DETECTION AND QUANTITATION OF HAZARDOUS CHEMICALS IN ENVIRONMENTAL MATRICES USING PAPER SPRAY MASS SPECTROMETRY

Volume 1

by

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
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To Nolan, my husband and best friend.

Thank you for the constant support on all of our adventures.

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LIST OF ABBREVIATIONS

Acetylcholinesterase	AChE
Alkyl Methylphosphonic Acid	AMPA
Area Under the Curve	AUC
Atmospheric Pressure Chemical Ionization	APCI
Bioconcentration Factor	BCF
Carbon Tetrachloride	CCl_4
Central Nervous System	CNS
Chemical Warfare Agent	CWA
Chemical Weapons Convention	CWC
Coefficient of Determination	R^2
Collision Induced Dissociation	CID
Cyclohexyl Methylphosphonic Acid	CHMPA
Delta	δ
Desorption Electrospray Ionization	DESI
Diisopropyl Methylphosphonate	DIMP
Dimethyl Methylphosphonate	DMMP
Direct Analysis in Real Time	DART
Drugs of Abuse	DOA
Electron Ionization	EI
Electrospray Ionization	ESI
Ethyl Methylphosphonic Acid	EMPA
Gas Chromatography – Mass Spectrometry	GC-MS
High Energy Collision Induced Dissociation	HCD
High Performance Liquid Chromatography	HPLC
High Resolution Mass Spectrometry	HRMS
Higher Energy Collisional Dissociation	HCD
Isobutyl Methylphosphonic Acid	iBuMPA
Isopropyl Methylphosphonic Acid	IMPA

Kappa	κ
Limit of Detection	LOD
Limit of Quantitation	LOQ
Liquid Chromatography Tandem Mass Spectrometry	LC-MS/MS
Liquid-Liquid Extraction	LLE
Mass Spectrometry	MS
Mass to Charge	*m/z*
Matrix Effects	ME
Membrane-Inlet Mass Spectrometry	MIMS
Mu	μ
Negative Ion Mode	NIM
Paper Spray Mass Spectrometry	PS-MS
Parallel Reaction Monitoring	PRM
Part Per Billion	ppb
Part Per Trillion	ppt
Pharmaceutical Based Agents	PBAs
Pharmaceuticals and other Personal Care Products	PPCPs
Pinacolyl Methylphosphonic Acid	PinMPA
Positive Ion Mode	PIM
Process Efficiency	PE
Quick, Easy, Cheap, Effective Rugged, and Safe	QuEChERS
Recovery	RE
Salting Out Liquid-Liquid Extract	SALLE
Selected Reaction Monitoring	SRM
Solid Phase Extraction	SPE
Tandem Mass Spectrometry	MS/MS
Trimethyl Phosphate	TMP
Triple Quadrupole	QqQ
Wastewater Treatment Plants	WWTP
Water-Based Epidemiology	WBE
Weapons of Mass Destruction	WMD

ABSTRACT

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Title: Detection and Quantitation of Hazardous Chemicals in Environmental Matrices using Paper Spray Mass Spectrometry
Committee Chair: Nicholas Manicke

Paper spray mass spectrometry (PS-MS) is an ambient ionization technique that has been proven useful in many types of investigative analyses. However, the use of this technique with regards to environmental samples has been largely unexplored since the technique's development. In this work, paper spray mass spectrometry was utilized to detect and quantify compounds for environmental, forensic and chemical defense applications. Due to the sensitive nature of some projects, the work was split into two volumes. Volume 1 focuses on the detection of pharmaceuticals in soil using paper spray (Chapter 2) and the detection of chemical warfare agent (CWA) simulants and CWA hydrolysis products (Chapter 3). Volume 2 focuses on the detection and quantitation of fentanyl analogs in environmental matrices. Chapter 5 focuses on the rapid analysis of fentanyl analogs in soil matrices. The following chapter evaluates the ability of PS-MS to detect low concentrations of fentanyl analogs in water (Chapter 6). Throughout this work, paper spray has proven to be an effective, rapid alternative to chromatography for the analysis of environmental samples.

CHAPTER 1. PAPER SPRAY MASS SPECTROMETRY

Mass spectrometry (MS) is an indispensable analytical technique utilized for many investigative analyses. Mass spectrometers operate by sorting and isolating ions based on their mass to charge ratio (*m/z*). The specificity of the technique can be further improved by fragmenting ions by tandem mass spectrometry (MS/MS) and associating a fragment ion to its precursor. However, before analysis takes place, the molecules must first be ionized. Ionization techniques fall into two categories, hard and soft ionization, based on the extent of molecular ion fragmentation. The most common hard ionization technique is electron ionization (EI), in which a sample in the gas phase is bombarded causing the molecules to fragment. This technique is most often associated with gas chromatography – mass spectrometry (GC-MS). However, EI can only be used for samples in the gas phase[1]. Coupling high performance liquid chromatography (HPLC) with mass spectrometry posed significant technological challenges[2]. It was not until the early 1990s with the advent of electrospray ionization (ESI) and improvements to atmospheric pressure chemical ionization (APCI) that HPLC-MS became routine[1, 3-4].

ESI, a soft ionization method, occurs when a solution flows through ESI needle under atmospheric pressure. A voltage is applied to the to the needle (typically 2-5 kV), which results in a potential difference between the needle and the inlet of the mass spectrometer. The applied voltage causes droplets to be charged on the tip of the needle. Once Coulombic repulsion of the positively or negatively charged solvent exceeds the surface tension of the solvent (i.e. reaches the Rayleigh Limit), a spray of charged droplets is formed. The plume of charged droplets formed during this process is known as a Taylor cone[8]. Through a combination of Columbic repulsion and solvent evaporation, gas phase analytes are created which are drawn into the atmospheric pressure inlet of the mass spectrometer[5-7]. The resulting ions vary based on the polarity of the applied voltage and are drawn into the inlet of the mass spectrometer. The mechanism of Taylor cone formation can be seen in Figure 1.

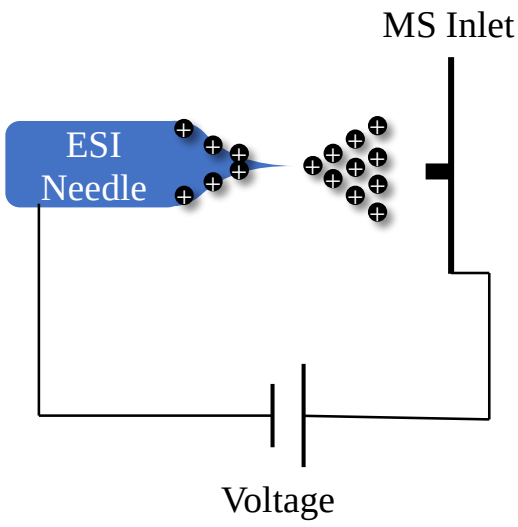


Figure 1. Taylor cone formation at the tip of an ESI needle

GC-MS and HPLC-MS are considered "workhorses" in diagnostic, forensic, and environmental laboratories for the analysis of complex matrices. Both techniques utilize a chromatographic column to separate and concentrate compounds prior to mass spectral analysis. Gas chromatography is only compatible with volatile or semi-volatile analytes; therefore, samples often require extraction and derivatization prior to analysis. When using liquid chromatography, sample preparation procedures are also typically necessary to remove matrix interferences, incompatible solvents, and prevent column clogging. Unless there are already protocols in place, extensive resources are utilized to optimize and validate the separation parameters – i.e. to determine the proper temperature or solvent gradient to increase separation[9-11]. Although GC and LC methods are useful for the detection and quantitation of trace amounts of various analytes in complex matrices, they both require sample preparation which makes them less time efficient and dampens their use in some fields.

A significant amount of recent research has been focused on making mass spectrometry a viable tool outside of traditional laboratories and by individuals with limited training. Unfortunately, typical mass spectrometers are large, complex, expensive instruments and are difficult to transport. However, Cooks, et. al at Purdue University is at

the forefront of designing and evaluating the applications of miniature mass spectrometers[12]. In addition to making mass spectrometers more accessible, the ionization method can also be adapted to better suit rapid, in-field analyses. Ambient ionization techniques are novel because samples can be directly analyzed by the mass spectrometer without the extensive sample preparation required for chromatography. They are ideal for field sampling and analysis because they are used in open-air without requiring a vacuum. Ambient ionization techniques such as desorption electrospray ionization (DESI)[13], direct analysis in real time (DART)[14] and paper spray mass spectrometry (PS-MS)[15-17] (the focus of this work) address the need for the analysis of complex matrices with little to no sample preparation[18].

Paper spray mass spectrometry is an ambient ionization technique that is an alternative to typical chromatographic separation or immunoassays[15-16, 19-20]. This technique is similar to ESI because it is a soft ionization technique that creates a plume of sample after applying a voltage. However, PS-MS diverges from ESI by the way that samples are stored, prepared, and extracted. In traditional PS-MS, a sample dries onto chromatography paper that is cut into a triangular shape, after which a solvent is applied that wicks through the paper by capillary action[16]. The solvent dissolves the analytes of interest, leaving the matrix behind on the paper. Voltage is then applied to the paper either creating a positive or negative electric field. The paper spray ionization mechanism can be seen in Figure 2.

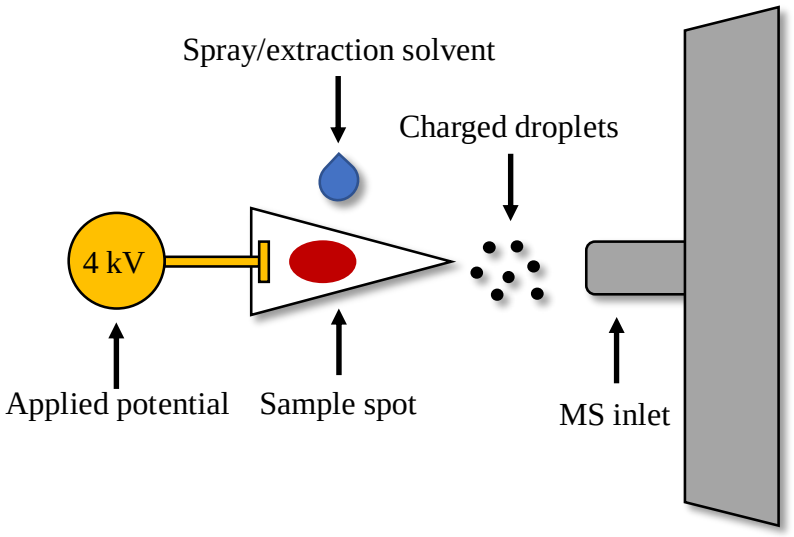


Figure 2. Paper Spray Ionization Mechanism. A potential is applied to the paper, pre-wetted with solvent. The applied potential can be either positive or negative creating positive or negative ions respectively

Because PS-MS does not utilize chromatographic separation prior to mass spectral analysis, the burden is placed on a mass spectrometer's ability to distinguish analytes with similar mass to charge ratios. Although paper-spray mass spectrometry has been used in conjunction with numerous types of mass spectrometers, the work presented in this thesis utilized a high-resolution mass spectrometer (HRMS) and a triple quadrupole (QqQ) mass spectrometer.

The high-resolution mass spectrometer used in this work was a Q-Exactive hybrid quadrupole orbitrap from Thermo Fisher Scientific. This instrument is capable of a resolving power >100,000 and high mass accuracy – i.e. <1 ppm. This provides a significant advantage for PS-MS in comparison to low resolution instruments. Due to the lack of sample preparation, many matrix components have the potential to interfere with the analyte signal or increase blank signal in a low-resolution instrument, increasing the limits of detection. However, the orbitrap design limits the effects of these variables (Figure 3). The Q-Exactive utilizes a quadrupole to filter precursor ions, a higher energy collisional dissociation (HCD) cell to be used for fragmentation, and an orbitrap for high resolution and accurate mass detection of both the precursor and fragment ions. In an orbitrap, small amounts of ions orbit around a central spindle electrode, while also oscillating between two

endcap electrodes[21-22]. The differences in oscillating frequency eventually cause ions to separate by their mass for detection. This principle is why the orbitrap is able to be highly specific – i.e. ±0.0005 *m/z*.

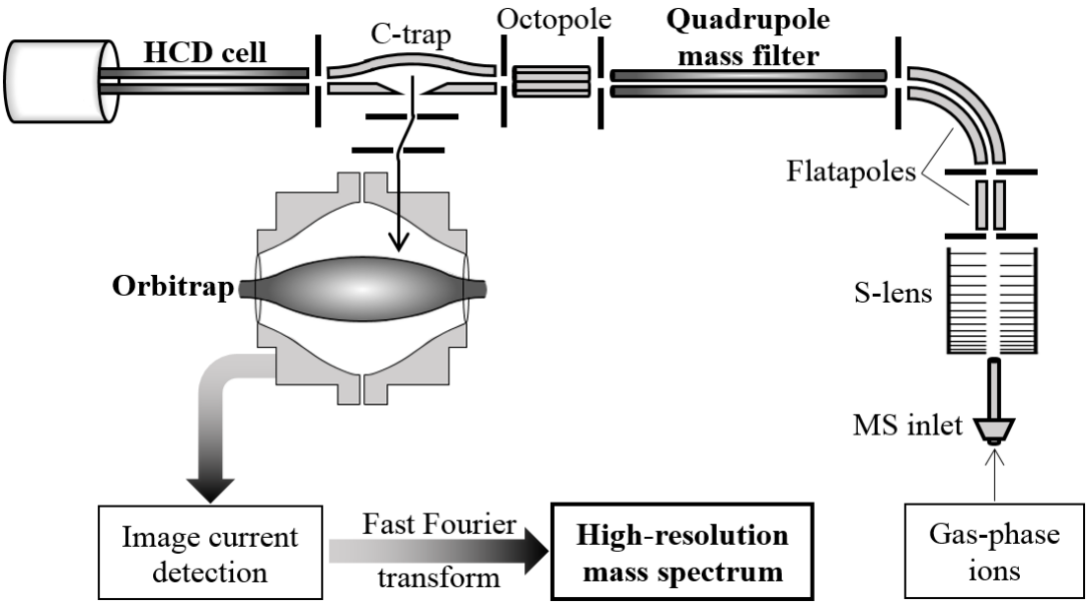


Figure 3. Schematic of the Q-Exactive Focus mass spectrometer[23]

In addition to the Q-Exactive mass spectrometer, a triple quadrupole mass spectrometer (QqQ) was also utilized. This instrument is a low-resolution instrument because it can only filter ions between ±0.5 *m/z*. Due to its lack in specificity, there is the potential for high background signal from other compounds with similar *m/z* or matrix interferences. However, this limitation is often overcome by tandem mass spectrometry[24]. A QqQ is designed with three separate quadrupoles (Figure 4). In MS/MS mode, the first quadruple filters the precursor mass, similar to the quadrupole in the Q-Exactive. In the second quadrupole, nitrogen gas collides with the precursor ions, causing them to fragment. This phenomenon is known as collision induced dissociation (CID). After the precursor ion fragments, the third quadrupole can be utilized to filter for specified fragment ions. Even if there is a matrix interference with a similar *m/z* as the precursor ion, it is less likely that there with be a fragment ion with the same *m/z* as the analyte. The specificity can be further improved by looking for more than one fragment ion from a single precursor. By

looking for both a quantitative fragment, as well as, a confirmatory fragment, this can lend further confidence that the instrument is in fact detecting the analyte and not just noise.

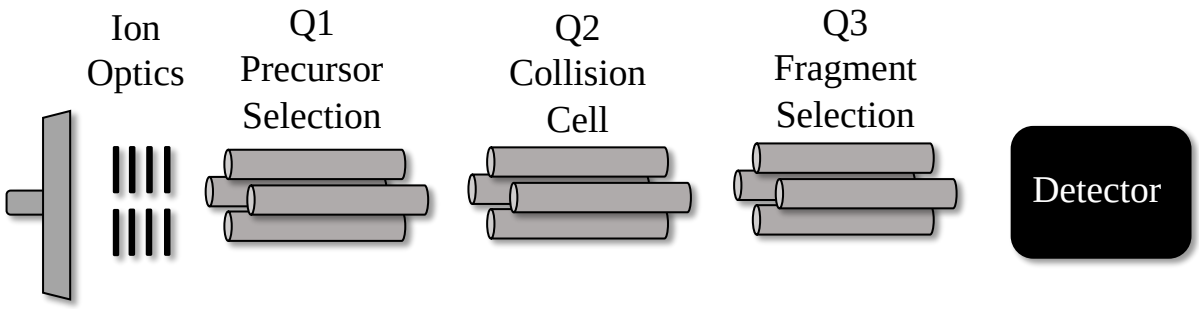


Figure 4. Schematic of a triple quadrupole (QqQ) mass spectrometer

Paper is a versatile and inexpensive substrate that can be modified to optimize sampling conditions. There are already many commercial uses of paper substrates as diagnostic tools, such as pregnancy tests and finger-stick glucose monitoring devices[15]. Similar to other paper-based sampling methods, paper spray mass spectrometry has a wide range of applications ranging from clinical, forensic, environmental, and more. However, PS-MS was originally developed in 2010 as a response to the growing need for rapid, sensitive and inexpensive techniques for detecting compounds outside of a laboratory setting with increased automation[15]. Due to mass spectrometry's irrefutable ability to detect and quantitate compounds in complex matrices, it is a useful analytical tool in many settings. In order to improve the accessibility of mass spectrometry both inside and outside of a laboratory, an ionization technique utilizing a paper substrate as the means of sampling and extraction was developed. The first published application of PS-MS was the analysis of pharmaceuticals in dried blood spots[19]. In their work, the quantitative performance of PS was evaluated by spiking blood sampled with therapeutic drugs such as, citalopram, amitriptyline, sunitinib, and telmisartan. They were able to obtain accurate and precise result without sample preparation.

This work focuses on the application of paper spray mass spectrometry for forensically relevant problems. Although PS-MS was originally developed to be useful in a clinical setting, clinicians face similar problems with sampling as forensic scientists. For example, the ability to detect emerging drugs of abuse in bodily fluids is a growing issue

that plagues both clinicians and toxicologists. PS-MS has already been shown to be an effective screening method for bodily fluids. In 2011, Manicke et al. first applied PS-MS for the analysis of therapeutic drugs in dried blood spots[19]. It was only a matter of time before this technology was applied to forensics[20, 25-26]. In these examples, PS-MS was utilized for the analysis of dried blood spots and were able to detect low ng/mL or part per billion (ppb) concentrations of over 100 drugs of abuse. Amphetamines, opiates, benzodiazepines, barbituarates, and more were included in the screenings. There have also been studies detecting drugs of abuse (DOA) in both plasma[27-28] and urine[27, 29] using paper spray.

In addition to the monitoring of pharmaceuticals and drugs of abuse, PS-MS has also been utilized for chemical defense applications. It was first used to detect and quantitate chemical warfare agent (CWA) simulants and CWA hydrolysis products in both blood and urine[30]. PS-MS is advantageous for this application because it eliminates sample preparation and has the potential to be utilized in the field. In addition to monitoring CWA simulants and hydrolysis products in biological matrices, it was advantageous to determine whether this method could also be used for air quality monitoring after a suspected CWA attack[31]. In the work by Dhummakupt et al. CWA simulants were aerosolized and captured on a paper spray cartridge. In typical PS-MS, a liquid sample is pipetted onto the paper substrate. In this work, the aerosol was pulled through the paper, and the sample was despositied onto the fibers for MS analysis. They were able to detect the simulants at mg/m^3 concentrations using this method which would be beneficial for in-field analysis.

Since its development in 2010, research regarding PS-MS has grown rapidly. The technique has now been used to detect analytes in various matrices such as biological fluids[15-16, 19, 23, 26-29, 32-33], areosols[31], foodstuffs[34-35], environmental samples[36] and many more. Although there has been extensive research into paper spray mass spectrometry, using PS-MS for environmental monitoring is lagging behind the other areas. The goal of this study was not only to detect compounds in various environmental samples, but to primarily prove that paper-spray has the capability to be utilized for solid samples. The work presented herein focuses on forensic and chemical defense applications of PS-MS for environmental samples.

Volume 1 discusses the detection of organophosphates, pharmaceuticals and drugs of abuse in soil using PS-MS. Volume 2 outlines the use of PS-MS to detect and quantify fentanyl analogs in soil and water.

CHAPTER 2. ENVIRONMENTAL FORENSICS VIA PAPER SPRAY MASS SPECTROMETRY

Introduction

Forensic science is a discipline that utilizes scientific principles to analyze evidence of a crime to be used in a court of law. Environmental forensics includes the analysis of environmental samples to identify and determine the extent of contamination. Morrison and Hone[37] defined environmental forensics to be, "the systematic and scientific evaluation of physical, chemical and historical information for the purpose of developing defensible scientific and legal conclusions regarding the source or age of a contaminant release into the environment." Environmental contamination can have a wide-reaching effect on a population. Companies and individuals can and have been held legally responsible for improperly disposing of chemicals or the negligent release of chemicals into the environment. A few of the most public and significant crimes of environmental contamination in recent years are the Flint, Michigan water crisis and the Deepwater Horizon oil spill.

In 2014, the city of Flint, Michigan began using the Flint River as its primary drinking water source, replacing Lake Huron. The Flint River pipe system was primarily lead pipes and the water from the river was not treated with corrosion inhibitors. The aging lead pipes were eventually corroded by the water and lead began to leach into Flint's drinking water[38-40]. Immediately after the water source switch, the Flint residents began to notice changes to the color, smell, and overall water quality. Residents also complained of rashes and hair loss[40]. A resident sent her water to an independent laboratory at Virginia Tech for analysis, where they analyzed the water by inductively coupled plasma – mass spectrometry (ICP-MS)[39]. Water samples were also analyzed by the Environmental Protection Agency (EPA)[41]. Lead is a potent neurotoxin, and in the years following the water crisis, resident children of Flint had increased blood lead levels (BLL) potentially affecting their long-term health[40]. The lack of prompt action by the government of Flint led to criminal charges, as well as multiple lawsuits regarding their negligence[42-43].

Another example was the Deepwater Horizon oil spill. Deepwater Horizon was an oil drilling rig that had a blowout which resulted in the release of over 4 million barrels of

oil into the Gulf of Mexico[44]. In addition to the oil spill, 2 million gallons of dispersants were also introduced into the ecosystem to aid in clean-up procedures[45]. The oil spill affected both land and sea environments and even destabilized the economy of the surrounding area due to the damage to tourism and the seafood industry. There were significant penalties for British Petroleum (BP) resulting from the widespread affect it had on the surrounding environment. This oil spill cost BP over $145 billion dollars since the incident occurred in 2010[46].

The previously mentioned cases of environmental forensics were high profile cases and had direct, visible consequences. There is also a concern about small molecules and their accumulation in the environment over time. Pharmaceuticals and drugs of abuse are used and disposed of by humans every day. There has been increasing concern with the fate, persistence, and ability to detect these compounds in the environment[47-65]. In addition to environmental accumulation, there is also research suggesting that the number of users in a population can be estimated using wastewater analysis[53-54]. In addition to the potential long-term environmental impact, the detection of clandestine laboratories is a significant concern due to their mobility. Individuals operating clandestine laboratories often dispose of their chemical waste in the environment. The chemical waste contains precursors, by-products, in addition to the desired product all of which could be dangerous[66]. Although pharmaceutical and DOA accumulation in soil may not have direct implications for the environmental health and safety of a population, it is necessary to be able to detect trace amounts of these harmful chemicals in the environment.

The work presented herein focuses specifically on the monitoring of pharmaceuticals and drugs of abuse in soil. Many pharmaceuticals and drugs of abuse have high pKa's and lipophilicity leading to adsorption to soil matrices[67]. These characteristics are associated with high bioconcentration factors (BCF) and bioaccumulation factors (BAF), which lead to the accumulation of these chemicals in both environmental and biological matrices[67-68]. Typical analysis methods include LC-MS[48, 66, 69] and GC-MS[70-71], but as mentioned previously, complex matrices, such as soil, require extensive extraction procedures prior to analysis. Ambient ionization techniques have proven to be valuable when analyzing complex environmental matrices[31, 36, 72].

This chapter focuses on the detection and quantitation of 11 different pharmaceuticals and drugs of abuse in soil using paper spray mass spectrometry. The analytes selected represent multiple classes including central nervous system (CNS) depressants, opiates, stimulants, and tricyclic antidepressants. The analytes were spiked into three different soil matrices, a Richfield clay loam, sand, and Sassafras sandy loam. These three soils represent different morphologies of soil with different characteristics. The samples were loaded into an automated paper spray cartridge for analysis on a high-resolution mass spectrometer. To improve quantitation of the analytes in soil, an offline extraction was also performed. Analytes were detected at part per billion concentrations in all three soil types using both extraction methods. Lastly, the adhesion to the soil was also monitored to evaluate the natural degradation of the analytes over time.

Experimental Methods

Chemicals and Materials

High performance liquid chromatography grade acetonitrile and 88% formic acid were purchased from Fisher Scientific (Hampton, NH, USA). Alprazolam, amitriptyline, clonazepam, cocaethylene, cocaine, hydrocodone, ketamine, 3,4–Methylenedioxymethamphetamine (MDMA), methamphetamine, morphine, and phencyclidine (PCP) were purchased from Cerilliant (Round Rock, TX, USA). Internal standards d_5-alprazolam, d_3-cocaine, d_3-methadone, d_{11}-methamphetamine, d_3-morphine, d_3-timipramine were also purchased from Cerilliant. Magnesium sulfate and sodium chloride salts were purchased from Fisher Scientific (Hampton, NH, USA) and Sigma Aldrich (St. Louis, MO, USA) respectively. Soil samples were provided by Dr. Simini at the U.S. Army Combat Capabilities Development Command (CCDC) Chemical Biological Center, (Aberdeen Proving Ground, MD, USA). An automated Velox360 paper spray source and compatible cartridges were purchased from Prosolia Incorporated (Indianapolis, IN, USA). The automated paper spray source has two methods to dispense the solvent. One pump dispenses 3μL of solvent into the front window of a cartridge. The second pumps 10μL of solvent into the solvent well. The amount of solvent as well as the timing between

dispenses can be modified depending on the sample needs. A longer delay between solvent dispenses will prevent overflow in the solvent well.

Preparation of Soil Samples

Working solutions of the pharmaceuticals were prepared at 10X concentration by diluting stock solutions in acetonitrile. Soil (250 mg) was moistened with 225 μL of water and 25 μL of the working solution. Soil samples were allowed to equilibrate for at least 12 hours after spiking. Three soils, Richfield clay loam, sand and sassafras sandy loam, were utilized in this study due to their differing characteristics. (See Table 1 for an overview of the soil properties). According to the United States Department of Agriculture (USDA), Richfield clay loams are commonly seen in states with prevalent agriculture such as Kansas, Nebraska, and Montana[73]. Sassafras sandy loam soil is commonly found near coastal plains. Sand is a coarse-textured soil that contains little to no organic material.

Table 1. Soil characteristics

Soil	Texture	pH	Organic Material	Sand	Silt	Clay
Richfield	Clay Loam	7.4	3.3%	30%	43%	27%
Sand	Sand	5.9	0.0%	99.2%	0.55%	0.20%
Sassafras	Sandy Loam	4.9	2.3%	54.9%	28%	17.8%

Paper Spray of Pharmaceutical Residues Directly from Soil

25 mg of soil was weighed into the solvent well of an automated paper spray cartridge obtained from Prosolia, Inc. (Figure 5). An internal standard (10 μL) consisting of a mixture of d_5-alprazolam (60 ng/mL), d_3-cocaine (200 ng/mL), d_3-methadone (60 ng/mL), d_{11}-methamphetamine (200 ng/mL), d_3-morphine (120 ng/mL), and d_3-timipramine (100 ng/mL) was added directly on top of the soil in the solvent well. The cartridges were then loaded into the automated Velox 360 paper spray source (Prosolia,

Inc.). The automated source dispensed 90 μL of acetonitrile with 0.1% formic acid to the solvent well directly onto the soil. The solvent wicked through the soil sample and onto the paper positioned below the soil sample. A spray voltage of +4.5 kV was then applied to the paper to commence paper spray ionization.

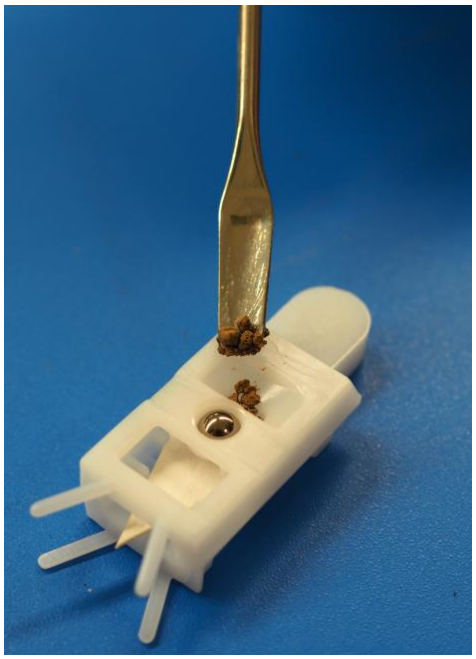


Figure 5. Prosolia paper spray cartridge solvent well filled with soil

Stability and Degradation Study

Five grams of clay loam, sand, and sandy loam were spiked with analyte at a middle concentration in the calibration curve. After a 12-hour incubation period, the samples were first analyzed as a control followed by rinsing the soil with three 1 mL aliquots of water. This was done to rinse away any residual analyte not bound to the soil. After the addition of each aliquot, the sample was vortexed and supernatant removed. In order to return the soil to the same consistency as the first analysis, 500 μL of water was added to the soil. The sampling procedure and analysis was repeated after the washing steps. The washed soil was then stored and analyzed at 7, 13, 22 and 28 days after the initial day when the soil was washed.

Salting Out Liquid-Liquid Extraction

Three soil types, Richfield clay loam, sand, and sassafras sandy loam were extracted using a salting out liquid-liquid extraction (SALLE) method. This technique was modified from the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method[47-48, 50, 74]. The QuEChERS extraction method is commonly used in industry to extract organic analytes from soil[74]. An organic solvent is added to extract the analytes. Then, salts are added to remove residual water in the sample. Lastly, dispersive solid phase extraction material is utilized to further clean up the sample.

After the 12-hour incubation period, 475 μL of acetonitrile, 200 mg of ammonium sulfate and 50 mg of sodium chloride were added to the pharmaceutical soil samples. The samples were shaken and vortexed until cooled to room temperature. The acetonitrile layer was pipetted into a clean vial and spiked with 25 μL of the same IS solution utilized for the direct extraction. The extract solution (10 μL) was pipetted onto paper spray cartridge for analysis. The paper spray and MS parameters were the same as those used for the direct soil analysis.

Mass Spectrometry and Data Analysis

Samples were analyzed using a Q-Exactive Focus Mass Spectrometer (Thermo Scientific Inc., San Jose, CA) with the ion transfer tube at 320 °C, resolution of 35,000, and the S-lens set to 50.The instrument was operated in MS/MS mode using an inclusion list with an isolation width of $\pm$0.5 *m/z*. The collision energies and fragment ions were optimized for the compounds and can be found in Table 2. Due to the simultaneous elution of all analytes of interest off of the paper, the plot of signal vs. time is known as a chronogram. Once a voltage is applied to the wetted paper, a sharp increase in signal occurs which remains consistent until the voltage is turned off or the solvent is depleted. Chronograms were integrated using Tracefinder v. 3.3. Calibration curves were constructed and fit with a 1/x weighted least square regression. For direct soil analysis, the limits of detection were determined to be the lowest calibrator concentration with a signal to blank ratio greater than 3 for the ion transition specified in Table 2. For the SALLE

method, the limits of detection were calculated by dividing the standard error of the y-intercept by the slope and multiplying by 3.

Table 2. Fragmentation and analysis parameters

Analyte	Description	CE (V)	Transition (*m/z*)	Internal Standard	Conc. Range (ng/g)
Alprazolam [M+H]+	Pharmaceutical	45	309.0902→281.0712	Alprazolam-d5	0.4-160
Amitriptyline [M+H]+	Pharmaceutical	28	278.2000→191.0852	Trimipramine-d3	0.7-320
Clonazepam [M+H]+	Pharmaceutical	45	316.0484→214.0416	Alprazolam-d5	1-480
Cocaethylene [M+H]+	Metabolite	25	318.1700→196.1328	Cocaine-d3	2-800
Cocaine [M+H]+	DOA	30	304.1543→182.1170	Cocaine-d3	2-800
Hydrocodone [M+H]+	Pharmaceutical	30	300.1594→199.0752	Morphine-d3	0.7-320
Ketamine [M+H]+	DOA	20	238.0993→125.0152	Methadone-d3	4-1600
MDMA [M+H]+	DOA	25	194.1176→135.0438	Methamphetamine-d11	2-800
Methamphetamine [M+H]+	DOA	10	150.1277→91.0546	Methamphetamine-d11	2-800
Morphine [M+H]+	Pharmaceutical	30	286.1000→201.0905	Morphine-d3	1-480
PCP [M+H]+	DOA	13	244.2060→159.1166	Methadone-d3	0.7-320

Results and Discussion

Direct Extraction of Pharmaceuticals in Soil

Soil samples were loaded into the solvent well of a paper spray cartridge and the extraction/spray solvent was applied directly to the soil via the automated source. As the solvent wicked through the paper, the analytes were extracted from the soil matrix. Once a potential was applied, ionization ensued, and the precursor and subsequent fragment ions were detected by the mass spectrometer. Paper spray mass spectrometry does not include

chromatographic separation, therefore, the graphs of signal vs. time in paper spray mass spectrometry are referred to as chronograms. The extracted ion chronograms were automatically integrated using the instrument's Xcalibur Quan software to determine the areas under the curve (AUC) (Figure 6).

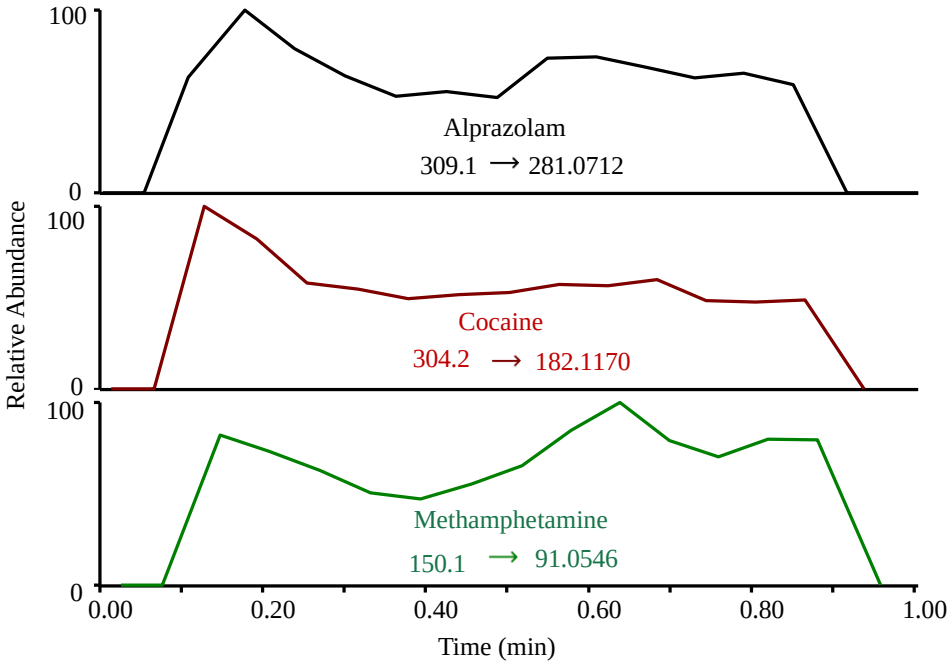


Figure 6. Extracted ion chronograms for alprazolam, cocaine and methamphetamine in Richfield Clay Loam

The tandem mass spectra obtained from direct soil analysis are mixed spectra combining fragment ions from target molecules as well as background chemicals from the soil. At lower concentrations, fragment ions from the pharmaceuticals may be relatively minor components of the spectra. Nevertheless, robust detection can be achieved by detecting characteristic MS/MS fragment ions at high mass accuracy. This can be seen for the MS/MS spectra obtained for direct soil analysis of a set of drugs at low ppb concentrations in Figure 7.

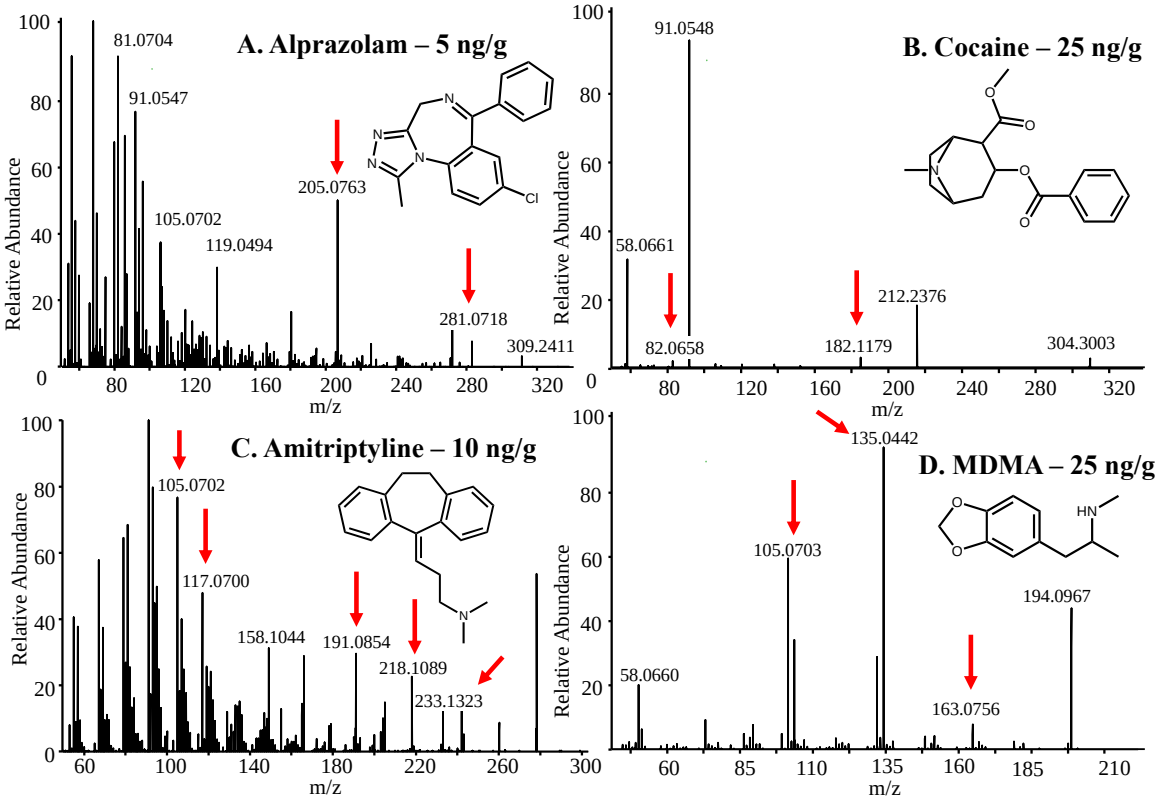


Figure 7. MS/MS spectra for alprazolam and MDMA in clay loam (A, D), cocaine in sand (B) and amitriptyline in sandy loam (C). Selected fragment ions arising from the drug targets are indicated with red arrows.

At a cocaine concentration of 25 ppb in sand, for example, the *m/z* 182.1178 fragment peak was still visible with high mass accuracy (Figure 7B). MS/MS spectra for alprazolam and MDMA in Richfield clay loam and amitriptyline in sassafras sandy loam can be seen in Figure 3A, 3D and 3C respectively. The AUC of a characteristic fragment ion was utilized for the preparation of calibration curves (Figure 8).

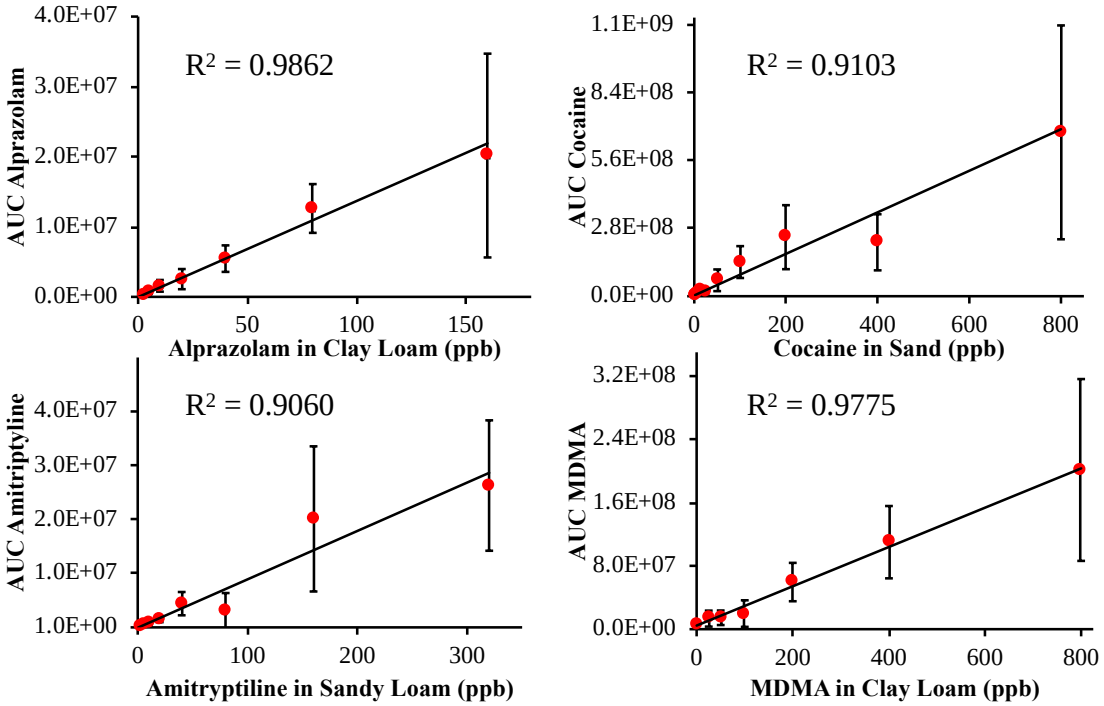


Figure 8. Select calibration curves for the extraction of pharmaceuticals from soil. Each data point is an average of three replicates with the standard deviation of the replicates represented by error bars

The limits of detection and correlation coefficients for the calibration curves are detailed in Table 3. There was a positive linear correlation between the analyte AUC and the concentration, however, the variability and linearity were not adequate for quantitation. This method was designed to be a rapid screening procedure with minimal sample preparation. The lack of soil homogenization and internal standardization prior to analysis introduced variability in replicate analyses and decreased linearity. The speed and simplicity of the method still make it an effective method for rapid screening of pharmaceuticals in heterogeneous soil matrices. Pseudo-internal standardization was investigated by spiking isotope labeled analogs onto the soil samples after weighing them into the paper spray cartridges. This did not improve the linearity or precision, although the pseudo-internal standards would still be useful for quality control. Morphine was not detected consistently in the Richfield clay loam samples. This could be due to morphine's tendency to degrade in aqueous solutions at a higher pH[75]; Richfield clay loam had the highest soil pH (Table 1).

Table 3. Limits of detection (LOD) and calibration curve correlation coefficients (R^2) for direct analysis of pharmaceuticals in soil by paper spray MS

	Richfield Clay Loam		Sand		Sassafras Sandy Loam	
	LOD (ppb)	R^2	LOD (ppb)	R^2	LOD (ppb)	R^2
Alprazolam	2.5	0.98	0.3	0.98	2	0.90
Amitriptyline	0.6	0.96	0.6	0.92	2	0.90
Clonazepam	0.9	0.91	0.9	0.99	0.9	0.83
Cocaethylene	6	0.99	2	0.91	12	0.94
Cocaine	12	0.94	2	0.91	6	0.90
Hydrocodone	0.6	0.98	0.6	0.68	5	0.91
Ketamine	3	0.97	3	0.68	12	0.88
MDMA	25	0.97	6	0.95	25	0.87
Methamphetamine	25	0.98	3	0.93	25	0.85
Morphine	NA	NA	0.9	0.62	4	0.80
PCP	5	0.98	2.5	0.88	40	0.88

In addition to the proof of concept direct extraction, an experiment was performed to determine if the pharmaceuticals and DOA adhered to the soil. A 1-gram soil aliquot was spiked with analyte and analyzed before and after rinsing the soil with water. This was done to rinse away any analytes that were not bound to the soil. The washed soil samples were then stored and analyzed at different intervals within a 28-day period. As seen in Figure 9, the analyte signal did decrease after washing the soil and over the 28-day period. However, many of the analytes were still detectable. Morphine had the lowest overall signals and more rapidly decreased to below the detection limit. The sand and sassafras sandy loam samples had similar decreasing trends.

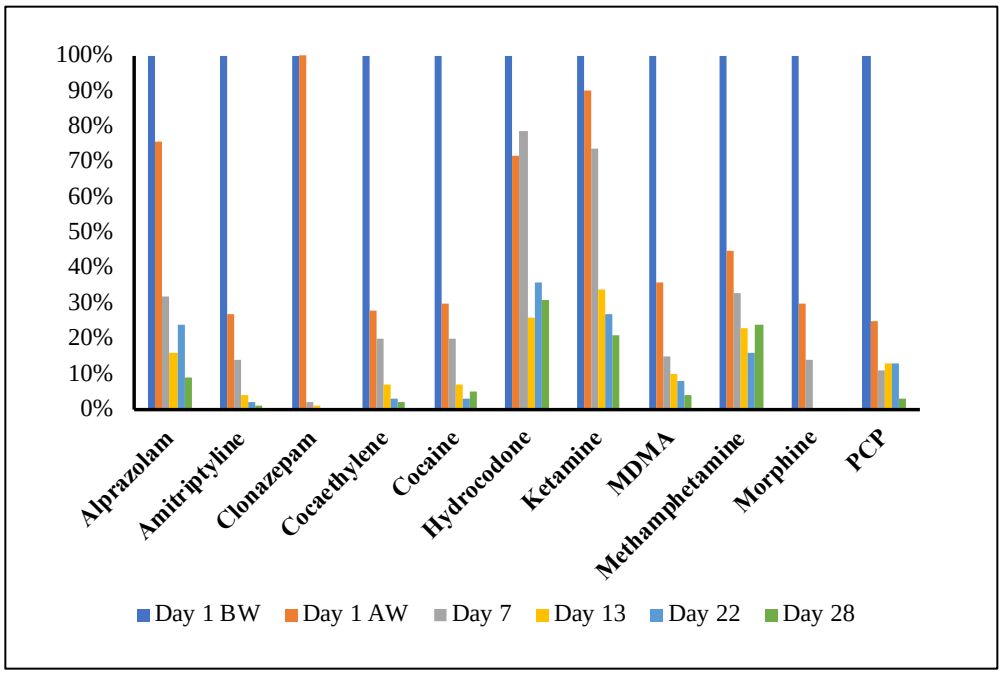


Figure 9. Richfield clay loam stability and degradation study. Day 1 BW (before wash) was sampled normally after a 12-hour incubation period. Day 1 AW (after wash) was after the addition of water to wash away analyte not absorbed or weakly absorbed to the soil. The same samples were analyzed on days 7, 13, 22 and 28. The day 1 before washing analyte signals were normalized to 100% with the subsequent days were being relative to the initial signal.

Salting Out Liquid-Liquid Extraction of Pharmaceuticals in Soil.

In order to improve quantitation, a salting out liquid-liquid extraction (SALLE) was devised to extract the analytes from the soil matrix prior to paper spray MS analysis. This extraction procedure was adapted from the QuEChERS extraction method. In a QuEChERS extraction method, acetonitrile followed by a high concentration of salts are added to moist soil. Our method differed from a typical QuEChERS extraction because we did not use dispersive solid phase extraction (SPE) material to further clean up the samples. Even without the dispersive SPE material, the SALLE method significantly improved both the precision and the linearity for the analysis of soil samples. Calibration curves for alprazolam in clay loam, cocaine in sandy loam and methamphetamine in sand can be seen in Figure 10. The correlation coefficients were greater than 0.96 for all of the analytes in the three soil types indicating that the concentration and analyte response are linearly correlated (Table 4).

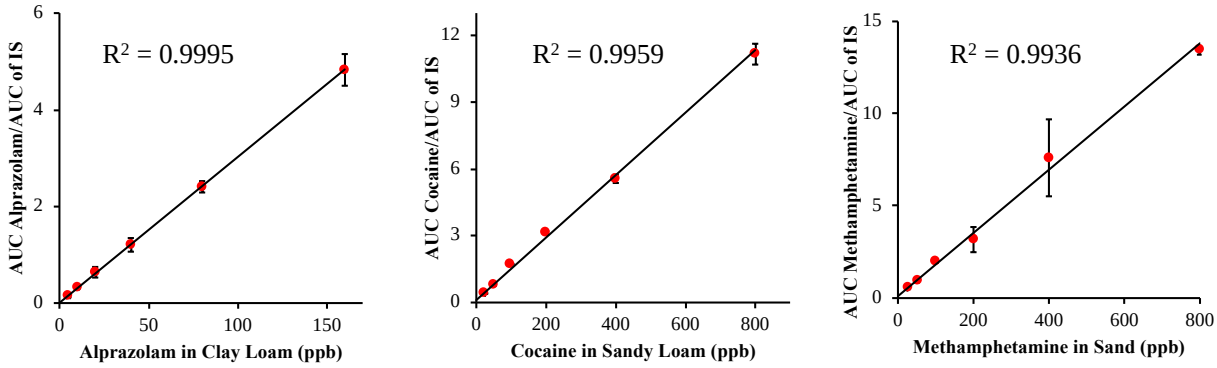


Figure 10. Select calibration curves for pharmaceuticals extracted using SALLE method. The points are an average of three replicates

Table 4. Correlation coefficient and LOD for Drugs in Soil Extracted using the SALLE Method

	Richfield Clay Loam			Sand			Sassafras Sandy Loam		
	LOD (ng/g)	R^2	Relative Slope Error	LOD (ng/g)	R^2	Relative Slope Error	LOD (ng/g)	R^2	Relative Slope Error
Alprazolam	1	0.99	1.1%	3	0.99	3.2%	2	0.99	2.7%
Amitriptyline	15	0.96	9.0%	10	0.98	5.6%	4	0.99	2.1%
Clonazepam	12	0.99	4.5%	10	0.99	4.1%	12	0.99	4.6%
Cocaethylene	38	0.96	8.8%	25	0.98	5.9%	8	0.99	1.9%
Cocaine	35	0.97	8.2%	22	0.98	5.1%	14	0.99	3.2%
Hydrocodone	8	0.99	4.5%	5	0.99	2.8%	10	0.98	5.7%
Ketamine	39	0.99	4.6%	46	0.98	5.4%	24	0.99	2.9%
MDMA	33	0.97	7.8%	25	0.98	5.8%	10	0.99	2.4%
Methamphetamine	23	0.98	5.5%	17	0.99	2.0%	7	0.99	1.7%
Morphine	10	0.99	3.9%	5	0.99	1.8%	13	0.99	5.0%
PCP	4	0.99	2.1%	5	0.99	2.9%	6	0.99	3.7%

Conclusion

In this study, a fast and efficient analysis method was developed for detecting pharmaceuticals and drugs of abuse in soil using paper spray mass spectrometry. Soil samples were added to the solvent well of a paper spray cartridge and analyzed by HR tandem mass spectrometry without additional preparation. This direct extraction method is beneficial because, unlike traditional soil analysis, it did not require any sample preparation prior to analysis. Pharmaceuticals and drugs of abuse were detected in three different soil matrices down to low ppb levels using this method. Using the direct extraction, a stability study was performed to assess the ability to detect pharmaceuticals and drugs of abuse over time. The signals did decrease over time for all of the analytes. This could be due to chemical degradation via oxidation or hydrolysis of the analytes as well as microbial degradation. More testing would be necessary to narrow down the cause of the degradation. In addition to the direct extraction method, we also reported an offline SALLE offline extraction in conjunction with paper spray MS to improve quantitative performance for the detection of pharmaceuticals in soil. This extraction method resulted in greater precision and correlation coefficients greater than 0.96. The paper spray methods outlined in this study have the potential to be utilized rapid screening methods for environmental samples contaminated with pharmaceuticals or drugs of abuse.

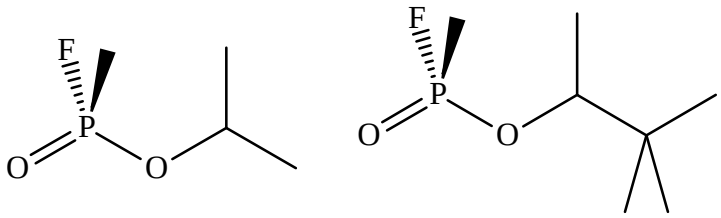
CHAPTER 3. DIRECT SOIL ANALYSIS OF CHEMICAL WARFARE AGENT SIMULANTS AND HYDROLYSIS PRODUCTS IN SOIL

Introduction

Weapons of mass destruction (WMD) are any chemical, biological or radiological substance that is utilized with the sole purpose to inflict substantial harm to life. Chemicals weapons like phosgene, chlorine, and mustard gases were first utilized in modern warfare during World War I[76]. This war is often called the "chemist's war" due to the increased reliance on scientific advances regarding chemical weapons[77]. The massive casualties both on and off of the battlefield sparked the need to control the use of these deadly weapons. Following the 1918 armistice, the Geneva Protocol was enacted, which prohibited "the use in war of asphyxiating, poisonous or other gases, and of bacteriological methods of warfare[76]". Although the Geneva Protocol was signed by 30 countries in 1925, the protocol neglected to include measures prohibiting the stockpiling or synthesis of chemical weapons[78].

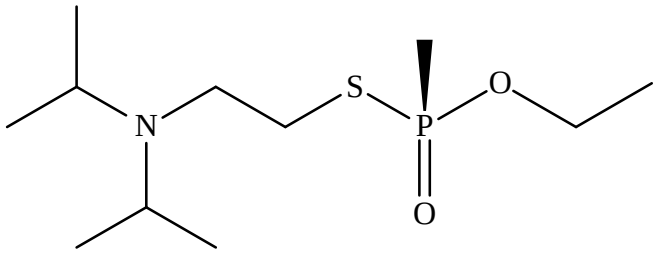
As a result of this omission, many countries continued researching, testing, and stockpiling chemical weapons. During the interwar period between WWI and II, there were significant advances in chemical weapon technology[79]. One of the most significant advances was by the German scientist Otto Bayer, who was researching organophosphorus insecticides. This group of insecticides operate by inhibiting acetylcholinesterase (AChE) activity leading to a build-up of acetylcholine, which can cause death from respiratory failure[80-81]. His work led to the synthesis of the G-series ("German") nerve agents, specifically, sarin and tabun (Figure 11). Although the Germans stockpiled nerve agents during WWII, they were not used on the battlefield. The second, and more dangerous, group of nerve agents, the V-series ("Venemous"), was also discovered when researching insecticides. In 1954, British scientists stumbled on a group of organophosphate esters that had the potential to be used as chemical warfare agents[76]. The G and V-series CWAs differ by their substituent groups. As shown in Figure 11, G-series agents contain a fluorine and the V-series contain a sulfide group respectively.

G Series



sarin soman

V Series



vx

Figure 11. Structures of O-isopropyl methylphosphonofluoridate (sarin), O-Pinacolyl methylphosphonofluoridate (soman) and O-ethyl-S-(2-diisopropylamino)ethyl methylphosphonothiolate (VX)

It was not until the Chemical Weapons Convention (CWC) treaty was signed and ratified in 1992 that countries began making efforts to reduce the stockpiles of chemical weapons[82]. By 2014, 190 countries had signed the treaty[79]. However, there have been reported uses of chemical weapons as recently as 2013-2018 during the Syrian Civil War[83-85]. The use of chemical weapons was confirmed by analyzing both biological and environmental samples, the latter being the focus of the work presented in this chapter. Environmental persistence, degradation and chronic effects of CWAs has been a significant concern in recent years[86-98]. Mass spectral techniques have primarily been utilized for the analysis of chemical warfare agents in both biological and environmental samples[86, 88, 94, 99-100]. Gas chromatography methods are most common due to the volatility of the CWAs, however, these techniques are not amenable to in-field analyses due to the required sample

preparation and vacuum requirements[30]. Organophosphorus chemical warfare agents hydrolyze rapidly in the environment to alkyl methylphosphonic acids (AMPA). The reaction mechanism can be seen in Figure 12. Ideally, a single method would be able to detect not only intact CWA but also hydrolysis products. CWA hydrolysis products are nonvolatile making them less GC-friendly and require a separate analytical technique, such as LC-MS, or derivatization[101]. Derivatization, specifically, increases sample preparation time and has been shown to make quantitation problematic[30]. Recently, ambient ionization techniques have been evaluated for their potential to decrease sample preparation and increase sample throughput. For example, techniques such as DESI[13], DART[102] and PS-MS[30-31] etc. have shown their potential to detect CWAs in complex matrices.

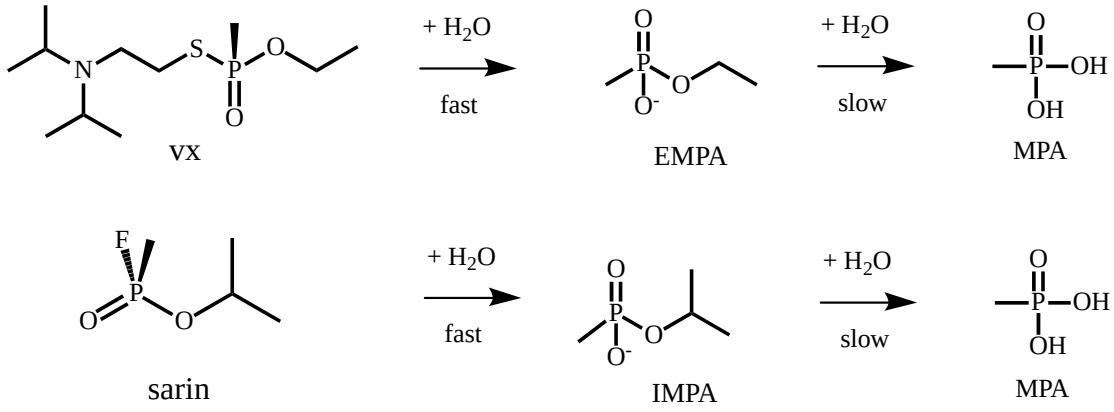


Figure 12. Hydrolysis reaction for VX and sarin. The first hydrolysis reaction occurs quickly creating alkyl methylphosphonic acids. The reaction from AMPAs to methylphosphonic acid is much slower

The work presented herein aims to develop a paper spray mass spectrometry method to analyze both simulants of G-series CWAs as well as the AMPA hydrolysis products. PS-MS was previously applied to the analysis of chemical warfare agent simulants and hydrolysis products in biological matrices. In the work by McKenna et al., chemical warfare agent simulants were detected at single digit ppb concentrations in both blood and urine[30]. The authors also optimized the solvent for negative ionization of the acidic CWA hydrolysis products. Because these hydrolysis products are more likely to form negative $[M-H]^-$ molecular ions, they are more amenable to negative ion mode MS analysis. Negative ion mode (NIM) research has lagged behind its positive counterpart due to its instability. In negative ion mode a negative potential is applied to the sample and the

onset of a phenomena known as corona discharge is much lower in negative ion mode (NIM) versus positive ion mode (PIM)[103]. Corona discharge occurs when gases and solvent molecules are ionized near the electrically charged tip of the ESI source or paper tip in PS-MS[104-105]. The electrical discharge breaks down the Taylor cone and decreases or eliminates analyte signal. The work presented by McKenna et al. optimized a solvent composition that included carbon tetrachloride. By including a chlorine-containing organic solvent as an electron scavenger, the corona discharge decreased for the analytes tested, resulting in a more stable spray[30].

In addition to detecting CWAs and CWA hydrolysis products in biological samples, detection of trace amounts of these compounds in the environment is also important31. The ability to detect the hydrolysis products in soil could provide evidence that chemical warfare agents were stored or deployed in an area[95]. This work describes a paper spray mass spectrometry method for the detection of CWA simulants and the CWA hydrolysis products in soil using PS-MS. In order to analyze both the simulants and hydrolysis products, a dual-polarity ionization method was developed to accommodate the different chemistries of the compounds. Paper spray has proven to be a valuable ambient ionization technique to sample complex matrices after a CWA attack.

Experimental Methods

Chemicals and Materials

HPLC grade methanol and Optima grade ammonium hydroxide were purchased from Fisher Scientific (Hampton, NH, USA). Chemical warfare agent simulants, trimethyl phosphate (TMP), diisopropyl methylphosphonate (DIMP), and dimethyl methylphosphonate (DMMP), as well as, carbon tetrachloride (CCl_4) were purchased from Sigma Aldrich (St. Louis, MO, USA). The isotopically labeled internal standard d_9 – TMP was purchased from Cerilliant (Round Rock, TX, USA) while the $^{13}Cd_3$ - DIMP internal standard was obtained from Dr. Bob Williams and Mark Alvarez at the Los Alamos National Laboratory (Los Alamos, NM, USA). Mixtures of cyclohexyl methylphosphonic acid (CHMPA), ethyl methylphosphonic acid (EMPA), isobutyl methylphosphonic acid (iBuMPA), isopropyl methylphosphonic acid (IMPA), and pinacolyl methylphosphonic

acid (PinMPA) were also purchased from Cerilliant (product number NAx8-CAL). A mixture of the stable isotopically labeled hydrolysis products, $^{13}C_6$-CHMPA, d_5-EMPA, $^{13}Cd_3$-iBuMPA, $^{13}C_3$-IMPA, $^{13}Cd_3$-iBuMPA, and $^{13}C_6$-PinMPA, were also purchased from Cerilliant (product number NAx8-IS). Soil samples were provided by Dr. Simini at the U.S. Army Combat Capabilities Development Command (CCDC) Chemical Biological Center, (Aberdeen Proving Ground, MD, USA). An automated Velox360 paper spray source and compatible cartridges were purchased from Prosolia Incorporated (Indianapolis, IN, USA).

Preparation of Soil Samples

Chemical warfare agent simulant working solutions were prepared in methanol at 50X concentration via serial dilution of stock solutions. Hydrolysis product working solutions were purchased in concentrations of 5000, 2500, 1250, 625, 250, 125, 63, and 25 ng/mL. Soil samples (250 mg) were mixed with water (235 μL) and the working solutions (5 μL of simulant and 10 μL of hydrolysis product working solutions). The soil samples were then allowed to equilibrate for at least 12 hours. Final concentrations of simulants in soil were 3200, 1600, 800, 400, 200, 100, and 50 part per billion of soil. Final soil concentrations of the hydrolysis products were 200, 100, 50, 25, 10, 5, and 1 ppb of soil.

Paper Spray of Chemical Agent Simulants and Hydrolysis Products Directly from Soil

As with the pharmaceutical experiment outlined in Chapter 2, 25 mg of a soil sample was added to the paper spray cartridge solvent well. Two soil types, Richfield clay loam and sand, were utilized in this portion of the study. An aliquot of an IS solution was added on top of the soil. The IS solution consisted of $^{13}C_6$-CHMPA, d_5-EMPA, $^{13}Cd_3$-iBuMPA, $^{13}C_3$-IMPA, $^{13}Cd_3$-iBuMPA, and $^{13}C_6$-PinMPA at a concentration of 125 ng/mL plus 4000 ng/mL of d_9 – TMP and $^{13}Cd_3$ - DIMP in methanol. An extraction/spray solvent of 95:5 methanol:carbon tetrachloride with 0.01% ammonium hydroxide was utilized for these samples. Carbon tetrachloride and ammonium hydroxide were introduced into the solvent composition to reduce the tendency of discharge and promote ion formation in NIM, respectively[30]. The mass spectrometer was operated in the positive ion mode from 0-

0.45 min and the negative ion mode from 0.6-1.25 min (Figure 13) with spray voltage ± 4 kV. In between the polarity change, the voltage was set to 0 kV to allow for automatic integration of the chronograms. Extracted ion MS/MS chronograms for two simulants at 3200 ppb and two hydrolysis products at 200 ppb from soil are shown in Figure 13.

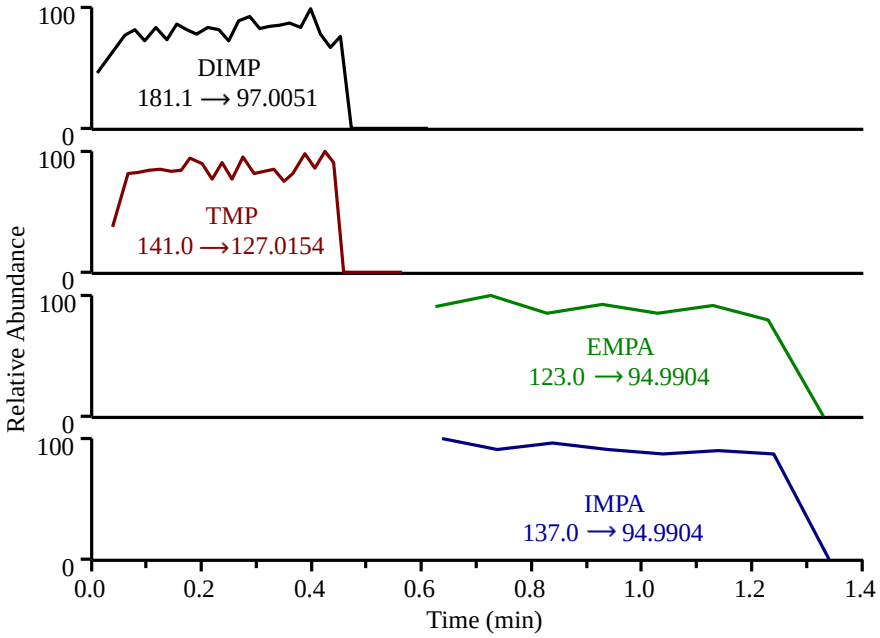


Figure 13. Extracted ion chronogram of DIMP, TMP, EMPA and IMPA at the highest calibrator concentration in Richfield clay loam

Mass Spectrometry and Data Analysis

Samples were analyzed using a Q-Exactive Focus Mass Spectrometer (Thermo Scientific Inc., San Jose, CA) with the ion transfer tube at 320 °C, resolution at 35,000 and the S-lens set to 50.The instrument was operated in MS/MS mode using an inclusion list with an isolation width of ±0.5 *m/z*. The collision energies and fragment ions were optimized for the compounds and can be found in Table 5 .

Table 5. Fragmentation and analysis parameters

Analyte	Description	CE (V)	Transition (*m/z*)	Internal Standard	Conc. Range (ng/g)
TMP $[M+H]^+$	Simulant	15	141.0311→127.0154	TMP-d_9	50-3200
DIMP $[M+H]^+$	Simulant	20	181.0988→97.0051	DIMP[C_{13}]-d_3	50-3200
DIMP $[M+Na]^+$	Simulant	10	203.0807→161.0334	DIMP[C_{13}]-d_3	50-3200
DMMP $[M+H]^+$	Simulant	15	125.0362→111.0205	TMP-d_9	50-3200
EMPA $[M-H]^-$	MPA	12	123.0217→94.9904	EMPA-d_5	1-200
IMPA $[M-H]^-$	MPA	14	137.0373→94.9904	[13C]IMPA-d_3	1-200
iBuMPA $[M-H]^-$	MPA	16	151.0530→94.9904	EMPA-d_5	1-200
CHMPA $[M-H]^-$	MPA	23	177.0686→94.9904	[13C]CHMPA-d_6	1-200
PinMPA $[M-H]^-$	MPA	21	179.0843→94.9904	[13C]PinMPA-d_6	1-200

The instrument was calibrated at least once in a seven-day period in both positive and negative ion modes to prevent drift in the mass spectrometer. In positive ion mode, the instrument was calibrated using the calibration solution purchased from Fisher Scientific (Hampton, NH, USA). The negative ion mode calibration solution did not calibrate the mass spectrometer with sufficient accuracy at lower *m/z* (<100). The calibration solution was therefore spiked with salicylic acid (*m/z* 137.122) and butyric acid (*m/z* 87.106) to improve mass accuracy for the hydrolysis product fragment ions.

Data analysis was carried out using Tracefinder v. 3.3 (Thermo Fisher Scientific) with a 5-ppm mass precision. The calibration curves were fit with a 1/x weighted least square regression. For direct soil analysis, the limits of detection were determined to be the lowest calibrator concentration with a signal to blank ratio greater than 3.

Results and Discussion

Direct Extraction of Chemical Warfare Agent Simulants and Hydrolysis Products

Chemical warfare agent simulants and hydrolysis products were analyzed directly from soil samples without an offline extraction or sample clean-up step. The analytical run was split into two portions: 30 s in PIM for detection of chemical warfare agent simulants which, like CWA, more readily form positive ions and NIM, which was more effective for the organophosphonic acid hydrolysis products. The MS/MS spectra for 2 simulants and 2 hydrolysis products can be seen in Figure 14. The peaks highlighted in red correspond to the fragment ions used for identification of each compound. The *m/z* 97 fragment for DIMP corresponds to the loss of the two alkyl ester groups from the DIMP parent ion, whereas the *m/z* 127 fragment for TMP results from the loss of a methyl group from the precursor ion. Both alkyl methylphosphonic acids shown, EMPA and IMPA, fragment into methylphosphonic acid.

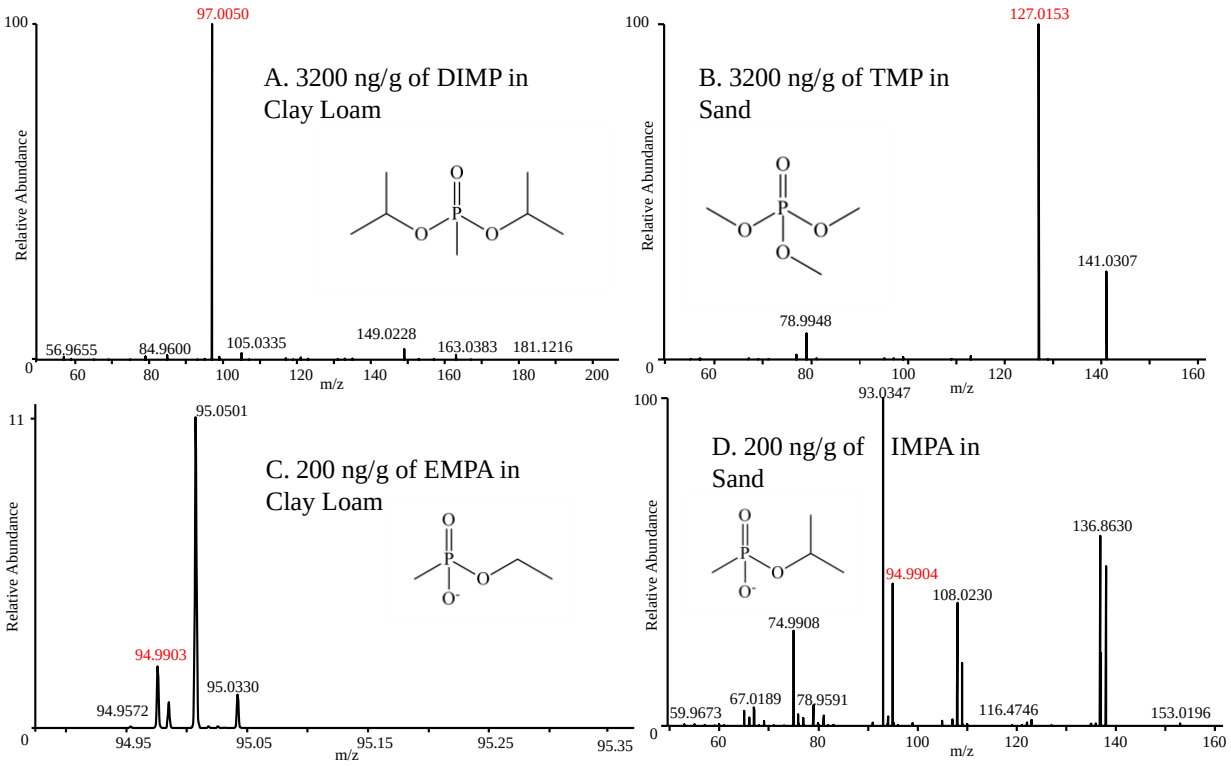


Figure 14. MS/MS spectra for DIMP and EMPA in clay loam and TMP and IMPA in sand

The calibration curves are summarized by their correlation coefficient and relative error of the slope in Table 6; calibration curves for select simulants and hydrolysis products are shown in Figure 15. The detection limits for the simulants were all around 50 ppb in the two soil types tested. The LODs were lower for the hydrolysis products, ranging from 1 to 5 ppb (Table 6). These detection limits are low enough for practical applications. A publication by Baygildiev et al. used a hydrophilic interaction with liquid chromatography–tandem mass spectrometry (LC-MS/MS) to measure the concentrations of various alkyl methylphosphonic acid compounds in soil and dust samples from a former chemical weapons plant, which had been closed and decontaminated at least 25 years prior[95]. IMPA concentrations (when detectable) ranged from 150 to 8720 ppb and PinMPA ranged from 180 to 2780 ppb. iBuMPA was detected in a single sample at 11 ppb. The detection limits obtained for direct soil analysis by paper spray MS are below all of these concentrations.

Table 6. Regression parameters and limits of detection (LOD) for direct analysis of chemical agent simulants and hydrolysis from soil by paper spray MS

	Richfield Clay Loam			Sand		
	LOD (ng/g)	R^2	Relative Slope Error	LOD (ng/g)	R^2	Relative Slope Error
DMMP $[M+H]^+$	50	0.99	3.6%	50	0.97	7.7%
DIMP $[M+H]^+$	50	0.99	4.4%	50	0.95	9.3%
TMP $[M+H]^+$	50	0.99	4.3%	50	0.97	7.0%
CHMPA $[M-H]^-$	1	0.79	22.9%	5	0.88	18.1%
EMPA $[M-H]^-$	5	0.76	28.0%	5	0.69	33.0%
iBuMPA $[M-H]^-$	1	0.77	24.0%	5	0.57	43.3%
IMPA $[M-H]^-$	5	0.83	22.2%	5	0.79	25.6%
PinMPA $[M-H]^-$	1	0.87	17.0%	1	0.89	15.6%

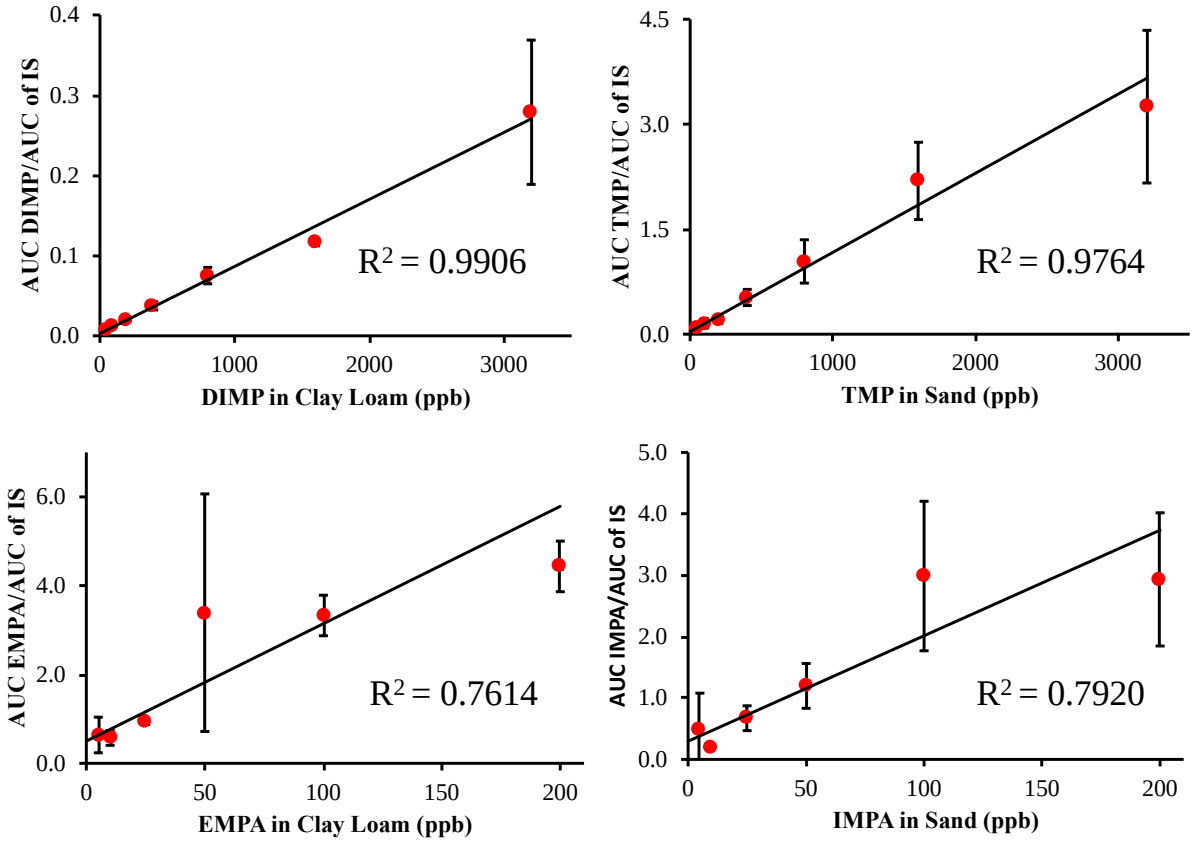


Figure 15. Calibration curves for select simulants and hydrolysis products in soil. The calibration curves have a positive correlation, but the curves are not robust enough for accurate quantitation

Conclusion

In this study, a fast, efficient analysis method was developed for detecting chemical warfare agent simulants and chemical warfare agent hydrolysis products in soil. Soil samples were added to the solvent well of a paper spray cartridge and were analyzed using a high-resolution mass spectrometer. This direct extraction method is beneficial because, unlike traditional soil analysis, it did not require any sample preparation prior to analysis. In addition to the lack of sample preparation required for this method, a dual polarity, targeted screening method was utilized to extract and ionize the simulants and hydrolysis products. Although 1.25 minutes is a typical run-time for paper spray methods, by splitting the method into separate polarities the method is limited by how many MS/MS scans can be accomplished before the switch occurs. There is the potential for improvement, but this

method was designed as a screening technique not a replacement for chromatographic separation requiring offline extraction and sample preparation procedures. This paper spray method has the potential to be utilized a rapid screening method for environmental samples contaminated with chemical warfare agents.

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VITA

Sarah Dowling (née Tockstein) was raised in Belleville, IL. In 2012, she began her collegiate career at the University of Kansas where she won the Owen M. Maloney Scholarship Award for Outstanding First Year Chemistry Students. In 2014, Sarah transferred to Western Kentucky University where she joined Dr. Rajalingam Dakshinamurthy's research group. The project she focused on was the synthesis of antibiotic-conjugated gold nanoparticles to combat multi-drug resistant bacteria. Sarah co-authored three papers with this research group. After graduating summa cum laude with a Bachelor of Science in Chemistry and a Bachelor of Arts in Criminology in 2017, she began graduate work at Indiana University - Purdue University Indianapolis. Sarah completed her Master's thesis research in Dr. Nicholas Manicke's laboratory. Her project focused on the analysis of pharmaceuticals, drugs of abuse, chemical warfare agents and chemical warfare agent hydrolysis products in environmental matrices.