PAPER-BASED AMBIENT IONIZATION MASS SPECTROMETRY TECHNIQUES FOR REGULATORY, ENVIRONMENTAL, AND AGRICULTURAL CHALLENGES

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ABSTRACT

Steven L. Reeber: Paper-Based Ambient Ionization Mass Spectrometry Techniques for Regulatory, Environmental, and Agricultural Challenges
(Under the direction of Gary L. Glish)

Sample collection techniques based on the use of a paper substrate have been used in a variety of applications, perhaps most notably in neonatal screening using dried blood spots. These techniques provide a simple and inexpensive way of collecting material for later chemical analysis. The utility of paper-based sample collection is dramatically enhanced by combining it with ambient ionization techniques for mass spectrometry, generating gas phase ions directly from the paper substrate without the sample preparation and separations typically employed. These ions may then be analyzed by a mass spectrometer to detect the compounds of interest.

In this work, two ambient ionization methods are explored for the ionization of samples collected on paper substrates. Paper spray ionization is an ambient ionization technique in which a spray of charged droplets is generated from a piece of paper cut to a sharp point. A custom paper spray ion source has been designed and built, and used to explore the potential of paper spray ionization-mass spectrometry for pesticide residue analysis applications. Additionally, the first commercial paper spray ion source has recently been released. An evaluation unit was characterized and compared to the custom paper spray ion source. Using this commercial system, automated methods were developed and used for analysis of pesticides in a variety of matrices, including residual impurity analysis of

pesticide formulations. These formulations are highly challenging matrices that typically require sample clean-up and the use of separation techniques; using paper spray ionization a simple dilution in acetonitrile was sufficient to enable analysis.

In addition to paper spray ionization, a novel ionization technique was developed to ionize compounds collected on paper matrices. This technique, nib-based electrospray ionization (nibESI) avoids the need to cut the paper to a sharp point by generating the electrospray from a sharpened fountain pen nib. This technique is characterized and applied to the analysis of therapeutic drugs and nicotine in a variety of different matrices including serum and saliva.

Soli Deo gloria

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	Acetic acid
ABS	Acrylonitrile butadiene styrene (a type of plastic)
ACN	Acetonitrile
API	Atmospheric pressure inlet
BSTFA	N,O-Bistrifluoroacetamide
C	Concentration
CID	Collision-induced dissociation
cm	Centimeter(s)
Conc.	Concentration
Da	Dalton(s) – unit of mass equal to one atomic mass unit
DART	Direct analysis in real time (an ionization technique)
DESI	Desorption electrospray ionization
ESI	Electrospray ionization
eV	Electron-volt(s) (unit of energy)
FT-ICR	Fourier transform-ion cyclotron resonance (a type of mass analyzer)
g	gram(s)
GC	Gas chromatography
GC-MS	Gas chromatography coupled to mass spectrometry
He-Ne	Helium-neon
HPLC	High-performance liquid chromatography
IPA	Isopropyl alcohol
IS	Internal standard

kV	Kilovolt(s)
L	Liter(s)
LC	Liquid chromatography
LC-MS	Liquid chromatography coupled to mass spectrometry
LCD	Liquid crystal display
LTPI	Low temperature plasma ionization
m^2	Square meter(s)
MeOH	Methanol
min	Minute(s)
Mk.	Mark (indicating a design iteration)
mL	Milliliter(s)
mM	Millimoles-per-liter (millimolar)
mm	Millimeter(s)
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MS^3	Three stages of mass analysis, with two rounds of controlled dissociation
m/z	Mass-to-charge ratio, in daltons per elementary charge unit
n	Number of measurements or experiments
nESI	Nanoelectrospray ionization
ng	Nanogram(s)
nibESI	Nib-based electrospray ionization
nM	Nanomoles-per-liter (nanomolar)

PEEK	Polyether ether ketone (a type of plastic)
P/N	Part number
ppb	Parts-per-billion
ppm	Parts-per-million
ppt	Parts-per-trillion
QC	Quality control
RSD	Relative standard deviation
sec	Second(s)
SRM	Selected reaction monitoring
Std. Dev.	Standard deviation
TIC	Total ion current
TMCS	Trimethylchlorosilane
USEPA	United States Environmental Protection Agency
V	Volts
V_{pp}	Peak-to-peak voltage (amplitude of alternating current)
XIC	Extracted ion current
10x	Ten-fold
100x	One hundred-fold
3D	Three dimensional
<	Less than
>	Greater than
#	Number
$	United States dollars (at the time of this writing)

%	Percent (parts-per-hundred)
°C	Degrees Celsius
µL	Microliter(s)
µm	Micrometer(s)
µM	Micromoles-per-liter (micromolar)

CHAPTER 1: PAPER-BASED SAMPLING METHODS FOR ANALYTICAL CHEMISTRY

1.1 Introduction to Paper-Based Sampling Methods

Analytical sample collection techniques based on porous sampling media have a long and varied history, from filtration-based methods to forensic swabs[1,2] to dried blood spots.[3,4] Paper in particular is a useful and inexpensive tool for sample collection, and significant research efforts have been invested in the development of paper-based sampling techniques for a wide range of applications. Perhaps the most familiar of these applications is dried blood spot collection for neonatal screening.[3,4] This technique uses a thick filter paper as a sample collection medium, which is used to collect fresh whole blood from a heel prick. A dried blood spot collection card is shown in Figure 1.1. After drying at room temperature, the samples can be stored for several weeks without notable degradation, and can be shipped much more easily than their equivalent in liquid form.[4,5] This simple, low-cost sample collection method has been a significant element in the development of universal newborn screening for inborn disorders, providing a significant contribution to public health.[6]

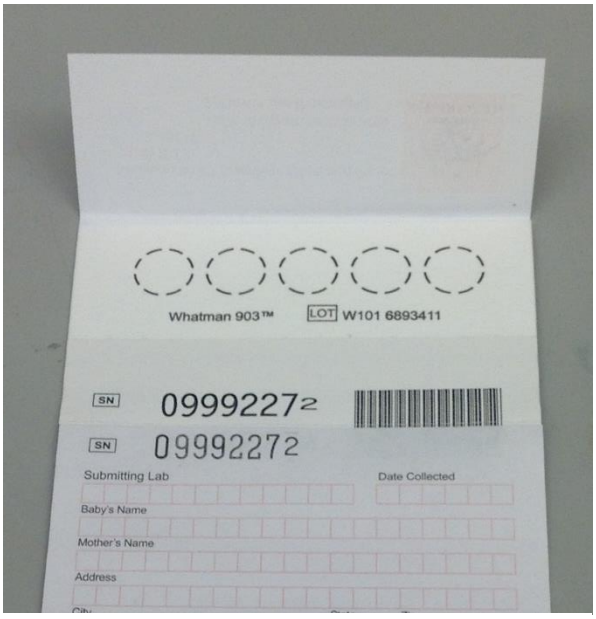

Figure 1.1: A dried blood spot collection card used for newborn screening. Blood is applied to each of the dashed circles and dried.

Novel paper-based sampling tools continue to be developed for applications in this area, such as the Noviplex sample collection card recently released by Novilytic, LLC.[7] These cards build on conventional dried blood spot collection by incorporating a filtering membrane on top of the paper disc on which the sample is collected and dried.[8] This filter membrane is engineered to separate cells from plasma without lysing the red blood cells. Only the plasma is collected on the paper disc; the rest of the material is discarded. Because the number of red blood cells in a sample of blood varies between individuals, the presence of these cells can lead to errors in analytical results when investigating compounds present in plasma. The Noviplex cards avoid this problem by excluding the red blood cells from collection.[7,8]

The vast majority of paper-based sampling applications at present are limited to the areas of clinical assays and

Figure 1.2: Noviplex plasma collection card. Top: The Noviplex card with filter membrane. Bottom: The paper sampling disc with the filter membrane removed.

forensic/security testing. The extension of paper as a sampling tool to other areas thus far has remained of primarily academic interest, and is directly connected to the development of new techniques for the analysis of samples collected on paper. The work presented herein is aimed at the application of paper-based sampling to agricultural and environmental applications in particular, through the development of ambient ionization-mass spectrometry techniques that enable analysis of samples on paper directly, with little to no sample preparation. It is necessary first, however, to consider the established methods used for the analysis of these samples and how they might be improved.

1.2 Conventional Analysis of Samples Collected on Paper

Conventional methods for analyzing samples collected on paper material rely on first removing the sample from the paper substrate, typically by elution/extraction in solvent. Biological samples, such as the dried blood spots used for newborn screening, are generally processed by punching out a section of the spot and incubating it in solvent to extract the compounds of interest.[4,6] Additional sample preparation steps are frequently employed, such as liquid/liquid extraction, solid phase extraction, or derivatization.[4,6]

After any sample preparation, the samples are typically analyzed by mass spectrometry, generally with gas or liquid chromatography (GC or LC) used as a separation technique prior to mass analysis.[4] Chromatography serves to separate the analyte from potentially interfering species in the sample at the cost of increased analysis time and expense. This can be of great importance in complex matrices such as blood or urine, where significant ionization suppression is frequently observed in the absence of a pre-ionization clean-up or separation step.[9–11] Selectivity in conventional assays is derived from the combination of chromatographic separations and tandem mass spectrometry (MS/MS).

Chromatography provides selectivity through reproducible retention times – the analyte consistently requires the same time to elute from the column, and any signal that does not correspond to this retention time may be excluded.

The selectivity of MS/MS derives from the dissociation chemistry of the analyte. An ion is isolated based on its mass-to-charge ratio (*m/z*) and excited, typically through collisions with noble gas atoms.[12,13] The internal energy of the ion is increased in this manner until the ion undergoes unimolecular dissociation to form one or more product ions. The product ion distribution is highly consistent for a given parent ion and internal energy: under the same excitation conditions, the same parent ion should produce the same product ions in the same ratios each time. Selectivity is typically obtained in MS/MS experiments by specifying particular parent→product transitions and monitoring only those during an experiment.[14] This type of experiment is particularly suited to the triple quadrupole mass spectrometer, where the first quadrupole is used to select the parent ion, the second as a collision cell for excitation of the ion, and the third to select the product ion. This operating mode is called "selected reaction monitoring" (SRM), or if the instrument is set to switch between multiple parent→product transitions, "multiple reaction monitoring" (MRM).[14] Similar experiments may be performed using other mass analyzers such as quadrupole ion traps, although in this case the three stages of parent isolation, excitation, and detection of product ions (ion traps do not generally select a particular product ion, but detect all the product ions in the trap) are performed sequentially in a single mass analyzer.

There have been ongoing efforts to reduce the time and expense entailed in the analysis of these samples by various means, including combining experiments into multiplexed assays,[15–18] switching between multiple liquid chromatography columns to

enhance duty cycle,[17,18] and development of automated extraction systems.[19] Multiplexed assay development is the most straightforward approach, measuring multiple analytes through one LC-MS or GC-MS experiment. This can be very effective, but requires that the sample preparation techniques used be suitable for all analytes simultaneously.[15] The use of column switching is essentially a duty cycle improvement.[17] Since the mass spectrometer is generally the most expensive component of the analytical apparatus and the analytes typically elute during only a fraction of the LC run time, an overall improvement in duty cycle may be obtained by using multiple LC columns with staggered start times, switching which column is connected to the mass spectrometer.[17] This does not reduce the consumables cost or sample preparation time per sample, but enables the analysis of more samples in a given amount of mass spectrometer time.

Automated extraction systems are a relatively recent development, used to automate the entire extraction, sample preparation, and analysis process. They have thus far been applied only to dried blood spot analysis. These systems operate by clamping a dried blood spot card between two nozzles and flowing solvent through the card to extract the analyte.[19] The analyte may then be mixed with an internal standard, subjected to other automated sample preparation procedures, and then analyzed by LC-MS.[19] These systems are expensive, but eliminate the hands-on sample preparation used in most other methods.

A more radical approach to reducing the time and expense entailed in the analysis of samples collected on paper substrates is the development of new ionization techniques to directly produce gas phase ions from the paper without separate extraction and sample preparation. This approach has been remarkably fruitful over the past decade, and it is this overall principle that has motivated the work presented here.

1.3 Ionization Techniques for Mass Spectrometry

1.3.1 Conventional Ion Sources

In a typical LC-MS experiment, the analyte is dissolved in a liquid solvent, separated from other species in a liquid chromatography column, and ionized by electrospray ionization (ESI) to yield gas phase ions which are then analyzed in a mass spectrometer. Electrospray ionization was developed in the 1980s and has become the standard atmospheric pressure ionization technique for many analyte classes.[20] The operating principles of electrospray ionization are based on a spray of charged droplets containing molecules of the analyte dissolved in solvent.[14,21] The solution of analyte in solvent is pumped through a conductive capillary with a sharp tip, and a potential difference of several kilovolts is applied between the capillary and the inlet to the mass spectrometer. The electric field is particularly intense at the sharp tip of the capillary. The intense electric field produces a Taylor cone from the liquid flowing out of the capillary. Charged droplets are ejected from the cone and travel towards the inlet of the mass spectrometer, accelerated by the electric field. Most modern ESI sources employ a nebulizing gas to assist in spray formation; this gas flow is directed through a nozzle surrounding the ESI capillary emitter and improves the stability of the electrospray.[14]

The spray of charged droplets is typically generated at ambient pressure. As the charged droplets traverse the distance between the emitter and the mass spectrometer inlet, the solvent in the droplet evaporates.[14] This leaves behind a shrinking droplet with increasing charge density. Eventually, the droplet reaches the Rayleigh limit, the point at which the Coulombic repulsion between charges is equal to the surface tension,[22] and the droplet

ruptures in a “Coulombic explosion.” This rupture forms a number of smaller droplets, which repeat the process. Eventually, the solvent is evaporated entirely, with the remaining charge carriers (in positive ion mode, typically protons, but in some cases metal ions or other adducts) bound to the analyte.[14,21] It is this charged species that is detected by the mass spectrometer.

To enhance the evaporation of solvent, most mass spectrometers employ a flow of heated gas (“desolvation gas” or “dry gas”), a heated inlet system, or both. The Bruker HCTultra, the primary mass spectrometer used in chapters 2, 3, and 6 of this dissertation, uses a flow of heated nitrogen, typically 5 L/min, to both directly aid in desolvation and heat the inlet capillary. Thermo Scientific mass spectrometers, in contrast, typically do not employ a desolvation gas flow and instead directly heat the inlet capillary.

An atmospheric pressure interface of some sort is needed to allow gas phase ions formed by electrospray ionization to enter the vacuum system of the mass spectrometer for mass analysis. For all the instruments used in this work, the inlet system consists of a conductance limit to which a voltage is applied; this may be a cone-shaped metal skimmer, as used in some Waters mass spectrometers, the metallic capillary used in Thermo Scientific instruments, or a resistive or insulating glass capillary with metallized ends, as used in the Bruker HCTultra.

While these inlet systems are generally designed for operation with the commercial electrospray ion sources included with the mass spectrometers, they may be used with a wide variety of ionization techniques that operate at ambient pressure. All that is required to operate with these ion sources is that the safety interlocks preventing operation with the atmospheric pressure source region open be disabled. These instruments may therefore be

easily adapted to use the various ambient ionization techniques developed for analysis of compounds with minimal sample preparation, including those used to directly ionize compounds from paper substrates.

1.3.2 Ambient Ionization

Ambient ionization is a blanket term for a range of methods used to generate gas phase ions for mass spectrometry at ambient conditions (i.e., atmospheric pressure, near-ambient temperature) with minimal or no sample preparation and no pre-ionization separations.[23] A wide range of methods have been developed over the past decade, based on various operating principles such as electrical discharges and plasmas,[24–27] electrospray variants,[28,29] thermal desorption,[25] and acoustic nebulization,[30,31] among others.[23,32] These techniques are used with samples in several forms, such as compounds deposited on a surface,[25,33] dissolved in a liquid, or aerosolized through various means.[34] Relatively few, however, are well suited to analysis of samples absorbed in paper.

Techniques such as desorption electrospray ionization (DESI) or low temperature plasma ionization (LTPI) which are primarily used for surface analysis are most effective when analytes are present on a relatively non-porous surface, rather than absorbed in a porous substrate. Similarly, ambient ionization methods like “direct analysis in real time” (DART) that rely on thermal desorption to volatilize analytes prior to ionization in the gas phase are not as effective for analytes absorbed in porous matrices as for samples on non-porous surfaces (e.g., glass capillary surface, wire mesh). Preliminary experiments investigating the ionization of samples collected on paper substrates using DESI, LTPI, and DART met with little success, as the available analyte at the surface of the paper is rapidly depleted. Ambient ionization of samples absorbed in paper matrices is better accomplished

through techniques dedicated to these matrices, particularly two methods: paper spray ionization and extraction spray ionization.

1.3.3 Ambient Ionization from Paper Matrices

Paper spray and extraction spray ionization are both variants of electrospray ionization in which the analyte is introduced on a paper substrate. Extraction spray ionization employs a drawn glass capillary like those commonly used for nanoelectrospray ionization (nESI), into which a small section of paper (to which the analyte has been applied) is inserted.[35,36] When the capillary is filled with solvent, the analyte is extracted from the paper and is then ionized when an electrospray is generated from the tip of the drawn glass capillary through application of a suitable voltage. This technique has several key advantages – it may be used with any sort of paper that can be cut to fit into the capillary, the paper is thoroughly wetted with solvent, and the drawn glass capillary is a well-defined and easy to work with spray emitter – and it has been used with several analytes in matrices of varying complexity, from water to blood.[36] However, the fact that this technique requires the insertion of the paper substrate into a narrow capillary is a significant drawback. Either the paper used for sample collection must be quite small, which limits the amount of sample that may be applied to it, or it must be cut to fit, which causes sample loss and may introduce error due to variability in cutting. For applications where significant sample volume is limited, such as blood and most biological samples, this may not present significant limitations, but in non-sample-limited applications it may constrain the volume of sample that can be used, limiting the ability of this technique to detect low concentration species.

A more straightforward alternative for the analysis of samples dried on paper is paper spray ionization, which ionizes compounds directly from a paper substrate. Paper spray

ionization employs the substrate itself as a spray emitter – the paper is cut to a sharply pointed tip and wetted, and an electrospray is generated from the pointed tip upon application of a potential difference of several kilovolts (kV) between the paper and a counterelectrode.[29,37] The analyte is extracted from the paper into the solvent and ionized through an electrospray process.[38] This has the advantage of being able to use larger paper substrates than can be used in extraction spray ionization, although the substrate material selection is constrained to those papers that can be reproducibly cut to form sharp tips.[29,38] The tip sharpness is essential in paper spray ionization as the intense electric fields required to generate an electrospray are generally only practical at a sharp point. A more complete description of the mechanism of paper spray ionization is included in Chapter 2.

Paper spray ionization was developed in the Ouyang and Cooks research groups at Purdue University, and first described in the peer-reviewed literature in 2010.[29,37] Since then it has been applied to a wide range of applications, from protein analysis[39,40] to forensics,[41–43] and the ionization mechanism and effects of paper geometry have been characterized.[38] Much of the work with paper spray ionization has focused on analysis of biological samples, especially for potential clinical applications, building on conventional dried blood spot sampling.[44–46] Applications in this area include detection of drugs in blood and saliva,[41,44,47] direct analysis of tissue sections,[48] and measurement of acylcarnitines in urine, serum, and whole blood.[46,49] Work with other sample types includes forensic analysis of inks to detect forged documents,[43] detection of quaternary ammonium salts used as corrosion inhibitors in oils,[50] and measurement of cocaine residues on surfaces using paper swabs.[42]

1.4 Environmental and Agricultural Applications of Paper-Based Sampling

One area that has seen relatively limited development of paper based sampling techniques coupled to ambient ionization is agrochemical and environmental analysis. A common herbicide used in the United States, atrazine, has been used in several cases as a test analyte in water matrices,[29,35] and several studies have investigated the use of paper spray for measurement of various contaminants and endogenous species in foods,[51–56] but there has been no systematic effort to apply paper-based sampling to routine agrochemical measurements, particularly with samples collected and dried on paper for transportation.

Atrazine was among the first test analytes used with paper spray ionization, with reasonably good results (1 ng/mL limit of detection), but was only tested in solution (using high purity solvents), not as a dried sample collected on paper or in environmental matrices.[29] Similar experiments were performed with atrazine in river water and thiabendazole (a fungicide) in orange homogenate using extraction spray ionization, but again, no quantitative methods were developed for these analytes.[35] The most comprehensive attempt to demonstrate the potential of paper spray for measurement of pesticides was performed using both a surface collection approach, in which the surface of a fruit or vegetable was wiped with the paper, and direct analysis of food homogenates.[53] This study investigated five pesticides, and obtained workable limits of detection, but as with the above two studies there was no attempt to develop a functional quantitative method.[53]

Quantitative experiments have been performed using paper spray ionization for analysis of foodstuffs, but have been focused on detecting contamination or adulteration rather than routine regulatory analysis. Experiments in this area include the measurement of Sudan azo dyes in powdered chili pepper,[51] 4-methylimidazole in beverages and caramel,[54]

clenbuterol in meat,[52] and melamine in milk.[52] With the exception of the study of azo dyes in pepper,[51] these experiments were conducted using samples applied to paper and immediately ionized, so that the paper served primarily as a support substrate rather than a sample collection medium.[52,54]

1.5 Summary and Outline

Paper is an ideal sampling medium for many applications, and has been used successfully in the clinical environment for many years. The development of ambient ionization methods has enabled the mass spectrometric analysis of samples directly from paper media, without separate extraction and separation steps. These techniques have been applied to a wide range of samples, but there remains significant room for development, especially in the area of agrochemical analysis for regulatory and quality control purposes.

There has been little investigation of the potential for use of paper as a collection tool for environmental samples in a manner analogous to dried blood spot collection. This is one of several applications of paper-based sampling coupled to ambient ionization explored in this work. Other topics investigated include the direct analysis of agrochemical formulations for detection of trace cross-contamination by paper spray ionization and measurement of therapeutic drugs and nicotine in biofluids using a novel ion source based on a nib-shaped structure coupled to paper sampling media.

One of the major impediments to the use of paper spray ionization for many applications is the need to construct a custom ion source. While a basic paper spray source can be as simple as a metal clip connected to a high voltage power supply, a somewhat greater investment in terms of design time and manufacturing cost is necessary to achieve a reliable instrument. The design and characterization of such a paper spray ion source is

described in Chapter 2, and its use for the measurement of herbicides in environmental matrices such as water, soil extracts, and crop extracts is detailed in Chapter 3.

The first general-purpose commercial paper spray ion source, the Prosolia Velox 360, has recently been released. The ability to use paper spray ionization without the need to first construct and validate a custom ion source may make paper spray ionization a much more attractive tool for application-focused researchers. The operation of this ion source, its performance with agrochemicals in environmental matrices, and the procedures for performing automated paper spray experiments and data analysis are covered in Chapter 4, along with a comparison to the custom paper spray ion source described in Chapter 2.

Chapter 5 describes the use of the commercial paper spray source for the analysis of pesticide formulations. These formulations are a significant challenge for the analytical chemist, as they typically contain high concentrations of surfactants and other ingredients that can interfere with conventional LC-MS analysis, as well as very high concentrations (>10% by mass) of the active ingredients. This renders detection of cross-contaminants at part-per-million levels very difficult, and most analytical methods described in the peer-reviewed literature have focused on ensuring the correct amount of active ingredient is present, not on the detection of trace cross-contaminants. Paper spray is relatively insensitive to particulates and other components of many formulations that would interfere with conventional ESI, and can be used for the analysis of formulations samples with a minimum of sample preparation (generally dilution in a suitable organic solvent, such as acetonitrile).

Paper spray is a powerful tool for the analysis of samples collected on paper substrates, but it does impose some constraints, particularly in the geometry of the paper used. To achieve stable spray, the paper must be cut to a sharp point, which is susceptible to

damage and can entail some loss of material if the paper is cut after sample application. A novel ion source, based on a fountain pen nib, is described in Chapter 6. This ionization technique, nib-based electrospray ionization, or "nibESI," generates an electrospray from a sharpened fountain pen nib. Paper or other porous material to which the sample has been applied is mounted atop the nib, and the sample is eluted from the paper when solvent is applied. This ion source is tested using the recently developed Noviplex plasma sampling cards described at the beginning of this chapter. These cards collect a sample on a small paper disc, which would be challenging to analyze by paper spray ionization due to its small size and circular shape, requiring cutting to a sharp point, with intrinsic loss of material. Analysis by nibESI does not require cutting or reshaping the paper, avoiding the constraints imposed by paper spray ionization.

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CHAPTER 2: DESIGN AND CHARACTERIZATION OF A PAPER SPRAY ION SOURCE

2.1 Introduction

To investigate and develop ambient ionization techniques for environmental and regulatory applications, it was necessary to first obtain a suitable ion source. At the time these experiments were begun no commercialized paper spray ion source was available. It was therefore necessary to design and build a custom paper spray ion source. The development and characterization of the custom paper spray ion source (which differs substantially from the commercial system recently released by Prosolia, Inc.)[1,2] is described herein, along with a summary of the operating principles of this ionization technique. This custom paper spray ion source was developed to explore the use of paper spray ionization-mass spectrometry for measurement of agrochemicals in environmental and agricultural matrices; the specifics of this application are described in detail in Chapter 3. In keeping with this proposed application, the test analytes used with the custom paper spray ion source were primarily agrochemicals.

The custom ion source was developed in an iterative fashion, beginning with an extremely simple device and adapting it to address shortcomings and add desired features. The development and characterization of this system has progressed through three distinct generations, which will be referred to as Mark 1 (Mk. 1), Mk. 2, and Mk. 3. Although there are a number of differences between them in terms of features, construction, and

reproducibility, each generation of the ion source was based on the same fundamental design principles and ionization mechanism.

2.1.1 General Paper Spray Ion Source Design

Paper spray ionization functions by generating a spray of charged droplets from a paper substrate which has been cut to a sharp point.[3] The earliest paper spray ion sources consisted of only a metal clip in which a small, typically triangular piece of paper with a sharp point could be held.[3,4] A high voltage was applied to the paper via the clip, and a small volume of solvent (ranging from 5 to 100 µL, depending on paper size) was applied to the paper.[3,4] For applications such as dried blood spot analysis, a more complex ion source design was developed, employing a disposable plastic cartridge to hold the paper substrate.[5] Use of a disposable cartridge allows a greater degree of automation and easier manipulation of the paper substrate prior to analysis.[2]

Although a variety of source designs have been developed for paper spray,[3,5–7] the core elements have remained fundamentally unchanged. There must be a structure to hold the paper substrate in place in front of the mass spectrometer, which may be fixed or adjustable.[3,5] An electrode is required to apply a high voltage to the paper substrate.[8] Finally, a controlled volume of solvent must be applied to the substrate to generate a spray of droplets.[7] The paper substrate itself may also be considered a part of the ion source, as it serves as the emitter from which charged droplets are generated and the physical parameters of the paper (e.g., sharpness, tendency to fray, absorbency) can have significant effects on the intensity of the ion signal observed.[9]

2.1.2 Paper Spray Ionization Mechanism

Paper spray ionization is fundamentally an electrospray process, in which a spray of charged droplets containing the compound to be ionized are generated via application of an intense electric field to a solution of the analyte.[4,10,11] Electrospray ionization (ESI) has been thoroughly characterized over the past thirty years, and the overall mechanism in paper spray is essentially the same.[9,11] In conventional ESI, solvent containing dissolved analyte is pumped through a capillary or needle which is positioned near the inlet of a mass spectrometer. A potential difference is applied between the capillary and the inlet. At the tip of the capillary the electric field is very intense, producing forces sufficient to generate a spray of charged droplets, which is sustained by continuous pumping. In many ESI sources this is assisted by a coaxial flow of inert gas which provides additional pneumatic nebulization.[11] The primary difference between conventional electrospray techniques and paper spray ionization lies in the use of a porous substrate fed by capillary action as a spray tip for the ion source, rather than a tubular capillary.[4,9]

Paper spray is derived from an earlier variant of electrospray ionization which employed a porous wick as a substrate through which solvent and analyte travel and from which they are electrosprayed.[12] Like its predecessor, paper spray relies solely on the electric field to generate a spray of charged droplets.[9,12] In these techniques, rather than being pumped through a capillary, solvent travels through the porous substrate via capillary action. A high voltage (typically several kilovolts) is applied to the wetted substrate, producing a potential difference between the substrate and the inlet as for conventional ESI. To achieve a sufficiently intense electric field at the tip of the porous substrate to induce electrospray, a sharp point must be present.[9] In paper spray ionization this is typically done by cutting the

paper substrate to yield a pointed shape, such as a triangle or sharp-tipped teardrop. The sharpest corner of the shape is directed towards the inlet to the mass spectrometer. It is essential that the substrate be free of extraneous sharp points or fibers, as these may produce additional, uncontrolled jets of droplets resulting in signal loss.[5,9]

In paper spray ionization the solvent carrying the dissolved analyte wicks forward to the tip of the paper substrate and is sprayed as a jet of small charged droplets. The analyte may be either applied in the spray solvent and analyzed immediately, or applied to the substrate separately. In the second case the analyte is extracted from the substrate into the spray solvent and then ionized, while in the first case the analyte is already in the spray solvent. The abundance of solvent at the tip is a critical parameter for ensuring efficient paper spray ionization.[9] If the tip is too wet, then the droplets will tend to increase in size and behave unpredictably. In some cases, droplets may be too large to be effectively evaporated by the time they enter the mass spectrometer, interfering with detection of the ions of interest. Excessive solvent can also produce dripping from the ion source resulting in sample loss. Alternatively, insufficient solvent prevents the formation of a stable spray jet.[9] Generally, when insufficient solvent is present for electrospray to occur, no signal is observed. On occasion, however, a corona discharge may occur under these conditions, producing a somewhat different mass spectrum. Field ionization has also been proposed as a mechanism for the ionization observed to occur in some cases under low solvent conditions.[9]

2.2 Materials and Operating Parameters

2.2.1 Chemicals and Materials

All herbicide samples used as test analytes were provided by Syngenta Crop Protection, LLC (Greensboro, NC). Isotopically labeled atrazine (ethyl-d_5) was purchased

from C/D/N Isotopes (Pointe-Claire, QC, Canada). Isotopically labeled metolachlor (propyl-d_6) was purchased from Crescent Chemical (Islandia, NY). Solvents, such as acetonitrile (ACN) and methanol (MeOH), and additives such as acetic acid (AA) were purchased from Fisher Scientific (Fair Lawn, NJ). Unless otherwise stated, LC-MS grade solvents were used (Fisherbrand Optima). All papers used as substrates for paper spray ionization were Whatman brand filter papers, purchased from GE Healthcare Life Sciences (Little Chalfont, UK).

2.2.2 Instrument Parameters

All experiments described in this chapter were performed using a Bruker HCTultra ion trap mass spectrometer with the default electrospray emitter and housing removed, unless otherwise stated. The inlet system of this instrument consists of a resistive glass inlet capillary with metallized ends, which serves as the conductance limit between atmosphere and the vacuum system. The end of the inlet capillary is covered by a stainless steel spray shield. The high voltage used for electrospray ionization is applied to the spray shield and the end of the inlet capillary. Desolvation of ions from spray-based ionization techniques is aided by a flow of heated nitrogen (100-300 °C) between the inlet capillary and spray shield.

Instrument parameters were set using the automated optimization tool included with the instrument control software. All parameters were optimized for each analyte. The potential difference used for paper spray ionization was set through application of two

Table 2.1: Typical instrument settings for analysis of atrazine and metolachlor.

Parameter	Setting
Spray Voltage	3000 V
Inlet Capillary	-3833 V
Capillary Exit	158.3 V
Skimmer	46.2 V
Octopole 1 dc	17.26 V
Octopole 2 dc	6.06 V
Octopole RF Amplitude	179.2 Vpp
Lens 1	-9.0 V
Lens 2	-74.5 V
Trap Drive	44.1

voltages – a fixed high voltage, typically 3 kV, applied to the ion source using an external power supply, and a voltage of the opposite polarity applied to the inlet capillary using the internal instrument power supply, optimized using the automated tool. This was selected primarily for convenience in operation, as higher overall potential differences could be achieved in this manner than were possible through use of either power supply alone. Typical operating parameters for atrazine and metolachlor are shown in Table 2.1. All experiments described in this chapter were performed in positive ion mode.

2.3 Paper Spray Ion Source Development

Unsurprisingly, given how recently it was first described,[3,4] paper spray has only recently been commercialized in any functional form.[1,2] To investigate the potential of this technique for regulatory and environmental applications it was therefore necessary to design and construct a custom ion source. The key design criteria for this source were as follows. First, the source must be a flexible testbed for assessment of the effectiveness of a variety of paper types, solvents, and analytes. This ruled out the use of pre-loaded cartridges (difficulty of reconfiguring and cost of manufacture) and mandated the use of durable and solvent-tolerant plastics and metals in the design. Secondly, the source should be a modular and easily transported unit, compatible with a wide variety of mass spectrometers, particularly the Bruker HCTultra used for the bulk of the experiments with this source. Finally, the source should be easy to clean and robust, while still enabling fine adjustment in position as needed.

2.3.1 Mark 1 Ion Source

The custom paper spray ion source design was developed in an iterative process through several generations of functioning systems. The initial design (Mk. 1, shown in Figure 2.1) consisted of an alligator clip attached to the end of an insulated cable. The cable

was mounted in a plastic holder attached to a three axis translation stage. The paper substrate was cut as desired using scissors or a razor blade and inserted into the alligator clip. Solvent was applied manually using an autopipette. The spray voltage was applied using a modular high voltage power supply connected to the insulated cable. This design is extremely simple and inexpensive to produce, and could be easily mated with most mass spectrometers equipped with an atmospheric pressure inlet.

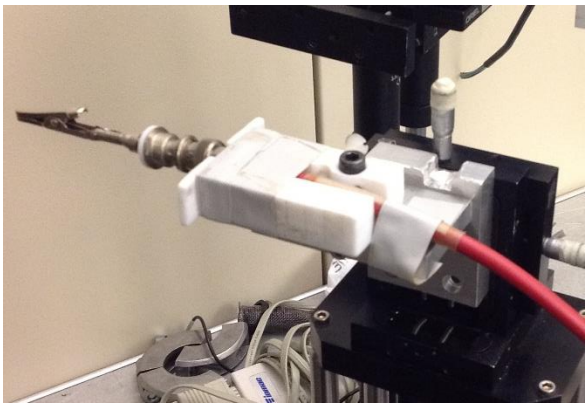

Figure 2.1: The Mk. 1 paper spray ion source.

Figure 2.2: Substrate cutting schematic for Mk. 1 and Mk. 2 ion sources. Sample was applied at the center of the dashed circle. Paper was cut along red triangle.

One of the first experiments performed with the Mk. 1 ion source demonstrated the limitations of this initial design and the need for modification. In this experiment, 50 µL aliquots of a 10 mM solution of atrazine (an herbicide commonly used in the United States) in ethanol (LC-grade) were applied to a Whatman #903 dried blood spot collection card and dried at room temperature. Triangular sections approximately isosceles in shape (1 cm base, 1 cm height) were cut from each sample spot (depicted in Figure 2.2) and inserted in the Mk. 1 paper spray ion source for analysis (the dashed circle in Figure 2.2 represents the approximate extent of the sample spot on the paper – a portion of the sample was not cut out). Spray solvent (99/1 MeOH/AA) was applied in 100 µL aliquots using an autopipette. The resulting total ion current (TIC) and extracted ion current (XIC) (protonated atrazine, *m/z* 216) traces for a single spot analyzed with two desolvation gas settings are shown in Figure 2.3. The data shown in panel A were

collected with a desolvation gas flow rate of 5 L/min, while the data in panel B were collected with a desolvation gas flow rate of 1 L/min. The desolvation gas temperature in both cases was set to 300°C. An aliquot of solvent was applied prior to each of the spikes in signal intensity observed (7 aliquots applied at 2 minute intervals for panel A, 5 aliquots applied at 6-7 minute intervals for panel B; one paper section used for each pane).

Clearly, in both cases significant atrazine remained on the substrate after the first aliquot of solvent was exhausted. Additionally, the significant increase in signal duration at the lower desolvation gas flow rate suggests that the primary reason for solvent exhaustion is evaporation, not consumption through spray from the tip. While the signal duration observed

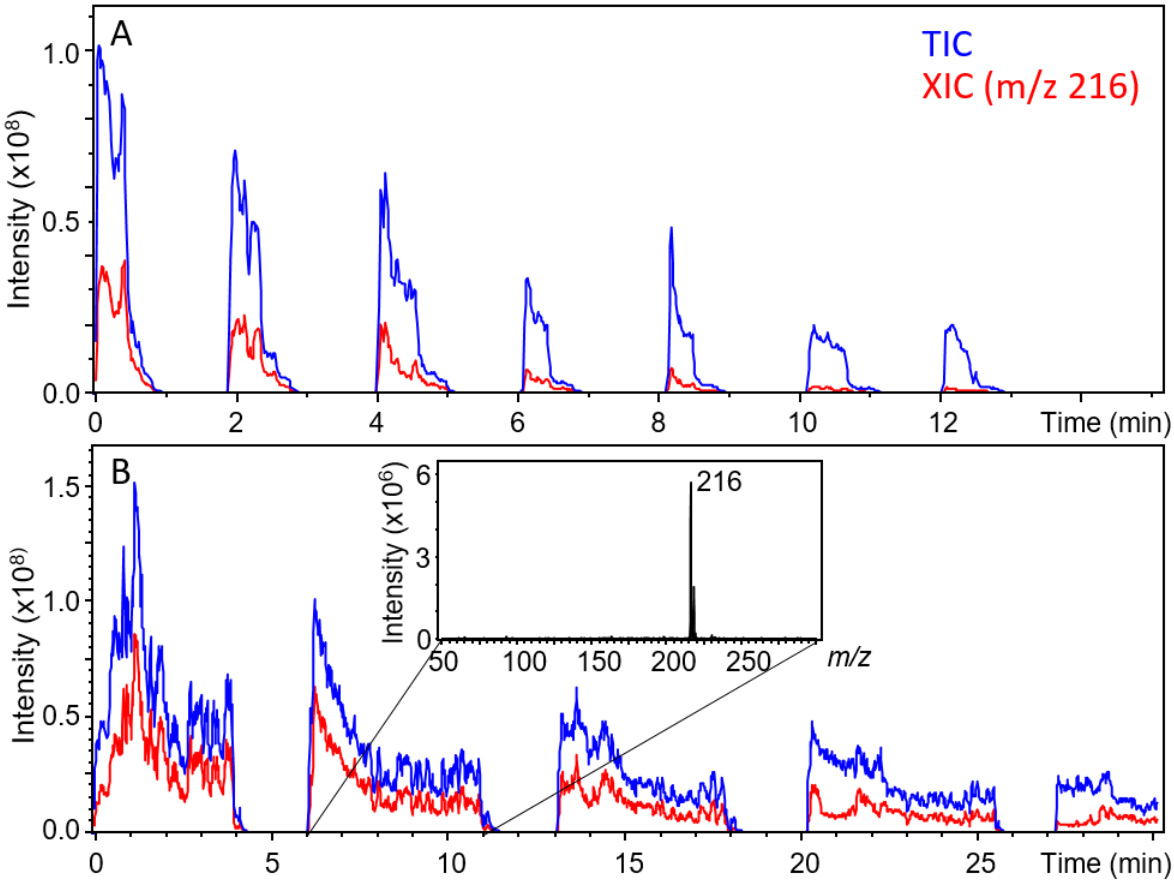

Figure 2.3: Total ion current and extracted ion current (protonated atrazine, m/z 216) using the Mk. 1 ion source. A: 5 L/min desolvation gas flow. B: 1 L/min desolvation gas flow. Inset: Averaged mass spectrum (6-11.1 min) showing the protonated atrazine peak.

with this source configuration was adequate for measurement, it was insufficient for rigorous instrument parameter optimization, which can take several minutes and requires consistent generation of ions. The ion source was therefore modified to address this problem, yielding the Mk. 2 paper spray ion source.

2.3.2 Mark 2 Ion Source

The second iteration of the ion source design, depicted in Figure 2.4, was based on the same alligator clip and mounting assembly as the Mk. 1 source. The alligator clip was modified to include a section of PEEK capillary tubing connected to a syringe pump. The tubing was attached to the upper (movable) jaw of the alligator clip using stainless steel ferrules and a wire wrapping which was soldered in place. When the paper substrate was inserted into the alligator clip, the end of the PEEK tubing was positioned directly above the back edge of the paper. A constant flow of solvent could thus be delivered using the syringe pump, maintaining a stable quantity of solvent on the substrate. This source design was expected to provide a more consistent signal without the need for manual solvent replenishment.

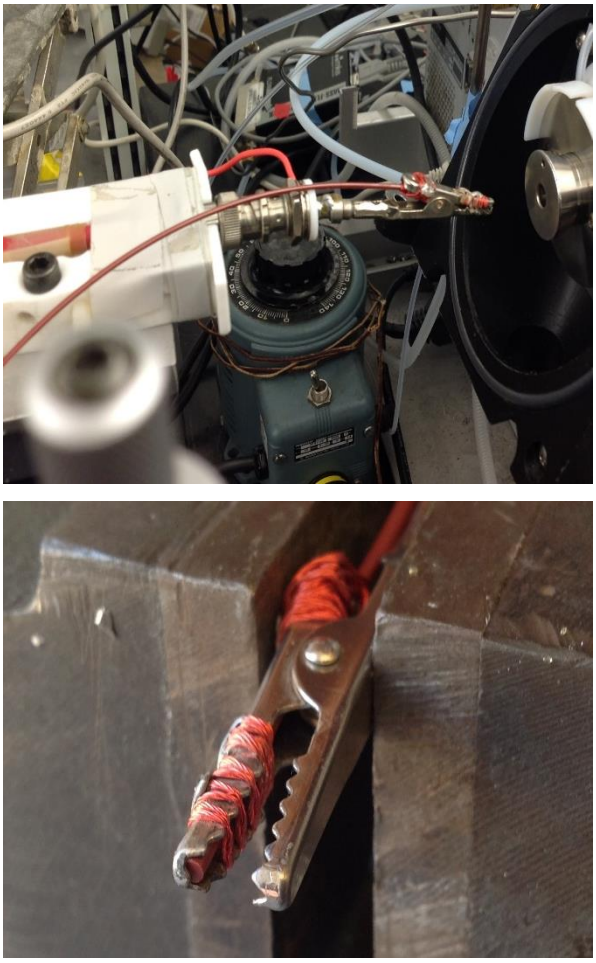

Figure 2.4: The Mk. 2 paper spray source. Top: The source in its mounting assembly, positioned in front of a mass spectrometer. Bottom: Detail of the alligator clip showing the position of the PEEK tubing.

2.3.3 Characterization of the Mark 2 Source

The Mk. 2 ion source was initially evaluated using samples of atrazine (1 mM in liquid chromatography grade ethanol) applied to #903 cards in 50 µL aliquots, dried, and cut as described above. The section cut from the card was inserted into the Mk. 2 ion source and analyzed using a Bruker Esquire 3000 ion trap mass spectrometer, an instrument nearly identical to the Bruker HCTultra mass spectrometer used for all other experiments. The desolvation gas flow at the Esquire 3000 inlet was set to 1 L/min and a temperature of 300 °C. Spray solvent consisting of 99/1 MeOH/AA was applied at a rate of 13.3 µL/min using a syringe pump connected to the PEEK tubing. 50 µL of solvent was applied initially to wet the paper using an autopipette. The total ion current trace and the extracted ion current trace for protonated atrazine (*m/z* 216) are shown in Figure 2.5, along with a representative mass spectrum from mid-run. Protonated atrazine could be consistently detected for fifteen

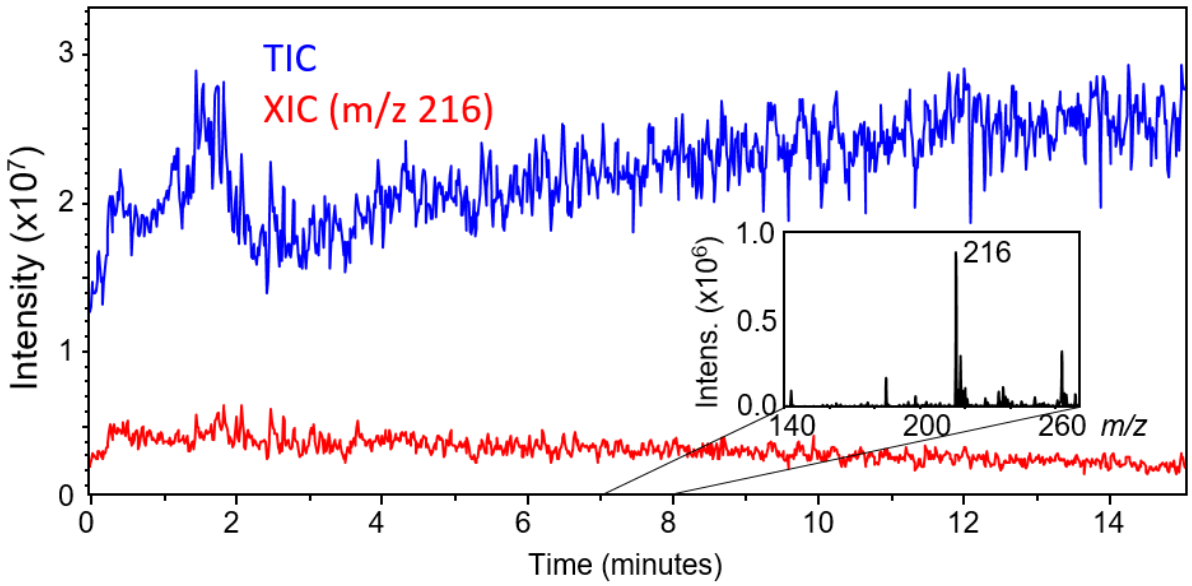

Figure 2.5: Total ion current and extracted ion current (protonated atrazine, m/z 216) traces for a sample of 1 mM atrazine applied to a #903 paper substrate and analyzed using the Mk. 2 paper spray ion source coupled to a Bruker Esquire mass spectrometer. Inset: Mass spectrum averaged from 7 to 8 minutes, showing the protonated atrazine peak.

minutes. The atrazine signal intensity is observed to decay slowly over the course of the analysis as the analyte is depleted.

The effect of a variety of spray solvents on signal intensity was investigated using the Mk. 2 ion source in an effort to achieve the lowest possible limit of detection. The primary test analyte employed for these experiments was atrazine, although metolachlor, another herbicide, was also used in some cases. Initial experiments were performed using 99/1 MeOH/AA, with ACS Certified grade solvents. A dramatic reduction in background ions was observed on switching to LC-MS grade solvents (Fisher "Optima" grade), which were used for all subsequent experiments. A further improvement was observed when acetonitrile was used in place of methanol. Figure 2.6 depicts the improvement observed; in identical samples of 1 ppm metolachlor in a wheat forage extract, one analyzed by paper spray using 99/1 MeOH/AA and one using 99/1 ACN/AA, the signal intensity of protonated metolachlor (*m/z* 284) is increased threefold when acetonitrile is used, while the absolute intensity of background ions did not increase, which is particularly advantageous in experiments using mass analyzers with a limited charge capacity, such as the ion trap mass spectrometer used here. LC-MS grade acetonitrile was therefore used as the primary solvent for all further experiments.

Mixtures of water and organic solvents were also tested, but were not observed to provide a significant improvement over simply 99/1 ACN/AA. In general, an organic fraction of at least 50% was required to achieve reliable paper spray ionization. This is consistent with previously published results for both paper spray and other electrospray based techniques, as the high surface tension of water impedes the formation of a spray of droplets. Paper spray experiments using solvents with a high aqueous fraction were observed to

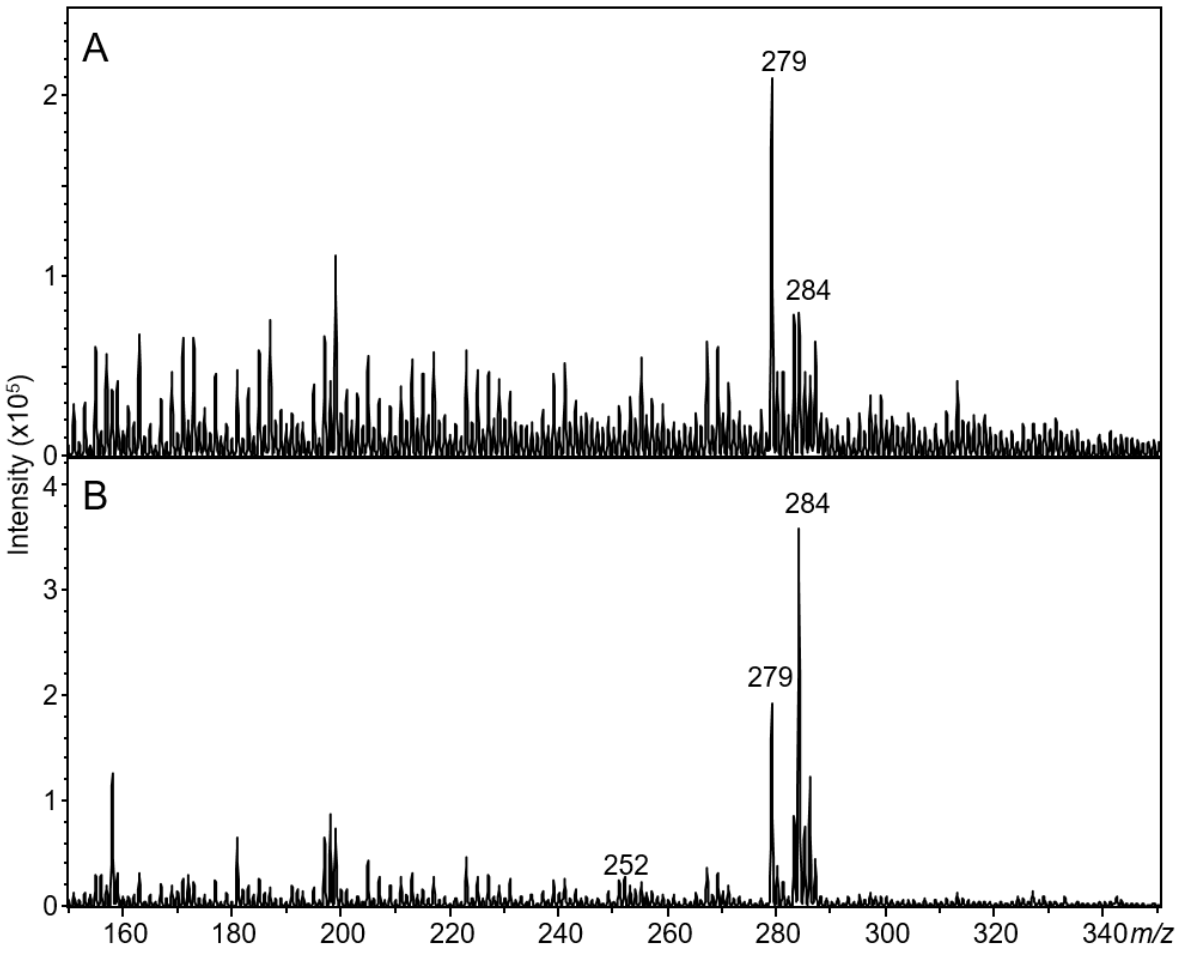

Figure 2.6: Paper spray ionization mass spectra of 1 ppm metolachlor in wheat forage extract. Protonated metolachlor is observed at m/z 284.
A: Using 99/1 methanol/acetic acid as spray solvent.
B: Using 99/1 acetonitrile/acetic acid as spray solvent.

produce large droplets which did not evaporate effectively and increased rates of electrical discharge (including arcing, which can damage the mass spectrometer).

While switching to LC-MS grade solvents yielded a significant reduction in background ions, and use of acetonitrile rather than methanol improved atrazine signal intensity remarkably, background ions were still observed. One possible source of background species is the substrate itself, which may have residual compounds present from manufacturing and packaging. Washing of the substrate prior to sample application was investigated as a potential solution to this problem. Washing procedures were tested using the

Mk. 2 paper spray ion source with #903 dried blood spot collection cards. Cards were held in a wire clip and rinsed three times on each side with methanol (approximately 1 mL per rinse). After drying at room temperature, 50 µL aliquots of an atrazine sample (50 µM in methanol) were applied and allowed to dry. Sections were then cut from the card as described above and inserted in the Mk. 2 ion source for paper spray ionization. Mass spectra of identical samples applied to washed and unwashed cards are shown in Figure 2.7. An approximately three-fold improvement in protonated atrazine signal intensity is observed, in addition to a dramatic reduction in the intensity of background species. Given the

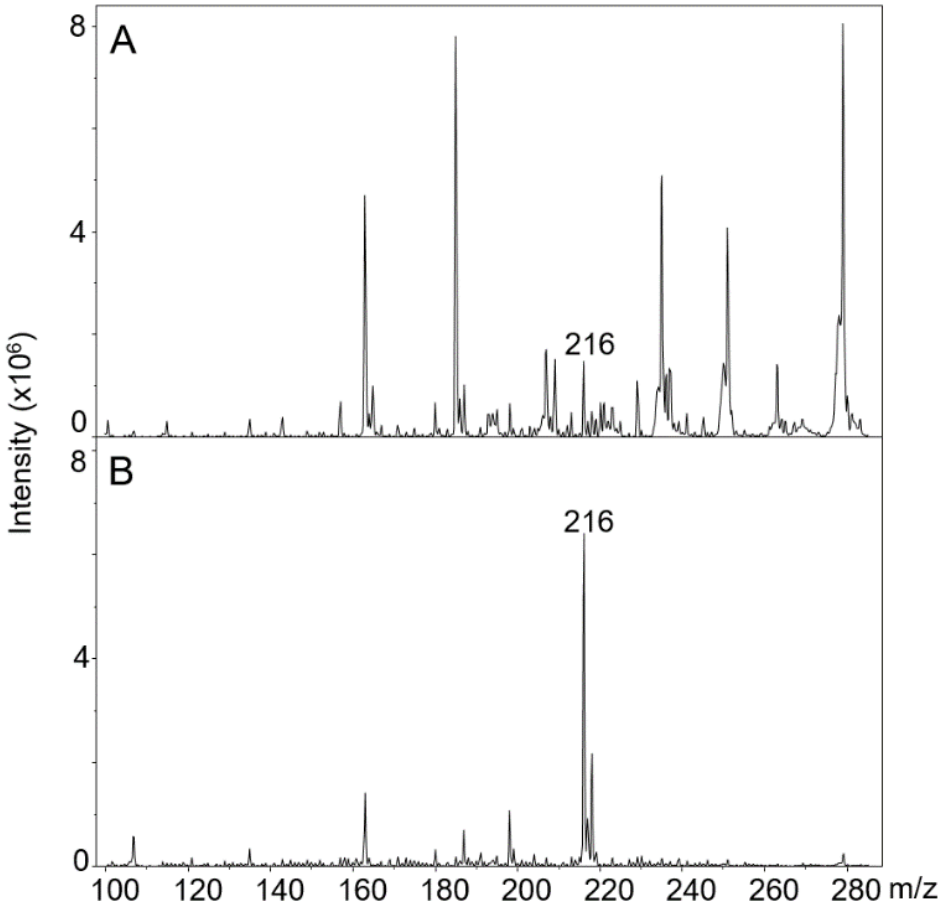

Figure 2.7: Paper spray ionization mass spectra of 50 µM atrazine samples analyzed using the Mk. 2 ion source. A: Sample applied to unwashed card B: Sample applied to card washed with spray solvent.

significance of the improvement observed, pre-washing substrates was adopted as a standard procedure for future experiments.

The Mk. 2 design represented a significant improvement over the Mk. 1 due to the ability to maintain signal consistently for an extended period of time. However, both alligator clip-based designs tended to warp the paper substrate due to uneven application of pressure, and it was very difficult to position the ion source reproducibly. Additionally, the alligator clip was extremely difficult to clean and gradually became corroded due to contact with the acetic acid used in the spray solvent to improve ionization efficiency. To address this issue a more systematic overhaul of the ion source design was undertaken.

2.3.4 Mark 3 Ion Source

The Mk. 3 paper spray ion source, shown in Figure 2.8, is designed for increased reproducibility, ease of cleaning, and more consistent pressure distribution across the paper substrate, while retaining the continuous solvent application features from the earlier designs. This device consists of two aluminum plates which are held together with a wire clip. The paper substrate is inserted between the two plates. The lower plate is notched to provide reproducible positioning, and both plates have a “U” shaped cutout to provide maximum support for the substrate while minimizing contact surface area. The lower plate is mounted in a plastic holder and held in place with a steel screw. The holder is in turn mounted on a three axis micrometer translation stage which is attached to a steel plate. The assembly, as shown in Figure 2.8, is positioned in front of the mass spectrometer on the instrument table and held in place with a simple C-clamp.

The requisite voltage for paper spray ionization is applied via a cable terminated in an alligator clip, which is clipped onto the exposed shaft of the screw (part e in Figure 2.8). A

modified stainless steel needle (Hamilton P/N 7780-04, large hub removable 22 gauge needle, point style 3) is affixed to the upper plate with epoxy and used to apply spray solvent to the paper substrate during ionization. One end of the needle (part g in Figure 2.8) is bent down through the U-shaped cutout in the upper plate so that the tip is in contact with the back of the paper substrate. The other end is connected to a syringe pump via a PEEK line. The desired solvent flow rate may be set at the syringe pump, and is typically in the range of 15-30 µL per minute, depending on the solvent composition and the temperature and flow rate of the desolvation gas.

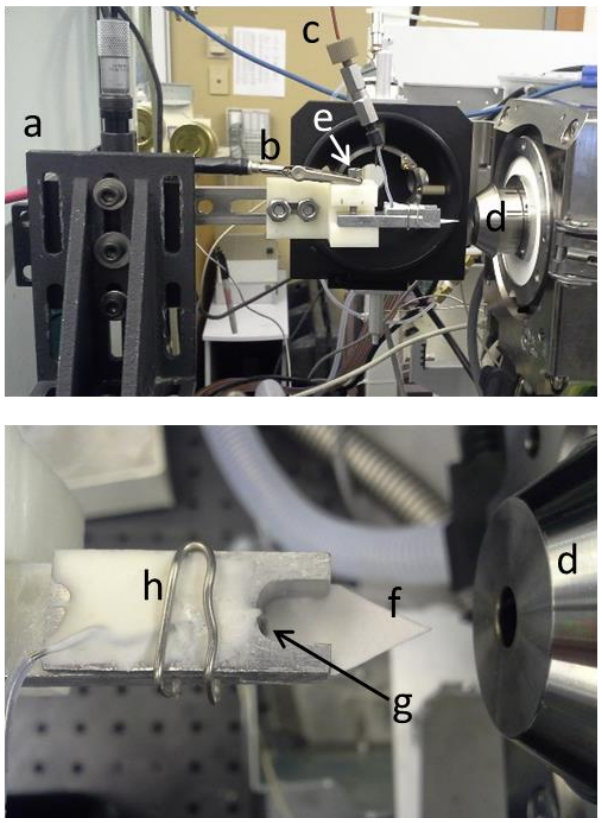

Figure 2.8: The Mk. 3 ion source.
Top: The fully assembled ion source with substrate positioned at the HCTultra inlet.
Bottom: Detail of the sample holder section.
Parts: a) 3-axis micrometer translation mount; b) High voltage contact; c) Solvent line; d) MS inlet; e) Mounting screw; f) Paper substrate; g) Solvent delivery needle; h) Wire clip.

The typical substrate geometry employed with the Mk. 3 paper spray source is an irregular pentagonal shape, which is depicted in Figure 2.9. The flat back edge allows for reproducible positioning between the two metal plates, and all corners except the sharp tip are covered by the plates, preventing undesirable spray jet formation. The corners of the plates themselves are rounded to avoid spray if they should become wetted. The substrate is prepared from sheets of paper (usually filter paper), cut using a razor blade and a template. Typically, the substrate is cut to form a 4 cm long strip, which is hung in a wire rack with the

pointed tip down. At this stage, the substrate may be washed, if desired, or sample may be applied immediately. Samples to be analyzed by paper spray ionization-mass spectrometry are applied using an autopipette, spotted between the corners of the triangular tapered section at the point marked "x" in Figure 2.9. Samples are dried hanging from the wire racks at ambient conditions and then cut 2 cm from the point (along the dashed line in Figure 2.9). The pointed section is inserted into the ion source for analysis, with the flat back edge positioned along the back of the holder and the sharp end pointing forward, towards the mass spectrometer.

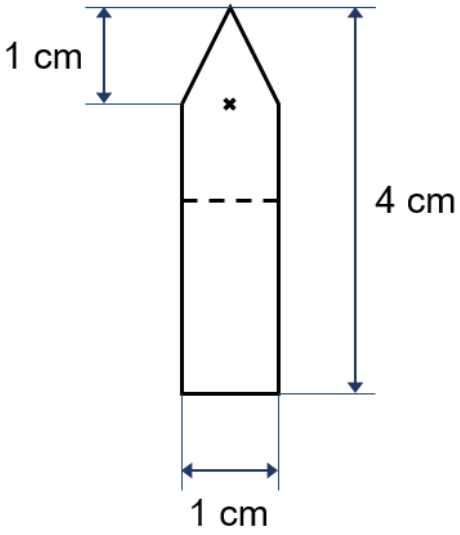

Figure 2.9: The paper spray substrate geometry used with the Mk. 3 ion source. The sample is applied at the point marked "x" in the diagram.

2.3.5 Characterization of the Mk. 3 Source

Visual inspection of paper strips inserted in the Mk. 3 ion source confirms that the substrate does not generally droop or warp when wetted. The source is straightforward to clean; for general cleaning it can simply be wiped with a tissue soaked in solvent, or soaked and sonicated for a more thorough cleansing. The primary problem remaining is the durability of the epoxy used to mount the solvent delivery needle to the upper plate of the sample holder. A variety of adhesives have been tested, with mixed results. Torr Seal vacuum epoxy (Varian/Agilent Technologies, Santa Clara, CA) was used initially, and yielded a very rigid epoxy bead, but became brittle and friable after repeated exposure to solvent. A more flexible and less expensive alternative is J-B KwikWeld epoxy (J-B Weld Co., Sulphur Springs, TX), which produces a very firm bond with the aluminum plate. This

epoxy is reasonably resistant to solvents, but is still subject to degradation if exposed repeatedly. Metal, plastic, or fabric tapes are not recommended as they tend to leave a residue and can contaminate the sample if in contact with solvent. Solder or brazing would be the ideal long-term solution, although they can be difficult when working with stainless steel and aluminum, especially with thin-walled tubes that can collapse when they become malleable at high temperatures.

The Mk. 3 paper spray ion source was initially tested with high concentrations of atrazine in LC-MS grade methanol. A mass spectrum of one of these samples (100 ppm atrazine in methanol) is shown in Figure 2.10, along with a MS/MS spectrum of protonated atrazine from the same sample. The product ions observed in MS/MS of protonated atrazine are consistent with the loss of one or both alkyl side chains from the secondary amines present in atrazine. Atrazine signal intensity was observed to be similar to that observed with the Mk. 2 ion source, but it was much easier to optimize the position and swap samples without disturbing the sample holder position using the Mk. 3 ion source.

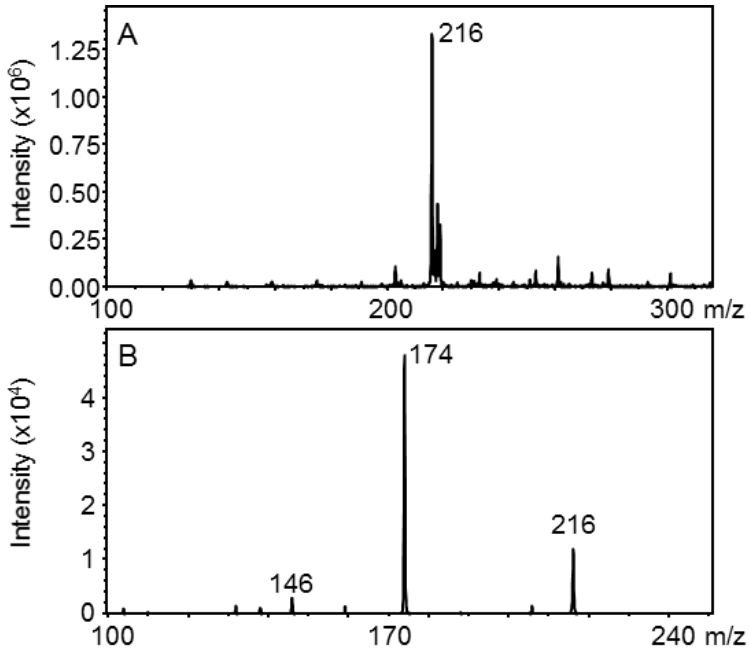

Figure 2.10: A) Mass spectrum of atrazine (100 ppm in MeOH) analyzed using the Mk. 3 ion source. B) MS/MS of the protonated atrazine ion from the same sample. The dominant product (m/z 174) arises from the loss of propylene, with a minor product (m/z 146) arising from loss of both propylene and ethylene.

Optimal spray position varied between samples due to the intrinsic irreproducibility of

the manual cutting method used to prepare the paper strips. In general, the best results are observed with the tip of the paper 5-10 mm from the mass spectrometer inlet capillary, centered horizontally on the inlet and slightly (about 1 mm) above or below center. At tip-inlet distances less than 5 mm, an electrical discharge can occur between the paper and the inlet, which can alter the ions observed and damage the substrate or mass spectrometer. Additionally, the evaporation of solvent due to the desolvation gas flow from the inlet is greater at short tip-inlet distances, requiring a higher solvent flow rate to compensate. At tip-inlet distances greater than 10 mm, signal may become intermittent or fail completely. Spray can typically be obtained at distances up to about 30 mm, but requires a significantly higher spray voltage and generally does not yield as stable ion signal as distances in the 5-10 mm range.

Given that samples are applied to paper substrates and dried prior to analysis by paper spray ionization, it is possible to apply multiple samples containing different compounds separately to a single strip. The effect of sequential analyte application was assessed using atrazine and a deuterated analog, atrazine-d_5 (ethyl-d_5). Three solutions were prepared: 1 ppm atrazine in acetonitrile, 1 ppm atrazine-d_5 in acetonitrile, and 1 ppm each atrazine and atrazine-d_5 in acetonitrile. Each solution was applied to washed #903 paper strips (100 µL aliquots) and allowed to dry. The strips were then analyzed by paper spray ionization-mass spectrometry, yielding the results shown in Figure 2.11. A clear pattern is observed, with the signal intensity of the protonated molecules being approximately equal when the two are mixed in the same solution, but differing dramatically when applied sequentially. The species applied second is consistently observed with a higher signal intensity than the species applied first. This behavior is observed regardless of sample matrix, and is contrary to some

previously reported results,[13] although the experimental conditions are significantly different (agrochemicals vs. pharmaceuticals, with a 50-fold greater sample volume for agrochemicals). It is worth noting that the signal intensity of the species applied first is not suppressed relative to the single solution case, rather, the signal intensity of the species applied second is enhanced by a factor of 1.5-2. The cause of this effect is not known at this time, but may be due to the second species preferentially depositing on the surface of the paper rather than deeper within the substrate.

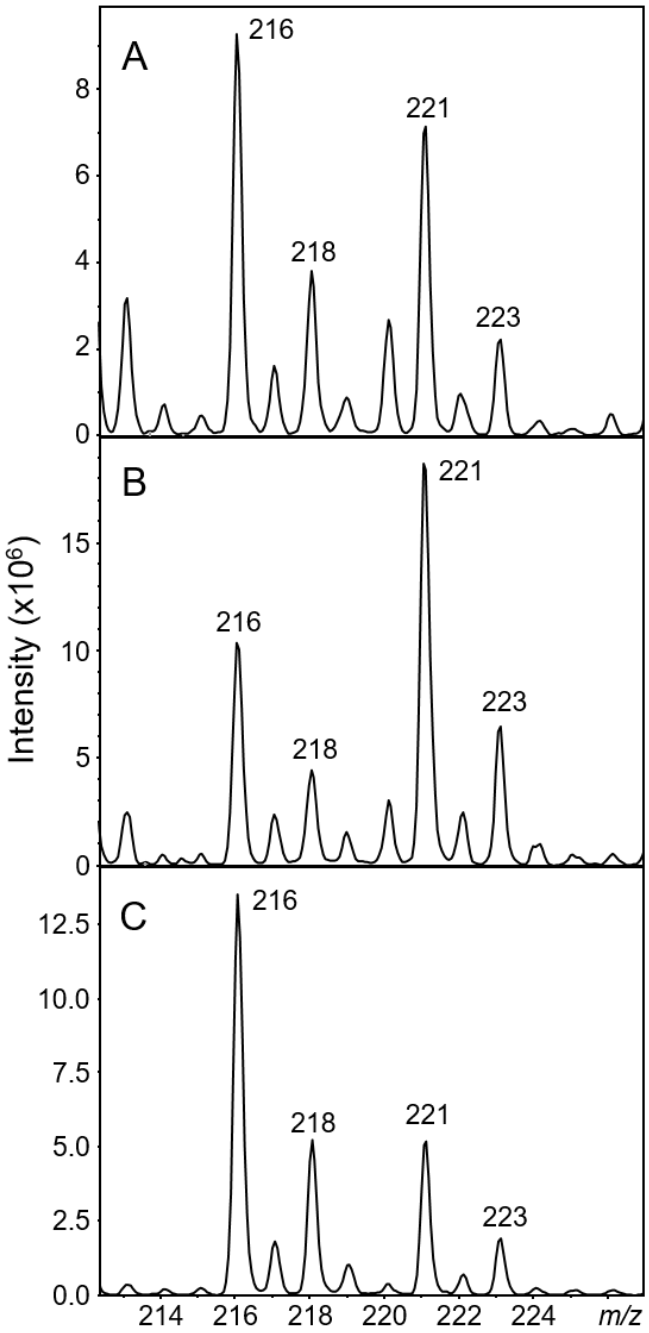

Figure 2.11: Paper spray mass spectra of A) atrazine (m/z 216) and atrazine-d_5 (m/z 221) applied in one solution, B) atrazine applied first, followed by atrazine-d_5, and C) atrazine-d_5 applied first, followed by atrazine. All spectra are zoomed in on the atrazine and atrazine-d_5 peaks.

2.3.6 Substrate Characterization

Initial experiments (using the Mk. 1 ion source) were performed using Whatman #903 dried blood spot collection paper. This paper, or similar grades of paper designed for the same purpose, have been used extensively in paper spray ionization. A range of other filter paper grades were investigated with the Mk. 2 ion source

to determine the ideal qualities of a paper spray substrate and select the most suitable material for the applications of interest (principally agrochemical measurement, described in detail in Ch. 3). Papers investigated varied in thickness, porosity, and hardness, and included Whatman #1 chromatography paper and #3, #4, #40, #41, #43, and #598 filter papers as well as #903 dried blood spot paper. No significant variation in the ions detected was observed using different paper grades, but some grades of paper were observed to yield higher overall signal intensity, and a stable ion signal was more easily obtained from some papers. The papers yielding the best overall performance were #598 filter paper and #903 dried blood spot paper.

The primary parameters determining the suitability of a paper grade for paper spray ionization are durability, sample capacity, and tip quality. The durability of the paper substrate is critical for paper spray ionization for two reasons; firstly, it reduces the likelihood of the paper sagging or otherwise becoming distorted due to soaking with spray solvent. Secondly, it reduces the chance of damage when transporting samples, which is crucial for applications involving sample collection in the field. Relatively thin, dense papers such as Whatman #40 (210 µm thick, 95 g/m^2),[14] or thicker papers such as #598 (320 µm thick, 140 g/m^2)[15] and #903 (500 µm thick) are generally more durable than lighter and more porous paper grades. The importance of sample capacity is application-dependent. Thinner, less absorbent papers may be suitable for sample-limited applications, while more absorbent papers such as Whatman #903 and #598 enable the application of larger volumes in cases where sample is abundant. Use of greater sample volumes was observed to dramatically increase signal intensity.

The quality of the substrate tip may be generally defined in terms of sharpness and ease of cutting. Most papers can be cut to yield a sufficiently sharp point to enable paper spray, but many, especially softer and more inhomogeneous papers, tend to yield a somewhat "fuzzy" tip, with many small fibers extending from the cut edges. Use of a more advanced cutting technique, such as a computer-controlled laser cutter, may ameliorate this tendency, but the cost of using such equipment was prohibitive. Harder and thinner papers tended to yield the highest quality tips. The best performing papers of those investigated were #598 and #903. The #903 dried blood spot paper was selected for experiments with the Mk. 2 and Mk. 3 ion sources due to its combination of excellent durability and sample capacity with acceptable tip sharpness. The #598 filter paper yielded a sharper tip and similar durability, but with significantly reduced sample capacity.

Oxidation and trimethylsilylation were also investigated as potential substrate preparation techniques. Oxidative treatment of paper substrates for paper spray ionization has been previously reported to yield significantly reduced background signal.[16] Strips of #903 paper were immersed in a solution of 0.1% nitric acid in water (16 mM HNO_3), covered, and placed in a 45°C water bath for 3 hours. The nitric acid solution was poured off and the strips were washed six times with water and allowed to dry at room temperature overnight (adapted from Su, *et al*).[16] A comparison of the strips treated with nitric acid and untreated strips washed with acetonitrile is shown in Figure 2.12. When used for paper spray ionization-mass spectrometry analysis of 100 ppb solutions of atrazine and metolachlor in acetonitrile with the Mk. 3 ion source, little to no reduction in background ion signal was observed, and only a moderate increase in the signal intensity of the protonated analytes. Given the increase in

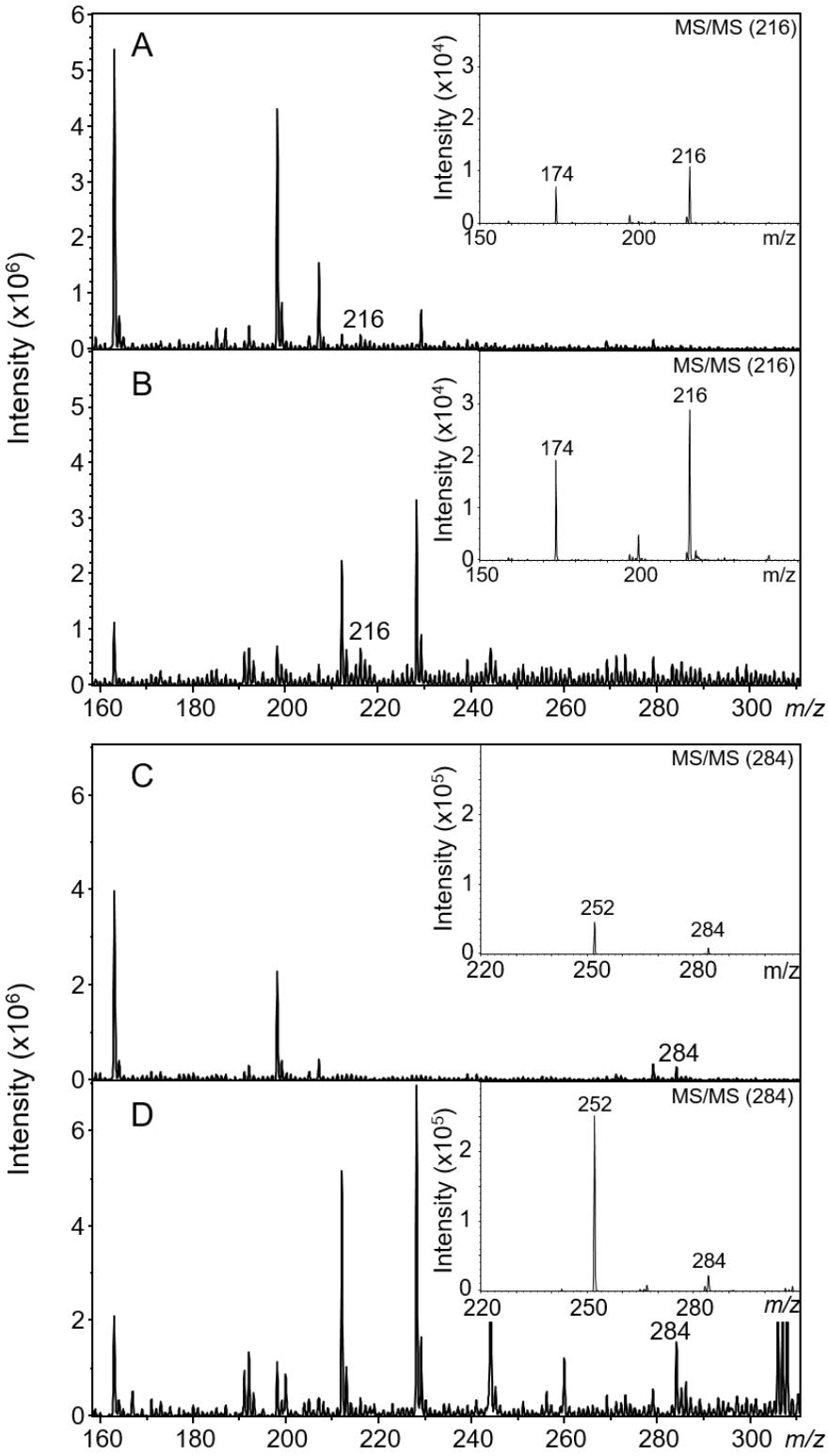

Figure 2.12: Paper spray mass spectra of atrazine (A and B) and metolachlor (C and D) applied to untreated (A and C) and nitric acid treated (B and D) paper substrates with inset MS/MS spectra.

complexity and lengthy reaction process required, treatment of substrate with nitric acid was not pursued further.

Trimethylsilylation of the paper substrate was investigated in an effort to control the binding of analyte molecules to the cellulose matrix. 10 strips of #598 paper were cut and weighed. Assuming the paper to be composed of 100% cellulose, the number of moles of glucose monomers in the sample was calculated. 4 molar equivalents of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (containing 1% trimethylchlorosilane [TMCS]) and 10 mL pyridine were placed in a beaker with the paper strips and covered with parafilm. The reaction mixture was placed in a 40°C water bath and allowed to react overnight (approximately 12 hours). The remaining liquid was removed and the strips were washed three times with acetonitrile (approximately 40 mL/wash). The strips were then allowed to dry overnight on a wire rack. Treatment of the strips with BSTFA produced a significant increase in rigidity and a slight increase in thickness, while rendering the surface of the paper somewhat hydrophobic. There was no notable degradation of tip quality based on visual observation.

Paper spray ionization-mass spectra of atrazine/metolachlor/propazine samples on treated and control strips are shown in Figure 2.13. A moderate increase in signal intensity is observed for all analytes, most notably metolachlor. Background signal is not significantly reduced. Due to the hydrophobicity of the treated paper, alternative solvent mixtures containing less polar components were investigated. The best results were observed for 80/20/0.1 ACN/acetone/AA, which yielded approximately a 5-fold increase in signal intensity when used with both treated and untreated paper. However, the increased volatility of this solvent blend requires an increase in solvent flow rate (>30 μL/min) for sustained use.

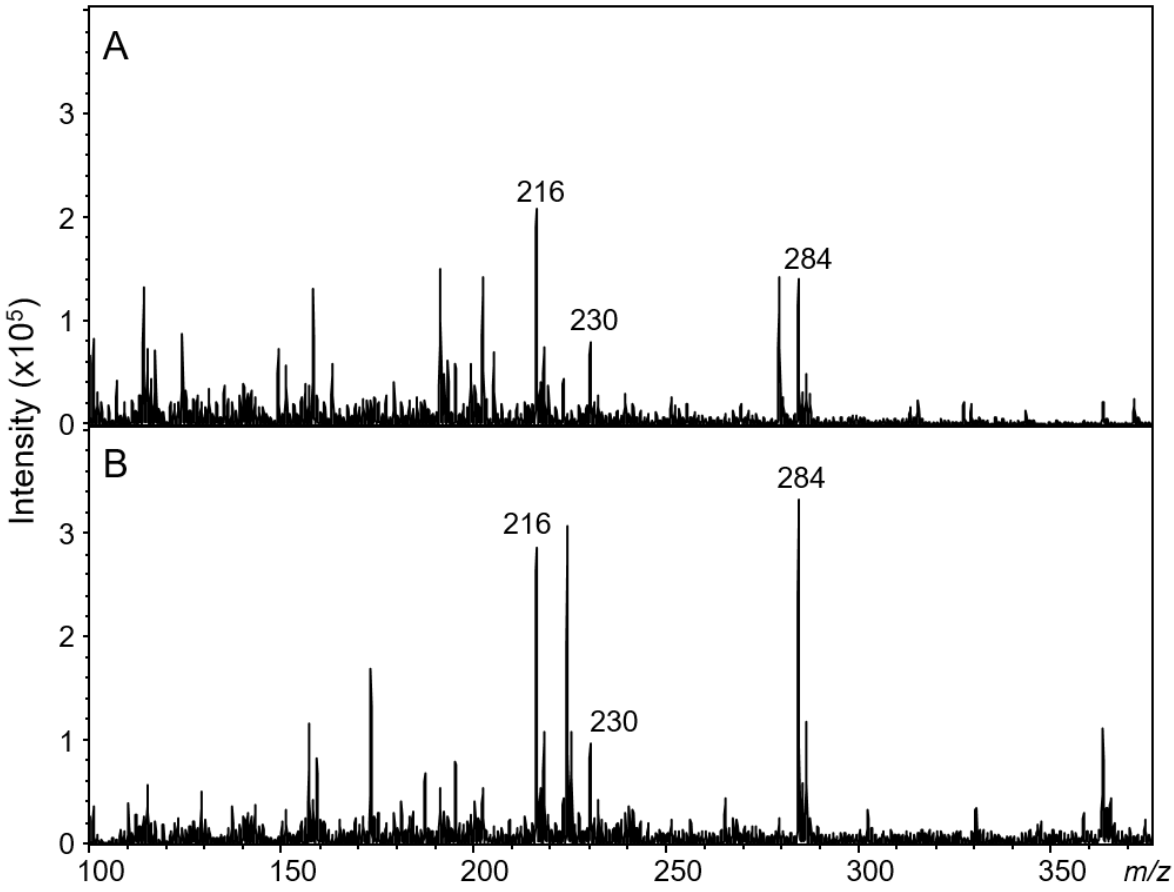

Figure 2.13: Paper spray ionization mass spectra of atrazine (1 ppm, m/z 216), propazine (250 ppb, m/z 230) and metolachlor (1 ppm, m/z 284) on untreated paper (A) and trimethylsilylated paper (B) substrates.

Overall, although pretreatment of the paper substrate can yield some improvement in signal intensity, it was determined that oxidative treatment and trimethylsilylation were not suitable for incorporation in standard operating procedures due to the increased complexity and preparation time required. Washing the substrate prior to sample application, however, is recommended as it is a simple and relatively quick method of reducing background ion signal.

2.4 Summary and Conclusions

A custom paper spray ion source has been developed and its performance investigated, primarily using the common herbicide atrazine. The device, in its final

configuration, consists of an aluminum sample holder mounted on a three-axis micrometer translation stage, with a fitting for the continual replenishment of solvent on the paper spray substrate, allowing for stable signal for several minutes from one sample, up to fifteen minutes or more depending on the quantity of analyte applied. The ion source has been tested with a variety of solvents, with the best performance observed using 99/1 acetonitrile/acetic acid or 80/20/0.1 acetonitrile/acetone/acetic acid. A range of filter papers were investigated as substrates, with Whatman #903 dried blood spot paper and #598 filter paper performing best when large sample volumes (>50 µL per replicate) are available.

While this device is not in itself particularly novel, it does incorporate several features not seen in most previously developed paper spray devices. Firstly, it incorporates continuous solvent replenishment, allowing for extended analyses. This feature has not been incorporated in most paper spray sources described in the scientific literature.[1,3,4,8] This ion source also uses a flat, U-shaped surface to hold the substrate in place, unlike the alligator clips and similar devices used in most other custom paper spray sources.[3,8,17] This sample holder design minimizes the surface area in contact with the paper, provides even support for the paper to prevent warping, and covers all corners of the paper except the tip, preventing undesired spray formation. The source as a whole provides the desired functionality to serve as a test platform for a variety of analytical applications, including the analysis of agrochemicals in environmental and agricultural samples.

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CHAPTER 3: MEASUREMENT OF HERBICIDES IN WATER AND CROP EXTRACTS BY PAPER SPRAY IONIZATION MASS SPECTROMETRY[1]

3.1 Introduction

Water monitoring and other widespread screening programs represent a critical tool for ensuring safe drinking water, protecting natural resources, and assessing the impact of herbicide use on our environment. However, conventional methods of water analysis require large volumes of liquid water samples (typically >10 mL per site, per collection, often as much as 1 liter) to be transported from field collection sites to the analytical laboratory at significant expense.[1–6] The cost of transporting liquid samples is particularly problematic for water monitoring programs targeting a wide area. An alternative to bulk liquid sample collection is sample depostion on an absorbent medium such as paper. The sample may then be dried and shipped at reduced cost.

Paper spray ionization is a natural choice for the measurement of dried samples on paper, as it eliminates the extraction required for most conventional analytical methods, reducing solvent consumption and analysis time. Paper spray has been employed for the direct analysis of several types of fresh samples, including foods, using the paper tip as a spray emitter.[7–10] The use of paper spray for analysis of dried samples, where the paper substrate is used for both sample collection and ionization, has focused on biological samples such as dried blood spots and dried urine samples.[11–13] Applications of dried sample paper

[1] This chapter previously appeared as an article in Analytical Methods. The original citation is as follows: Reeber, S.L., Gadi, S., Huang, S.-B., Glish, G.L. Direct analysis of herbicides by paper spray ionization mass spectrometry. *Anal. Methods*, **2015**, *7*, 9808-9816 DOI: 10.1039/c5ay02125a.

spray to food or agriculture analysis include investigation of coffee samples for origin discrimination,[14] detection of azo dyes in chili peppers,[15] and measurement of anti-inflammatory compounds in olive oil.[16] The use of paper spray ionization for measurement of part-per-million levels of fungicides in fruits has recently been demonstrated using both a wiping technique and by applying a homogenate to the paper and drying.[17]

Collection of water samples for paper spray analysis is trivial, requiring only that a known volume (in these experiments, 50 or 100 μL) be applied to a paper strip. The paper may be dried under ambient conditions and then packaged for transport by simply placing it in a plastic bag. As noted above, transportation of these dried samples would be much less costly and difficult than shipping samples for conventional methods, which typically call for collection of significantly larger sample volumes.[3–6] Paper spray of dried samples also involves minimal sample handling in the laboratory. Internal standards are applied to the paper strips, dried, and then the strips are analyzed without additional liquid handling or sample preparation. Analysis of non-liquid samples, such as soils and crops, is slightly more complex as it does typically require at least a crude extraction.

Paper spray ionization has several other advantages for analysis of environmental samples. It is immune to clogging, eliminating the need for filtration of samples containing dispersed solids. Once in the laboratory, analysis is rapid and straightforward, requiring no separation techniques and only approximately two minutes of instrument time per sample. All steps relating to preparation of the paper are carried out prior to application of the sample. The ion source used, described in detail in Chapter 2, is modular and may be implemented on most mass spectrometers designed for atmospheric pressure ionization techniques such as ESI or atmospheric pressure chemical ionization. Alternatively, paper

spray has been demonstrated in conjunction with a portable mass spectrometer for *in situ* analysis.[18] Although it would be inaccurate to describe paper spray ionization-mass spectrometry as a replacement or substitute for conventional LC-MS analysis in all cases (it remains limited in terms of limit of detection and has only recently been commercialized), it is a complementary analytical technique, particularly useful in applications calling for rapid analyses for screening of large numbers of samples that can be effectively collected and transported on paper substrates.

3.2 Instrumentation, Materials, and Methods

3.2.1 Materials

Environmental matrices (ground water, lake water, soil extracts, and crop extracts) and herbicide standards were provided by Syngenta Crop Protection, LLC (Greensboro, NC). Atrazine, propazine, and metolachlor were used as test analytes. Crop extracts were prepared by homogenization of 10 g of crop sample using a Polytron homogenizer, followed by extraction with 200 mL of 80/20 acetonitrile/water. Soil extracts were prepared by extraction from 20 g of soil sample with 200 mL of 80/20 acetonitrile/water. LC-MS grade acetonitrile (Fisherbrand Optima Acetonitrile) and glacial acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Deuterated atrazine (ethyl-d_5) was purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada). Deuterated metolachlor (propyl-d_6) and deuterated propazine (isopropyl-d_6) were purchased from Crescent Chemical (Islandia, NY). Whatman 903 paper, purchased from GE Life Sciences (Little Chalfont, UK), was used for all analyses. This paper is a standard dried blood spot collection paper used in neonatal testing, similar to the Whatman #31 ETF paper used in several paper spray experiments using blood samples.[8,11]

3.2.2 Instrumentation

Unless otherwise stated, experiments were performed on a Bruker HCTultra ion trap mass spectrometer coupled to the Mark 3 custom paper spray ion source described in Chapter 2. The electrospray ion source was removed from the HCTultra and the safety interlock overridden to allow operation with the custom paper spray ion source. Spray solvent was applied using a syringe pump at a rate of 15-35 μL/minute (usually 25 μL/minute, adjusted as needed to maintain stable spray without overloading the paper) and a voltage (typically 3.5 kV) was applied to the sample holder using a separate power supply. The instrument's ESI desolvation gas (nitrogen) was set to a temperature of 300°C and a flow rate of 1.0 L/min. Voltages applied to the mass spectrometer inlet and ion optics were optimized for each analyte using the automated optimization tool included with the instrument control software. Typical instrument operating parameters are listed in Table 3.1.

Table 3.1: Typical mass spectrometer operating parameters for paper spray ionization-mass spectrometry analysis of atrazine/metolachlor.

Parameter	Setting
Inlet Capillary	-3833 V
Capillary Exit	158.3 V
Skimmer	46.2 V
Octopole 1 dc	17.26 V
Octopole 2 dc	6.06 V
Octopole RF Amplitude	179.2 Vpp
Lens 1	-9.0 V
Lens 2	-74.5 V
Trap Drive	44.1
MS/MS Amplitude	1.40-1.70 V
Low Mass Cutoff (% of parent ion mass)	40%

All versions of the custom ion source design include exposed high voltages on both the mass spectrometer inlet and the sample holder. This presents a risk of electric shock to the user; care must be taken to avoid contact with the sample holder and mass spectrometer inlet while the source is energized.

3.2.3 Sample Preparation

Samples were applied to paper strips designed for use with the Mk. 3 ion source; strip geometry is described in detail in Chapter 2. Strips were washed three times on each side with approximately 1 mL volumes of LC-MS grade acetonitrile and dried at ambient conditions prior to sample applications. Samples were applied in 100 µL aliquots and allowed to dry for at least 30 minutes (longer drying times, up to 60 minutes, were required for samples in aqueous matrices). Some experiments were also performed using the Mk. 2 ion source; in these cases, samples were applied to washed Whatman #903 paper cards as described in Chapter 2. For experiments requiring internal standards, the analyte was applied first and allowed to dry. After the analyte was thoroughly dry, an equal volume of internal standard solution was applied and allowed to dry prior to analysis.

3.3 Herbicides in Environmental and Agricultural Matrices

Representative herbicides of two different classes were investigated for analysis in agricultural and environmental matrices using paper spray ionization. Triazines comprise a class of synthetic herbicides commonly used for the protection of corn and other crops,[19] such as blueberries[20] and triazine-tolerant canola,[21] from broadleaf weeds and grasses.[22,23] Atrazine is one of the most commonly used herbicides in the United States[19] and is also used in other countries, such as Australia[21] and Canada.[20] It is not currently permitted in the European Union,[24] but a similar triazine herbicide, terbuthylazine, is used in many of the same applications there.[25] Simazine and propazine are also herbicides in the triazine family, used for similar applications. The U.S. Environmental Protection Agency (USEPA) limits for triazine herbicides and their metabolites in crops range from 50 ppb in guava to 15 ppm in corn forage for animal feed.[26]

Metolachlor was selected as a representative of the chloracetanilide family of pesticides, which includes alachlor and acetochlor, among others. Metolachlor is frequently used in combination with atrazine for weed control in field crops, particularly corn.[23] Along with atrazine it may be observed in runoff in agricultural areas shortly after application,[23] and there is significant interest in monitoring the level of these herbicides in water matrices as well as soil and crops to ensure proper usage, minimize environmental impact, and guarantee the overall safety of the food and water supply.

The triazine herbicides atrazine and propazine can be detected in water samples and soil and crop extracts at concentrations in the part-per-billion range using the paper spray ion source described in Chapter 2. As observed during ion source characterization using triazine herbicides as test analytes, at part-per-million concentrations in simple matrices such as water samples protonated triazines are the dominant ions observed. Protonated atrazine is detected at *m/z* 216 and protonated propazine at *m/z* 230. A variety of ubiquitous background species are also observed. At lower concentrations, these background species are more abundant than the protonated triazines. An example of this is shown in Figure 3.1, using samples of atrazine and propazine spiked into surface water samples at 100 ppb. Here, the dominant ion in the mass spectra (inset) is a background species at *m/z* 198. Despite their low relative abundance, protonated atrazine and propazine are still readily detected, and the identity of these ions may be confirmed using MS/MS.

MS/MS of atrazine and propazine, as noted in Chapter 2 and shown in Figure 3.1, yields primarily the loss of propylene (-42 Da) from the isopropylamino side chain. The propylene loss product from atrazine is observed at *m/z* 174 and from propazine at *m/z* 188. An ion due to the loss of both side chains (loss of two propylene molecules from propazine,

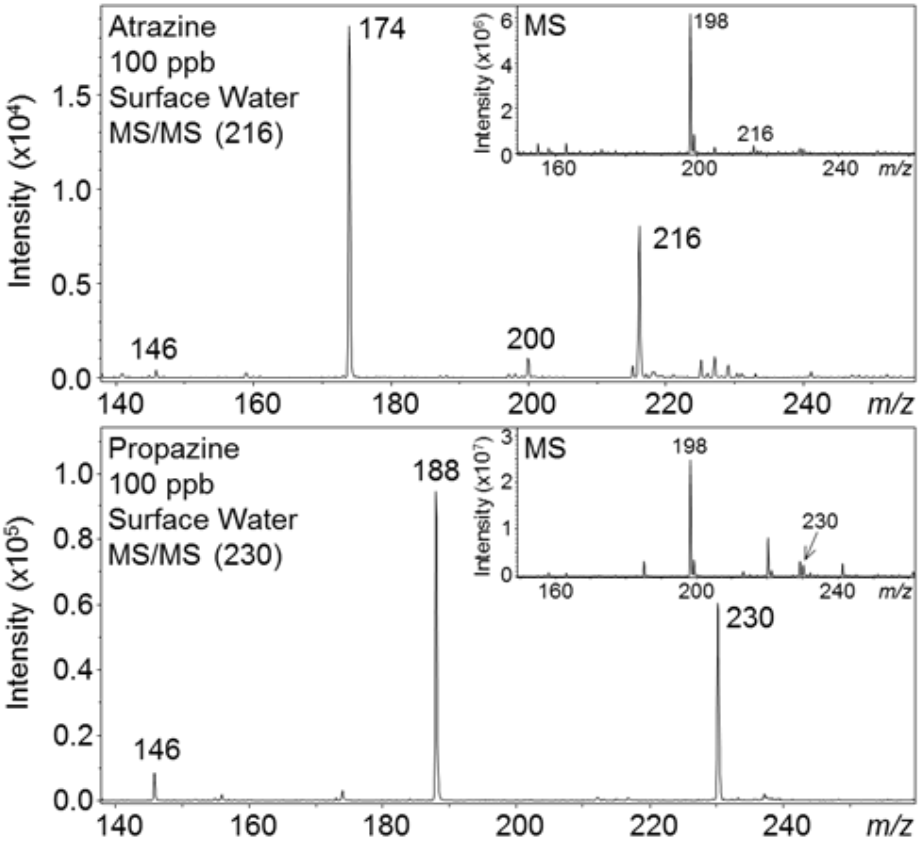

Figure 3.1: MS/MS spectra of 100 ppb atrazine (top) and propazine (bottom) in surface water with mass spectra inset. Protonated atrazine and propazine are observed at *m/z* 216 and 230, respectively.

loss of propylene and ethylene from atrazine) is detected in both cases at *m/z* 146. Atrazine and propazine may be detected using MS/MS at concentrations as low as 10 ppb (approximately 5 picomoles per 100 µL aliquot).

The presence of a background species isobaric to atrazine is indicated in the atrazine MS/MS spectrum by the presence of a product ion at *m/z* 200 which is not observed at higher atrazine concentrations. Because this species produces different product ions than atrazine it does not interfere directly with atrazine measurement, although if such ions are present in large quantities they may be a limiting factor in mass analyzers limited by charge capacity.

Measurement of herbicides in crops and soils is somewhat complicated by the need for extraction prior to application to the paper substrate, but the more complex matrices do

not seem to impose any significant difficulty in the detection of these species. A mass spectrum of a sample of wheat grain extract spiked with atrazine, propazine, simazine, and metolachlor (2 ppm each) is shown in Figure 3.2. All species are observed as protonated molecules, with virtually no background species of note. A moderate amount of fragmentation of metolachlor is observed, yielding the peak at *m/z* 252. This is due to the optimization of the instrument settings for measurement of atrazine; when tuned for optimum measurement of metolachlor significantly less fragmentation is observed.

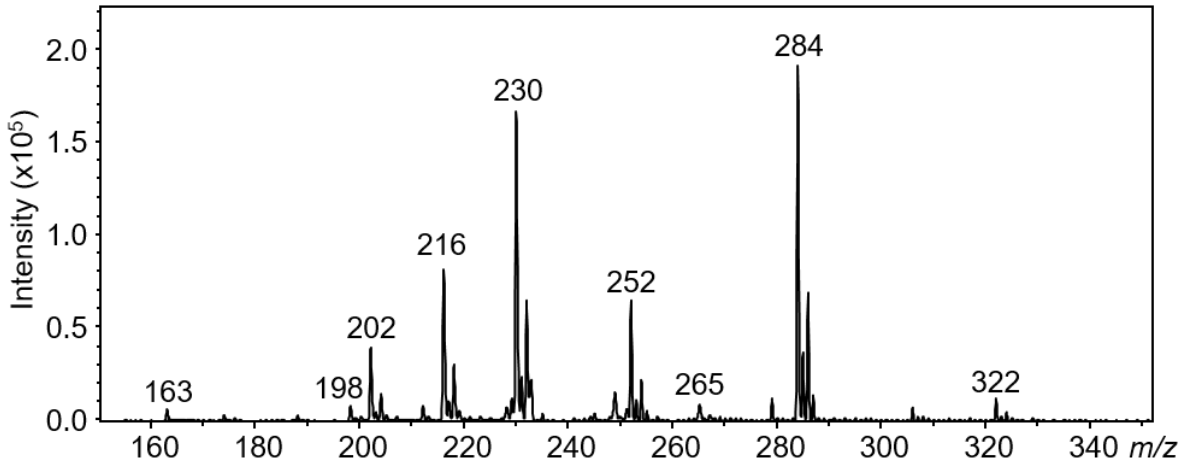

Figure 3.2: Paper spray ionization mass spectrum of wheat forage extract containing 2 ppm each simazine (*m/z* 202), atrazine (*m/z* 216), propazine (*m/z* 230), and metolachlor (*m/z* 284). Matrix species are observed at low intensity, with notable peaks at *m/z* 163, 198, 265, and 322. The ion at *m/z* 252 is a fragment ion derived from metolachlor.

Part-per-million level analyte concentrations in grain extracts can yield an overly optimistic view of the challenges of detecting herbicides in crop matrices. At lower concentrations and in other matrices, such as wheat forage or lettuce extracts, matrix ions are observed with significantly greater intensity. Mass spectra and MS/MS spectra of metolachlor samples at 1, 10, and 100 ppb concentrations in lettuce extract are shown in Figure 3.3. In the inset mass spectra, the significant abundance of a variety of matrix species is quite evident. However, despite the low relative abundance of protonated metolachlor, it

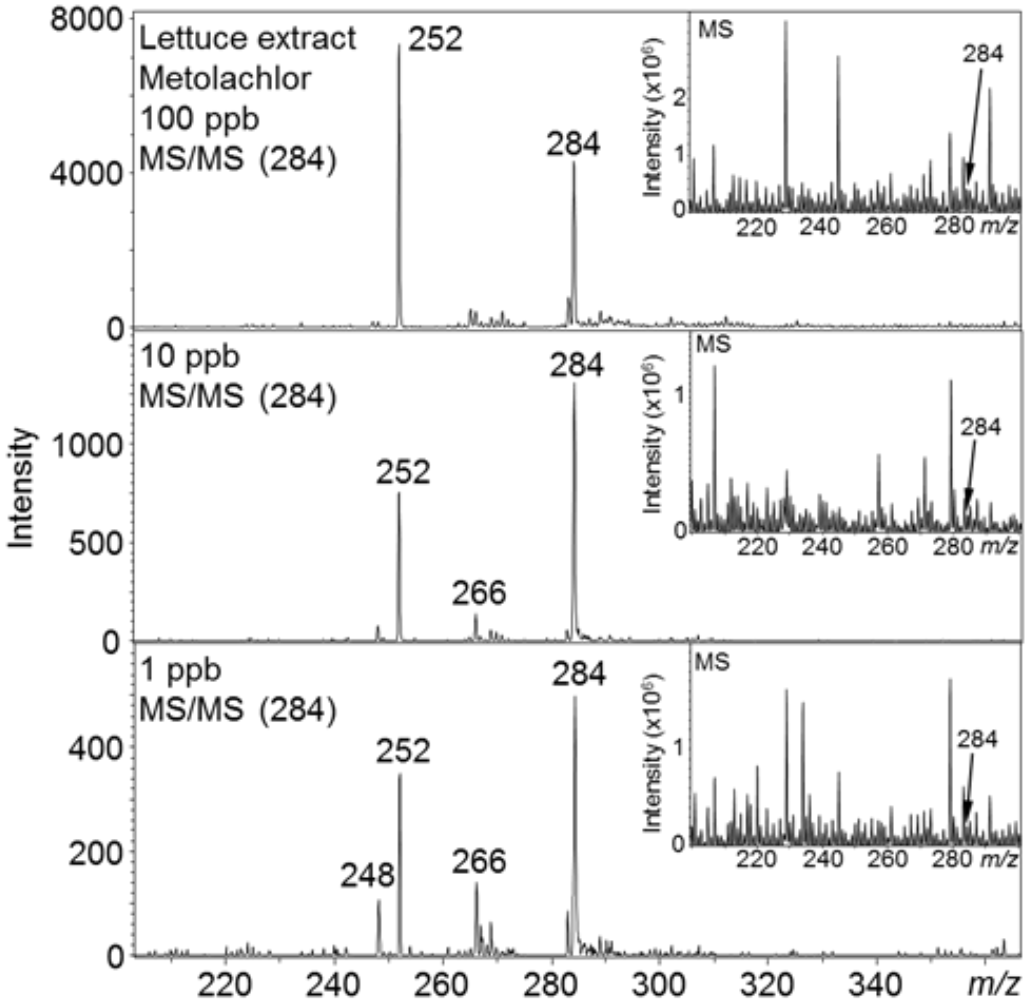

Figure 3.3: MS/MS spectra of lettuce extract samples containing 100, 10, and 1 ppb metolachlor, ionized by paper spray. Mass spectra are inset. The primary product ion from metolachlor is observed at *m/z* 252, while product ions from background species are detected at *m/z* 248 and 266.

can still be readily observed using MS/MS. The sole product ion produced from MS/MS of protonated metolachlor is the methanol loss product at *m/z* 252, consistent with data from previously published methods for LC-MS/MS analysis of metolachlor.[27,28] MS^3 can be used for further confirmation if needed, but was not necessary in the matrices used in these experiments. At lower concentrations, near 1 ppb, the presence of isobaric background ions is evident due to product ions not derived from metolachlor, observed at *m/z* 248 and 266 in the MS/MS spectrum shown in Figure 3.3. As in the case of the triazine herbicides, the presence

of an isobaric species does not directly interfere with measurement as different product ions are produced, confirmed using metolachlor-free control samples. Metolachlor can be detected using this method at concentrations as low as 100 ppt (35 femtomoles in a 100 µL aliquot).

Soil extracts similarly do not present a significant difficulty, with relatively few matrix species observed and no major matrix effects on the ionization of triazines or metolachlor. A mass spectrum of metolachlor spiked into a sample of soil extract is shown in Figure 3.4. As for wheat grain extract, there are some background species observed, but the dominant ion in the spectrum is protonated metolachlor. Neither soil nor crop extracts were filtered prior to analysis, but were simply decanted; some particulate matter remained suspended in the extract. As noted above, the presence of particulates does not pose the same problem for paper spray ionization as it would in the case of conventional electrospray ionization, as there is no capillary to clog. The particulates are generally retained on the paper and do not seem to influence the performance of the ion source.

3.4 Quantification of Herbicides

Quantification of herbicides may be performed using this method with the addition of a suitable internal standard. MS/MS is used to provide selectivity - the signal intensity ratio for the primary product ions of the analyte and internal standard for a range of herbicide concentrations is used to generate a calibration curve. Several methods have been employed for the addition of internal standards to samples for paper spray ionization. Ideally, the internal standard would be added to the sample prior to any sample processing, and thus compensate for inefficiencies in extraction or transfer. One approach to preparing paper spray samples in this manner utilizes small glass sampling capillaries pre-coated with internal

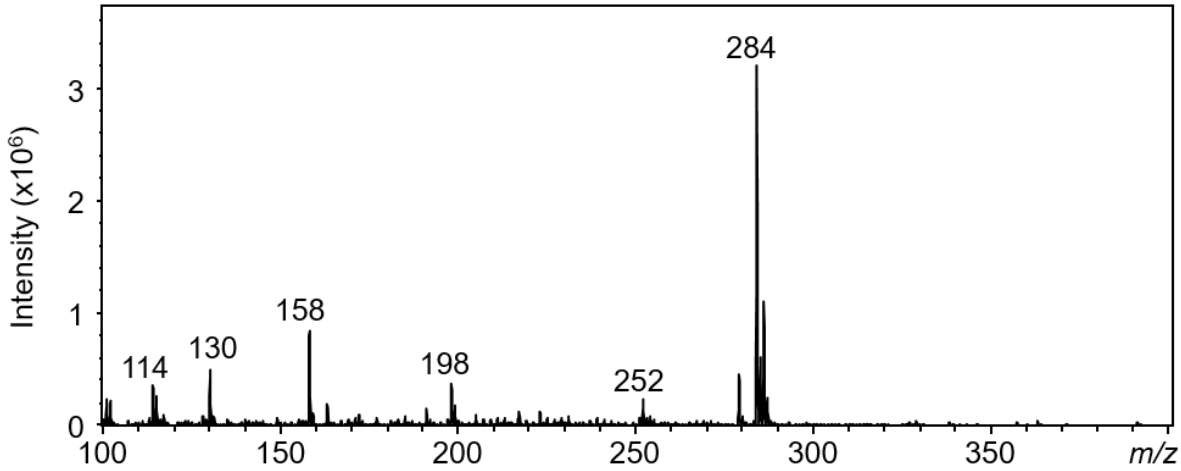

Figure 3.4: Paper spray ionization mass spectrum of metolachlor spiked into a soil extract to a concentration of 1 ppm.

standard.[29] This approach has the advantage of mixing the internal standard with the analyte before application to the paper, but requires that the analyte of interest be known at the time of application and is designed for very small sampling volumes (approximately 1 μL).[29] Additionally, if the samples are applied to paper strips in the field, this technique would require all relevant internal standards to be prepared and taken into the field with the technician (or farm hand) collecting samples. Internal standards may also be pre-applied to the strips and the analyte applied afterwards.[8,11] This procedure avoids the need for internal standard preparation by the field technician, but still requires that the identity of the analyte of interest be known in advance. Both the coated capillary and pre-applied internal standard approaches involve transporting the internal standards (often expensive isotopically labeled compounds) into the field, with the attendant hazards due to non-ideal storage conditions and limited shelf life.

The following experiments were conducted using the most general approach, in which the internal standard is added to the paper strips after application of the sample. In the case of field collection, the technician need only apply the sample to the paper strip, allow it to dry, and ship the samples to the analytical lab. Internal standards may then be applied as

needed for whatever analysis is desired. This method is the simplest and easiest approach, but does have some drawbacks, as the internal standard is not exposed to the same conditions as the sample for the entire period. Additionally, sequential application of the analyte and internal standard affects the signal intensity ratio, as described in Chapter 2. The impact of this effect is minimal, however, as long as the internal standard volume, concentration, and application procedures are reproducible.

Herbicide standards were dissolved in environmental matrices (surface water) to yield the desired concentration, applied to paper strips or cards, and allowed to dry completely at room temperature in air (minimum drying time 30 minutes). After the samples were completely dry, a solution of isotopically labeled internal standard (atrazine-d_5, metolachlor-d_6, propazine-d_6 as appropriate) was applied in LC-MS grade water and allowed to dry completely before the samples were analyzed by paper spray mass spectrometry. Quantitative experiments in soil and crop matrices were performed using the Mk. 2 ion source with samples applied to paper cards in 50 µL aliquots; experiments with water samples were performed using the Mk. 3 ion source with samples applied to paper strips in 100 µL aliquots.

Unlike in LC-MS experiments, paper spray ionization with continuous replenishment of the spray solvent does not produce a discrete peak in time. Rather, the analyte is eluted from the paper over a period of several seconds to tens of minutes, depending on the quantity present. At the concentrations and aliquot volumes employed in these experiments, the analyte signal stabilized within a few seconds after the application of solvent and high voltage, and a nearly constant signal could be observed. Quantitative experiments were

conducted by integrating the signal for two minutes beginning immediately after the signal was stable, analogous to a direct infusion experiment.

Calibration curves for atrazine in soil and crop extracts are shown in Figure 3.5. These experiments were performed using the Mk. 2 ion source, and do not reflect the improved sensitivity and reproducibility obtained with the Mk. 3 source. Regardless, a linear response is observed in these matrices across the part-per-million concentration range, without saturation issues at high concentrations. Calibration curves for atrazine, propazine, and metolachlor in environmental water matrices are shown in Figures 3.6, 3.7, and 3.8. These experiments, performed using the Mk. 3 ion source, cover the part-per-billion range. A linear response is observed over approximately three orders of magnitude for all samples; a wider range is not practical with a single internal standard concentration.

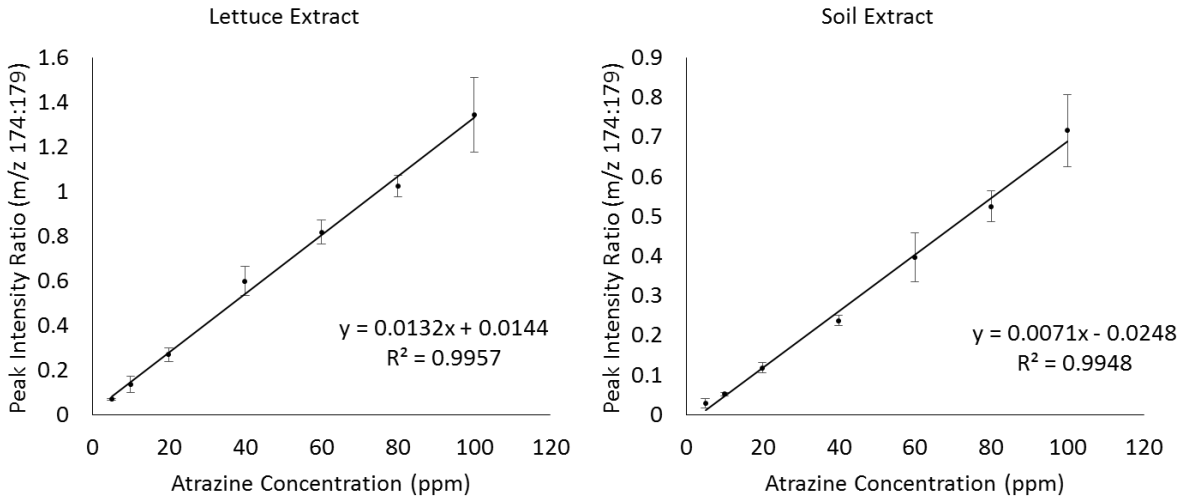

Figure 3.5: Calibration curves for atrazine in lettuce and soil extracts at concentrations from 5 ppm to 100 ppm. Measurements were made using the Mk. 2 ion source. Trend lines are calculated using an unweighted linear fit.

A preliminary investigation of signal intensity as a function of time did not indicate any significant changes due to storage on paper in dried form for up to one month, suggesting that this method may be viable for work with samples collected in the field and transported to

the analytical laboratory after drying. Samples of atrazine were applied to paper strips and stored at room temperature or at -20 °C. Sets of these strips were analyzed at regular intervals over the course of one month, and compared to samples prepared on the day of analysis. No significant variation was observed for either storage condition. However, additional study of suitable storage conditions is recommended prior to use in regulatory applications.

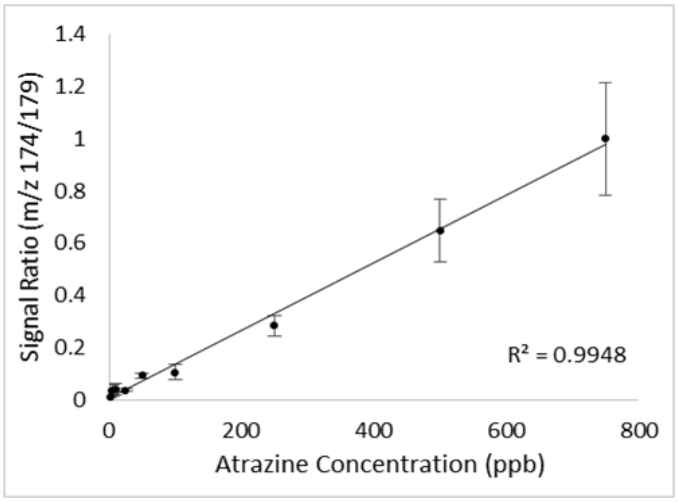

Figure 3.6: Calibration curve atrazine in surface water at concentrations from 1 to 750 ppb, measured using the Mk. 3 ion source. Trend line calculated using an unweighted linear fit.

The calibration curve for quantification of atrazine in surface water shown in Figure 3.6 ranges from 1 ppb to 750 ppb. Atrazine-d_5 (ethyl-d_5) was used as an internal standard at a concentration of 250 ppb. A linear response is observed over the entire range, with increasing imprecision as the concentration of atrazine increases. Because the variability observed increases with the concentration of analyte, 1/x and $1/x^2$ weighted linear least-squares fits were investigated (equations of linear fits are shown in Table 3.2). Quality control (QC) samples were tested at 3, 60, 150, and 400 ppb. QC results are listed in Table 3.3, showing results for the unweighted, 1/x, and $1/x^2$ weighted fits. Absolute error values are in the range of 1 ppb for all but the highest concentration QC samples, calculated using a $1/x^2$ weighting. Relative standard deviations (RSDs) of QC samples (n = 3) are below 15 % except for the lowest concentration (3 ppb). The limit of detection for atrazine, calculated as three times the standard deviation of the signal ratio in the blanks (n = 3) using $1/x^2$ weighting is 3.53 ppb.

Table 3.2: Linear fits for the atrazine calibration curve data presented in Figure 3.X, using three weighting parameters.

Weighting	Equation
Unweighted	Signal Ratio = 0.001295 x $C_{atrazine, ppb}$ + 0.007027
1/x	Signal Ratio = 0.001257 x $C_{atrazine, ppb}$ + 0.014122
$1/x^2$	Signal Ratio = 0.001804 x $C_{atrazine, ppb}$ + 0.010548

This limit is just above the concentration of the most dilute QC sample, explaining the high RSD for that measurement. However, this measurement is both precise and accurate between the limit of quantitation (five times the standard deviation in the blank, 9.78 ppb) and several hundred parts per billion, with accuracy and precision falling off at higher concentrations. The error at high concentrations may be remedied by the use of a higher concentration of

Table 3.3: Quality control measurements for atrazine in surface water. Values calculated using linear fits with three weighting parameters.

	Unweighted		**1/x**		**$1/x^2$**		
Concentration	Value	Error	Value	Error	Value	Error	% RSD
3 ppb	8.44 ppb	181 %	3.06 ppb	1.89 %	4.11 ppb	37.0 %	61.9%
60 ppb	86.7 ppb	44.6 %	83.8 ppb	39.6 %	60.3 ppb	0.58 %	3.7%
150 ppb	210 ppb	40.2 %	211 ppb	40.6 %	149 ppb	0.69 %	8.4%
400 ppb	456 ppb	14.1 %	465 ppb	16.1 %	326 ppb	18.6%	13.1%

internal standards; the calibration curves for atrazine at part-per-million concentrations in soil and crop extracts show similar results overall.

While the limits of detection and quantitation for atrazine are greater than the USEPA maximum contaminant limits in drinking water,[19,30] they are below the USEPA health advisory limits for both 7 year and single day exposures for children (50 ppb and 100 ppb, respectively).[19] Coupled with the low cost, minimal sample processing requirements, and short analysis time, this suggests that this method may be suitable for rapid response analysis in the case of contaminated water supplies to ensure water is safe for short-term human or livestock consumption. Additionally, this technique may be suitable for measurement of atrazine in post-application runoff, where concentrations are likely to exceed the year-round

average (to which the USEPA limits apply). This method involves collection of far less liquid than comparable conventional EPA methods, and requires significantly fewer liquid handling steps.[6,31]

The working range for this technique also includes the US regulatory limits for atrazine in crops (50-15000 ppb, depending on varieties),[26] and little impact on quantitation or signal intensity is observed when working with more complex matrices such as crop extracts. Since this method requires only the most rudimentary preparation from crop or soil samples (crude extraction, no filtration) it ought to be suitable for routine crop testing as well.

A direct comparison to infusion electrospray ionization using the same instrument was carried out using atrazine spiked into surface water samples. To each 200 µL surface water sample containing atrazine, 20 µL 500 ppb atrazine-d_5 in acetonitrile was added, along with 2 µL glacial acetic acid. The source gases were optimized manually to yield the most stable signal for protonated atrazine. Ion optics were optimized using the automated tuning method included with the instrument software. The limit of detection for atrazine in surface water using this technique was determined to be 30.3 ppb. In general, reproducibility was better for electrospray ionization than paper spray, but signal intensity was somewhat better for paper spray ionization. The primary limiting factor in this case is likely the sensitivity of the instrument, which is an older ion trap mass spectrometer. Better results using both ion sources would be expected for a modern triple quadrupole mass spectrometer due to the improved sensitivity and faster duty cycle.

Propazine exhibits similar performance to atrazine, as shown in Figure 3.7, with somewhat less of an increase in variability as the concentration is increased. In general,

propazine yields somewhat better signal intensity for a given concentration than atrazine, especially in MS/MS experiments due to the greater dissociation efficiency of propazine.

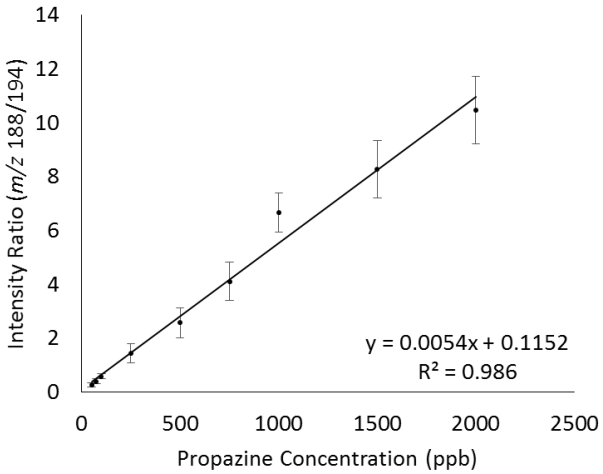

Figure 3.7: Calibration curve for propazine in ground water at concentrations from 50 ppb to 2000 ppb. The trend line was calculated using an unweighted linear least-squares fit.

A similar calibration curve for metolachlor at concentrations from 100 ppt to 500 ppb in surface water is shown in Figure 3.8. A linear response is observed over the full concentration range. Metolachlor-d_6 was used as an internal standard at a concentration of 75 ppb. QC samples at 750 ppt, 15, 100, and 250 ppb concentrations were measured and used to evaluate the accuracy and precision of the calibration. Measured values, RSDs, and absolute and relative errors for QC samples (n = 3) are listed in Table 3.4 and equations for weighted and unweighted linear fits in Table 3.5. Error values are similar to those observed for atrazine, but are more consistent across the concentration range. Relative standard deviations are generally higher for metolachlor than atrazine.

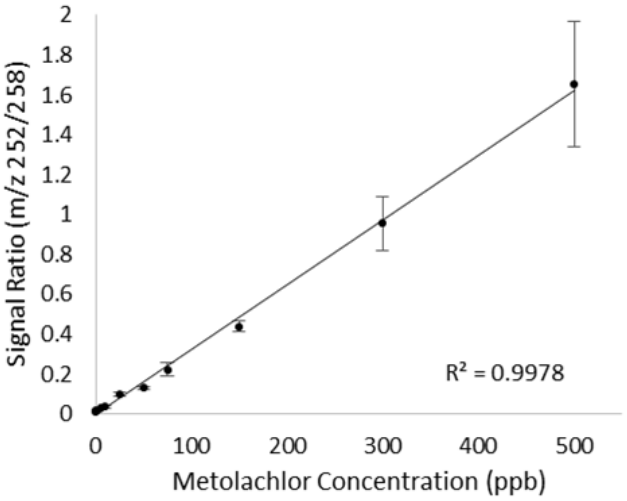

Figure 3.8: Calibration curve for quantification of metolachlor in surface water at concentrations from 100 ppt to 500 ppb. Trend line calculated with an unweighted linear least-squares fit.

Table 3.4: Quality control measurements for metolachlor in surface water, calculated for linear fits using three different weighting values.

	Unweighted		**1/x**		**$1/x^2$**		
Concentration	Value	Error	Value	Error	Value	Error	% RSD
750 ppt	4.42 ppb	490 %	399 ppt	46.9 %	480 ppt	36.0 %	6.06 %
15 ppb	20.9 ppb	39.5 %	17.6 ppb	17.2 %	14.3 ppb	4.22 %	34.1 %
100 ppb	96.1 ppb	3.86 %	95.9 ppb	4.08 %	77.5 ppb	22.5 %	10.4 %
250 ppb	244 ppb	2.32 %	250 ppb	0.05 %	202 ppb	19.2 %	23.3 %

Inter-day variation was assessed by analyzing three 250 ppb metolachlor QC samples over several days. The measured values (n = 3 for each day) were 195, 239, and 258 ppb with RSDs of 11.7 %, 15.9 %, and 18.9 % respectively. The inter-day variation (RSD = 14.1 %) is comparable to the intra-day variation, suggesting that the majority of the imprecision in these measurements is due to factors such as strip shape and edge variation or imprecise strip positioning.

The limit of detection for metolachlor, calculated in the same fashion as for atrazine, using the unweighted linear fit, is 1.38 ppb. The limit of quantitation is 1.70 ppb, calculated in the same manner (values calculated using the weighted fits are below zero due to the imperfection of the fit, although the 1/x weighting yields better accuracy overall for QC samples). These values are well below any relevant regulatory limits for metolachlor, such as the USEPA residue tolerances in crops and food commodities (20-20000 ppb)[32] as well as lifetime human health advisory limits for metolachlor in drinking water.[30]

The low limit of detection obtained by this method for metolachlor suggests that this technique ought to be suitable for most tasks with this analyte, though with the custom paper

Table 3.5: Linear fits for the metolachlor calibration curve data presented in Figure 3.X, calculated using three weighting values.

Weighting	**Equation**
Unweighted	Signal Ratio = 0.003255 x $C_{metolachlor,\ ppb}$ - 0.002880
1/x	Signal Ratio = 0.003125 x $C_{metolachlor,\ ppb}$ + 0.010268
$1/x^2$	Signal Ratio = 0.003874 x $C_{metolachlor,\ ppb}$ + 0.009652

spray ion source it remains too irreproducible for regulatory use. However, for routine investigative or other non-regulatory analysis of water, crop, or soil samples, or any other task where fast analysis, minimal sample processing, and low cost are important, paper spray ionization mass spectrometry appears to be a suitable tool for the measurement of metolachlor.

3.5 Summary and Conclusions

Paper spray ionization mass spectrometry is a powerful complement to conventional LC-MS/MS for targeted analysis applications, eliminating the need for sample cleanup and preparation. It is suitable for sample collection in the field, where it has the potential to reduce the mass and volume of samples to be transported to the analytical laboratory, thus reducing costs. Initial results with triazine herbicides and metolachlor indicate that the quantitative measurement of these herbicide residues in environmental and agricultural matrices is feasible at regulatory levels. The robust, rapid, and low cost nature of paper spray ionization make it an attractive alternative for high volume tasks such as quality monitoring of pesticide sprays, analysis of herbicide-damaged crops, and other field collection tasks where low cost and rapid response are high priorities. For compounds with low limits of detection, such as metolachlor, paper spray may be a suitable technique for the monitoring of contaminated water in cases of runoff or spills, although reproducibility is not yet suitable for routine regulatory drinking water testing.

The primary limitations of paper spray ionization for quantitative applications are its dependence on customized hardware, the limited reproducibility of the custom source and paper strips used, and the inability to employ a separation prior to ionization. A commercialized paper spray ion source has recently been released, which employs a variety

of features expected to improve reproducibility and ease-of-use; this source is characterized in Chapter 4. The lack of a separation is an ongoing difficulty, due to the presence of background species that can interfere with ionization or detection, but in the agricultural and environmental matrices tested sufficient selectivity was achieved by use of MS/MS. One option which has yet to be explored is the combination of paper spray ionization with post-ionization separation techniques such as ion mobility separations; while this approach is not investigated here, it may be a viable tool for future work with paper spray ionization of complex samples.

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CHAPTER 4: CHARACTERIZATION OF A COMMERCIAL PAPER SPRAY ION SOURCE

4.1 Introduction

The Velox 360 ion source is a commercialized paper spray ion source available from Prosolia, Inc. This product was launched in 2014 and is the only commercially available paper spray ion source at this time.[1,2] The Velox 360 is currently available for Thermo Scientific mass spectrometers only, although work is ongoing to adapt it to other instrument designs. The Velox 360 became available shortly after the successful demonstration of the applicability of paper spray ionization using a custom source to environmental and agricultural applications, described in Chapters 2 and 3. Evaluating this new commercial platform was the natural next step.

The Velox 360 has been demonstrated for a number of applications, particularly in the analysis of pharmaceuticals in biological matrices.[1,3] However, it has not been tested in environmental applications, which involve very different matrices, analytes, and relevant concentration ranges. It was therefore necessary to thoroughly characterize the Velox 360 and evaluate its potential as a tool in these applications. Additionally, this ion source is in the relatively early stages of production. These experiments therefore also served as a field test to identify any mechanical or design problems that may remain.

In addition to evaluating the Velox 360 ion source on its own merits, it is useful to consider it in comparison to the custom ion source described in Chapter 2. The two ion sources are based on the same principles, but are constructed in significantly different ways

and thus incorporate very different feature sets. The custom source is a simple, highly adjustable assembly designed for use with bare paper strips. It requires manual positioning and loading of the sample, but allows continuous solvent replenishment. The Velox 360 does not provide for as much adjustment in position or continuous solvent application, but has significant automation and a more robust mounting assembly. Comparison of the two sources allows for a practical assessment the relative importance of these features and the viability of the two sources for similar applications.

4.2 Chemicals and Equipment

A pre-production Velox 360 ion source and associated mounting hardware and control software was provided by Prosolia, Inc. (Indianapolis, IN) and was used for all experiments. This ion source is expected to be functionally identical to production units. All experiments were performed using Velox sample cartridges, which were also provided by Prosolia, Inc. Unless otherwise stated, all solvents and additives (e.g., acetic acid) were of LC-MS grade (Fisherbrand Optima grade, Fisher Scientific, Fair Lawn, NJ). Herbicides and herbicide metabolites used as test analytes were provided by Syngenta Crop Protection, LLC (Greensboro, NC). Atrazine-d_5 was purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada). Unless otherwise stated, experiments were performed using a Thermo Scientific LTQ-FT XL hybrid linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer operated in linear ion trap-only mode. All experiments

Table 4.1: Typical LTQ-FT XL instrument tuning parameters for analysis of atrazine using the Velox 360.

Instrument Parameter	Value
Capillary Temp	200 °C
Source Voltage	4.00 kV
Capillary Voltage	8.00 V
Tube Lens	60.00 V
Skimmer Offset	0.00 V
Multipole RF amp.	400.00 V_{pp}
Multipole 00 Offset	-4.75 V
Lens 0	-5.50 V
Multipole 0 Offset	-5.75 V
Lens 1	-11.00 V
Gate Lens Offset	-76.00 V
Multipole 1 Offset	-7.00 V
Front Lens	-6.50 V

were performed in positive ion mode. All mass spectrometer settings were controlled using the Thermo LTQtune instrument control software, and were optimized using the automated tuning tool. Typical instrument settings for analysis of atrazine using the Velox 360 are listed in Table 4.1.

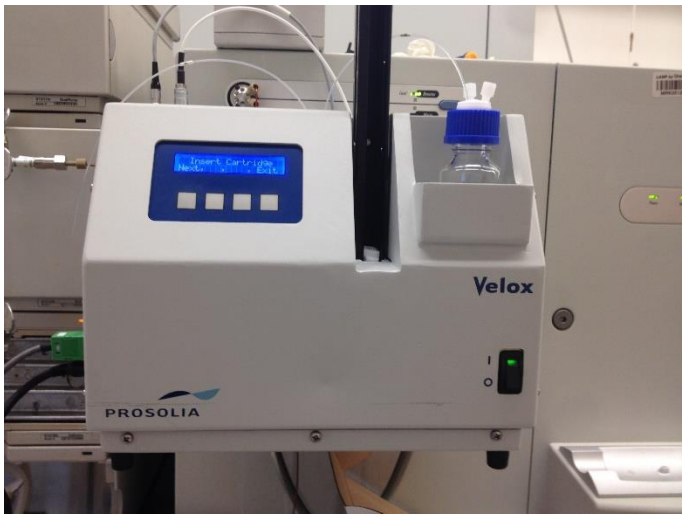

Figure 4.1: The Prosolia Velox 360 paper spray ion source, installed on a Thermo LTQ-FT XL mass spectrometer.

4.3 The Velox 360 Ion Source

The Velox 360 is a modular paper spray ion source currently available for Thermo Scientific mass spectrometers equipped with the Ion Max or Ion Max NG atmospheric pressure inlet (API) systems, which spectrometers designed to be used with electrospray ionization or other spray-based ionization techniques.[4] The Velox 360 ion source is shown in Figure 4.1, mounted at the inlet of the LTQ-FT XL mass spectrometer. The Velox 360 mounts on the front of the mass spectrometer at the atmospheric pressure inlet using a specialized mounting flange, shown in Figure 4.2.[4] The position of the ion source is fixed in two dimensions by the mounting assembly, but the distance between the source and the inlet of the mass spectrometer may be adjusted manually using a screw on the mounting flange ("f" in Fig. 4.2). The flange also connects to the high voltage and source interlock contacts on the mass spectrometer, feeding these to the ion source via two cables.[4]

The Velox 360 employs paper substrates mounted in plastic cartridges, which are prepared in bulk and sold by Prosolia, Inc. An example of a Velox sample cartridge is shown in Figure 4.3. The ion source consists of several component assemblies: the cartridge feed

system, carousel and motors, solvent delivery system, and onboard electronics.[4] The Velox 360 is shown with the front cover opened in Figure 4.4. The cartridge feed system employs a vertical magazine into which a stack of up to 40 cartridges may be loaded. The magazine is inserted into a loading port in the Velox 360 ("d" in Fig. 4.4). The stack of cartridges rests on a spring steel clip at the base of the magazine. A computer-controlled linear actuator ("e" in Fig. 4.4) is employed to retract the clip, allowing a single cartridge to fall into a plastic holder mounted on the carousel. The carousel is a circular steel plate with four plastic cartridge holders mounted on it ("f" in Fig. 4.4). The carousel rotates to move the cartridges from one position to another, driven by a small electric motor.

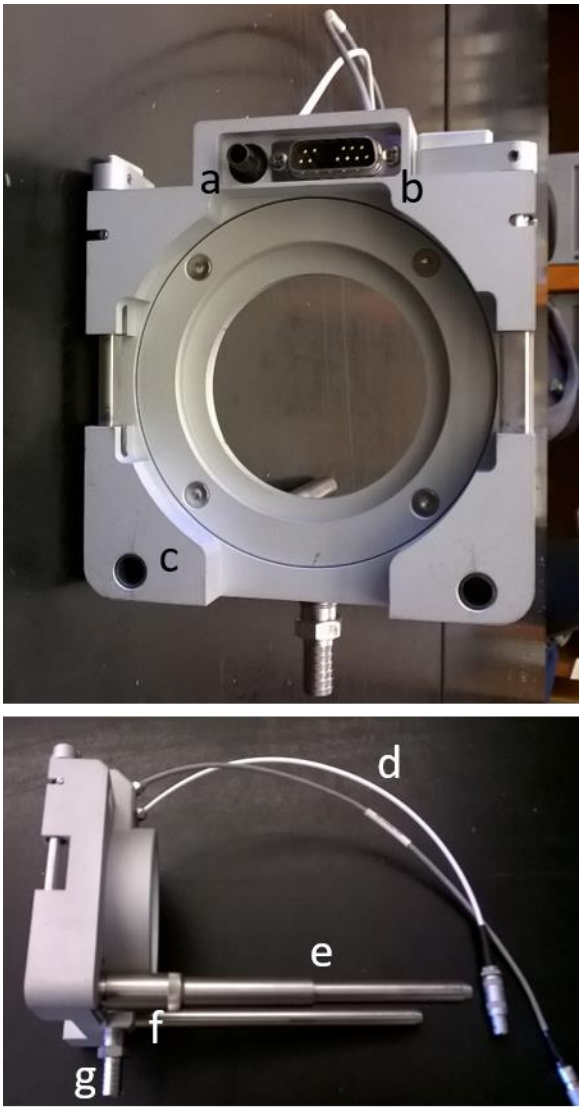

Figure 4.2: The Velox 360 mounting flange. Major parts: a) high voltage connection; b) interlock connection; c) alignment holes; d) high voltage and interlock cables; e) support rods; f) positioning screw; g) vent port.

There are several "stations" the cartridges are moved through. After being loaded onto the carousel, the cartridge is moved to the "sample dispense" station. At this position, the outlet of the solvent delivery system ("c" in Fig. 4.4) is positioned over the forward opening in the cartridge, and a solution may be applied if desired. This position is intended for use with standard solutions, such as internal standards or calibrants. The cartridge then moves slightly

to the “solvent dispense” station where the outlet of the solvent delivery system is positioned over the back opening of the cartridge. At this point the solvent for paper spray ionization is dispensed. At this station the cartridge is also positioned over a small fan, which may be used prior to application of the solvent to dry the paper if needed. From this position the cartridge moves forward to the “analysis” station, where it is positioned in front of the inlet to the mass spectrometer. A high voltage is applied at this position *via* a spring steel clip in contact with the ball bearing at the back of the cartridge. The high voltage is supplied by the mass spectrometer using the standard ion source voltage output connection. After analysis, the cartridge is moved to the final station, where it is ejected *via* a steel chute to a waste bin below the ion source.

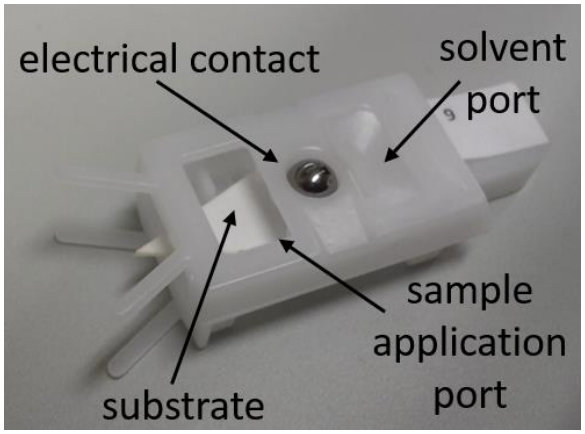

Figure 4.3: Paper spray cartridge for used with the Velox 360 ion source.

The solvent delivery system consists of two computer-controlled pumps, labeled pump A and pump B. Liquid is fed to both pumps through plastic tubing which extends outside the source housing to a small rack where bottles of solvent may be positioned. The 50 mL bottles included with the ion source provide enough solvent for analysis of several hundred cartridges. The outlets from both pumps are positioned next to each other, so that either or both pumps may be used to apply solvent to the cartridge at either position. Pump A dispenses solvent in 3 µL aliquots, while pump B dispenses in 10 µL aliquots. The number of aliquots per cartridge dispensed using each pump is set in the control software or manually

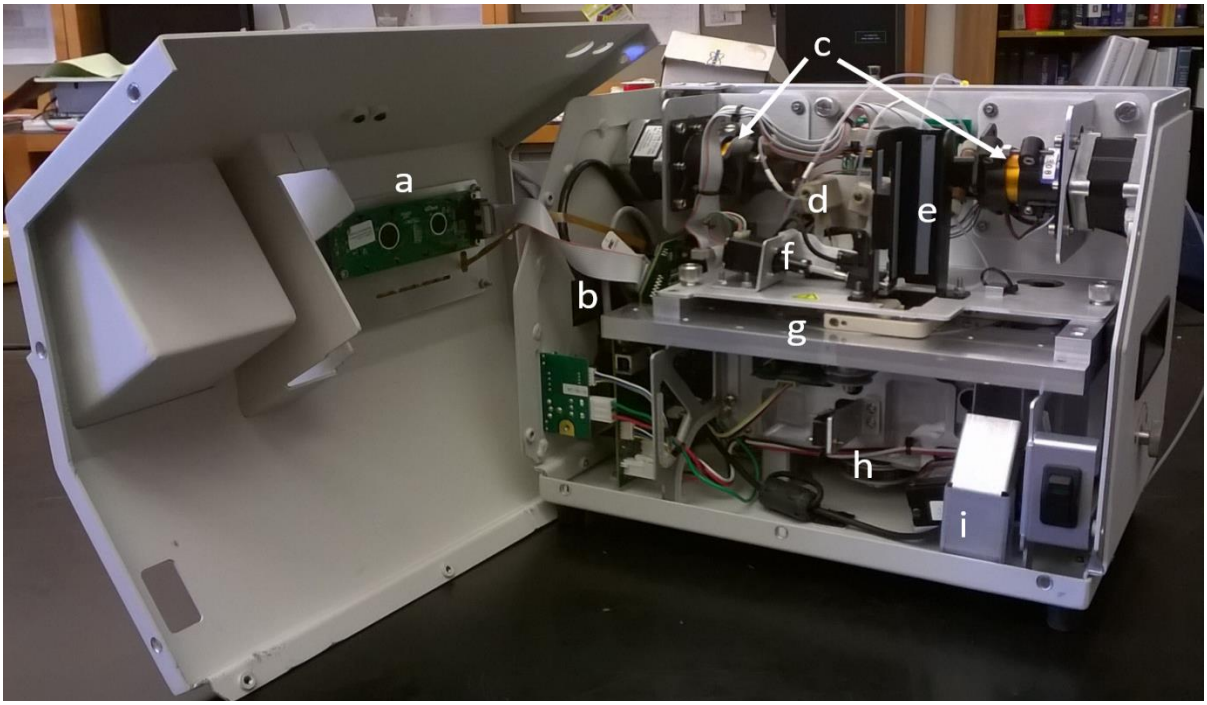

Figure 4.4: The interior of the Velox 360 ion source. Major assemblies are labeled: a) front panel interface; b) on-board computer; c) pumps; d) solvent dispenser; e) cartridge loading port; f) linear actuator; g) carousel with cartridge holders; h) carousel drive assembly; i) waste chute.

controlled by the user. In the experiments described below, pump B was used exclusively to dispense spray solvent. Pump A was not used.

All components of the ion source are controlled by an onboard computer system ("b" in Fig. 4.4). The computer may be controlled manually using a LCD soft panel on the front of the ion source ("a" in Fig. 4.4), or parameters may be uploaded from a computer workstation connected *via* a direct Ethernet connection. The Velox 360 is also designed to communicate with the mass spectrometer using a contact closure signal to trigger the start of data acquisition. This is identical to the system used to synchronize the mass spectrometer with a liquid chromatograph, and does not require any modification of the mass spectrometer.

Control software for the Velox 360 is included with the ion source. This software is designed for use with a Windows PC platform, typically the same workstation used to control the mass spectrometer. The software ("Velox Control") is used to set each of the parameters

of the ion source and upload them to the device. The control software can only adjust settings between experiments; real-time control of the ion source is handled by the on-board computer. Velox Control may be operated in either “analyst” or “supervisor” mode. Supervisor mode is password-secured, while analyst mode is the default mode of operation. Analyst mode is restricted to controlling parameters authorized by the supervisor user. In supervisor mode the user may adjust all software-controlled parameters and set which parameters are available to users at the analyst level of access.[5]

Once the parameters set using Velox Control are uploaded to the ion source, the device may be operated in automatic mode. In this mode of operation, the Velox 360 will feed cartridges continuously, analyzing each one and ejecting it into the waste bin. It will continue feeding cartridges onto the carousel until the magazine is empty. The ion source typically uses a contact closure signal to trigger data collection by the mass spectrometer, and can also be configured to wait for a “ready” signal from the mass spectrometer before applying solvent to the cartridge.[5] The ready signal functionality is not available on all mass spectrometers; in the experiments described below a 10 second delay was used instead to ensure adequate time for the mass spectrometer and data system to prepare for the next run.

4.4 Testing Methodology

The primary mass spectrometer used for testing the Velox 360 ion source was a Thermo Scientific LTQ-FT XL hybrid instrument. This instrument is a hybrid linear ion trap/Fourier transform ion cyclotron resonance instrument (FT-ICR). As noted above, experiments were conducted using the linear ion trap analyzer. Quantitative experiments were also conducted using a Thermo Scientific TSQ Ultra triple quadrupole mass spectrometer to investigate the suitability of the paper spray ion source for quantitative

experiments using a typical analytical platform for routine quantitative environmental and regulatory measurements.

The primary test analytes employed in these experiments were triazine herbicides and their metabolites. Experiments were initially conducted using simple matrices (water, organic solvents) to evaluate the general performance and reliability of the Velox 360 and determine appropriate instrument settings, solvent mixtures, and the like. A direct comparison was then carried out between the Velox 360 and the custom ion source developed earlier. In these experiments, both ion sources were installed on the LTQ-FT XL and the same sample set analyzed using both sources. Samples used with the custom ion source were prepared as described in Chapter 2.

Throughout these experiments, all hardware errors and problems were investigated as they arose. All issues were reported to Prosolia staff, who provided troubleshooting support. In most cases, problems were easily resolved through minor maintenance procedures. Some minor design problems were noted and reported, and one major issue required the ion source be returned to Prosolia, Inc. for more in-depth repairs and maintenance. Design changes to eliminate these problems are expected to be incorporated in new versions of the Velox 360.

4.5 Characterization of the Velox 360

4.5.1 Preliminary Testing

Samples of atrazine and metolachlor dissolved in acetonitrile or water (LC-MS grade) were applied to cartridges and allowed to dry for at least 30 minutes. Paper spray ionization using the Velox 360 yielded mass spectra very similar to those observed with the custom ion source. As expected, the Velox 360 primarily yields protonated molecules when operated in positive mode, and little fragmentation is observed. MS/MS of protonated atrazine and

metolachlor yields results identical to experiments performed using electrospray ionization or the custom paper spray ion source. The typical instrument method employed with the Velox 360 ion source incorporates three "segments" to control application of the spray voltage to the ion source. When data collection begins, the spray voltage is set in the tune method to zero volts. After a short time, typically 0.1 minutes, the tune method is switched to the optimized settings, which incorporate a spray voltage of at least 3.5 kV. This method is employed for the majority of the experiment. Shortly before stopping data collection, the tune method is switched back to the initial setting, applying zero volts to the ion source. This three segment instrument method ensures that the formation of gas phase analyte ions begins after data collection has already started, and ensures a sharp beginning and end to the signal of interest. This is critical for the automation of data analysis, which is discussed later in this chapter. A typical total ion current (TIC) trace for the ionization of atrazine in a simple matrix using the Velox 360 is shown in Figure 4.5.

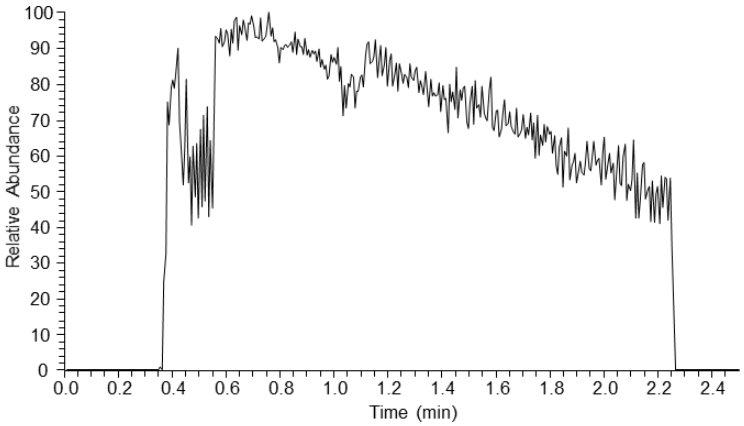

Figure 4.5: Total ion current trace for analysis of atrazine in water (20 ppm) using the Velox 360 paper spray ion source as described. In this experiment the voltage is switched on at 0.3 minutes and switched off at 2.2 minutes.

Initial experiments were performed using 99/1 acetonitrile/acetic acid as spray solvent, based on previous results with the custom paper spray source. However, slightly more reliable results were achieved using 90/10/0.1 acetonitrile/water/acetic acid. This improvement is likely due to a moderate reduction in volatility achieved by the addition of

water. This is important when working with the Velox 360 because it lacks the continuous flow of solvent used with the custom ion source. This is one of the primary advantages of the custom source, as the continuous application of solvent enables much longer experiments and enables the use of more volatile solvents by continuously replenishing the solvent lost to evaporation. Lacking this capability, the Velox 360 requires some care in the selection of solvents, and can only generate stable spray for approximately two minutes, depending on solvent and volume employed. The volume of solvent employed is constrained by the design of the cartridge. As depicted in Figure 4.3, the cartridge used with the Velox 360 has two openings through which solvent may be applied. As noted above, the spray solvent is generally applied through the back opening. This solvent application port is not a simple opening in the plastic shell, like the front opening, but is fully enclosed by plastic on the sides. This enables the port to serve as a small reservoir of solvent which will wick through the paper to the tip over the course of the experiment. The volume of this reservoir determines the quantity of solvent which may be applied, and therefore the maximum spray duration. In most experiments, 100 µL of solvent was used (maximum capacity approximately 150 µL).

As the Velox 360 requires the use of a proprietary cartridge design, which is sold pre-loaded with paper, it is not practical to use alternative paper substrates with this ion source. While the paper used in the cartridges is an excellent choice for paper spray ionization, its sample capacity is limited. In experiments with the Velox 360, 50 µL sample aliquots were applied to the paper rather than the 100 µL aliquots which were routinely used with the custom paper spray source. It is possible to load custom paper substrates into cartridges for

the Velox 360, but this would not be desirable for most high volume applications as it would require a significant amount of time spent on manual paper preparation.

A number of background ions are observed with significant intensity in experiments using the Velox 360. This is also the case in experiments with the custom paper spray ion source, but the ions observed differ significantly. A mass spectrum for a sample of atrazine (1 ppm in acetonitrile) analyzed using the Velox 360 is shown in Figure 4.6. Protonated atrazine is observed at *m/z* 216.1, along with a variety of background ions ranging from approximately *m/z* 60 to *m/z* 612. The most prominent background species observed are a pair of ions at *m/z* 604.3 and *m/z* 609.4. These species are consistently observed when using the Velox 360, but are not detected in experiments with the custom paper spray ion source, which suggests that they derive from either the Velox 360 itself or the paper or plastic in the cartridges. The identity of these ions has not yet been ascertained; MS/MS experiments have so far been inconclusive. Background ions observed at lower masses vary significantly in intensity and identity with the mass spectrometer tuning parameters and spray solvent; these ions are consistently lower in intensity than the species at *m/z* 604.3 and 609.4. It is notable that these species are observed with such intensity when the mass spectrometer is tuned to optimize the signal intensity of protonated atrazine. If the instrument were tuned for higher mass species the ions at *m/z* 604.3 and 609.4 would likely be detected with even greater intensity. However, although the background ions observed with the Velox 360 can interfere with measurement of some analytes at low concentrations, they do not present a significant difficulty, especially when using tandem mass spectrometry to provide selectivity.

Regardless of the constraints imposed by the Velox 360, a number of advantages of this ion source are immediately apparent. Because the paper substrate and cartridge assemblies are mass produced, the labor of preparing paper strips by hand is eliminated. Additionally, the automated laser cutting system employed by Prosolia, Inc. is far more precise than the manual cutting used to prepare paper for the custom paper spray source. The cartridges also provide protection to the paper substrates in transit, preventing damage to the fragile tip or contamination by contact with other surfaces. Use of the cartridges enables automated mechanical positioning of the paper in front of the inlet, with the ion source itself mounted on the inlet via a fixed mounting flange. This reduces variability due to changes in the position of the paper tip relative to the inlet of the mass spectrometer, and eliminates the need for repeated optimization of the sample position.

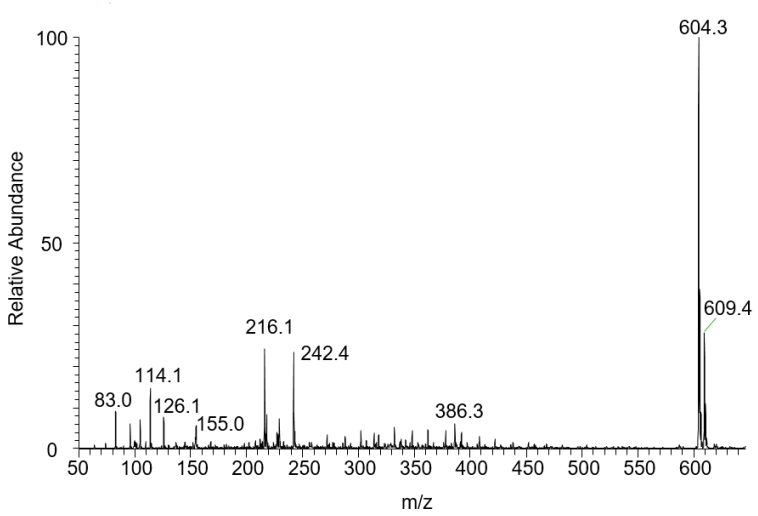

Figure 4.6: Mass spectrum of 1 ppm atrazine in acetonitrile, analyzed using the Velox 360, illustrating the major background ions observed. Ions at m/z 604.3 and 609.4 are observed consistently when using the Velox 360. A variety of ions at lower mass are also regularly observed, albeit at lower intensity.

4.5.2 Errors, Malfunctions, and Design Issues

A number of malfunctions and errors were encountered in early experiments with the Velox 360, ranging in severity from minor issues requiring only resetting a component to a consistent error that required an overhaul by Prosolia engineers. Of the issues encountered,

the least severe was an intermittent “stacker position unknown” error. This error refers to the linear actuator used to load cartridges from the vertical magazine into the ion source. If for some reason the linear actuator fails to retract fully or return completely to its initial position, as may happen if a cartridge is improperly loaded, this error may occur. It is readily dealt with by eliminating the source of the problem, such as a jammed cartridge, and resetting the position of the stacker using the diagnostic tools available *via* the instrument soft panel. This issue can generally be attributed to user error.

A more persistent and difficult to resolve problem was observed immediately upon installing the Velox 360 on the LTQ-FT XL mass spectrometer. Signal was intermittent and very low intensity at expected spray voltages (3-4 kV), and remained unstable at significantly elevated voltages (up to 8 kV) although signal intensity improved. Upon investigation it was observed that the spray voltage measured at the output from the mounting flange was significantly different from the voltage set in the instrument control software. At a voltage setting of 4 kV, the measured voltage output from the mounting flange was only 1.5 kV. Testing the Velox 360 with the mounting flange on another instrument yielded performance as expected at 5 kV, indicating that the problem was likely at the interface between the flange and our mass spectrometer. Investigating further, it was discovered that the high voltage contact at the mass spectrometer inlet had been displaced slightly, yielding poor electrical contact with the pin in the mounting flange. The high voltage contact in Thermo Scientific mass spectrometers is mounted in a flexible insulator. Through use, the contact may be displaced back into the instrument housing. This can be corrected fairly easily by removal of the front housing of the mass spectrometer and pushing the high voltage cable forwards, returning the contact to its original position.

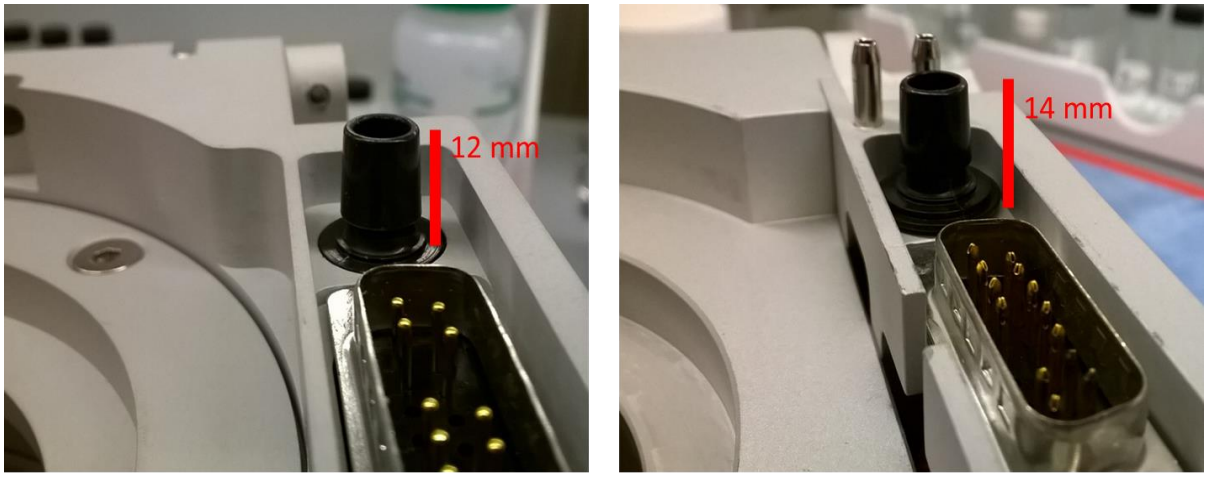

Figure 4.7: Differences between the high voltage contacts on the Velox and Thermo ESI sources. The high voltage contact extends 2 mm further from the face of the flange on the ESI source.

The ultimate cause of this malfunction is a slight difference between the design of the Thermo Scientific electrospray ionization source and the mounting flange of the Velox 360. The difference between the two is shown in Figure 4.7: the entire high voltage contact assembly extends approximately 2 mm further from the surface of the flange in the Thermo Scientific ESI source than in the Velox 360 mounting flange. The extra 2 mm in the ESI source is sufficient to maintain good contact even when the high voltage contact on the mass spectrometer is slightly displaced. This difference was reported to Prosolia, Inc. and is expected to be incorporated in future revisions of the Velox 360 mounting flange design.

The most persistent issue which arose was a “cartridge position unknown” error which began to occur shortly after receipt of the Velox 360. This error indicates that the carousel has not completed its rotation to the next position properly. If this error occurs while operating in manual mode, simply pressing “next” will frequently cause the carousel to rotate to the proper position and allow the experiment to continue. However, in automatic mode the error cannot be handled without stopping the experiment, causing the loss of any cartridges which may already have been wetted. The failure of the carousel to complete its rotation

properly is likely due to friction between the cartridges and the metal clip used to apply the spray voltage, exacerbated by loose pulleys in the carousel drive system (shown in Figure 4.8). Tightening the pulley mounting clamps in the drive system reduced but did not eliminate the problem, and the friction between the clip and cartridge could not be easily adjusted. The ion source was shipped to Prosolia, Inc. and overhauled by engineers there. Several minor issues were corrected, and the drive pulleys and belts were adjusted. After this, the problem was greatly reduced, and found to occur very infrequently. There appears to be some minor slippage of the carousel over time, but routine calibration is all that is required to prevent this from developing into a significant problem. Prosolia engineers have indicated that they plan to modify the pulley assembly design to incorporate a locking, D-shaped fitting between the pulleys and the drive shafts, which should minimize slippage.

These errors and malfunctions, while certainly problematic, were not significant enough to impede testing of the ion source. All hardware problems were effectively alleviated with the assistance of Prosolia engineers, and have not recurred with significant frequency. Our assessment is that these reflect the relatively early stage of commercialization of this technology, and the reliability of the Prosolia paper spray ion sources is likely to improve as further effort is made to refine and enhance the design.

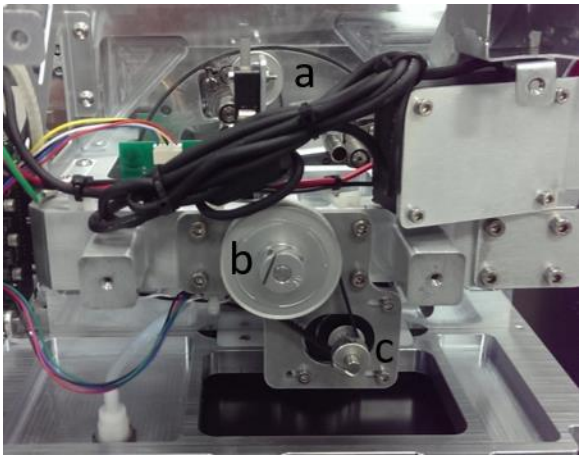

Figure 4.8: The Velox 360 carousel drive system. a) carousel; b) carousel drive pulley mounted on drive shaft; c) drive motor with pulley.

4.5.3 Direct Comparison to a Custom Paper Spray Source

A direct comparison of the Velox 360 and the custom paper spray ion source described in Chapter 2 was performed, with both ion sources installed on the LTQ-FT XL mass spectrometer. Solutions of atrazine in water were prepared at concentrations ranging from 1000 to 0.75 parts-per-billion (ppb) and spotted onto pre-cut and washed paper strips (100 µL aliquots, used with the custom paper spray ion source) and Velox cartridges (50 µL aliquots). Samples were dried at room temperature and an equal volume of 250 ppb atrazine-d_5 in LC-MS grade water was applied. Samples were analyzed after the internal standard solution was completely dry. The mass spectrometer was operated in what the Thermo instrument control software refers to as "selected reaction monitoring" mode, switching between MS/MS of protonated atrazine (*m/z* 216) and protonated atrazine-d_5 (*m/z* 221), and the signal intensity ratio of the primary product ions (*m/z* 174 and 179 from atrazine and atrazine-d_5, respectively) was plotted against atrazine concentration to yield the calibration curves shown in Figure 4.9.

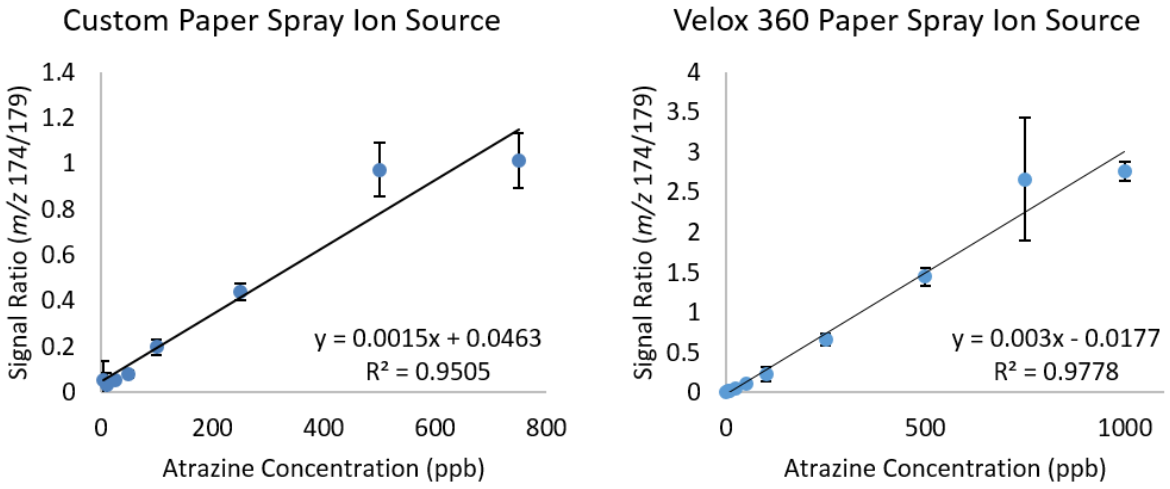

Figure 4.9: Calibration curves for measurement of atrazine in water using the custom paper spray source (left) and the Velox 360 ion source (right). Error bars represent one standard deviation.

Linearity and signal intensity were dramatically better when using the Velox 360, especially at low concentrations (<10 ppb) with a relatively concentrated internal standard (250 ppb). The reproducibility of the Velox 360 is far better than that of the custom paper spray source. With the exception of the point at 750 ppb, all relative standard deviations for the Velox ion source were less than 15%, with most below 10%. The cause of the high standard deviation for the 750 ppb measurement is unknown. To assess the viability of the ion sources for measurement of significantly lower concentrations, similar experiments were performed using an internal standard concentration of 1 ppb and analyte concentrations from 0.75 to 5 ppb. A sense for the relative reproducibility of the two sources can be gained from the data shown in Table 4.2, below, which includes the measured signal intensity ratios for the low concentration experiments. The average relative standard deviation for measurements below 5 ppb using the Velox 360 was 5.7%, compared to 21.3% for the custom source. This is consistent with expectations based on the greater consistency of positioning, paper shape, and solvent application for the Velox 360.

Based on the low concentration results, the limit of detection (mean signal in the blank plus three times the standard deviation of the blank, converted to parts-per-billion

Table 4.2: Atrazine/atrazine-d_5 signal ratios at low concentrations, measured using the custom paper spray ion source and the Velox 360.

	Custom Paper Spray Ion Source			Prosolia Velox 360 Ion Source		
Conc. (ppb)	Mean Signal Ratio	Std. Dev.	%RSD	Mean Signal Ratio	Std. Dev	%RSD
Blank	0.39	0.08	20.7%	1.11	0.08	6.97%
0.75	0.56	0.12	21.1%	1.20	0.14	12.0%
1.0	0.65	0.05	8.1%	1.26	0.11	8.87%
1.5	0.78	0.38	48.6%	1.37	0.04	3.00%
2.0	1.07	0.17	15.6%	1.41	0.02	1.64%
3.0	1.24	0.14	11.16%	1.38	0.13	9.07%
5.0	1.61	0.38	23.9%	1.75	0.04	2.36%

using the calibration curve) for atrazine using the Velox 360 is 1.6 ppb, while the limit of detection using the custom source is 0.76 ppb. However, it is very difficult to obtain reliable measurements using the custom source; considerable operator skill is required in positioning it for maximum analyte signal intensity. Additionally, the ideal position differs from strip to strip due to subtle variation in the tip shape introduced by the intrinsic variability of the manual cutting method used with the custom ion source, so some adjustment can be necessary between samples. The Velox 360, in contrast, requires no adjustment between samples and is mounted directly on the mass spectrometer, ensuring consistent positioning. These experiments were conducted while troubleshooting the voltage application problem observed with the Velox 360. The slightly better limit of detection for the custom source may be due to a combination of the malfunctioning high voltage contact, which interfered with proper operation of the Velox 360 during these experiments, and the greater sample volume capacity of the custom source. The sample capacity difference is primarily a function of the paper material. If the volume used with the custom source is reduced to that used with the Velox 360, a moderate reduction in signal intensity would be expected based on previous experiments with the custom source.

The Velox 360 is far easier to use than the custom paper spray source even if, in the case of ongoing carousel errors, it can only be operated in manual mode. The automated sample loading and positioning in the Velox 360 is a major advantage, as it eliminates the manual positioning of the paper in the metal sample holder of the custom source and also does not require frequent adjustments to maintain the optimized paper position in front of the mass spectrometer inlet. The availability of mass-produced cartridges also dramatically reduces the time required for preparation of samples relative to the custom source, as well as

improving the reproducibility of the paper geometry. The disadvantages of the Velox 360 are the lack of a continuous flow of solvent, which limits experiment time, the relative lack of flexibility entailed in the use of mass-produced cartridges, and the cost of the cartridges, which at the time of this writing cost between $4.95 and $6.95 apiece.[6] The operational disadvantages are negligible relative to the significant improvements in reproducibility, ease of use, and automation the Velox 360 provides. The simplicity of the custom source, while minimizing frustrating mechanical and software malfunctions, also renders it impractical to automate in any significant way. The Velox 360 is, therefore, strongly recommended as an alternative to the custom paper spray source described in Chapter 2 if the consumables cost can be justified for the application in question.

4.5.4 Analysis of Multiple Analytes

Samples containing both atrazine and propazine were analyzed to confirm that measurement of atrazine was not affected by the presence of a similar compound. Atrazine and propazine solutions were prepared at concentrations of 400 ppb in water. Samples were prepared in three ways: a) 50 µL atrazine solution applied to cartridges and dried, followed by 50 µL of 250 ppb atrazine-d_5 solution (no propazine); b) 50 µL of a solution containing both atrazine and propazine at 400 ppb each was applied and dried, followed by atrazine-d_5 solution; c) 50 µL atrazine solution was applied and dried, followed by 50 µL propazine solution, followed finally by 50 µL atrazine-d_5 solution. Analysis of the three sample sets based on the calibration curve presented above yielded the data presented in Table 4.3.

Atrazine samples prepared without propazine yielded an average measured value of 401 ppb, a remarkably accurate concentration measurement. Error values were greater for samples including propazine, but never exceeded 15% for the average of three

measurements. The increasing deviation from the actual value was not random, but consistently yielded measured values greater than the target concentration. While the stated composition of the propazine indicated that it may contain some trace atrazine contamination, no significant atrazine signal is observed in experiments conducted using propazine alone, suggesting that the increased signal for atrazine is not due to contamination. The cause of the elevated atrazine signal in experiments with propazine is unknown. Regardless, the presence of propazine did not cause significant difficulty in detecting and quantifying atrazine with reasonable accuracy.

Table 4.3: Measured concentrations of atrazine in 400 ppb samples prepared with and without propazine present (400 ppb propazine).

Sample	Measured Conc. (ppb)	% Error
Atrazine Only	401.3	0.32 %
Atrazine+Propazine mixed	441.6	10.4 %
Atrazine+Propazine applied separately	457.6	14.4 %

4.5.5 Triple Quadrupole Experiments

To investigate the performance of the Velox 360 ion source with instruments other than the LTQ-FT XL, particularly platforms commonly used for quantitative analysis, a series of experiments were performed using a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer. Atrazine, simazine, and three metabolites (desisopropyl-atrazine, desethyl-atrazine, and desethyl-, desisopropyl-atrazine) in water were used as test analytes. The instrument parameters were set based on a previously published liquid chromatography-electrospray ionization-tandem mass spectrometry method[7] and further tuned using the instrument automatic tuning tools. Due to constraints on instrument time, it was not possible to rigorously optimize all settings for each analyte measured. The tuning settings used are listed in Table 4.4, and analyte-dependent settings in Table 4.5.

Excellent results were observed for atrazine, simazine, and desethyl-atrazine in water. Desisopropyl-atrazine and desethyl-, desisopropyl-atrazine performed poorly in all matrices. This is thought to be due to poor ionization and especially poor dissociation efficiency. The cause of the poor ionization efficiency is not clear, but it may be due to strong binding to the paper substrate. Similar poor ionization efficiency for desethyl-atrazine, desisopropyl-atrazine, and desethyl-, desisopropyl-atrazine has been observed with the custom paper spray ion source; this is not a function of the particular ion source, but the ionization technique.

Table 4.4: TSQ Quantum Ultra tuning parameters.

Parameter	Setting
Spray Voltage	3700 V
Capillary Temperature	300 °C
Capillary Offset	35 V
Tube Lens Offset	75 V
Q1 Peak Width	0.70
Q3 Peak Width	0.70
Scan Width	0.01

Calibration curves were generated for all analytes except for desethyl-, desisopropyl-atrazine using atrazine-d_5 (250 ppb in water) as an internal standard, and are shown in Figure 4.10. Limits of detection ranged from below 1 ppb for atrazine, simazine, and desethyl-atrazine to 38 ppb for desisopropyl-atrazine (summarized in Table 4.6). Limits of detection observed with the triple quadrupole platform were better than those observed previously using either paper spray ion source with the LTQ-FT XL or the custom ion source with the Bruker HCTultra. The linearity and reproducibility are better for all analytes than previously

Table 4.5: Analyte-dependent settings used for analysis of atrazine, simazine, and metabolites with the Velox 360 and TSQ Quantum Ultra triple quadrupole mass spectrometer.

Analyte	Parent Ion	Product Ion	Dwell Time (sec)	Collision Energy (eV)
Atrazine-d_5	221.100	179.100	0.010	20
Atrazine	216.100	174.100	0.020	20
Simazine	202.100	132.000	0.050	22
Desethyl-atrazine	188.050	145.950	0.050	20
Desisopropyl-atrazine	174.050	132.000	0.100	20
Desethyl-, desisopropyl-atrazine	146.000	104.000	0.100	20

observed with either ion source, likely due to the intrinsic sensitivity and duty cycle advantages of the triple quadrupole instrument design for quantitative experiments. Even desisopropyl-atrazine, which is detected with relatively poor sensitivity, can be measured effectively across two orders of magnitude using this ion source and mass spectrometer. Additionally, this mass spectrometer is several years old, and has been superseded for high sensitivity analyses by newer triple quadrupole instruments. It is likely that even lower limits of detection may be obtained with newer, more sensitive instrumentation.

Table 4.6: Limits of detection for atrazine, simazine, and metabolites measured using the Velox 360 coupled to the TSQ Quantum Ultra mass spectrometer.

Analyte	Calculated Limit of Detection
Atrazine	-0.06 ppb*
Simazine	-0.03 ppb*
Desethyl-atrazine	0.33 ppb*
Desisopropyl-atrazine	38 ppb

*Practical LOD approximately 0.5 ppb

4.5.6 Automated Data Analysis

An automated data analysis method for data collected using the Velox 360 ion source was developed using the Thermo Scientific Xcalibur mass spectrometer control and data analysis software package. The automated data analysis options in the Xcalibur software are designed for use with chromatographic peaks. The peak detection algorithms employed are not designed for use with ion sources, like paper spray ionization, which yield a minute or more of signal duration. However, by switching the spray voltage on after data collection begins and off before it ends, a "peak" of sorts is obtained and the software may be set to detect it. The key settings are in the "detection" tab of the processing method builder, shown in Figure 4.11. The baseline window should be set to as large a value as possible, which directs the program to search for the beginning and end of the peak over the maximum time

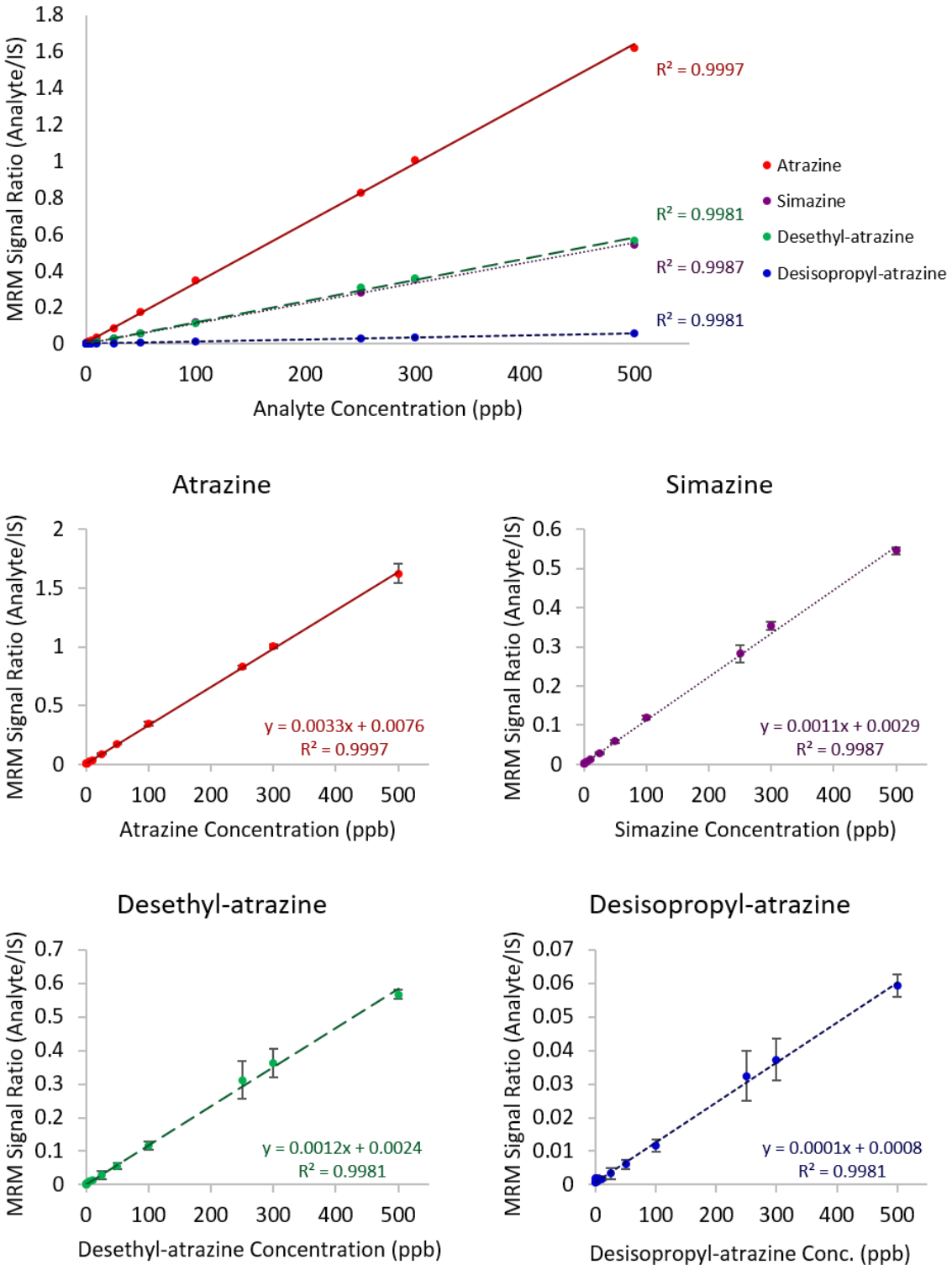

Figure 4.10: Calibration curves for atrazine, simazine, desethyl-atrazine, and desisopropyl-atrazine in water generated using the Velox 360 ion source coupled to a Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer. The top panel shows all analytes simultaneously (error bars omitted for clarity). The four lower panels show each analyte with error bars representing one standard deviation.

range. The peak noise and area noise factors should be set as small as possible. These settings adjust the peak signal threshold and peak edge detection threshold.

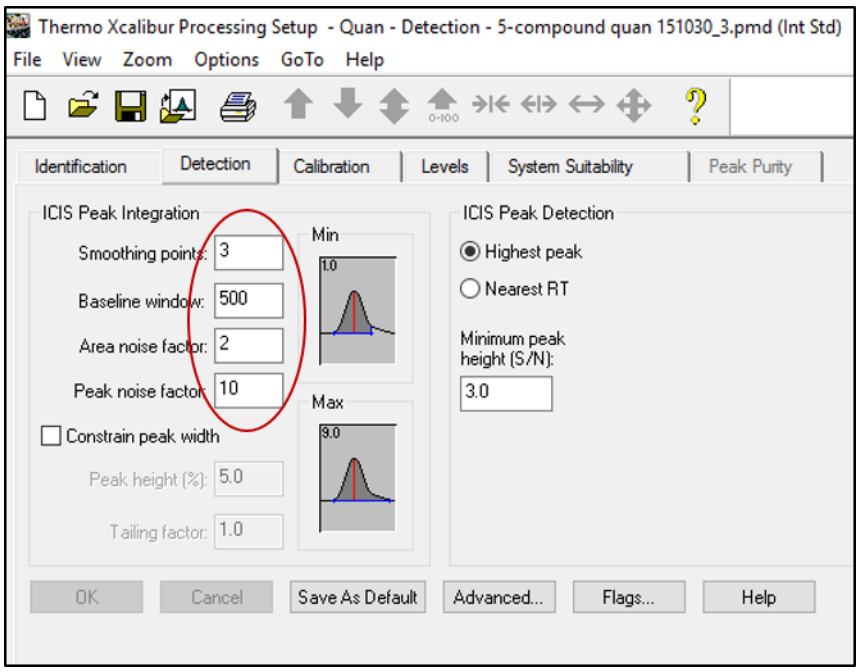

Figure 4.11: The detection tab of the Thermo Xcalibur processing method development program. The circled parameters must be adjusted to achieve automated peak integration.

In the "advanced" settings accessible from the detection tab, shown in Figure 4.12, the multiplet resolution should be set to the largest possible value and the minimum peak width set to a value reflecting the number of scans collected, which is instrument dependent. Setting the multiplet resolution high prevents the program from dividing the signal into multiple "peaks". Because only one peak is present, and its w idth is known, these settings should be set to large values to prevent misidentification of multiple

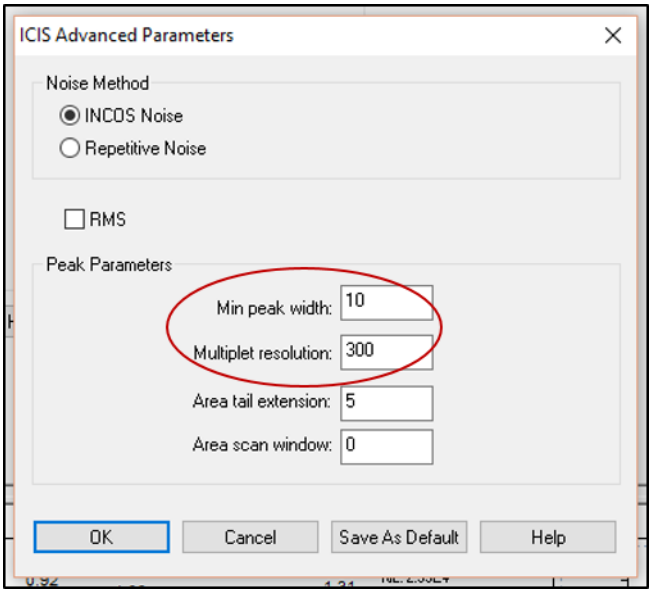

Figure 4.12: The advanced peak detection parameters in Xcalibur. The indicated settings must be changed to ensure that the signal is considered a single peak for data analysis purposes.

peaks. A set of typical settings for automated analysis of data collected using the Velox 360 with the Thermo Scientific TSQ Quantum Ultra is shown in Table 4.7.

Table 4.7: Typical settings for automated signal integration for data collected using the Velox 360 with a triple quadrupole mass spectrometer.

Parameter	Value
Peak detection algorithm	ICIS
Smoothing points	3
Baseline window	500
Area noise factor	2
Peak noise factor	10
Constrain peak width	OFF
ICIS peak detection	Highest peak
Minimum peak height (S/N)	3.0
Noise method	INCOS Noise
RMS	OFF
Min peak width	75
Multiplet resolution	300
Area tail extension	5
Area scan window	0

This method enables the automatic analysis of quantitative data collected using the Velox ion source in the same manner as LC-MS data would be analyzed. The algorithm does not identify the signal as a single peak with complete efficiency, and in some cases manual integration is required, especially when designing a new method. Regardless, the time required is dramatically reduced compared to manual data analysis by exporting extracted ion current traces into a spreadsheet, which is the primary data analysis method used with the custom paper spray source.

4.6 Summary and Conclusions

The first commercially available paper spray ion source, the Velox 360 produced by Prosolia, Inc. has been investigated and characterized with several pesticides of regulatory interest. This ion source, while still open to improvement in some mechanical details, is certainly suited to use in qualitative and quantitative analyses of common pesticides such as the triazine family of herbicides. The Velox 360 is compatible with current and recent model Thermo Scientific mass spectrometers equipped with an atmospheric pressure inlet. Quantitative data collected using the Velox 360 coupled to these instruments is amenable to automated data processing using the same tools used for peak identification and integration in liquid chromatography-mass spectrometry experiments, although some adaptation is

required. The Velox 360 is also suited to the simultaneous analysis of multiple analytes, particularly when coupled to a triple quadrupole instrument operated in multiple reaction monitoring mode.

Several mechanical problems and minor design flaws were identified; all were reported to Prosolia, Inc. and resolved with assistance from Prosolia engineers. The majority of these problems are amenable to correction through minor design changes. Despite these difficulties, the Velox 360 is far easier to use than the custom ion source described in Chapter 2, and provides significantly better overall performance. In particular, the reproducibility of the Velox 360 is dramatically better than the custom ion source, an improvement that derives from the more precise mounting system, reproducible sample positioning, and reliable mass produced sample cartridges. The only significant feature lacking in the Velox 360 is provision for the continuous application of solvent, which would be advantageous in performing instrument tuning and experiments investigating the behavior of samples over a longer period of time than is practical with the current design. However, this is not an essential feature, and the improvements in reproducibility, automation, ease of use, and expert support are sufficient to justify use of the Velox 360 rather than a custom ion source for routine analytical applications.

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CHAPTER 5: PAPER SPRAY IONIZATION-MASS SPECTROMETRY FOR ANALYSIS OF COMMERCIAL PESTICIDE FORMULATIONS

5.1 Introduction

Pesticide formulations are an exceptionally challenging matrix for the analytical chemist. Designed to ensure the stability and effective distribution of the active ingredients of the pesticide, the exact compositions of these formulations are not publically disclosed.[1,2] Pesticides may be dispensed in a variety of ways and as such there are a large number of different classes of formulations, ranging from finely-divided powders or dusts to organic solvent-based solutions to be emulsified with water, or even microencapsulated particles suspended in solvent.[1–3] While the composition of these matrices has been carefully engineered to deliver the desired quantity of pesticide to the target efficiently and in a controlled fashion, many formulations are effectively incompatible with conventional ionization techniques for mass spectrometry. Inspection of public documents such as safety datasheets reveals that many formulations include significant quantities of surfactants, emulsifiers, and wetting agents.[2,4,5]

These chemicals play a number of critical roles, such as ensuring effective mixing with water, controlling droplet size in the spraying process, and influencing the interactions between the droplet and the plant surface.[1] However, many surfactants are known to produce significant signal suppression in electrospray ionization-mass spectrometry, interfering with analysis of minor components of the solution.[6] Some surfactants have been identified as compatible with electrospray ionization, but these are the exceptions rather than the rule.[7]

Similarly, the presence of significant quantities of salts and particulates can pose major problems for spray-based ionization techniques, as these can not only alter the mass spectrum through ionization suppression but also cause clogging of spray emitters and contamination of ion source surfaces.

Interfering species can be excluded to some extent by desalting, filtration, and extraction-based sample preparation procedures. However, these sample clean-up procedures increase analysis cost, due to the need for consumables such as solid-phase extraction cartridges and filtration membranes, and analysis time due to the hands-on nature of most sample preparation options. Alternative approaches have been investigated, including spectroscopic analytical techniques (particularly Fourier transform-infrared spectroscopy), but without sample preparation prior to measurements these techniques are generally applicable primarily to the active ingredients and other components present at high concentrations in the formulation, with limits of detection near 0.1 % by mass.[8,9] More sensitive spectroscopy methods and chromatography-based techniques are highly dependent on pre-injection sample preparation to eliminate interfering species, and have generally been aimed at ensuring the correct quantity of active ingredient is present rather than investigating trace impurities,[10–16] or have only been tested with relatively simple formulations.[17]

Paper spray ionization-mass spectrometry is an alternative approach to the analysis of pesticide formulations which mitigates many of these problems, particularly those relating to clogging or fouling of spray emitters. Unlike the metal, glass, or fused silica emitters used in most variants of electrospray ionization, paper spray employs a porous substrate as a spray emitter.[18] The paper emitter is not limited to a single flow path like typical electrospray emitters based on tubular capillaries. Instead, liquid wicks forward to the sharp tip by

capillary action, moving along a variety of different paths through the interior of the paper and along the surface.[18] Paper spray therefore is not subject to "clogging" by particulate matter or salt precipitates, as there is no single channel to be blocked.[19]

Paper spray ionization has also previously been employed with other troublesome matrices, such as algae,[20] whole blood,[21–23] and urine,[23,24] although some matrix components, such as salts, have imposed limitations in some cases.[23] Based on the general high tolerance of paper spray ionization for complex and otherwise difficult to analyze matrices, quality assurance in pesticide formulations seemed a natural application area. Since the same factory and equipment may be used for the production of multiple pesticides, it is essential that testing methods be in place to detect any trace level cross-contamination of products. The simple paper-based sampling and automated operation offered by the newly commercialized paper spray source described in Chapter 4 offers a potential solution to the problem of rapid quality assurance analysis of pesticide formulations, and the part-per-billion level limits of detection in water and solvent matrices suggest that detection of part-per-million level impurities in more complex matrices ought to be attainable.

5.2 Materials and Methods

Samples of pesticide formulations, formulation blanks (matrix only, no active ingredient), and pesticide standards were provided by Syngenta Crop Protection, LLC (Greensboro, NC). Deuterated atrazine was purchased from C/D/N Isotopes (Pointe-Clair, QC, Canada), and deuterated metolachlor was purchased from Crescent Chemical (Islandia, NY). Solvents and acetic acid were of LC-MS grade (Fisherbrand Optima) and were purchased from Fisher Scientific (Fair Lawn, NJ).

Experiments were conducted using both the custom paper spray ion source described in Chapter 2 and the Velox 360 commercialized paper spray ion source described in Chapter 4. The same Whatman #903 filter paper and Velox sample cartridges described earlier were employed for all analyses. Experiments using the Velox 360 ion source were carried out using two instruments: a Thermo Scientific LTQ-FT XL linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer operated in ion trap-only mode, and a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer. The custom paper spray ion source was used in only one experiment, coupled to a Waters Quattro LC triple quadrupole mass spectrometer.

Samples of pesticide standards were spiked into formulation matrices at part-per-million concentrations. Aliquots of formulations spiked with pesticides were applied to paper strips or Velox cartridges for analysis as described in Chapters 2 and 4. Most experiments were performed using atrazine as a test analyte; instrument parameters were the same as those used for measurement of atrazine in simpler matrices (see Chapter 4). Unless otherwise stated, 90/10/0.1 acetonitrile/water/acetic acid was used as spray solvent for experiments with the Velox 360 ion source.

5.3 Preliminary Experiments

The first experiments carried out investigating the potential use of paper spray ionization for quality assurance analysis of pesticide formulations employed the custom paper spray source with a Waters Quattro LC triple quadrupole mass spectrometer, operated in full scan mode. A sample of the Karate EC insecticide formulation, containing the active ingredient lambda-cyhalothrin, was spiked with malathion (18.33 ppm), another common insecticide, simulating a sample of Karate product containing a residual impurity from cross-

contamination in manufacturing. The Karate EC formulation is an oily liquid that forms an emulsion in water; the safety datasheet indicates that it contains naphthalene, mineral oil, and petroleum-based solvents in addition to proprietary ingredients.[4] A 50 µL aliquot of the spiked Karate EC formulation was applied to a pre-washed strip of #903 paper and allowed to dry for five minutes before analysis (strips washed as described in Chapter 2). Samples were ionized using the custom ion source using 80/20/0.1 acetonitrile/water/formic acid (all Optima grade) as spray solvent at a flow rate of 20 µL/min and 5 kV spray voltage. A mass spectrum of the Karate EC formulation spiked with 18.33 ppm malathion is shown in Figure 5.1. While the identity of the base peak and the low mass species present was not determined, both the active ingredient and the simulated contaminant were detected as protonated molecules. The intensity of the peak believed to be protonated malathion (*m/z* 331) was poor, but discernable. The fact that a part-per-million level component could be detected at all in the presence of a highly complex matrix and 13.1% active ingredient suggested that further

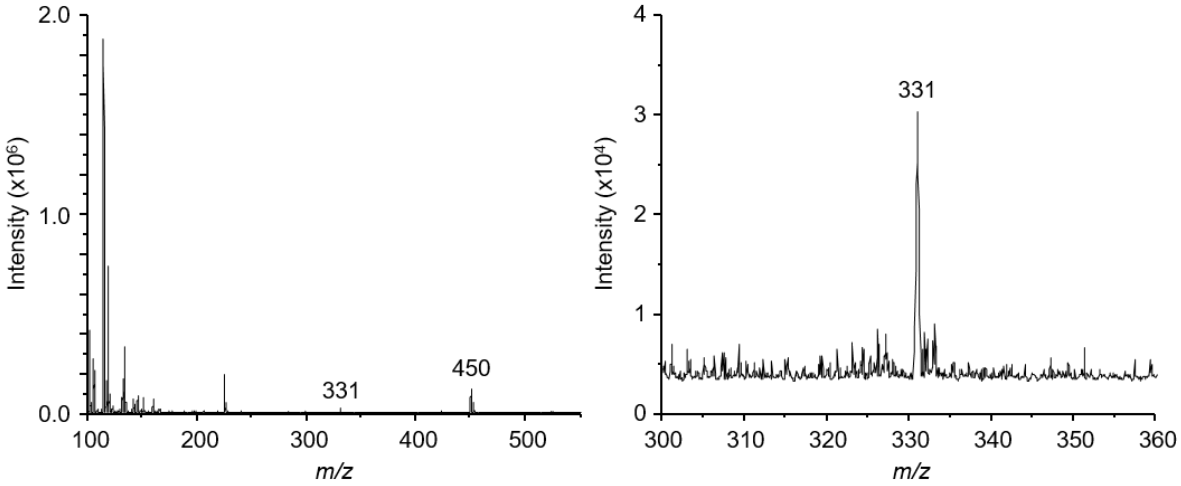

Figure 5.1: Left: Mass spectrum of Karate EC formulation spiked with 18.33 ppm malathion. The active ingredient, cyhalothrin, is observed as a protonated molecule at m/z 450. Right: Zoom on the region of the spectrum from m/z 300 to 360. The simulated contaminant, malathion, is believed to be observed as a protonated molecule at m/z 331.

investigation of paper spray for detection of trace contaminants in pesticide formulations would be worthwhile.[4]

5.4 Formulation Blanks

To more systematically evaluate the potential of paper spray ionization-mass spectrometry for quality assurance analysis of pesticide formulations, experiments were conducted using a variety of formulations without the active ingredients ("formulation blanks"). A selection of formulation blanks were provided by Syngenta Crop Protection, LLC. The exact compositions of these formulation blanks were not of interest for these experiments, but a general idea of the types of compounds present could be obtained from safety datasheets. Typical ingredients include surfactants, glycerol, and petroleum-based solvents. All formulation blanks investigated were liquids; no particulate-based blanks were used.

Samples of formulation blanks without added analytes were applied to Velox sample cartridges and analyzed to determine whether these matrices were compatible with the Velox 360 ion source. Four of the six formulation blanks yielded reasonably stable mass spectra; examples are shown in Figure 5.2. The two formulation blanks which failed to produce stable ion signal were both relatively viscous organic matrices. These solvents did not evaporate at room temperature and were effectively immiscible with the acetonitrile/aqueous spray solvent. The formulation blanks that did yield stable ion signal were generally less viscous and tended to evaporate, at least in part.

The matrix ions observed vary dramatically between the different matrices. Most matrices investigated yield a variety of relatively intense ions between 80 and 250 Da, but in some cases a broad distribution of ions at higher masses is observed. The identity of the

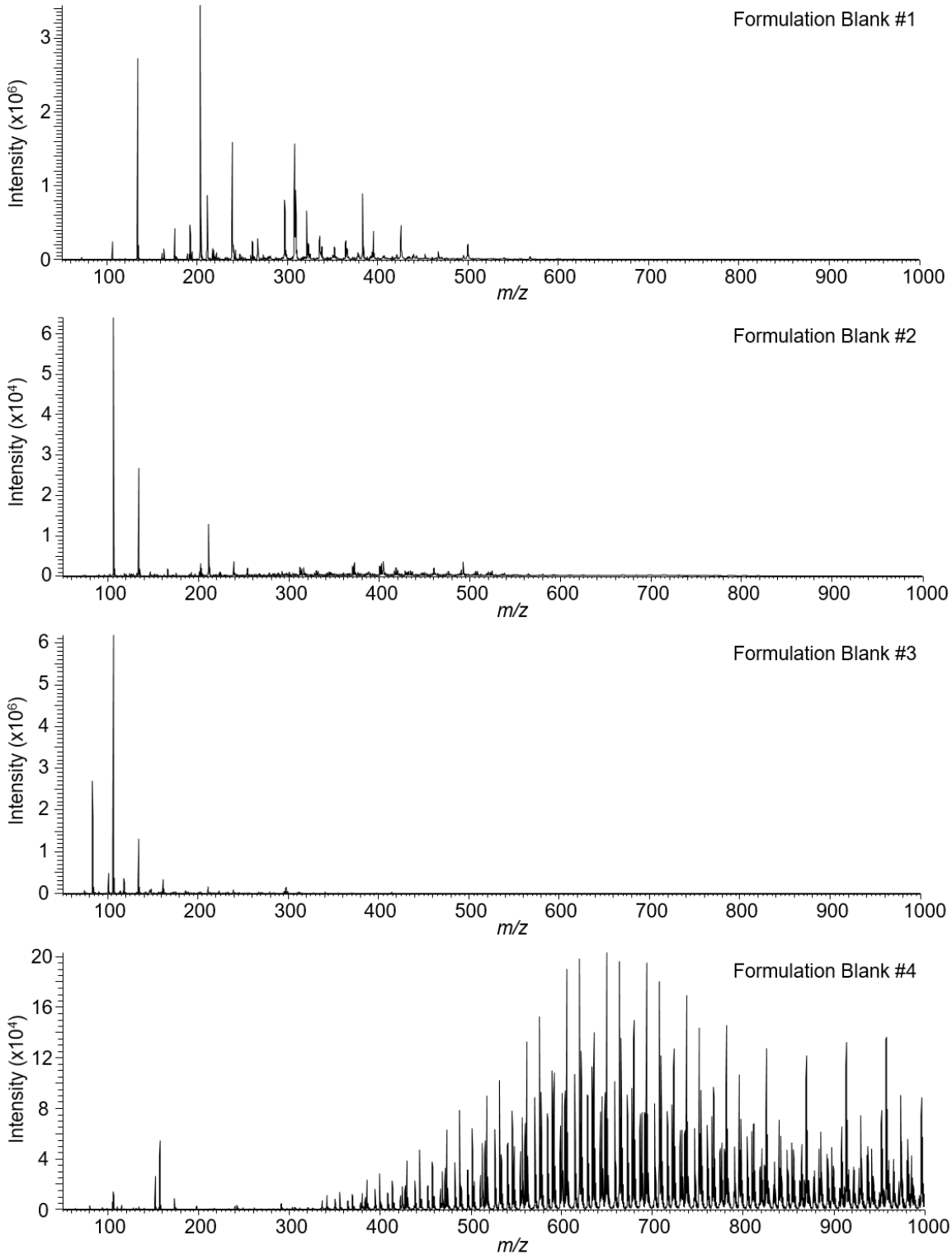

Figure 5.2: Mass spectra of four formulation blanks analyzed using the Velox 360 ion source.

background species was not investigated as this was not relevant to the project objectives. In general, few matrix species are observed in the 200-300 Da region where many pesticides of interest are observed (e.g., atrazine, propazine, metolachlor, thiamethoxam). The primary difficulties presented by the matrix ions are ionization suppression and the limited ion capacity of the linear ion trap mass spectrometer used in most of these experiments. A greater problem is the poor miscibility of the spray solvent with some formulations. While it may be possible to achieve some success in these cases by adjusting the spray solvent composition, dilution or other sample preparation may be required.

The feasibility of measuring atrazine and similar pesticides at trace levels in pesticide formulations was investigated using the four formulation blanks which did generate stable ion signal when analyzed by paper spray using the Velox 360. Initial testing of formulation blanks spiked with atrazine at part-per-million levels were not promising. A representative example of the challenge of detecting atrazine in these matrices is the analysis of 10 ppm atrazine in one of the least challenging matrices, formulation blank #3. A mass spectrum of this sample analyzed using the Velox 360 ion source is shown in Figure 5.3. This spectrum is quite similar to that shown in Figure 5.2; there is no notable protonated atrazine peak visible upon first inspection (*m/z* 216). Even zooming in around *m/z* 216 (Figure 5.3, inset) only a very small peak at the mass of protonated atrazine can be discerned. Results obtained using tandem mass spectrometry are slightly more promising. MS/MS of *m/z* 216 from samples of formulation blank #3 containing 10 ppm atrazine yields the spectrum show in Figure 5.3b. The dominant product ion is *m/z* 174, from the neutral loss of propylene, and minor products are observed at *m/z* 188 and 146 corresponding to the loss of ethylene and both ethylene and propylene, respectively. However, the overall signal intensity is very low and a number of

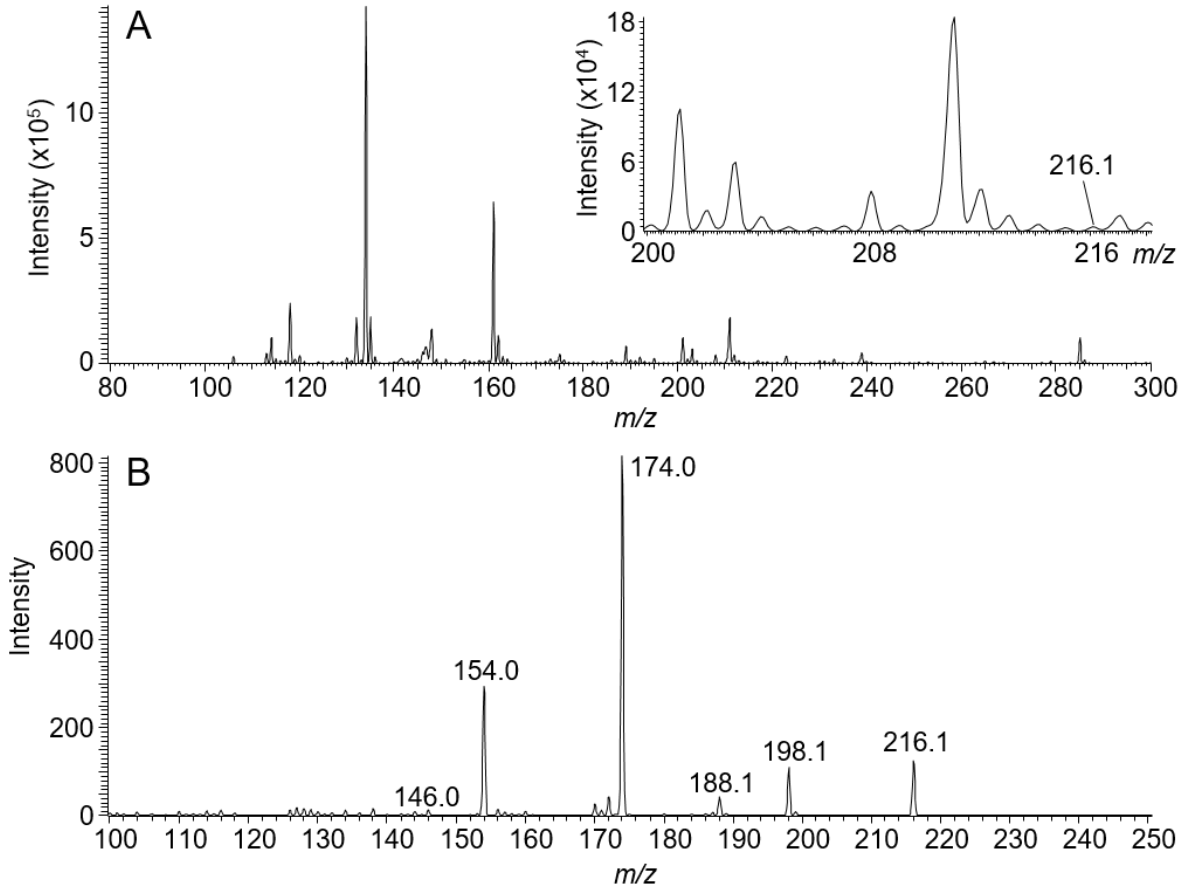

Figure 5.3: Mass spectra of formulation blank #3 spiked with atrazine (10 ppm) and ionized using the Velox 360 paper spray ion source. A) Mass spectrum with inset zoom around m/z 216. B) MS/MS of protonated atrazine (m/z 216).

other product ions are observed which do not correspond to known atrazine dissociation products. This suggests two conclusions. Firstly, the sensitivity of this method for detection of atrazine is significantly reduced by the presence of the formulation blank. Second, there is another species present at *m/z* 216 which is responsible for the product ions such as *m/z* 154 and 198 which do not correspond to known collision-induced dissociation products from protonated atrazine. Both of these factors are problematic, but not insurmountable.

To address the difficulties arising from the formulation matrices, two methods were investigated. First, in an effort to separate the heavy organic solvents which are immiscible with the spray solvent, samples were washed with hexanes (HPLC grade, Fisher Scientific,

Fair Lawn, NJ) after application to the paper substrate. The hexanes should be miscible with the organic solvents, but will not dissolve atrazine or similar polar pesticides. Hexanes are also sufficiently volatile that any remaining solvent should evaporate from the paper before analysis. To confirm that the hexanes would not wash away the analyte, samples of atrazine dissolved in acetonitrile were applied to paper strips, allowed to dry, and washed with 1 mL hexanes. Washing was performed by inserting the strips into a wire clip with the pointed tip hanging down, followed by rinsing with hexanes on both sides using a pipette. The volume of hexanes was sufficient to thoroughly wet the paper with excess solvent flowing down from the tip of the paper into a catch basin. After washing, any remaining hexanes were allowed to completely evaporate from the strips. The washed samples were then analyzed using the custom paper spray ion source and compared to samples that were not washed with hexanes. The hexane wash did not affect the signal intensity for protonated atrazine. Washing with hexanes was then tested using the heavy organic-based formulation blanks that did not generate stable signal in initial experiments. A slight improvement was observed in experiments using the custom paper spray ion source, but not when working with the Velox 360 ion source. The difference in performance is likely due to the continuous solvent application capability of the custom ion source, which allows enough time for the solvent to wick through the paper even impeded by remaining matrix components. Regardless, the minor improvement obtained was not worth pursuing further.

The second method investigated to reduce the impact of the formulation blank matrix was simple dilution. Samples of formulation blanks spiked with atrazine were diluted 10- or 100-fold in acetonitrile and then applied to paper substrates for analysis by paper spray ionization-mass spectrometry. A mass spectrum of a sample of formulation blank #5, an oil-

based formulation blank, spiked with atrazine and metolachlor (50 ppm each), diluted 10-fold and applied to Whatman #598 filter paper and ionized using the custom paper spray ion source is shown in Figure 5.4. Without dilution no signal at all could be observed using this matrix; the hydrophobic, non-volatile oleic acid substrate did not evaporate and prevented free flow of the spray solvent. After 10-fold dilution a stable mass spectrum is observed, with clear protonated atrazine and metolachlor peaks (*m/z* 216 and 284, respectively). MS/MS experiments yield unambiguous atrazine and metolachlor product ions (*m/z* 174 and 146 for atrazine and *m/z* 252 for metolachlor, shown in Figure 5.4 B and C, respectively). The protonated atrazine and protonated metolachlor signal intensity is somewhat lower than would be expected in a water or acetonitrile matrix, but is easily high enough for reliable operation. Similar results are observed with other matrices using the custom paper spray ion source.

Results were not as favorable using the Velox 360 paper spray ion source. Testing with 10-fold diluted formulation blanks spiked with atrazine and metolachlor yielded a moderate improvement over undiluted formulations, but atrazine and metolachlor were observed at extremely poor signal intensities. However, samples diluted 100-fold in acetonitrile yielded dramatically higher atrazine and metolachlor signal intensity as well as much more stable signal overall. This is believed to be due to a reduction in ionization suppression after dilution, as the species expected to interfere with ionization, such as surfactants, are much lower in concentration after dilution. Additionally, solvents that are poorly miscible with the spray solvent will be significantly dispersed, reducing their effects on flow of solvent through the paper. Velox 360-LTQ MS/MS spectra of protonated atrazine

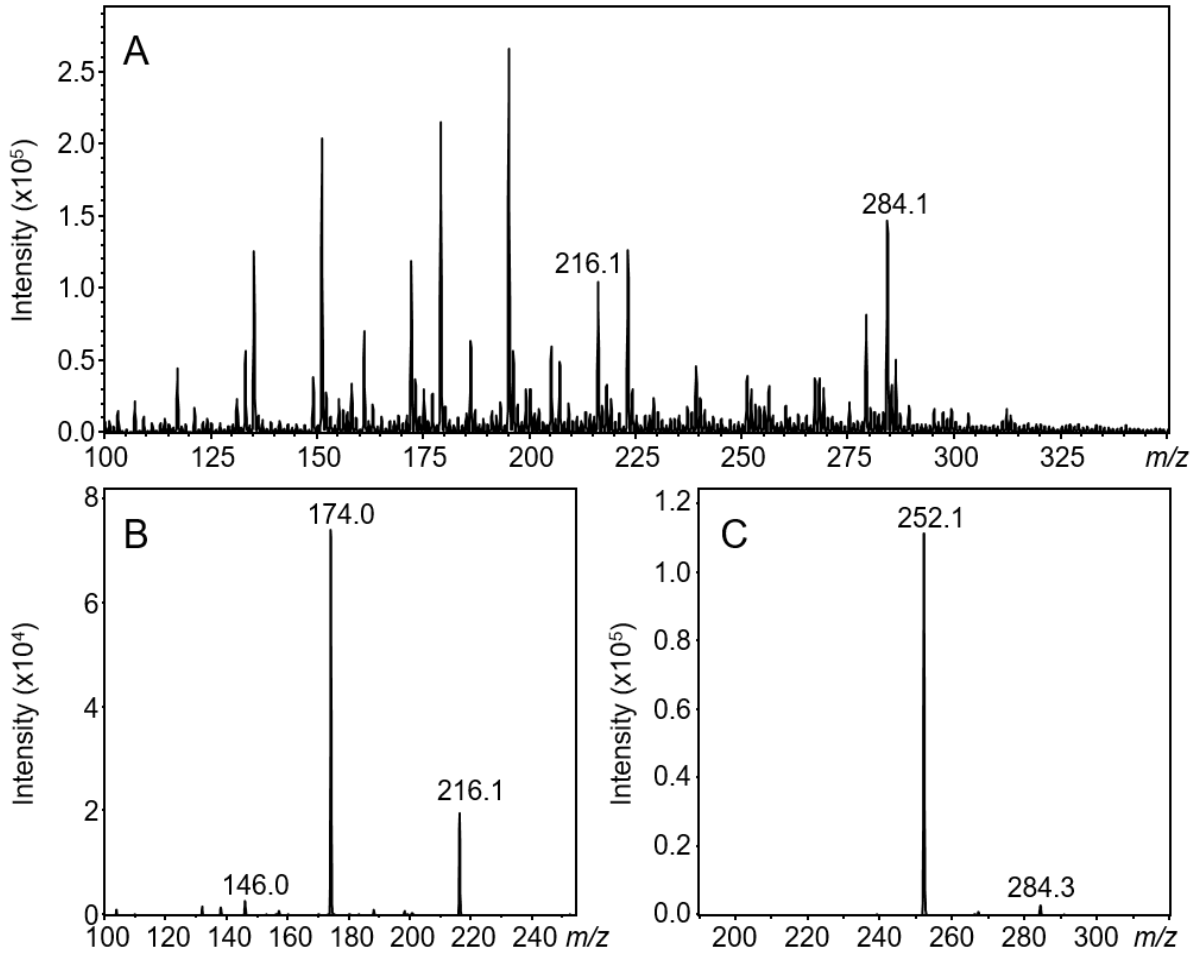

Figure 5.4: Mass spectra of atrazine and metolachlor spiked into formulation blank #5 (50 ppm each), diluted 10x and ionized using the custom ion source. A) Mass spectrum; a variety of matrix species and protonated atrazine (m/z 216) and metolachlor (m/z 284) are observed. B) MS/MS of protonated atrazine. C) MS/MS of protonated metolachlor.

and protonated metolachlor from 10-fold and 100-fold diluted samples of formulation blank #6 containing 50 ppm atrazine and metolachlor are shown in Figure 5.5. This matrix, like formulation blank #5, did not yield stable signal without dilution.

While the 10-fold dilution of formulation blank #6 spiked with atrazine and metolachlor yielded the expected product ions for atrazine and metolachlor, the signal intensity was far too low to be analytically useful. In contrast, after 100-fold dilution, the signal intensity of the atrazine and metolachlor product ions increased by a factor of 200-2000, reaching levels suitable for quantitative experiments.

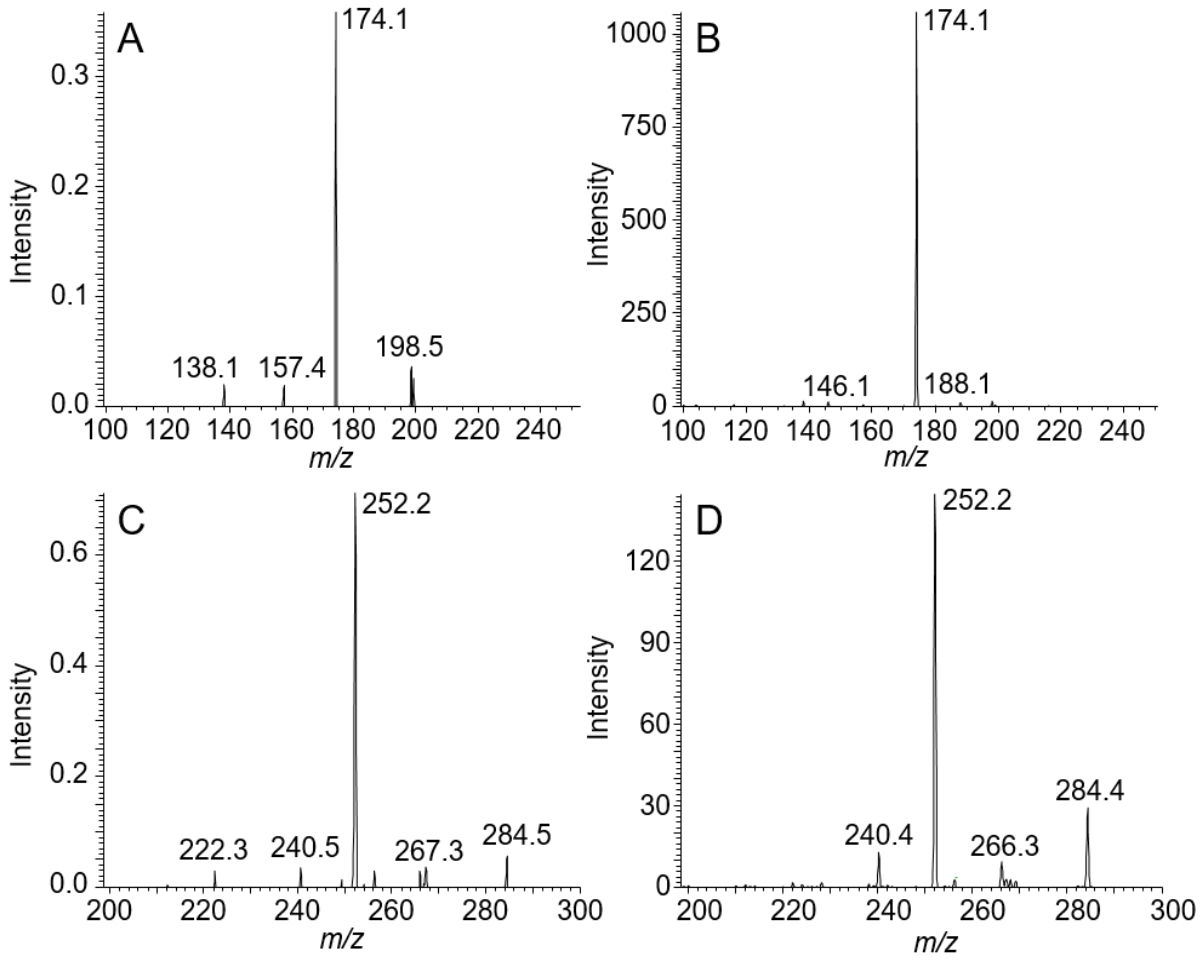

Figure 5.5: MS/MS spectra of samples of formulation blank #6 spiked with atrazine and metolachlor (50 ppm each) and diluted 10x and 100x in acetonitrile. A) Protonated atrazine, 10x dilution. B) Protonated atrazine, 100x dilution. C) Protonated metolachlor, 10x dilution. D) Protonated metolachlor, 100x dilution.

5.5 Formulations with Active Ingredients

Several samples of commercial pesticide formulations containing active ingredients were provided by Syngenta Crop Protection, LLC and spiked with other pesticides at part-per-million concentrations to simulate contaminated products. As with formulation blanks, the samples of commercial formulations were initially tested by direct, undiluted application to paper substrates for paper spray ionization-mass spectrometry analysis. Similar difficulties in achieving stable ion signal were encountered; the total ion current trace for a sample of

Quilt XCEL is shown in Figure 5.6. Quilt XCEL is a commercial fungicide formulation containing the active ingredients azoxystrobin and propiconazole; the formulation matrix includes propylene glycol and 1-octanol, but a large fraction of the formulation is not publically disclosed.[5] As Figure 5.6 illustrates, although some ion signal was observed spray was intermittent, pulsing from virtually no signal to intense bursts of ions. Similar behavior was observed in some of the more challenging formulation blank matrices.

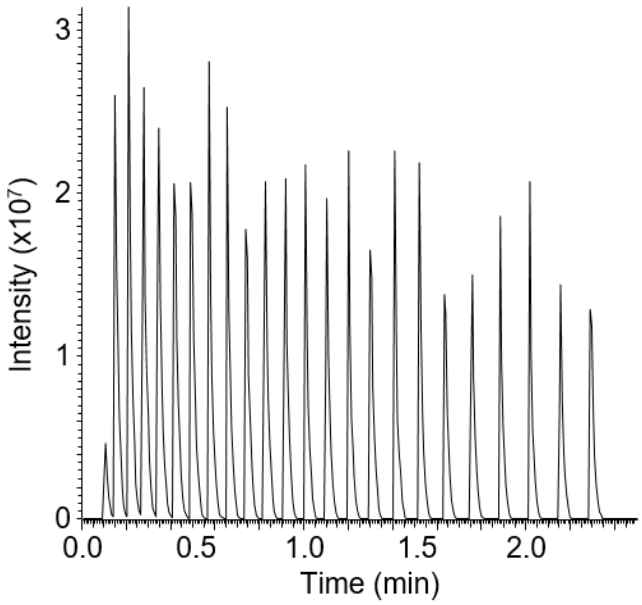

Figure 5.6: Total ion current trace for paper spray ionization-mass spectrometry of a sample of Quilt XCEL fungicide, ionized using the Velox 360.

Based on the results with formulation blanks, simple sample preparation methods based on dilution were expected to improve the stability of the ion signal in analysis of formulation samples containing active ingredients. Samples of 10- and 100-fold diluted formulations containing active ingredients applied to Velox sample cartridges were observed to generate stable ion signal when analyzed using the Velox 360 ion source; in general, the best results were obtained if samples were not dried after application to the sample cartridge but analyzed immediately. Indeed, in a quality control environment there would be no particular need for drying of the sample for transport as it would be desirable to minimize the lag between production and analysis to identify contaminated samples as rapidly as possible.

In general, the 100-fold dilution was preferable both in terms of signal stability and analyte ion intensity. The total ion current traces for samples of Quilt XCEL spiked with 50 ppm atrazine and diluted 10- and 100-fold are shown in Figure 5.7. In contrast to the intermittent signal observed from undiluted Quilt XCEL samples, in both the 10-fold and 100-fold dilution continuous ion signal is observed. However, in the 10-fold dilution sample significant variation is observed over time. The signal is initially somewhat irregular, with significant change in total ion signal between scans, leveling out towards the end of the run. In contrast, the 100-fold dilution sample yields highly consistent total ion signal throughout the analysis time. Additionally, the maximum total ion signal observed in the 100-fold dilution is an order of magnitude higher than the maximum total ion signal observed in the 10-fold dilution. In both cases, however, the actual ions observed do not vary significantly over the course of the run.

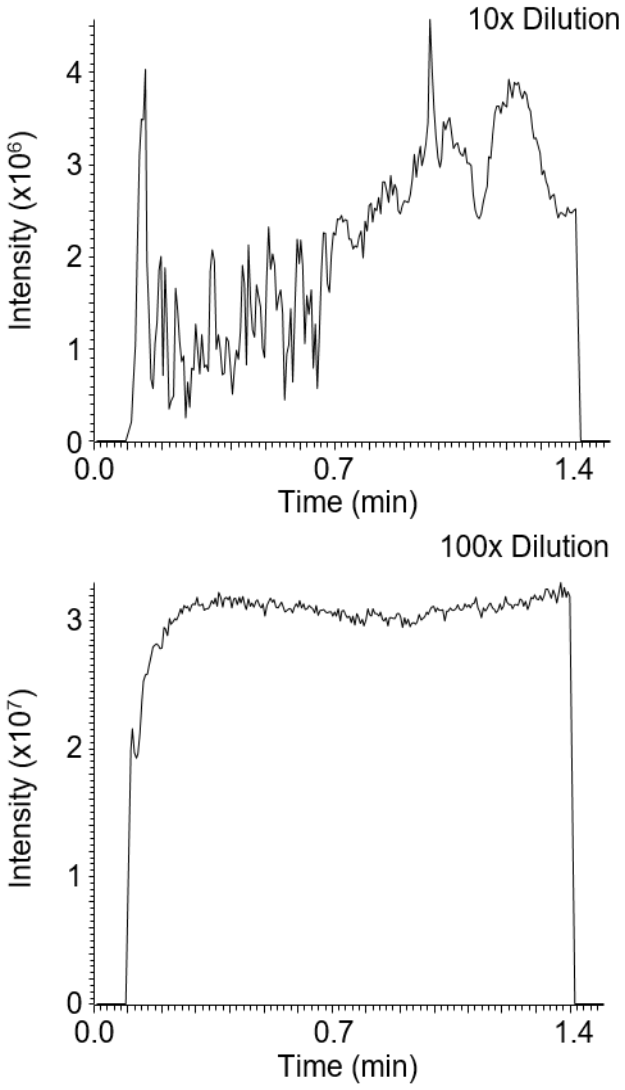

Figure 5.7: Total ion current traces for Quilt XCEL formulation samples spiked with atrazine (50 ppm) to simulate a contaminated product. Top: 10x dilution in acetonitrile followed by paper spray ionization. Bottom: 100x dilution in acetonitrile followed by paper spray ionization.

Representative mass spectra from each of these samples are shown

in Figure 5.8. Aside from an overall greater signal intensity in the 100-fold dilution than the 10-fold dilution, the principal difference between the two spectra is the near elimination of the distribution of ions between *m/z* 450 and 750. As might be expected, the dominant peak in both samples is *m/z* 342 - protonated propiconazole, one of the active ingredients in Quilt XCEL. The other active ingredient, azoxystrobin, is observed at much lower intensity at *m/z* 404. The peak at *m/z* 426 may be sodium-cationized azoxystrobin. In both cases, a small peak is observed at *m/z* 216 due to the atrazine added to the formulation as a simulated contaminant. Mass spectra zoomed to show this portion of the mass range are also shown in Figure 5.8. The intensity of the protonated atrazine peak is greater by a factor of about 16 in the 100-fold dilution than in the 10-fold dilution, consistent with observations in formulation blanks.

Samples of Quilt XCEL prepared in the same fashion (spiked with part-per-million quantities of atrazine, then diluted 100-fold) were analyzed using the Velox 360 coupled to a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer to investigate the potential viability of this technique using a typical platform for routine quantitative analysis. Three concentrations of atrazine in Quilt XCEL were used (1 ppm, 35 ppm, and 70 ppm) in addition to a "blank" containing no atrazine. Atrazine-d_5 was added in the dilution solvent (10 ppm final concentration) as an internal standard. Analysis parameters were the same as those described in Chapter 4 for the analysis of atrazine using the Velox 360 with the Thermo Scientific TSQ Quantum Ultra mass spectrometer. Analysis of these samples yielded the plot shown in Figure 5.9. While sensitivity (and therefore the practical limit of detection) are much poorer for atrazine in Quilt XCEL than in water or other simple matrices, the reproducibility of the atrazine internal standard product ion signal ratio was not

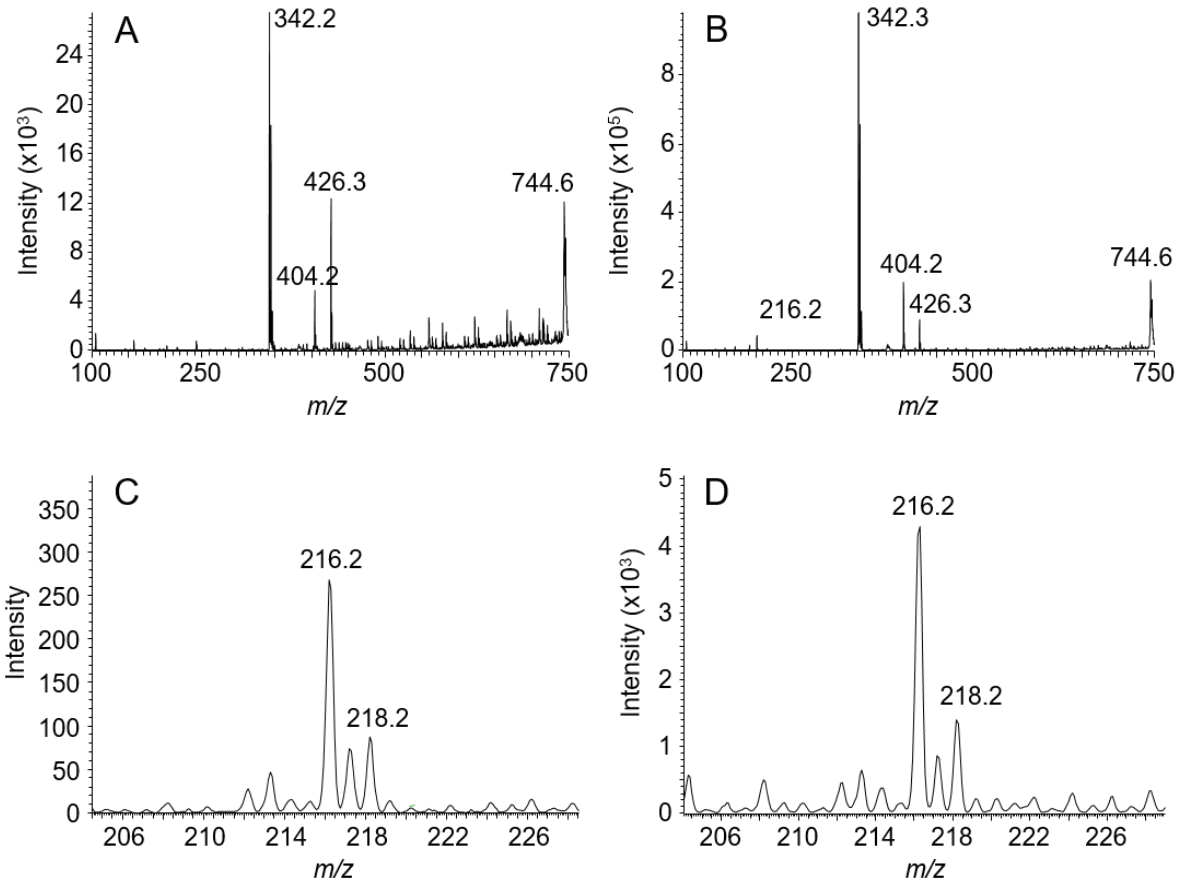

Figure 5.8: Mass spectra of Quilt XCEL samples spiked with atrazine (50 ppm) to simulate a contaminated product, diluted 10x (A and C) and 100x (B and D) in acetonitrile. A and B: Mass spectrum (100 to 750 Da). C and D: Zoom on protonated atrazine peak (m/z 216.2).

significantly worse. A general linear trend is clearly apparent, although there are insufficient points to reliably define a calibration curve.

In an effort to develop a more complete calibration curve, experiments using the same procedure were performed using the Thermo Scientific LTQ-FT XL. The results of a preliminary investigation using the usual spray solvent, 90/10/0.1 acetonitrile/water/acetic acid, are shown in Figure 5.10a. While a general trend is apparent, linearity is poor, especially at concentrations below 10 ppm. Several alternative solvent mixtures were investigated to determine if the use of alternative organic solvents in which atrazine is highly

soluble might improve the signal intensity for protonated atrazine. The results for two of these solvent blends are shown in Figure 5.10b and 5.10c.

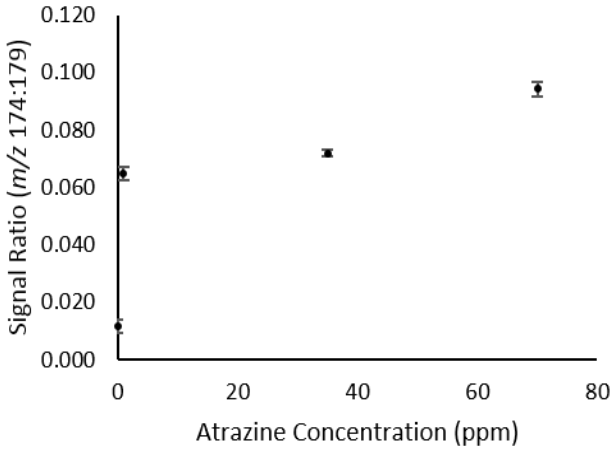

Figure 5.9: Atrazine spiked into Quilt XCEL samples analyzed using the TSQ Quantum Ultra triple quadrupole mass spectrometer after 100x dilution in acetonitrile.

A distinct improvement in linearity relative to 90/10/0.1 acetonitrile/water/acetic acid is observed with both 70/20/10/0.1 acetonitrile/isopropanol/water/acetic acid and 70/20/10/0.1 acetonitrile/acetone/water/acetic acid, although the reproducibility in all cases leaves much to be desired. Additionally, the atrazine signal intensity in the blank is significantly higher for both of the alternative solvent blends, which is undesirable. The practical limit of detection for all three blends remains approximately 10 ppm. However, it certainly appears possible to achieve at least approximate quantitation of "contaminants" at concentrations between 10 and 100 ppm in pesticide formulations with or without the presence of active ingredients using a simple dilution-based sample preparation procedure.

Dilution of pesticide formulations does have some disadvantages as a sample preparation method for paper spray ionization. The most obvious, perhaps, is that the trace components to be measured are diluted at the same time. For part-per-million level analytes this does not seem to be a significant problem, as the signal intensity gained by the dilution of the interfering species in the matrix is greater than the intensity lost through dilution, but it may limit the utility of this technique for analysis of analytes at lower concentrations. Another difficulty that can arise, depending on the formulation, is due to the imperfect

miscibility of the pesticide formulations and the acetonitrile used as dilution solvent. While some formulations dissolve or are dispersed more-or-less homogeneously in the acetonitrile, others form an unstable emulsion or contain particulates which settle out rapidly. Worse, in some cases formulation components which are effectively dispersed or dissolved in the original formulation aggregate when mixed with acetonitrile, forming a clearly separate globule or sediment. In cases like this, mixing with acetonitrile may be better characterized as an extraction rather than dilution, and care will need to be taken to ensure that the analyte of interest is efficiently extracted into the dilution solvent and not trapped in a semi-solid, inaccessible matrix. This has not been a major problem thus far, but it may be worth investigating methods to ameliorate this effect.

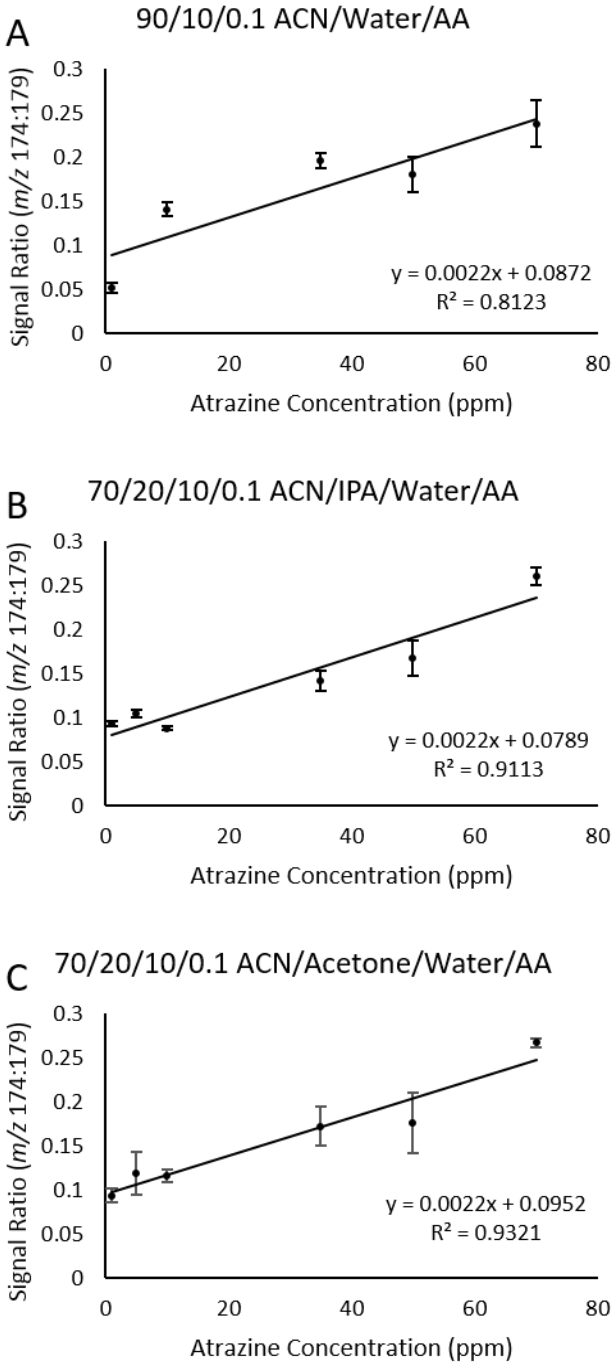

Figure 5.10: Calibration curves for atrazine spiked into Quilt XCEL, analyzed using several spray solvents.

One further problem warranting additional investigation is the transfer of material from the paper substrate to the surface of the mass spectrometer inlet during paper spray ionization experiments. Three general problems of this type have been observed: the contamination of the mass spectrometer inlet by high concentration species such as the active ingredients of pesticide formulations, clogging and contamination of the inlet by particulates from pesticide formulations, and the transfer of fine paper lint to the surface of the inlet, which may also contribute to clogging. All three of these issues appear to be related to the distance between the tip of the paper substrate and the mass spectrometer inlet. At short tip-inlet distances (<4 mm) transfer of liquid material from samples loaded with undiluted non-volatile liquid formulations can be visually observed. Similarly, at such distances a layer of white material (believed to be paper lint as it has been observed even in blanks) gradually appears on the outer surface of the inlet as paper spray experiments are conducted. Contamination of the inlet with active ingredients or analytes is observed as carryover between samples and appears much more frequently at tip-inlet distances less than 4 mm.

The likelihood of problems related to unintended transfer of material from the paper substrate to the inlet surface can therefore be reduced by simply increasing the tip-inlet distance. Greater tip-inlet distances generally require increased spray voltages, which limits the range of potential positions, but operation of the Velox 360 at tip-inlet distances of 4 and 6 mm is certainly viable. Operation at less than 4 mm is strongly discouraged, and some contamination or material transfer may still occur at 4 mm, although observed carryover is vastly reduced at 4 mm relative to 2 mm. Dilution of formulation samples in acetonitrile also reduces contamination, especially when combined with brief centrifugation to separate particulates from the supernatant.

5.6 Summary and Conclusions

Pesticide formulations present a unique challenge to the analytical chemist, and to the mass spectrometrist in particular, due to the high concentrations of compounds generally inimical to chromatography and conventional spray-based ionization techniques. While active ingredients, with concentrations in the percent range, can be measured using a variety of analytical techniques, it is more difficult to measure trace contaminants in the part-per-million range. Paper spray ionization-mass spectrometry provides a simple, rapid, and commercially available solution to this problem. The paper substrates used in paper spray ionization are not susceptible to the same problems with clogging and contamination as electrospray capillary emitters, and the automation features of the commercial ion source used enable rapid, bulk analysis with relatively little user input required.

Paper spray-based analytical methods were developed using atrazine and metolachlor as simulated contaminants spiked into formulation blanks and commercial products. Difficulties were encountered for some matrices, particularly those containing significant quantities of high molecular weight organic solvents that were immiscible with the acetonitrile-based spray solvent used. Additionally, significant quantities of matrix ions were observed in both pesticide formulations containing active ingredients and formulation blanks containing only the inactive matrix components. The presence of these species causes a dramatic reduction in the ionization efficiency of trace analytes. In general, these matrix effects could be substantially dealt with using a simple dilution-based sample preparation procedure, which also provides a convenient means for the addition of an internal standard for quantitative analysis.

While true quantitative analysis of trace contaminants in pesticide formulations has not yet been demonstrated with this technique, preliminary experiments indicate that a linear response is observed for 10 to 100 ppm atrazine in a pesticide formulation, even in the presence of active ingredient, using an isotopically labeled internal standard and multiple reaction monitoring experiments. Overall, given the challenging nature of these matrices and the level of sample preparation necessary for conventional analyses, paper spray ionization is a viable analytical method for detection of trace contaminants in pesticide formulations. Additional work is needed to produce a fully quantitative method and to validate the technique for a wider range of matrices and contaminants, but the general utility of the technique for quality assurance is clear.

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CHAPTER 6: NIB-BASED ELECTROSPRAY IONIZATION FOR SIMULTANEOUS ELUTION AND IONIZATION FROM PAPER SAMPLING MEDIA

6.1 Introduction

In recent years much research has been focused on the development of ambient ionization techniques designed to circumvent much of the sample preparation involved in conventional analytical methods used with paper sampling media. The principal ambient technique designed for analysis of samples collected on paper media is paper spray ionization, which is described in detail in the preceding chapters and has been used for analysis of a wide variety of samples in a range of different matrices.[1–4] Paper spray ionization imposes significant constraints on the sampling medium as it must be capable of being cut to a sharp point.[5,6] Any stray fibers or additional sharp corners may yield undesired sprays of droplets, wasting sample and solvent.[6] If the paper is cut prior to application of the sample, the resulting fragile tip must be protected from damage lest the analysis be compromised. This has typically been accomplished by encasing the paper in a plastic cartridge, which enables relatively safe storage and transportation at the cost of increased bulk, weight, and expense.[7,8] If the paper is cut to suit after application of the analyte, either excess paper will be included (if the spot is small enough to cut around) or a portion of the sample will be lost. Inclusion of excess paper is not a major problem, but is still undesirable. It increases the surface area of the substrate, and thus increases evaporative loss of solvent, and it may introduce contaminants or matrix species without increasing the quantity of analyte. Sample loss due to cutting of the paper is a more serious concern, particularly in the

case of small sampling media such as Noviplex cards (5 mm diameter paper discs). Instead of paper spray, desorption-based ionization techniques such as DART, low temperature plasma ionization (LTPI), or desorption electrospray ionization (DESI) may be employed. However, these are not ideal for use with samples collected on paper substrates as they are limited to sampling either compounds on the surface of the sample medium (LTPI and DESI) or relatively volatile species (DART and LTPI).[9–12]

An alternative option for rapid analysis of samples on paper media is extraction spray, a technique in which a small portion of the paper is inserted into a drawn glass capillary along with the spray solvent.[13,14] Compounds from the paper are extracted into the solvent and, when a suitable voltage is applied, ionized through nano-electrospray (nanoESI) from the sharp tip of the drawn glass capillary.[13] This technique is intuitive and highly suitable for samples on paper, as it avoids the surface sampling and volatility biases of desorption techniques and unites the extraction and electrospray process in a single step. However, it requires that the sampling media be cut into small sections which can be inserted into a glass capillary,[13,14] which causes sample loss and may be difficult to perform reproducibly.

Nib-based electrospray ionization (nibESI) is a novel approach for the ionization of samples collected on paper substrates, similar to extraction

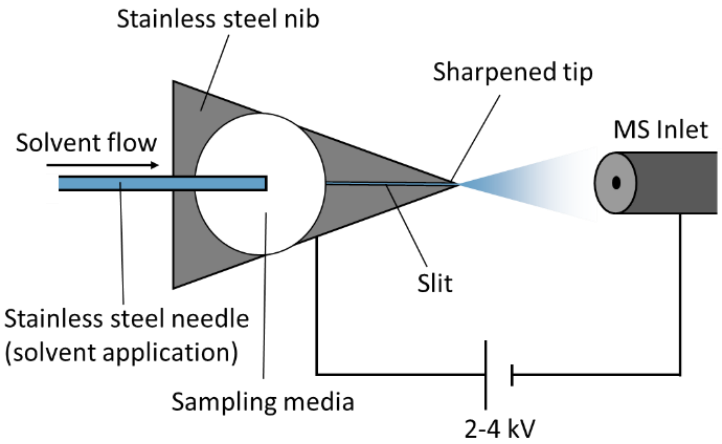

Figure 6.1: Diagram of nibESI source. Electrospray is generated from the sharpened tip of a stainless steel nib. Analyte applied to a porous sampling material is eluted and flows through the slit in the nib to the tip where it is ionized.

spray and paper spray ionization. This technique utilizes a modified fountain pen nib as a support for the paper sampling medium, with the sharpened metal tip of the nib functioning as an electrospray emitter. A diagram of the nibESI ion source is shown in Figure 6.1. Solvent flowing across the paper substrate serves to extract the analyte and immediately flows between the tines of the nib to the tip, drawn forward by capillary action. A high voltage applied to the stainless steel nib provides a sufficiently intense electric field at the sharp point of the nib to generate an electrospray, yielding gas-phase ions for mass analysis. Nib-like structures have been employed in two previously reported ion sources: a variety of paper spray ionization in which a sharpened paper tip is held between the tines of the nib,[15] and a nanoESI variant where a microfabricated nib-like emitter is used for conventional nanoESI.[16,17] The ion source described herein differs from both of these as it employs a porous substrate from which the sample is eluted, while generating the electrospray from the tip of the metal nib, rather than the paper.

Because the metal nib serves as the electrospray emitter, the geometry of the sampling media is of little importance. This approach also eliminates the size limitations imposed by extraction spray. The primary requirements in this technique are: 1) firm contact between the sampling media and the slit in the nib to ensure continuous and efficient liquid flow, 2) efficient wetting of the sampling media by the solvent, and 3) sufficient solvent flow to overcome evaporative losses over the surface area of the sampling media. In this chapter we present the design and characterization of a nibESI ion source, and demonstrate nibESI-MS detection of several compounds in matrices of varying complexity.

6.2 Experimental Materials, Methods, and Equipment

6.2.1 Materials

Stainless steel fountain pen nibs (EF Pen & Ink Sketch, Art Alternatives/MacPherson's, Emeryville CA) were purchased from a local office supply store. Nibs were flattened in a steel vice and ground to a sharp point (<50 µm tine tip width, as shown in Figure 6.2) using an abrasive grinding wheel. A plastic holder for the nib was fabricated by 3D printing with a uPrint SE printer using acrylonitrile butadiene styrene (ABS) plastic. A needle used to apply solvent was adapted from a blunt tip large-hub removable needle (Hamilton, 22 ga., tip type 2). LC-MS grade methanol, water, acetic acid (Fisherbrand Optima) and adult bovine serum were purchased from Fisher Scientific (Fair Lawn, NJ). Nicotine, nicotine-d_4, lidocaine, and atropine were purchased from Sigma-Aldrich (St. Louis, MO). Noviplex sample collection cards were provided by Shimadzu Scientific Instruments, Inc. Saliva samples were donated by one of the authors (a non-smoker).

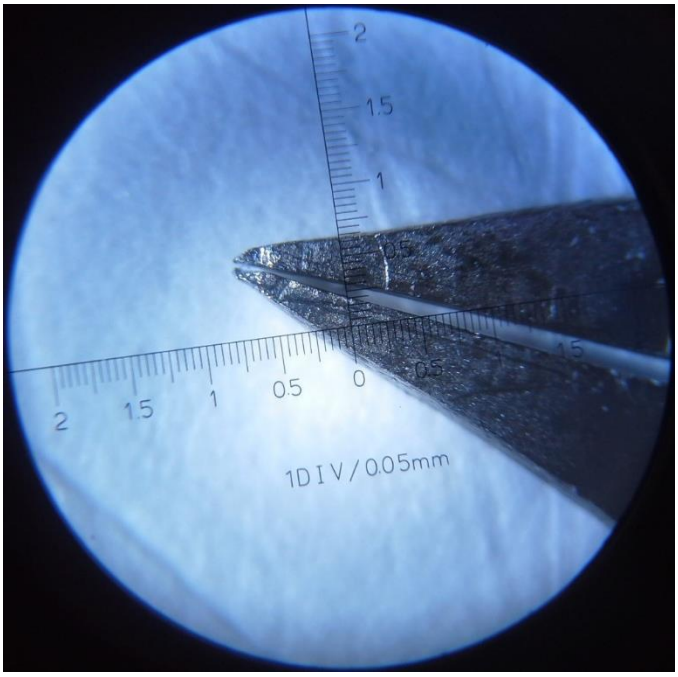

Figure 6.2: Optical microscope image of the tip of the sharpened fountain pen nib used in the nibESI ion source.

6.2.2 Ion Source Design

A sharpened stainless steel fountain pen is inserted into a plastic holder as shown in Figure 6.3. The nib is held in place by a stainless steel screw inserted into a threaded hole in

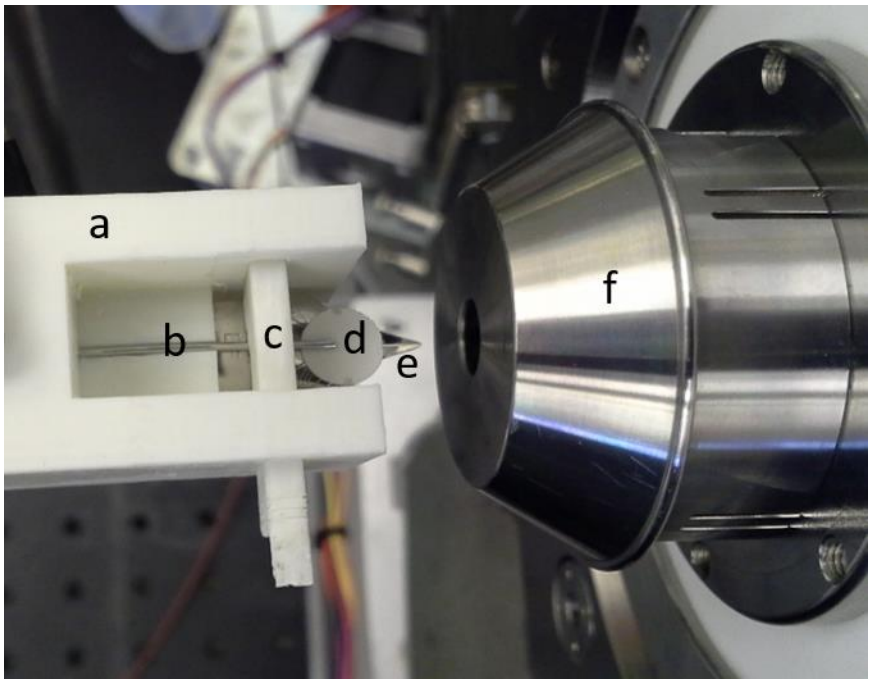

Figure 6.3: The nibESI source positioned at the inlet to the Bruker HCTultra mass spectrometer. a) 3D printed ABS plastic holder; b) solvent delivery needle; c) locking bar; d) Noviplex sampling disc; e) nib; f) mass spectrometer inlet assembly.

the plastic holder. This screw also serves as an electrical contact for application of a suitable voltage to the nib. A stainless steel needle is inserted through a hole in the back of the plastic holder, positioned so that the end of the needle is just forward of the breather hole in the nib. The back end of the needle is connected to a PEEK capillary using a stainless steel union. A syringe pump is used to deliver solvent via the PEEK tubing and stainless steel needle. The sample collection media, such as the collection disc of a Noviplex card, is inserted on top of the nib. The media is held between the tip of the steel needle and the nib surface. A plastic locking bar is inserted through the plastic holder, forcing the stainless steel needle down into firm contact with the sampling medium and locking the needle-sample-nib assembly in place.

To elute analytes from the sampling medium, solvent (90/10/0.1 methanol/water/acetic acid unless otherwise stated) is applied at a rate of 10 µL/minute using a syringe pump. The solvent saturates the sampling medium and flows through the slit in the

nib to the sharp tip. A potential difference is generated between the nib and the inlet to the mass spectrometer, inducing a spray of droplets from the sharp tip of the nib. In the experiments described here, 500 V was applied to the nib, and negative 2-3.5 kV was applied to the inlet, producing a total potential difference of 2.5-4 kV. As described below, spray may be generated by applying the entire spray potential to the nib and grounding the inlet, but due to the design of the mass spectrometer employed, it was more feasible to operate with the high voltage applied to the inlet in this case.

All mass spectra were obtained using the nibESI ion source coupled to a Bruker HCTultra quadrupole ion trap mass spectrometer. Typical instrumental parameters are listed in Table 6.1. Experiments to determine the onset voltage for various solvent mixtures were performed using a flat, electrically grounded steel plate as a counter-electrode. Spray was observed in these experiments by illuminating the tip of the nib with a portable helium-neon laser, enabling visual observation of light scattering from the plume of droplets.

Table 6.1: Typical Bruker HCTultra operating parameters for experiments with the nibESI ion source.

Parameter	Value
Ion Polarity	POSITIVE
Inlet Capillary Voltage	-3800 V
Dry Gas Temperature	300 °C
Dry Gas Flow Rate	0.50 L/min
Capillary Exit	141.7 V
Skimmer	19.3 V
Octopole 1 DC	4.27 V
Octopole 2 DC	0.80 V
Octopole RF Amplitude	137.5 Vpp
Lens 1	-6.5 V
Lens 2	-70.0 V
Trap Drive	17.9
ICC Target	100000
Averages	10
Rolling Averaging	OFF

6.2.3 Sample Preparation

Analytes were dissolved in suitable solvents (water or acetonitrile) and spiked into the desired matrices (water, adult bovine serum, saliva). Samples were applied to Noviplex cards using an autopipet. Noviplex sample collection cards consist of a paper disc on a polymer support, covered by a removable filter

membrane.[18] Samples applied through the membrane are filtered to remove cells and other particulate matter as the liquid components wick down to the paper disc.[18] The filter membrane is then peeled off and discarded, and the sample collected on the paper disc is allowed to dry at ambient conditions. Some samples were also applied with the filter membrane removed. In this case, the Noviplex card simply acts as a paper sample collection medium.

6.3 Results and Discussion

6.3.1 Solvents and Voltages

One of the central challenges in any spray-based ionization technique is the generation of a spray of droplets. Conventional ESI sources typically employ a nebulizing gas to assist in the formation of a stable spray of charged droplets. This is not used with methods such as nanoESI or paper spray ionization. These techniques employ electrical forces alone, generating a jet of charged droplets due to the very intense electric fields at the extremely sharp tip (in the case of nanoESI) or fine fibers (for paper spray). A nebulizing gas flow cannot be readily employed with nibESI, so a spray must be generated through intense electric fields. Surface tension is expected to play a significant role in this process, as a low surface tension solvent will more readily produce a spray of droplets than a high surface tension solvent, which will tend to remain as a single mass on the nib. Surface tension is also known to be a major factor in the performance of fountain pen inks: lower surface tension inks tend to flow down the nib readily while high surface tension inks are prone to feed problems.

The effects of the applied voltage and solvent composition on observed spray was investigated using a variable voltage applied to the nib and a grounded planar counter-

electrode. The spray was detected by visualization using a low-power helium-neon laser beam passing between the tip of the nib and the counter-electrode. Spray was deemed to occur if light scattering from a spray plume was visually observed. An example of a spray plume illuminated with a He-Ne laser is shown in Figure 6.4. A potential difference of at least 2 kV was necessary to generate a spray under all circumstances. The lowest spray onset voltages were observed with solvent mixtures containing 25% water or less. Solvent mixtures containing greater than 50% water were not observed to produce a spray plume at voltages up to 5 kV. The use of more precise machining techniques to produce a finer point on the nib may enable the use of lower voltages or enhance the efficiency of ionization by increasing the local electric field density at the tip.

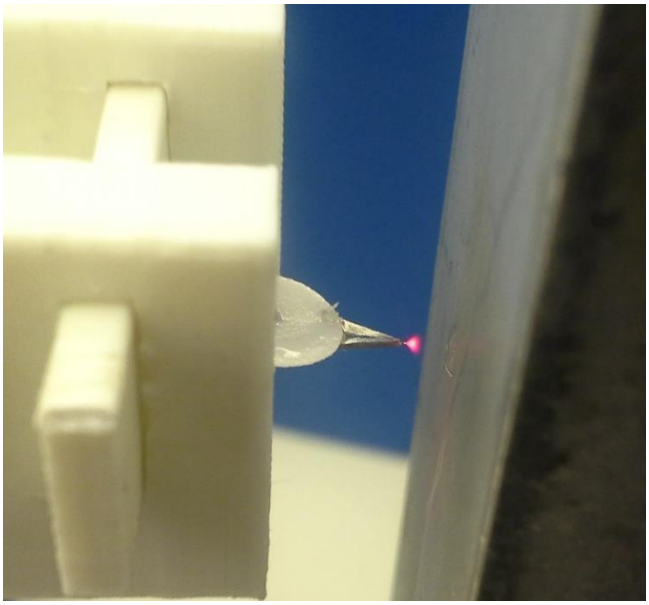

Figure 6.4: Spray plume from nibESI source illuminated with a helium-neon laser.

Greater organic content in the spray solvent, and thus lower surface tension, is observed to produce more efficient flow through the nib to the sharpened tip. High water content solvent blends (>50% water) produce beading on the surface of the nib rather than flow through the slit. In these cases, spray is not observed until the solvent drop on the surface of the nib has become large enough to reach the tip. This is consistent with the behavior of fountain pen inks. In commercial inks, surfactants are commonly employed to adjust the surface tension. For nibESI, organic solvents are employed to achieve the same

effect without the background signal and ion suppression frequently observed when common surfactants such as sodium dodecyl sulfate are employed in mass spectrometry.[19,20] It may be possible to achieve similar results using ESI-compatible surfactants such as perfluorinated organic acids[20] or by modifying the surface chemistry of the nib to increase wettability.[21–23]

Due to the exposed surface of the paper sampling medium and the open flow of solvent through the slit in the nib a flow rate of 8-10 µL/minute is necessary to achieve consistent spray and thorough wetting of the sample. At lower flow rates too much solvent is lost to evaporation to generate consistent spray. At higher flow rates more solvent is applied than is lost to evaporation and electrospray, and a build-up of solvent is observed on the upper or lower surface of the nib. This is undesirable as it may lead to dripping or unpredictable spray of droplets too large for effective desolvation, interfering with mass analysis. The balancing of solvent flow versus spray and evaporation is particularly difficult when working with mass spectrometers (such as the Bruker HCTultra used in these experiments) that employ a flow of heated gas to aid in desolvating ions generated by spray-based techniques and prevent the entrance of solvent molecules into the vacuum system. The gas flow and temperature must be sufficiently high to accomplish desolvation and maintain the temperature of the inlet, but at excessive levels it causes significant evaporation of solvent from the paper and impedes effective ionization.

Firm contact between the paper and the slit in the nib is also essential for efficient fluid transfer. This is achieved through the use of a plastic locking bar, which is inserted across the top of the nib, clamping the solvent delivery needle in place. When the paper sampling medium is inserted between the needle and nib and the locking bar is inserted, the needle is pressed down onto the paper directly above the slit, ensuring that solvent can flow

from the paper into the slit, and thence to the sharp tip where spray is generated. The locking bar is positioned behind the sampling media, avoiding contact with the sample or wetted surfaces.

6.3.2 Preliminary Testing with Mass Spectrometry

Preliminary testing of the nibESI ion source coupled to a mass spectrometer was performed using solutions of therapeutic drugs in water. 25 µL aliquots were applied to Noviplex cards with the filter membrane removed. After drying, samples were analyzed as described above, using 99/1 methanol/acetic acid as elution solvent at a flow rate of 8 µL/min. As shown in Figure 6.5, lidocaine, a common local anaesthetic, is readily detected as a protonated molecule (*m/*z 235) at a concentration of 10 µM in a water matrix. Collision induced dissociation experiments were performed to confirm the identity of the ion at

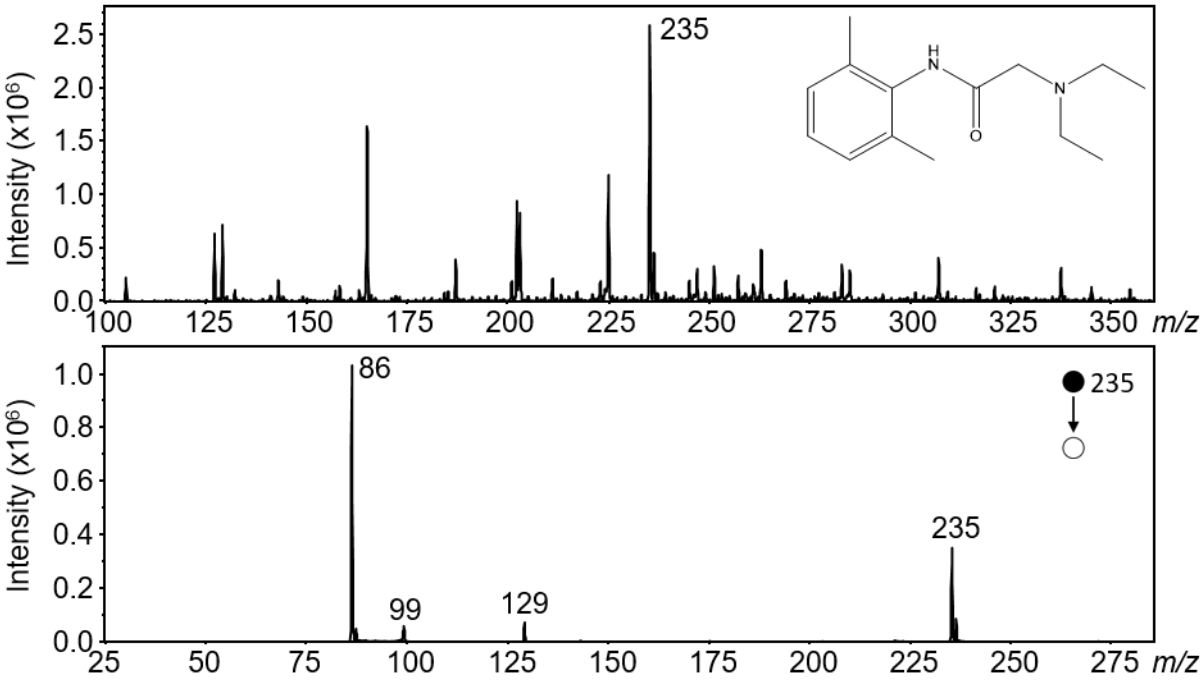

Figure 6.5: NibESI mass spectra of 10 µM lidocaine in water, dried on Noviplex cards with the filter membrane removed. Top: Mass spectrum of lidocaine. Protonated lidocaine is observed at *m/z* 235. Bottom: MS/MS spectrum of protonated lidocaine. The dominant product ion, *m/z* 86, is likely $C_5H_{12}N^+$.

m/z 235, also shown in Figure 6.5. The primary dissociation product is *m/z* 86, consistent with a $C_5H_{12}N^+$ product ion from the tertiary amine moiety in lidocaine.

A mass spectrum of atropine (100 µM in water) from a Noviplex card, prepared as described above, is shown in Figure 6.6. Atropine is also observed as a protonated molecule, at *m/z* 290. A CID MS/MS spectrum of the ion at *m/z* 290 is also shown in Figure 6.6. The primary product ions observed in MS/MS spectra of protonated spectrum are *m/z* 260 and 124. The ion of *m/z* 260 arises from a neutral loss of 30 daltons, presumably CH_2O.

The origin of the background species observed in nibESI mass spectra of samples in water matrices is not clear. The range of background species observed is exemplified in the mass spectrum shown in Figure 6.6, with a range of background ions detected at mass-to-charge values ranging from *m/z* 105 to 466. These ions are consistent with those observed in experiments with blank Noviplex sampling cards (the nibESI source requires a porous

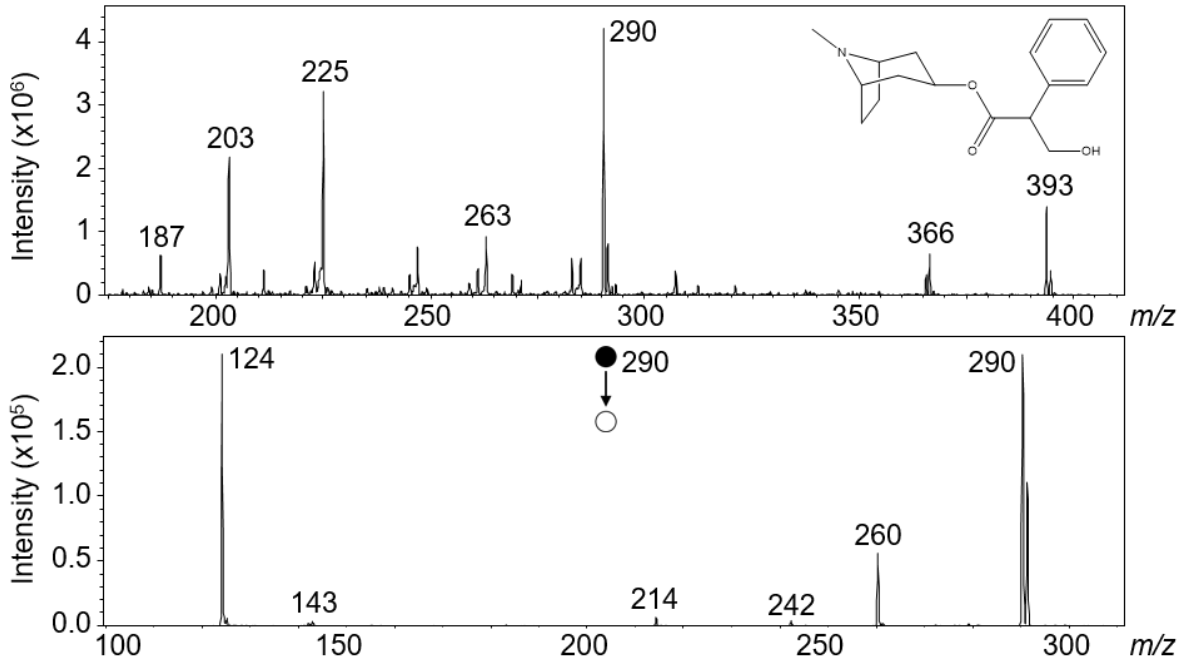

Figure 6.6: NibESI mass spectra of 100 µM atropine in water, dried on Noviplex cards with the filter membrane removed. Top: Mass spectrum of atropine. Protonated atropine is observed at m/z 290. Bottom: MS/MS spectrum of protonated atropine.

substrate to achieve liquid flow; experiments without one produce little to no signal). This paper sampling medium is one potential source of background signal; it is packaged in contact with both a plastic support structure and a complex membrane composed of unspecified materials. Either of these components may cause some limited contamination of the paper sampling disc.

6.3.3 Biological Matrices

Samples of therapeutic drugs dissolved in human saliva and adult bovine serum were applied to Noviplex cards in 25 µL aliquots; experiments were conducted with samples applied through the filter membrane as well as without the membrane. After drying, samples were analyzed as described above. No significant difference in matrix ion signal was observed based on the presence or absence of the filter membrane, suggesting that for matrices with relatively low particulate or cell content, a filtration stage may not be necessary or beneficial prior to ambient ionization.

Lidocaine is often employed during minor surgery to minimize pain without requiring general anesthesia. Measurement of lidocaine in oral fluid has been demonstrated as an effective and non-invasive alternative to blood measurement. A mass spectrum of lidocaine in human saliva is shown in Figure 6.7, along with a MS/MS spectrum showing dissociation products from collision induced dissociation of the protonated molecule. Significant signal suppression is observed in saliva relative to water (approximately 10-fold lower signal for a 10-fold more concentrated sample), likely because of the abundant background species in the saliva matrix. The MS/MS spectrum is effectively identical to that observed in a water matrix.

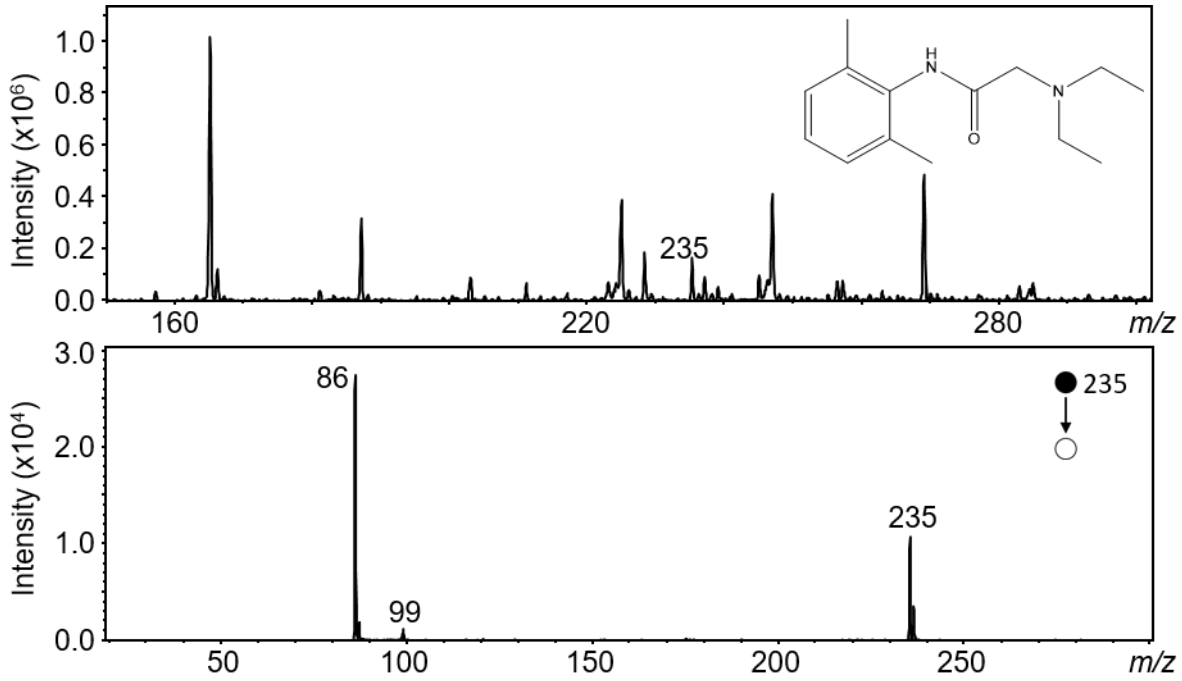

Figure 6.7: NibESI mass spectra of lidocaine (100 µM) in human saliva, applied to Noviplex card through the separation membrane. Top: Mass spectrum, with protonated lidocaine visible at m/z 235. Bottom: MS/MS of protonated lidocaine.

As the Noviplex sample collection cards were primarily designed for collection of blood samples, samples of adult bovine serum spiked with lidocaine and atropine were also investigated. 25 µL aliquots were applied through the separation membrane in accordance with the instructions provided on the Noviplex cards. NibESI mass spectra of lidocaine and atropine in adult bovine serum are shown in Figure 6.8. Both species are observed as protonated molecules. MS/MS spectra of both species were identical to MS/MS spectra obtained using a water matrix. Matrix species are present in significant abundance, which appears to suppress ionization of the analytes to a similar extent as in saliva.

6.3.4 Quantification of Nicotine in Saliva

Nicotine is frequently measured in saliva,[24–28] and salivary nicotine correlates well with urinary and blood nicotine levels.[29] To investigate the potential applicability of nibESI to quantitative applications, samples of control saliva donated by one of the authors (a

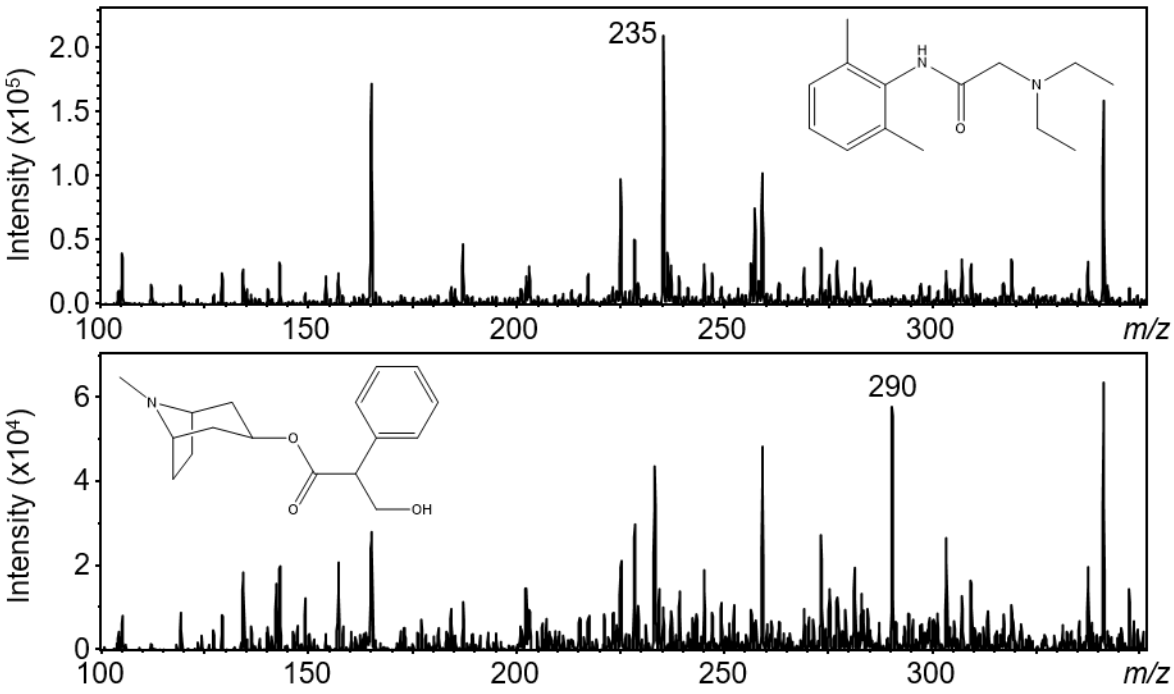

Figure 6.8: Mass spectra of lidocaine (top) and atropine (bottom) in adult bovine serum (100 µM). Both species are observed as protonated molecules, at m/z 235 and 290, respectively.

nonsmoker) were spiked with a stock solution of nicotine in water to yield a concentration ladder. Each 1 mL sample was spiked with 20 µL of internal standard solution (500 µM nicotine-d_4 in acetonitrile) and aspirated and vortexed to mix. 25 µL aliquots were applied to Noviplex cards with the filter membrane removed and allowed to dry at ambient conditions.

Samples of saliva spiked with nicotine were analyzed by MS/MS. A mass spectrum of nicotine and nicotine-d_4 in saliva is shown in Figure 6.9, along with a MS/MS spectrum *m/z* 163 (protonated nicotine). Switching between MS/MS of nicotine and nicotine-d_4 yields results similar to a MRM experiment on a triple quadrupole mass spectrometer. Signal was integrated over two minutes. Plotting the ratio of the major product ion from nicotine to the equivalent product ion from nicotine-d_4 (m/z 132 and 136, respectively) versus the concentration of nicotine yields the calibration curve shown in Figure 6.10. A linear response is observed across the concentration range investigated, from 500 nM to 100 µM. The limit

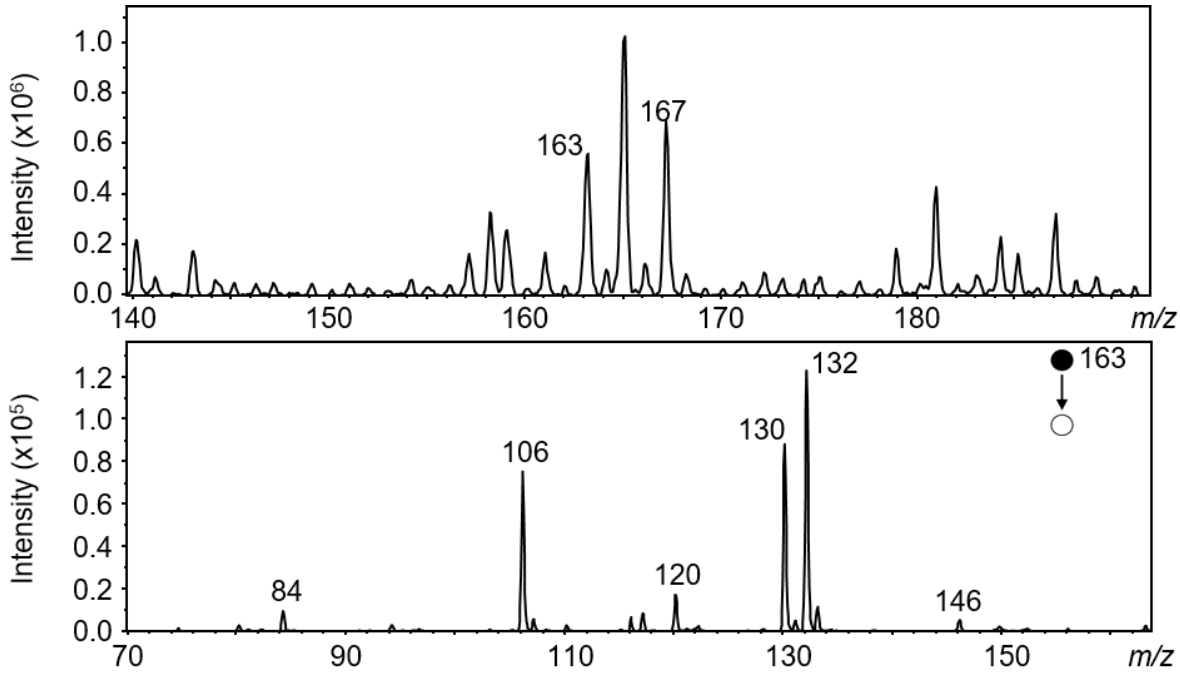

Figure 6.9: NibESI mass spectra of nicotine in saliva. Top: Mass spectrum of nicotine and nicotine-d_4, observed at *m/z* 163 and 167, respectively. Bottom: MS/MS spectrum of protonated nicotine.

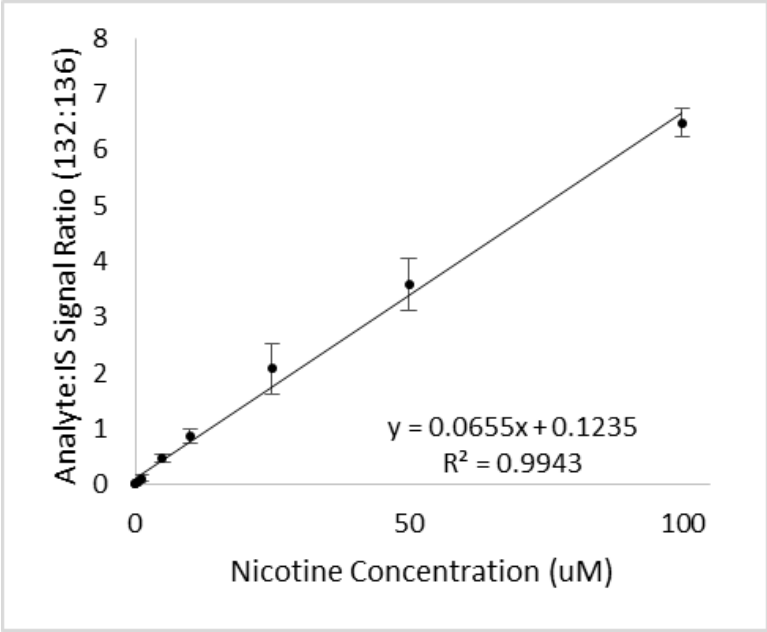

Figure 6.10: Calibration curve for measurement of nicotine in saliva using nibESI-MS/MS. Each point is the average of three measurements. Error bars represent one standard deviation.

of detection for nicotine was calculated to be 129 nM, or 3.2 picomoles deposited on the Noviplex card. The linear dynamic range for this method includes the relevant salivary nicotine concentrations for smokers with a range of cigarette consumption habits and levels of cigarette consumption,[24,29] while the limit of detection is above the level of nicotine observed in non-smokers.[29]

Nicotine represents a straightforward analyte for saliva analysis via nibESI due to its high basicity and excellent solubility in typical solvents for nibESI. More challenging analytes will likely require optimization of solvent composition to maximize elution efficiency. The primary tools available for optimization of ionization efficiency in nibESI on a compound-dependent basis are solvent composition, electrospray voltage, and source parameters such as desolvation gas flow/temperature. Some optimization of the ion source position is necessary when installing it on a mass spectrometer, but once installation is completed no significant adjustment is necessary.

6.4 Conclusions

The use of paper and other porous media for collection of analytical samples is a powerful tool, but one which typically requires additional sample processing steps to enable mass spectrometric analysis. Nib-based electrospray ionization represents an alternative technique analogous to paper spray ionization, allowing the analyte to be directly eluted from the substrate and ionized by electrospray without additional sample handling or preparation. NibESI eliminates the substrate geometry requirements of paper spray ionization by generating an electrospray from the tip of a sharp metal nib, on which the sample is placed (no need for the paper to be cut to a sharp point). This enables the use of sampling media that would otherwise be difficult to work with. This work has demonstrated nibESI as an

analytical technique for samples collected on Noviplex cards, but it is expected to be amenable to use with other media, such as fiber-based swabs, punches from paper cards, or small fabric samples. Like paper spray or extraction spray, nibESI may be coupled to most mass spectrometers with a suitable atmospheric pressure inlet system. NibESI has been demonstrated in qualitative and semi-quantitative applications, and is expected to be suitable for most analytes compatible with paper spray ionization. This technique presents a viable alternative for rapid analysis of samples collected on porous media when a rapid and low-preparation method is desirable, especially if the geometry of the sampling media is incompatible with paper spray or extraction spray.

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CHAPTER 7: FUTURE DIRECTIONS FOR PAPER-BASED SAMPLING WITH AMBIENT IONIZATION-MASS SPECTROMETRY

7.1 Summary

Paper-based sampling methods coupled to mass spectrometry represent a powerful tool for environmental, regulatory, and agricultural applications. The utility of paper-based sampling is significantly enhanced when these sample collection media are coupled to ambient ionization techniques that can generate gas-phase ions for mass analysis with minimal sample preparation or separations. Paper spray ionization is the intuitive choice, generating ions via an electrospray directly from the paper itself.[1–4] A custom paper spray ion source was developed to investigate the utility of this technique for analysis of agrochemicals in particular. When a commercial paper spray ion source became available, the Velox 360, it was characterized and compared to the custom paper spray source. Using both the custom-built and commercial paper spray ion sources, methods for analysis of samples of pesticides collected on paper were developed for a variety of matrices, from relatively simple water samples to agricultural extracts to highly challenging pesticide formulations.

The custom paper spray source was developed through three design iterations, producing a final design that is modular and easily adapted to most mass spectrometers designed for electrospray ionization. This source was investigated using atrazine, propazine, and metolachlor as test analytes in water, crop extracts, and soil extracts. Quantification of these pesticides was performed using deuterium-labeled internal standards, with good results at concentrations from the part-per-million level to 1 part-per-billion (or below).

Using the Prosolia Velox 360 commercial paper spray ion source, methods for automated paper spray ionization-mass spectrometry analysis and data analysis were developed for pesticides in water and crop samples. Building on the results in these relatively simple matrices, paper spray ionization was investigated as a tool for the analysis of commercial pesticide formulations for trace level cross-contamination. These matrices are highly challenging for mass spectrometry, but measurement of part-per-million level simulated cross-contaminants was achieved using paper spray ionization-mass spectrometry after diluting spiked formulations 10- or 100-fold in acetonitrile. Preliminary quantitative experiments using these methods have met with some success, suggesting rapid analysis of these highly complex matrices.

One of the main constraints imposed by paper spray ionization is the requirement that the paper geometry include a sharp point, which can complicate transportation of samples, due to the need to protect the sharp tip, or if papers are cut after sample application, can lead to loss of some sample in the cutting process. A new ionization technique was developed to circumvent this limitation, based on a fountain pen nib. This design is compatible with a variety of different paper geometries, as it employs a sharpened metal nib to generate an electrospray rather than a pointed piece of paper. The paper is instead mounted atop the nib and the analyte eluted in solvent, reaching the tip of the nib by capillary action. This ion source (nibESI) was tested using therapeutic drugs and nicotine in water, serum, and saliva deposited on paper sample collection discs. The potential of this method for quantitative applications was demonstrated using nicotine in saliva from 500 nM to 100 µM, with a calculated limit of detection of 129 nM, sufficient to distinguish between smokers and non-smokers based on previously reported salivary nicotine concentrations[5] and covering the

relevant levels for smokers with a variety of different use patterns.[5,6] Significant work remains to be done, but the initial characterization of nibESI suggests that it has significant potential as a tool for analysis of a wide variety of samples on paper or other porous media.

7.2 Ongoing Work with Pesticide Formulations

The primary focus of ongoing research with the Prosolia Velox 360 ion source continues to be the challenge of analyzing pesticide formulations for trace-level cross-contaminants. The work presented here has focused on proof-of-concept experiments using triazines and metolachlor as test analytes; moving forward, it is necessary to extend this work to include a variety of other pesticides and related compounds of interest as potential cross-contaminants. Two species of particular interest at this time are azoxystrobin, a fungicide, and thiamethoxam, an insecticide. These compounds have been detected in preliminary experiments, but significant work remains to be done to optimize instrumental parameters and solvent conditions to maximize sensitivity and achieve effective quantification at the low part-per-million level.

An additional challenge arising from work with these matrices containing highly concentrated active ingredients is the difficulty of avoiding cross-contamination between samples. This is less of a problem for paper spray than electrospray ionization as the sample does not come into direct contact with the permanent liquid handling path (as the sample is contained within a disposable cartridge), but cross-contamination can still occur. A common site of contamination is the mass spectrometer inlet. Not all of the nebulized material from the spray is transferred into the vacuum system; a non-trivial quantity seems to be deposited on the inlet and outer shielding. These components are easily cleaned (in many cases, they

may be replaced without venting the mass spectrometer), but the build-up of material causes variations in signal intensity and can degrade instrument performance over time.

Dilution of samples seems to reduce the frequency of inlet contamination, as does centrifugation of samples containing suspended material prior to application on paper. These procedures do not solve the problem of contamination, but merely slow it. Ongoing investigation of suitable sample preparation, application, and elution techniques that may reduce the risk of contamination is essential to the success of this technique for routine use with matrices containing highly concentrated species like pesticide formulations, particularly for quantitative applications. In some cases contamination has been observed which does not appear to be related to the inlet; it is possible that some contamination may be occurring within the paper spray ion source. Work is currently underway to identify the site of contamination and develop procedures to prevent future contamination of the ion source.

7.3 Future Prospects for Paper Spray Ionization

Paper spray ionization is in a critical stage of its development at present. With a commercial paper spray ion source available, which is compatible with at least one of the major mass spectrometer manufacturers' instruments, this technique is poised for a significant expansion of its user base, transitioning from an object of academic interest among instrument builders to a viable tool employed by applications-oriented analytical chemists. However, there remain significant hurdles to be overcome. Currently, the Velox 360 paper spray ion source is only directly compatible with Thermo Scientific mass spectrometers.[7] While it is not overly difficult to adapt it for use with other instruments, it is not a "plug-and-play" tool for them and has not been rigorously tested to confirm full

compatibility. Hardware to easily interface the Velox 360 with other instruments, beginning with Bruker ion trap mass spectrometers, is currently under development.

An additional barrier to the widespread adoption of paper spray ionization is cost: the consumables cost is presently quite high, as noted in Chapter 4, and the purchase price of the Velox 360 is substantial. To address the problem of consumables cost in particular, ongoing experiments are investigating the possibility of recycling the plastic cartridges used with the Velox 360. By removing the paper, thoroughly cleaning the plastic and metal components, and then reassembling the cartridges with new paper, much of the cost of replacement cartridges can be avoided (especially if pre-cut paper can be obtained in bulk), albeit at the cost of some additional labor in the recycling process. Preliminary experiments have been promising, but comprehensive validation will be necessary before recycled cartridges are deemed suitable for regulatory applications.

The commercial cartridges used with the Velox 360 are only available with one type of paper, which, while adequate for general use, may not be ideal for all applications. At a small scale, this can be addressed by using experimental paper in the cartridge recycling process, rather than simply reloading used cartridges with the standard paper. Two general approaches may be valuable here: the investigation of alternative commercial papers for use with the Velox 360 and the chemical modification of paper to control its properties directly. There are a wide variety of commercial filter papers available, as discussed in Chapter 2, and the utility of these papers is expected to vary based on the application. Sample-limited applications, where maximum sensitivity is needed to get the most out of a small volume, may benefit from a paper substrate with relatively low sample capacity but a very sharp tip. Alternatively, for applications where the sample is relatively abundant (such as the

environmental applications described here), better overall performance may be obtained by using a thicker, more absorbent paper with somewhat reduced tip sharpness, but on which a larger volume of sample may be deposited.

Modification of the paper itself is a more complex process, but has been explored in a number of previous studies. These efforts range from simple deposition of silica particles on the surface of the paper,[8–10] to the trimethylsilyl modification described in Chapter 2, to the use of more exotic materials such as carbon nanotubes.[11] While the results of the trimethylsilylation experiments were not particularly promising, there remains a great deal of room for development of modified papers to enhance sensitivity or selectivity in paper spray ionization. One of the great advantages of paper spray ionization in this regard is the ease with which cellulose-based papers can be chemically modified. The abundance of hydroxyl groups in cellulose means this material is amenable to a wide range of functionalization reactions, from modification with acidic or basic groups to the addition of large organic moieties.[12] The potential of chemically modified papers as substrates for paper spray ionization has only been explored in a preliminary fashion,[8,9,13] and not at all with a commercial ion source.

Paper spray ionization-mass spectrometry has significant potential as an analytical technique, especially if barriers to entry such as the significant initial investment in the ion source and the ongoing cost of consumables can be overcome. With the development of automated paper spray analyses, this technique represents a powerful tool applicable to a wide range of applications. There remain several areas of interest for ongoing research improving the capabilities of paper spray ionization, particularly the development of improved and alternative substrates for more sensitive and/or selective measurements.

7.4 Future Directions in NibESI

Preliminary work with nib-based electrospray ionization has demonstrated the potential of this technique for the ionization of compounds deposited on paper discs, but significant work remains to be done to improve the sensitivity and reproducibility of the technique and the robustness of the hardware. One of the difficulties with the current prototype is its imprecise mounting and positioning assembly – the nibESI assembly is positioned in front of the mass spectrometer manually using a post and clamp. A better mounting assembly could be easily constructed, enabling more reproducible positioning of the nib relative to the inlet. It would also be useful to redesign the 3D printed holder in which the nib is mounted. The current design is adequate, but has several minor flaws rendering it susceptible to degradation and damage over time. Additionally, the use of a different plastic would be desirable, as the ABS plastic used in the current holder is not compatible with acetonitrile. The 3D printer used to produce this part is compatible with more chemically inert plastics such as polyethylene and polypropylene with some adaptation; experiments to investigate a suitable material and employ it in an improved holder design are strongly recommended.

The nib employed in the nibESI ion source is a modified fountain pen nib, ground to a fine point. This grinding process was performed using a hand-held rotary tool with a grinding wheel, which produced an irregular, scored surface in the nib. The effect of this surface roughness is not known, but there are a variety of methods available which might be employed to polish the surface including fine sandpaper, buffing with diamond polishing paste or various grades of alumina or silica grit, and for an extremely fine surface polish, electropolishing or electroplating. It should also be possible to obtain a more reliable

commercial supply of relatively sharp nibs; the nib used in these experiments was a typical writing nib, which required significant grinding to be effective. Alternative approaches such as laser-cutting steel to produce the desired shape or purchasing commercial nibs that are already fairly sharp, such as the finest grades of calligraphy nibs, may allow for effectively interchangeable nibs instead of the unique and irreproducible hand-made nibs used currently.

Initial testing of the nibESI source employed therapeutic drugs and nicotine as test analytes in water, saliva, and adult bovine serum. Future work with this ion source should expand the range of matrices to include whole blood, urine, plasma (collected using the Noviplex card from whole blood), and environmental matrices. Additional analyte classes worth investigating would include endogenous species in biological matrices such as acylcarnitines, peptides, proteins, and small molecule biomarkers. Pesticides and organic pollutants would also be of interest in environmental matrices.

The primary advantage that nibESI has relative to paper spray ionization is that nibESI can accommodate a much broader range of substrate geometries than paper spray. This may be extended to include matrices other than paper, such as cotton swabs, fiberglass filters, or other porous media that, while useful as sample collection tools, are incompatible with paper spray ionization, or can be used only with difficulty. This suggests a range of other applications for nibESI, including analysis of forensic swabs for drug or explosives residues, analysis of aerosol residues collected on filters, and potentially direct tissue analysis. These materials can simply be positioned atop the nib, held in position with the solvent delivery needle. When solvent is applied through the needle, it will soak the sample and wick down to the nib in the same manner as for a paper substrate. This broad range of

applications has not been realized yet, but would be fairly straightforward to investigate given the easily reconfigured design of the nibESI ion source.

7.5 Conclusions

Paper-based sample collection techniques are a powerful tool for a wide range of applications, and their utility is significantly enhanced when coupled to ambient ionization techniques. Paper spray ionization is the most intuitive choice for analysis of samples collected on paper, particularly given the availability of a commercial ion source. A custom-built and a commercial paper spray ion source were characterized; both ion sources were determined to be viable tools for the measurement of pesticides in a variety of matrices at part-per-billion concentrations.

The custom paper spray ion source is significantly more flexible as a research tool, as it can easily be adapted to nearly any mass spectrometer with an atmospheric pressure inlet and is capable of extended experiments because of its continuous solvent application capabilities. The commercial ion source, while at present only directly compatible with Thermo Scientific mass spectrometers, yielded significantly better reproducibility and is far more user-friendly than the custom source. The automated methods developed using the commercial ion source provide a particular advantage, enabling the use of paper spray ionization for high throughput applications without the substantial labor requirements of the custom ion source.

The use of paper spray ionization for the measurement of simulated residual impurities in pesticide formulations has also been demonstrated. While these matrices are quite challenging to analyze by conventional liquid chromatography-mass spectrometry, simple dilution in acetonitrile is sufficient to enable analysis by paper spray ionization-mass

spectrometry. This approach has been successful in detecting atrazine in a variety of formulation matrices at part-per-million concentrations, although linearity remains difficult to achieve. The presence of active ingredients in the pesticide does not cause significant difficulty in the detection of simulated residual impurities, but can produce contamination of the inlet system. Although further development is needed to eliminate this problem and provide additional improvements to linearity and sensitivity, the applicability of paper spray ionization to the analysis of these challenging matrices has been confirmed.

In an effort to eliminate the geometry restrictions imposed by paper spray ionization while still directly ionizing compounds collected on a paper substrate, a new ionization technique was developed using a metal nib. Initial testing using therapeutic drugs and nicotine in a variety of biological matrices indicates that this ion source has significant potential as a tool for qualitative and quantitative analysis of samples collected on paper regardless of the shape of the substrate. This approach is therefore expected to be suitable for use with a variety of substrates that at present are difficult to work with a paper spray, such as forensic swabs or punches from dried blood spot cards.

The overall outlook for paper-based sampling coupled to ambient ionization is excellent. This approach provides simple, rapid, and relatively low-cost sample collection and analysis for a wide variety of applications, including the analysis of pesticides in environmental matrices and commercial formulations as described here. These ambient ionization techniques have thus far been primarily restricted to academic research with custom-built hardware, but with the development of commercial instrumentation capable of automated analysis, paper spray ionization is poised for broader acceptance. There is significant room for further development of techniques and equipment for analysis of

samples collected on paper, and as paper spray ionization matures as a commercialized technique, demand for more sensitive, more selective, and more versatile tools for analysis of these samples can only be expected to increase.

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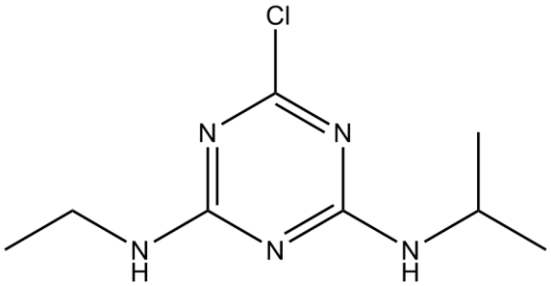
APPENDIX A: MOLECULAR STRUCTURES OF ANALYTES AND INTERNAL STANDARDS

A.1 Pesticides

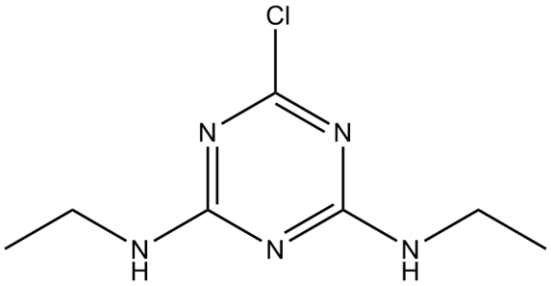
Atrazine:

Formula: $C_8H_{14}ClN_5$
Molar mass: 215.68 g/mol
Monoisotopic mass: 215.09 Da
Structure:



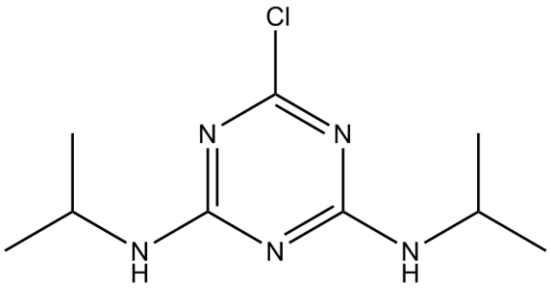
Simazine:

Formula: $C_7H_{12}ClN_5$
Molar mass: 201.66 g/mol
Monoisotopic mass: 201.08 Da
Structure:



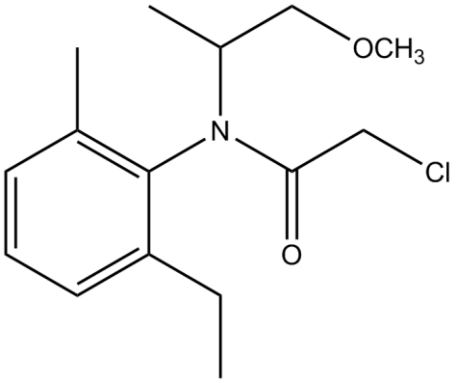
Propazine:

Formula: $C_9H_{16}ClN_5$
Molar mass: 229.71 g/mol
Monoisotopic mass: 229.11 Da
Structure:



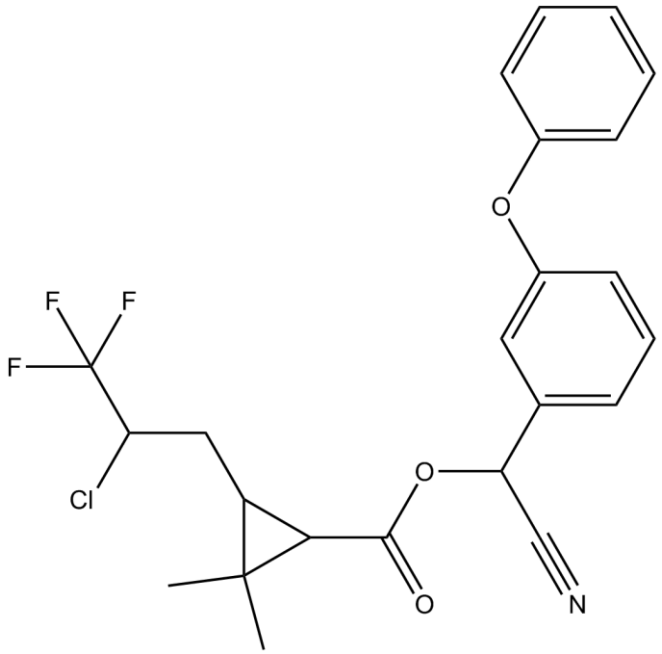
Metolachlor:

Formula: $C_{15}H_{22}ClNO_2$
Molar mass: 283.79 g/mol
Monoisotopic mass: 283.13 Da
Structure:



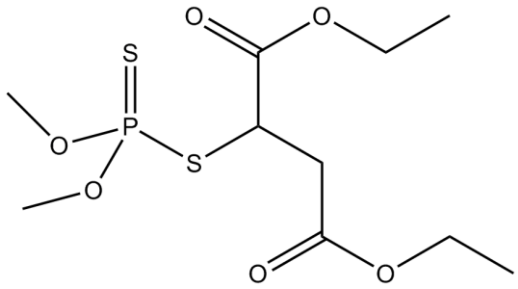
λ-cyhalothrin (mix of stereoisomers):

Formula: $C_{23}H_{19}ClF_3NO_3$
Molar mass: 449.85 g/mol
Monoisotopic mass: 449.10 Da
Structure:



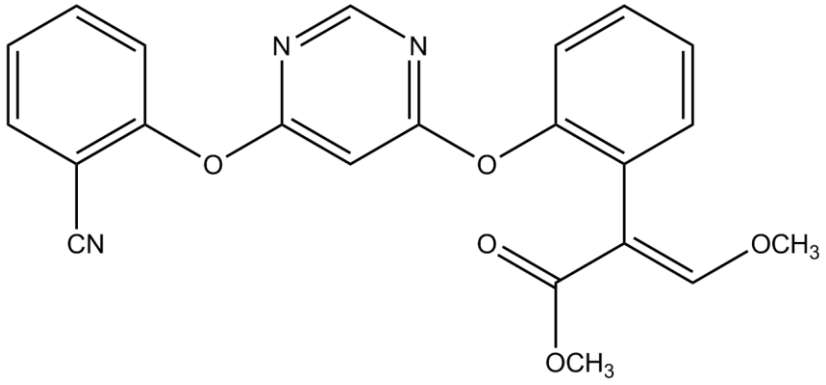
Malathion:

Formula: $C_{10}H_{19}O_6PS_2$
Molar mass: 330.36 g/mol
Monoisotopic mass: 330.04 g/mol
Structure:



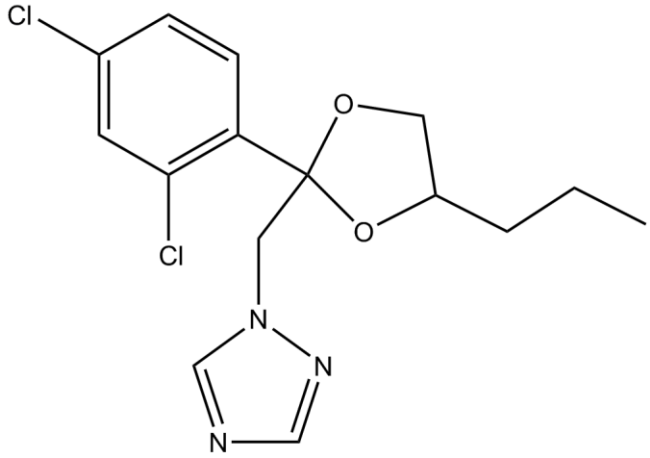
Azoxystrobin:

Formula: $C_{22}H_{17}N_3O_5$
Molar mass: 403.39 g/mol
Monoisotopic mass: 403.12 Da
Structure:



Propiconazole:

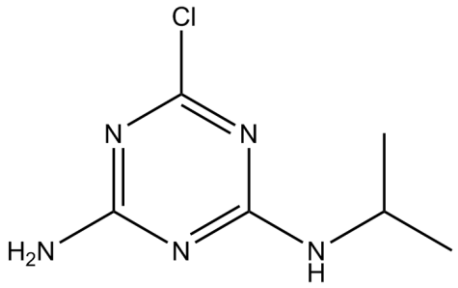
Formula: $C_{15}H_{17}Cl_2N_3O_2$
Molar mass: 342.22 g/mol
Monoisotopic mass: 341.07 Da
Structure:



A.2 Pesticide Metabolites/Decomposition Products

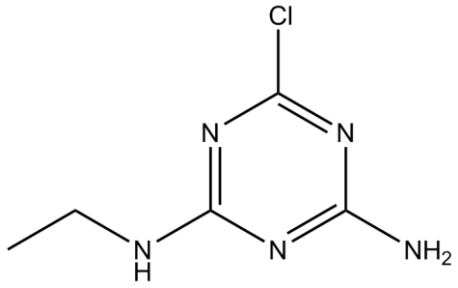
Desethyl-atrazine:

Formula: $C_6H_{10}ClN_5$
Molar mass: 187.63 g/mol
Monoisotopic mass: 187.06 Da
Structure:



Desisopropyl-atrazine:

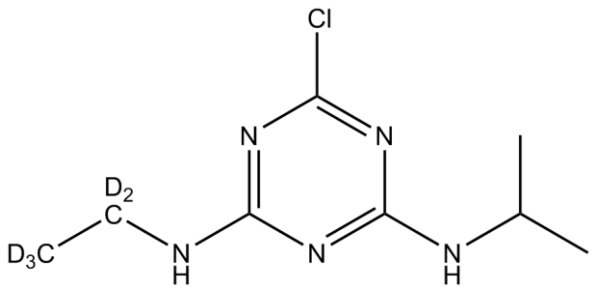
Formula: $C_5H_8ClN_5$
Molar mass: 173.60 g/mol
Monoisotopic mass: 173.05 g/mol
Structure:



A.3 Deuterated Pesticides

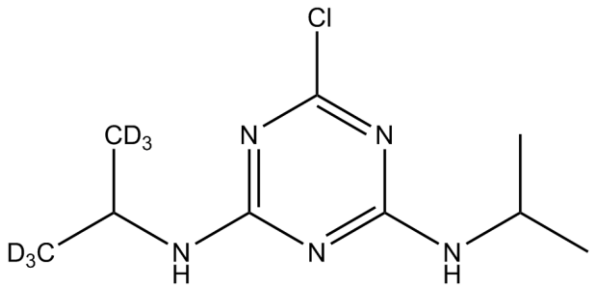
Atrazine-d_5:

Formula: $C_8H_9D_5ClN_5$
Molar mass: 220.71 g/mol
Monoisotopic mass: 220.13 Da
Structure:



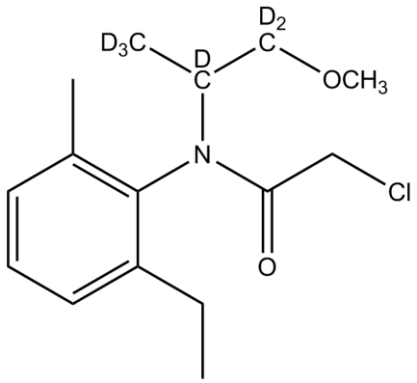
Propazine-d_6:

Formula: $C_9H_{10}D_6ClN_5$
Molar mass: 235.75 g/mol
Monoisotopic mass: 235.15 Da
Structure:



Metolachlor-d_6:

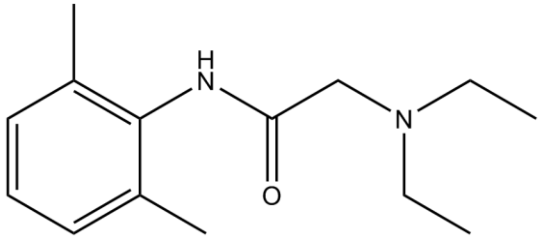
Formula: $C_{15}H_{16}D_6ClNO_2$
Molar mass: 289.83 g/mol
Monoisotopic mass: 289.17 Da
Structure:



A.4 Drugs

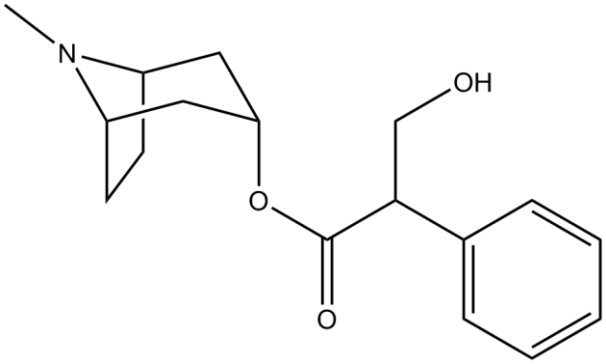
Lidocaine:

Formula: $C_{14}H_{22}N_2O$
Molar mass: 234.34 g/mol
Monoisotopic mass: 234.17 Da
Structure:



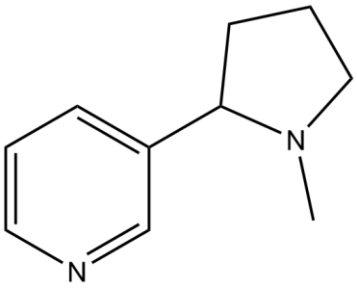
Atropine:

Formula: $C_{17}H_{23}NO_3$
Molar mass: 289.37 g/mol
Monoisotopic mass: 289.17 Da
Structure:



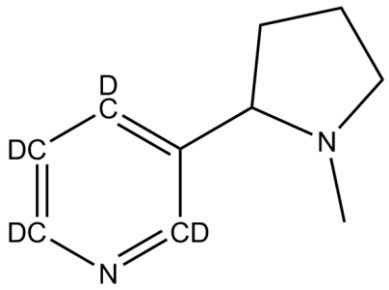
Nicotine:

Formula: $C_{10}H_{14}N_2$
Molar mass: 162.23 g/mol
Monoisotopic mass: 162.12 Da
Structure:



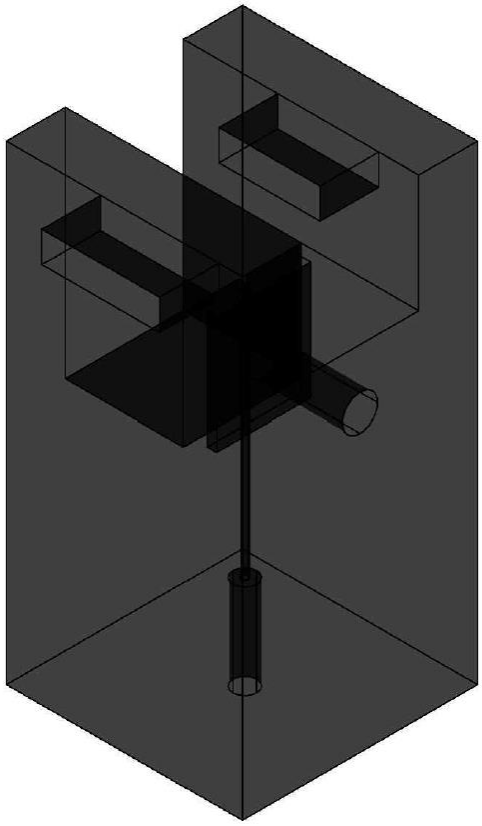
Nicotine-d_4:

Formula: $C_{10}H_{10}D_4N_2$
Molar mass: 166.26 g/mol
Monoisotopic mass: 166.14 Da
Structure:

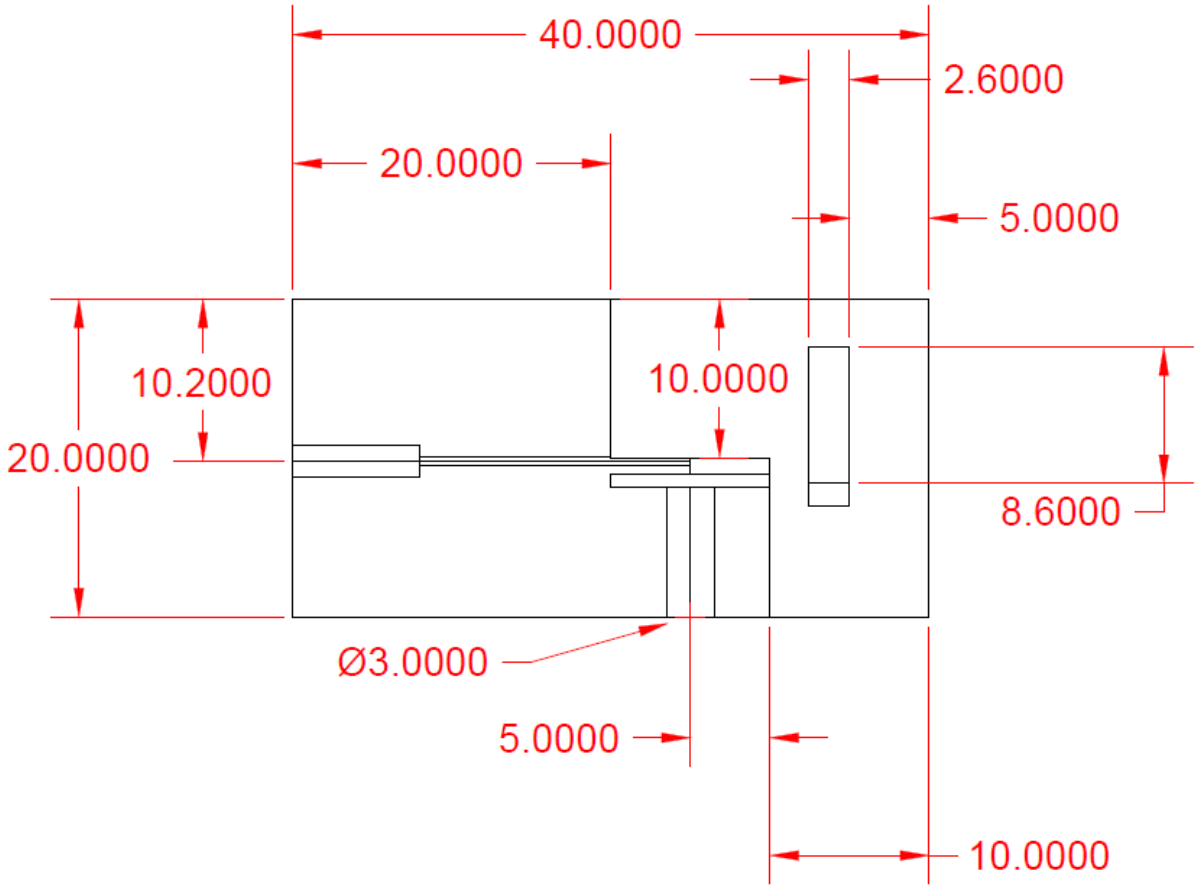


APPENDIX B: TECHNICAL DRAWINGS

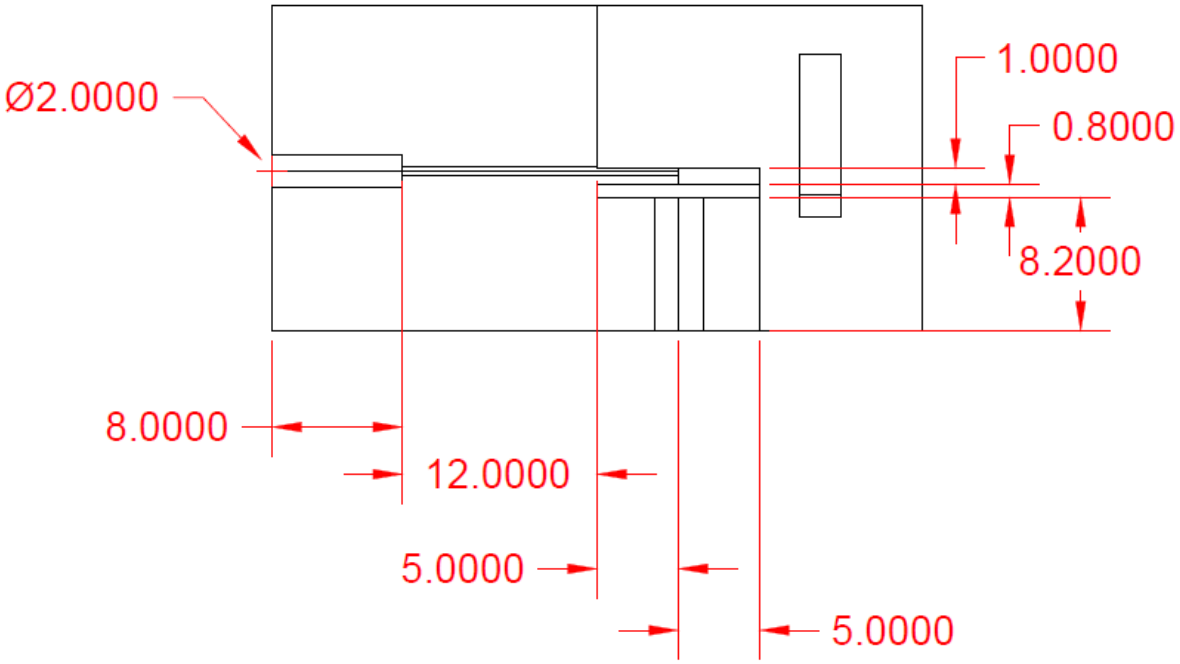
Components of the nibESI source were designed in AutoCAD 2014 and constructed by 3D printing. The technical drawings for these parts are shown below. All dimensions are in millimeters. The material used was ABS plastic. This was the best available material at the time, but is not recommended for future work with parts that may be exposed to solvents. It is strongly recommended that a more solvent-compatible plastic, such as polypropylene, be employed whenever possible.



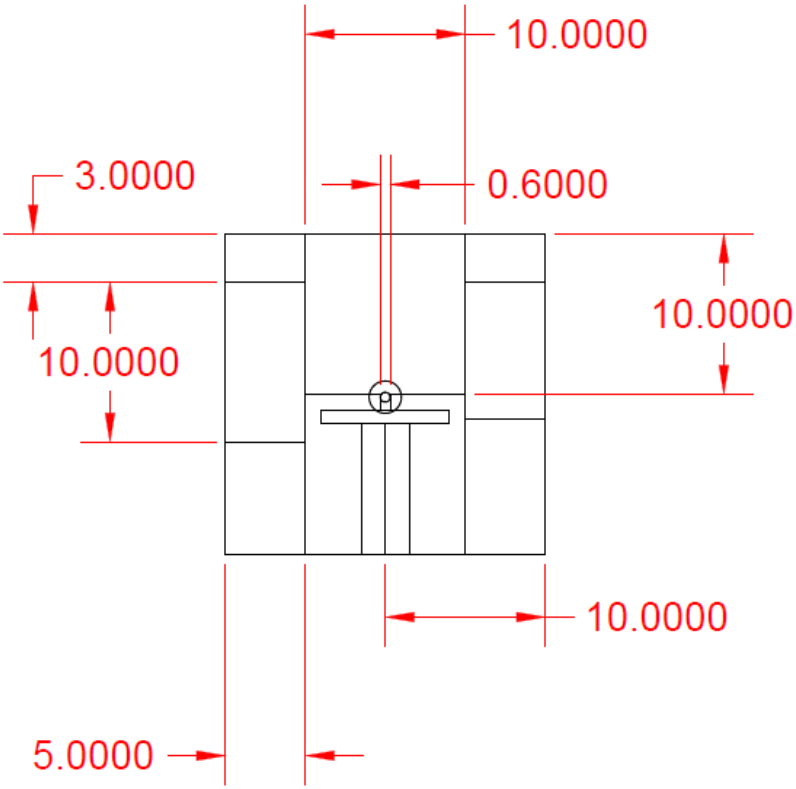
3-dimensional “x-ray” view of the nibESI holder model.



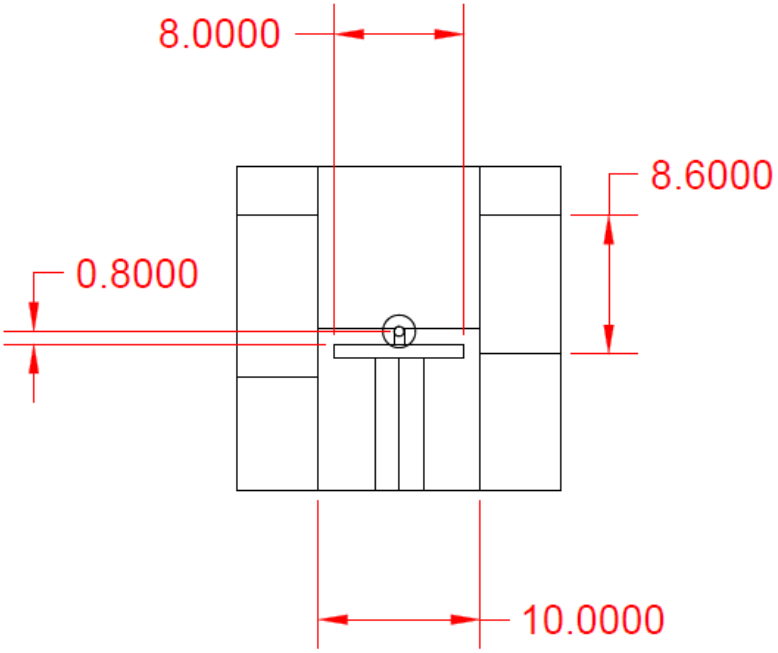
Side view of the nibESI holder. The part consists of a rectangular prism with sections removed. Through the center of the block, running lengthwise, there is a cylindrical hole for a needle. There is a large cut away section at the front (right) with holes for the nib, a locking pin, and a screw to be inserted.



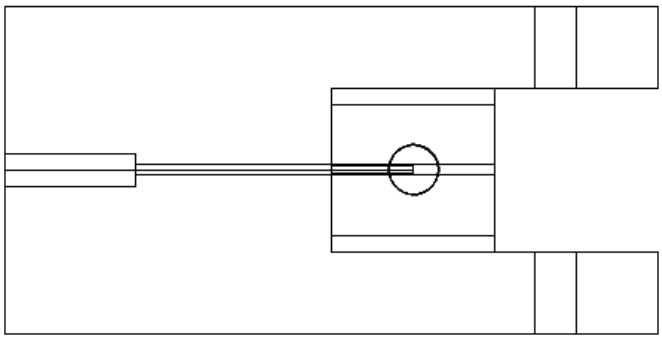
Side view of the holder with additional dimensions labeled.



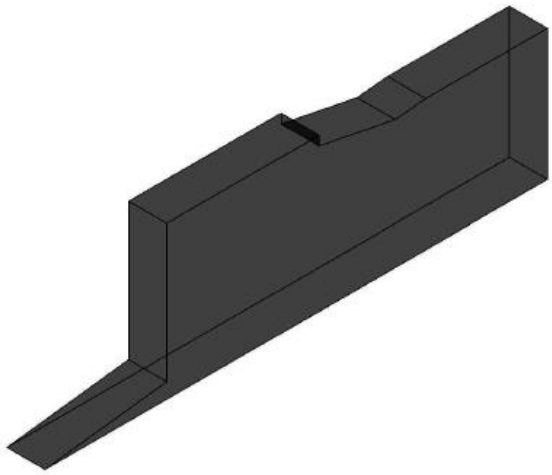
Front view of the holder.



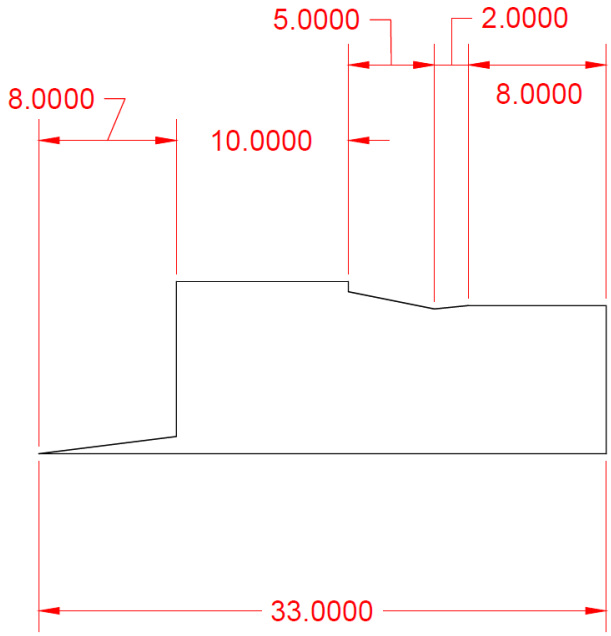
Front view of the holder. The 8 mm wide slot for the nib is visible in the center of the drawing, just below the small hole for the needle.



Top view of the holder. The hole for the screw that fixes the nib in place is visible at the end of the hole for the needle, just right of center.

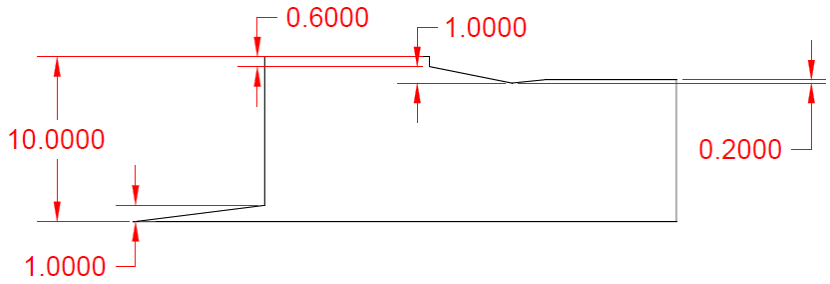


3-dimensional “x-ray” image of the locking pin used to hold the needle-paper-nib assembly together.

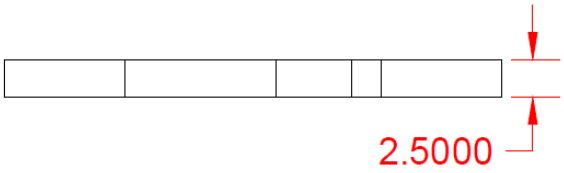


Side view of the locking pin with horizontal dimensions. The pin is shown inverted here. It is inverted and inserted through the larger of the two rectangular holes near the front of the nib holder. The notch in the pin locks into place over the needle, holding it down. The narrow

end fits into the other rectangular hole in the nib holder, holding the pin in place. The narrow triangular section at left is used as a handle to retract the pin from the holder.



Side view of the locking pin with the vertical dimensions labeled.



Top view of the locking pin.