[43]. Therefore, this method requires delicate engineering and parameter optimization for in-field analysis. Another drawback of IMS technique is the high potential of false positive caused by chemical interference in a highly contaminated environment, leading to other limitation of this technique for in-field analysis[43]. As ambient techniques advanced, paper spray ionization (PSI) becomes an ideal candidate to replace or complement IMS trace detection for military and national security purposes. It outperforms IMS because of the significantly better selectivity and detection limits owing to MS detection. At the same time, PS-MS analysis can be easily performed under ambient condition without being affected by environmental factors. Due to PSI versatility and simplicity, it is of great interest the coupling of PSI to the portability of miniatured mass spectrometers to make feasible the real-time on-site screening.

With the potentials of PS - MS in drug screening, the purpose of this thesis is to improve the application of this method by investigating different sampling methods and devices to enhance sensitivity and ease of application in forensics and customs. Chapter two focuses on the usage of pressure-sensitive adhesive (PSA) paper as a low-cost, readily available alternative to the traditional paper substrates. A fast and sensitive method is developed with the aid of PSA paper in order to perform drug screening on exterior or interior surfaces. To better understand the application of the PSA paper during the sampling procedures, a variety of synthetic drugs with a wide range of logP and pKa, from fentanyl analogs to synthetic cannabinoids, are chosen for the experiment. At the same time, the reproducibility of the study and detection limits of ten drug compounds are determined. In chapter three, PSA paper collection followed by a paper spray mass spectrometry assay is performed on BaySpec Continuity portability mass spectrometer. "Realistic" scenarios are also considered, in which drug residues are collected from a variety of surfaces using PSA paper, including cardboard, plywood, t-shirt, and office paper. From this, the functionality of PSA paper during the sample collection coupled with paper spray - MS for on-site drug screening is evaluated. In another project, chapter four shows an alternative cartridge design in which a SPE column can be snapped into a cartridge compatible with an existing automated paper spray system. Previous research depicts the use of an integrated solid-phase-extraction (SPE) column for sensitivity improvement[35,36]. However, this integrated format has several limitations, including difficulties in transporting the entire cartridge and requirement for a new cartridge just for the SPE.

The new snap-in SPE outperforms the previous integrated approach due to greater flexibility in preconcentration and volume control device with improved efficiency during on-site sample collection and transportation.

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CHAPTER 2. PRESSURE-SENSITIVE ADHESIVE PAPER FOR LOW-COST COLLECTION AND ANALYSIS OF DRUG RESIDUES ON SURFACES

2.1 Abstract

The continuous growth of the drug market and drug trafficking has overwhelmed forensic and customs laboratories. Various screening assays have been developed to detect and quantify illicit drugs in biofluid. However, such tests are not readily available to screen for the drugs on non-porous and porous surfaces for forensic and border control purposes. The work presented in this chapter evaluates the use of paper substrates coated with pressure-sensitive adhesive (PSA) for collection of drugs on various surfaces that are commonly encountered in forensic and customs investigations. Following collection on the PSA substrate, the sample was then analyzed using paper spray mass spectrometry. Both sample collection and analysis occur on a single ticket, making this workflow simple enough to be performed in the field on a portable mass spectrometer. A mixture of ten drugs, including acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11, sampled from asphalt, cloth, concrete, glass, and aluminum surfaces, were detected and quantified in this study. The assay was performed on a conventional benchtop linear ion trap mass spectrometer. It was determined that PSA paper outperformed paper without adhesive and also showed its ability to collect residues even after being used several times during sample collection. Moreover, the detection limits of ten compounds ranged from 2 ng to 10 ng. When coupled with paper spray mass spectrometry, this novel sampling tool allows a simple, yet rapid and effective collection procedure and screening method in general.

2.2 Introduction

After dropping 17% from 2017 to 2018 for the first time in almost two decades, drug overdoses deaths began increasing again in the months preceding the COVID-19 pandemic and accelerated even further after disruptions set in[1]. There were over 81,000 drug overdose deaths during the 12-month period ending in May 2020, the largest ever recorded in the US over any 12-month period[2]. The misuse and abuse of drugs, from prescription pain relievers to synthetic opioids and cannabinoids, has become a serious crisis that has no conclusive solution. Without any doubt, drug screening has served as an important tool for harm reduction in order to combat the opioid crisis around the world. Various drug tests have been developed to detect the presence or absence of specific drugs as well as drug metabolites in biological samples for addiction identification, doping control, workplace drug testing and postmortem toxicology[3-5]. The use of mass spectrometry (MS) allows for rapid, accurate screening and confirmation for many drug categories in blood, plasma, urine, and oral fluid samples[6-9].

Despite the popularity of drug testing in biological specimens, there are scant numbers of works that focus on detecting these chemicals on porous surfaces. This is especially troubling when drug trafficking and smuggling directly fuel the opioid crisis, causing drug contamination on surfaces in different ways. Needless to say, a globally interconnected supply chain has significantly complicated the traditional drug tracing process of law enforcement authorities. The drug packages arriving through the mail can originate from the black market, a part of Dark Web, and have a close relation to cryptocurrencies[10,11]. After purchase on the Dark Web, illicit substances are shipped through postal services and private carriers all over the world with low chances of being seized at border controls[10]. However, drug residues may be present on the surfaces of such parcels sent via sea or air cargo.

Besides the cargo coming from the cybercrime black market, human drug couriers can also have drug contamination on surfaces like on passengers' identity documents, clothing, or luggages[12]. To avoid drug screening at border controls or airport customs, drug smugglers use several methods to discreetly hide drugs for transportation, including taping the drug packages around bodies or in many cases, body packing. Even with advanced packaging procedures, the human drug couriers can still face life-threatening complications such as drug absorption and toxicity due to packages' rapture or gastrointestinal blockage[13]. As a result, a rapid drug screening

method on porous absorbent surfaces of the suspects' belongings can provide earlier intervention to prevent deadly consequences of body packing at the border and customs.

While ion mobility mass spectrometry is commonly used for airport security, ambient ionization, a new MS technique, can be a better substitute due to its greater sensitivity and selectivity. The advent of ambient ionization techniques in general and paper spray – mass spectrometry (PS – MS) in particular opened the door for direct chemical and biological analysis in the field[14-16]. This is especially relevant for forensic applications because it is necessary to analyze raw samples and detect compounds of interest at low concentration[17]. Surface sampling by combining swabbing with paper spray mass spectrometry has been demonstrated for detection of drugs[18], explosives[19], and protein toxins[20]. With no sample pretreatment and simple procedure, PS-MS also takes advantage of low-cost paper substrate with small sample and solvent volumes required. These makes PS-MS a versatile direct analysis method for on-site drug detection and crime scene investigation.

There is a lot of flexibility in creating a PS-MS assay, from paper modification to extraction methods of choice, with the hope of enhancing analyte detection. In the literatures, paper substrates have been coated with carbon, silica, polymers, and even metal powders in order to reduce the binding of the analytes to the substrate and also improve analyte ionization[20,21]. However, these modifications can take time, effort, and money to prepare the paper substrates. In this work, pressure-sensitive adhesive paper was investigated for its compatibility and efficiency when coupled with rapid PS-MS analysis is investigated. This approach is a better alternative to substrate coatings for border controls because pressure-sensitive adhesive papers (PSAs) are commercially produced, easily obtained, and require no activation by water, solvent, or heat[22]. PSAs are primarily acrylic-based elastomers with or without added tackifiers. PSAs are found in many consumer products, from masking tapes, labels, pressure-sensitive adhesive dress wounds, to even tile flooring. Due to its simplicity, PSA paper can further be used for different purposes, including PS-MS analysis. Here, PSA-coated paper is utilized with paper spray MS for the collection and analysis of microscopic traces of chemical evidence.

In this work, a single paper ticket with repositionable PSA serves as both a sampling tool for drug residues collection on surfaces and also as the paper substrate for paper spray ionization. Both porous and non-porous surfaces, like glass, aluminum, cloth, asphalt, concrete, and cardboard, were used to investigate the application of PSA papers for PS-MS. A mixture of ten compounds

was spotted onto the PSA paper to compare the effects of adhesive vs non-adhesive. Collection efficiency and detection limits were also determined both directly on paper and from sampling surfaces by dabbing the paper tickets onto contaminated surfaces.

2.3 Method

2.3.1 Materials

Clonazolam, cocaine, fentanyl, heroin, ketamine, methamphetamine, methylone, U-47700, and their deuterated standards, cocaine-d3, fentanyl-d5, heroin-d9, methamphetamine-d11, U-4770-d6, were purchased from Cerilliant (Round Rock, TX, USA). Acetyl fentanyl standard in powder form, XLR-11 and its d5 were purchased from Cayman Chemical (Ann Arbor, MI, USA). High-performance liquid chromatography (HPLC) grade solutions of acetonitrile, formic acid, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Finally, PSA paper (Post-It® Notes, 3M) in several formats was purchased from an online retailer, while all-purpose flour was sourced locally.

2.3.2 Preparation of Working Solutions

Using the powdered acetyl fentanyl drug standard, acetyl fentanyl stock solution was prepared in methanol (1 mg/mL). The concentration of clonazolam, cocaine, fentanyl, heroin, ketamine, methamphetamine, methylone, and U-47700 were 1 mg/mL, while the concentration of XLR-11 was 100 µg/mL. For the experiments comparing adhesive and non-adhesive paper as well as evaluating the robustness of PSA paper in sample collection, a working solution was prepared from ten individual stock solutions at 50 ppm. A mixture of 50:50 water:acetonitrile was selected for sample spotting to minimize absorption into porous surfaces. Calibrators for detection limits determination were prepared using a serial dilution, in which the working solution prepared above was sequentially diluted from 50 ppm to 0.1 ppm to make 10 calibrants. From that, the amount of drug spotted at these calibrant concentrations were in the range of 0.6 to 300 ng. A 50-ppb internal standard solution containing six internal standards was made in methanol with 0.1% formic acid. All solutions and calibrants were stored at -20°C when not in use.

2.3.3 PSA Paper Spray Tip Preparation

Three common types of repositionable PSA paper (normal home/office, ruggedized outdoor, and recycled) were evaluated. Normal home/office Post-It® Notes were used for all subsequent experiments because they showed the highest and most consistent signal among the three paper types tested. The PSA paper was prepared in stacks of seven. The paper was cut into 0.5 x 1 cm strips, and a triangular (~33°) shaped tip was cut from one end using a razor blade. The top and bottom paper layers were discarded to remove any possible contamination, and the remaining five layers were used for sampling/analysis. This separation was done just prior to surface sampling to avoid contamination during storage and handling. Only the rectangular portion of the paper ticket contained adhesive while the triangular tip did not (Figure 2.1). Cutting the paper spray tickets such that the triangular tip was adhesive-free produced sharper, more reproducible spray tips.

2.3.4 Drugs Deposit and Samples Collection

Five surfaces were used to evaluate and compare the functionality of the PSA paper and non-adhesive paper, including asphalt, cloth, concrete, glass, and aluminum. Aliquots of the working solution (6 μL) were spotted onto each surface and allowed to dry completely. Compressed nitrogen gas was blown onto surfaces after the drug deposit to remove any loose particles. Five replicates of the sample were created by dabbing the adhesive paper tickets, adhesive or non-adhesive, onto the dried drug spots of each surface to collect drug residues. Ten blank samples were also prepared by dabbing five adhesive paper tickets and five non-adhesive paper tickets onto non-drug areas of each surface. Next, 3 μL aliquots of the deuterated internal standard solution were added to the paper spray tickets and let dry before the analysis. The separate steps for addition of the drug and deuterated standards were done to mimic the experiments using real life sampling of drug powders off surfaces.

To investigate the effect of sampling order when dabbing a single PSA ticket multiple times, two sampling sequences were tested to mimic the most extreme scenarios: the paper tip was either first dabbed seven times on non-drug areas of surfaces then one time on a dried drug spot or vice versa (contaminated area first followed by seven clean areas). On each surface, samples and blanks

were prepared in five replicates for each sampling sequence. The sample preparation step was finished by adding 3 µL aliquots of the deuterated internal standard solution onto the tickets.

Two sets of detection limits were determined. For the first set, 3 µL aliquots of the calibrants were spotted on the paper tips with three µL of the internal standards added in order to determine the detection limits when using PSA papers. The second set of detection limits were determined by spotting 6 µL aliquots of the calibrants on to surfaces, followed by sample collection using PSA paper tips. Calibrants and blank samples were made in triplicate for each surface. Finally, 3 µL aliquots of the deuterated internal standard solution were added and allowed to dry completely.

2.3.5 Paper Spray – Mass Spectrometry and Data Analysis

A milled Delrin cartridge was made to hold the paper spray tickets while providing an electrical contact between the high voltage source and the paper tips during the analysis (Figure 2.1). PSA paper tickets or non-adhesive tickets, depending on the experiments, were placed inside this cartridge and 3-4 mm from the MS inlet. A 65 µL volume of the spray solvent (acetonitrile with 0.1% formic acid) was added dropwise into the solvent well of the cartridge, allowing the paper tickets to completely wet to the tips. The detailed PS-MS experiments were performed on a Thermo LTQ XL mass spectrometer, in which a voltage of +4.5kV was applied to the paper ticket for 1.5 minutes to induce the plume of ions. Capillary temperature was set at 300°C. Both full MS and MS/MS data were acquired in positive ion mode, with the mass ranging from *m/z* 50 to 500 and collision-induced dissociation (CID) values listed in Table 2.1. The two chosen fragment ions used for MS/MS analysis were ones yield the most stable and highest peaks during tuning.

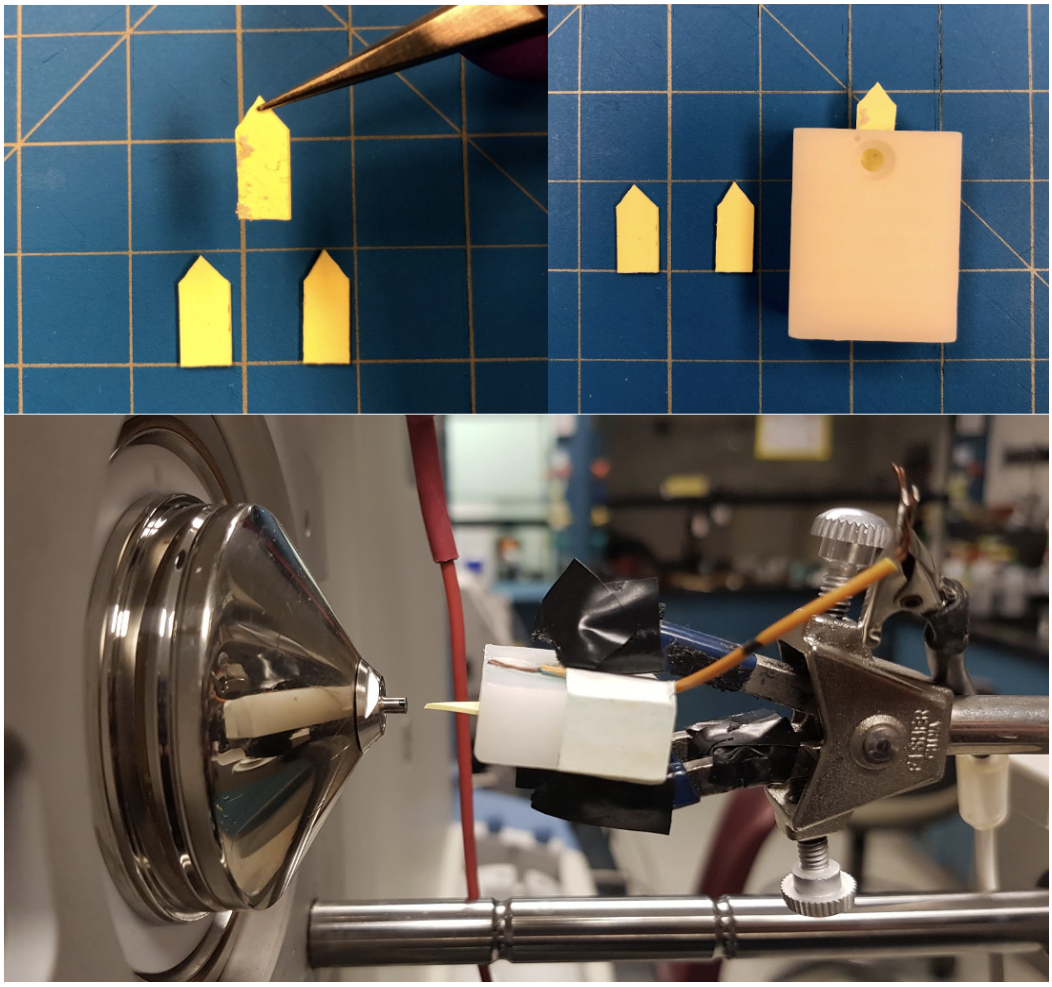

Figure 2.1 A) Paper spray tip preparation with sample collected on concrete surface, B) paper tip inserted into milled Delrin cartridge and C) placed 3-4 mm from the inlet.

Table 2.1 Ten analytes being investigated with their molecular formulas, precursor ions, quantifying and confirming ions, and CID values.

Analytes	*Chemical Formula*	*Precursor m/z*	*Fragment m/z**	*CID (eV)*
Acetyl fentanyl	$C_{21}H_{26}N_2O$	323.20	**188.1,** 105.1	33
Clonazolam	$C_{17}H_{12}ClN_5O_2$	354.07	**308.1,** 326.0	30
Cocaine	$C_{17}H_{21}NO_4$	304.15	**182.1,** 150.0	35
Fentanyl	$C_{22}H_{28}N_2O$	337.20	**188.2,** 105.0	40
Heroin	$C_{21}H_{23}NO_5$	370.16	**328.1,** 211.1	55
Ketamine	$C_{13}H_{16}ClNO$	238.10	**220.1,** 179.0	45
Methamphetamine	$C_{10}H_{15}N$	150.13	**119.0,** 91.0	50
Methylone	$C_{11}H_{13}NO_3$	208.10	**190.1,** 160.0	50
U-47700	$C_{16}H_{22}Cl_2N_2O$	329.11	**284.0,** 203.6	40
XLR-11	$C_{21}H_{28}FNO$	330.22	**232.1,** 125.0	42

* Bold items indicate the quantifier ion for each analyte

Data analysis was performed using Tracefinder v. 3.3 software (Thermo Fisher Scientific). Ten analytes and six internal standards with their fragment ions' peaks were integrated for the quantification of the drug residues. From that, calibration curves of ten compounds were graphed using the 1/x weighted least squares. With this approach, more weight would be put towards the lower concentration of the curve, counteracting the larger variance at high concentrations. Finally, limits of detection (LOD) of ten drug compounds were calculated using equation 1:

$$LOD = 3 \times S_b/m \quad (1),$$

in which S_b was the standard deviation of blank signals, and m was the calibration curve's slope. All statistics were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and R (Vienna, Austria).

2.4 Results and Discussion

2.4.1 Method Development

Analyte Selection

A wide variety of drug compounds with a wide range of logP and pKa were selected for the experiment to investigate the effects of the PSA papers and how they interact with the analytes of interest during sample collection. Their physical properties were documented in Table 2.2 below, in which some compounds have both strongest acidic and strongest basic pKa due to Zwitterionic effect in their molecular structures.

Table 2.2 Analytes properties from Drugbank of eight compounds of interest. Properties of acetyl fentanyl and XLR-11 were unavailable.

Analytes	*LogP*	*pKa (acid)*	*pKa (basic)*	*Physiological Charge*
Clonazolam	2.96	17.54	4.09	0
Cocaine	2.28	N/A	8.85	1
Fentanyl	3.82	N/A	8.77	1
Heroin	1.55	N/A	9.10	1
Ketamine	3.35	18.78	7.45	1
Methamphetamine	2.24	N/A	10.21	1
Methylone	1.91	7.74	N/A	0
U-47700	3.91	N/A	9.2	0

PSA Paper Selection

Different types of Post-it® Notes were tested to determine which substrate produces stable spray and high ion efficiency with low ion suppression. Original Post-it® Notes, colored papers, and super sticky papers were all tested as potential substrates. Among these, colored papers tended to cause dye contamination (Figure 2.2A), while super sticky Post-it® Note picked up most of the drug residues, leaving "cleaner" spots on surfaces after sample collection (Figure 2.2C). Therefore, PS-MS analysis utilizing super sticky notes showed an increase in analyte signals in general. From

these data, yellow super sticky Post-it® Notes were selected as paper substrates for further PS-MS analysis.

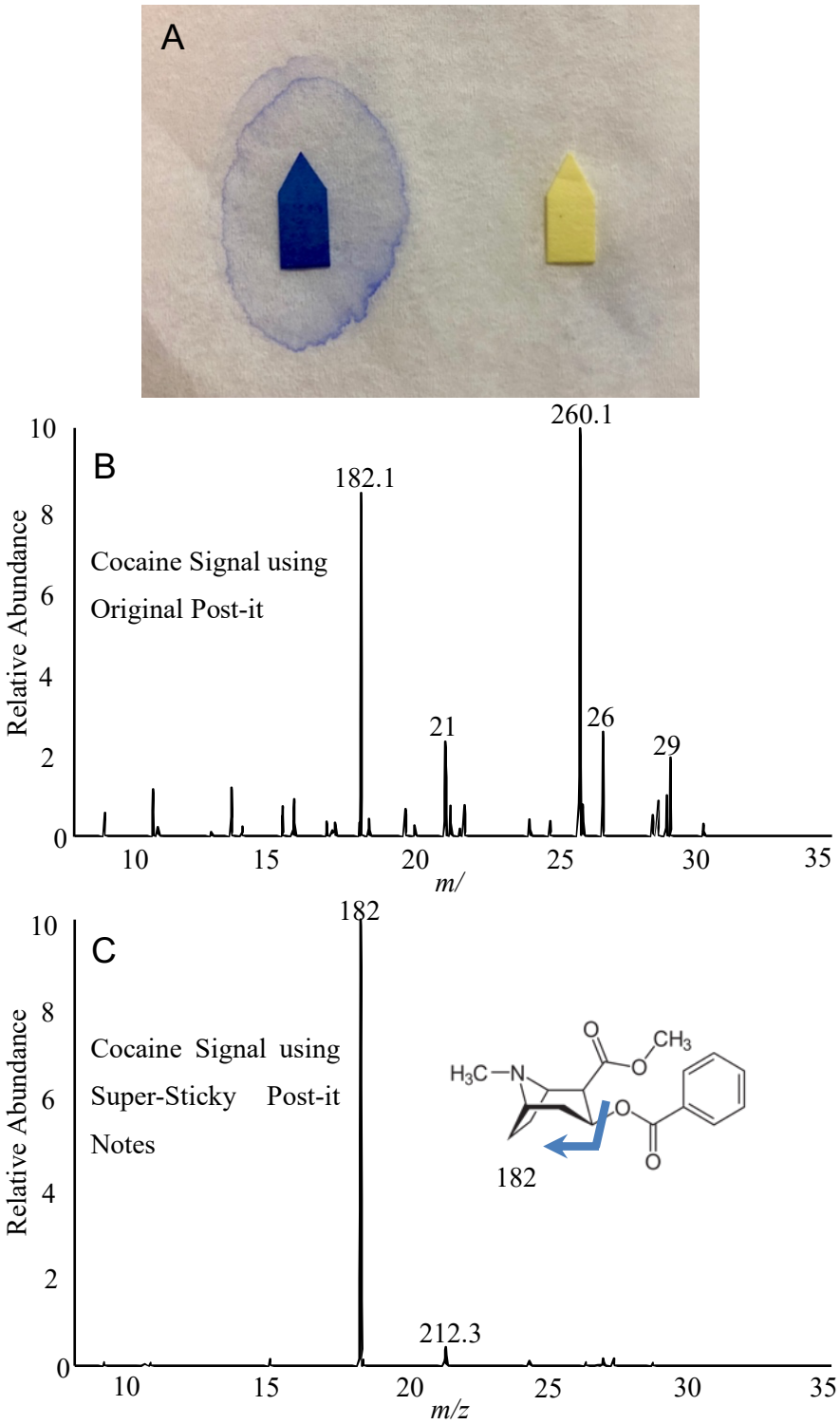

Figure 2.2 A) Dye from colored Post-it® Notes compared to non-colored super sticky notes, cocaine mass spectra using B) original and C) super sticky papers.

2.4.2 Adhesive Versus Non-Adhesive Paper

To assess the impact of the adhesive on drug residue collection, identical paper with and without pressure sensitive adhesive was used for collection. In general, the signal-to-blank (S:B) ratios of the ten investigated compounds improved by a factor of between two and 1000 with adhesive paper compared to non-adhesive, depending on surface and the analyte (Table 2.3). Wilcoxon rank-sum tests was performed in R to determine if the S:B ratios obtained for adhesive paper were significantly improved compared to non-adhesive. Almost all of the p values calculated from these tests were below 0.05, indicating a statistically significant difference between using adhesive and non-adhesive paper tickets on both porous and non-porous surfaces (Table 2.3). For example, on asphalt surface, when S:B ratios of all compounds were higher with PSA paper, only 70% of the p values were below 0.05. On the other hand, on concrete surface, all ten compounds had higher S:B ratios when using adhesive paper with all of their p values below 0.05. Cloth was also a porous material that indicated an improvement in signal response with PSA paper substrate. S:B ratios of eight out of ten drug compounds were significantly enhanced with all of them having p values below 0.05. Ketamine and methylone were two compounds that worked better with non-adhesive paper tips; however, their p values were above 0.05, indicating no significant difference.

Adhesive paper tips showed a consistent improvement in drug response across all surfaces relative to non-adhesive paper. The highest median improvement was found for concrete surfaces (23× higher S:B), showing the ability of PSA paper in collecting residues off porous and uneven surface. On the glass surface, S:B ratios of all ten compounds significantly increased with adhesive paper, especially XLR-11, which was not even detectable when using non-adhesive paper tickets. Higher S:B ratios on the aluminum surface also indicated a signal response improvement in nine out of the ten compounds, with all of the p values below 0.05. Kruskal-Wallis rank sum test was performed in R to assess the difference in factor improvements among five surfaces. The results indicated no significant difference in factor improvement between surfaces ($p = 0.2$). In other words, the increase in signal response when using PSA tickets compared to non-adhesive paper can be observed on both porous and non-porous surfaces.

There was a total of eight drug-surface combinations that were not detectable with non-adhesive paper at the level studied here (300 ng) but were detectable by PSA-coated paper. S:B ratios of clonazolam, heroin, ketamine, and methamphetamine were lower compared to other compounds, possibly due to poor ion recovery and ionization; however, they still showed a

significant improvement with adhesive paper on all five surfaces. Taken together, these results indicate that inclusion of the adhesive significantly improves detection sensitivity for drug residues from a variety of surface types.

Table 2.3 Heatmap showing adhesive and non-adhesive S:B ratios comparison at 300 ng. The darker the color, the higher the detection limit. P-values obtained from Wilcoxon rank-sum test indicated significant improvement in signal response when using adhesive paper on all surfaces with some exceptions. P-values of Krusal-Wallis rank-sum test indicated no significant difference in factor improvement between surfaces. S:B ratios below 3 (in red ink) were considered not detectable.

Analytes	*S:B ratio*														
	Asphalt			*Cloth*			*Concrete*			*Glass*			*Aluminum*		
	Adhesive	*Non-adhesive*	*p*	*Adhesive*	*Non-adhesive*	*p*	*Adhesive*	*Non-adhesive*	*p*	*Adhesive*	*Non-adhesive*	*p*	*Adhesive*	*Non-adhesive*	*p*
Acetyl fentanyl	2E4	33	0.03	4E5	1E3	0.02	514	3	0.01	2E3	23	0.03	4E3	53	0.01
Fentanyl	4E3	24	0.01	9E4	1E2	0.02	82	3	0.00	580	13	0.02	682	17	0.01
Cocaine	2E3	115	0.01	1E4	7E3	0.03	1E3	22	0.00	3E3	185	0.01	3E3	305	0.00
Clonazolam	71	27	0.06	881	123	0.04	56	4	0.00	182	13	0.03	151	48	0.01
Heroin	9	2	0.01	1E3	81	0.03	19	1	0.00	22	2	0.00	40	4	0.01
Ketamine	12	3	0.95	247	1E3	0.1	37	2	0.00	87	4	0.02	300	71	0.00
Methamphetamine	16	8	0.03	2E4	629	0.00	194	18	0.00	41	25	0.01	86	131	0.2
Methylone	38	9	0.04	1E3	2E3	0.09	41	3	0.01	329	19	0.00	94	18	0.01
U-47700	903	116	0.06	5E5	1E4	0.01	2E3	14	0.04	6E3	60	0.00	1E4	849	0.01
XLR-11	158	21	0.05	1E4	2E3	0.04	249	3	0.00	8E3	0.4	0.00	6E3	28	0.00
Kruskal-Wallis Test for Surfaces	chi-squared = 6.15			Df = 4			p-value = 0.2								

2.4.3 Dabbing Sequences Comparison

During real-world sampling, it may be desirable to dab a piece of evidence in multiple places to increase the chance of drug residue detection. Two dabbing sequences were compared to determine if the order of dabbing drug-contaminated versus drug-free areas significantly impacted detectability. Two extremes were tested. In the "dab first" sequence, the PSA paper tickets were dabbed once on the dried drug residue (300 ng of each drug), and then dabbed seven times on non-contaminated areas of the surfaces. In sequence "dab last", the paper tips were first dabbed seven times on clean areas of the surfaces, and then once on the drug spots. Generally speaking, whether the drug residue was dabbed first or last in the sequence had little effect on drug detection (Figure 2.3). The median factor change for dabbing first versus last was 1.25 with a range of 0.25 to 4.50.

Of the 50 drug-surface combinations investigated, only four showed statistically significant differences between dabbing first and last. All of those instances occurred for collection off aluminum. It was observed that some of the adhesive remained on the aluminum surface during dabbing, suggesting that there was less adhesive remaining on the paper at the end of the collection sequence. However, with "dab last" sequence on concrete surface, the PSA tickets actually collected loose concrete particles first, leaving less adhesive portion to collect drug residues after that. Therefore, on these two surfaces, "dab first" sequence resulted in higher signal response.

Although it has been shown that different dabbing methods may lead to different signal response outcomes, most of their p values were above 0.05. In other words, dabbing the PSA paper ticket multiple times did not significantly affect the ability to collect drug residues off of surfaces during the sampling process. As a result, a single PSA ticket can be dabbed multiple times on different areas of the same piece of evidence, such as a mail package or a piece of luggage, for sample collection.

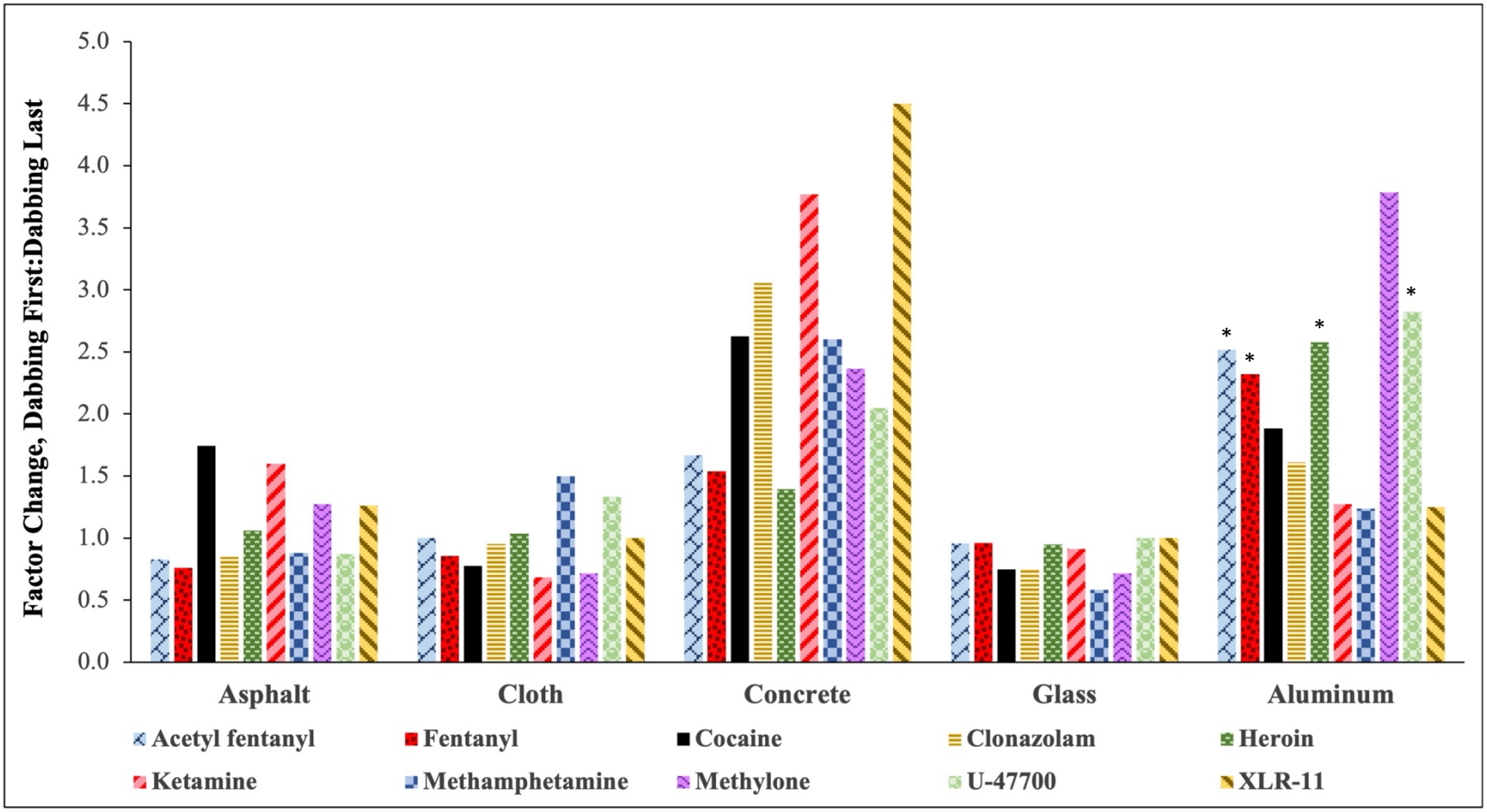

Figure 2.3 Factor change in S:B ratios for dabbing the drug residues first compared to dabbing last in a sequence of eight dabs. *Drug compounds have a significant difference in S:B ratios between two dabbing sequences.

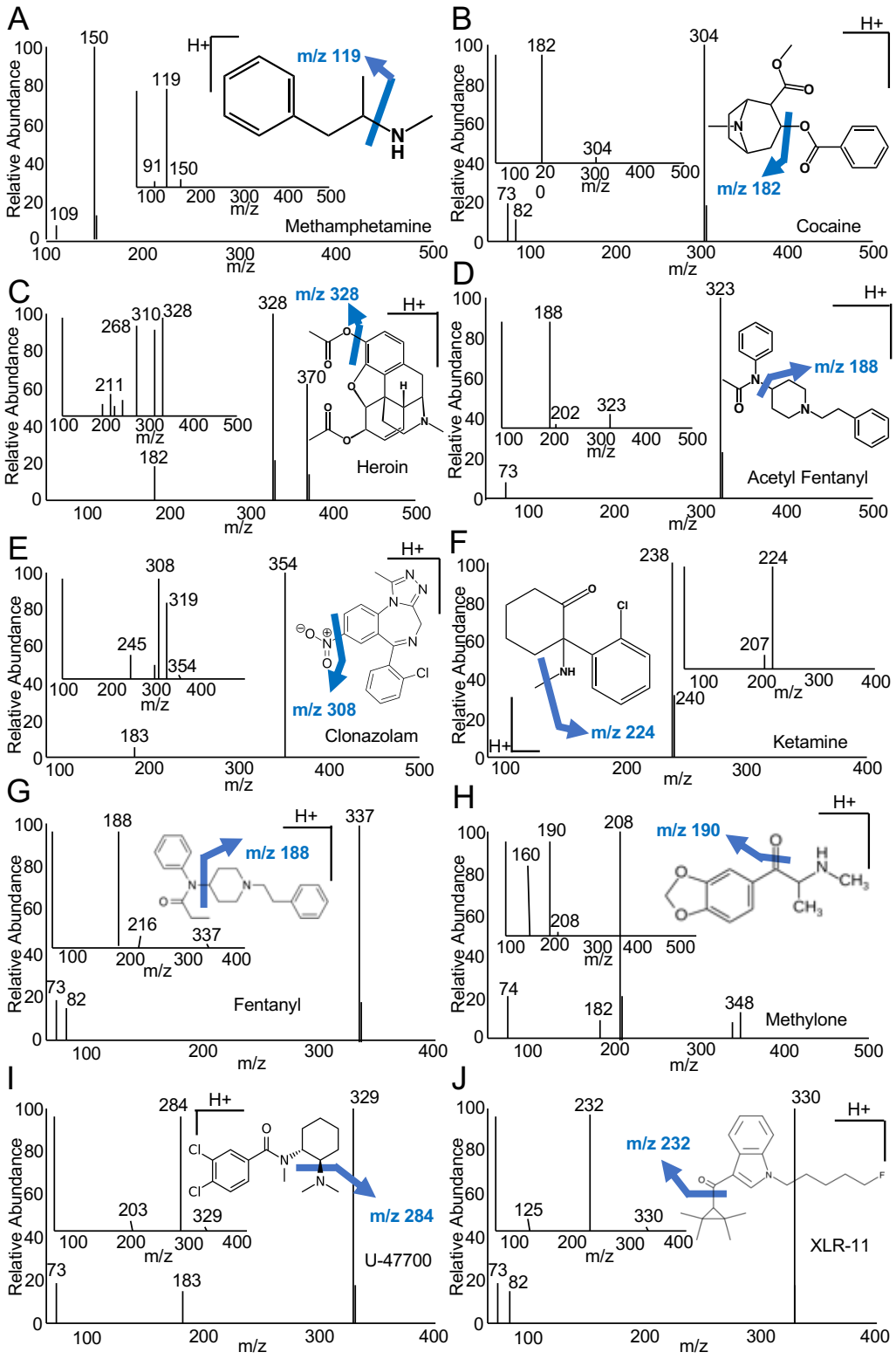

Figure 2.4 Full MS and MS/MS data collected for ten drugs. Insets show the molecular structure and fragmentation pattern for A) methamphetamine, B) cocaine, C) heroin, D) acetyl fentanyl E) clonazolam, F) ketamine, G) fentanyl, H) methylone, I) U-47700, and J) XLR.

2.4.4 Detection Limits Determination

Collision energies were determined to maximize the primary product ion, ideally while the precursor ion peak was still present. Using these optimized collision energies, full MS and MS/MS data are shown for each drug in Figure 2.4 along with the corresponding MS/MS fragmentation. Calibration curves were generated from neat standards spotted directly on the PSA paper (Figure 2.5). The error bars represent the standard deviation of triplicate measurements. The variability of the calibration curves was higher than in typical paper spray MS quantitative methods because true internal standardization was not performed – the deuterated standards were spotted onto the paper separately rather than mixed with the drug standards. As a result, this method is appropriate for screening and semi-quantitative analysis only. On-paper LODs determined from the neat solution on paper were calculated from the calibration curves; all ten investigated drugs had detection limits in the 10 – 110 pg range (Table 2.4). Calibration curves of ten drugs on five surfaces were also graphed, with their coefficients of determination (R^2) recorded in Table 2.4. As expected, the sampling limits of detection (LOD) of ten compounds were higher and the R^2 lower compared to spotting drug solutions directly on-paper because of incomplete recovery and higher variability associated with collection from surfaces.

Despite varying LODs on different surfaces, LODs of all ten compounds were much lower compared to the amount detected in the literature, in which the drugs were wiped from contaminated benches, door handles, and storage bins, and quantified using LC/MS/MS[23,24]. The discrepancy between LODs for non-porous and porous surfaces under similar experimental conditions was shown clearly in Table 2.4. In particular, LODs on asphalt ranged from 0.1 to 5.3 ng and on concrete from 0.1 to 10.8 ng. Furthermore, several analytes showed a consistency in high detection limits on porous surfaces, such as clonazolam with the highest LOD of 10.8 ng on concrete, heroin with the highest LOD being 8.4 ng on concrete, and ketamine being detectable at 5.3 ng on asphalt. On the other hand, the low ng detection limits, from 0.05 to 2.5 ng, obtained by sampling on aluminum and glass surfaces indicated higher recovery of drug residues from these surfaces. Surprisingly, detection limits of ten compounds were in the low ng range on cloth surface, from 0.16 to 2.6 ng, despite being a porous surface. It was observed that during drug standards deposition, the solution beaded up on the cloth surface, leading to more drug residues staying on the surface instead of wicking through like on asphalt and concrete. This explains for the increase in signal response and detection limits for the cloth surface. Despite the fluctuation on different

surfaces, detection limits of most of the compounds were at ng quantities, with the exception of two compounds being detectable at 8.4 ng and 10.8 ng. This approach proved to be sensitive enough for drug detection and quantification in forensic and border control applications.

To assess the repeatability of the sampling process, standard solutions containing all ten compounds were spotted onto five surfaces followed by PSA paper collection. In general, porous surfaces tended to show larger variation in signal response. Among the five surfaces, asphalt and cloth showed the greatest relative standard deviation (%RSD) in the analyte/IS response ratio, with the median %RSD found to be 76 and 63 respectively (Figure 2.6). The variability was lower for nonporous surfaces, especially on aluminum with a median %RSD of 37. U-47700 had higher variability compared to the other compounds, namely on cloth, concrete, and glass.

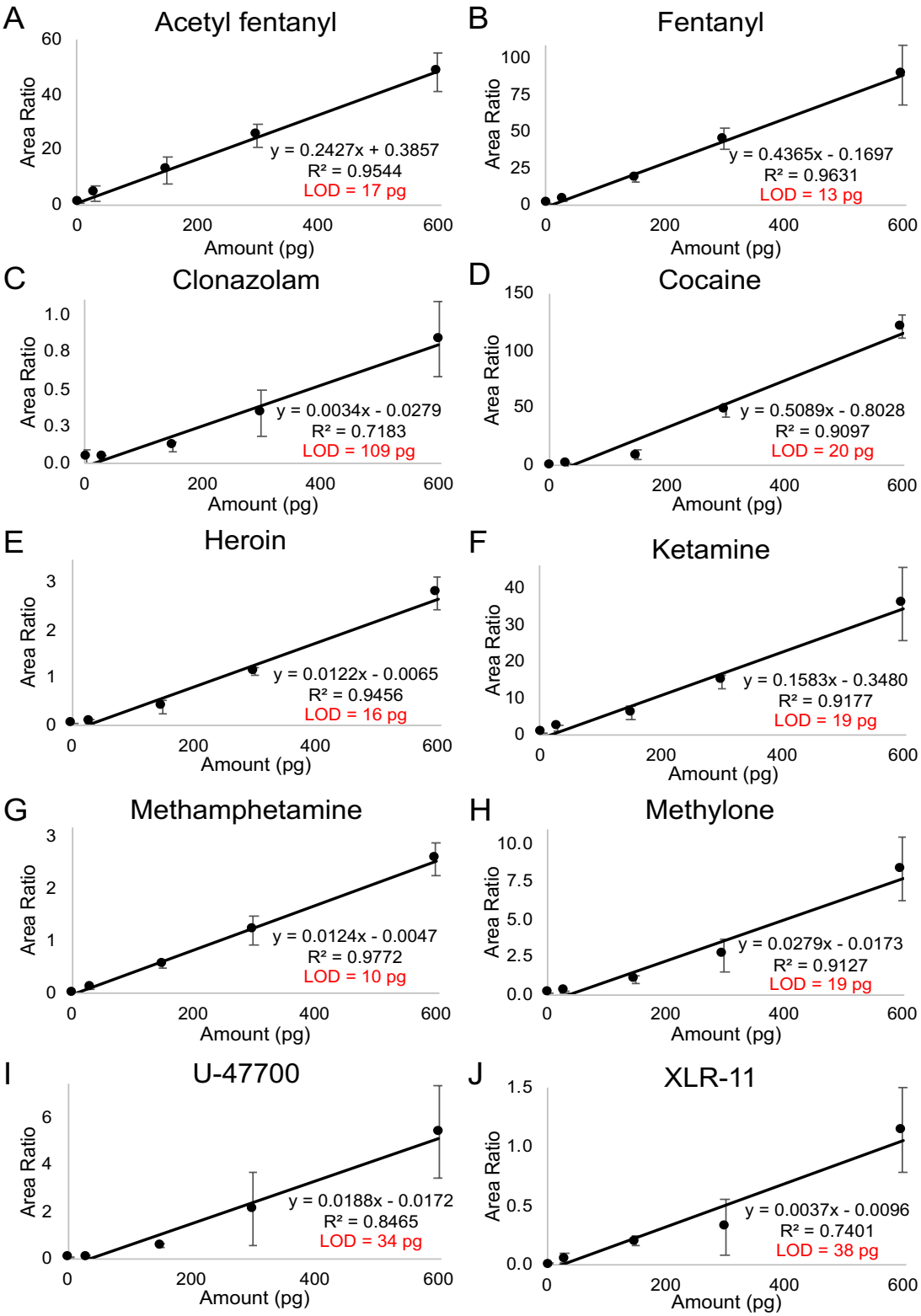

Figure 2.5 On-paper calibration curves of A) acetyl fentanyl, B) fentanyl, C) clonazolam, D) cocaine, E) heroin, F) ketamine, G) methamphetamine, H) methylone, I) U47700, and J) XLR-11. Data points represent the average response ratio of the triplicate runs. Error bars are ± the standard deviation of these averages.

Table 2.4 Heatmap of limits of detection of ten compounds determined using two methods and their calibration curves' R2 values with %RSD. Again, the darker the color, the higher the detection limit.

Analytes	*Neat on Paper*		*Sampling on Surfaces*									
			Asphalt		*Cloth*		*Concrete*		*Glass*		*Aluminum*	
	R^2	*LOD (pg)*	R^2	*LOD (ng)*	R^2	*LOD (ng)*	R^2	*LOD (ng)*	R^2	*LOD (ng)*	R^2	*LOD (ng)*
Acetyl fentanyl	0.95	17	0.81	0.8	0.83	0.2	0.65	0.2	0.87	0.1	0.89	0.1
Fentanyl	0.96	13	0.83	0.5	0.81	1	0.68	1	0.87	0.8	0.87	0.4
Clonazolam	0.72	109	0.67	2	0.84	2	0.50	11	0.79	0.1	0.76	1
Cocaine	0.91	20	0.67	0.4	0.89	0.3	0.54	0.6	0.93	0.1	0.93	0.05
Heroin	0.95	16	0.83	5	0.86	0.8	0.83	8	0.79	1	0.81	4
Ketamine	0.92	19	0.71	5	0.91	2	0.37	4	0.78	0.4	0.87	0.5
Methamphetamine	0.98	10	0.67	0.2	0.78	2	0.51	0.6	0.82	2	0.89	2
Methylone	0.91	19	0.76	4	0.92	0.3	0.43	4	0.72	0.3	0.79	0.8
U-47700	0.85	34	0.83	0.3	0.92	0.4	0.49	0.1	0.85	0.2	0.72	0.05
XLR-11	0.74	38	0.73	0.4	0.85	3	0.49	0.7	0.83	0.7	0.83	0.2

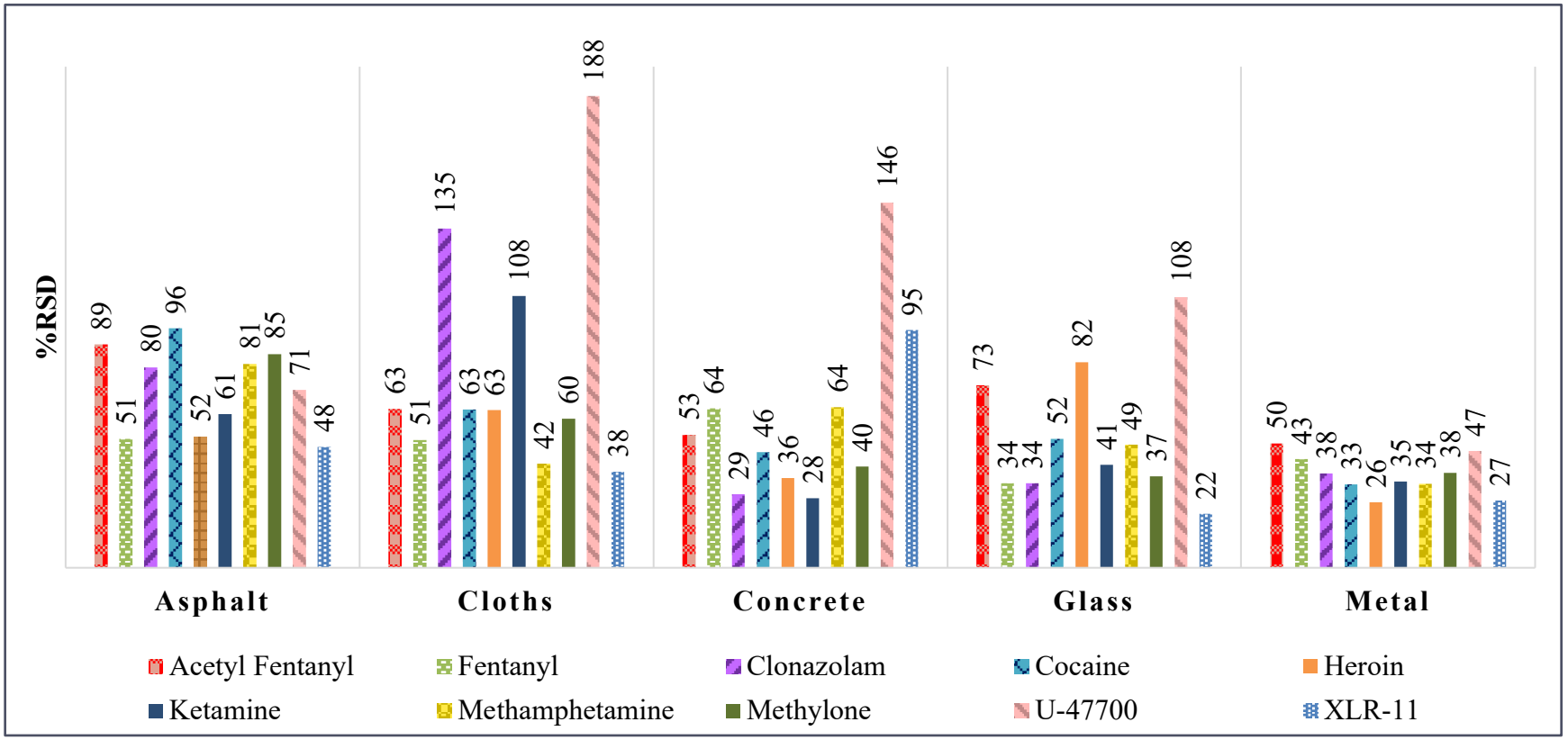

Figure 2.6 Relative standard deviation (%RSD) of ten drug compounds on five surfaces.

2.5 Conclusions

This chapter introduces a new affordable means of combining sample collection and paper spray analysis utilizing a single PSA-coated paper ticket. Ten illicit drugs including acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11 were sampled on five surfaces to generate their calibration curves as well as determine the detection limits. By showing higher signal response and S:B ratios compared to non-adhesive paper, no significant difference between dabbing sequences, and detection limits at ng quantities, PSA paper has proved its robustness and efficiency in drug residue collection. PSA coated paper, combined with PS-MS, enables ease of sampling procedure and analysis in the field. For future works, interference and recovery study are necessary to further assess the functionality of PSA in the presence of different interferences such as dust, humidity, and common cutting agents, as well as to determine the amount of drugs remained on the paper ticket. In addition, there is a need to couple this approach with portable MS to investigate the PSA paper's compatibilities with PS-MS analysis in the field, and to further enhance its functionalities and potentials to be used for forensics and customs.

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CHAPTER 3. PRESSURE-SENSITIVE ADHESIVE COMBINED WITH PORTABLE MASS SPECTROMETER FOR DRUG SCREENING ON SURFACES

3.1 Abstract

With the development and complexity of the drug market today, there is a need to develop portable techniques for real-time, on-site analysis, especially in forensic investigations. Gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS) are two commonly used analytical techniques in drug screening. However, portable GC-MS still has several disadvantages, such as complicated procedure time-consuming sample preparation, difficulties analyzing nonvolatile compounds, and small chemical library[1]. These difficulties could be overcome using paper spray ionization, an ambient ionization technique, coupled with portable MS that offers rapid, simple, yet sensitive and effective forensic drug screening method. In this work, the combination of paper spray - mass spectrometry (PS-MS) and pressure-sensitive adhesive (PSA) paper was investigated for on-site detection of illicit substances on surfaces. The assay was implemented on Continuity Transportable Mass Spectrometer provided by BaySpec. A total of ten illicit substances (acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11) were screened from five surfaces and their detection limits were determined to be 10 ng and below. Several realistic scenarios were performed to evaluate the effectiveness of PSA paper in drug collection, including cocaine residues off cardboard and on fingers after being washed with soap and water, acetyl fentanyl on clothing, methamphetamine on plywood, and XLR-11 off office paper. The obtained mass spectra and results indicated the novel approach's potentials in drug screening on both porous and non-porous surfaces for forensic and border control purposes.

3.2 Introduction

Since the first ambient ionization technique described by the Cooks group in 2004, an increasing number of ambient methods have been developed to bypass the complexity of instrument size and set ups, sample preparation and separation, as well as lengthy analysis times[1-3]. Allowing direct sampling and ionization of compounds of interest under atmospheric condition, ambient ionization mass spectrometry gives rise to rapid, real-time, sensitive, and cost-effective analytical techniques. In order to utilize ambient ionization techniques in the field and eliminate the time and resources needed to transport samples, various types of mass spectrometers have been miniaturized and further developed into portable instruments. The miniaturization of the mass spectrometers proceeded via three stages, starting from miniaturization of mass analyzers to the miniaturization of the mass spectrometers as a whole, including the control and pumping system, and finally the total MS analytical systems for in situ analyses[4].

Being an important part of a mass spectrometer, mass analyzers such as time-of-flight (TOF)[5], quadrupole[6,7], and ion trap[8,9] were first developed for portable MS as they could be small in size, and easy to manufacture, but still maintain adequate performance. To miniature a TOF analyzers, the flight tube must be shortened which causes a decrease in mass resolution. As a result, space and time-focusing devices such as infinite flight path in multiturn TOF and reflectron analyzers[5,10], or spiral orbit trajectory[11,12] and electrostatic multi-pass mirrors help compensate for the short flight tube[13,14]. On the other hand, quadrupole mass analyzers are well-suited for portable MS systems due to their small size, weight, and low cost, and more modest vacuum requirement[15]. Moreover, being capable of tandem MS with higher selectivity and sensitivity is another advantage of quadrupole analyzer in portable instruments[16]. Among these analyzers, ion traps were considered to be most favored when miniaturized MS was first developed. This is due to the fact that as mass spectrometer with beam-type mass analyzers is miniaturized, more pressure is required to enhance mass resolution. Meanwhile, ion traps are more flexible and can operate at higher pressure (10^{-3} torr), which is achievable with small pumping systems[17]. In addition, ion trap analyzers surpassed others by being capable of performing multistage MS experiments up to MS^n, thus proving accurate ionic structure information[18,19]. At the same time, to improve the ion storage of the portable MS during trapping, several approaches have been proposed, including toroidal rf ion trap and rectilinear ion trap[20,21]. Without any doubt, these developments have brought portable mass spectrometers out of the lab and closer into the field for analysis.

Vacuum system plays an important role in reducing background signal and avoiding intermolecular collision events[4]. Unfortunately, vacuum systems are usually large, heavy, and energy-consuming, which is not suitable for portable MS. Various miniaturizations of the vacuum system have been reported. For example, Gao, Cooks, and Ouyang developed Mini 10 handheld rectilinear ion trap mass spectrometer with miniature rough and turbomolecular pumps of only 5L/min and 11 L/s pumping speeds, respectively[22]. Or in the work of Riter *et al*., a miniature membrane introduction mass spectrometry (MIMS) was introduced, in which a membrane was positioned inside the vacuum system in order to monitor organic compounds in aqueous media[23]. In the work of Yang *et al*., a 50mm x 35mm home-made ion getter pump was built to work alongside a roughing pump to maintain high vacuum of up to 10^{-7} torr[24]. New challenge emerged with the development of miniaturized vacuum system since analytes that are ionized under ambient conditions need to be transferred to the high vacuum environment for analysis. This led to the introduction of the discontinuous atmospheric pressure interface (DAPI), which opens and closes periodically to control the number of ions getting into the mass analyzer and avoid large flows[25]. These advancements allowed atmospheric pressure ionization to be performed in portable mass spectrometers with small pumping system, mentioning DESI, DART, and PSI[26-29].

Gaining its popularity for bypassing complex sample preparation and analyses, paper spray-mass spectrometry (PS-MS), a well-established ambient technique, has been coupled to portable instruments for the analyses various samples[30-32]. For example, PS-MS and the commercial Mini β Portable MS have been utilized to rapidly identify and confirm the presence of fentanyl and fentanyl analogs on surfaces of forensic relevance[30]. The use of PS-MS and the Mini 12 Miniature MS developed by Ouyang's group was also reported in the analysis of synthetic cannabinoids, as both trace amount on surfaces and substances in blood and urine[31]. In another case, a home-built handheld mini mass spectrometer was coupled with PS-MS for therapeutic drug screening in dried blood spots[32]. Limits of quantification for the tested drugs were determined to be from 10 to 20 ng/mL, which is sufficient to cover their dosage range in blood and plasma[32]. The combination of PS-MS and miniaturized mass spectrometer has become more accessible for analysis in the field to detect and quantify illicit substances of forensic relevance.

In this work, an affordable means of combining sample collection and paper spray analysis utilizing a single pressure-sensitive adhesive (PSA) coated paper ticket was performed on BaySpec Continuity mass spectrometer, investigating the suitability of the PSA paper substrate on a portable

system. The Continuity uses a linear ion trap (LIT), which allows for the compact size of the system. Ten illicit drugs including acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11 were sampled on five different surfaces to determine their detection limits on these surfaces as well as to generate their calibration curves. As a proof-of-concept to demonstrate the method's practicability in the field, these PSA paper spray tips were pressed in contact with the contaminated surfaces for sample collection and later used for PS-MS analysis.

3.3 Method

3.3.1 Materials

Samples of street cocaine, heroin, and methamphetamine were obtained from a local forensic chemistry laboratory. Cocaine-d3, fentanyl-d5, heroin-d9, methamphetamine-d11, U-4770-d6, were purchased from Cerilliant (Round Rock, TX, USA). Acetyl fentanyl standard in powder form, XLR-11 and its deuterated standard (d5) were purchased from Cayman Chemical (Ann Arbor, MI, USA). High-performance liquid chromatography (HPLC) grade solutions of acetonitrile, formic acid, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Finally, all-purpose flour and Post-it® Super Sticky Note used as PSA papers were sourced locally.

3.3.2 Preparation of Working Solutions and Calibration Solutions

Using the powdered acetyl fentanyl drug standard, acetyl fentanyl stock solution was prepared in methanol (1 mg/mL). The concentration of clonazolam, cocaine, fentanyl, heroin, ketamine, methamphetamine, methylone, and U-47700 stock solutions were 1 mg/mL, while the concentration of XLR-11 was 100 ug/mL. Three working solutions, each contained three to four drug compounds, were prepared by diluting the stock solutions to 25, 30, or 70 ppm in 50:50 water:acetonitrile with 0.1% (v/v) formic acid (Table 3.1).

Calibrants for limits of detection (LOD) determination were prepared using a serial dilution, in which the working solutions prepared above were sequentially diluted six times to make six calibrants. A 7-ppb internal standard solution containing six internal standards was made in methanol with 0.1% formic acid. All solutions and calibrants were stored at -20°C when not in use.

Table 3.1 Drug compounds in three working solutions and concentration (ppm) of calibrants at six levels.

Solutions	*Analytes*	*Cal 1*	*Cal 2*	*Cal 3*	*Cal 4*	*Cal 5*	*Cal 6*
Solution 1	**Methamphetamine**	0.8	1.6	3.1	6.3	12.5	25
	Cocaine						
	Fentanyl						
Solution 2	**Ketamine**	0.9	1.9	3.8	7.5	15	30
	Acetyl fentanyl						
	U-47700						
Solution 3	**Methylone**	2.2	4.4	8.8	17.5	35	70
	XLR-11						
	Clonazolam						
	Heroin						

3.3.3 Preparation of PSA Paper Tickets

The PSA paper was prepared in stacks of seven. The paper was cut into 0.5 x 1 cm strips, and a triangular (~33°) shaped tip was cut from one end using a razor blade. The top and bottom paper layers were discarded to remove any possible contamination, and the remaining five layers were used for sampling/analysis. This separation was done just prior to surface sampling to avoid contamination during storage and handling. Only the rectangular portion of the paper ticket contained adhesive while the triangular tip did not. Cutting the paper spray tickets such that the triangular tip was adhesive-free produced sharper, more reproducible spray tips.

3.3.4 Calibrants Preparation and Drug Deposition for "Realistic" Scenarios

To prepare the calibrants for detection limit determination, 3 µL aliquots of the calibrants containing all ten drug compounds were spotted on the paper tips and allowed to dry completely. After that, 3 µL of the internal standards was added to the paper tickets and allowed to dry before the analysis. Calibrants and samples were prepared in triplicate.

To demonstrate the concept of PSA sample collection combined with portable MS, PSA paper collection was used to collect and detect drug residues from various surfaces for "realistic" scenarios. The collection process was followed by paper spray analysis on a portable MS system.

Testing was done on unpurified acetyl fentanyl, street cocaine, heroin, methamphetamine, and XLR-11.:

1) In the first case, to simulate a street drug sample in which a fentanyl analog would be a relatively minor component, 1 mg of acetyl fentanyl was cut with 30 mg of all-purpose flour and approximately 200 μg was rubbed between two gloved fingers. A t-shirt was then touched in two different places and the second location was dabbed with the PSA paper for sample collection.
2) In the second scenario, a small pinch of street cocaine sample (~200 μg) was rubbed between two bare fingers. A piece of cardboard was touched five times on different areas and the last area touched was sampled with the PSA paper tickets.
3) To further assess the effectiveness of PSA papers during sample collection in a more challenging scenario, the cocaine-contaminated finger was dabbed again, but this time on the skin, after touching the cardboard and being washed thoroughly with soap and water.
4) Residues of street heroin were deposited on concrete surface by first rubbing a small amount of the street sample between two bare fingers. A piece of concrete was touch five times, and the last area touched was sampled with the PSA paper for analysis.
5) In the next scenario, in order to determine if the PSA substrate could be damaged by rough surfaces during sampling process, street methamphetamine was rubbed between two fingers and dabbed twice onto a piece of plywood. The second area touched was sampled using PSA paper tickets.
6) To explore the feasibility of testing mail nondestructively by the PSA paper, 4 μg of the synthetic cannabinoid XLR-11 was applied to office paper as a solution to mimic drug smuggling through a soaking and spraying process. Contaminated area of the paper was dabbed once with the PSA paper tickets for analysis.

Blank samples were made in triplicate for each scenario with 3 μL aliquots of the deuterated internal standard solution (10ppm) added to all PSA paper tickets and allowed to dry completely before the PS-MS assay.

3.3.5 Portable Mass Spectrometer Calibration, PS-MS analysis, and Data Processing

The experiments were performed on a portable BaySpec Continuity mass spectrometer (33 cm × 33 cm × 43 cm W × H × L). Before the analysis, the mass spectrometer was calibrated using

a standard solution containing six compounds: methamphetamine, methylone, cocaine, heroin, 25I-NBOMe, and reserpine to cover a mass range from *m/z* 100 to *m/z* 600. The solution was infused with a Chemyx Fusion 100T syringe digital pump at 0.001 µL/min with a 500 µL Hamilton syringe for electrospray ionization. A voltage of 4.0kV was applied to the syringe needle using a metal insulated alligator clip. Funnel voltage was set at -1450V and RF level was 150V. In the calibration page on the MS, the observed *m/z* and theoretical *m/z* values of six compounds were input into a pre-loaded script. After clicking the "Calibrate *m/z*" button, the calibration was saved into the same script and the instrument was ready for PS-MS analysis.

A milled Delrin cartridge was made to hold the paper spray tickets while providing an electrical contact between the high voltage source and the paper tips during the analysis (Figure 3.1). PSA paper tickets were placed inside this cartridge and approximately 1 mm from the MS inlet. A spray solvent (acetonitrile with 0.1% formic acid) volume of 80 µL was added dropwise into the solvent well of the cartridge, allowing the paper tickets to completely wet to the tips. During the analysis, as the PSA paper tickets tended to curl up due to the high voltage, and because the portable MS inlet was smaller compared to benchtop MS, manual adjustment was required to make the tip of the paper ticket pointed towards the center of the inlet during the run. A voltage of +4.0kV was applied to the paper ticket for 20 seconds to obtain full MS spectra, and then for another 1 minute to perform MS/MS analysis of five compounds used for "realistic" scenarios. Both full MS and MS/MS data were acquired in positive ion mode, with the mass ranging from *m/z* 50 to 400 and MS/MS parameters being optimized for each compound. These settings can be found in Table 3.2.

Data were autosaved into Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and later processed using MATLAB (Natick, MA, USA). To generate calibration curves, ten analytes and their internal standards' peaks were integrated based on the intensity counts saved in Excel. A custom MATLAB code was developed to integrate the intensity of each peak. From that, limits of detection (LOD) of ten drug compounds were calculated using equation 1:

$$\mathrm{LOD} = 3 \times S_b / m \qquad (1),$$

in which S_b was the standard deviation of blank signals, and m was the calibration curve's slope.

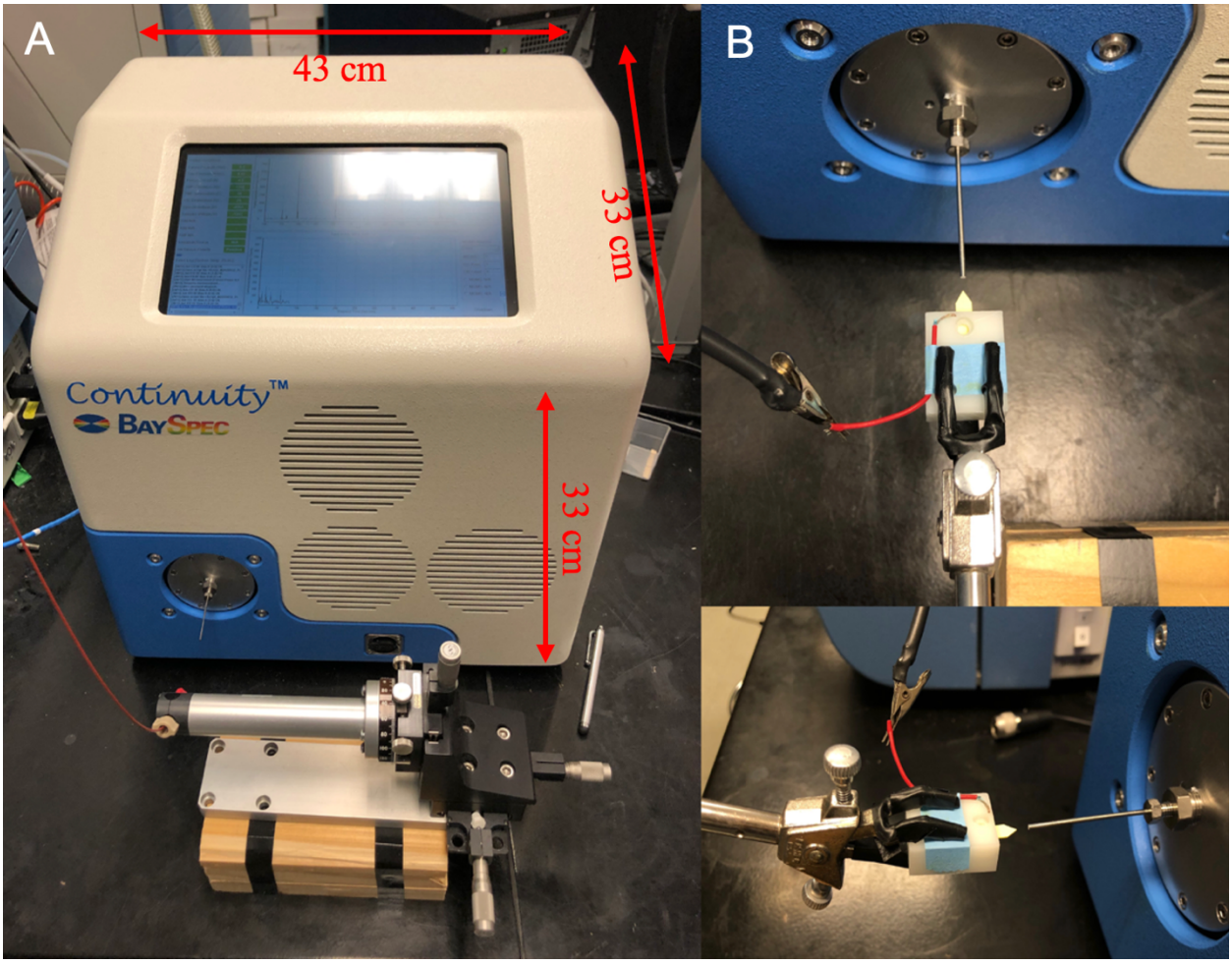

Figure 3.1 A) BaySpec Continuity Mass Spectrometer with electrospray ionization set-up for calibration and optimization and B) PS set-up with PSA paper ticket inserted into Delrin cartridge and positioned 1 mm from the MS inlet.

Table 3.2 Parameters used for MS and MS/MS analysis, including Detector Anode, RF level, ISO Frequency Center, ISO Frequency Width, and CID level of ten compounds. Some drugs were not analyzed by MS/MS on the portable MS; MS/MS-related settings for those analytes are indicated with a dash.

		MS mode			MS/MS mode			
Solutions	***Analytes***	***Precursor m/z***	***Fragment m/z***	***Detector Anode (V)***	***RF Level (V)***	***ISO Freq. Center (kHz)***	***ISO Freq. Width (kHz)***	***CID level (V)***
Solution 1	**Methamphetamine**	150.1	119.2	-1450	150	160	4	0.75
	Cocaine	304.6	182.3		150	76	4	0.65
	Fentanyl	337.4	-		-	-	-	-
Solution 2	**Ketamine**	238.1	-	-1450	-	-	-	-
	Acetyl fentanyl	323.8	188.5		150	72	4	0.60
	U-47700	329.5	-		-	-	-	-
Solution 3	**Methylone**	208.7	-	-1600	-	-	-	-
	XLR-11	330.2	232.2		150	70	4	0.70
	Clonazolam	354.3	-		-	-	-	-
	Heroin	370.2	268.8		250	107	2	0.85

3.4 Results and Discussions

3.4.1 Optimization of Instrument Settings

Detector Anode, RF Level, and ISO Frequency

The LIT of BaySpec Continuity mass spectrometer utilizes a funnel comprised of a series of thin, coaxial disks with varying inner diameters (Figure 3.2). These are aligned in a row to make it better equipped for capturing and directing the ions towards the mass analyzer using radio frequency (RF) field and direct current (DC) voltages. The RF waveforms push the ions off of the plates and keep them near the centerline of the funnel, while the DC voltages help achieve an axial push and direct the ions towards to mass analyzer[33]. The RF level and DC voltage applied to the funnel during the analysis varied depending on the analytes as some of them needed higher RF level and lower voltage to be trapped. Especially in the case of isolating and fragmenting heroin, a RF level of 250 V was necessary as heroin's molecular mass was higher compared to other analytes even though the mass range of the Continuity MS is *m/z* 50 to *m/z* 1200. Once a signal has been established, its intensity can be increased or decreased by adjusting the gain of the detector using detector anode, which is the voltage applied to the channeltron detector. Among ten analytes, methylone, XLR-11, clonazolam, and heroin required a detector anode voltage of -1600V, much lower than other compounds, to produce more intense, visible peaks.

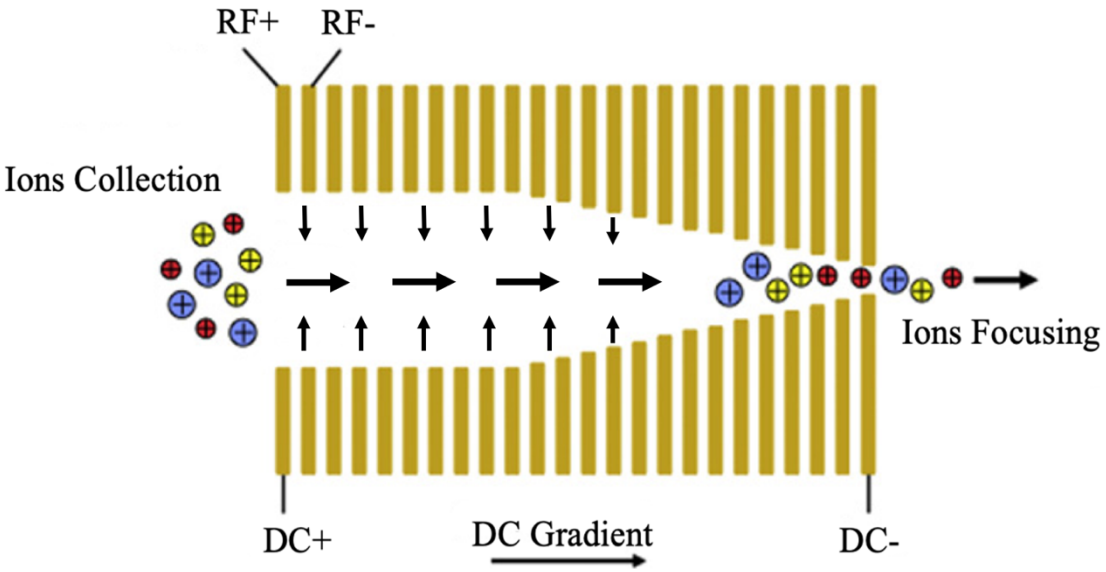

Figure 3.2 Schematic of the ion funnel with RF potentials of equal magnitude and opposite phase applied to alternate electrodes and DC potentials applied to each electrode.

Two ISO frequencies were used for isolating the MS peaks for MS/MS fragmentation: the ISO Freq Center adjusts the notch filtered noise that is applied onto the ring electrodes to eject unwanted ions in radial direction, and ISO Freq Width is the isolation window width. In theory, with the same RF level, the higher molecular mass, the lower ISO frequency. However, among five analytes used in "realistic" scenarios, heroin was the trickiest to perform fragmentation. Therefore, heroin required a completely different set of parameters during MS/MS analysis, including much higher RF level that led to higher ISO Freq Center even though its molecular mass is the highest of the five compounds.

Distance Tolerance of Paper from MS Inlet

In most studies utilizing PS-MS, the paper tickets were arranged ~3 to 4 mm from the MS inlet[34]. Nonetheless, in this study, due to a much smaller MS inlet of the BaySpec Continuity mass spectrometer compared to benchtop ones (0.25 mm vs. 0.55 mm)[35], a distance of 3 to 4 mm from the paper tip to the inlet did not result in any MS or MS/MS peaks during analysis, while a distance of 1 mm or below was likely to cause discharge. After several trials, the highest signal stability was observed when the paper ticket was positioned ~1.5 to 2 mm away from the MS inlet.

3.4.2 Calibration Curves and Detection Limits

Calibration curves were generated from neat standard to determine the LODs for acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11 (Figure 3.3). In these calibration curves, the error bars indicated the magnitude of one standard deviation above and below these values to better visualize the fluctuation of the calibrants' signal-to-blank ratios at each concentration. A greater level of signal response variability was observed at higher concentrations, which directly affected the coefficients of determination (R^2) of the calibration curves. From these S:B ratios of the precursor ions peak, a rough estimate of the feasible detection limits was provided for each drug compound using Equation 1 (Table 3.3). Six analytes' calibration curves had coefficients of determination (R^2) above 0.75, yet clonazolam, heroin, methylone, and XLR-11 had relatively poor calibration curves with their R^2 values ranging from 0.34 to 0.69.

Overall, the detection limits of ten compounds were in the low ng range, in which LODs of seven drug analytes were from 2 ng to 6 ng. It is noteworthy that the three lowest LODs were of

methamphetamine, acetyl fentanyl, and fentanyl, which were around 2 ng. These results have shown that the method using PSA paper tickets coupled with the BaySpec Continuity MS has great potential for detecting commonly abused, traditional drugs, as well as novel synthetic opioids such as fentanyl analogs. On the other hand, XLR-11, heroin, and methylone were three analytes that had higher LODs, being 5.4 ng, 10.2 ng, and 8.1 ng respectively. Low ion recovery of these analytes led to weaker peaks observed during the analysis, which then required lower detector anode voltage to increase signal intensity. As a result, the highest LOD belonged to heroin since it was more challenging to ionize, trap, and focus compared to other drug compounds. Nonetheless, the LODs of ten drugs were still in low ng range, demonstrating the ability of PSA tickets with portable mass spectrometer to detect and achieve low detection limits for a wide variety of illicit substances.

Table 3.3 M/z ranges of ten compounds used for peak integration in MATLAB and signal-to-blank ratios of six calibrants, along with their calibration curves' coefficients of determination (R2) and detection limits determined from the curves.

Analytes	*m/z range*	*S:B ratios*						R^2	*LOD (ng)*
		Cal 1	*Cal 2*	*Cal 3*	*Cal 4*	*Cal 5*	*Cal 6*		
		2.4 ng	**4.8 ng**	**9.3 ng**	**18.9 ng**	**37.5 ng**	**75 ng**		
Methamphetamine	148.0-152.0	7.5	8.8	18.4	21.0	58.7	106.7	0.793	**2**
Cocaine	303.0-306.0	2.6	3.8	8.9	9.7	20.8	31.5	0.864	**3**
Fentanyl	323.0-326.0	5.0	8.7	12.4	28.0	38.6	77.2	0.855	**2**
		2.7 ng	**5.7 ng**	**11.4 ng**	**22.5 ng**	**45 ng**	**90 ng**		
Acetyl Fentanyl	337.0-340.0	2.2	5.7	11.3	25.1	29.5	66.6	0.747	**2**
Ketamine	238.0-242.0	5.0	4.1	7.2	18.7	27.0	67.9	0.917	**3**
U-47700	329.0-333.0	3.4	3.4	5.2	12.7	15.7	33.4	0.871	**3**
		6.6 ng	**13.2 ng**	**26.4 ng**	**52.5 ng**	**105 ng**	**210 ng**		
XLR-11	329.0-331.0	7.3	5.7	10.2	15.4	26.2	45.0	0.551	**5**
Heroin	369.0-372.0	4.2	3.0	4.0	6.5	16.6	24.1	0.689	**10**
Methylone	206.0-210.0	2.6	3.5	4.3	8.1	17.5	52.4	0.341	**8**
Clonazolam	352.0-356.0	4.1	6.4	7.0	9.2	18.0	50.1	0.354	**6**

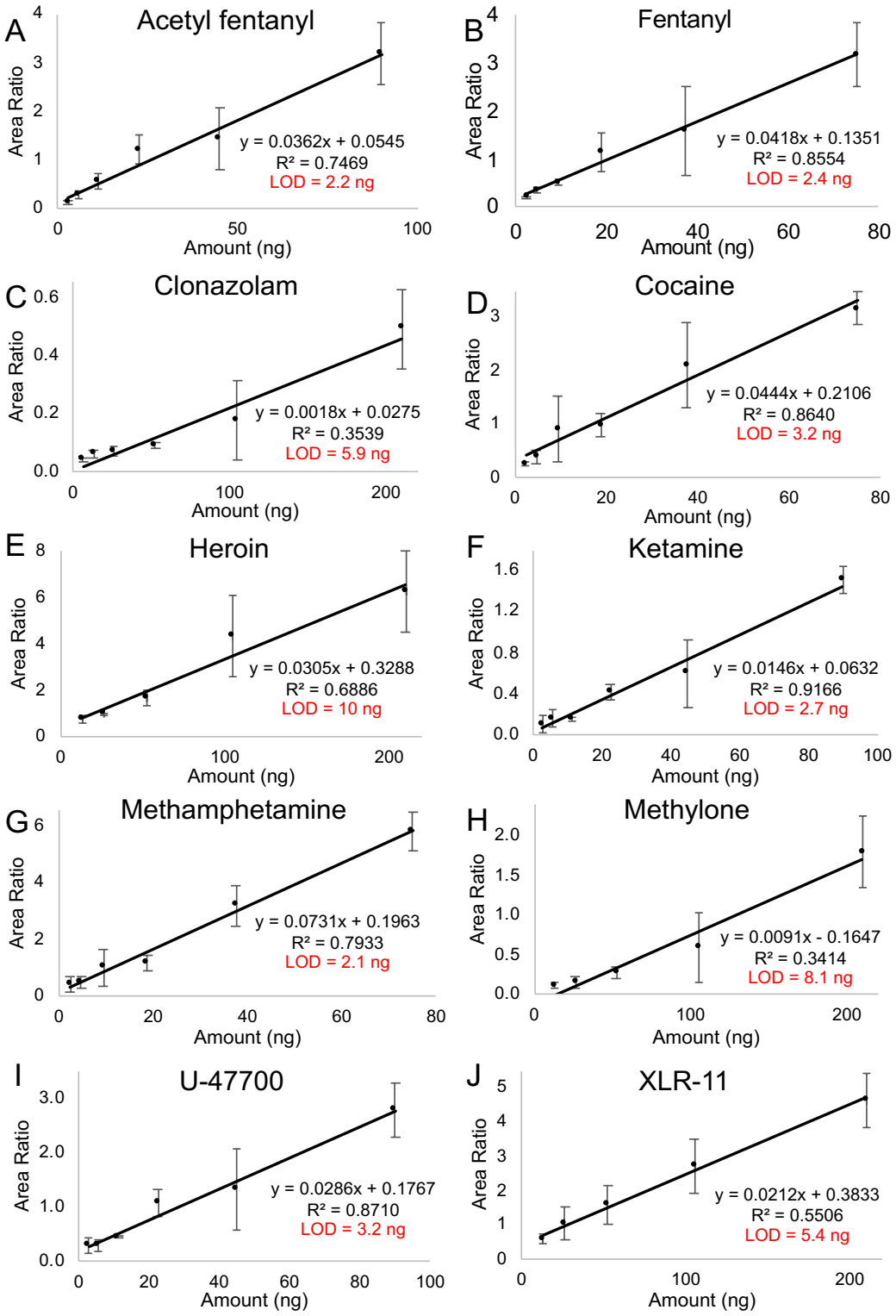

Figure 3.3 Calibration curves of ten drugs of abuse on portable MS for A) acetyl fentanyl, B) fentanyl, C) clonazolam, D) cocaine, E) heroin, F) ketamine, G) methamphetamine, H) methylone, I) U47700, and J) XLR-11. Data points represent the average response ratio of the triplicate runs. Error bars are ± the standard deviation of these averages.

3.4.3 "Realistic Scenarios" on Surfaces

PSA sampling of drug residues from surfaces followed by PS-MS on the portable mass spectrometer was performed to simulate real-world sample collection. In the first "realistic" scenario in which a cotton t-shirt was contaminated with acetyl fentanyl cut with all-purpose flour, full MS spectra were collected showing acetyl fentanyl's intact molecular ion at *m/z* 323, its internal standard at *m/z* 342 (Figure 3.4A). Its characteristic fragment ion detected at *m/z* 188 in MS/MS mode arises from the N-phenethylpiperidine moiety, a commonly generated ions by other fentanyl analogs[36,37].

In the second scenario, in which cocaine was sampled from a piece of cardboard, an intense peak could be found at *m/z* 304, corresponding to the $[M+H]^+$ ion of cocaine. Peak of cocaine-d3 (*m/z* 307) was also seen in full MS mode but with much lower relative abundance. The identification of cocaine peak was further confirmed by the presence of the fragment ion at *m/z* 182 in the MS/MS spectrum, arising from the neutral loss of benzoic acid (Figure 3.4B). During the analysis, both of cocaine's precursor ion signal and its fragment ion signal were strong, indicating the ease of detection even after touching the surface multiple times. This led to the next scenario, where the cocaine-contaminated finger was washed thoroughly with soap and water. Although cocaine's signal was weaker with varying amounts of background noise, clear detection of cocaine residues was still obtained with a visible peak of its $[M+H]^+$ ion at *m/z* 304. Moreover, the presence of cocaine residues was confirmed by an intense fragment ion peak at *m/z* 182, which could be distinguished from the background noise using MS/MS mode (Figure 3.4C).

In the next experiment, street heroin was clearly detected on the concrete surface. An intense peak of heroin's molecular ion was observed at *m/z* 370, along with its internal standard at *m/z* 379, but with much lower relative abundance. Despite the challenge in performing MS/MS analysis of heroin, the presence of heroin residues was confirmed by the fragment ion and the precursor at *m/z* 268 and *m/z* 370, respectively (Figure 3.4D).

For the next application, street methamphetamine sample was detected from a rough piece of plywood. In full MS, methamphetamine's molecular ion was seen at *m/z* 150, right next to its internal standard's peak at *m/z* 161 in full scan (Figure 3.4E). MS/MS spectrum confirmed the presence of methamphetamine, indicated by the intense peaks of its fragment ions at *m/z* 91 and 119 with low background noise. Here, the collection substrate was not damaged because the PSA-coated paper is simply dabbed on surface rather than wiping or rubbing.

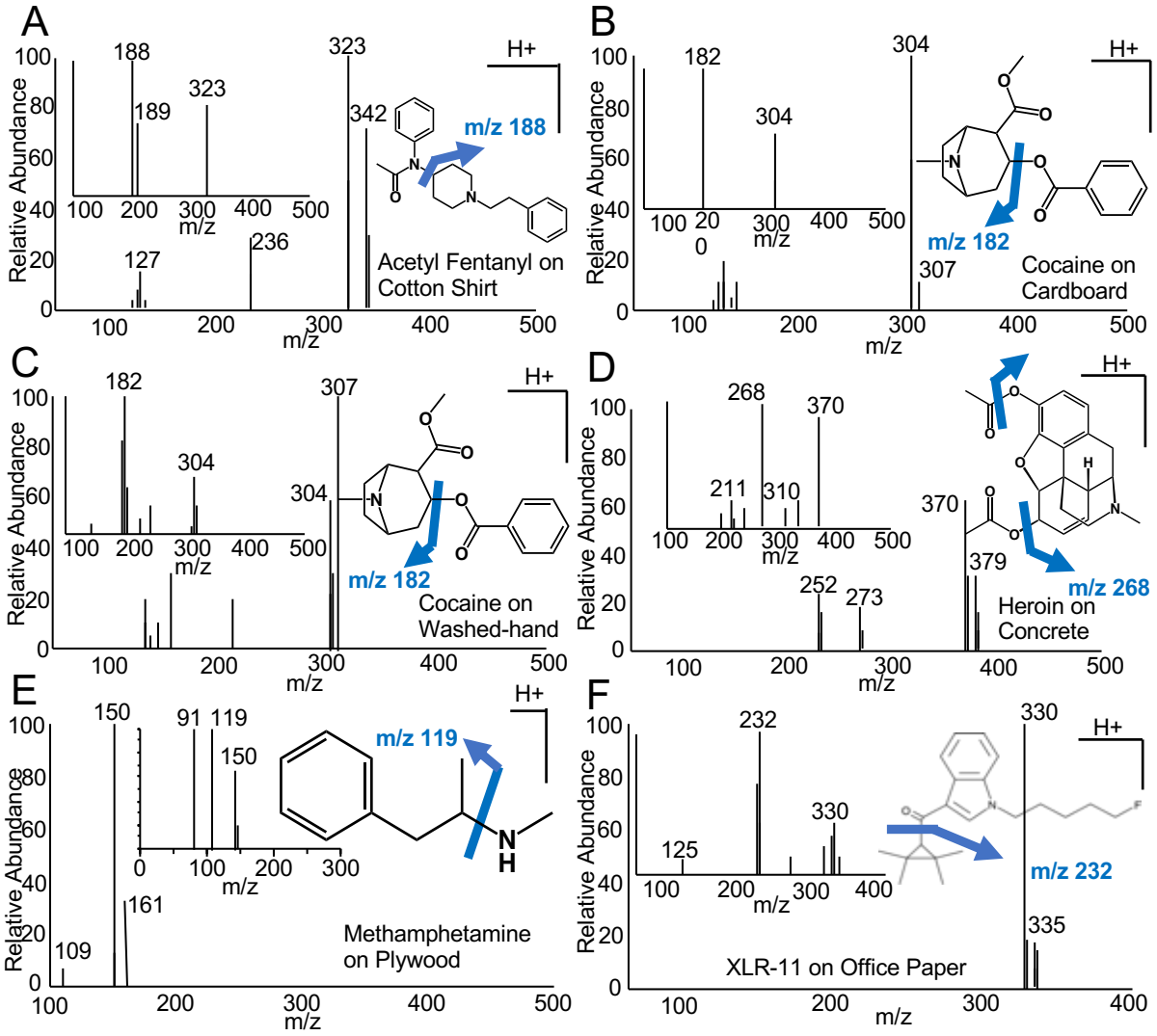

Figure 3.4 Full MS and MS/MS data for five compounds used in "realistic" scenarios. Insets show the molecular structure and fragmentation pattern for A) acetyl fentanyl on cloth, B) cocaine on cardboard, C) cocaine on washed hand, D) heroin on concrete surface, E) methamphetamine on plywood, and F) XLR-11 on office paper.

In the final application, due to the increase in synthetic cannabinoids smuggling into prisons *via* contaminated mail, XLR-11 was deposited onto office paper to test the feasibility of testing mail nondestructively using the PSA paper spray technique. XLR-11 residues were easily detected, with an intense peak of the molecular ion appearing at *m/z* 330 and its internal standard at *m/z* 335 (Figure 3.4F). The presence of XLR-11 was confirmed as indicated by the major product ion at *m/z* 232 obtained from the precursor ion at *m/z* 330 in MS/MS spectrum. Cleavage of the C-C bond between carboxamide group and the cycloalkane gave rise to N-fluoropentylindole acylium ion at *m/z* 232, a characteristic fragment of many other synthetic cannabinoids[38].

The data acquired from the "realistic scenario" experiments have demonstrated the robustness and potential of PSA paper tickets to collect street drug sample residues on multiple porous and rough surfaces. In this method, the surfaces were sampled nondestructively, followed by the detection and confirmation of the drug presence through both full scan and MS/MS analysis on a portable MS, even after extreme scenarios such as touching the surfaces multiple times or washing hands thoroughly.

3.5 Conclusions

This work demonstrated a cost-effective, yet efficient method which combined sample collection utilizing the PSA paper sampling technique and paper spray - MS analysis performed directly on the same ticket using the Continuity portable mass spectrometer. Calibration curves were generated for ten different illicit drugs including acetyl fentanyl, fentanyl, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, and XLR-11. The portable MS was able to detect these substances at low nanogram quantities, ranging from 2 to 10 ng. To evaluate this method's applicability in forensics and customs field work, several "realistic" scenario applications were performed. This method was used to detect acetyl fentanyl on cloth, cocaine on cardboard and washed hands, heroin on concrete surface, methamphetamine on plywood, and XLR-11 on office paper. The detection and confirmation of these drug residues on all tested surfaces shows the potential of PSA sampling method coupled with portable mass spectrometer for detecting commonly abused, traditional drugs, as well as novel synthetic opioids such as fentanyl analogs and synthetic cannabinoids. To make this technique more universally available to forensic investigations, robust and scalable sampling devices will need to be developed to be compatible with various miniaturized mass spectrometers.

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CHAPTER 4. DEVELOPING SNAP-IN SOLID-PHASE-EXTRACTION CARTRIDGE FOR AUTOMATED SYSTEM

4.1 Abstract

Paper spray mass spectrometry is one of a few methods that allows rapid analysis with high quantitative accuracy and precision at low concentration. In previous research, paper spray with integrated solid-phase extraction (SPE) has shown improvement in sensitivity. However, the integrated format has several limitations, including difficulties in transporting the entire cartridge and requirement for a new cartridge just for the SPE. Therefore, this study shows a major improvement in the cartridge design, in which a snap-in SPE, being compatible with the existing Velox system, is utilized in the paper spray mass spectrometry assay. The snap-in SPE outperforms previous approach due to greater flexibility in preconcentration and volume control device, as well as the efficiency during on-site sample collection and transportation. Recovery, quantitative performance, and detection limits were examined for 11 drugs: Acetyl Fentanyl, fentanyl, cocaine, clonazolam, methamphetamine, methylone, heroin, XLR-11, AB-CHMINACA, U-47700, and 25I-NBOMe. Calibration curves were generated, leading to the detection limits of 11 compounds being in low nanogram per milliliter range, some at even sub-nanogram level. These results demonstrated the potential of the novel sampling device with snap-in SPE column for ease of sample collection, and at the same time for detection limits improvement.

4.2 Introduction

Designer drugs, or new psychoactive substances (NPS), have emerged as a major problem all over the world. With no legitimate industrial or medical use, the misuse and abuse of these illicit drugs have contributed to increase in the number of deaths by overdose[1]. Designer drugs are structural or functional analogs of controlled substances that have been illicitly produced to imitate the pharmacological effects of the traditional drugs[1]. Examples of these include synthetic phenethylamines, such as synthetic cathinone or other synthetic hallucinogens, and synthetic cannabinoids, which can be found in herbal incense products or in liquid form[1]. Opioid overdoses, especially from fentanyl and its analogs, also contribute to a majority of deaths[2,3]. These fentanyl analogs can have potencies ranging over several orders of magnitude, from less potent to 200 times more than fentanyl, making it harder to control the dosage[4,5]. With the dosage being poorly controlled and side effects being rarely studied, these synthetic drugs pose a serious threat to the health of people who use drugs[5].

Paper spray – mass spectrometry (PS-MS) is a fast, cheap and simple method for illicit drug screening as well as therapeutic drug monitoring[6-8]. Sample analysis by PS-MS is performed by spotting a liquid sample onto a triangular-shaped paper substrate, followed by the addition of a spray solvent to extract the analytes and addition of high voltage to generate a plume of charged droplets. Earlier applications of PS-MS in clinical chemistry and forensic toxicology involved detecting and quantifying drugs and drug metabolites directly from dried biofluids[6-8]. However, in some cases, its sensitivity and specificity can be poor due to matrix effects from the biofluid samples[9]. In particular, detection limits of analytes poorly ionized or extracted are significantly higher than hydrophobic and basic analytes[10]. Hence, PS-MS is sometimes coupled with different sample extraction methods, such as liquid-liquid extraction using hydrophobic layer on paper substrate[11] and solid phase extraction on a cartridge in order to achieve lower detection limits[12]. In previous research, a paper spray mass spectrometry cartridge with integrated solid-phase extraction (SPE) was developed, allowing extraction of target molecules and removal of selective interference to overcome matric effects[11]. Overall, paper spray with integrated SPE had less ionization suppression and higher recovery compared to direct paper spray for the tested drugs[12]. Compared to direct paper spray analysis of dried plasma spots, the integrated solid phase extraction enhanced PS-MS analysis by lowering the detection limits significantly by up to a factor of 70 for some drugs[12]. Unfortunately, the integrated format still has several limitations, including

requirement for a new cartridge design just for the SPE materials, difficulties in transportation of the cartridge itself, and the risk of paper tip contamination or damage during shipping[12]. This leads to further development of a conceptual cartridge design for a snap-in SPE column that is compatible with Velox 360 PaperSpray automated system (Figure 4.1).

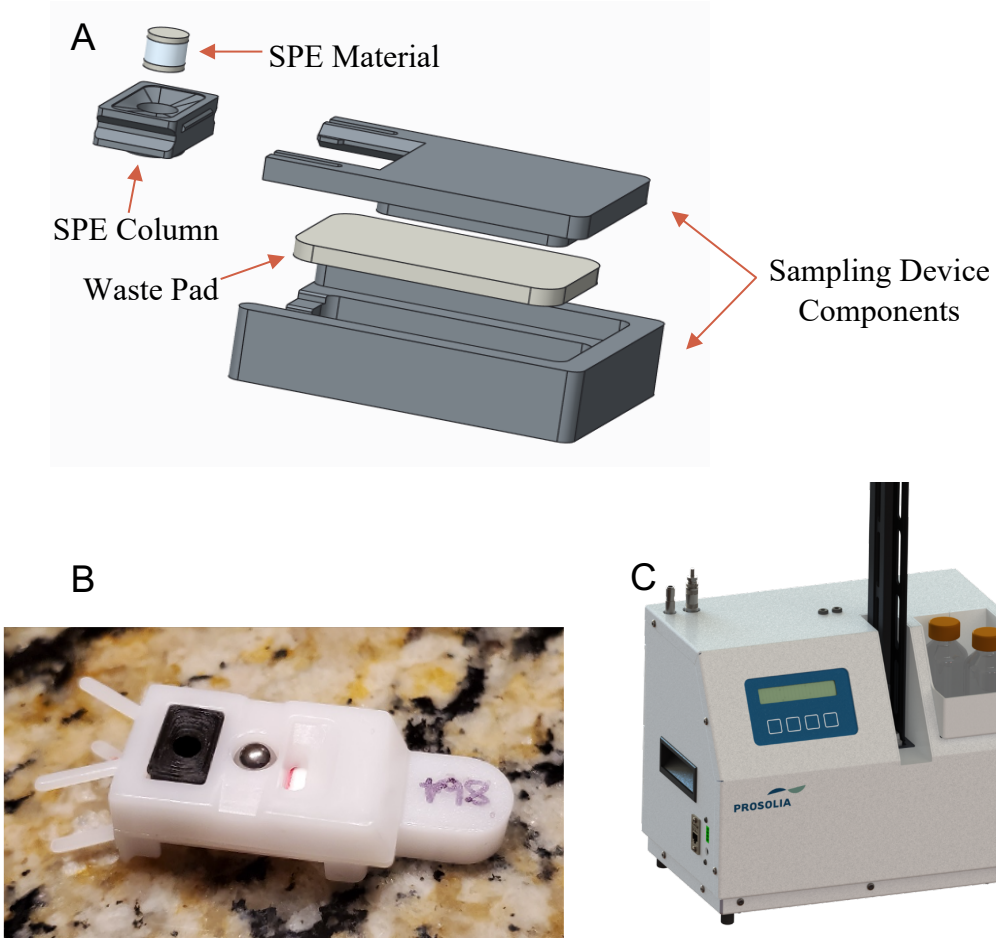


Figure 4.1 A) Exploded view of the snap-in SPE column and sampling device, B) SPE column inside paper spray cartridge, and C) Velox 360 PaperSpray Automated system.

Due to the small size of the snap-in SPE to fit into the Velox paper spray cartridge, a sampling device was designed to hold the column during sample loading and an adsorbent waste pad beneath it to collect biofluid waste (Figure 4.1A). The column has a funnel shape solvent inlet in order to hold the fluid sample as well as spray solvent during the PS-MS analysis. After the sample is loaded and allowed to wick through the SPE material, the column is removed from the sampling device and placed into a Velox paper spray cartridge, and the waste pad is disposed (Figure 4.1B).

The Velox cartridge containing the SPE column with precut paper ticket is then be positioned in front of the MS inlet for analysis. With this design, the column can be more easily transferred, the paper tickets will not be contaminated or damaged during the process, and the assay can be performed using an automated system.

This work evaluates the functionality of the new snap-in SPE column using human blood plasma. Testing is done on 14 drug compounds: 25I-NBOMe, AB-CHMINACA, acetyl fentanyl, carfentanil, fentanyl, remifentanil, clonazolam, cocaine, heroin, ketamine, methamphetamine, methylone, U-47700, XLR-11. Detection limits and calibration curves were also determined and graphed for these compounds.

4.3 Methods

4.3.1 Materials

Analytes and their deuterated internal standard (ISTD), except acetyl fentanyl, XLR-11 and its d5, were purchased from Cerilliant (Round Rock, TX, USA). Acetyl fentanyl, XLR-11 and XLR-11 d5 were obtained from Cayman Chemical (Ann Arbor, MI, USA). Pooled human plasma came from Fisher Scientific (Waltham, MA, USA) and stored at -20 °C. High-performance liquid chromatography (HPLC) grade solutions of acetonitrile, formic acid, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Whatman grade 31 ET was purchased from GE Healthcare Life Sciences (Pittsburgh, PA, USA) and Strata-XL SPE tubes were obtained from Phenomenex (Torrance, CA, USA). Polypropylene (PP) filament was from Verbatim (Chiyoda, Tokyo, Japan).

4.3.2 Making Sampling Devices and Snap-In SPE Columns

The sampling devices and snap-in SPE columns were modeled in Sketchup and 3D printed using a polypropylene filament on an Ultimaker 2+ extended (Geldermalsen, Gelderland, Netherlands). Inside the sampling devices, 31ET chromatography paper was used as the waste pad. For the column, Strata X as the SPE material was packed between a 3.0 mm Whatman ET 31 paper punch on top and a 3.0 mm nylon punch on the bottom.

4.3.3 Preparation of Working Solutions and Calibrators

Using the powdered acetyl fentanyl drug standard, acetyl fentanyl stock solution was prepared in methanol (1 mg/mL). The concentration of 25I-NBOMe, AB-CHMINACA, clonazolam, cocaine, fentanyl, heroin, ketamine, methamphetamine, methylone, and U-47700 standard solutions were 1 mg/mL, while the concentration of carfentanil, remifentanil, and XLR-11 were 100 µg/mL. Limits of detection (LODs) in plasma were evaluated by analyzing calibration curves with SPE extraction. Five working calibration solutions were prepared by diluting the stock standards in acetonitrile with 0.1% (v/v) formic acid to 40, 200, 800, 2000, and 8000 ng/mL. An internal standard solution (ISTD) was prepared from the internal standard stock solutions at 500 ng/mL. Calibration curves were prepared by spiking 25 µL of working calibration solutions and 10 µL of the ISTD solution into 965 µL of blank plasma so that the final concentrations of the plasma calibrators were 1, 5, 20, 50, 200 ng/mL. Five measurements were made at each concentration as well as eight blank measurements. 50 µL of plasma spiked with drug solution and the ISTDs was pipetted onto the SPE column for extraction and allowed to dry overnight. After drying, the SPE columns were removed from the sampling devices and inserted into the Velox paper spray cartridges for analysis.

4.3.4 Paper Spray Analysis and Data Processing

Mass spectral data was acquired using a Q Exactive Orbitrap mass spectrometer (ThermoFisher Scientific Inc., San Jose, CA, USA). Velox paper spray cartridge with a paper ticket below the snap-in SPE column was placed in front of the inlet. A total volume of 65 µL spray solvent (acetonitrile with 0.1% formic acid) was added dropwise into the SPE column, providing sufficient time for the solvent to wick through the column and to the tip of the paper. Spray voltage was applied for a period of 1.5 min, providing MS/MS data in PRM mode. The spray voltage was set to +4.0 kV for all drugs of abuse spanning a typical scan range of 80-500 *m/z*. The m/z resolution was set at 35,000, which lies in the middle of the range offered by the instrument. Capillary temperature was 300 °C. All collision-induced dissociation (CID) values are listed in Table 4.1.

Data analysis was performed using Tracefinder v. 3.3 software (Thermo Fisher Scientific). Ten analytes and six internal standards with their fragment ions' peaks were integrated for the

quantification of the drug residues. From that, calibration curves of ten compounds were graphed by plotting the response ratio of the analyte to its internal standard. The response ratio was calculated from the area-under-the curve (AUC) of selected fragment ions in MS/MS mode. Calibration curves were fitted to a straight line using the 1/x weighted least squares. With this approach, more weight is given to lower concentrations on the curve to counteract the greater absolute variance in the response ratio at high concentrations. Finally, limits of detection (LOD) of ten drug compounds were calculated using equation 1:

$$\mathrm{LOD} = 3 \times \mathrm{SE}/\mathrm{m} \qquad (1),$$

in which SE was the standard error of the intercept, and m was the calibration curve's slope.

Table 4.1 Data for drug compounds and their calibration curves.

Analytes	*Chemical Formula*	*Precursor m/z*	*Fragment m/z**	*CID (eV)*	*R^2*	*LOD (ng/mL)*
25I-NBOMe	$C_{18}H_{22}INO_3$	428.07	**121.06** 91.05	30	0.992	2
AB-CHMINACA	$C_{20}H_{28}N_4O_2$	357.23	**259.14** 241.13	40	0.963	4
Acetyl fentanyl	$C_{21}H_{26}N_2O$	323.20	**188.14** 105.08	45	0.998	0.8
Carfentanil	$C_{24}H_{30}N_2O_3$	395.23	**335.2** 113.06	30	0.998	0.5
Clonazolam	$C_{17}H_{12}ClN_5O_2$	354.07	**308.08** 326.00	50	0.957	1
Cocaine	$C_{17}H_{21}NO_4$	304.15	**182.12** 150.00	35	0.997	0.5
Fentanyl	$C_{22}H_{28}N_2O$	337.20	**188.17** 105.00	40	0.995	0.2
Heroin	$C_{21}H_{23}NO_5$	370.16	**328.08** 211.08	45	0.981	4
Ketamine	$C_{13}H_{16}ClNO$	238.10	**220.08** 179.00	45	0.981	3
Methamphetamine	$C_{10}H_{15}N$	150.13	**119.00** 91.00	45	0.966	0.2
Methylone	$C_{11}H_{13}NO_3$	208.10	**190.08** 160.00	20	0.969	4
Remifentanil	$C_{20}H_{28}N_2O_5$	377.21	**345.18** 317.18	30	0.997	1
U-47700	$C_{16}H_{22}Cl_2N_2O$	329.11	**284.00** 203.58	30	0.997	0.1
XLR-11	$C_{21}H_{28}FNO$	330.22	**232.11** 125.00	50	0.947	0.1

* Bold items indicate the quantifier ion for each analyte.

4.4 Results and Discussion

4.4.1 Limits of Detection Using Snap-In SPE Column

Because no chromatography is performed in paper spray, the term chronogram is used here rather than chromatogram. An example of a total ion chronogram of spiked plasma acquired using the described instrument method is shown in Figure 4.2A. A full cycle of the inclusion list, encompassing all targeted compounds, was completed within ~1 min. Zero-intensity scan was at the beginning and the end of the scan, which was obtained by turning off the volage, to perform automatic peak integration through the TraceFinder software later. The MS/MS spectrum of acetyl fentanyl (collision induced dissociation of *m/z* 323) is shown in Figure 4.2B, showing the generation of the quantifier ion at *m/z* 188.

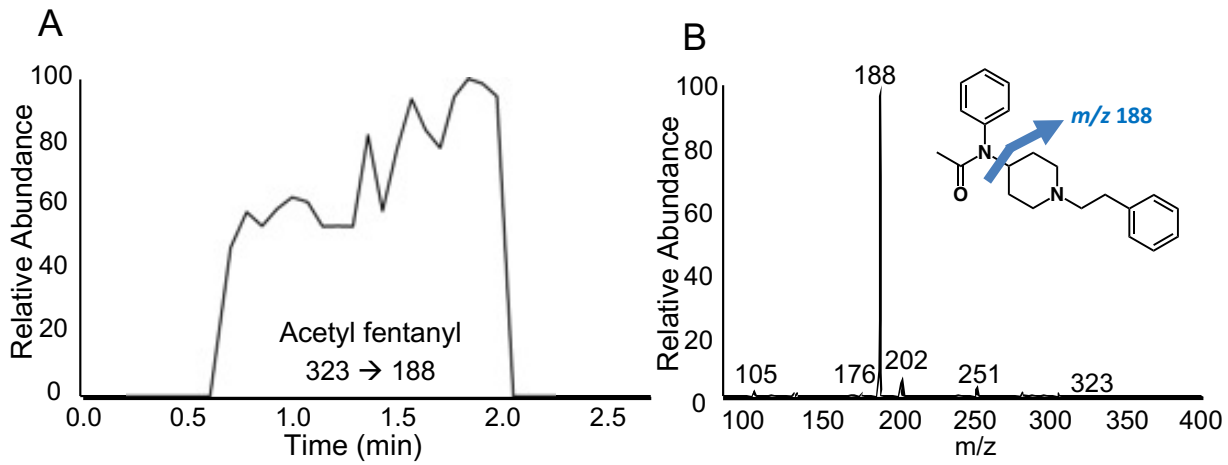


Figure 4.2 A) Extracted ion chronogram and B) MS/MS spectrum of acetyl fentanyl in spiked plasma.

Fourteen compounds were successfully quantitated, and their five-point calibration curves are shown in Figure 4.2. The correlation coefficient (R^2) for each calibration curve was 0.95 and above, indicating good linearity. The detection limits (Table 4.1) are below the concentration normally encountered in forensic toxicology[13-15]. Among 14 drug compounds, AB-CHMINACA, clonazolam, and XLR-11 showed larger variation in signal response, especially at higher concentration, leading to lower R^2 values. At the same time, AB-CHMINACA, heroin, and methylone stood out as having much higher LODs compared to other 10 drugs, all at 4 ng/mL. Eight other compounds, including fentanyl analogs, synthetic cannabinoid and opioid, could be

detected at sub-ng range. These results indicate the potential of the new snap-in SPE column in performing quantitative analysis of illicit drugs in plasma using Velox automated system.

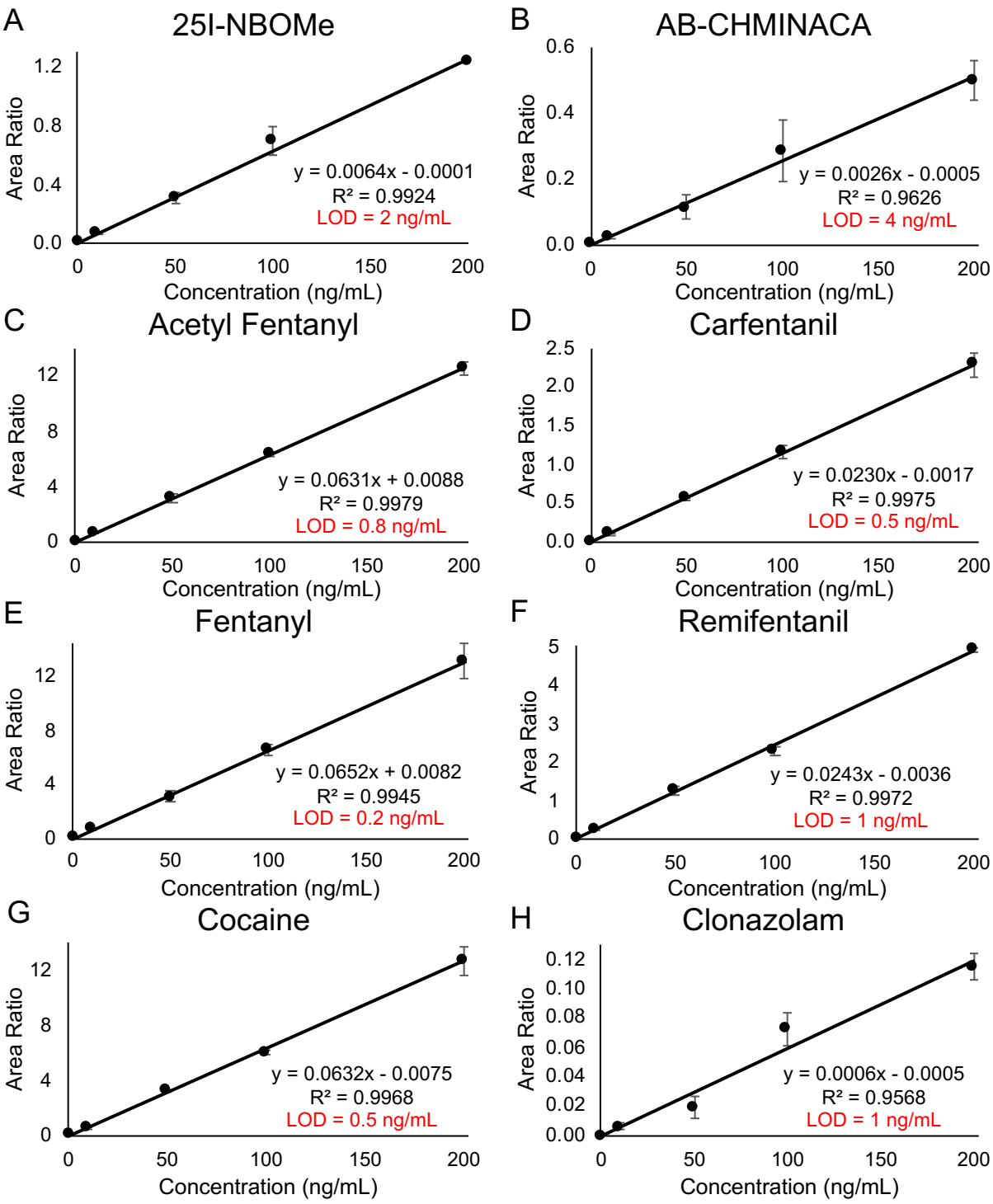
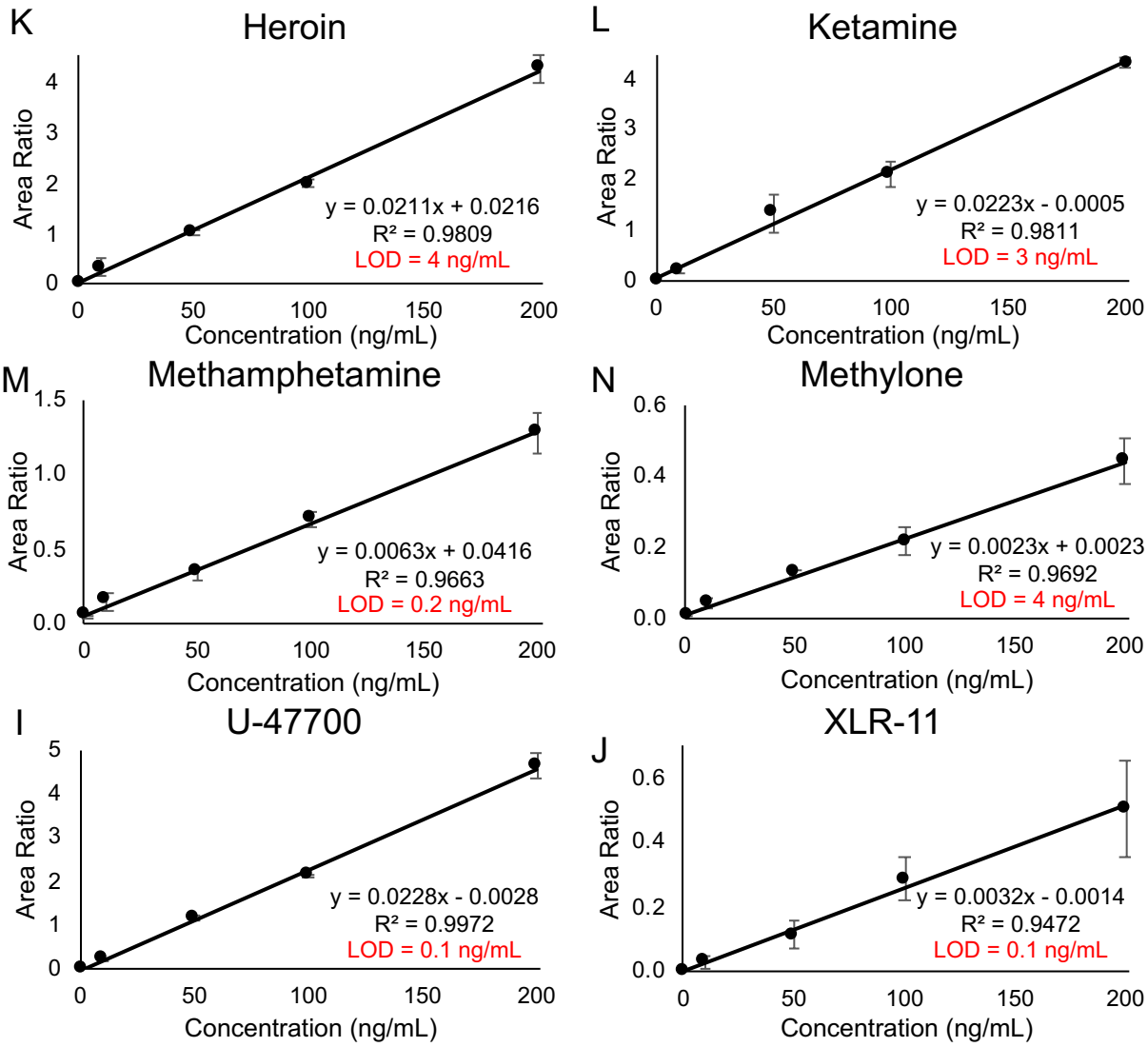


Figure 4.3 Calibration curves of 14 drugs of abuse. Data points represent the average response ratio of the triplicate runs. Error bars are ± the standard deviation of these averages.

Figure 4.3 Continued



4.4.2 Future Works

Further Snap-In SPE Column Development

Future efforts should focus on improving the snap-in SPE column in order to hold more SPE material, which eventually leads to more sample for analysis. More importantly, new paper spray tip shape will be developed to be smaller in size with less paper behind the sample. This way, sample will easily wick to the tip of the paper *via* capillary action and not to the back of the cartridge. As a result, the sensitivity of this method can be improved significantly.

4.5 Conclusions

A new snap-in SPE column and sampling device were developed to be compatible with Velox automated system. Here, the SPE column can be more easily transferred, and the assay can be performed using an automated system by removing the SPE column from the sampling device and inserting into Velox paper spray cartridge. Quantitative analysis was carried out by generating calibration curves for fourteen drug compounds. All drug compounds showed good linearity when an internal standard was spiked into the sample. Detection limits were also calculated based on the calibration curves, yielding most LODs in the sub-ng/mL level. Indeed, this work showed an improvement in the paper spray cartridge with integrated SPE, allowing the use of an "all-in-one" sampling device on an automated system. Future work will focus on further developing the snap-in SPE column to hold more SPE materials and sample, as well as to have smaller paper spray ticket for higher sensitivity.

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CHAPTER 5. OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Overall Conclusions

Paper spray – mass spectrometry (PS-MS) has garnered attention in the analytical community due to its simplicity, versatility, cost effectiveness, and more importantly, sensitivity. Mechanistically, solid or liquid sample is spotted to the front of a triangular paper tip, followed by a suitable solvent applied to wet the paper from the rear in order to extract analytes of interest. Subsequently, the analysis is initiated by applying a high voltage, either positive or negative, to the paper that leads to the production of charged ions. In addition to simple procedure described above, the ability to analyze a wide range of molecules has opened the door for the analysis of drugs and metabolites, explosives, and proteins in biofluids[1,2], environment[3], forensic[4], and food safety[5] using PS-MS.

On the other hand, the ionization event and signal stability can be affected by various factors, such as paper type, treatment and shape, sample-to-paper ratio, the angle of the paper spray tip, distance between the paper tip and the mass spectrometer inlet, as well as solvent used[6,7]. As a result, lots of research has been done to investigate and optimize these parameters for further enhancement of signal stability and detection limits, which are summarized elsewhere[8]. In this work, different sampling devices were investigated for the qualification and quantification of illicit drugs using PS-MS to improve sensitivity and detection limits. In chapter 2 and 3, a novel sampling tool was investigated for paper spray mass spectrometry. A commercially produced paper coated with pressure-sensitive adhesive (PSA) was used as both sampling tool and paper substrate for the PS-MS analysis. Chapter 3 demonstrated the use of PSA paper on a benchtop mass spectrometer to collect and detect ten illicit drugs on common surfaces such as asphalt, cloth, concrete, glass, and aluminum. Detection limits of ten compounds were also determined to be at ng quantities. More importantly, by showing higher signal response and S:B ratios compared to non-adhesive paper as well as no significant difference between different dabbing scenarios, PSA paper has proved its robustness and efficiency in drug residue collection. To bring this approach closer to drug screening in the field, PSA paper was coupled with a portable mass spectrometer for "realistic" scenario applications in chapter 4. Acetyl fentanyl, cocaine, heroin, methamphetamine, and XLR-11 were successfully detected and confirmed on cloth, cardboard and washed hand, concrete

surface, plywood, and office paper, respectively. These results signify the potential of PSA sampling method in detecting commonly abused drugs as well as novel synthetic opioids and synthetic cannabinoids on surfaces for security and border control purposes. In other words, with further development, these novel sampling tools can certainly be ideal alternatives to other traditionally used technique for drug screening.

Finally, in another project in chapter 4, a combination of new snap-in SPE column and sampling device was developed to be more easily transferred, and to allow the use of an automated system by removing the SPE column from the sampling device and inserting into Velox paper spray cartridge. Fourteen drug compounds from different drug classes, including fentanyl analogs to synthetic cannabinoids, were spiked into drug-free plasma for PS-MS quantitative analysis. Calibration curves of fourteen compounds showed good linearity with R^2 values of 0.95 and above. At the same time, detection limits of the analytes determined from their calibration curves were all below 5 ng/mL, eight of them were even at sub-ng/mL range. These results indicated the ability to detect trace amounts of drugs in plasma using the snap-in SPE column with Velox automated system.

5.2 Future Directions

5.2.1 PSA Sampling

Interference Study

Because the adhesive is non-selective, there is risk that large amounts of surface detritus or cutting agents can interfere with sample collection. As a result, an interference study is needed to investigate the matrix effects of different interferents on analyte signal response.

In preliminary experiments, three sugars were used as interferents to simulate cutting agents (3% w/v), including inositol, lactose, and mannitol, while the surfaces being tested were cloth, glass, and aluminum. The result shows that these interferents do not hinder the sample collection and identification or lead to a loss of signal during ionization (Figure 5.1). Among a total of 30 cases being investigated, no significant difference in analyte detection was observed in 27/30 cases ($p > 0.05$). Interferent only significantly decreased drug collection in 1/30 case, while enhanced drug collection in 2/30 cases. Signal response of all ten compounds tend to decrease on glass surface yet increase on aluminum surface. On the other hand, signal response of all compounds on

porous surface like cloth was not affected by the present of the interferents. This could be due to the treatments on these surfaces, as well as the interaction between the compounds, interferent, and surface structure. These preliminary data demonstrated the low impact of matrix interferences on the PSA paper collection despite the interferences being 1000× higher in amount (by mass) than the drug target.

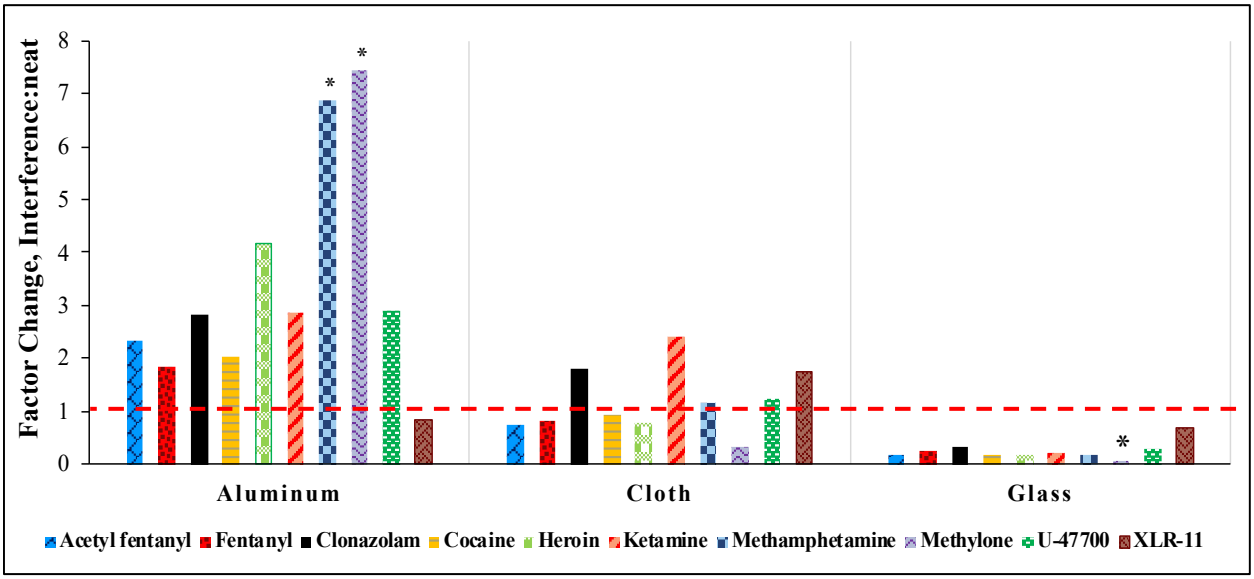

Figure 5.1 Factor change in the peaks' area-under-curve (AUC) of ten compounds on three surfaces. *Drug compounds with asterisks have a significant difference in AUC compared to samples without interferents ($p < 0.05$).

Other than sugars, interference from the surfaces such as dust and liquid droplets should also be investigated since these interferences can hinder the functionality of the adhesive materials during sample collection. In addition, structural analogs need to be taken into consideration to help inform data interpretation, as well as produce accurate results. Potential interferences can arise from isomeric or isotopic parent ion overlap between drug compounds having common fragment ions. For example, isopropylbenzylamine, a legal industrial isomer commonly used to dilute methamphetamine, exhibits the same chemical formula and molar mass as methamphetamine[9]. Because these two compounds share fragment ions at *m/z* 91 and *m/z* 150, signal response or area under the curves of the product ions during MS/MS analysis can be much higher, leading to false positive results[9]. With that in mind, a list of cutting agents will be determined and investigated

further, naming phenacetin, nicotinamide, ephedrine, paracetamol, caffeine, phenolphthalein, piracetam, nicotinamide, and starch.

Recovery Study

The interaction between the PSA paper substrate and compounds of interest will be determined in a recovery study. Recovery refers to the percentage of the analyte that is extracted from the matrix, in this case, the PSA paper itself. Here, the study can be done by spotting a drug mixture on the PSA paper in the amount of either 225 ng (level 1) or 450 ng (level 2), followed by mass spectrometry analysis. After running PS – MS, five replicates at each level will be extracted offline in methanol solution containing internal standard using a sonicator. A calibration curve of concentration (100 ppb to 2500 ppb) versus analyte/internal standard ratio will be prepared to determine the amount of drug residues remained on the PSA ticket after the first PS – MS analysis.

5.2.2 Snap-In SPE Column

Improvement of 3D Printed SPE Columns and Paper Tickets/Waste Pad

The snap-in SPE column will be further improved to hold more SPE materials by making the column wider and the funnel deeper to hold more fluid sample and spray solvent. Figure 5.2 shows 3D design of the new column with wider and deeper funnel, with the column width increases from 2.5 mm to 3.5 mm. More importantly, as polypropylene (PP) often has warping properties and poor layering adhesion upon cooling, this filament makes it challenging to 3D print. To improve the quality of the 3D printed SPE column, other filaments that are also organic-solvent friendly yet easier to 3D print such as PP GF30 and PAHT CF15 can be used as substitute for PP filament[10]. In particular, PP GF30 is a composite filament filled with 30% glass fiber for chemically and environmentally resistant that is easier to 3D print with than other PP filaments thanks to the enhanced interlayer adhesion[11,12]. PAHT CF15 is also 15% carbon fiber reinforced thus stiffer, being suitable for 3D printing of demanding applications[13].

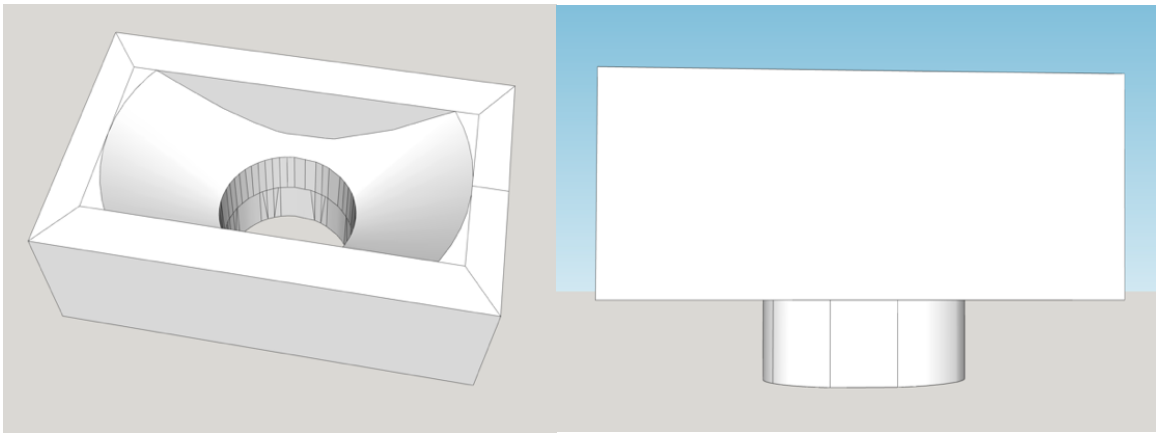

Figure 5.2 3D design of new snap-in SPE column to hold more SPE materials.

At the same time, the method's sensitivity can be improved by using paper ticket with smaller size (by 50%), while different types of Whatman filter paper used as waste pad will be tested to help shorten the extraction time. For example, Whatman Grade 1 and 4 Qualitative Filter papers are standard grade, medium to high-flow rate filter papers used for fast filtration[13]. This way, plasma sample can wick through the SPE column and elute faster on the waste pad underneath, allowing more rapid extraction and larger sample volume for analysis.

Although studies have shown that PS – MS can be an alternative to chromatography-based methods in analytical laboratories for traditional xenobiotic and biomolecule analysis, it cannot analyze compounds that require chiral separation or comprehensively analyze a broad range of chemicals with high sensitivity[14]. With the aim of sensitivity improvement, paper substrate used as paper spray tickets in this study can go under further treatments like silanization and glass-fiber enforcement, combined with the use of different spray solvents or solvent mixtures. These modifications have been shown to aid the ion recovery and ionization, improving the signal-to-blank ratios of different drug classes during biofluid analysis as a whole[15].

In short, PS – MS has become a popular and widely used ambient ionization technique thanks to its simplicity, rapidity, and versatility. Yet, substantial work is still needed to improve this technique's sensitivity and standardization among laboratories, bringing it closer to clinical works from research applications. In addition, the combination of PS with portable/miniaturized mass spectrometers still needs to undergo significant forensic and clinical studies to validate its overall robustness and evaluate its practicality in different settings.

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