FORENSIC SCIENCE APPLICATIONS UTILIZING NANOMANIPULATION-COUPLED TO NANOSPRAY IONIZATION-MASS SPECTROMETRY FOR THE ANALYSIS OF ULTRA-TRACE ILLICIT DRUGS

Nicole Wallace, B.S.

Thesis Prepared for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

December 2010

APPROVED:

Guido F. Verbeck, Major Professor
Stephen A. Cooke, Minor Professor
William E. Acree, Chair of the Department of Chemistry
James D. Meernik, Acting Dean of the Robert B. Toulouse School of Graduate Studies

Wallace, Nicole. Forensic Science Applications Utilizing Nanomanipulation-Coupled to Nanospray Ionization-Mass Spectrometry for the Analysis of Ultra-Trace Illicit Drugs. Master of Science (Chemistry - Analytical Chemistry), December 2010, 48 pp, 2 tables, 18 figures, chapter references.

Presented in this thesis are two methods that are coupled to the instrumentation for the recovery and analysis of ultra-trace illicit drug residues. The electrostatic dust lifting process is coupled with nanomanipulation-nanospray ionization to retrieve drug particles off of hard surfaces for analysis. For the second method, drug residues from fingerprint impressions are extracted followed by analysis.

The methodology of these hyphenated techniques toward forensic science applications is applied as to explore limits of detection, sensitivity, and selectivity of analytes as well as immediacy and efficiency of analysis. The application of nanomanipulation-coupled to nanospray ionization-mass spectrometry toward forensic science based applications is considered as future improvements to trace and ultra-trace analysis.

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my advisor Dr. Guido F. Verbeck for sharing his time and knowledge in helping me complete this research project as well as the opportunity to do this project. I would also like to thank my research group members, Billy Hoffman, Stephen Davila, Ubisha Joshi, J.D. Fox, Barbara Walton, Aaron Jesseph, and Aaron Hart for their support and assistance in completing this project. I would also like to thank Edward E. Hueske for his support and assistance as well in this project. Lastly, I thank the University of North Texas at Denton Department of Chemistry for their support and assistance throughout my graduate career.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Nanomanipulation-coupled to nanospray ionization-mass spectrometry (NSI-MS) has proven to be a valuable instrumental tool toward trace and ultra-trace analysis. The coupling of this tool with a range of analytical techniques makes it very versatile in application. Forensic science applications in particular benefit from the hyphenated methodology of this tool. Forensic trace analytical techniques towards the analysis of illicit drugs can be improved as to allow a step by step procedure that is immediate and ultimately providing quality results. A desired advantage of this instrumental method is that the ultra-trace quantity of the sample is not a limitation for analysis. The introduction of nanomanipulaion-coupled to nanospray ionization-mass spectrometry with the utilization of two other trace gathering techniques will be discussed in this thesis.

The second chapter of this thesis discusses the electrostatic dust lifting process as a means to collect ultra-trace particles of illicit drugs from surfaces. Matrix effects such as background contamination are studied and explored as well to examine possible interference of particulates to the detection of desired analytes. Raman spectroscopy is implemented to examine the adherence of particulates to the metalized mylar film used in the electrostatic lifting process. UV fluorescence is coupled to nanomanipulation to provide distinguishing verification of analytes among a matrix. Ion intensity counts from the ESI-MS and NSI-MS spectra of the drug lifts are analyzed to observe sensitivity from the extraction of the analytes.

The third chapter discusses the proof of concept with fingerprint residue extraction utilizing nanomanipulation-nanospray ionization-mass spectrometry. The casting of live skin tissue from a finger is implemented to facilitate the analysis of fingerprint residue from friction

ridge surface impressions. Drug residues from the fingerprint impressions are extracted with nanomanipulation followed by nanospray ionization. Matrix effects such as fingerprint powders used in latent print development are studied to examine possible interferences to the detection of desired analytes. UV fluorescence is coupled as well to distinguish the analyte from the matrix. Mass spectra are analyzed to observe the MH^+ peaks of the drug analytes extracted from the fingerprint impressions.

CHAPTER 2

ULTRA-TRACE ANALYSIS OF ILLICIT DRUGS FROM TRANSFER OF ELECTROSTATIC LIFT

Abstract

This article introduces a method of collecting and analyzing drug residues that integrates both electrostatic lifting and nanomanipulation-coupled to nanospray ionization mass spectrometry. The application of this hyphenated technique exhibits a useful means of collection and extraction of drug residues with ease and efficiency along with decreased limits of detection. From this method, it is shown how increased sensitivity of analysis and lower limits of detection for drug analysis can be achieved. The same principles that allow lifting of dust prints by electrostatic lifting can be applied to lifting drug residues. Probing of the drug residues by nanomanipulation occurs directly from the lift, which provides a great platform for extraction. Nanomanipulation-coupled to nanospray ionization-mass spectrometry has been used for the extraction of trace analytes in previous experiments and is known as a very sensitive technique for the detection of ultra-trace residue. This method demonstrates the electrostatic lifting of drug residue particles from a surface followed by extraction and ionization with nanomanipulation-nanospray ionization. The utility of this novel methodology allows for a more productive analysis when presented with ultra-trace amounts of sample.

Introduction

The extraction of ultra-trace analytes using nanomanipulation-coupled to nanospray ionization-mass spectrometry (NSI-MS) has proven to be a very effective tool. Extraction of trace drug particulates from fibers has been documented using this method [1] as well as the

extraction of peptides from individual beads.[2] Both of these techniques employed nanomanipulation-coupled to NSI-MS and showed high reproducibility when compared with traditional methods of analysis yielding quality results. This method of analysis exhibits extreme sensitivity with limits of detection in the picogram range.[1] This demonstration of sensitivity holds the potential for ultra-trace extraction of analytes from a myriad of matrices.

The very nature of matrix effects has been an issue for trace drug analysis. It remains a prominent issue in biological samples toward the limits of detection/quantitation in biological fluid based matrices.[3] Many factors, such as the instrumental methods involved, the ionization method employed, or sample preparation procedures, can influence the overall effectiveness of the analysis. Trace analysis of contaminants from food products carries the same burden even after an extensive cleanup procedure of the samples has been performed.[4] These factors limit the overall quality of analysis, producing results that are not reliable. In general, accurate quantitation of ultra-trace analytes is difficult to obtain due to these limitations.

Ultra-trace techniques differ according to the type of analysis being executed. Many techniques utilized for ultra-trace analysis have been developed to overcome the issue of matrix effects. However, the actual quantitation of ultra-trace amounts varies depending on the type of sample being analyzed. There is no clear cut definition of the word and so most scientists refer to the quantity being under 1 ppm.[5] Innovative techniques developed for ultra trace work in drug analysis include instrumental methods such as affinity probe capillary electrophoresis[6] and supercritical fluid extraction.[7] Through affinity probe capillary electrophoresis, drug quantitation can be achieved in the picomolar range from biological matrices.[6] Supercritical fluid extraction has been combined with solid phase extraction to detect ultra-trace amounts of drug metabolites from plasma [7]. Various methods utilizing mass spectrometry include techniques where solid-

phase extraction is -coupled to liquid chromatography-coupled to electrospray-tandem mass spectrometry for the detection of illicit drugs and their metabolites from sewage water.[8] This method provides an automated system of analysis where high throughput is achievable while maintaining sensitivity in the nanogram range. Liquid chromatography-electrospray ionization techniques are utilized more for the analysis of drugs due to the superb structuring detail of molecules that can be obtained from spectra when drugs are analyzed in various matrices.[9] Liquid chromatography-coupled to quadrupole-time-of-flight mass spectrometry for the detection of illicit drugs in oral fluids presents another type of liquid chromatography-mass spectrometry method utilized for drug detection.[10] These methods are an improvement of older conventional methods in regards to precision, accuracy, and selectivity. However, the actual application of these techniques is still a tedious detailed analytical process requiring a good deal of time for sample cleanup and preparation along with a suitable amount of time to complete analysis.

Other improvements made in ultra-trace drug analysis that will emphasize immediacy with accuracy include such techniques as optical chemical imaging .[11] Solid surface luminescence of minute drug amounts from matrices can be achieved. With this technique, the need for sample preparation is eliminated while maintaining detection limits in the picogram range. Raman spectroscopy has been used to detect quantities of street cocaine from the surface of human tissue matrices.[12] This method is very efficient for detection of trace analytes. An improvement of this method is surface enhanced Raman scattering (SERS) where narcotics on a metal surface can be detected in the attogram range.[13] The only drawback of this method is that it requires a very controlled environment in order to maintain the chemical and electronic effects of analysis due to the specialty of the SERS method which focuses on the substrate being utilized

along with the manner of analyte adsorption. There is no uniform method for performing SERS; it varies according to the analyte. The coupling of Raman spectroscopy with IR has proven to be beneficial for immediate quantitation of cocaine among mixtures.[14] Discrimination of the concentration of various components in a solid mixture can be determined with this method. The only limitation regarding this method is the large amount of sample needed to obtain a reasonable amount of accuracy. Surface probing techniques such as desorption electrospray ionization (DESI) provide a rapid and direct approach to ultra-trace analysis eliminating the need for sample preparation.[15] DESI releases charged droplets onto the surface for ionization of analytes. Direct analysis in real time (DART) is another high throughput surface method similar to DESI that releases excited state gas molecules for ionization of the analytes on the surface.[16] Although high sensitivity is attainable with DESI and DART, it requires a suitably large amount of surface area for analyte ionization. Further improvements with these methods could be possible with the implementation of imaging techniques.

The imaging capabilities that accompany mass spectrometric analysis serve as a great tool toward various types of analyses. These imaging capabilities can provide valuable information on spatial and chemical facets of analysis.[17] Most imaging mass spectrometry methods are employed in biological applications for the chemical imaging of analytes in tissues and protein extraction. Imaging mass spectrometry has made significant headway in the study of proteomics.[18]The various imaging techniques employed have enhanced the study of cell and gene function at the protein level. Imaging techniques not only can facilitate the probing of analytes, but also can provide a means of verifying the geometric and luminescent properties of particular analytes among a matrix using fluorescence microscopy.[19] This can be used as a way of determining the identity of the analytes being studied. Fluorescence microscopy techniques

are utilized very often in protein studies. These techniques serve as a great tool for the spectral imaging of fluorescing proteins in molecular samples, where imaging of the structural network of proteins within a molecule can be obtained by coupling fluorescence microscopy with Fourier Transform spectroscopy. [20] Fluorescence microscopy is commonly applied toward the analysis of solid phase analytes to determine and verify the characteristic luminescent properties unique to the analyte.[19]

The application of the electrostatic dust lifter towards the retrieval of particulates on various surfaces has been utilized mostly towards the lifting of prints at crime scenes. Figure 2.1 displays an image taken of a footwear impression made at a crime scene. Documentation of dust prints at crime scenes is essential to police investigations for it provides evidence of footwear impressions made by the suspect.[21] This method of retrieval has shown tremendous results toward identifying print patterns from footwear showing high sensitivity for footwear identification. [22] The same method of particle retrieval is applied toward lifting drug residues. The electrostatic lifting process utilizes a static electricity instrument that provides a voltage to a metallic film for the lifting of dust particles.[23] This process is applied to drug residues where the metalized plastic film is placed over the residue sample and is lifted by applying voltage to the film with a high voltage probe. Normally, crime scene photographs are taken of footwear impressions after a lift has been done since the dust particles are not permanently affixed to the film. They only adhere to the film for a limited amount of time. The image of the impression is usually the sole purpose of doing the electrostatic lifting process as they are kept for evidence. However, in the method being introduced here, the film lifts are packaged in airtight plastic bags following the completion of an electrostatic lift. The packaging allows for transfer to the nanomanipulator for extraction. The ability to electrostatically lift ultra-trace amounts of drug

residues from crime scenes offers a more practical approach toward the collection of drugs for analysis.

The application of nanomanipulation-nanospray ionization to the extraction of ultra-trace drug residues retrieved by the electrostatic lifting process can provide a more efficient means of performing analysis on drug quantities in the picogram to attogram range. The samples are adequate in size and quantity for this type of analysis, providing ample signal for detection. Particle extraction from a matrix is achieved allowing deconvoluted spectra to be produced as to determine the identity of the analyte. This innovative and simplistic method of drug analysis allows for high quality analysis of ultra-trace residues of illicit drugs while eliminating the tedious, multi-step sample preparation process.

Materials

The illicit drugs electrostatically lifted and ultimately analyzed in this method included rock cocaine (0.0043 g total), crystal methamphetamine (0.0033 g total) and black tar heroin (0.0034 g total). All drugs were provided by the University of North Texas Police Department (Denton, TX). The method was validated using ~0.0025 g of caffeine (Alfa Aesar, Ward Hill, MA) per lift. Powder cocaine (0.0030g total) was used in the preliminary studies. Each sample was mixed with a sand based soil (0.0021g per sample). Each sample was also mixed separately with potting soil (0.0021g per sample) for matrix studies. Prior to electrostatic lifting, half the mass of each analyte was reserved for Raman spectroscopy and UV fluorescence. The solvent mixture used for dissolution and extraction of samples was a 1:1(v/v) solution of optima LC/MS methanol (Fisher Scientific, Fair Lawn, NJ) and Millipore water (Millipore, Billerica, MA) with 1% glacial acetic acid (Malinkrodt Chemical Co., Phillipsburg, NJ). The methanol used was

doubly distilled to eliminate any impurities in the solvent. The electrostatic lifting of each sample was done with an Electrostatic Dust Print Lifter (Kinderprint (Model No.3C), Martinez, CA) utilizing metalized plastic films for each lift. The nanomanipulator (Zyvex, Richardson, TX) -coupled to a MultiZoom AZ100 microscope (Nikon, Melville, NY) along with a PE2000b four-channel pressure injector (MicroData Instrument Inc., S. Plainfield, NJ) was used for extraction of the analytes from the film lifts. The mass spectrometric analysis was done on a LCQ DECA XP Plus (Thermo Finnigan, San Jose, CA) with a nanospray ionization source (Proxeon Biosystems, Odense, Denmark). Raman spectroscopy lift studies were carried out on a T64000 (Jobin Yvon, Cedex, France) -coupled with a Coherent Innova 90 argon ion laser (Laser Innovations, Santa Paula, CA) with a 488 nm line. Prior to completing Raman spectroscopy, the films were coated with a 50 nm layer of gold (93.999% purity) to enhance the Raman signal. The film lifts also underwent UV fluorescence studies with a Nikon Eclipse E600 (Nikon, Melville, NY) microscope attached to an EXFO X-cite 120 fluorescence illumination system (EXFO, Canada). A TE2000U Inverted Microscope was utilized for the UV fluorescence studies of crystal methamphetamine.

Methods

Electrostatic Lifting

The Kinderprint Electrostatic Dust Print Lifter is utilized in this method for the electrostatic lifting of the drug residues from a smooth table surface. The electrostatic lifter is a high voltage power unit (120 v/60 Hz) with an adjustable voltage. This power unit was utilized along with a high voltage probe used for lifting and a ground lead, which connects to a ground source. Each drug/soil sample and the caffeine/soil sample were electrostatically lifted at a

medium voltage range for 30 seconds off of the surface of a table. The ground lead was connected to the metal leg of the table for a ground source. Each lift was done with a 1.5 cm x 1.5 cm section of metalized plastic film. The metalized side of the plastic film is where the charge takes place with the high voltage probe during lifting. After each electrostatic lift was completed, the films were folded over and packaged separately in airtight plastic bags for transfer to the nanomanipulator.

Nanomanipulation

Each lift collected was placed under the Multi-Zoom AZ100 microscope for viewing and probing of drug and caffeine residues from each of the mixtures. A metal fixture was used to secure each film lift. Viewing and probing of each sample took place with an AZ Plan Fluor 2x objective. Image capturing was done on NIS Elements software, which was -coupled to the microscope with a CCD camera.

The nanomanipulator mounted to the microscope consists of four nanopositioners controlled in either coarse or fine mode. The range of motion and the resolution achievable is dependent on the mode of manipulation. The coarse mode has a range of motion of 12 mm in the X and Z-axes and 28 mm in the Y-axis with a translational resolution of 100 nm. In the fine mode of operation, the range of motion is 100 µm in the X and Z-axes and 10 µm in the Y-axis. The course mode utilizes nanospray capillary tips coated with Pt that perform the injection and extraction of the analytes. All of the electrostatic lifts were manipulated with the coarse mode of operation. The PE2000b pressure injector provides the pressure required to do the injection/aspiration process through the nanospray capillary tips.

Analyte injection/extraction process was carried out with 10 µL of solvent loaded into the nanospray capillary tips. After the analytes were located among the soil particles, the nanospray capillary tip was landed on the desired particle of interest at ~1 µm away. An injection of the solvent mixture (1:1 (v/v) methanol/water, 1% acetic acid) was performed at a pressure of 25 psi for dissolving process of the analyte. After 10 seconds of dissolution, the solvent/analyte solution was aspirated into the nanospray capillary tip at a pressure of 40 psi. The nanospray capillary tip was then transferred to the nanospray ionization source for nanospray ionization mass spectrometric analysis (NSI-MS). An ionization voltage of 2.5 kV was applied for analysis on the mass spectrometer. After NSI-MS analysis took place, a wash of the remaining analyte/soil mixture was carried out with 200 µL of solvent mixture (1:1 (v/v) methanol/water, 1% acetic acid) and then centrifuged down for 30 seconds to separate the soil particles from the drug solution. Analysis with electrospray ionization mass spectrometry (ESI-MS) was conducted afterwards. An ionization voltage of 4.0 kV was applied along with a syringe pump flow of 10 µL/min. This procedure was repeated for each drug/soil mixture lift.

Raman Spectroscopic Analysis

Metalized plastic films (1.5 cm x 1.5 cm) coated with 50 nm of gold (93.999% purity) were utilized in the lifting of each drug analyte without the soil matrix in order to do qualitative studies of each particular lift for Raman spectroscopy. The T64000 Groupe Horiba with a factory built stereomicroscope attached was utilized to gain spectra of each analyte lifted. Each lift was held in place by metal stage holders while being viewed under a 100x objective. They were imaged with a CCD camera along with the appropriate software. The spectroscopic analysis of

each lift was done with an argon ion laser at 488 nm. The procedure was repeated for each analyte/soil mixture.

UV Fluorescence Analysis

The drug/soil (sand based) mixture lift was viewed with the E600 microscope -coupled to a fluorescence illumination system. An objective of 4x was used to observe the illumination of the drug particles among the soil matrix on the films. Images were captured with a CCD camera along with appropriate software. Crystal methamphetamine particles with potting soil were viewed under the TE2000U Inverted Microscope -coupled to a fluorescence illumination system using an objective of 10x/0.30.

Results and Discussion

In preliminary studies using caffeine, a caffeine/sand-based soil mixture was electrostatically lifted and underwent nanomanipulation-coupled with nanospray-ionization mass spectrometry (NSI-MS). The caffeine/sand-based soil mixture was completely lifted onto the metalized plastic film during the electrostatic lifting process. The caffeine particles on the lift can be seen among the soil particles viewed through the microscope (Figure 2.2a). A spectrum was obtained from the injection/extraction process of the caffeine particles from the lift utilizing NSI-MS. The amount of solvent used for injection/extraction was less than 10 µl utilizing a total analysis run time of less than two minutes. The MH^+ peak for caffeine appears at m/z 195.13. Figure 2.3 shows this peak at a leading intensity. Preliminary studies also included a powder cocaine/sand based mixture as well following the same procedure as the caffeine sample. Figure 2.4 shows the NSI-MS and the ESI-MS spectrum of the powder cocaine/sand-based soil mixture.

The caffeine extraction from the soil matrix shows that single crystal extraction provides ample signal for detection of analytes through nanomanipulation-coupled to NSI-MS. The metalized plastic film lift provides a great platform for the extraction of particles as well as an illuminating colorful background under the light source of the microscope. The caffeine/sand-based soil particles appear enhanced due to this illuminating background thus facilitating the probing of the analytes among the soil matrix. The powder cocaine extraction from the soil matrix also shows exceedingly well results confirming how well the single crystal extraction process facilitates analysis. The ESI-MS wash of the powder cocaine lift demonstrates how ESI-MS alone does not show high selectivity toward the extraction of powder cocaine crystals as does nanomanipulation-coupled to NSI-MS.

All three drug/sand-based soil mixtures underwent the same process as the caffeine and powder cocaine extractions. The sample amount reserved for each drug lift completely adhered to the film through the electrostatic lifting process for the sand based soil mixtures; no particles were left behind on the table surface. The potting soil mixtures underwent the same process described above. However, the potting soil particles were not lifted in their entirety for each lift. In addition to nanomanipulation-coupled to NSI-MS, a wash was performed on the remaining analyte/soil mixtures from each lift and underwent electrospray ionization-mass spectrometry (ESI-MS) for comparison of both methods of ionization. Nanomanipulation -coupled to NSI-MS analysis was not done on the drugs mixed with the potting soil due to the size of the soil particles. Particles could not be extracted with the nanospray capillary tips. However, ESI-MS studies were done on the washes. Figure 2.2 (a-e) displays the images captured of the lifts. The spectra taken from ESI-MS and NSI-MS mixed with the sand based soil are shown in Figures 2.5-2.7. Figure 2.8 (a-c) shows the ESI-MS spectra of the drugs/potting soil mixtures. Studies

with the caffeine and rock cocaine were extended to performing MS-MS analysis to observe the breakdown products of these analytes as to eliminate false positives that could occur from background matrices. Figure 2.9 displays the MH+ peaks for each analyte along with their collision induced dissociation (CID) products, making the identification near-absolute.

A study of the MH^+ peaks were done for each drug lift to observe the sensitivity and detection limits of each ionization method in the extraction of the analytes from each lift. Figure 2.5 shows the MH^+ peak for rock cocaine in both NSI-MS and ESI-MS spectra leading at m/z 304.33. When observing the intensity for the rock cocaine spectra, the intensity appears to be higher for the NSI-MS spectrum by 6 times that of ESI-MS. For the leading MH^+ peak at m/z 150.2 in the crystal methamphetamine spectra, the NSI-MS spectrum shows intensity 1.5 times higher than the ESI-MS spectrum (Figure 2.6). In Figure 2.7, the leading MH^+ peak at m/z 370.33 for black tar heroin has intensity 6 times higher in the NSI-MS spectrum than the ESI-MS spectrum. This is the same magnitude for the rock cocaine spectra. Overall intensity for NSI-MS analysis appear higher for the drug extractions showing that sensitivity of these analytes are greatly enhanced with the nanospray ionization method. The study is continued with the MH^+ peak areas of each drug to understand how detection limits are affected with both modes of ionization. The ion count ratios were calculated for each drug lift to determine how well the particles were detected. Table 2.2 shows these ratio calculations of the each drug lift with sand-based soil and potting soil in NSI and ESI mode.

Area calculations were taken for each MH^+ peak in order to get a peak to background ratio. Table 2.1 displays the area calculations for the MH^+ peaks along with the background area for each drug. According to the table, black tar heroin shows a peak to background ratio of 19.8% for ESI-MS while its NSI-MS spectrum shows a ratio of 40.8%. For the rock cocaine, the

peak to background ratio from ESI-MS is 44.1% while NSI-MS shows a ratio of 65.5%. Crystal methamphetamine has a peak to background ratio of 48.2% while NSI-MS shows a ratio of 69.2%. An average area increase of 21% is observed for the NSI-MS analysis of each drug. This study shows that detection limits are actually lower with NSI-MS.

The ESI-MS spectra of the drugs with potting soil in Figure 2.8 displays the MH^+ peaks for each drug along with peaks from the potting soil particles. These spectra indicate how efficiently electrostatic lifting retrieves particles from the table surface. The particle sizes of the potting soil did not allow passage through the nanospray capillary tips. The drugs dissolved quickly into the potting soil and therefore could not be extracted. However, sensitivity of the ESI-MS analysis is seen from looking at the spectra. Rock cocaine and crystal methamphetamine MH^+ peaks show higher intensities than the black tar heroin. The black tar heroin MH^+ peak may have interference from the potting soil particles. Adequate intensity is shown for this peak even with the interferences.

The electrostatic lift studies were done with Raman spectroscopy to investigate the adherence of the analytes to the metalized plastic film. In order to obtain the Raman spectra for the drug lifts, the metalized plastic films were plated with 50 nm of gold (93.999% purity) on the non-metalized side of the film. As before, the lifts were completed on a table surface. In the electrostatic lifting process for rock cocaine, a medium voltage range was applied in the lifting process for ~30 seconds. Two of the particles were left behind while the others lifted onto the film. Figure 2.10a and 1.10c shows the image captured along with the Raman spectrum obtained from the lift. The procedure was repeated for crystal methamphetamine and black tar heroin. In the electrostatic lifting process for crystal methamphetamine, two attempts were required in order for the majority of the particles to adhere to the lift. Two to three particles were left behind

on the table surface from the original amount. Figures 2.10 (b) and (d) show the captured image of the crystal methamphetamine on the gold plated film along with the Raman spectrum obtained.

The electrostatic lifting process for the black tar heroin required four attempts to lift the drug particles. Only one particle from the original amount adhered to the film. No results could be obtained for the Raman spectroscopic analysis of the black tar heroin. No detection of analyte could be seen nor could an image be captured in good quality. The rock cocaine and crystal methamphetamine particles were favorable toward Raman spectroscopic analysis as can be seen from their spectra. The gold plated films, in some instances, required more attempts to lift the analytes and did not retrieve all of the particles present, both Figures 2.10 (c) and (d) display obvious detection of analyte present from the lifts. Raman spectroscopic studies were extended to analyze rock cocaine in a blind soil mixture in order to verify detection of the analyte from the matrix. Figure 2.11 displays the image of rock cocaine crystal along with its spectrum. Raman imaging can be performed on peaks of interest, utilizing the nanomanipulator-coupled to MS for near-absolute identification with CID.

UV fluorescence studies were done in addition to Raman spectroscopy studies in order to differentiate the drug particles from the soil matrix on the lifts. Figure 2.12(a) shows the RGB fluorescence of the rock cocaine/sand-based soil mixture. The RGB fluorescence exhibits the rock cocaine particles among the soil mixture. The imaging of the rock cocaine particles is clearly seen from the soil matrix with the RGB fluorescence. The RGB fluorescence image of the crystal methamphetamine particle is clearly seen among the potting soil mixture shown in Figure 2.12 (b). This analysis was done using the inverted microscope for crystal methamphetamine since fluorescence could not be seen with the crystal methamphetamine on the film lift most

likely due to the black background of the film. No images were taken of the black tar heroin particles. Fluorescence could not be seen with the black tar heroin among the soil particles.

Conclusion

The introduction of electrostatic lifting with nanomanipulation-coupled to nanospray ionization mass spectrometry demonstrates effectively how ultra-trace amounts of drug particles can be lifted and extracted with ease, eliminating need for a preconcentration technique. The quantity of particles lifted through the electrostatic lifting process is sufficient for NSI-MS analysis. The nanomanipulator-coupled to the microscope provides an efficient means of extraction by bringing the nanospray tip to the sample. As can be seen from the drug spectra, single crystal extraction provides ample signal for detection for these analytes. Higher selectivity is achievable with this method as opposed to ESI-MS alone. Nanomanipulation-coupled to NSI-MS provides the benefit of being able to select the analyte(s) of interest among a matrix. The imaging software utilized provides an enhanced capability to locate the analytes among the matrix in order to complete extraction. Although these studies did not reveal any information for the black tar heroin, it can be understood that the crystalline forms of these analytes are most preferable for lift and detection with microscopy. The Raman spectroscopic studies still indicate that the electrostatic lifting process is effective in lifting ultra-trace particles, even the black tar heroin. The data from the NSI-MS spectra on the drug lifts verify the parent ion peaks of the drugs. The MS-MS spectra provide qualitative verification of the drug analytes according to their degradation products. Both the NSI-MS and MS-MS spectra demonstrate the enhanced sensitivity and selectivity of utilizing mass spectrometry, providing non-contestable results as to the identity of the analytes. The UV fluorescence imaging studies exhibited the drug particles

among the soil matrix. The utilization of this imaging technique can be applied in the extraction of analytes in mixtures where the matrix appears to dominate under normal microscopic view. In addition to this imaging technique, the capabilities to distinguish the unique geometric shapes of drug crystals could be applied as well to the nanomanipulation method.

The utilization of nanomanipulation-coupled to NSI-MS has already proven to be advantageous in the analysis of trace particles from previous experimental studies. The lifting of drug particles from the electrostatic process provides an innovative means to collect drug residues in ultra-trace amounts. This process combined with nanomanipulation-coupled to NSI-MS displays a new technique of collection and extraction where the quantity of the particles is not a limitation for analysis. The usage of this combined method could be an improvement in many types of trace analysis.

Table 2.1 The table displays the peak to background area ratio calculations for both NSI-MS and ESI-MS analysis of black tar heroin, crystal methamphetamine and rock cocaine.

Drugs	**m/z Background Range**	**m/z MH+ Peak Range**	**MH+ Peak Area**	**Bkg. Area**	**Peak to Bkg. Area Ratio**
Black Tar Heroin(ESI-MS)	300.07 to 400.0	369.53 to 370.87	7.45E+06	3.77E+07	1.98E-01
Black Tar Heroin(NSI-MS)	300.07 to 400.0	369.53 to 370.88	3.86E+07	9.47E+07	4.08E-01
Rock Cocaine(ESI-MS)	200.07 to 500.0	303.47 to 304.80	1.64E+07	3.71E+07	4.41E-01
Rock Cocaine(NSI-MS)	200.07 to 500.1	303.47 to 304.87	1.15E+08	1.76E+08	6.55E-01
Crystal Meth(ESI-MS)	100.07 to 300.0	149.47 to 150.73	1.42E+07	2.94E+07	4.82E-01
Crystal Meth(NSI-MS)	100.07 to 300.1	149.33 to 150.8	2.21E+07	3.20E+07	6.92E-01

Table 2.2 Ion count ratios calculated for each drug lift. The table displays the ratios for drug lifts done with both potting soil and sand-based soil with NSI-MS and ESI-MS.

Drug/(Sand-Based Soil) NSI-MS	**NL**	**TIC**	**Ratio**
Black Tar Heroin	8.06E+07	2.51E+09	3.21E-02
Rock Cocaine	2.34E+09	4.02E+09	5.82E-01
Crystal Methamphetamine	4.92E+07	6.71E+08	7.33E-02
Drug/(Sand-Based Soil) ESI-MS	**NL**	**TIC**	**Ratio**
Black Tar Heroin	1.55E+07	7.74E+08	2.00E-02
Rock Cocaine	1.06E+08	1.91E+09	5.55E-02
Crystal Methamphetamine	4.95E+07	7.95E+08	6.23E-02
Drug/(Potting Soil) ESI-MS	**NL**	**TIC**	**Ratio**
Black Tar Heroin	3.07E+08	1.06E+10	2.90E-02
Rock Cocaine	2.76E+08	4.40E+09	6.27E-02
Crystal Methamphetamine	6.41E+07	2.03E+09	3.16E-02

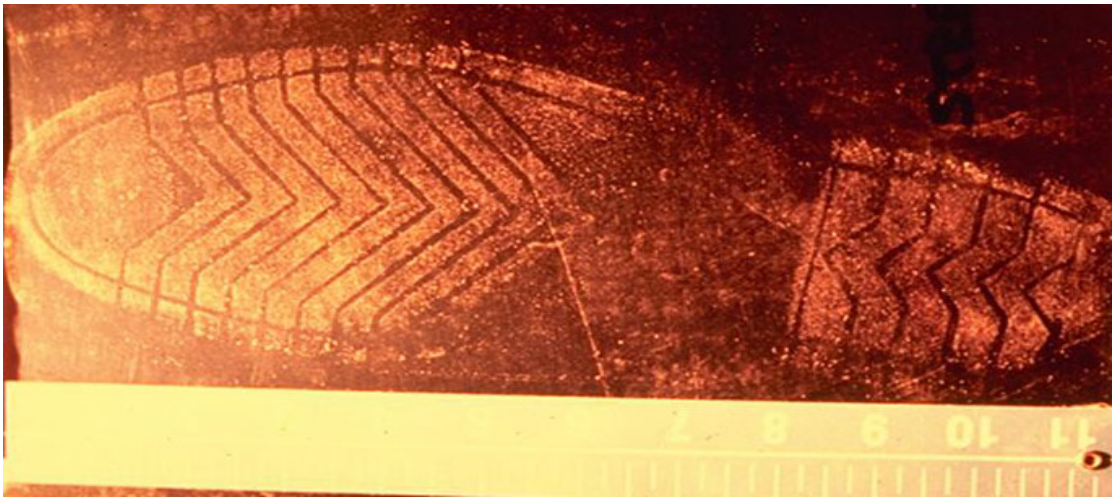


Figure 2.1 Photograph taken of a tread wear pattern from a shoe made at a crime scene. The electrostatic dust lifter was utilized to generate this pattern from the tread of a shoe.

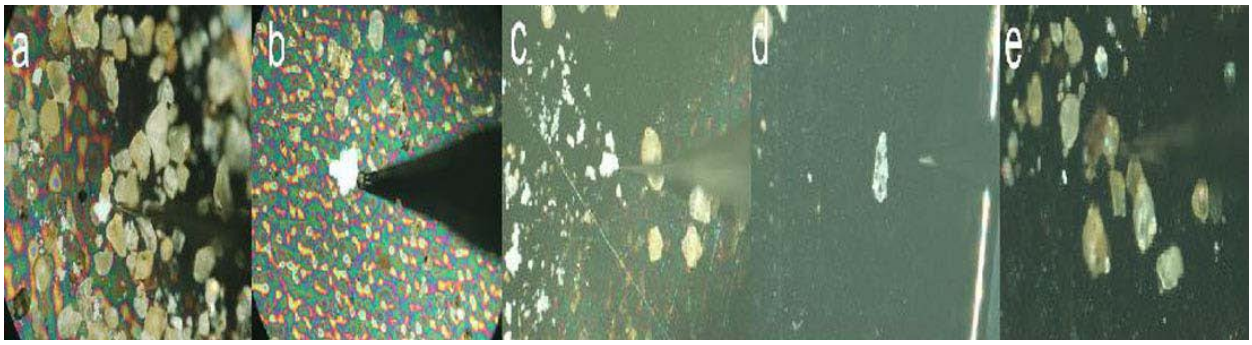


Figure 2.2 Images captured of the drug/sand-based soil lifts ready for the injection/extraction process. The nanospray tip can be seen landed within micrometers of the particles. (a) caffeine/soil lift (b) powder cocaine/soil lift (c) rock cocaine/soil lift (d) crystal methamphetamine/soil lift (e) black tar heroin/soil lift.

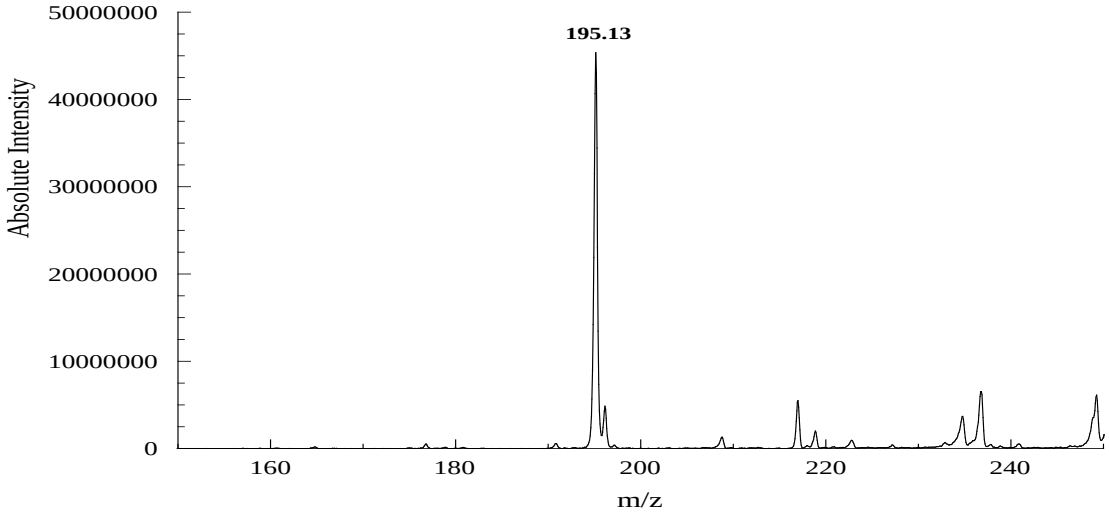


Figure 2.3 The NSI-MS spectrum of the caffeine extraction from the sand-based soil mixture. The MH^+ peak for caffeine is leading at m/z 195.13.

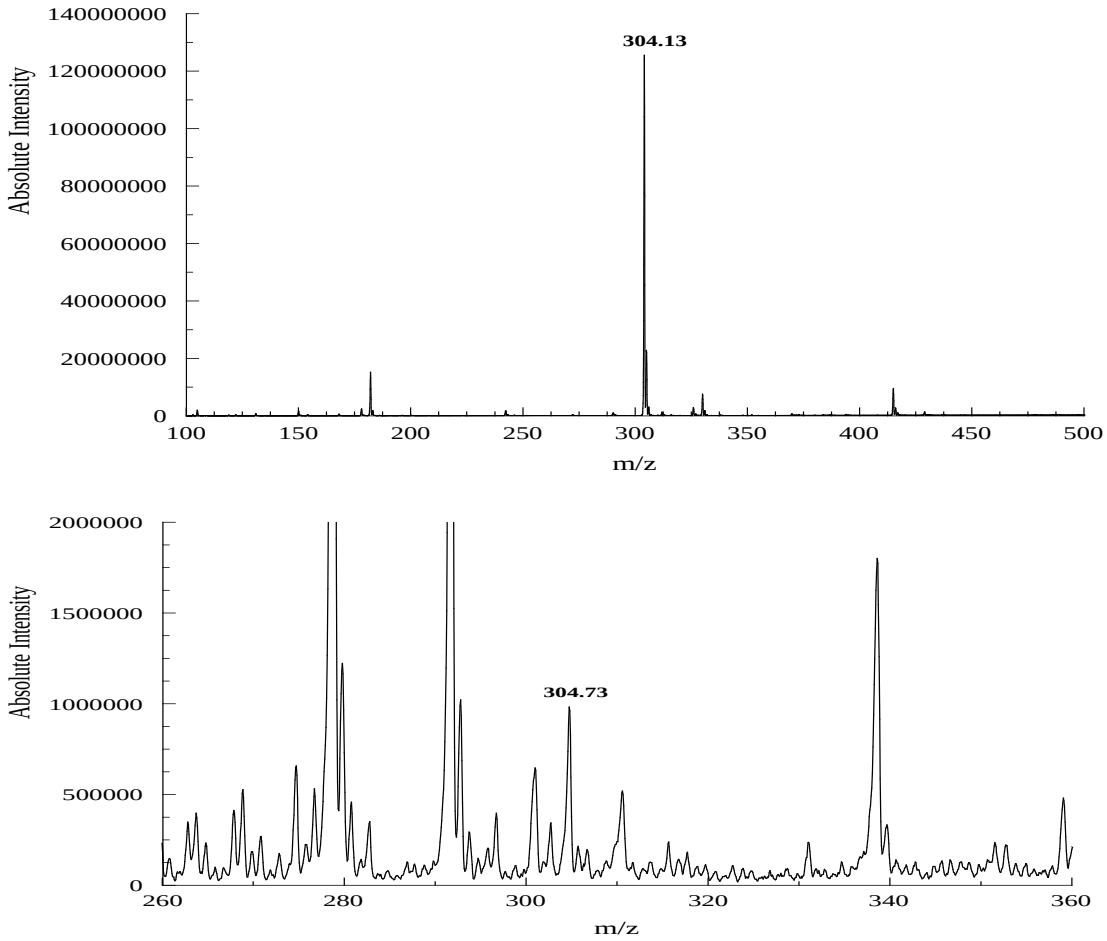


Figure 2.4 The powder cocaine spectra utilizing NSI-MS and ESI-MS. The NSI-MS spectrum of powder cocaine at a leading intensity with its MH^+ peak at m/z 304.13 (top spectrum). The ESI-MS spectrum of the powder cocaine/sand-based soil wash from the lift (bottom spectrum). The MH^+ peak for powder cocaine in the bottom spectrum appears at m/z 304.73 within the grass region.

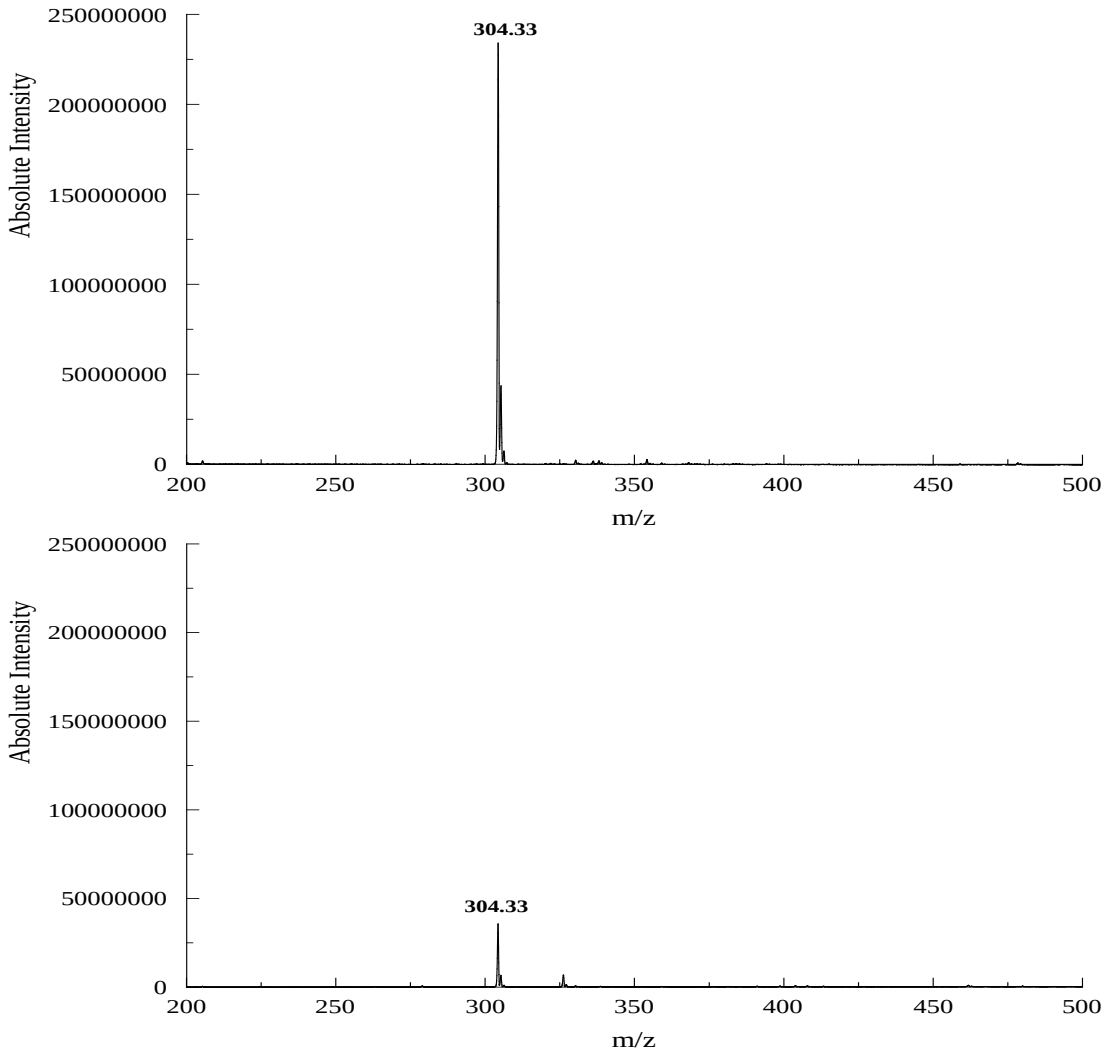


Figure 2.5 The rock cocaine spectra utilizing NSI-MS and ESI-MS. The NSI-MS spectrum of the rock cocaine extraction from the lift at a leading intensity with its MH^+ peak at m/z 304.33 (top spectrum). The ESI-MS spectrum of the rock cocaine/sand-based soil wash (bottom spectrum). The MH^+ peak for rock cocaine in the bottom spectrum appears at m/z 304.33 at a lower intensity.

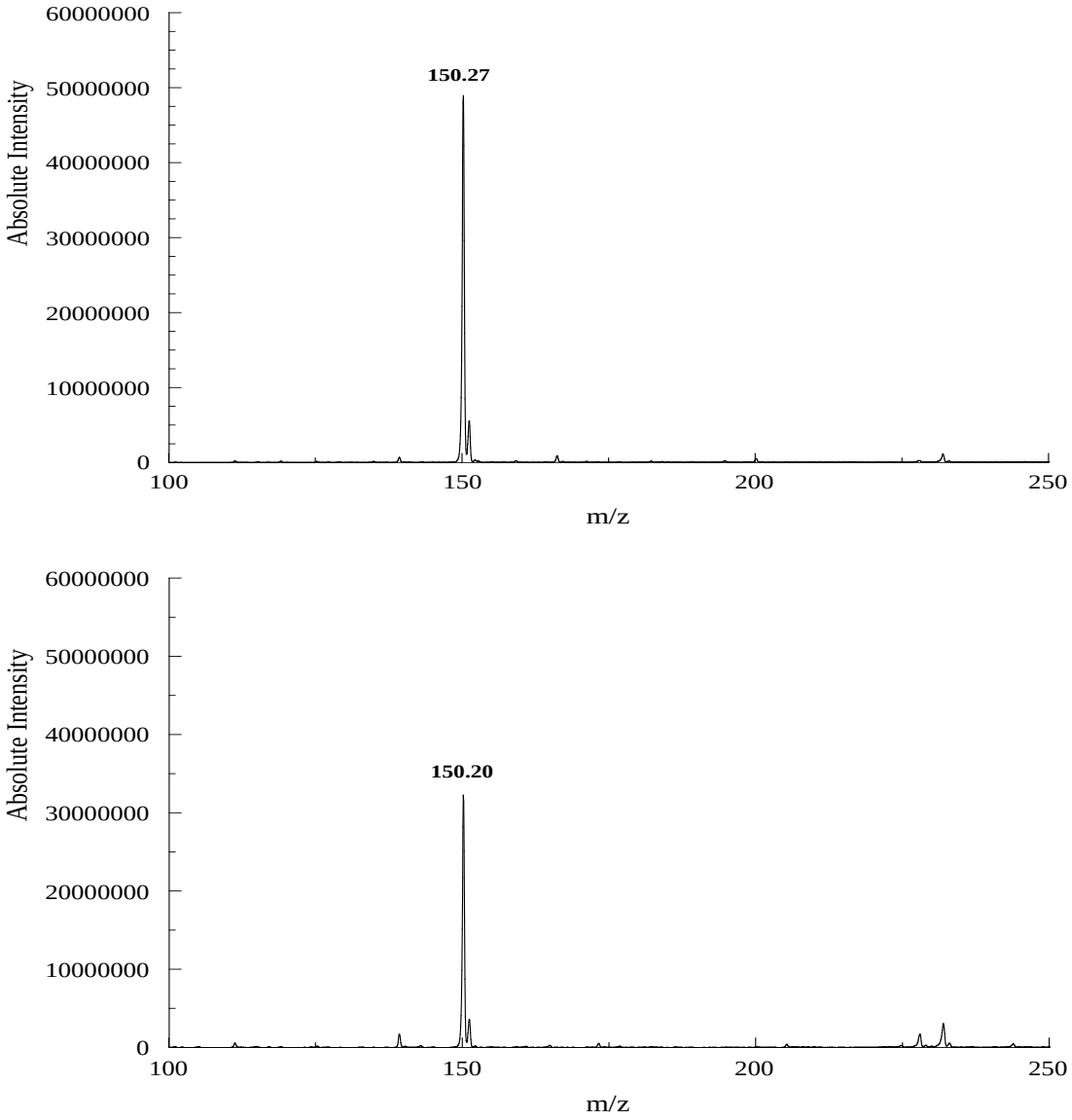


Figure 2.6 The crystal methamphetamine spectra utilizing NSI-MS and ESI-MS. The NSI-MS spectrum of the crystal methamphetamine extraction from the lift at a leading intensity with its MH^+ peak at m/z 150.20 (top spectrum). The ESI-MS spectrum of the crystal methamphetamine/sand-based soil wash (bottom spectrum). The MH^+ peak for crystal methamphetamine appears at m/z 150.20 at a lower intensity.

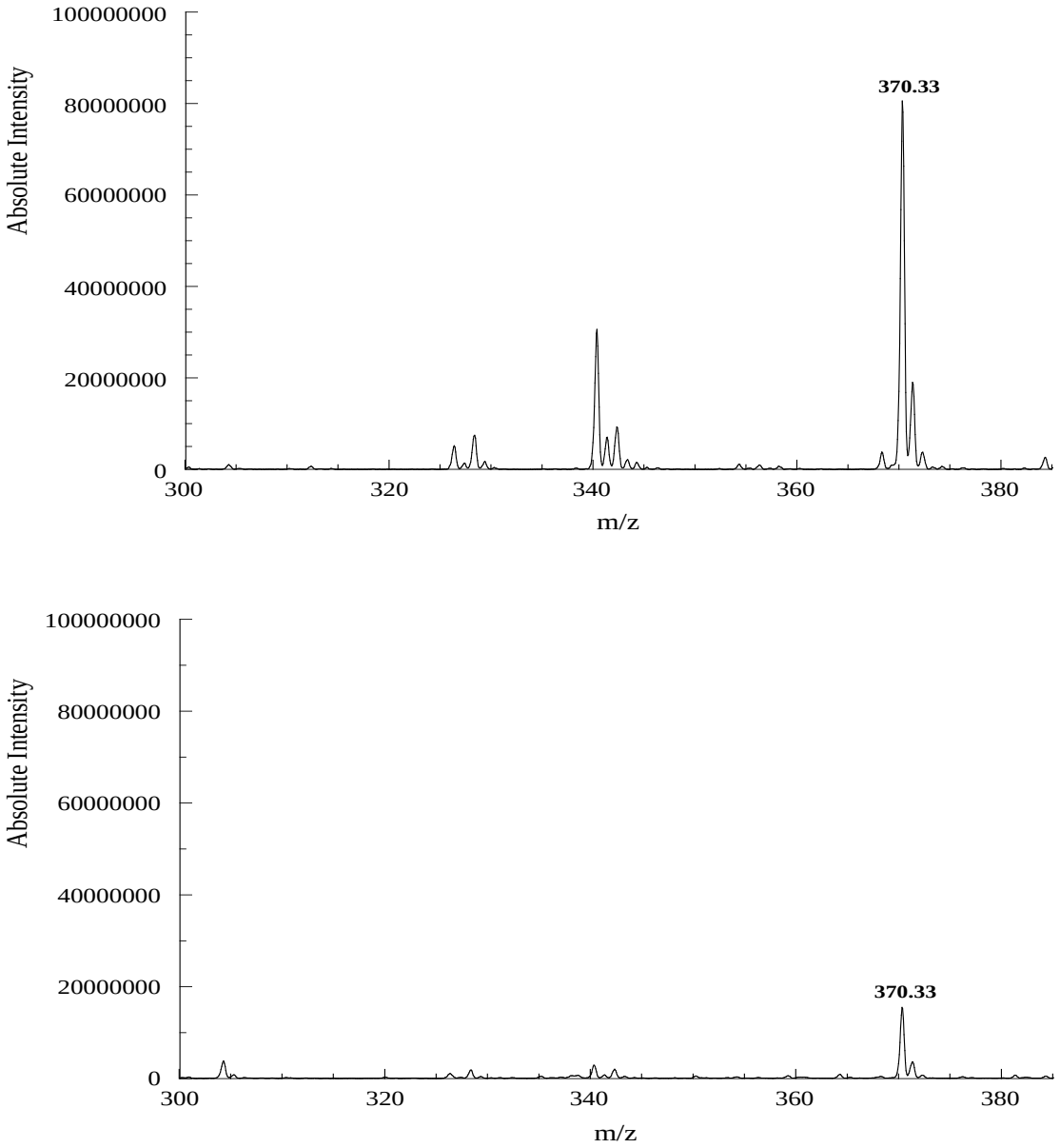
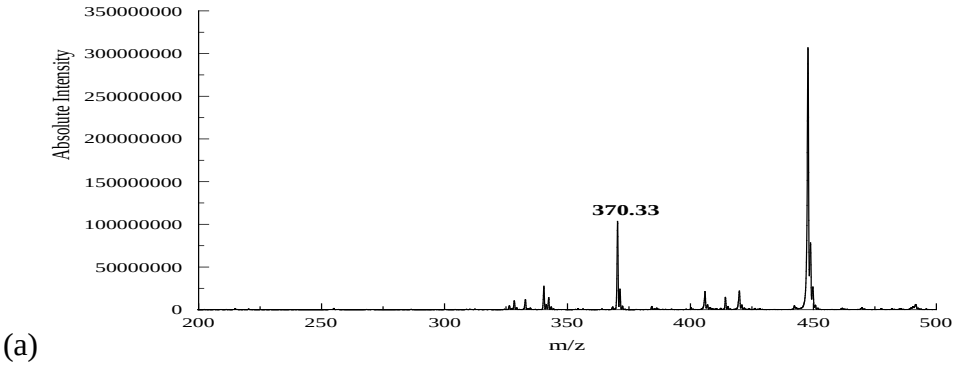
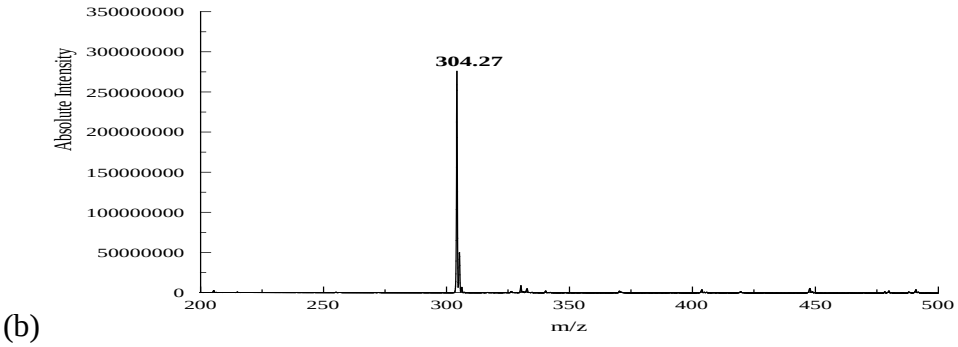


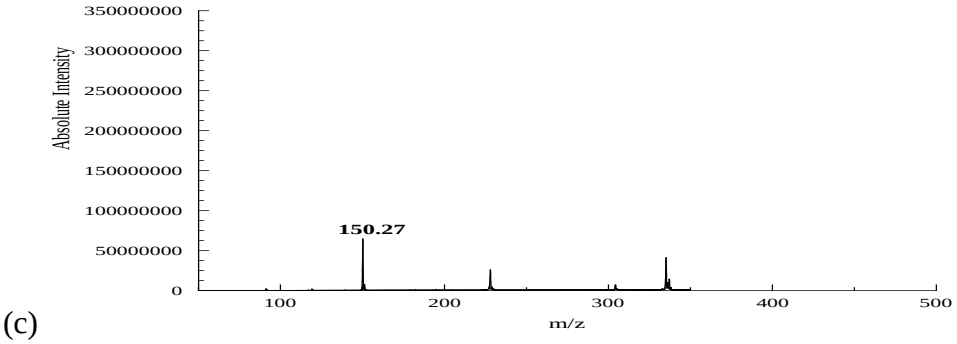
Figure 2.7 The black tar heroin spectra utilizing NSI-MS and ESI-MS. The NSI-MS spectrum of the black tar heroin extraction from the lift at a leading intensity with its MH^+ peak at m/z 370.33 (top spectrum). The ESI-MS spectrum of the black tar heroin/sand-based soil wash (bottom spectrum). The MH^+ peak for the black tar heroin appears at m/z 150.20 at a lower intensity.



(a)

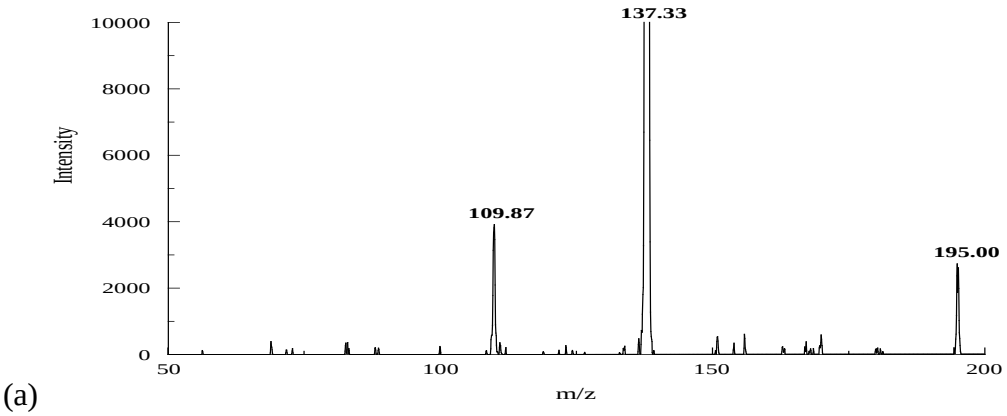


(b)

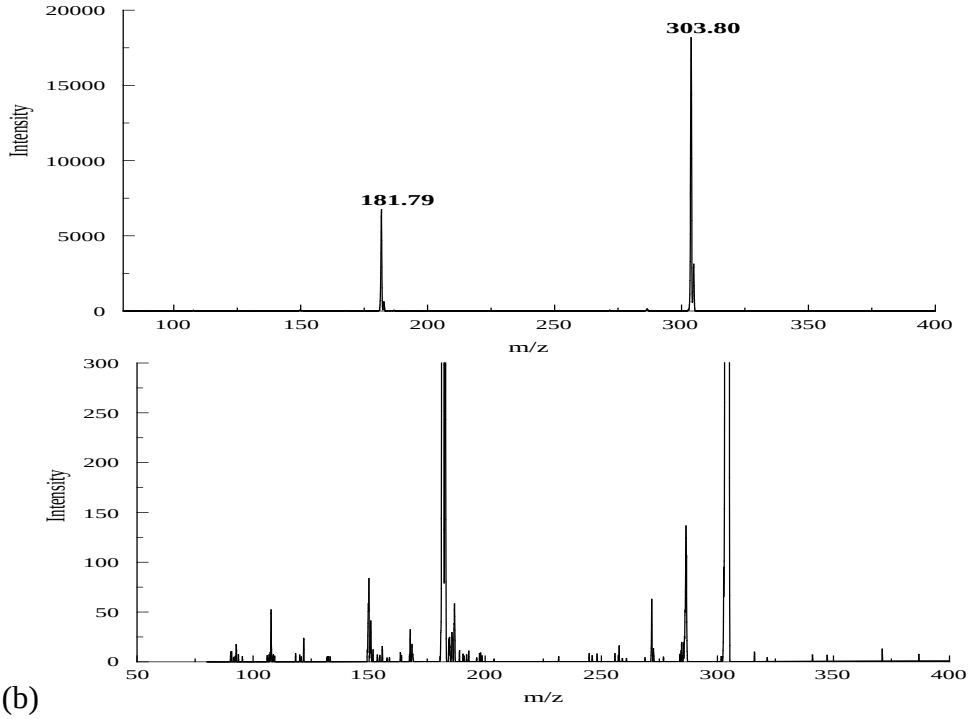


(c)

Figure 2.8 The ESI-MS spectra for drug/potting soil washes. (a) The black tar heroin/potting soil wash from the lift with the MH^+ peak appearing at m/z 370.33 along with peaks from the potting soil. (b) The rock cocaine/potting soil wash from the lift with the MH^+ peak appearing at m/z 304.27. (c) The crystal methamphetamine/potting soil wash from the lift with the MH^+ peak appearing at m/z 150.27.



(a)



(b)

Figure 2.9 The MS-MS spectra of caffeine and rock cocaine. (a) The caffeine MS-MS spectrum. The MH^+ peak for caffeine appears at m/z 195.00 along with its degradation products. (b) The rock cocaine MS-MS spectra. The MH^+ peak for rock cocaine appears at m/z 303.80 along with one of its degradation products at m/z 181.79 (top spectrum). The bottom spectrum displays an expanded view of the degradation products of rock cocaine at lower intensities.

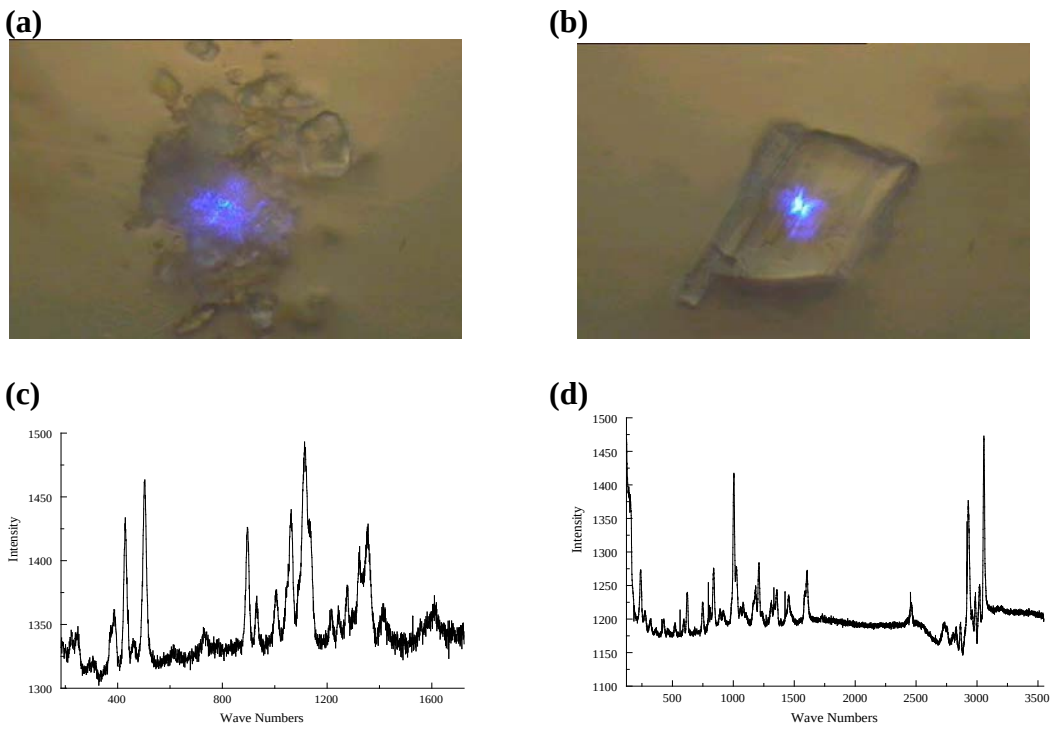


Figure 2.10 Rock cocaine and crystal methamphetamine images and Raman spectra. (a) The image captured of the rock cocaine particles on the gold plated lift. (b) The image captured of the crystal methamphetamine particle on the gold plated lift. (c) The Raman spectrum of the rock cocaine particles. (d) The Raman spectrum of the crystal methamphetamine particle.

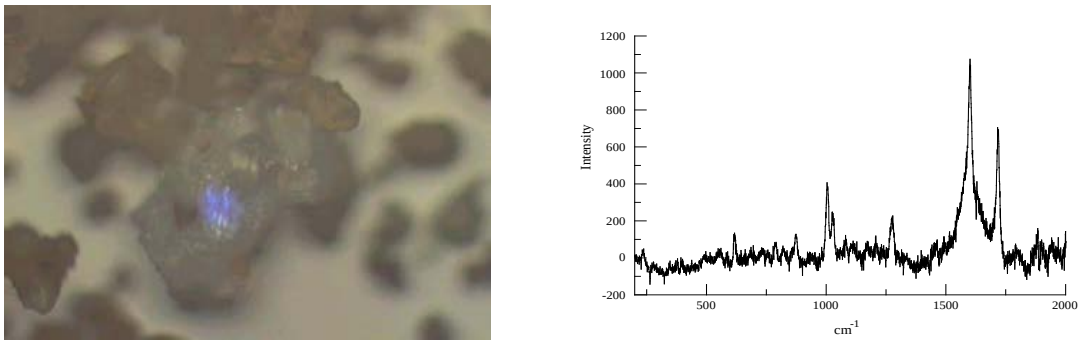


Figure 2.11 The image captured of the rock cocaine particle among the soil mixture (left). The Raman spectrum obtained of the rock cocaine particle on the gold plated lift (right).

(a) **(b)**

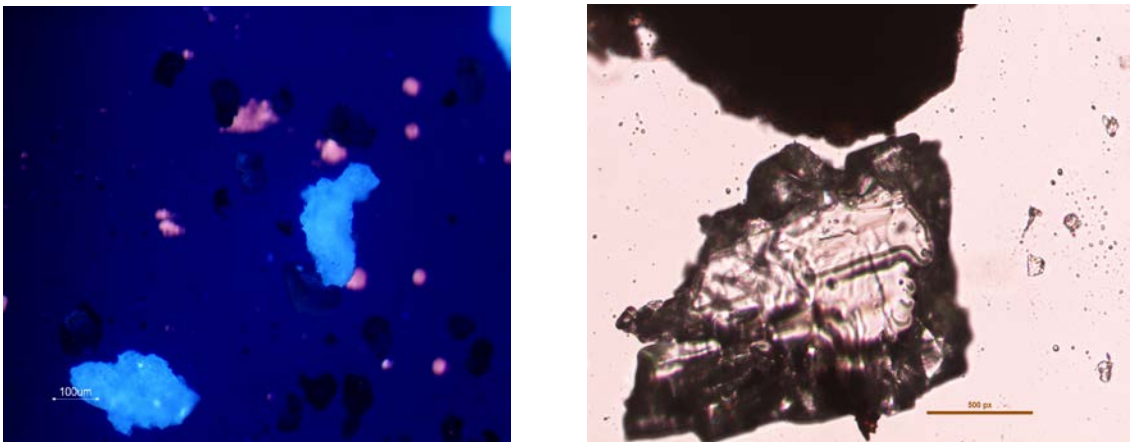


Figure 2.12 The RGB images of rock cocaine and crystal methamphetamine. (a) The rock cocaine particles among a sand-based soil mixture. (b) The crystal methamphetamine particle among a potting soil mixture.

Chapter Reference

1. Ledbetter, N. L.; Walton, B. L.; Davila, P.; Hoffmann, W. D.; Ernest, R. N.; IV, G. F. V., Nanomanipulation-Coupled Nanospray Mass Spectrometry Applied to the Extraction and Analysis of Trace Analytes Found on Fibers*. *Journal of Forensic Sciences 9999* (9999).

2. Brown, J. M.; Hoffmann, W. D.; Alvey, C. M.; Wood, A. R.; Verbeck, G. F.; Petros, R. A., One-bead, one-compound peptide library sequencing via high-pressure ammonia cleavage coupled to nanomanipulation/nanoelectrospray ionization mass spectrometry. *Analytical Biochemistry 398* (1), 7-14.

3. Dams, R.; Huestis, M. A.; Lambert, W. E.; Murphy, C. M., Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. *Journal of the American Society for Mass Spectrometry* 2003, *14* (11), 1290-1294.

4. Hajslová, J.; Zrostlíková, J., Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices. *Journal of Chromatography A* 2003, *1000* (1-2), 181-197.

5. Ortner, H. M., Ultratrace analysis — Facts and fiction. *Fresenius' Journal of Analytical Chemistry* 1992, *343* (9), 695-704.

6. Tim, R. C.; Kautz, R. A.; Karger, B. L., Ultratrace analysis of drugs in biological fluids using affinity probe capillary electrophoresis: Analysis of dorzolamide with fluorescently labeled carbonic anhydrase. *Electrophoresis* 2000, *21* (1), 220-226.

7. Liu, H.; Cooper, L. M.; Raynie, D. E.; Pinkston, J. D.; Wehmeyer, K. R., Combined supercritical fluid extraction/solid-phase extraction with octadecylsilane cartridges as a sample preparation technique for the ultratrace analysis of a drug metabolite in plasma. *Analytical Chemistry* 1992, *64* (7), 802-806.

8. Postigo, C.; Lopez de Alda, M. J.; Barcelo, D., Fully Automated Determination in the Low Nanogram per Liter Level of Different Classes of Drugs of Abuse in Sewage Water by On-Line Solid-Phase Extraction-Liquid Chromatographyâˆ'Electrospray-Tandem Mass Spectrometry. *Analytical Chemistry* 2008, *80* (9), 3123-3134.

9. Smyth, W. F., Recent studies on the electrospray ionisation mass spectrometric behaviour of selected nitrogen-containing drug molecules and its application to drug analysis using liquid chromatography-electrospray ionisation mass spectrometry. *Journal of Chromatography B* 2005, *824* (1-2), 1-20.

10. Mortier, K. A.; Maudens, K. E.; Lambert, W. E.; Clauwaert, K. M.; Van Bocxlaer, J. F.; Deforce, D. L.; Van Peteghem, C. H.; De Leenheer, A. P., Simultaneous, quantitative determination of opiates, amphetamines, cocaine and benzoylecgonine in oral fluid by liquid chromatography quadrupole-time-of-flight mass spectrometry. *Journal of Chromatography B* 2002, *779* (2), 321-330.

11. Fisher, M.; Bulatov, V.; Schechter, I., Fast analysis of narcotic drugs by optical chemical imaging. *Journal of Luminescence* 2003, *102-103*, 194-200.

12. Ali, E.; Edwards, H.; Hargreaves, M.; Scowen, I., Raman spectroscopic investigation of cocaine hydrochloride on human nail in a forensic context. *Analytical and Bioanalytical Chemistry* 2008, *390* (4), 1159-1166.

13. Ryder, A. G., Surface enhanced Raman scattering for narcotic detection and applications to chemical biology. *Current Opinion in Chemical Biology* 2005, *9* (5), 489-493.

14. Ryder, A. G.; O'Connor, G. M.; Glynn, T. J., Quantitative analysis of cocaine in solid mixtures using Raman spectroscopy and chemometric methods. *Journal of Raman Spectroscopy* 2000, *31* (3), 221-227.

15. Takáts, Z.; Wiseman, J. M.; Cooks, R. G., Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. *Journal of Mass Spectrometry* 2005, *40* (10), 1261-1275.

16. Cody, R. B.; LaramÃ©e, J. A.; Durst, H. D., Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Analytical Chemistry* 2005, *77* (8), 2297-2302.

17. Rubakhin, S. S.; Jurchen, J. C.; Monroe, E. B.; Sweedler, J. V., Imaging mass spectrometry: fundamentals and applications to drug discovery. *Drug Discovery Today* 2005, *10* (12), 823-837.

18. Aebersold, R., Mass spectrometry-based proteomics. *Nature* 2003, *422* (13), 198-207.

19. Morris, M. D., *Microscopic and Spectroscopic Imaging of the Chemical State*. Marcel Dekker, INC.: New York, 1993; Vol. 16, p 1-21.

20. Zimmermann, T.; Rietdorf, J.; Pepperkok, R., Spectral imaging and its applications in live cell microscopy. *FEBS Letters* 2003, *546* (1), 87-92.

21. LeMay, J., Evidence Beneath Your Feet. *Law Enforcement Technology* 2006, *33* (3), 42-45.

22. Craig, C. L., Evaluation and Comparison of the ElectrostaticDust Print Lifter and the Electrostatic Detection Apparatus on the Development of Footwear Impressions on Paper. *Journal of Forensic Sciences* 2006, *51* (4), 1556-4029.

23. (Japan), N. P. A. T., An Electrostatic Method of Lifting Footprints. *International Criminal Police Review* 1973, (272), 287-292.

CHAPTER 3

ULTRA-TRACE DRUG RESIDUE ANALYSIS FROM FINGERPRINTS USING NANOMANIPULATION-COUPLED TO NANOSPRAY IONIZATION-MASS SPECTROMETRY

Abstract

The extraction of ultra trace drug residues from fingerprints can provide significant forensic value to an investigation, yet the ability to retrieve and analyze these residues can be an exigent task. Ultra trace drug residue analysis is a method that has prompted many innovative instrumental techniques to be developed to attain high quality results from such a limited quantity. The only drawback of most of these innovative instrumental methods is that they require a preconcentration technique or a tedious, detailed instrumental procedure in order for analysis to be effective. Fingerprints present a great means of extracting drug residues for analysis with nanomanipulation-coupled with nanospray ionization mass spectrometry. The methods employed for lifting fingerprints can be modified as to allow nanomanipulation of ultra trace residues from friction ridge impressions made by the fingers or palms of hands. Nanomanipulation-coupled with nanospray ionization-mass spectrometry is an instrumental technique that allows for the direct probing of trace analytes. This technique achieves low detection limits from small sample volumes without the need for preconcentration. Direct extraction occurs from the sample using a nanospray tip followed by nanospray ionization by the mass spectrometer. Immediate analysis is accomplished by the elimination of a multi-step instrumental procedure. This technique of ultra trace drug residue analysis presents a valuable approach to performing drug analysis and can be applied to other types of ultra trace analysis work.

Introduction

The retrieval of drug residues in ultra-trace amounts poses a challenging task for most trace analysts to due mostly to instrumental limitations. The instrumental method used for analyzing ultra-trace amounts can often be a hindrance to obtaining productive results. Detection of extremely small quantities is usually beyond most operational standards of instruments preventing results in good quality. [1]Matrix effects have a role in the outcome of results as well. Background contamination has always been an issue for trace analysis work. Much expertise of the instrument is required along with sufficient prep time in order to carry out an analytical procedure out will allow the analyst to achieve quality results. The overall process can still result in much loss of the desired analyte due to the pretreatment of the sample, not allowing enough of the analyte to be detected by the instrument.[1] This issue has prompted the development of new techniques combined with instruments to achieve quality results that include higher sensitivity and selectivity of analytes. However, the retrieval of drug residues from fingerprint impressions is a method that does not receive much attention. Although there are many techniques that can image the friction ridge detail of fingerprint impressions, few actually advance their techniques to the extraction of particulates contained by impressions.

The electrostatic lifting method is applied toward the retrieval of soil particulates and other particles outlining the footwear impressions made on surfaces. Visual image recording of these dust prints is an essential part of the process for it provides documentation of the evidence.[2]Although the electrostatic lifting process is focused mainly on developing footwear impression detail, it gives an example of how imaging of the dust prints is the ultimate goal. Traditionally, fingerprinting makes use of different types of powders specific to developing fingerprints off of various surfaces in order to record friction ridge detail. This established

method is still very common and used quite frequently to develop fingerprints although other new methods have been developed. Fingerprint developing methods over the years have evolved from such techniques as chemical fuming to laser light enhancement to improve the overall image quality of the print and to facilitate the recording of the print off of various surfaces and surface angles. [3] There have been studies conducted on lipid secretions deposited from the eccrine glands of fingers to examine how it influences print visibility over time.[4] Degradation products of prints due to extreme heat have been studied as well to formulate reagents for latent print development specific to these conditions. Both studies were explored to aide in the design of effective fingerprint developing techniques. Overall fingerprinting methods generally are geared toward providing detailed imaging of friction ridge patterns to be captured photographically and used as evidence for investigations.

Research has been done on latent fingerprint residue to explore its chemistry further along with its degradation products over time. The modification of GC-MS method is a technique that has been implemented to facilitate the preparation of residue components of fingerprints in order identify analytes present.[5] This method was geared toward identifying the organic and inorganic compounds of latent print residue and was extended to understanding the degradation products over time. Another method using GC-MS was experimented with as an attempt to identify a person according to their latent print residue chemistry along with any residue that may be found according to daily habits.[6] It was carried out to see if a pool of suspects could be narrowed down. Both GC-MS methods provide qualitative data but require much preparation of the latent print residues before instrumental analysis can commence.

Spectroscopic instrumental methods are used quite often for trace analysis work. These methods have been coupled with techniques to detect analytes from trace amounts of residues.[7]

Chemical imaging of trace amounts of residue from fingerprints has been implemented in the identification of analytes according to their chemical signature exhibited in spectrums through the use of infrared spectromicroscopy. [8] However, surface areas that prints residues are deposited on will vary which may not be ideal for infrared spectroscopic techniques. Hyphenated Raman spectroscopy methods have been utilized frequently for analysis of trace drug amounts. Raman spectroscopy has been applied toward the analysis of cocaine residues found on human nails.[9] Experimental results were collected on the cocaine analyte deposited on the human nail matrix. Quantitative analysis on cocaine mixtures have been done with Raman spectroscopy using near-infrared excitation to perform principal component analysis.[10] Spectral variations were examined to determine their influence over concentration ranges. Another spectroscopic technique for analysis of narcotics is involves using surface enhanced Raman scattering for the increased detection of trace amounts of particles.[11] This method was implemented to improve Raman spectroscopy in regards to sample amounts. Qualitative limitations of the analyte may prevail with both spectroscopic methods due to the surface needed to obtain adequate analysis. The geometric shape or chemical form of the analyte could pose as a complication as well. Another issue for these methods is that most impressions are made at the scene of the crime; it is not always a simple task to retrieve these particles for analysis. Transfer of extremely trace amounts can often lead to partial or total loss of the analyte or interest.

Nanomanipulation-coupled with nanospray ionization is an instrumental method that provides a straight forward efficient approach to ultra-trace drug analysis. Single crystal extraction provides ample signal for detection of analyte residue. This hyphenated technique has been applied to the extraction of analytes found in swatches of fiber.[12] Detection of cocaine and caffeine has been successfully achieved with this method. It was also been applied in the

extraction of peptides from biological beads producing spectra that is reproducible to traditional techniques used.[13] Mass spectrometry is acknowledged for its sensitivity and selectivity, proving remarkable qualitative characterization of analytes. Nanospray ionization enhances these instrumental qualities of sensitivity and selectivity with extremely trace amounts of sample. Nanomanipulation of the analyte is direct and does not require any preconcentration techniques, allowing immediate analysis to initiate. This technique is minimally destructive and offers the ability to utilize optical imaging techniques to improve the identification of chemical analytes. The coupling of this instrumental method to the extraction of ultra-trace drug residues provides an exceptional approach to identifying analytes in ultra-trace quantities.

Materials

The solvents utilized in this experimental method for dissolution and extraction of samples was a 1:1(v/v) solution of optima LC/MS methanol (Fisher Scientific, Fair Lawn, NJ) and Millipore water (Millipore, Billerica, MA) with 1% glacial acetic acid (Malinkrodt Chemical Co., Phillipsburg, NJ). The methanol used was doubly distilled to eliminate any impurities in the solvent. The method was validated using ~0.0025g of caffeine (Alfa Aesar, Ward Hill, MA). A sample of rock cocaine (<0.005g) was provided by the University of North Texas Police Department (Denton, TX). The fingerprint cast for the friction ridge impressions was made using mikrosil silicone casting material (Evident Crime Scene Products, Union Hall, VA). The fingerprint powders (Lynn Peavey Company, Lenexa, KS) were utilized for the development of the fingerprint impressions. Pam cooking spray oil (ConAgra Foods, Inc, Omaha, NE) was used to mimic natural secretions from the glands of the fingers. The nanomanipulator (Zyvex, Richardson, TX) -coupled to a Multi-Zoom AZ100 microscope (Nikon, Melville, NY) along

with a PE2000b four-channel pressure injector (MicroData Instrument Inc., S. Plainfield, NJ) was used for extraction of the analytes. The mass spectrometric analysis was done on a LCQ DECA XP Plus (Thermo Finnigan, San Jose, CA) with a nanospray ionization source (Proxeon Biosystems, Odense, Denmark). The TE2000U inverted microscope (Nikon, Melville, NJ) was used for UV fluorescence studies on the fingerprint samples.

Methods

Fingerprint impressions were made on microscopic glass slides utilizing a finger cast made of silicone casting material. The casting of a live hand was done with this silicone casting material in order to impress the friction ridge detail from the skin tissue onto the casting material. Casting was done in order to avoid the use of living skin tissue to retrieve residue from the drugs. Cooking spray oil was applied sparsely on the fingerprint casts to imitate the oils secreted from the glands on the fingers. The fingerprint impressions made on the glass slides with drug and caffeine residues underwent microscopic and nanomanipulation-coupled to nanospray ionization-mass spectrometric (NSI-MS) analysis. Background spectrums of the fingerprint powders, cooking oil and adhesive tape lifts were analyzed as well using both NSI-MS and electrospray ionization-mass spectrometry (ESI-MS).

Casting of a Fingerprint

The molding of a live hand was done with silicone casting material in line to build a finger cast. Silicone casting material was used to generate a cast that was flexible in form. The casting material was extruded and applied to a live hand. After the casting was allowed to dry completely, it was peeled back toward the fingers slowly retaining the form of the whole hand. In

order to prevent mixing and hardening of the second casting, a butter based cooking spray oil was misted into the interior of the cast where the fingers were situated to maintain lubrication for a second casting. The second casting was extruded into the cavity of two of the fingers from the casted hand and allowed to dry completely. Two fingerprint casts were made in order to have a spare cast. The fingerprint casts were removed carefully by pulling back the original casts.

Fingerprint Impressions

Cooking spray oil was applied evenly to the surface of a fingerprint cast in scant amounts to imitate the natural secretions from the eccrine glands of the finger. The fingerprint cast was then impressed into 2 to 3 particles of caffeine on a microscopic glass slide. In turn the fingerprint cast containing the caffeine particles was impressed onto another slide. The second slide underwent nanomanipulation followed by NSI-MS analysis. The same procedure was followed for the rock cocaine sample. In addition to the procedure for the rock cocaine sample, the second slide was developed with red fluorescent fingerprint powder.

Nanomanipulation-Coupled to Nanospray Ionization-Mass Spectrometry

Fingerprint impression slides were placed under the Multi-Zoom AZ100 microscope for probing of drug and caffeine residues. Probing of the analytes took place with an AZ Plan Fluor 2x objective. Image capturing was done with NIS Elements software via a CCD camera coupled to the microscope.

The nanomanipulator mounted to the microscope contains four nanopositioners controlled in two different modes, coarse and fine. The resolution and range of motion both vary according to the mode of manipulation. The range of motion for the coarse mode is 12 mm in the

X and Z-axes, and 28 mm in the Y-axis along with a translational resolution of 100 nm. In the fine mode of operation, the range of motion is 100 µm in the X and Z-axes, and 10 µm in the Y-axis. The course mode utilizes nanospray capillary tips coated with Pt that perform the injection/extraction of the analytes. Analytes from the fingerprint impressions were manipulated with the coarse mode of operation. The PE2000b pressure injector connected to the nanomanipulator provides the pressure required to do the injection/extraction process via the nanospray capillary tips.

The analyte injection/extraction process was carried out with 10 µL of solvent loaded into the nanospray capillary tips. After the analytes were located from the fingerprint impression, the nanospray capillary tip was landed on the desired particle of interest at ~1 µm away. An injection of the solvent mixture (1:1 (v/v) methanol/water, 1% acetic acid) was performed at a pressure of 23 psi for dissolving process of the analyte. After 10 seconds of dissolution, the solvent/analyte solution was aspirated into the nanospray capillary tip at a pressure of 60 psi. The nanospray capillary tip was then transferred to the nanospray ionization source for nanospray ionization mass spectrometric analysis (NSI-MS). An ionization voltage of 2.5 kV was applied for analysis on the mass spectrometer using positive mode. A background spectrum was collected for the adhesive tape lift following the same procedure above with and without the analyte present.

Electrospray Ionization–Mass Spectrometry Analysis

Background collections were done on the orange fluorescent, red fluorescent, white and black fingerprint powders. A concentration of 1 mg/ml was prepared from each powder. From that concentration, a dilution of 1/10 was prepared from each sample before spectra collection commenced on ESI-MS. An instrument voltage of 4 kV was used with a flow of 5 µl/min. Each

sample was run in the positive mode. Background spectrums were also done on the cooking spray oil utilizing the same procedure as the fingerprint powders.

UV Fluorescence Analysis

The fingerprint impression slides were viewed under the TE2000U Inverted Microscope coupled to a fluorescence illumination system using an objective of 10x/0.30. UV fluorescence images were captured with a CCD camera utilizing NIS elements software.

Results and Discussion

The fingerprint casting process provided the friction ridge detail necessary to execute the experiment. Friction ridge detail matching that of the live finger tissue was impressed onto the casting material successfully. Although the impression was not flawless, sufficient detail was replicated in order to carry out the impression process. Figure 3.1 displays the image of the casted fingerprint with friction ridge detail recorded.

Extraction of caffeine particles was achieved from the fingerprint impression made on the glass slide. Figure 3.2 displays the image captured of the fingerprint impression along with the NSI-MS spectrum of the caffeine extraction. The MH^+ peak of caffeine at m/z 195.20 is clearly seen demonstrating detection of the analyte.

For the fingerprint impression made with the rock cocaine sample, red fluorescent fingerprint powder was used to develop this impression in order to examine any matrix effects from the powder. Figure 3.3 (a) displays the image of the developed fingerprint impression. The rock cocaine particles can be seen within this image. Figure 3.3 (b) displays the NSI-MS spectrum of the rock cocaine extraction from the fingerprint impression. The MH^+ peak for rock

cocaine is prominently seen at m/z 304.27. The red fluorescent fingerprint powder does not appear to influence the detection of rock cocaine from the extraction.

Background spectrums were generated on four different colored fingerprint powders along with the cooking spray oil used. The purpose of collecting the spectra was to determine if these materials had any effect on analyte detection. The fingerprint powder spectra were obtained in the same m/z range as the analytes. Figure 3.4 displays the spectra for the fingerprint powders, and cooking spray. For the orange and red fluorescent fingerprint powders, no prominent detection of any peak is seen therefore leading to the conclusion that there would most likely be no interference to analyte detection. From looking at the spectrum for the black fingerprint powder, the same conclusion can be made. The white fingerprint powder spectrum does exhibit prominent peak signals indicating that there would be possible interference to the analyte. The cooking spray oil spectrum does not exhibit any prominent peaks that would possibly lead to any interference in common with the first three fingerprint powders mentioned.

The adhesive tape lift background was analyzed with NSI-MS analysis due to the difficulty of providing an effective ESI-MS analysis for an actual tape lift resin. The purpose of this background collection is the same as for the fingerprint powder and cooking spray oil. In addition to this background analysis, a sample of rock cocaine was extracted from this tape lift and analyzed as well. Figure 3.5 displays the NSI-MS spectra of both the adhesive tape lift with and without the rock cocaine sample. The spectrum showing just the extraction of the resin on the tape lift without the rock cocaine exhibits some prominent detection around m/z 370.00. The spectrum showing the rock cocaine extraction on the tape lift does exhibit detection of the analyte. The MH^+ peak of the rock cocaine is clearly seen at m/z 303.93. Significantly decreased detection is seen at the once prominent peak situated around m/z 370.00. Although, interference

can possibly occur at the peak situated around m/z 370.00, it does not appear to effect the detection of rock cocaine due to its MH^+ peak appearing at m/z 303.93.

UV fluorescence analysis on rock cocaine residue from the fingerprint impression was carried out to demonstrate coupling of another method to provide supplemental verification of analyte(s) present in a matrix. With the matrix being mostly the red fluorescent fingerprint powder, the rock cocaine fingerprint impression prior to extraction with nanomanipulation underwent UV fluorescence analysis. Figure 3.6 displays the red, green, blue, and RGB images of the rock cocaine among the red fluorescent fingerprint powder matrix. The RGB image clearly shows rock cocaine residue among the red fluorescent fingerprint powder. The blue fluorescence image appears to show a more enhanced image of the rock cocaine residue than the red and green fluorescence images.

Conclusion

The extraction of fingerprint residues utilizing nanomanipulation-coupled to nanospray ionization-mass spectrometry (NSI-MS) attests to becoming a valuable tool toward ultra-trace analysis of illicit drug residues. Although this technique is certainly an atypical approach to ultra-trace analysis, it does provide a more immediate method of analysis eliminating the need for sample preparation. Lower limits of detection can be achievable with this instrumental method demonstrating high sensitivity and selectivity of analytes as seen with the extractions of rock cocaine and caffeine from the fingerprint impressions.

Electrospray ionization-mass spectrometry studies of the fingerprint powders and cooking spray oil toward matrix effects show very little or no interference to the detection of the analytes with the possible exception of the white fingerprint powder. While the white fingerprint

powder does exhibit heighted peak intensities, the overall usage of white fingerprint powder for developing prints is not as frequent. The NSI-MS studies on the resin from the adhesive tape lift showed no possible interferences to the MH^+ peak range of the rock cocaine. Heightened peak intensity was observed in a m/z range other than the MH^+ peak range for the rock cocaine. However, decreased peak intensity from this m/z range was actually observed when rock cocaine was detected. This may indicate that the adhesive tape lift resin does not interfere with the detection of other analytes. UV fluorescence imaging analysis provided substantiation of the analyte residue imbedded in the red fluorescent fingerprint power matrix. The coupling of this optical imaging technique provides the additional capabilities at times needed to identify analytes of interest.

The application of nanomanipulation-coupled to nanospray ionization-mass spectrometry towards the analysis of fingerprint residues exhibits a favorable approach to identifying illicit drug residues. The adherence of these residues to material surfaces or the friction ridge surfaces of skin can be retrieved and analyzed in a competent manner. The coupling of this method with other techniques can be feasible as to allow improvement of the overall analysis process. Trace analysis work could move into the ultra-trace realm leading to better detection of analytes with no limitation to the amount of sample.

(a) **(b)**

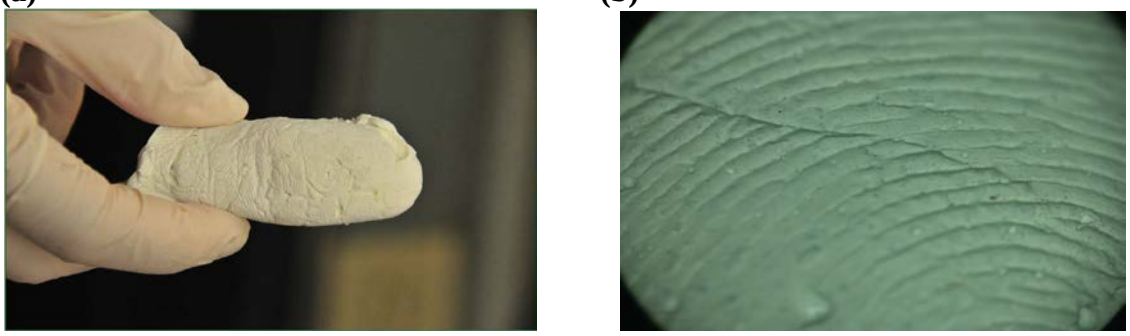


Figure 3.1 (a) Image displayed of the casted fingerprint. (b) Image of friction ridge detail recorded from casting.

(a) **(b)**

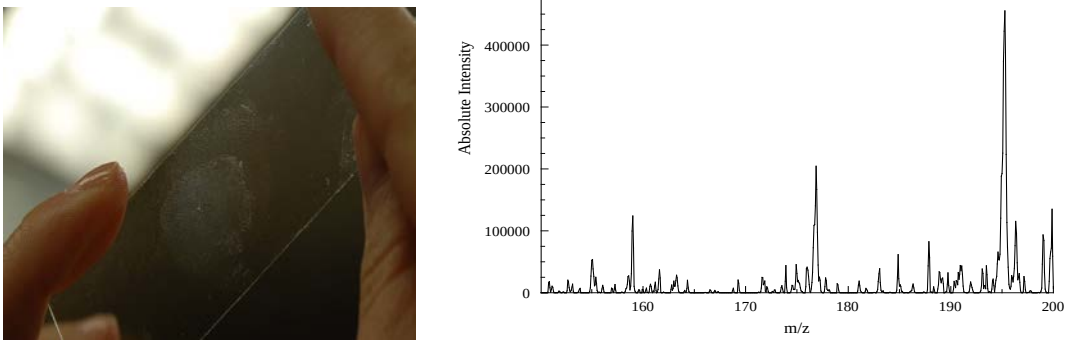


Figure 3.2 (a) Image captured of the fingerprint impression containing caffeine residue. (b) NSI-MS spectrum of the caffeine residue extraction. The MH^+ peak appears at m/z 195.20 at a leading intensity.

(a) **(b)**

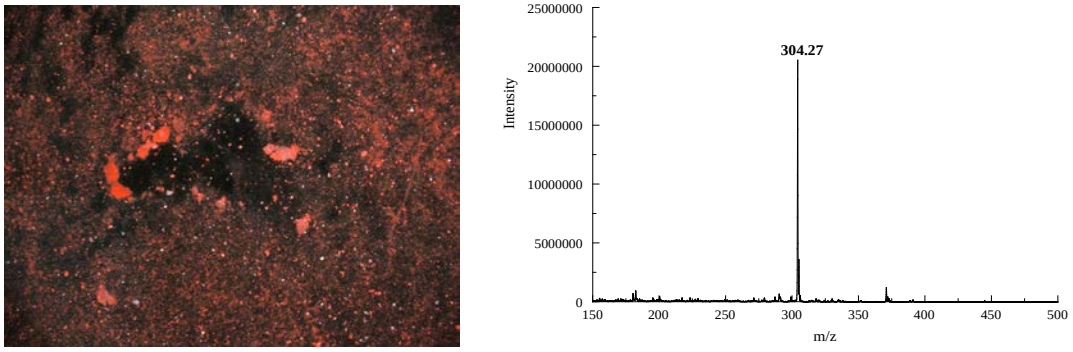


Figure 3.3 The cocaine residue extraction with NSI-MS (a) Image captured of the developed fingerprint impression containing cocaine residue. (b) The NSI-MS spectrum of the cocaine extraction from the developed fingerprint impression. The MH^+ peak for cocaine is prominent at m/z 304.27.

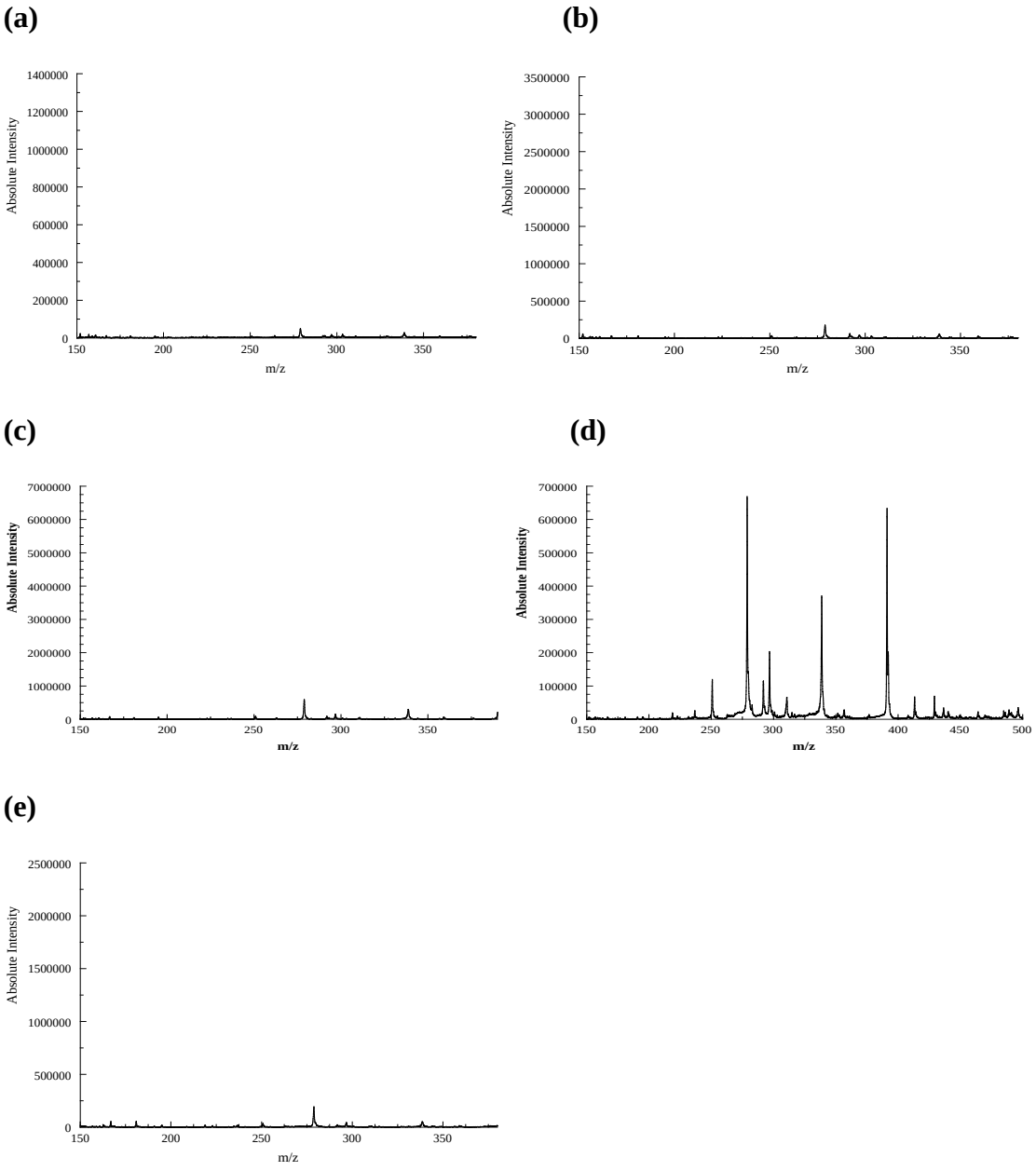
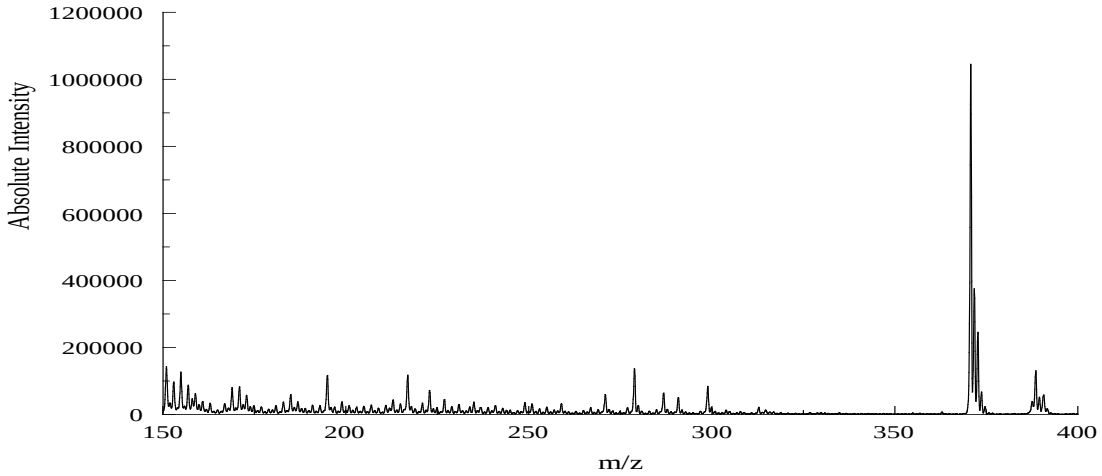


Figure 3.4 ESI-MS spectra on the fingerprint powders and cooking spray. (a) orange fluorescent powder (b) red fluorescent powder (c) black powder (d) white powder (e) cooking spray.

(a)



(b)

Figure 3.5 The NSI-MS spectra on the adhesive tape lift. (a) NSI-MS spectrum of the adhesive resin from the tape lift. (b) NSI-MS spectrum of the cocaine residue extraction from the adhesive tape lift. The MH^+ peak of cocaine is prominent at m/z 303.93.

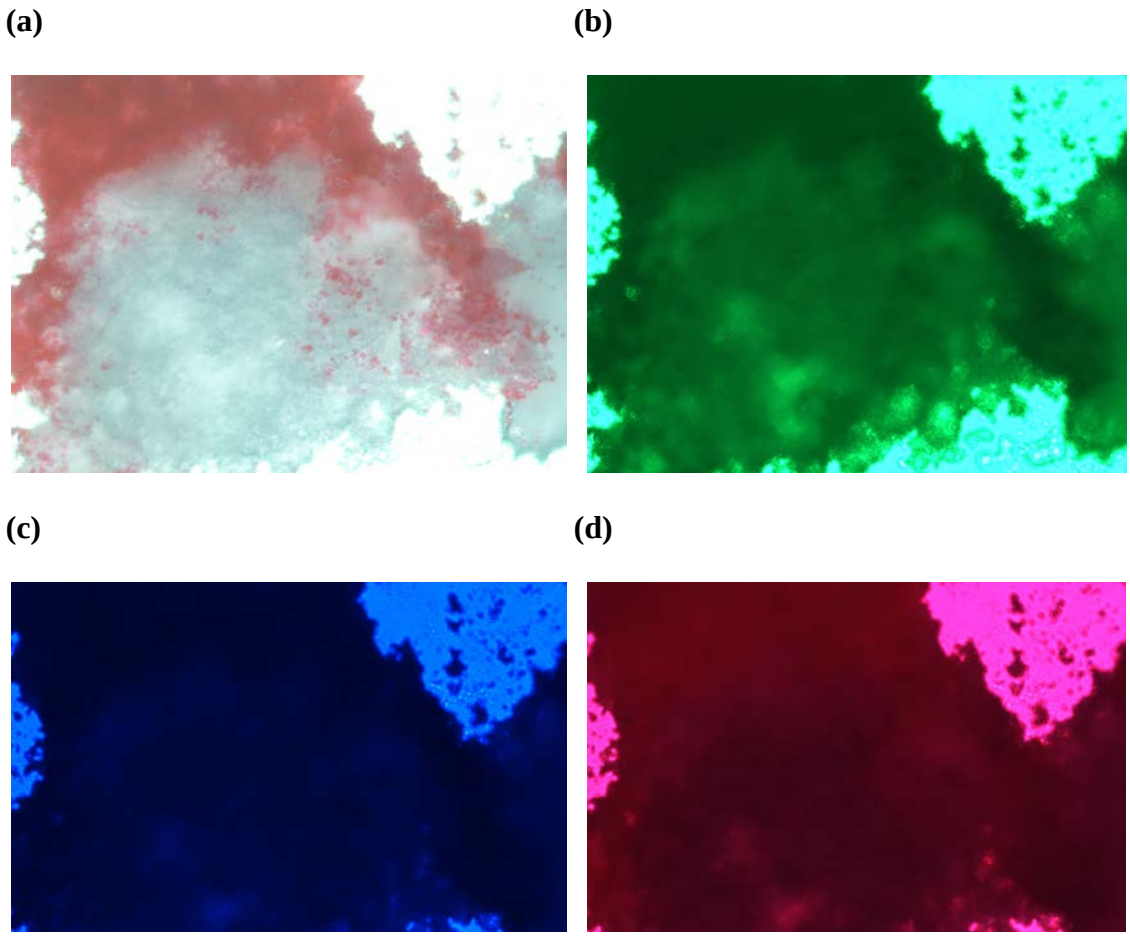


Figure 3.6 UV fluorescence images captured of the cocaine residue in red fluorescent fingerprint powder. (a) RGB image (b) Blue fluorescence (c) UV fluorescence (d) Green fluorescence.

Chapter References

1. Prichard, E.; Mackay, G.; Points, J., *Trace analysis: a structured approach to obtaining reliable results*. published for the Laboratory of the Government Chemist by the Royal Society of Chemistry: 1996.

2. LeMay, J., Evidence Beneath Your Feet. *Law Enforcement Technology* 2006, *33* (3), 42-45.

3. Lee, H.; Gaensslen, R., *Advances in fingerprint technology*. CRC Press: 2001.

4. Archer, N. E.; Charles, Y.; Elliott, J. A.; Jickells, S., Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Science International* 2005, *154* (2-3), 224-239.

5. Croxton, R. S.; Baron, M. G.; Butler, D.; Kent, T.; Sears, V. G., Development of a GC-MS Method for the Simultaneous Analysis of Latent Fingerprint Components*. *Journal of Forensic Sciences* 2006, *51* (6), 1329-1333.

6. Hartzell-Baguley, B.; Hipp, R. E.; Morgan, N. R.; Morgan, S. L., Chemical Composition of Latent Fingerprints by Gas Chromatographyâ€“Mass Spectrometry. An Experiment for an Instrumental Analysis Course. *Journal of Chemical Education* 2007, *84* (4), 689-null.

7. Morris, M. D., *Microscopic and Spectroscopic Imaging of the Chemical State*. Marcel Dekker, INC.: New York, 1993; Vol. 16, p 1-21.

8. Ashleigh Grant, T. J. W., Derek R. Holman, and Michael C. Martin, Identification of Recently Handled Materials by Analysis of Latent Human Fingerprints Using Infrared Spectromicroscopy. *Applied Spectroscopy* 2005, *59* (9), 1182-1187.

9. Ali, E.; Edwards, H.; Hargreaves, M.; Scowen, I., Raman spectroscopic investigation of cocaine hydrochloride on human nail in a forensic context. *Analytical and Bioanalytical Chemistry* 2008, *390* (4), 1159-1166.

10. Ryder, A. G.; O'Connor, G. M.; Glynn, T. J., Quantitative analysis of cocaine in solid mixtures using Raman spectroscopy and chemometric methods. *Journal of Raman Spectroscopy* 2000, *31* (3), 221-227.

11. Ryder, A. G., Surface enhanced Raman scattering for narcotic detection and applications to chemical biology. *Current Opinion in Chemical Biology* 2005, *9* (5), 489-493.

12. Ledbetter, N. L.; Walton, B. L.; Davila, P.; Hoffmann, W. D.; Ernest, R. N.; IV, G. F. V., Nanomanipulation-Coupled Nanospray Mass Spectrometry Applied to the Extraction and Analysis of Trace Analytes Found on Fibers*. *Journal of Forensic Sciences 9999* (9999).

13. Brown, J. M.; Hoffmann, W. D.; Alvey, C. M.; Wood, A. R.; Verbeck, G. F.; Petros, R. A., One-bead, one-compound peptide library sequencing via high-pressure ammonia cleavage coupled to nanomanipulation/nanoelectrospray ionization mass spectrometry. *Analytical Biochemistry 398* (1), 7-

CHAPTER 4

CONCLUSIONS AND FUTURE WORK

Throughout this thesis I have shown and discussed the results of nanomanipulation-coupled to nanospray ionization-mass spectrometry utilized with other trace analytical techniques. The results show both increased selectivity and sensitivity of analytes among various matrices. Further work should be done to analyze more types of matrices off of various surfaces. I have also shown and discussed how the instrumental method in regards to analyte extraction and analysis is very direct with immediate results.

The gathering techniques implemented with nanomanipulation-nanospray ionization-mass spectrometry demonstrate the versatility of applications toward trace and ultra-trace work with this instrumental method. Enhanced sensitivity and selectivity of analytes was exhibited from spectra showing detection from single crystal extraction as being sufficient. The directness and efficiency of the hyphenated techniques ultimately demonstrated good quality results. The methodologies employed with nanomanipulation-nanospray ionization-mass spectrometry appear to have much potential in various types of trace and ultra-trace work. This instrumental method provides proof of its ability to improve or replace conventional methods of trace and ultra-trace analysis. Future work will be applied in detecting ultra-trace quantities of pre-blast and post-blast explosive residues along with other types of trace analysis in the field of forensic science.