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TITLE: Incubation and Growth of Life Sciences, Medical and Biotechnology Businesses in Proteomics, Genomics, Medicine, and Dentistry

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14. ABSTRACT

Trace level detection of chemical warfare agent simulants and biological toxins by desorption electrospray ionization(DESI) has been demonstrated. The detection of several chemical agent simulants as well as peptide and fungal toxins was shown at picogram levels from a variety of surfaces and in the presence of potential matrix interferences. In addition, the detection of intact bacterial cells was also demonstrated. Smears of cells taken from cultures were analyzed yielding characteristic mass spectra for the different species studied. Ions arising from samples of Pseudomonas aeruginosa have been successfully identified as quinoline intercellular signaling molecules. Ions from other species have not yet been identified. Finally, a prototype DESI wand was developed for the sampling of object not accessible by the standard mass spectrometer interface. The device extended approximately 20 cm from the mass spectrometer and was equipped with an array of both DESI spray heads and ion collection tubes, enabling higher surface area scanning than is possible with a single spray head/ion collection tube combination

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Introduction

The research reported here is an initial investigation in the utility of desorption electrospray ionization for the rapid and sensitive detection of chemical and biological warfare agents. Using appropriate simulants, the studies described in this report establish a baseline in terms of the ability of desorption electrospray ionization to detect these classes of compounds. The studies performed here provide the initial steps towards developing a novel and nascent technique for chemical analysis for the future use in the protection of people from chemical and biological threats.

Body

Biological and chemical warfare are defined as the intentional use of organisms or toxic agents to harm or kill people. While not a new phenomenon, the attack on the World Trade Center and the finding of anthrax and ricin-laden letters in the US mail system have heightened awareness of the risks and potential devastating consequences of bioterrorism. Mitigation of these risks requires that the nation have systems for rapid detection and identification of chemical and biological toxins (e.g., cyanide, ricin) and infectious microorganisms (e.g., *Varicella* (smallpox virus), *Francisella tularensis*, *Bacillus anthracis*). Many different assay formats for detection of these agents and organisms have been tried including use of classical microbiological methods, lateral flow assays, traditional ELISA, nucleic acid detection, time resolved fluorescence, and mass spectrometry (MS). Development of practical surveillance systems is difficult because:

- Not all detection and identification technologies are suitable for field use
- The number and diversity of potential pathogens and toxins is huge
- The location of the toxic agent is frequently difficult to pinpoint due to lack of immediate signs of exposure or presence of symptoms that mimic those of common diseases.

The need for rapid, sensitive, and selective detection methods for chemical and biological threats in complex samples is clear. Analysis of environmental samples presents a daunting challenge to analytical chemists due to the complexity of the sample matrix relative to the often times trace amounts of material of interest. Mass spectrometry (MS) is generally considered to be the ‘gold standard’ analytical analysis method due to its speed, sensitivity, and selectivity, and to this end, there has been a

significant focus on the development of methods based on MS to meet this challenge. Thus far, the high cost and large size of the instruments systems has hampered adoption of MS methods. Miniaturization of mass spectrometers[1-4] is one strategy for making MS more accessible for field analysis. However, the traditional methods for sample analysis used with MS require extensive sample cleanup and preparation prior to introduction of the sample into the mass spectrometer for analysis. The recent introduction of desorption electrospray ionization (DESI) has offered the possibility to analyze condensed phase samples, in many cases without the need for sample preparation, and do so under ambient conditions. DESI has been shown to be compatible with both small molecules such as explosives and chemical warfare agents to peptides, proteins, and other biomolecules. This work will explore the role DESI might play in the detection and identification of chemical and biological toxins and microorganisms which could be used for terrorism purposes from common surface materials in the presence of environmental matrices.

Desorption Electrospray Ionization

Mass spectrometry has significant advantages in speed, sensitivity and specificity over other methods of chemical analysis and its broad applicability has proven valuable in many different scientific fields; however, MS is limited in part by the requirements for samples to be prepared prior to analysis. In most cases, the sample must be placed in vacuum for analysis (e.g matrix-assisted laser desorption/ionization - MALDI) or dissolved or extracted in a solvent and sprayed in atmosphere (e.g. electrospray ionization - ESI) into the mass spectrometer. The requirement for the sample to be introduced into the vacuum system poses potential problems with contamination, speed of analysis, and the ability to provide true *in situ* measurements. Recent advances in mass spectrometry have taken the analysis of samples outside of the vacuum environment and into atmospheric pressure where the sample is maintained under ambient conditions.[5] Desorption electrospray ionization (DESI),[6, 7] developed in the laboratory of Professor R. Graham Cooks at Purdue University and now commercialized by Prosolia, Inc., is the principal method in this new family of ionization methods. Figure 1 shows a photograph of the Prosolia Omni Spray™ ion source used throughout these studies mounted onto a ThermoElectron LTQ MS. Other methods in this group of ionization techniques include

electrospray laser desorption ionization (ELDI),[8] direct analysis in real time (DART™),[9] and the atmospheric-pressure solids analysis probe (ASAP).[10]. Desorption electrospray ionization minimizes the requirements for sample preparation by enabling the investigation of samples in their native environment, where the sample is free for further chemical or physical manipulation. In this new method, charged droplets and ions produced from the electrospray are directed by a high velocity gas jet to the surface bearing the analyte. The charged droplets impact the surface where the analyte is dissolved into the electrically charged droplets. Secondary droplets ejected from the surface are subsequently collected in the ion transfer tube or atmospheric inlet of a standard commercial mass spectrometer and are mass-analyzed.

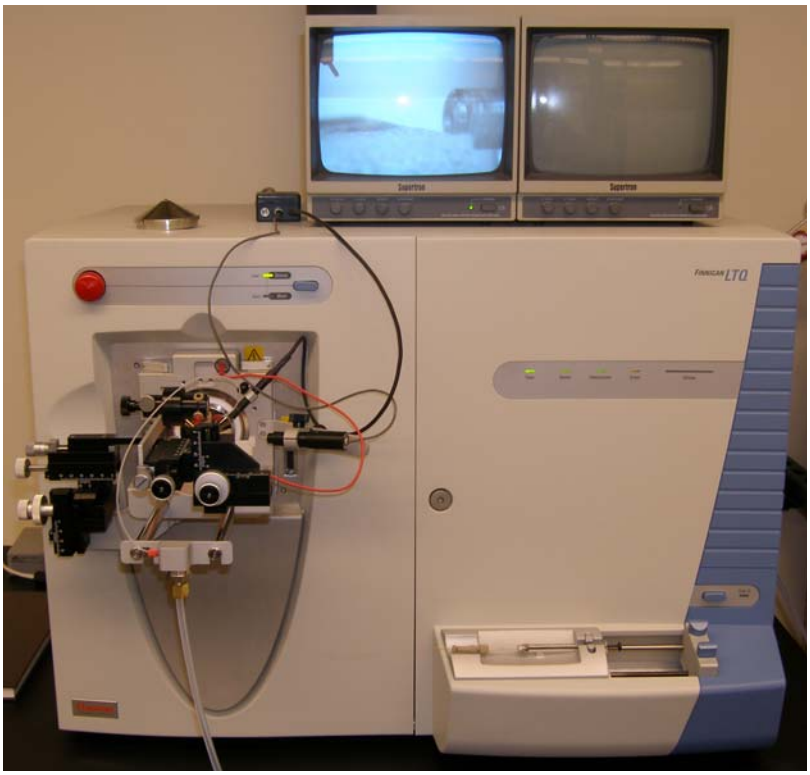


Figure 1 – Photograph of the Prosolia Omni Spray™ ionization source mounted onto the ThermoElectron LTQ MS system

Research Summary

The research performed during the duration of the grant period focused on four areas related to chemical and biological warfare agent detection, chemical agent detection, biological toxin detection, biological agent detection, and investigation in a

DESI 'wand' device. Each of these areas was treated independently and the results from each area will be discussed below. Although each area of research was treated independently, only changes in the operating conditions of the DESI source and mass spectrometer were necessary to optimize for the different types of analytes.

For the detection of the different classes of chemical agents and toxins, three general sets of experiments were performed as outlined below.

1). Calibration studies on various surfaces

The first set of experiments performed for the different classes of compounds involved the optimization of the DESI source for the particular type of compound to be analyzed. This includes both the optimization of source parameters as well as the optimization of instrumental parameters including ion optics voltages, collision energies, etc. These experiments also aimed to determine the limits of detection for each compound studied through the construction of a standard curve from various surface materials.

2) Experiments with mixtures of compounds

The second set of experiments performed for each class of compound was used to determine the effects of various combinations of compounds from within that particular class on the response of a selected individual compound. These experiments consisted of binary and ternary mixtures of equal amounts of individual compounds over a range of concentrations, as well as varying amounts of one compound of a binary mixture with the concentration of the other compound held constant.

3) Experiments with matrix interferences

The third set of experiments performed on each class of compounds was used to determine the effects of various chemical matrices on the detection of the various classes of compounds studied. In this set of experiments, common household chemicals were used in an attempt to 'mask' the compound of interest and to determine their effect on the results obtained for 'clean' samples.

Laboratory Preparation

As outlined in the original proposal, one goal of this funding was to aid in the development and growth of small business associated with the Indiana Research and

Technology Corporation. As such, a laboratory environment in which these studies could be performed was designed and constructed during the first two months of funding. This included the purchase of two mass spectrometer systems, a ThermoElectron LTQ linear ion trap MS and a ThermoElectron TSQ Quantum Discovery Max triple quadrupole MS. The instruments were chose to offer complementary capabilities through the different mass analyzers employed in each mass spectrometer and for compatibility with Prosolia's Omni Spray™ Ion source. Briefly, the LTQ MS, installed in mid-November of 2005, is capable of analyzing ions of a mass/charge range of 20-4000 Thompsons (1 Thompson, Th, = 1 AMU/elementary charge[11]). The ion trap is thus useful in the detection and identification of peptide toxins and proteins as the multiply charged ions from these types of analytes typically fall between in the range of 1000-4000 Th. In addition, the instrument can also perform tandem mass spectrometry (MS^n) experiments[12] to add to the selectivity and confidence of a chemical identification.[13] The TSQ MS, installed in mid-December 2005, is capable of analyzing ions with a mass to charge range between 15-1500 Th. This instrument is also capable of MS^n experiments, several of which are difficult to perform on the LTQ. The fast scanning capability of the TSQ permits for rapid switching between MS^n experiments which generated data characteristic of particular compounds. This allows for several analytes to be monitored in fast succession.

Chemical Agent Detection

The initial experiments performed focused on the first area of research, the application of DESI to the detection of chemical warfare (CW) agents on common surface materials. For these studies, several CW agent simulants were chosen and the limits of detection for each were determined on both a frosted glass surface and an addition surface material. The simulants chosen, dimethyl methylphosphonate (DMMP, simulant for Saran (GB)), diethyl methylphosphonate (DEMP, simulant for GB) , diethyl phosphoramidate (DEPA, simulant for Tabun (GA)), pinacolyl methylphosphonate (PMP, simulant for Soman (GD)), 2-(butylamino)-ethanethiol (2-BAET, simulant for VX), and 2-chloroethyl ethylsulfide (2-CEES, simulant for Sulfur Mustard (HD)), were based on their prior use as described in several literature sources.[14, 15] The simulants

were obtained as neat compounds from Sigma Aldrich (St. Louis, MO) and used without additional purification. Solutions of each simulant were prepared in methanol at concentrations ranging from 10 mg/mL to 100 pg/mL. Samples were prepared by depositing a 1 μL aliquot of the appropriate standard solution onto the test surface materials and allowing the spot to air dry at room temperature, leaving the residual simulant on the test surface at amounts ranging from 100 fg to 1 μg.

A Prosolia Omni Spray™ ion source was used to perform the analysis of each sample spot by DESI using the conditions summarized in Table 1 on the ThermoElectron TSQ Quantum Discovery Max using single reaction monitoring (SRM) mode. Data was collected from 30 seconds to 1 minute and each concentration level was repeated in triplicate. Standard curves were constructed and limits of detection were determined using the background signal present when analyzing the surface with no analyte present. Table 2 summarizes the limits of detection studies for these CW agent simulants. The results of this limit of detection study show the sensitive detection of the tested CW simulants from both a 'standard' frosted glass surface and from various other surface materials. An effort was made to vary the types of surfaces chosen such that a variety of common materials would be tested.

Figure 2 shows an example of the typical data collected for this study. From the raw data file, an extracted ion chromatogram (EIC) was created for the product ion monitored during the SRM experiment. This EIC was then integrated during the entire data collection time, with the area used to plot a standard curve. The standard curve obtained for the determination of DMMP from leather is shown in Figure 3. Generally, the limits of detection were determined to be on the order of 100's of picograms for the compounds tested. One exception to this is the soman simulant pinacolyl methylphosphonate (PMP). The sensitivity towards this analyte was much lower than the others studied. This can be explained by the lower proton affinity of the molecule relative to the other compounds studied.

Parameter	Setting
ES voltage	4.5 kV
Solvent flow rate	3-5 μl/min, 1:1 Methanol/Water
Gas pressure	100 PSI
Distance from tip to surface	~5 mm
MS inlet temperature	270° C
Spray Impact angle (α)	60°
Sample to capillary distance	~1.5 mm

Table 1 – Experimental conditions for limit of detection determination for CW agent simulants

Also, of particular interest in these results is the detection of 2-butylamino-ethanethiol (2-BAET, molecular weight 133 g/mol). It was expected that a protonated molecule of 2-BAET would be detected at m/z 134, however, a much stronger ion was present at m/z 265. Oxidation of the thiol, presumably due to atmospheric oxygen, leads to the formation of a disulfide bond between two molecules of 2-BAET, which when protonated during the DESI experiment gives rise to the ion at m/z 265. Because this ion had a much stronger signal than that of the protonated 2-BAET, it was used to identify the presence of 2-BAET in these experiments. It should be noted that the LODs determined on the second surfaces tested were all lower than that determined on the frosted glass surface. This is due to the reduction of background signal after cleaning residual simulants from the instrument following the first round of analysis from the standard glass surface. In all cases, at high levels of simulants (100 ng-1 μg) are deposited onto surfaces for analysis, the high vapor pressure of the compounds studied caused the instrument to respond even when the location of the deposited sample was not interrogated by the DESI spray plume. Although this is not the intended function of the DESI experiment, the interaction of the vapor phase simulants with the DESI spray gives the technique the ability to ionize these molecules from the gas phase in a manner consistent with secondary electrospray ionization[16] and in the condensed phase via DESI.

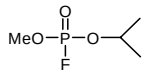
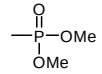
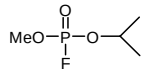
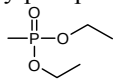
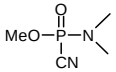
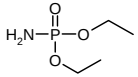
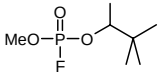
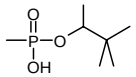
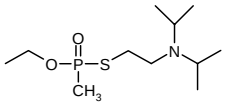
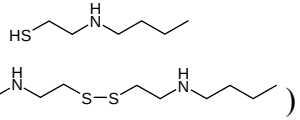
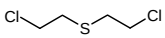
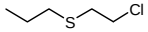
CW agent	Simulant	LOD (pg) on frosted glass	LOD (pg) (surface indicated)	SRM Transition	Collision Energy (V)
Saran (GB) 	Dimethyl methylphosphonate (DMMP) 	158	126 (Leather)	125 → 93	20
Saran (GB) 	Diethyl methylphosphonate (DEMP) 	303	193 (Cotton Canvas)	153 → 97	20
Tabun (GA) 	Diethyl phosphoramidate (DEPA) 	710	35 (Yellow Envelope Paper)	154 → 98	20
Soman (GD) 	Pinacolyl methylphosphonate (PMP) 	1190000 (1.19 µg)	1780 (PVC)	181 → 97	18
VX 	2-(Butylamino)-ethanethiol (2-BAET) 	87	1.7 (3003 Aluminum)	265 → 132	20
Sulfur Mustard (HD) 	2-Chloroethyl ethylsulfide (2-CEES) 	95	91 (Buna-N Rubber)	89 → 61	20

Table 2 – Summary of results for the detection of chemical warfare agent simulants from various surface materials

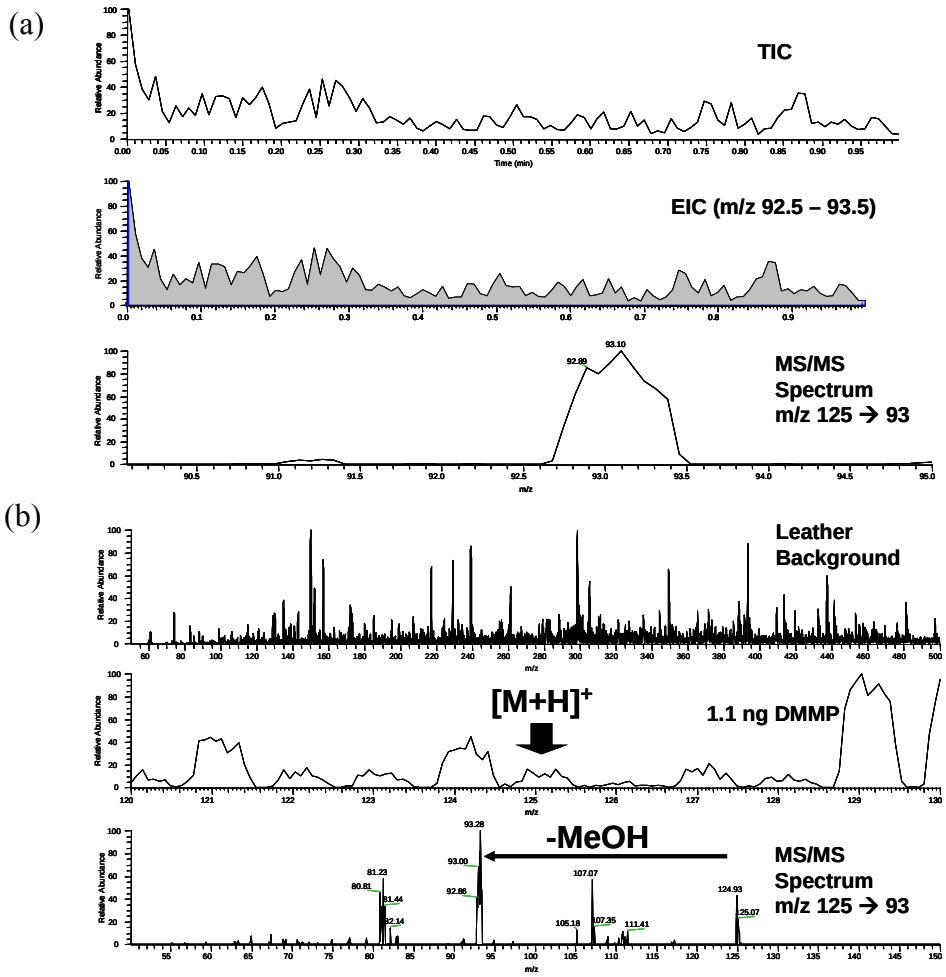


Figure 2 – (a) Data obtained for a 1.1 ng sample of DMMP on frosted glass surface showing the total ion chromatogram, and extracted ion chromatogram, and the product ion spectrum for the protonated DMMP molecule. (b) Data obtained for a 1.1 ng sample of DMMP on a leather surface showing a full scan mass spectrum and the product ion spectrum for the protonated DMMP on leather

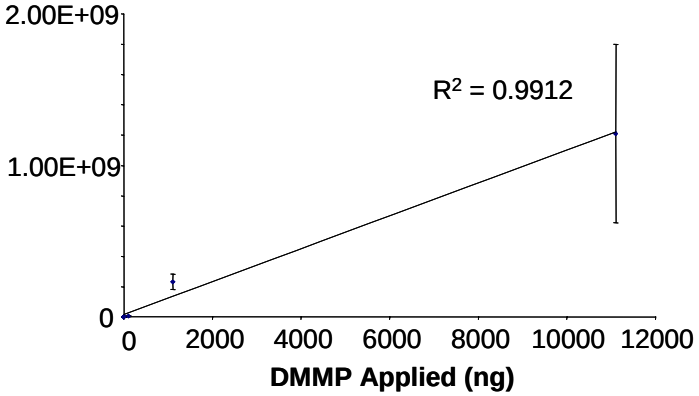


Figure 3 – Standard curve for the determination of DMMP from a leather surface

In addition to the determination of the LODs of these compounds on relatively 'clean' surfaces, the limit of detection for DMMP was also evaluated in the presence of other chemicals, intended to simulate common chemical compounds that may be found in environmental matrices. Solutions of 2% (v/v) blue window cleaner (Office Depot brand), vinegar, and germicidal bleach were prepared in methanol as well as a solution of 2% WD-40 in dichloromethane and used as the diluents for this study. A full standard curve was generated for DMMP in the 2% window cleaner solution using the same experimental method described above. This data was then used to choose a concentration level to test with the other matrices. The LOD was determined to be 2.8 pg for DMMP in the 2% window cleaner solution. Solutions were then prepared at the 100 ng/mL level (100 pg applied to the surface) in the other matrices and tested against interfering with the detection of DMMP. In all cases, DMMP was easily detected in the presence of the matrix, as summarized in Table 3.

Sample Matrix (containing 100 pg DMMP)	**Average Instrument Response**
DMMP Standard	127376
Blue Window Cleaner	116998
White Vinegar	595036
Bleach	589482
WD-40	1236112

Table 3 – Instrument response for the detection of DMMP in the presence of interfering matrices. Detection of DMMP was successful in all matrices tested

The final study on the detection of chemical simulants in mixtures was performed. The first experiment of the study was performed to show the simultaneous analysis of three of the CW agent simulants in one sample. A solution was prepared in which each simulant was present at twice the limit of detection level shown in Table 1. This sample was then analyzed using DESI from a frosted glass surface. The TSQ Quantum mass spectrometer was set up in multiple reactions monitoring mode (MRM) to sequentially perform the appropriate SRM transition for each of these compounds. Data was collected for 20 scans of each SRM transition (30 seconds total). The results of this experiment (Table 4) show the successful detection of all three simulant in the same sample. However, the signal intensity from the 2-CEES is significantly lower than had been

expected. This effect is though to be due to the decomposition of the solution used in this experiment and the high vapor pressure of the compound, rather than a suppression effect due to the presence of the other two simulants.

	2-CEES	DEMP	DEPA
Background	210	2067207	58313
Sample 1	387	12897312	4864775
Sample 2	6195	13432044	2747154
Sample 3	1561	12685821	2942207
Corrected Average	2504.3	10937852	3459732

Table 4 – Signal intensity of components in the tertiary mixture of CW agent simulants

A second experiment with mixtures was performed to test for suppression of a low concentration simulant in the presence of an increasing concentration of a second simulant. For this experiment, DMMP and 2-BAET were chosen. The concentration of DMMP was held constant at twice the limit of detection found in Table 1 while the concentration of 2-BAET was varied from 1x to 1000x the concentration of the DMMP. The sample for each concentration level was analyzed in triplicate As can be seen in Figure 4, the signal due to DMMP is relatively constant as the concentration of 2-BAET is increased.

The set of experiments described here have underlined the applicability of DESI in detection of CWA simulants at trace levels, demonstrating the ionization techniques ability to detect these simulants in mixtures and in samples that have been masked with common matrices. Although several studies must be performed to access the ruggedness of DESI, in particular in conjunction with field portable mass spectrometers or ion mobility spectrometers, these experiments should serve as a proof of concept for the application of DESI to such an important area.

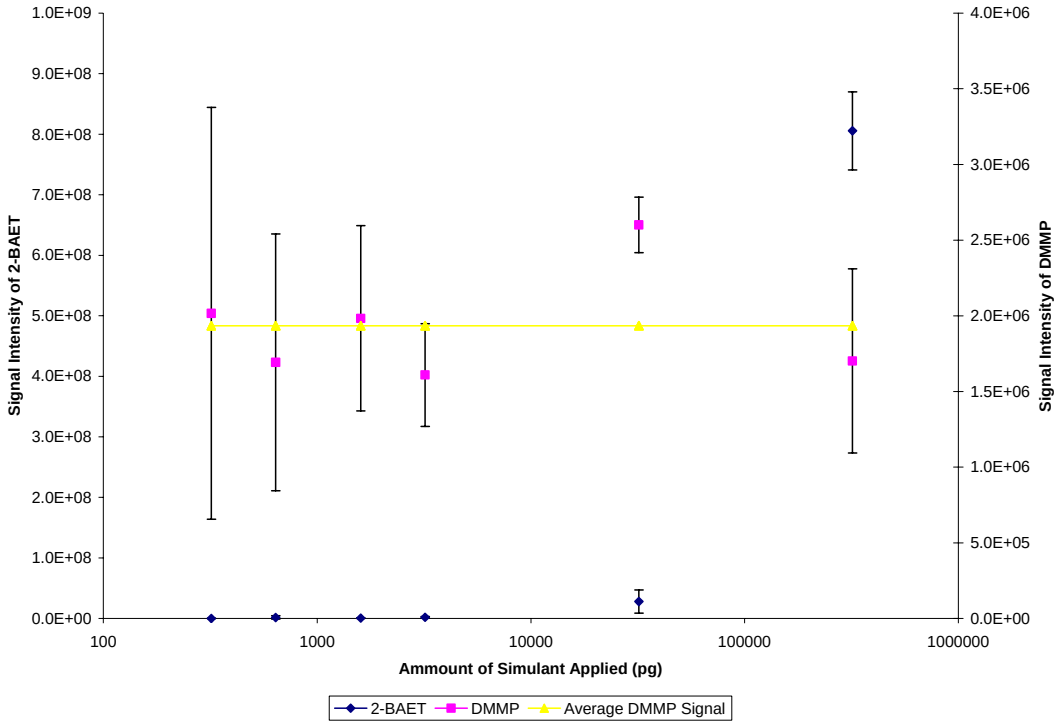


Figure 4 – Plot of DMMP and 2-BAET signal obtained during mixture study. The line represents the average DMMP signal for all concentrations

Peptide and Biological Toxins – Peptide Toxins

The second area of research pursued focused on the use of DESI for the detection of toxins of biological origin from common surface materials. These studies included both small molecule and peptide toxins. The experiments described here were designed to investigate the behavior of three peptide and fungal toxins in the DESI experiment. The overall aim of the study was to evaluate DESI detection of toxins from various surfaces and experimentally determine their practical limits of detection, the possible interference effects due to mixtures and matrices.

DESI has been shown to be able to ionize molecules with a large range in both mass and chemistry (i.e. polar to non-polar molecules). The source conditions which favor ionization of large molecules such as peptides and proteins can be slightly different from those used for ionization of smaller molecules such as chemical warfare agents. As such, several preliminary trials were performed in order to determine the optimal settings for both the mass spectrometer and the DESI interface (data not shown). Spray angle, flow rate, voltages, temperature, etc., once determined were used for all of the

experiments which followed. The optimized settings used for the peptide studies as well as the settings used for chemical agent detection are summarized in Table 5. The three peptides used in the study were bradykinin, melittin, and mastoparan. These peptides were chosen because of their relatively low levels of toxicity when compared to other peptide toxins such as conotoxins. Bradykinin is a plasma kinin, which are normal constituents of blood, and are the most potent vasodilator autacoids in mammals. At very low concentration, they increase capillary permeability, produce edema, evoke pain and reflexes by acting directly on nerve endings, contract or relax various smooth muscles, and elicit many other responses. Due to these vasodilatation properties, Bradykinin can produce a drastic, sharp fall in blood pressure, which at high concentrations leads to circulatory collapse and death. Bradykinin can also cause respiratory distress in asthmatics as well as intense burning pain when applied to exposed tissue or injected into the skin. Kinins that resemble Bradykinin are also found in wasp stings. Bradykinin is a nine amino acid peptide of molecular weight approximately 1060. The two major ion peaks at m/z 1060 and 531 correspond to $[M+H]^+$ and $[M+2H]^{+2}$, respectively. Mellitin is a strongly basic (pI=12.4) 26 residue peptide of molecular weight 2847.5, with a net charge state of +5 at pH 7.0. It is the major component of bee venom, particularly from the honey bee, *Apis mellifera,* and constitutes 40-50 percent of the dried venom. Mastoparan is a 14 amino acid peptide of molecular weight 1479.9. At pH 7.0, the net charge state is +3, or m/z 494. Although fairly hydrophilic, it is a strong base, with pI = 10.8. Mastoparan is a peptide component of wasp venom isolated from *Vespula lewisii.*

All three peptides were obtained from Sigma-Aldrich and used without further purification. Stock solutions for each peptide were prepared by weighing 1 mg of each peptide and diluting with 1 mL of methanol/water (50% v/v) solution. Standard solutions for this and the subsequent studies were prepared at levels ranging from 100 μg/mL to 100 pg/mL by serial dilutions of 1mg/mL stock solutions using methanol/water. The DESI experiments were performed using the ThermoElectron LTQ mass spectrometer, operated in full scan mode to collect ions from the multiple charge states typically generated from peptides in DESI.

Parameter	Setting for peptides	Setting for CW
ES voltage	4.5 kV	4.5 kV
Solvent flow rate	1.0 µl/min, 1:1 methanol/water	1.0 µl/min, 1:1 methanol/water
Gas pressure	100 PSI	100 PSI
Spray impact angle (α)	65°	60°
MS inlet temperature	180° C	270° C
Tube lens voltage	150 V	71 V
Emitter to sample distance	~3 mm	~5 mm
Sample to capillary distance	~2 mm	~2 mm

Table 5 – Optimized operating parameters for the detection of peptides using DESI. The optimized settings for detection of chemical warfare agents is also shown for comparison

In order to evaluate the suitability of DESI for the detection of peptides toxins located on common surface materials, the sensitivity of the technique (and associated mass spectrometer) was determined through the collection of standard curves. From these standard curves, it is also possible to determine the limits of detection for the method. Data for standard curves was collected by spotting the peptide solutions at appropriate concentrations in 1 µL volumes, directly onto the surface of interest and allowed to dry at room temperature. Spots from each concentration level were analyzed in triplicate. Blank measurements (used for limit of detection calculation) were made without spotting any solution by directly analyzing the surface of interest.

Data colleted under the source conditions described in Table 5 were used for the construction of standard curves for each peptide on the different surface materials. For each sample spot analyzed, the highest three peak intensities were averaged to obtain an instrument response. Since peptides have the potential for multiple charging due to the presence of several basic sites on the molecule, the sum of the average intensities of the peaks corresponding to the charge states seen for each peptides was used to plot the standard curves, although only the most favored charge state could also be used. The instrument response was corrected for the average background signal corresponding to the ion(s) of interest in the sample spots. Figure 5 shows a representative mass spectrum of 100 pg mastoparan analyzed from the surface of a VISA card. In this case, the intensities of the 3+ and 2+ charge states are summed and used to plot the standard curve.

Figure 6 shows the standard curve for bradykinin from the standard glass surface. The data points plotted are the sum of the $[M+H]^+$ and the $[M+2H]^{2+}$ ions, corrected for contributions of background chemical noise. The standard curves generated in all other experiments are similar. A response factor at each concentration was calculated by dividing the signal intensity by the amount of peptide spotted. The average response factor was then determined by averaging of the response factors at the individual concentration levels. Finally, the limit of detection was determined by calculation of an average response factor multiplied by the 3x standard deviation of the blank signal. Table 6 summarizes the limits of detection determined in this portion of the study for the three peptides spotted on a standard glass surface, as well as various other common materials.

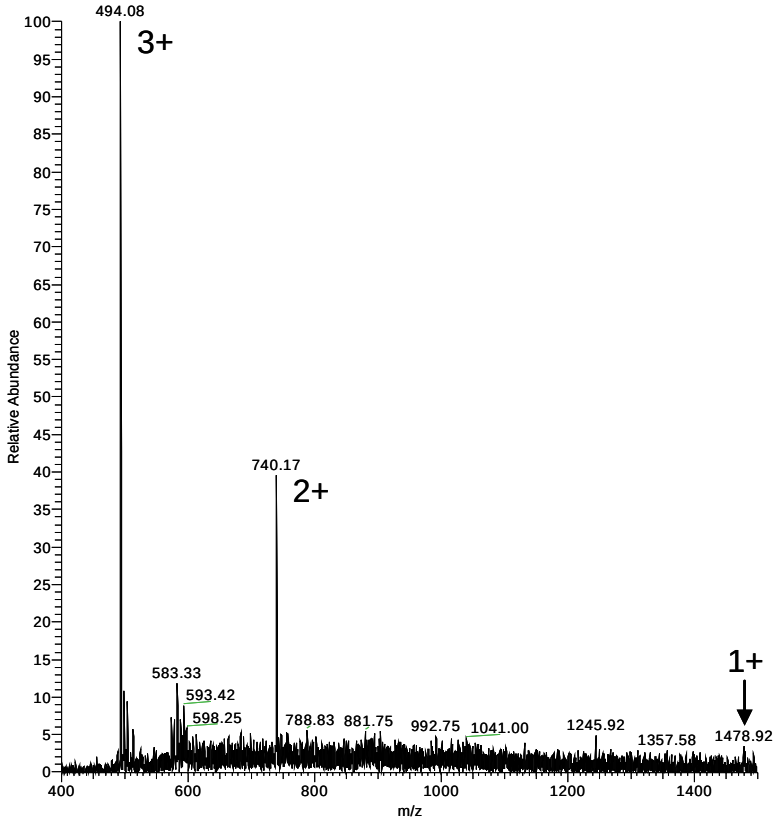


Figure 5 – Mass spectrum of 100 pg mastoparan deposited onto a VISA card

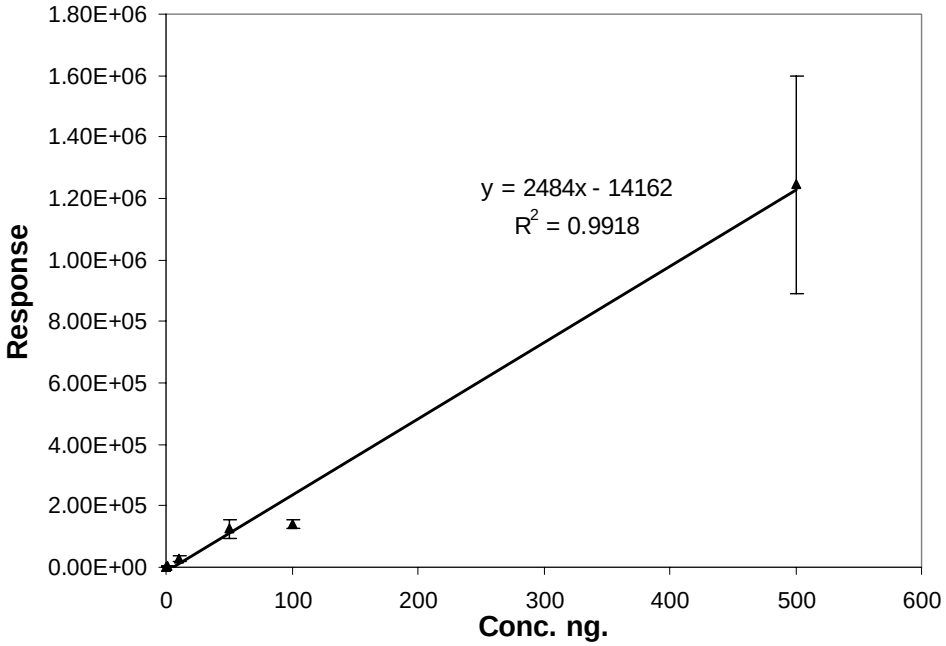


Figure 6 – Standard curve generated for bradykinin analyzed by DESI from a microscope glass slide

The second study undertaken with the peptide toxins was designed to determine the effects of various combinations of peptides on individual compound response. These experiments consisted of binary and ternary mixtures of equal amounts of individual compounds over a range of concentrations, as well as the effects of varying amounts of one peptide upon the responses of a binary mixture when the concentration of one peptide was held constant. Figure 7 demonstrates the ability of DESI to simultaneously detect all three peptides present on a glass surface. Although not performed here, the spectrum can be deconvoluted to obtain a single peak per peptide to identify each molecular mass.

Peptide	LOD (pg) on glass	LOD (pg) (surface indicated)
Bradykinin	2.0	30 (Frosted Glass)
Bradykinin	- - -	140 (Plastic)
Bradykinin	- - -	3.3 (Polyethylene Foam)
Bradykinin	- - -	4900 (Filter Paper)
Melittin	36	62 (Poly-methylmethacrylate)
Melittin	- - -	140 (LDPE)
Mastoparan	160	620 (Viton Rubber)
Mastoparan	- - -	430 (VISA Card Plastic)

Table 6 – Limits of detection for peptides studies from several common surface materials

To investigate the effects of increasing the surface concentration of one peptide relative to another, two experiments were performed using binary mixtures of peptides. In the first, mastoparan was held at 10 ng per spot, while the level of melittin was changed from 50 to 100 ng. Although limited in the number of data points collected, the data shows no ion suppression effects at a 10 fold excess of melittin relative to the mastoparan. The second experiment performed held bradykinin constant at 10 ng per spot while increasing the concentration of melittin from 1 to 100 ng per spot. The data collected shows no decrease in the signal due to bradykinin with increasing the level of melittin. A slight upward trend is noted, however, this is thought to be due to errors in the experiment rather than an enhancement of ionization.

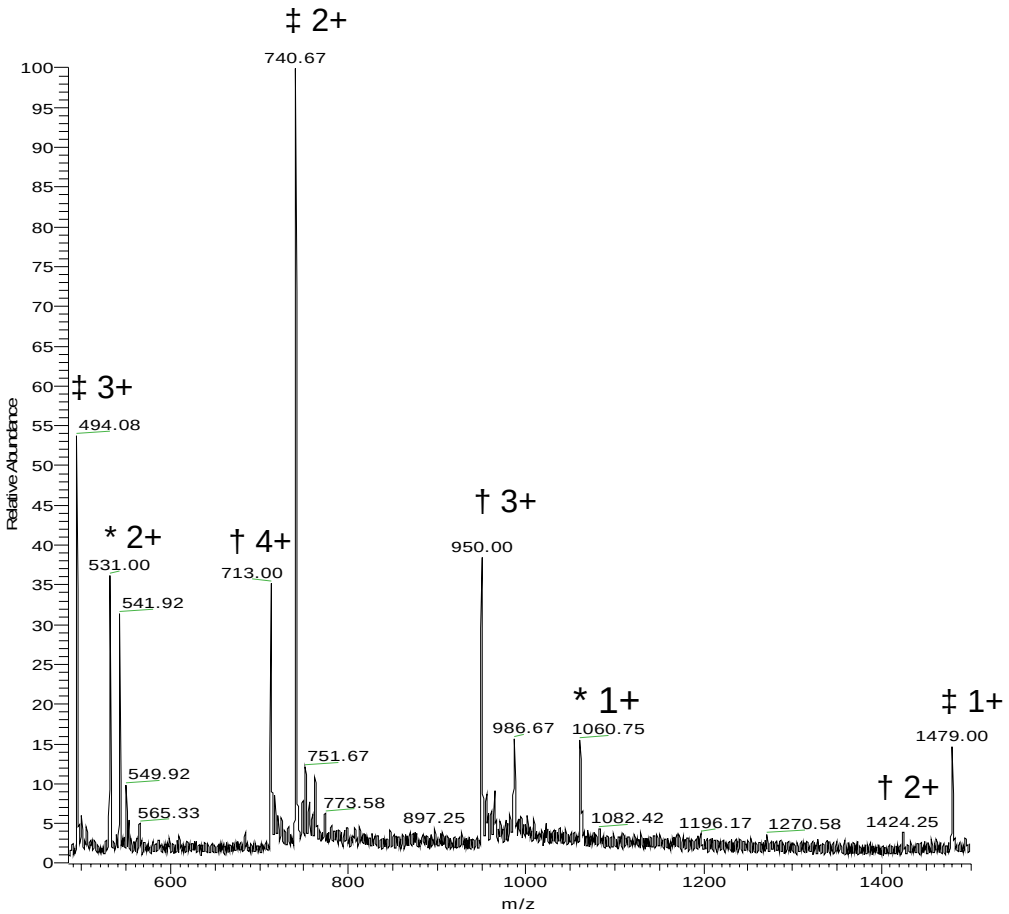


Figure 7 – Mass spectrum of a mixture of 100 ng each bradykinin (*), melittin (†), and mastoparan (‡). The charge state of each ion is indicated in the figure

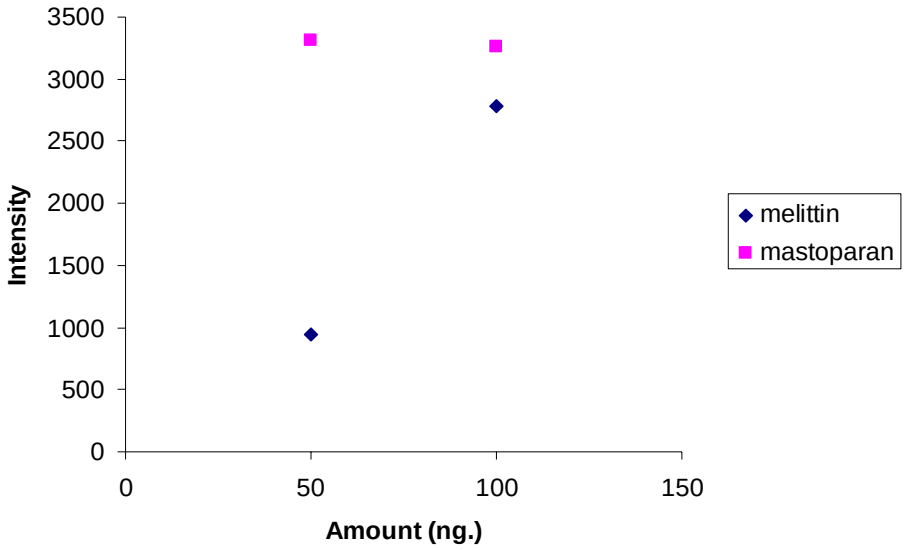


Figure 8 – Variation of the signal intensity of mastoparan with increasing levels of melittin. The mastoparan level was held constant at 10 ng

The final study performed with peptide toxins was to determine the effects of matrix interferences was studied using mastoparan, spotted onto a glass surface from matrix solutions. Solutions of 2% (v/v) blue window cleaner (Office Depot brand), vinegar (acetic acid), and germicidal bleach in methanol and 2% WD-40 in dichloromethane were prepared and used as the diluents for this study. Peptide/matrix standards were then made in the same manner as the original standards, except the 2% matrix solutions were used for dilution of the stock solution rather than methanol/water.

The results for this study are presented in Table 7. For these analytes (in contrast to the CW simulants) the presence of bleach did lower sensitivity (e.g. lowered instrument response) and caused the limit of detection to increase to over 2 ng present in the sample spot. It was noted that adduct formation in the case of bleach was more prominent than in samples without a matrix. Because we have not considered adduct ions in out data analysis, theses ions cause a loss of instrument response for the ions that are monitored. It is also though that the basic nature of the bleach solution might play a roll. This effect is likely small compared to the problems seen in the extensive formation of adducts. As evidence of this, samples containing 2% window cleaner, basic due to its ammonia content, showed a slight improvement in the limit of detection. The solutions containing 2% acetic acid showed a marked improvement in the limit of detection. This

effect is not unexpected as the peptide deposited on the surface would be positively charged at low pH, which then only requires liberation from the surface without the additional need for ionization. In the case of the 2% WD-40 solutions, no peptides were detected up to levels of 1μg deposited onto glass. It is thought that the low polarity of the solvent (hydrophobicity) may have forced the peptide out of solution and to the walls of the glass storage vial.

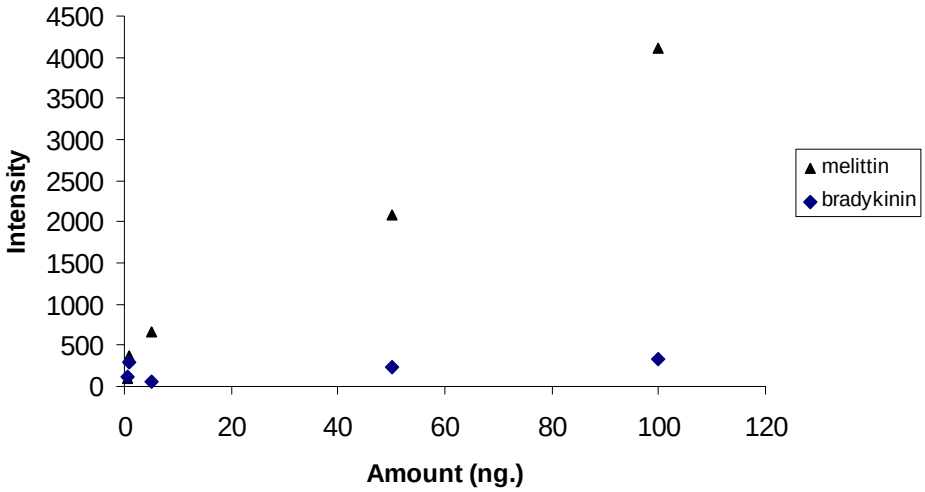


Figure 9 – Variation of the signal intensity of bradykinin with increasing levels of melittin. The bradykinin level was held constant at 10 ng

Peptide	Matrix	LOD (pg) on glass
Mastoparan	2% Bleach	2300
Mastoparan	2% Acetic Acid	42
Mastoparan	2% Glass Cleaner	110
Mastoparan	2% WD-40	DND

Table 7 – Limit of detection determined for mastoparan for a glass surface in the presence of interfering matrices (DND – did not detect)

Peptide and Biological Toxins – Fungal Toxins

Fungal toxins were chosen as a second toxin of biological origin due to the differences in mass, structure, and chemistry when compared to both chemical warfare agents and peptide toxins. Fungal toxins present a possible threat for weaponization as the methods for large scale cultivation of fungi known as well as techniques for purification of such

compounds. The detection of fungal toxins may also play a role in the analysis of food products and animals feeds for fungal infestation. The toxins chosen for this study include ergotamine from *Claviceps paspali*, fumonisin B1 from *Fusarium moniliforme*, and aflatoxins B1 and G1 from *Aspergillus flavus.*

All toxins studied were purchased from Sigma Aldrich and used without further purification. Solutions of each toxin were prepared by serial dilutions in concentration ranging from 1 mg/mL to 100 pg/mL. Samples were prepared by depositing a 1 μL aliquot of the appropriate standard solution onto the test surface material and allowing the spot to air dry at room temperature. The spot was then analyzed by DESI using the conditions summarized in Table 8 on the ThermoElectron TSQ Quantum Discovery Max using single reaction monitoring (SRM) mode. In this mode of operation, an ion formed from the analyte of interest using the DESI source (typically protonated or sodiated molecules in these studies), is mass selected from the background ions and subjected to high energy collisions. A specific fragment ion, characteristic of the analyte of interest, is then mass selected and detected. Using this method, one can selectively separate signals due to the analyte of interest over the chemical background thereby vastly improving the specificity of detection and identification. Data was collected from 30 scans (representing 45 seconds of data acquisition per spot) and each concentration level was repeated in triplicate. Data for the SRM transition monitored was integrated for the entire time period the sample was monitored. Standard curves were constructed and limits of detection were determined using the background signal present when analyzing the surface with no analyte present.

Parameter	**Setting for fungal toxins**
ES voltage	5.0 kV
Solvent flow rate, Solvent composition	2.0 μl/min, 1:1 methanol/water
Gas pressure	150 PSI
Spray impact angle (α)	52°
MS inlet temperature	250° C
Tube lens voltage	75 V
Emitter to sample distance	~2 mm
Sample to capillary distance	~1.5 mm

Table 8 – Optimized operating parameters for the detection of fungal toxins using DESI

Figure 10 represents a typical data file collected for ergotamine at 100 pg deposited onto a polymethyl methacrylate (PMMA) surface showing both the total ion chromatogram (top) and the mass spectrum (bottom). The summary of the limits of detection determined in this study is given in Table 9 with the surface material from which the toxins were detected is indicated. For all toxins, except for aflatoxin G1, the protonated molecule was selected as the precursor ion for SRM. For aflatoxin G1, the sodiated molecule was present in the mass spectrum at a higher abundance than the protonated molecule, thus the sodiated ion was chosen as the precursor ion for this toxin. In general, the limits of detection determined were in the low to mid picogram level of material present in the sample spot. These results are similar to those determined for both the chemical warfare agent simulants and the peptide toxins.

Toxin	**LOD (pg) on PMMA**	**LOD (pg) (surface indicated)**	**SRM Transition**	**Collision Energy (V)**
Ergotamine	9.45	- - -	582.2→223.3	35
Ergotamine	- - -	6.46(glass)	582.2→223.3	35
Fumonisin B1	71.0	- - -	722.5→334.4	35
Aflatoxin B1	5.39	- - -	313→269	35
Aflatoxin B1	- - -	17.3 (LPDE)	313→269	35
Aflatoxin G1	30.8	- - -	351→307	35
Aflatoxin G1	- - -	221.8 (ORV)	351→307	35

Table 9 – Limits of detection for the fungal toxins studied from several common surface materials. PMMA – polymethyl methacrylate, LPDE – low density polyethelyene, ORV – oil resistant vinyl

To demonstrate the capabilities of DESI-MS for the detection and identification of toxins in mixtures, mixtures of aflatoxins B1 and G1 were prepared with the concentration of aflatoxin G1 held constant at 500 pg per sample spot and aflatoxin B1 varied from 5 to 50000 pg per spot. The integrated instrument response is plotted in Figure 11. At high excess of aflatoxin B1, there is some ionization suppression of the constant level of aflatoxin G1. The suppression seen is likely due to the high levels of total analyte present in the sample.

Finally, mixtures of toxins with potential interfering matrix compounds were studied. Solutions of ergotamine were prepared in a 2% Office Depot brand Blue Window Cleaner ranging from 10 ng/mL to 100 μg/mL. Samples were spotted in 1 μL

aliquots onto a PMMA surface and analyzed in triplicate under the conditions described above. A standard curve for ergotamine in the presence of 2% window cleaner is shown in Figure 12. Using a blank measurement of the window cleaner solution, the limit of detection for this experiment is calculated to be 170.7 pg. This experiment shows a slight increase in the limit of detection for ergotamine when prepared in the 2% window cleaner solution. Results of the blank measurements show an increase in signal with no ergotamine present in the sample, indicating some background contamination of the instrument with ergotamine from previous studies results in some of the increase in the limit of detection.

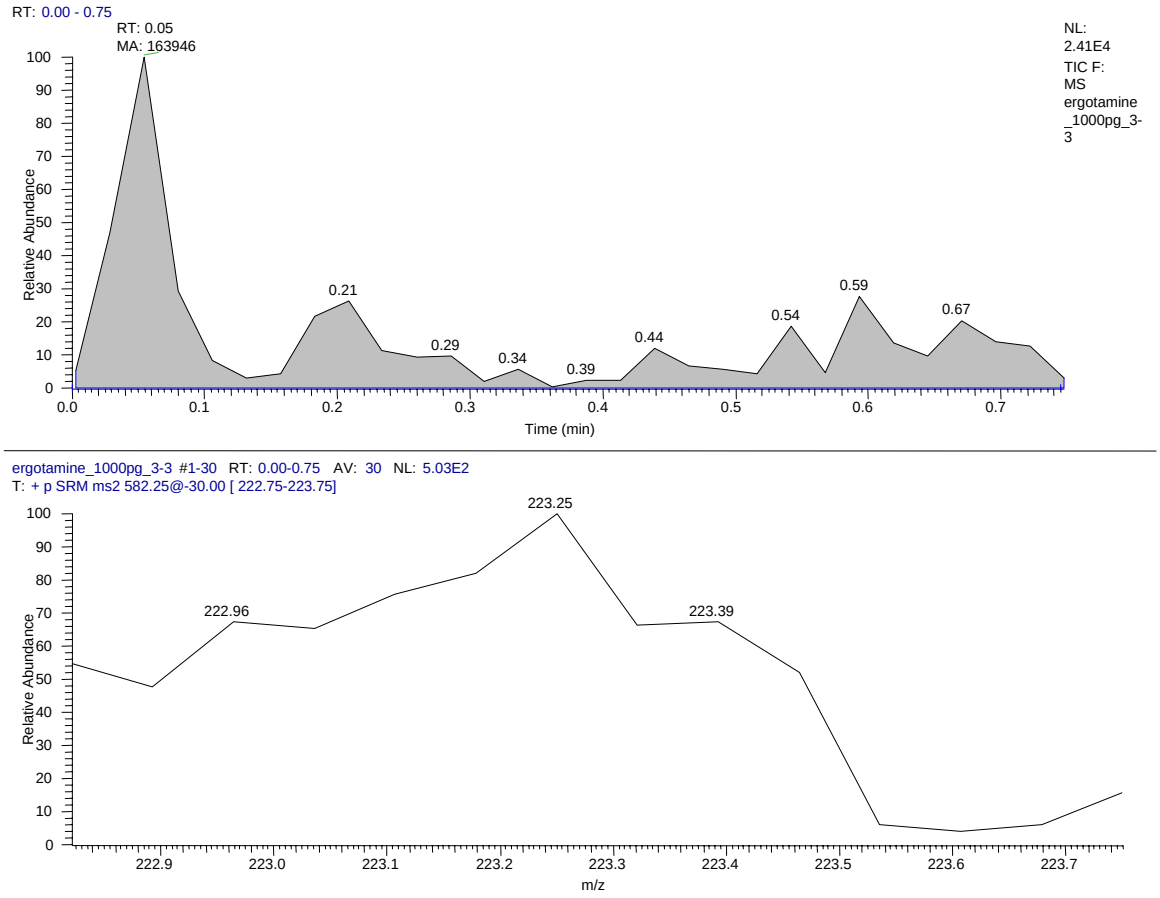


Figure 10 – Representative data file collected for 100 pg sample of ergotamine analyzed from a PMMA surface. The top trace is the total ion chromatogram for the SRM transition 522→223. The bottom trace is the averaged mass spectrum recorded over the m/z range 222.8-223.8

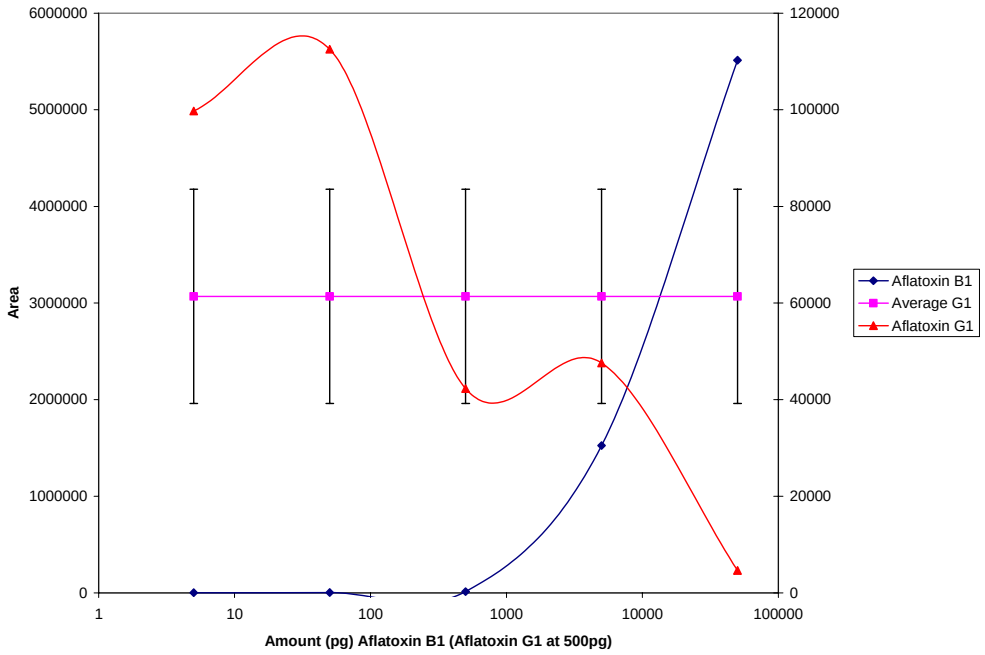


Figure 11 – Instrument response for mixtures of aflatoxins B1 and G1. The average and standard deviation of the response for aflatoxin G1 is plotted as squares. Note some suppression effects at high excess of aflatoxin B1 relative to aflatoxin G1

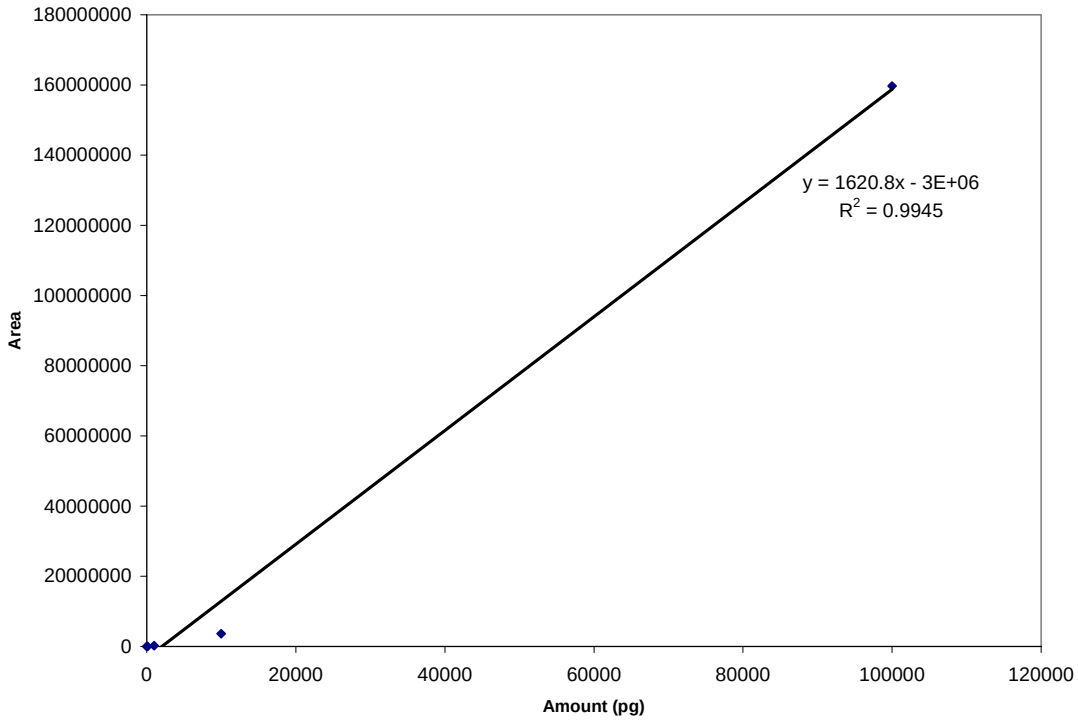


Figure 12 – Standard curve generated for ergotamine in 2% blue window cleaner for 10 to 100,000 pg per sample spot

As a demonstration of DESI in the presence of other possible interfering matrix compounds, solutions of 10 and 100 ng/mL aflatoxin G1 were prepared in ~1% JP-8 jet fuel and 10 and 100 ng/mL aflatoxin B1 in 2% vinegar were prepared. The solutions were analyzed using the same procedure described above and a summary of the results is presented in Table 10. As indicated above, some matrix effects are noted in the case of ergotamine in the presence of widow cleaner. As a departure from earlier studies with the chemical warfare agent simulants and peptide toxins, these compounds all show some effects due the presence of the matrix compounds. Given that these matrices contain acids and bases (vinegar and window cleaner, respectively) it is likely that the reduced response is due to degradation of the toxins by acid/base hydrolysis. The samples of aflatoxin Gl prepared in 1% JP-8 fuel also show a significant matrix effect. At the 100 pg level, the instrument response is approximately 2X greater than that of the blank (2839 and 4871, respectively). At this level, the JP-8 fuel is greater than 100,000 fold excess over the toxin. When the sample spot dries, this amounts to analyzing the toxin from an oil spot on a surface, which is quite a challenge for any analytical method.

Toxin	**Response w/ no matrix**		**Response with matrix**	
	10 pg	100 pg	10 pg	100 pg
Ergotamine (2% Windex)	3371	20890	10758.7*	10249*
Aflatoxin B1 (2% vinegar)	3560*	27804	4956	6455
Aflatoxin G1 (1% JP-8)	18518	262794	2358*	4871

Table 10 – Summary of results obtained while analyzing fungal toxins with various matrix compounds. Values marked with an * represent those with less than 2x increase in signal from the blank

Intact Bacteria Detection

The final phase of research for this grant was to investigate the capabilities of DESI for the detection and identification of biological warfare agents, in particular, intact microorganisms. Currently, several groups are pursuing the detection of intact microorganisms using MS with various means of introducing the intact cells for analysis.

Early work on microorganism detection with MS resulted from the adaptation of older gas chromatography/flame ionization detector methods for the profiling of fatty acids released from membrane phospholipids.[17] Similarly, carbohydrate profiles of whole cell hydrolysates have also been used to identify bacteria by GC/MS.[18] With the advent of ESI and MALDI, researchers had changed the focus of detection to peptides, proteins, and nucleic acids derived from bacterial cells for identification.[19, 20] These methods encompass the sequencing of proteins, pattern recognition of protein profiles, and detection of genetic material amplified via the polymerase chain reaction (PCR). In all cases, sample pretreatment is necessary prior to analysis by MS. The use of DESI as both the sampling and ionization technique for the detection of bacterial cells may allow for the direct analysis of intact cells with little to no sample pretreatment. Although reports of bacterial detection using DESI have been published, these studies have been limited in scope.[6, 21] The results described here have been collected as the initial data necessary for the expansion of this type of study in our laboratory.

Parameter	**Setting**
ES voltage	5.0 kV
Solvent flow rate, Solvent composition	2.0 µl/min, 1:1 methanol/water
Gas pressure	100 PSI
Spray impact angle (α)	55°
MS inlet temperature	250° C
Tube lens voltage	125 V
Emitter to sample distance	~2 mm
Sample to capillary distance	~1.5 mm

Table 11 – Operating parameters for the detection of intact bacteria using DESI

Five cultures of bacteria were obtained for this study. The bacteria obtained included three species, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. These species include both Gram positive and Gram negative bacteria. Also obtained were two strains of *E. Coli*, DH5α and DH1. The *E. Coli* DH1 strain contains a plasmid gene which is induced in the presence of isopropyl β-D-1-thiogalactopyranoside (IPTG) to cause the bacteria to form beta-galactosidase. The inclusion of 5-bromo-4-chloro-3-indolyl- beta-D-galactopyranoside (X-gal) to the growth media caused the colony to take

on a blue color when the gene is induced. In these experiments, the bacteria was cultured in both the presence and absence of IPTG (i.e. forming both colorless and blue colonies) to investigate any differences in the activation of this gene.

The cultures of *E. Coli* were grown on LB (Luria-Bertani) medium in standard Petri dishes at 37°C. The *P. aeruginosa* and *B. cereus* were grown on a soybean trypsin medium at 37°C. The cultures were analyzed immediately after growth to avoid contamination or changes to the bacteria due to aging of the samples. Initial experiments were performed using the DESI source conditions described by Cooks et al.[21] Samples of each bacteria culture were taken from the culture plate using a sterile inoculation loop and smeared onto either glass or Teflon coated glass slides. The slides were then mounted in the slide holder of the DESI source and analyzed using the ThermoElectron LTQ mass spectrometer using the conditions in Table 11.

Previous work with microorganisms as well as animal tissues has shown strong instrument response from lipid species in the positive ionization mode, and lipids and fatty acids in the negative ionization mode. It was hypothesized that we would see similar results to those previously described, but failed to see a strong response for the lipids. Analysis of intact bacteria with no sample treatment did not show the expected lipid species; rather several small molecule peaks were noted. Figure 13 shows the data collected for each bacterial sample and the instrument background for comparison. Several ions can be seen arising from the different species analyzed. To better see the ions arising from the bacterial cells, the data from the previous figure has had the background signal subtracted and is shown in Figure 14. It can bee seen that unique signatures for each species were obtained from these small molecule components between 150-500 Th.

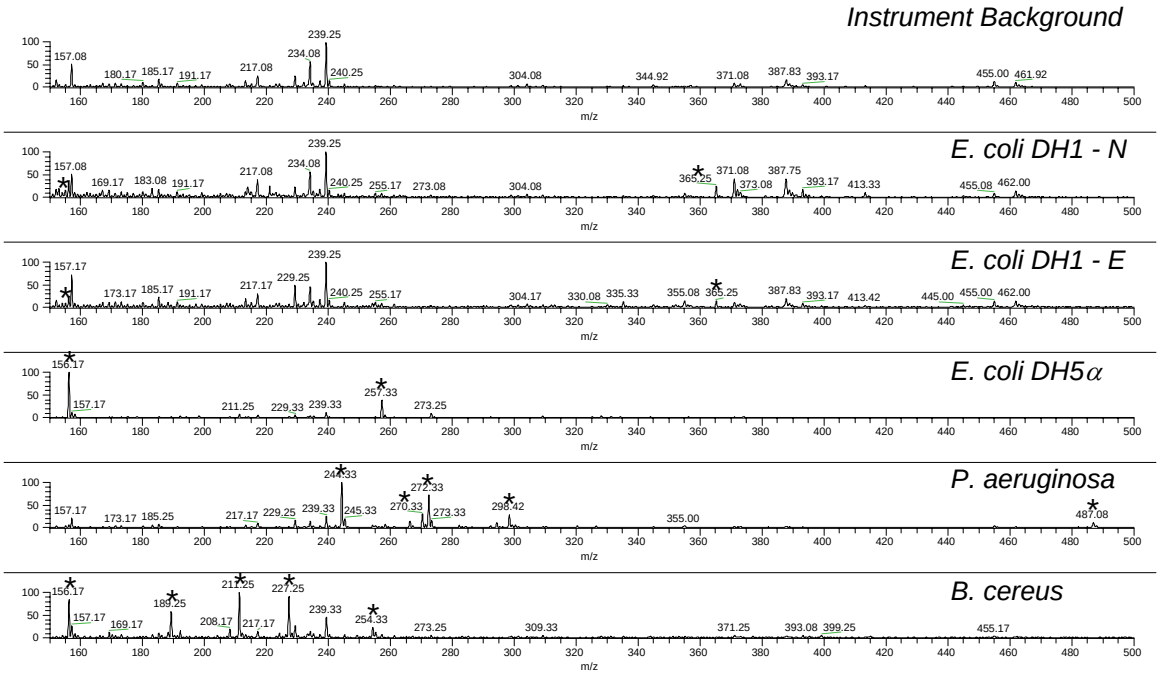


Figure 13 – Mass spectra collected from intact bacteria smeared onto a glass surface. Ions arising from bacteria indicated with an asterisk (*)

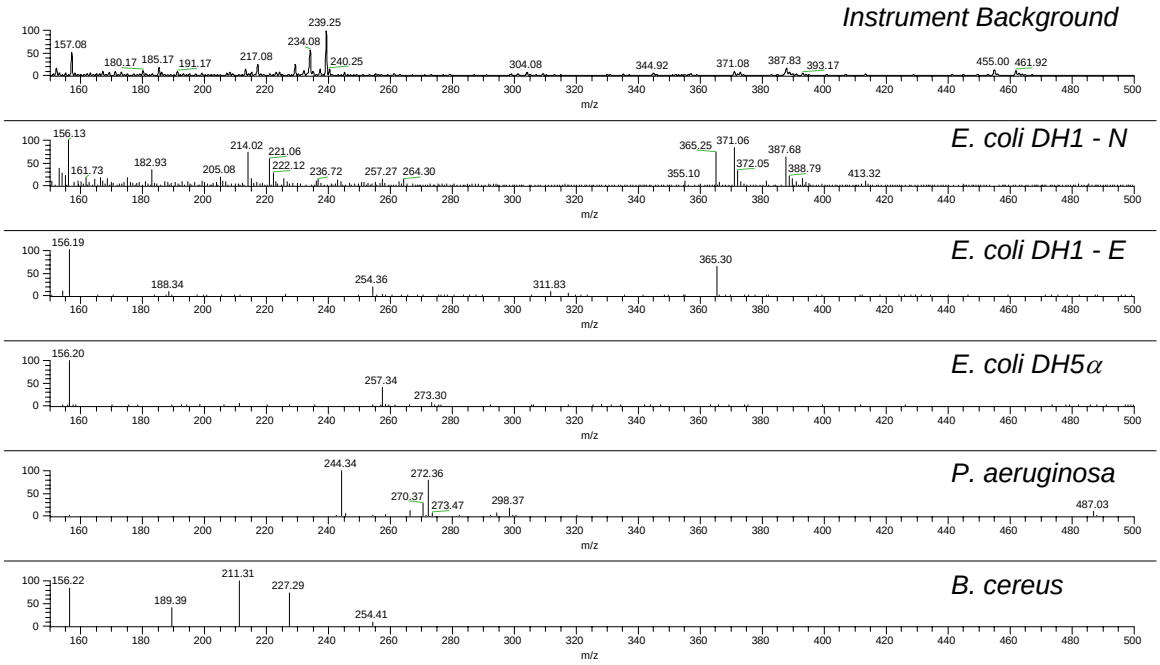


Figure 14 – Background subtracted mass spectra of intact bacterial cells

The data from *P. aeruginosa* was investigated in an attempt to identify the major components seen in the mass spectrum. Pseudomonads, and *P. aeruginosa* in particular, are well known for the production and excretion of quinolines for intercellular signaling. Tandem MS data was acquired and compared to fragmentation data available in the literature[22] for the tentative identification of the compounds seen in our studies. Positive identification of 4-hydroxy-2-heptylquinoline (HHQ) at m/z 244, 4-hydroxy-2-nonylquinoline (HNQ) at m/z 272, 3,4-dihydroxy-2-heptylquinoline (pseudomonas quinolone signal, PQS) at m/z 260, and 4-hydroxy-2-undecenylquinoline at m/z 298, as well as 4-hydroxy-2-nonenylquinoline at m/z 270. Representative product ions mass spectra from 4-hydroxy-2-heptylquinoline (HHQ) at m/z 244, 4-hydroxy-2-nonylquinoline (HNQ) at m/z 272 are shown in Figure 15. The ions arising from the other species have not yet been identified.

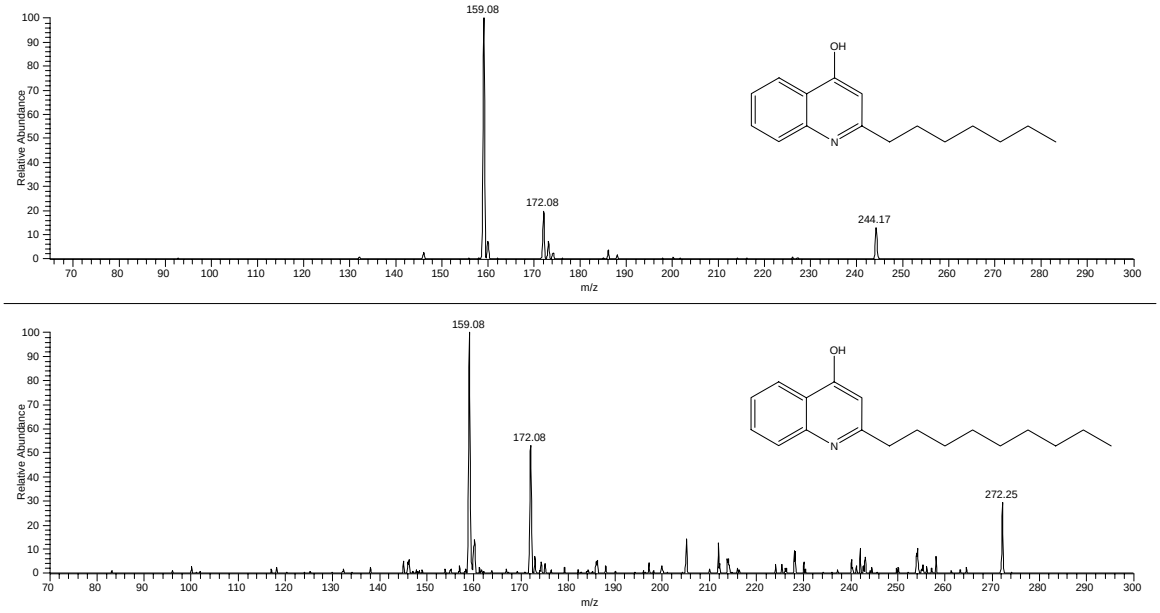


Figure 15 – Product ion mass HHQ (top) and HNQ (bottom) arising from P. aeruginosa. The product ions at m/z 159 and 172 Th are characteristic of 4-hydroxy-2-alkylquinolines

Reproducibility of the technique was assessed by sampling the *P. aeruginosa* culture at three additional locations from the agar plate and immediately analyzing as described above. The mass spectra of the four samples taken are shown in Figure 16. The mass spectra are very reproducible in terms of both peak locations and peak shapes. The overall intensity of the signal varied for each sample, averaging an intensity of 2.26×10^3 (arbitrary units) with a standard deviation of 781.5 (34.5% RSD). The bacterial

sample is assumed to be homogeneous across the agar plate, as such variation in signal is thought to be due to unequal sampling (actual mass of bacteria sampled was not measured).

The lack of response for lipids as previously described has caused some concern that differences in the growth media suppress ionization of the lipids. Previously described experiments using DESI for detection of microorganisms relied upon growth of the bacteria in a minimal media broth, followed by isolation and washing of the cells to eliminate contamination from the media. As no such effort was made in the initial analysis of these bacteria, additional samples were scraped from the cultures and washed in either water, methanol, or 50/50 methanol/water. To wash, a small sample of the bacteria was placed into an eppendorf tube followed by a 0.5 mL aliquot of the wash solution. The mixture was vortexed for 15 seconds and then centrifuged at 14000 RPM for 5 minutes. The supernatant was pipetted from the tube and saved for further analysis. The washing procedure was repeated for a total of three washes with the supernatant from each wash combined. For each bacterial sample, the washed cells were sampled from the tube and smeared onto a glass slide for analysis. To analyze the supernatant, the collected solutions were evaporated until ~200 μL remained. A 1 μL aliquot was then spotted onto a Teflon surface for analysis.

Washing the cells prior to analysis revealed several features of the spectrum not seen in the cells directly analyzed from the agar. The various washes each brought out different components of the bacteria. The bacterial samples washed in water showed little change from the unwashed cells. Samples washed in methanol showed some changes in the mass spectra. For all samples, cells analyzed after the methanol wash showed groups of ions at higher masses, particularly in the ranges of 500-800 Th and 1100-1400 Th as seen in Figure 17. Previous analysis of biological tissues have shown rich distributions of lipids in these mass ranges,[21, 23, 24] bringing some agreement between earlier studies of microorganisms by DESI-MS. It should be noted that although the lipid profiles were not seen to the extent described by others in the unwashed cells, the data obtained for small molecules provides a complement to the lipid data obtained by others. In actual use, the analysis of the small molecules may be more desirable than lipids as transitioning such methods to field use with miniature instruments will likely limit the

upper mass limit that can be analyzed (typically ca. 500 Th for current miniature mass spectrometers). For either case, the ruggedness of the methods needs to be further investigated to learn the advantages and disadvantages of both approaches.

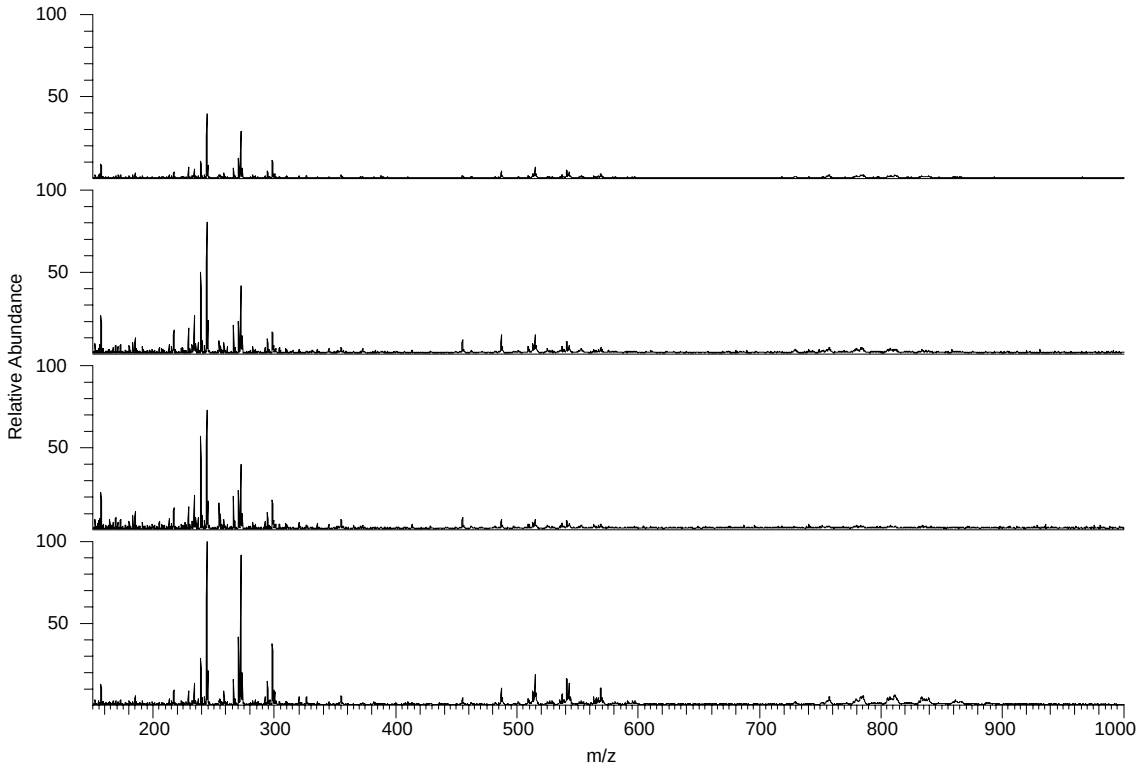


Figure 16 – Reproducibility of DESI spectrum of *P. aeruginosa* for samples taken from different locations in the culture. Each spectra plotted on the same scale for signal response comparison

Automated classification routines were not investigated at this point in the study. To properly train and test the algorithms, data needs to be collected from several different cultures for each bacteria to study the variance inherent to the microorganisms. This will include studies of reproducibility between samples from the same agar plate (inter-culture), several generations of a particular bacterium (intra-culture), age of culture (phase of growth), growth in different media and under different conditions, etc. in addition to collection of data from several other species. Without this knowledge, robust classification methods cannot be developed.

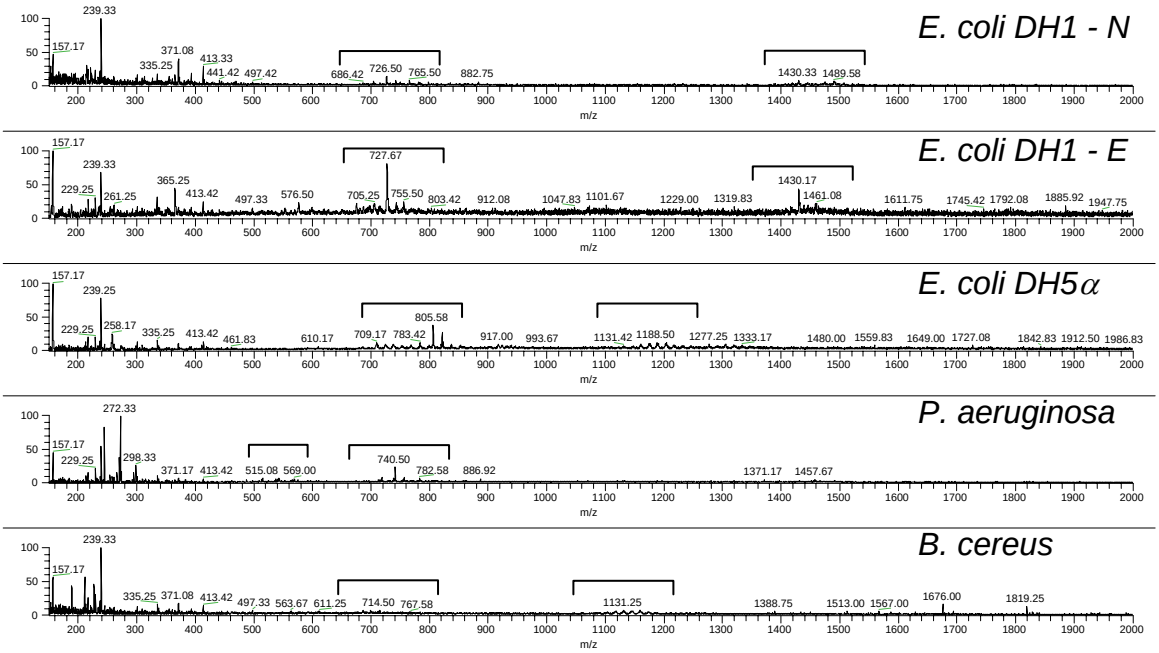


Figure 17 – DESI-MS spectra of intact bacterial cells after washing with methanol. Characteristic peak distributions indicated below brackets

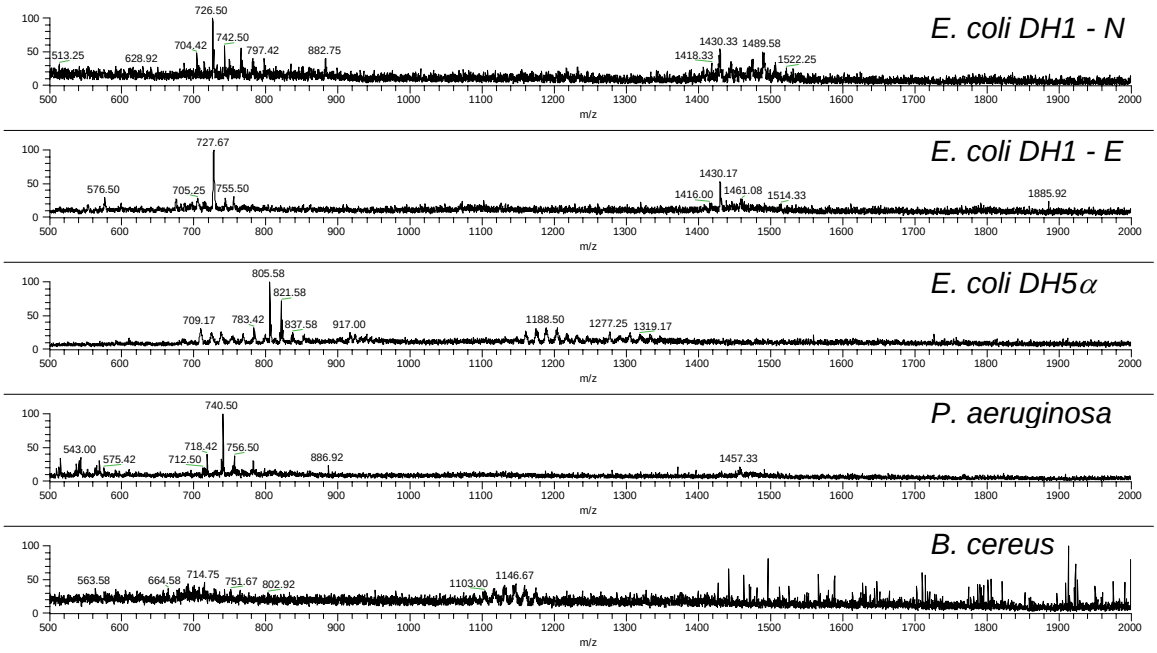


Figure 18 – Zoom of lipid regions of the mass spectra of methanol washed bacterial cells also shown in Figure 17

These studies, as well as other using DESI for the detection of microorganisms, have shown the ability to directly analyze intact bacteria without the need to extensive sample preparation. In these cases, the data obtained for different species and strains of bacteria have shown distinctions in the mass spectra, opening up the possibility of identification of these bacteria by DESI-MS. In addition, prior studies to identify the quinoline signaling molecules in *P. aeruginosa* have required long sample preparation procedures and long LC/MS times. With DESI, five of these compounds were identified directly from samples taken right from the agar plate. Current experiments with linked tandem MS experiments (precursor ion scans) will allow for the detection of additional, lower concentration qunoline molecules with specific fragmentation pathways. Studies of the time-dependent expression of the signaling molecules during culture growth are also possible with this method.

DESI 'Wand'

DESI, as it is currently practiced, limits one to the analysis of only a small area (~0.75-0.80 mm^2) of surface at any one time. In addition, the best sensitivity for the technique is obtained with the sample located within 1-2 mm of the inlet of the mass spectrometer. Due to the small sampling area and the need to have the instrument in close proximity to the sample, a DESI 'wand' device has been proposed to overcome these limitations. The ideal DESI wand would allow for both higher surface area sampling and the capability to sample objects at a distance from the mass spectrometer, as well as be made of flexible materials so that it can be easily positioned for analysis of different objects. To this end, we have developed a prototype wand which allowed for the collection of data necessary to further develop this technology.

To accomplish the requirements above, prototype DESI wand device was designed and constructed. A cross-section and diagram of the device is shown in Figure 19. The device extends the collection point for ions approximately 20 cm from the front of the mass spectrometer. In addition, the extension has been coupled to a funnel and three ion collection tubes. Each collection tube is positioned to collect ions generated from a sample through three DESI spray heads. A photograph of the three spray heads arranged in an aluminum holder is given in Figure 20. These two parts can be combined

to form a three channel DESI spray head to enable the sampling of larger areas relative to the single spray DESI source.

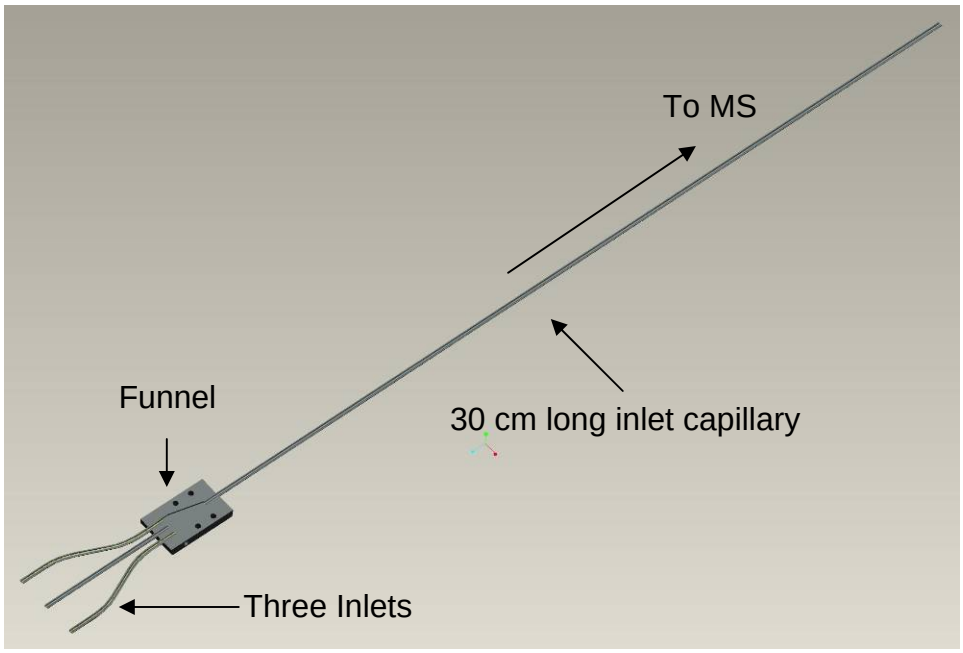


Figure 19 – Extended length, triple inlet device designed and used in these studies

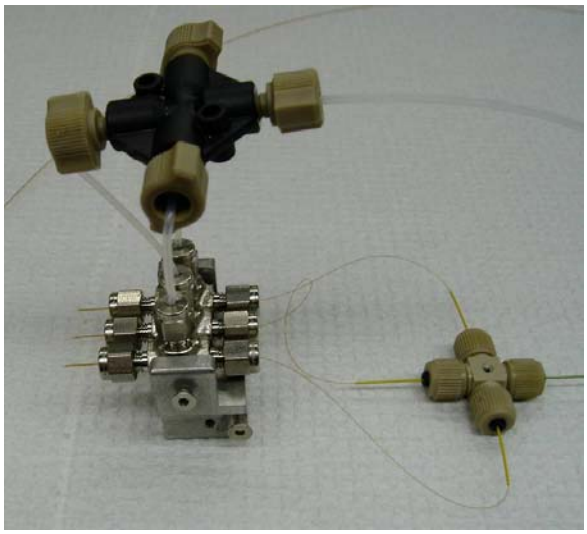


Figure 20 – Triple electrospray device designed for the three channel ion inlet

The ion collection device was constructed by silver soldering stainless steel tubing (1/16 inch outer diameter, 0.030 inch inner diameter) into a machined stainless steel 'funnel'. This allows for the transition of three ion sampling channels into the single inlet of the LTQ mass spectrometer. An adapter was also machined which allows

for the standard inlet capillary of the LTQ to be replaced easily by the extended ion inlet. Each spray head of the apparatus was built from a 1/16th inch stainless steel Swagelok tee as described by Cooks et al.[7]

Initial testing of the device was performed in order to find the effect of inlet length on the sensitivity of the instrument. Several compounds were chosen to cover different types of molecules expected to be analyzed in the future. Table 12 summarizes the results of this study using a polytyrosine solution (tyr_1, tyr_3, tyr_6 molecular weights 181.19, 507.54, 996.07 Da), the peptide bradykinin (molecular weight 1060.2 Da), and the protein lysozyme (chicken egg white, molecular weight 14306 Da). In general, the use of the inlet extension has decreased the sensitivity of the instrument by approximately 10 - 100X when sampling through only one of the three inlets off the device. Some sensitivity is regained (approximately 10X) when sampling ions through all three inlets of the extension.

The results obtained fall in line with a previous study of ion transport through capillaries by Sunner et al.[25] As expected, there is some loss of ions due to collisions with the walls of the tubing during ion transport. Also noted is the disproportionate loss of small ions (high mobility) relative to the loss of larger ions (lower mobility). This can be seen in the case of the polytyrosine solution where there is a great loss for the m/z 182 ion than for the larger polytryosine ions. Ions traveling through the capillary inlet experience repulsion due to like charges, and as such, are forced to the calls of the capillary at rates dependent upon their ion mobilities. Increasing the time that ions spend within the capillary will affect both the total ion current transferred as well as the relative abundance of ions based upon mobility. In addition, a third effect noted was the charge state shifting seen in the case of lysozyme. Using the standard inlet, the most abundant charge state seen for lysozyme is the 10+ charge state at m/z 1431. When the extended inlet is installed, the most abundant charge state shifts to 8+ charge state at m/z 1788. With shifting of charge states in the case of lysozyme, the sum of the signals due to each charge state of lysozyme can be used as an indication of total ion loss. This effect is also due to the increased transfer time through the capillary inlet. In this case, charge (proton) stripping by other compounds contained within the sampled gases can shift the charge states of proteins to lower charge states.

In this set of experiments, there was approximately a 20x loss in the total detected signal when sampling ions in a single channel of the extended inlet. This was reduced to ~10x decrease sampling ions into all three channels of the inlet. Unfortunately, the next lower charge state was above the upper mass limit of the mass spectrometer, so if it is present, it cannot be accounted for in these results.

Further testing of the three inlet DESI wand proceeded by coupling the multiplexed spray head to the three inlet ion collection tube. The Figure 21 shows the extended inlet as currently configured with the triple spray DESI spray head and heating system to aid in ion desolvation. In order to eliminate the need for linear movements to allow for the positioning of the sample, DESI spray head, and mass spectrometer ion inlet capillary, the geometry of the DESI source was changed. The standard DESI source operated using a spray impact angle of approximately 60 degrees with a collection angle of approximately 10 degrees. The extended inlet used an impact angle of approximately 80 degrees and a collection angle of 90 degrees. The differences in spray/collection angle are illustrated in Figure 22. The spray heads and inlet capillaries are fixed in relation to each other to eliminate the micromanipulators of the standard source. Estimates using rhodamine dye from red permanent marker show the signal intensity using the modified angles is comparable to that expected using the conventional angles after accounting for ion losses in the extended capillary.

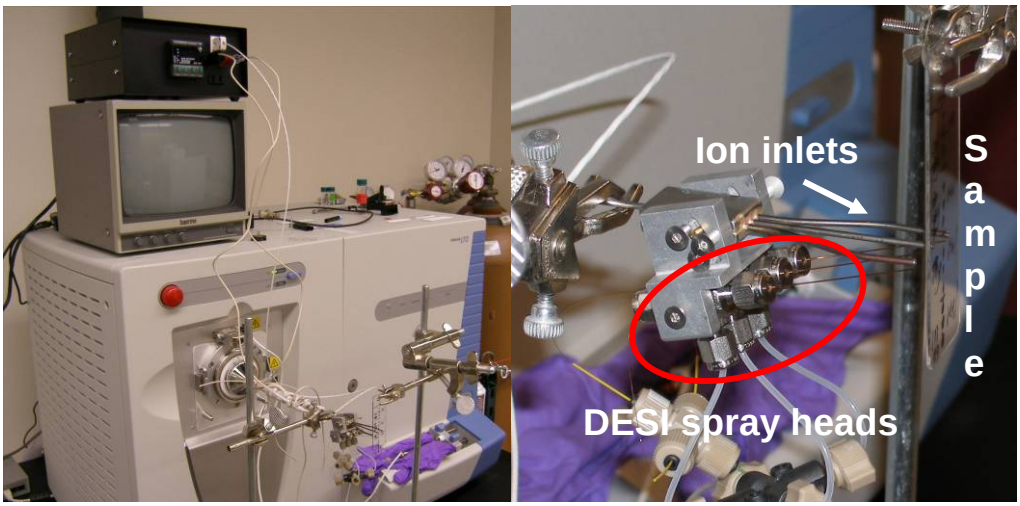


Figure 21 – Photographs of the tripe-spray DESI wand on the Thermo Electron LTQ mass spectrometer (left). Close up of the spray head/ion collection region (right).

	Standard Inlet	Left Extended	Center Extended	Right Extended	Triple Spray (2.5 ul/min)	Triple Spray (7.5 ul/min)
Polytyrosine						
Tyr1 (182)	2.81E+05	2.85E+03 (98.99)	2.49E+03 (99.11)	2.52E+03 (99.10)	3.79E+03 (98.65)	4.17E+03 (98.52)
Tyr3 (508)	2.52E+06	8.21E+04 (96.74)	6.76E+04 (97.32)	6.80E+04 (97.30)	1.21E+05 (95.20)	1.33E+05 (94.72)
Tyr6 (997)	1.35E+06	6.05E+04 (95.52)	5.62E+04 (95.84)	5.00E+04 (96.30)	1.04E+05 (92.30)	1.12E+05 (91.70)
Bradykinin						
2+ (531)	1.29E+04	3.87E+03 (70.00)	3.46E+03 (73.18)	2.53E+03 (80.39)	1.08E+05 (-737.21)	1.00E+05 (-675.19
1+ (1060)	9.13E+03	3.90E+02 (95.73)	3.29E+02 (96.40)	2.59E+02 (97.16)	7.32E+02 (91.98)	1.23E+03 (86.53)
Lysozyme						
11+ (1301)	1.10E+04	0.00E+00 (100.00)	0.00E+00 (100.00)	0.00E+00 (100.00)	0.00E+00 (100.00)	0.00E+00 (100.00)
10+ (1431)	1.17E+05	2.43E+02 (99.79)	3.11E+02 (99.73)	1.05E+02 (99.91)	4.59E+02 (99.61)	3.54E+02 (99.70)
9+ (1590)	7.61E+04	7.93E+03 (89.58)	8.23E+03 (89.19)	5.17E+03 (93.21)	9.30E+03 (87.78)	9.80E+03 (87.12)
8+ (1788)	1.08E+04	1.12E+04 (-3.70)	1.13E+04 (-4.63)	8.23E+03 (23.80)	1.42E+04 (-31.48)	1.35E+04 (-25.00)

Table 12 – Summary of results obtained using the multiple inlet extension on the LTQ mass spectrometer. The % loss for each ion monitored is indicated in parentheses

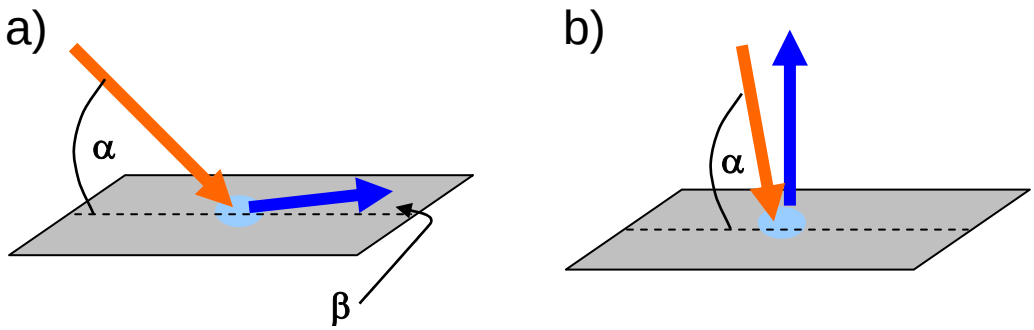


Figure 22– a) Geometry of standard DESI source showing the spray impact angle (α) and ion collection angle (β) b) Geometry of the DESI triple-spray apparatus showing modified angles

In an attempt to quantify the effect of the extension on DESI, the limit of detection for mastoparan and bradykinin were determined from a glass surface using the previously developed methods. Samples ranging from 10 to 100000 pg were analyzed as single spots (i.e. only one of the three spray heads analyzed the sample). Table 13 summarized the results of a limited study to this end. When accounting for ion losses measured in the previous report (100-1000x), the results of these experiments show there is an increase in limit of detection for bradykinin, however, the limit of detection for mastoparan has decreased. It should be noted that even though the limit of detection based on measurements made of a blank has improved for mastoparan over the previously reported level, but no signal from either the mastoparan or the bradykinin was detected until the 10 ng level. Also seen in this study was a decrease in chemical background noise (Figure 23) when using the extended capillary inlet. These results are complimentary to those seen by Cooks and coworkers[26] when working with capillary inlet extensions for non-proximate detection.

Peptide	**Previous LOD**	**Expected LOD with extension**	**Estimated LOD with extension**
Bradykinin	2.0 pg	200 pg	1199 pg
Mastoparan	160 pg	16000 pg	122 pg

Table 13– Expected and estimated limits of detection for peptide toxins using the extended ion transfer capillary.

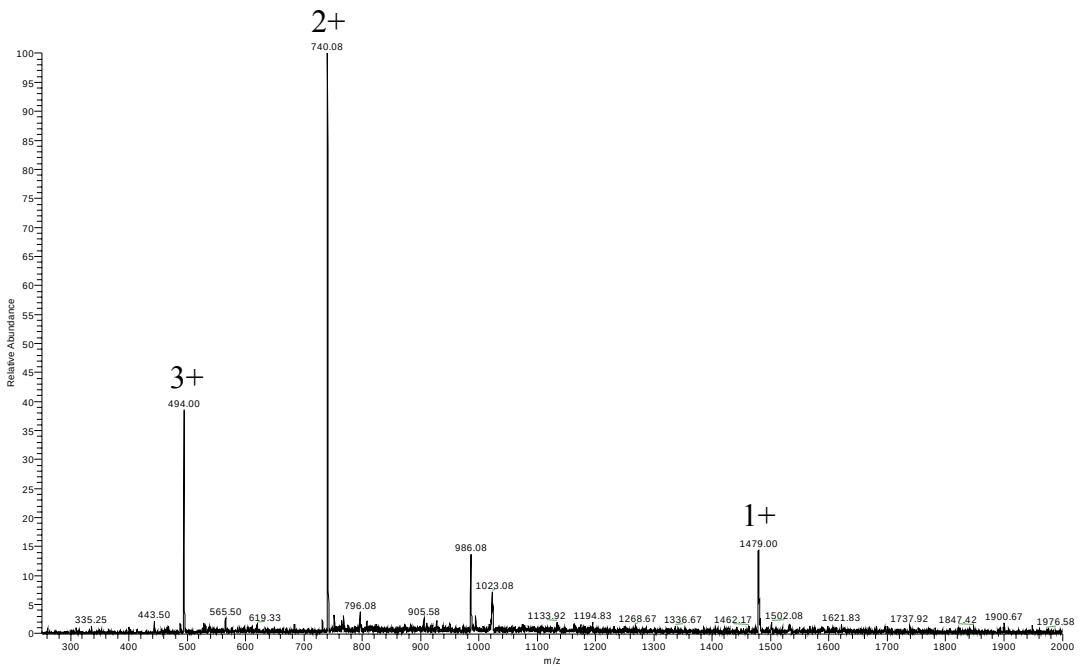


Figure 23– Mass spectrum collected for a 10 ng sample of mastoparan analyzed from a glass surface. Note the lack of chemical noise in the collected data

Key Research Accomplishments

- Established laboratory to perform research described in this report
- Development of DESI methods for the detection of several chemical warfare agent simulants
- Development of DESI methods for the detection of several toxins of biological origin
- Initial development of DESI methods for the detection and identification of intact bacteria
- Prototype DESI wand developed and tested, including high surface area scanning via spray head and collection tube arrays

Reportable Outcomes

- Presentation on research given at the April 2006 TATRC PLR
- Proposal for the continuation of funding based on the bacterial work has been submitted to TATRC (3/07)
- Proposal submitted and accepted to Edgewood Chemical Biological Center BAA for the continuation of chemical agent detection and miniaturization of DESI for portable MS systems
- With additional data to be taken, the results of the bacterial analysis will be prepared for publication

Conclusions

The research accomplished during the granting period has led to the development of DESI methods for the detection of chemical and biological warfare agents from a variety of common surfaces. Efforts were made to examine the effects of both matrix interferences and the presence of similar compounds on the detection of targeted compounds. To this end, common chemicals such as blue window cleaner (Windex) and bleach were used in an attempt to mask the detection of the compound of interest. The sensitivity and selectivity of MS coupled with the simplicity of DESI for direct analysis of the surfaces containing the compounds studied yielded detection limits on the order of 10s-100s of picograms for both chemical and biological toxins with little to no effect from interferences. These results are significant in terms of the ability to *directly* detect chemical threats from surfaces without the need for extraction or other sample preparation.

Future work using DESI for trace level detection should focus on expanding the studies described in the report in several aspects. Studies with the toxins should be expanded to include other simulants as well as surface materials and interfering compounds. In addition, the quantitative precision of these methods was not tested in the reported studies, and should be performed in follow-up studies. Finally, transitioning these methods to the field through the coupling of DESI to miniature mass spectrometers for *in situ* detection of chemical and biological threats is of interest. Collaboration with academic and commercial groups developing miniature mass spectrometers is desired to transfer methods developed here to field use.

A prototype DESI wand was developed which extended the inlet of the instrument to approximately 20 cm from the face of the instrument as a first step towards enabling stand-off detection capabilities to mass spectrometers Experiments performed to assess the loss of signal intensity have shown a loss of approximately 10-100X versus the standard ion sampling tube. The wand was designed with an array of three ion collection tubes in order to allow high surface area sampling when coupled to a DESI spray head array. The ability to sample larger surface areas is important for the efficient detection of chemical and biological agents, as well as other threats such as explosives.

Several variations of the DESI wand device can be envisioned for future development. Of these variations, perhaps the most desirable change will be towards flexible tubing to allow for true freedom on sampling. Alignment of the DESI spray heads to the ion collection tubes proved to be difficult in the current version of the DESI wand. Future versions of the wand should address this issue to better fix the alignment of these parts. To improve the high surface area sampling ability of the DESI wand, additional spray heads and ion pickups, placed closer together then in the current design are necessary. Inclusion of ion focusing devices to help improve ion transmission through long ion transfer tubing would also be valuable.

Also studied in this project was the applicability of DESI for the direct detection and identification of intact bacterial cells. Samples of several species and strains of bacteria were sampled directly from an agar plate and smeared onto a sample slide, followed by analysis by DESI-MS. Ions formed from several small molecules were detected from the various samples analyzed. The masses of ions arising from the different species and strains were different by the species or strain, opening up the possibility for automated identification of bacteria via statistical classification and pattern matching techniques. The direct identification of bacteria without the need for traditional taxonomic classification by DESI-MS under ambient conditions offers an analogous method to classification by MALDI-MS, without the need for sample preparation and introduction of the bacterial sample into the vacuum system of the mass spectrometer. For *P. aeruginosa*, the major ions seen have been identified as compounds based on the 4-hydroxy-2-quinoline intercellular signaling molecules produced by this species. Ions arising from other species have not yet been identified.

Additional studies for the detection and identification of microorganisms are warranted. Expansion of the initial studies performed here will include the addition of several species/strains of bacteria to build a database of bacterial DESI mass spectra. To properly build such a database, further understanding of the changes in the bacterial mass spectra due to differing growth conditions, growth phase of cultures, age of culture, etc. is necessary. With the database of mass spectra, automated classification methods can then be investigated.

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Appendix A

Publications derived from this funding:

No publications or meeting abstracts have yet been prepared

Appendix B

List of personnel receiving pay from this funding:

Mark S. Long
Kevin J. Boscacci
Brian C. Laughlin
Justin M. Wiseman
Timothy Pyle
Katia Rothhaar
Cynthia J. Helphingstine