Urinalysis Screening of Drugs in Adulterated Samples via Direct Analysis in Real Time -- High Resolution/ Mass Spectrometry (DART-HR/MS)

2019

Bianca E. Olivieri

University of Central Florida

Find similar works at: https://stars.library.ucf.edu/honorstheses

University of Central Florida Libraries http://library.ucf.edu

Part of the Biochemistry Commons, and the Chemistry Commons

Recommended Citation

Olivieri, Bianca E., "Urinalysis Screening of Drugs in Adulterated Samples via Direct Analysis in Real Time -- High Resolution/ Mass Spectrometry (DART-HR/MS)" (2019). Honors Undergraduate Theses. 538.
https://stars.library.ucf.edu/honorstheses/538

This Open Access is brought to you for free and open access by the UCF Theses and Dissertations at STARS. It has been accepted for inclusion in Honors Undergraduate Theses by an authorized administrator of STARS. For more information, please contact lee.dotson@ucf.edu.
URINALYSIS SCREENING OF DRUGS IN ADULTERATED SAMPLES VIA DIRECT ANALYSIS IN REAL TIME – HIGH RESOLUTION/MASS SPECTROMETRY

by

BIANCA E. OLIVIERI

B.S. University of Central Florida, 2019

A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program in the Department of Chemistry in the College of Sciences at the University of Central Florida
Orlando, Florida

Spring Term
2019

Major Professor: Candice Bridge
ABSTRACT

Current screening methods for drug analysis with urine samples include examination of the sample with an immunoassay. These methods are used to determine the concentration of drug metabolites contained within the sample prior to further confirmatory testing. Drug testing plays a crucial role in maintaining safe workplace environments and safety of individuals. However, a positive result can lead to heavy consequences for the employee including suspension or removal from the workplace. Therefore, a majority of individuals add commonly known products into the sample to evade detection by developing a false negative result. Although specimen integrity examinations are performed to identify tampering of the sample, these results are typically biased on the experience of the examiner. The purpose of this study was to develop an analytical screening technique that will detect the drug of interest as well as the presence of any additional products that may be added into the sample via Direct Analysis in Real Time – High Resolution/Mass Spectrometry (DART-HR/MS) which is an ambient ionization source that produces fast mass spectrum results that can provide semi-quantitative information of the target metabolite concentration. Although there are various studies that indicate the ability of the DART to detect drug compounds, there are no known studies that have examined how real-world urine samples are analyzed. Additionally, there are no current studies that take into consideration adulteration of the urine sample using the DART method. The results obtained in the study showed the ability
for DART to identify molecular protonated peaks indicative of dextroamphetamine and/or the presence of masking agents. While the other target drugs could not be identified using this method, the identification of dextroamphetamine, adulterant products and the deuterated internal standard show promise in using this as a screening technique prior to confirmatory tests. Future work is currently being conducted to optimize the protocol for the evaluation of THC, cocaine and benzodiazepines.
ACKNOWLEDGEMENTS

I would like to thank my amazing mentors who have entrusted me in this project, Dr. Bridge and Dr. Mari. Thank you for not only believing in me to become a member of your research group, but also for allowing me to grow into my own person and giving me the confidence to pursue my dreams. I am also thankful for the support of my laboratory peers. Thank you to my HIM thesis committee for taking the time to complete this journey with me. And lastly, thank you to my family and friends who have always supported my endeavors and lifted me up when I felt overwhelmed or needed words of encouragement.
DEDICATION

This project is dedicated to my biggest supporter. –Love, your Mariposa.
# TABLE OF CONTENTS

**CHAPTER 1: INTRODUCTION**

1

**CHAPTER 2: BACKGROUND**

3

**CHAPTER 3: INSTRUMENTATION**

6

**CHAPTER 4: STATISTICAL ANALYSIS**

9

**CHAPTER 5: DESIGN OF STUDY**

11

**CHAPTER 6: ELISA AND TEST STRIPS RESULTS**

19

**CHAPTER 7: DART RESULTS**

23

**CHAPTER 8: CONCLUSION**

42

**REFERENCES**

44
LIST OF FIGURES

Figure 1. Mechanism of Direct Analysis in Real Time (DART)\textsuperscript{18} .................................................. 8
Figure 2. DART- HR/MS SPE-it Fiber Blank .......................................................... 23
Figure 3. DART-HR/MS THC Sample Positive and Negative Mode Comparison ..........24
Figure 4. DART- HR/MS THC Sample pH Comparison .............................................25
Figure 5. DART-HR/MS Cocaine Sample Negative and Positive Mode Comparisons .. 26
Figure 6. Structure of Ecstasy Provided by NIST ....................................................27
Figure 7. DART- HR/MS Amphetamine Sample pH Comparison............................28
Figure 8. DART- HR/MS Adulterated Amphetamine Sample – Bleach ......................30
Figure 9. DART- HR/MS Adulterated Amphetamine Sample – Vinegar .................33
Figure 10. DART- HR/MS Adulterated Amphetamine Sample – Sodium Nitrite .......35
Figure 11. DART-HR/MS Adulterated Amphetamine Sample – Eye drops ...............37
Figure 12. DART-HR/MS SPE-it Fiber and Eye drop Solution Results .....................37
Figure 13. DART - HR/MS Adulterated Amphetamine Sample – Drano\textsuperscript{®} ..........39
Figure 14. DART-HR/MS Benzodiazepine Sample #2 Positive and Negative Mode Comparison ........................................................................................................... 41
LIST OF TABLES

Table 1. Drugs of Interest and Important Characteristics .............................................. 12
Table 2. Participant Samples and Important Characteristics ........................................ 13
Table 3. List of adulterants and their active ingredients ............................................... 15
Table 4. Statistical Values for the Concentration of Dextroamphetamine versus Adulterant Concentration; Bleach (B), Vinegar (V), Sodium Nitrite (N), Eye drops (E), Drano® (D), Average (Avg.), Standard Deviation (Std. Dev.) .......................................................... 40
LIST OF EQUATIONS

Equation 1. Confidence Interval for Samples .......................................................... 9
Equation 2. Upper Confidence Level ......................................................................... 9
Equation 3. Lower Confidence Level ......................................................................... 9
LIST OF ABBREVIATIONS

• THC........................................... Tetrahydrocannabinol
• DART – HRMS.............................. Direct analysis in real time – high resolution mass spectrometry
• ELISA....................................... Enzyme linked immunosorbent assay
• PCC......................................... Pyridinium Chlorochromate
• AC6 ......................................... AdultaCheck® 6
• In7........................................... Intect™ 7
• [M+H]+.................................... Protonated molecular peak
• SPE-it Fibers.............................. Solid-phase microextraction tips fibers
• CI............................................. Confidence Intervals
CHAPTER 1: INTRODUCTION

The formation of the Mandatory Guidelines for Federal Workplace Drug Testing in 1988 has increased the number of individuals required to complete a drug test to ensure a safe workplace environment. However, this does not limit drug testing to only places of employment, but also extends to court proceedings, medical environments, rehabilitation and athletic programs. The drug testing process involves a preliminary screening of a urine sample through an immunoassay technique, where if the result is positive the sample is evaluated further by a confirmatory technique such as gas chromatography – mass spectrometry. Due to the heavy consequences that may arise as a result of a positive response, individuals will often add common household products into the urine matrix in an attempt to manipulate the screening technique to produce a false-negative result thereby avoiding confirmatory testing. A variety of these products include bleach, Drano®, vinegar etc. To combat this increase of adulterant use, analysts often evaluate the samples using specimen integrity tests those most commonly used being adulterant test strips. Previous research conducted by this group has exemplified that the current screening techniques produce false-negative results in respect to certain adulterants and their concentration levels in the urine sample. Additionally, current evaluation of samples by specimen integrity tests are highly subjective to analyst interpretation.

With the advancement of the forensic science community, a new approach is necessary to eliminate the producibility of false-negative results and limit the subjectivity in current screening techniques. Ambient mass spectrometry has shown promise in the
field of forensics and in this study, it is proposed that the use of direct analysis real time – high resolution mass spectrometry (DART-HRMS) can be used as a screening method for urinalysis. The specific objectives of this study are:

- The development of a screening technique using DART to identify drug/metabolite peaks as well as additional peaks indicating adulteration.
- Comparative analysis of unadulterated and adulterated urine samples containing drugs/metabolites of interest on DART and an immunoassay, examining the concentration of drug metabolites in response to an increase of adulteration.
- Quantification of drug concentrations in real world samples via the DART method.
CHAPTER 2: BACKGROUND

Individuals who may abuse drugs are at risk for drug dependence, erratic behavior, psychiatric disorders, and heart/circulatory problems. Therefore, various agencies have proposed drug testing guidelines to prevent the misuse of these substances. Drugs that are included in common testing protocols include: tetrahydrocannabinol (THC), cocaine, amphetamines, and benzodiazepines. While there are a variety of sampling mediums, using urine is not as invasive as other methods, while maintaining the drug metabolites in the sample for an extended period of time. The detection range for amphetamine and cocaine is 2 to 4 days, and chronic use of THC and benzodiazepines may persist in the sample for up to a month.

However, with current drug screening methods, the urine sample can be manipulated with the addition of products \textit{(in vitro)} producing a false-negative result. Immunoassay techniques that have been reviewed for manipulation of the urine sample with adulterant products include radioimmunoassay (RIA), enzyme multiplied immunoassay technique (EMIT), fluorescence polarization immunoassay (FPIA), and enzyme-linked immunosorbent assay (ELISA). In these studies, certain products demonstrated abnormal results. At a 10 % v/v concentration eye drops, liquid bleach, and vinegar interfered with the detection of THC metabolites using the FPIA method. Additionally, the use of eye drops as an adulterant resulted in false-negative results for THC assays using EMIT.

Due to the increased use of adulterants, examiners will use a form of a specimen integrity test to ensure that there is no manipulation of the urine sample potentially causing
a false negative or positive result. While specimen integrity test often includes evaluation of the sample visually, by pH, and/or temperature, a majority of adulterants do not produce these effects. Adulterant test strips use reagent pads that interact with chemical substances, nitrite, creatinine, glutaraldehyde, pyridinium chlorochromate (PCC), specific gravity and pH, to develop a color change indicating in vitro addition. Additionally, another benefit is adulterant test strips can be easily purchased in many drug stores or through online vendors. Adulterant test strips that have been analyzed in previous research include AdultaCheck® 6 (AC6) and Intect™ 7 (In7). In a study conducted by Dasgupta et al., AC6 and In7 were compared for their ability to detect nitrite, PCC, and bleach with responses compared to potassium iodide spot tests. The results revealed that the test strips were able to detect the adulterants and at times were able to differentiate concentration levels, with In7 being superior to AC6 in sensitivity. Another study evaluated adulterant detection between In7, AdultaCheck® 4 and Mask Ultra Screen by adding adulterants to spiked drug samples. In conclusion, In7 proved to be the most effective of the three in detecting bleach, vinegar, PCC and nitrite.

In a previous study conducted by the Bridge research group, the use of ELISA and adulterant test strips In7 and AC6 exemplified that certain adulterants were able to evade detection. These results will be further discussed in chapter 6, however what these outcomes concluded was the need for a new, more robust screening technique with the prevention of analyst subjectivity.

The introduction of DART-HRMS as a screening technique for urinalysis has shown promise due to the ability of the instrument to provide information regarding the
components within the sample as a mass spectrum. Additionally, DART is capable of analyzing and providing high mass accuracy of compounds within a few seconds with minimal sample preparation. In previous literature, the DART has exhibited the ability to detect drugs of interest, drug metabolites, and chemical substances.\textsuperscript{18, 19, 20} Prior studies focused on evaluation of urinalysis by spiking either synthetic or clean urine samples with the drugs standards of interest, whereas in this study the focus is the evaluation of real-world urine samples containing metabolized drugs of abuse.
CHAPTER 3: INSTRUMENTATION

Current screening techniques for drug analysis in urine samples often consists of an immunoassay technique. The one specifically used in this protocol was enzyme linked immunosorbent assay (ELISA). Competitive ELISA is a heterogeneous immunoassay technique that focuses on use of enzyme-linked antigens to compete with the antigen of the sample for antibody sites whereas other techniques use enzyme-linked antibodies.\textsuperscript{10, 21} The 96 well plate contains antibody with a specific binding site for the target analyte and the enzyme conjugate. The sample is then added into the well plate along with quality controls and standards. The standards will provide an informative standard curve to exemplify if pipetting was accurate and precise by the R\textsuperscript{2} value. After the addition of the urine sample, an enzyme labeled conjugate is added into the wells and the conjugate competes with the antigen for the binding site during the first incubation period. Following the first incubation, a washing procedure is performed using the Biotek\textregistered ELISA washer to remove any unbound components. A substrate is then added into each well which creates a color reaction during the second incubation period. The optical density of the color reaction can then be read using a Biotek\textregistered plate reader at absorbances of 450 and 630 nm. The ELISA protocol used for this study was provided by Randox ELISA well plate manufacturers.

Due to the number of individuals who manipulate the urine samples with adulterants to alter the immunoassays capability to detect the target analyte(s), analysts use specimen integrity tests. These tests detect when the sample has additional products by a variety of methods such as smell, appearance, and chemical reactions. Of these a
common method is using adulterant test strips which have reaction pads that interact with the sample creating a color change that exhibits particular chemicals that are typically not seen in a urine sample are present.

Direct analysis in real time – high resolution mass spectrometry is an ambient ionization technique used to analyze samples at high mass accuracy within a few seconds and limiting the amount of required sample preparation.\textsuperscript{22} The mechanism of the DART (Figure 1) begins with a helium gas source that enters the instrument and interacts with the needle electrode forming a glow discharge producing cations, anions, electrons and helium metastables which are neutral electronically excited atoms. These species then flow through the grounded electrode which separates the charged species from the metastables allowing only the metastables to enter the second chamber. In the second chamber there is a gas heater, heating the metastables to the desired temperature required for analyzing the sample. The heated metastables then flow through the exit grid electrode which can be biased towards a negative or position ionization mode depending on the requirements for evaluation. In a positive ionization mode, a water cluster protonates the sample to form a protonated sample whereas in negative ionization mode, the water cluster removes a proton from the sample giving a deprotonated molecular peak.\textsuperscript{22} As the metastables exit the apparatus, they interact with the sample located in the sample gap to ionize and desorb the sample for introduction of ionized atoms into the mass spectrometer inlet. The sample is then broken down into components and a mass spectrum indicating the protonated molecular peaks ([M+H]\textsuperscript{+}) for individual compound analysis.
Prior literature acknowledged that direct sampling of urine samples on DART leads to the reduction in the detection of drug metabolites within the sample due to the high concentration of creatinine and urea. For this particular method, solid-phase microextraction tips fibers were necessary in decreasing the urine matrix components such as creatinine that could inhibit the detection of the drug metabolite in question. Ionsense® SPE-it fibers extraction technique isolates the analyte of interest and allows direct analyzation on the DART system. Another feature of the SPE-it fiber is the ability to use it in conjunction with a linear rail system. The use of a linear rail system allows for reproducible results and minimization of error seen in manual introduction of the sample into the sample gap.

Figure 1. Mechanism of Direct Analysis in Real Time (DART)
CHAPTER 4: STATISTICAL ANALYSIS

An objective of this study was to evaluate how the increase of adulterant concentration affects the detectable concentration of the target drug metabolite. Specifically for this study, confidence intervals calculated by Equation 1 were conducted to provide information for the most probable value for the sample mean ($\mu$). The critical value $t$ was obtained for a 95% confidence level with a degree of freedom of 1 which provided a $t$-value of 12.71. This large critical value was obtained due to the small sample set of each adulterated urine sample being run in duplicates, however this was the statistical test allowing for the comparison within a data sample set. To obtain a range of the confidence intervals (CI) the upper confidence level and lower confidence level were obtained by using Equations 2 and 3.

*Equation 1. Confidence Interval for Samples*

$$CI = \frac{t - \mu}{\sqrt{n}}$$

*Equation 2. Upper Confidence Level*

$$Upper\ confidence\ level = Average + CI$$

*Equation 3. Lower Confidence Level*

$$Lower\ confidence\ level = Average - CI$$

Measurements were performed for all adulteration levels and the confidence intervals for each adulterant concentration were compared to the unadulterated sample.
If there was intertwining values between the adulterant level and unadulterated sample, the difference in the antigen concentration was not considered statistically significant.
CHAPTER 5: DESIGN OF STUDY

As previously stated the novelty of this project is the use of real-world urine samples to evaluate the detection of illicit drugs metabolized through the body. Therefore, samples were collected by anonymous volunteers under UCF IRB no. SBE-16-12568. In addition to providing the samples, volunteers filled out a questionnaire detailing information pertaining to the drugs and/or prescriptions taken, the history of use, chronic or recreational use, the amount consumed, and the time of recent drug use. Other information that was obtained from the survey included the age, gender and additional consumption of caffeine products within the week. Samples were stored in a biological hazard freezer until needed for evaluation.

The drugs of interest and their main metabolites for this study included: tetrahydrocannabinol (11-nor-9-carboxy-Δ9-THC), cocaine (Benzoylcgonine), amphetamines (Dextroamphetamine), and benzodiazepines (Diazepam). However, for this particular paper the main discussion of results will focus on the evaluation of amphetamine real world samples. To evaluate the DART experimental method and semi-quantify the drugs in the real-world samples, internal standards and drug standards were purchased (Cerilliant Corporation, Round Rock, TX). The internal standards of interest included (-)-11-nor-9-Carboxy-Δ9-THC (T-018), (-)-11-nor-9-Carboxy-Δ9-THC-D3 (T-004), (-)-Δ9-THC (T-005), (-)-Δ9-THC-D3 (T-003), Cocaine (C-008), Cocaine-D3 (C-004), Benzoylcgonine (B-004), Benzoylcgonine-D3 (B-001), (±) – Amphetamine (A-007), (±) – Amphetamine-D5 (A-002), Diazepam (D-907), Diazepam-D5 (D-902), Nordiazepam (N-
905), and Nordiazepam-D5 (N-903). All information pertinent to the drugs of interest in this study are found in Table 1.

Table 1. Drugs of Interest and Important Characteristics

<table>
<thead>
<tr>
<th>Drug of Interest</th>
<th>Major Metabolite</th>
<th>Internal Standard(s) for DART</th>
<th>Cut-off Level&lt;sup&gt;25&lt;/sup&gt; on DART (g/mol)</th>
<th>Target [M+H]&lt;sup&gt;+&lt;/sup&gt; on DART (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>11-nor-9-carboxy-Δ9-THC</td>
<td>(-)-11-nor-9-Carboxy-Δ9-THC-D3</td>
<td>50 ng/ml</td>
<td>345.4446 and/or 315.4617</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Δ9-THC-D3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>Benzoylecgonine</td>
<td>Benzoylecgonine-D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>300 ng/ml</td>
<td>304.3529 and/or 290.3264</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>Dextroamphetamine</td>
<td>(±) – Amphetamine-D&lt;sub&gt;5&lt;/sub&gt;</td>
<td>1000 ng/ml</td>
<td>136.2062</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Diazepam</td>
<td>Diazepam-D&lt;sub&gt;5&lt;/sub&gt;</td>
<td>200 ng/ml</td>
<td>145.1732 and/or 285.7402</td>
</tr>
</tbody>
</table>

For the examination of real-world samples on ELISA, kits were purchased from Randox laboratories (Kearneysville, WV, USA) for each of the drugs of interest. Samples that identified having a high potential for the detection of the target drugs based on their concentration were selected and examined using the ELISA protocol provided by the manufacturer. Samples that exhibited the target compound above the concentration cut off limit for the immunoassay were chosen for downstream analysis. Sample collection
for the DART consisted of identifying samples in which the volunteers indicated drug use of a target drug in the study. Samples were evaluated using the SPE-it fiber protocol provided by Ionsense (Saugus, MA, USA) and optimized parameters. Samples used for both portions of the study are found in Table 2.

Table 2. Participant Samples and Important Characteristics

<table>
<thead>
<tr>
<th>Participant Number</th>
<th>Drug(s) Disclosed</th>
<th>Amount of Drug Ingested</th>
<th>Drug Use Prior to Sample Collection</th>
<th>Target Drug Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Marijuana</td>
<td>Chronic User</td>
<td>1 hour</td>
<td>THC</td>
</tr>
<tr>
<td>23</td>
<td>Marijuana, Cocaine</td>
<td>Chronic User</td>
<td>2 hours, 2 days</td>
<td>Cocaine</td>
</tr>
<tr>
<td>20</td>
<td>Marijuana, Cocaine, Adderall</td>
<td>Recreational</td>
<td>Not provided, 20 mg</td>
<td>Amphetamines</td>
</tr>
<tr>
<td>11</td>
<td>Marijuana, Wellbutrin, Topamax, Abilify&lt;sup&gt;®&lt;/sup&gt;, Ativan (Lorazepam)</td>
<td>Chronic User, 300 mg, 75 mg, Not provided, 1 mg</td>
<td>~14 hours</td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>DART-HR/MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Marijuana</td>
<td>Chronic User</td>
<td>1 hour</td>
<td>THC</td>
</tr>
<tr>
<td>98</td>
<td>Marijuana</td>
<td>Recreational</td>
<td>1 day</td>
<td>THC</td>
</tr>
<tr>
<td>99</td>
<td>Marijuana</td>
<td>Chronic User</td>
<td>~12 hours</td>
<td>THC</td>
</tr>
<tr>
<td></td>
<td>Substance</td>
<td>Type</td>
<td>Dosage</td>
<td>Duration</td>
</tr>
<tr>
<td>---</td>
<td>-----------</td>
<td>------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>100</td>
<td>Marijuana</td>
<td>Recreational</td>
<td>N/A</td>
<td>THC</td>
</tr>
<tr>
<td>95</td>
<td>Marijuana, Molly, Ecstasy, Cocaine</td>
<td>Recreational</td>
<td>N/A</td>
<td>Cocaine</td>
</tr>
<tr>
<td>31</td>
<td>Cocaine</td>
<td>“1 Bump”</td>
<td>2 days</td>
<td>Cocaine</td>
</tr>
<tr>
<td>32</td>
<td>Marijuana, Adderall, Cocaine</td>
<td>Recreational</td>
<td>20 mg</td>
<td>Cocaine</td>
</tr>
<tr>
<td>25</td>
<td>Marijuana, Adderall, Cocaine</td>
<td>Chronic</td>
<td>10 mg</td>
<td>Cocaine</td>
</tr>
<tr>
<td>20</td>
<td>Marijuana, Adderall, Cocaine</td>
<td>Recreational</td>
<td>20 mg</td>
<td>Cocaine</td>
</tr>
<tr>
<td>14</td>
<td>MDMA, Cocaine</td>
<td>“2 beans (Tesla)”</td>
<td>1 week</td>
<td>Cocaine</td>
</tr>
<tr>
<td>93</td>
<td>Marijuana, Molly, Cocaine, Ecstasy, Adderall</td>
<td>Recreational</td>
<td>N/A</td>
<td>Amphetamines</td>
</tr>
<tr>
<td>2</td>
<td>Marijuana, Wellbutrin, Topamax, Abilify®, Ativan (Lorazepam)</td>
<td>Chronic User</td>
<td>300 mg, 75 mg</td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>11</td>
<td>Marijuana, Wellbutrin, Topamax, Abilify®, Ativan (Lorazepam)</td>
<td>Chronic User</td>
<td>300 mg, 75 mg</td>
<td>Benzodiazepines</td>
</tr>
</tbody>
</table>
In previous literature, studies have identified common household products that are used to adulterate the urine samples screened for drugs of abuse. Adulterants that have been recognized for manipulation of immunoassay techniques and are used in this study include: bleach\(^4, 23\), Drano\(^4, 26\), vinegar\(^4, 17, 27\), Naphcon-A eye drops\(^4, 14, 23, 26\), table salt\(^28, 29\), and sodium nitrite\(^17, 30\) which is an active ingredient in the adulterant whizzies. The active ingredients of the adulterants in this study can be found in Table 3.

*Table 3. List of adulterants and their active ingredients*

<table>
<thead>
<tr>
<th>Adulterant/ Product</th>
<th>Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clorox(^\circledR) bleach</td>
<td>Sodium hypochlorite</td>
</tr>
<tr>
<td>Liquid Drano(^\circledR)</td>
<td>Sodium hypochlorite (5%)</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>White vinegar</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>Naphcon A eye drops</td>
<td>Naphazoline hydrochloride (0.025%)</td>
</tr>
<tr>
<td></td>
<td>Pheniramine maleate (0.3%)</td>
</tr>
<tr>
<td></td>
<td>Benzalkonium chloride (0.01%)</td>
</tr>
<tr>
<td>Table Salt</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Sodium nitrite (Whizzies)</td>
<td>–</td>
</tr>
</tbody>
</table>
To prepare the selected samples for adulterant analysis, the sample were obtained from the freezer and thawed at room temperature. For ELISA analysis, 1 mL aliquots were collected for each drug and each adulterant concentration of that particular drug target. A 200 µL sample was maintained to ensure the original concentration of the sample and all other samples adulterated were kept at a final volume of 200 µL. The adulterant concentrations ranged from 5, 10, 25, and 50 % v/v or w/v. Adulterant sample for 5 % v/v contained 190 µL of urine and 10 µL of adulterant to achieve the 200 µL volume. The remaining sample contained respective ratios of urine to adulterant product (10 % v/v – 180:20, 25 % v/v – 150:50, and 50 % v/v – 100:100). Certain adulterant products, Drano®, table salt and nitrite, were viscous or solid and were instead added into the sample using weight-by-volume calculations.

Unadulterated samples and samples of concentrations 5 and 25% v/v or w/v were evaluated on the test strips, Intect 7 (In7) and AdultaCheck 6 (AC6), by using a glass pipette to place 1 drop on each reagent pad. The pads were then examined for color change by comparison of the pad to the provided color chart at the specified time. Additional examinations were performed for eye drops at 50 % v/v due to their ability to decrease the detectable metabolite concentration while maintaining a normal color appearance at 25 % v/v on the reagent pads.

Regarding DART analysis, the adulterants that previously exemplified a decrease of detectable metabolite concentration on ELISA were evaluated on DART. To prepare the DART samples, the total volume for SPE-it fiber protocols was 1 mL. Therefore, aliquots of the urine samples were obtained and adulterated using volume-by-volume
calculations to achieve the 5 to 50 % v/v or w/v adulterant concentration range. An unadulterated sample was maintained to determine if the target metabolite or derivatives could be seen in the mass spectrum and for a comparison of adulterant effects as the adulterant concentration increased.

Prior to evaluation of the samples on DART, optimization studies were conducted for the DART protocol parameters including temperature, evaluation of the exit grid voltage, and adjustment of the linear rail distance and speed. To determine the optimal temperature for the detection of drug metabolites, real world samples containing their respective internal standards were analyzed from 250 to 400 °C at increments of 50. The ideal temperature was identified at 400 °C due to the amount of molecular peaks for identification of the sample components in the mass spectrum. Additionally, this temperature provided the most intense signal in terms of total ion count. While there is not a current clear understanding of the purpose of the exit grid voltage, it is proposed that adjustment of the exit grid voltage will highlight different peaks for the sample of interest. The exit grid voltage was examined at 50, 150, 250, 350, 450 and 530 volts using real world samples with internal standard(s). The exit grid voltage parameter of 250V provided the greatest amount of protonated molecular peaks ([M+H]+) which is essential for this study due to the need to identify not only the main drug metabolite, but also the presence of any potential adulterants of fragmentation of the drug metabolite or urine components (i.e. creatinine, urea). While the main purpose of the SPE-it fiber is to isolate the analytes of interest from the urine components, another purpose is to allow for reproducibility by using a linear rail system manufactured holding the SPE-it fiber railing.
The linear rail distance and speed was analyzed to determine at what parameters provided the best ionization of the target metabolite. The distance was evaluated from 1.5 to 2 cm distance from the MS inlet orifice to the ionization gap. Any further measurements will prove inadequate for this study due to the need of the sample to interact with the metastables and enter the inlet source while still maintaining space for the linear rail in the gap. Additionally, the speed of the linear rail through the ionization gap was examined between 0.2 and 0.5 mm/s. Any higher speed would minimize interactions of the sample to the metastable source or flow of ionized particles into the inlet. The parameters providing the best results for the linear rail was inlet distance of 1.5 cm and linear rail speed of 0.5 mm/s.
CHAPTER 6: ELISA and TEST STRIPS RESULTS

Immunoassays and specimen integrity tests are the current methods for urine screening for the detection of drugs and their metabolites. A previous study highlighted the effects of adulterant concentrations on ELISA, In7 and AC6. These results are discussed in this chapter to provide comparative analysis of current screening techniques to those developed in this study.

THC Results

The cut-off level indicated for the screening of THC is 50 ng/ml and examining the urine sample it had an original concentration of 94.0 ng/ml. Adulterants were added at various concentrations to determine if these adulterants would decrease the detectable concentration below the cut-off limit and at which adulterant concentration level would the adulterant be identified by the adulterant test strips. Bleach and Drano® both decreased the concentration to below 50 ng/ml at only a 5 % level, with bleach causing a decrease to 2.7 ng/ml. Evaluation by a student t-test revealed that these values were both considered significant in comparison to the original concentration. In consideration of this false-negative result, there was no detection of bleach or Drano® using AC6 at 5 %. Both adulterants were detected on AC6 at the higher 25 % concentration. However, using In7, bleach was detected at 5 % v/v on the PCC pad which is a reagent pad not available on AC6. Nitrite also decreased the detectable concentration of the metabolite at 5 % but did not have significant effect according to the student t-test until a 10 % w/v concentration level. Unlike bleach and Drano®, nitrite was detected on both adulterant test strips at 5%. Lastly, eye drops and vinegar decreased the concentration at 50 % potentially due to
dilution of the sample decreasing the amount of detectable antigen. Vinegar could be identified on both adulterant test strips at 25 %, but eye drops evaded detection at concentration of 5, 25 and 50 %. All other adulterants did not show an effect on the assay response.

**Cocaine Results**

Using the ELISA cocaine assay, the initial concentration of the urine sample containing cocaine metabolites was identified at 534.0 ng/ml. Similar to the results obtained on the THC assay, bleach and Drano® at 5 % level decreased the concentration of detected benzoylecgonine to below the 300 ng/ml cut-off level. However, according to the student t-tests, bleach did not have significance until 10 % v/v. Vinegar was able decrease the initial concentration to below 300 ng/ml at 10 % v/v by a 44% change. However, similar to Drano® there was no significance after performing a student t-test. For these three adulterants, only Drano® was identified at 5 % using AC6 on the oxidant pad. Whereas bleach and vinegar exemplified color responses at 25 % concentration. Drano® and vinegar created a color change at 5 % on In7, and again bleach only showed a color change at 25 %. On the ELISA assay, eye drops dropped the detected concentration of target metabolite below the cut-off at 50 % v/v. In contrast to the results of THC, using In7 the specific gravity pad exhibited a slight color change at 25 and 50 % v/v. Other adulterants were unable to decrease the detected benzoylecgonine below 300 ng/ml.
Amphetamine Results

The initial concentration of dextroamphetamine was above the upper threshold limit and therefore, it was only determined that the amount was above 1575 ng/ml. The only adulterant that decreased the concentration to below the cut-off level of 1000 ng/ml was Drano® causing a decrease by 97.7 %. Bleach and sodium nitrite both decreased the concentration at the 10 % level while vinegar and eye drops decreased at 25 % v/v. When evaluating these adulterants on AC6 bleach, Drano®, and nitrite were all detected at 5 % and vinegar displayed color change at 25 %. In7 showed a similar response, however at 5 % for bleach and Drano® there was only a slight color change in the PCC pad which can be considered subjective to analyst interpretation. Again, eye drops exhibited presence at 25% on the specific gravity pad of In7. This contributes to the theory that eye drops may decrease the metabolite concentration of the ELISA assay through a form of dilution of the sample.

Benzodiazepine Results

The sample identified of benzodiazepines use was examined for initial concentration with the ELISA benzo assay and was identified at 96.1 ng/ml, well below the cut-off threshold of 200 ng/ml. Therefore, adulterant concentrations were evaluated using student t-test for the difference of the metabolite concentration at the particular adulterant concentration compared to metabolite concentration at the unadulterated level. At 5% adulterant concentration, bleach and Drano® slightly decreased the initial concentration by 4.2 and 0.8 % respectively. At 50% both adulterants decreased the concentration by 28.6 and 13.4 % whereas, vinegar showed the greatest decrease in
concentration of all adulterants at 50 % with a 49.8 % decrease. However, the student t-test did not identify vinegar at 50 % to be significant. When evaluating the original sample on the adulterant test strips, there was indication of high specific gravity on In7 and therefore, specific gravity was not evaluated for the remaining analysis. At a 5 % level, AC6 was only able to identify nitrite, but at 25 % was able to detect color change from bleach, Drano® and vinegar. When using In7, Drano® was detected at the 25 % level. Neither adulterant test strip could identify eye drops at the 5, 25 or 50 % concentrations.
CHAPTER 7: DART RESULTS

As shown, current screening techniques for drugs of abuse (immunoassay and specimen integrity tests) can be manipulated to produce a negative response when common adulterants are added \textit{in vitro} to the urine matrix. Therefore, it was proposed that using an ionization technique called direct analysis in real time – high resolution mass spectrometry (DART-HRMS) would provide a higher quality screening technique to identify not only the drug/metabolite of interest, but additionally any adulterants present.

SPE-it Fiber Results

Prior to performing analysis on the urine samples on DART, a SPE-it fiber was analyzed using deionized water instead of urine. This was done in efforts to determine what protonated molecular peaks ([M+H]$^+$) would appear on the mass spectrum from interactions of the fiber with the metastabiles in the ionization gap. As shown in Figure 2, the spectrum exemplified peaks at 83.0600 [M+H]$^+$, 88.1120 [M+H]$^+$, 242.1313 [M+H]$^+$, and 241.1717 [M+H]$^+$.

![DART-HR/MS Results](image)

*Figure 2. DART-HR/MS SPE-it Fiber Blank*
THC Results

Samples containing THC metabolites were identified from the volunteer packets and evaluated with deuterated internal standard to determine if the metabolites and standard could be identified on the mass spectrum. As shown in Figure 1, all samples were evaluated in positive and negative ionization mode, but none of the samples had the characteristic peaks (Table 2). Samples collected specifically for DART examination (#99) did not display protonated molecular peaks at 345.4446 or 315.4617 g/mol. The sample used in the ELISA study was then evaluated in positive and negative ionization mode with no demonstration of the target peaks.

Figure 3. DART-HR/MS THC Sample Positive and Negative Mode Comparison
Upon recommendation from the developer at Ionsense, the pH was then evaluated for a clean sample spiked with THC internal standard at pH of ~10 compared to the pH of ~7. As shown in Figure 4, there are no indications of the target molecular peaks. Further work will be conducted to optimize the protocol for the identification of THC metabolites in real world samples.

![DART-HR/MS Results](image)

Figure 4. DART- HR/MS THC Sample pH Comparison

**Cocaine Results**

Cocaine sample #95 (Table 2) with the Benzoylecgonine-D₃ spiked internal standard was initially analyzed using the SPE-it/ DART protocol in positive ionization mode (Figure 5). However, any molecular peaks indicating cocaine metabolites were not present and instead a peak at 194. 1176 [M+H]+ appeared which after evaluation on Mass
Mountaineer was identified as Ecstasy (Figure 6). The assumption made based on this result was the masking effect of amphetamine metabolites to any present cocaine metabolites. This may be due to the nature of the SPE-it fiber which is PDMS coated to concentrate analytes of interest. However, there are only a few binding portions on the fiber and therefore, the analyte of higher binding affinity will bind to those binding sites before other potential analytes. The sample was then analyzed in negative ionization mode (Figure 5), but similar to positive mode no peaks presented information pertaining to cocaine metabolites. Any samples donated that indicated use of cocaine (Table 2) were then evaluated to determine if the metabolites were present, but none of the samples displayed metabolite or internal standard peaks.

![DART-HR/MS Results](image)

*Figure 5. DART-HR/MS Cocaine Sample Negative and Positive Mode Comparisons*
When analyzing the unadulterated and adulterated amphetamine samples there were a series of results that supported the original hypothesis of the detection of the analyte and presence of adulterants. Upon recommendation by Ionsense operators, the pH of the urine sample (# 93, Table 2) was evaluated at a pH of ~7 and pH of ~10. At pH of ~7, the dextroamphetamine peak at 136.2062 [M+H]$^+$ was not present, but when adjusting the pH to ~10 using 10N NaOH the dextroamphetamine was visible at 136.1136 [M+H]$^+$ (Figure 7). Therefore, all further evaluations for amphetamine sample #93 were tested with pH litmus paper and adjusted to a pH of ~10 prior to continuing the SPE-it protocol.
To detect the concentration of the drug without the manipulation of adulterants, an internal standard (amphetamine – D₅) was spiked into the urine sample to a cut-off level as indicated by the ELISA test kits (1000 ng/ml). An aliquot of the spiked sample was analyzed for identification of the amphetamine metabolite and the internal standard. The mass spectrum exemplified peaks at 136.11 [M+H]+ indicative of dextroamphetamine and 141.14 [M+H]+ of the amphetamine – D₅ internal standard. Upon completing a ratio of the dextroamphetamine peak to the deuterated internal standard and multiplying this by the cut-off factor of 1000 ng/ml, the average concentration was calculated as 11975.19 ng/ml (Table 4).
Evaluating the adulterated amphetamine samples of bleach, as the adulterant concentration increases the relative intensity of the amphetamine peak decreases (Figure 8). The dextroamphetamine peak is visible until reaching a concentration of 50% v/v of bleach. However, after evaluating the abundance values for both duplicate trials the average concentration at 5 and 25% adulterant levels are 7401.471 ng/ml and 6291.235 ng/ml respectively and thereby well above the 1000 ng/ml cut-off limit (Table 4).

Vinegar however, was detected throughout all levels of adulterant concentration (Figure 9). An additional factor when evaluating the vinegar spectrums, was the consistency of the concentration of dextroamphetamine to the internal standard during the increase of adulterant concentration (Table 4).

Similar to the results observed using vinegar as the adulterant, sodium nitrite was detected through all adulterant concentration levels (Figure 10). Additionally, the concentration of dextroamphetamine remained above the 1000 ng/ml cut off level after calculating the average concentration from the internal standard (Table 4).
Figure 8. DART-HR/MS Adulterated Amphetamine Sample – Bleach
Figure 9. DART- HR/MS Adulterated Amphetamine Sample – Vinegar
DART-HR/MS Results

Unadulterated Amphetamine Sample

Amphetamine 5% Sodium Nitrite

Amphetamine 10% Sodium Nitrite

Amphetamine 25% Sodium Nitrite

Amphetamine 50% Sodium Nitrite
The use of eye drops as an adulterant produced unexpected results (Figure 11). The expectation of using DART was the detection of either the drug metabolite or of other characteristic peaks that could deduce that an additional product was added into the matrix to manipulate testing procedures. Instead the eye drops did not exemplify additional peaks, but interacted with the SPE-it fiber increasing the relative intensity of the 241.17 [M+H]^+ peak. To confirm the interaction of eye drops solution with the SPE-it fiber, a run was conducted following the protocol, but instead substituting 1 ml of eye drop solution instead of urine. As predicted, the relative intensity of the 241.1717 [M+H]^+ peak present in the SPE-it fiber blank (Figure 2) increased when using eye drop solution as the sampling medium (Figure 12). The identity of these peaks are currently unknown, however, further research will be conducted to establish the identity.
As previously mentioned, the expectation of this study was the ability to detect peaks that could exemplify adulteration and as shown in the results of Drano® while the Dextroamphetamine peak and internal standard were not present, there are a variety of additional peaks that were not identified in the primary unadulterated sample (Figure 13). For case-work these results indicate that there is manipulation of the sample and the sample would not be appropriate for further evaluation by confirmatory techniques.
DART-HR/MS Results

Unadulterated Amphetamine Sample

Amphetamine 5% Drano®

Amphetamine 10% Drano®

Amphetamine 25% Drano®

Amphetamine 50% Drano®
Figure 13. DART - HR/MS Adulterated Amphetamine Sample – Drano®
Table 4. Statistical Values for the Concentration of Dextroamphetamine versus Adulterant Concentration; Bleach (B), Vinegar (V), Sodium Nitrite (N), Eye drops (E), Drano® (D), Average (Avg.), Standard Deviation (Std. Dev.)

<table>
<thead>
<tr>
<th>Adulterant (%)</th>
<th>Abundance Values for 136.11 [M+H]*</th>
<th>Abundance Values for 141.14 [M+H]*</th>
<th>Ratio Values (ng/ml)</th>
<th>Avg. (ng/ml)</th>
<th>Std. Dev</th>
<th>Upper Limit Confidence Level</th>
<th>Lower Limit Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
<td>Trial 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadulterated</td>
<td>59.4497</td>
<td>100</td>
<td>10.2999</td>
<td>5.501</td>
<td>5771.872</td>
<td>18178.51</td>
<td>11975.19</td>
</tr>
<tr>
<td>B 5%</td>
<td>23.2899</td>
<td>32.4998</td>
<td>2.6</td>
<td>5.56</td>
<td>8957.654</td>
<td>5845.288</td>
<td>7401.471</td>
</tr>
<tr>
<td>B 10%</td>
<td>49.23</td>
<td>37.2397</td>
<td>8.8403</td>
<td>5.3096</td>
<td>5568.816</td>
<td>7013.655</td>
<td>6291.235</td>
</tr>
<tr>
<td>B 25%</td>
<td>2.5</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B 50%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V 5%</td>
<td>73.9286</td>
<td>82.4387</td>
<td>8.6297</td>
<td>8.3106</td>
<td>8566.764</td>
<td>9919.705</td>
<td>9243.234</td>
</tr>
<tr>
<td>V 10%</td>
<td>57.8707</td>
<td>54.9393</td>
<td>6.8506</td>
<td>6.3209</td>
<td>8447.537</td>
<td>8691.689</td>
<td>8569.613</td>
</tr>
<tr>
<td>V 25%</td>
<td>50.6908</td>
<td>54.9603</td>
<td>8.3895</td>
<td>5.9801</td>
<td>6042.172</td>
<td>9190.532</td>
<td>7616.352</td>
</tr>
<tr>
<td>V 50%</td>
<td>79.4184</td>
<td>100</td>
<td>8.2306</td>
<td>15.9803</td>
<td>9649.163</td>
<td>6257.705</td>
<td>7953.434</td>
</tr>
<tr>
<td>N 5%</td>
<td>100</td>
<td>100</td>
<td>3.9</td>
<td>15.8302</td>
<td>25641.03</td>
<td>6317.04</td>
<td>15979.03</td>
</tr>
<tr>
<td>N 10%</td>
<td>100</td>
<td>100</td>
<td>12.4287</td>
<td>8.7225</td>
<td>8045.894</td>
<td>11464.6</td>
<td>9755.248</td>
</tr>
<tr>
<td>N 25%</td>
<td>100</td>
<td>70.1508</td>
<td>5.5402</td>
<td>10.5491</td>
<td>18049.89</td>
<td>6649.932</td>
<td>12349.91</td>
</tr>
<tr>
<td>N 50%</td>
<td>100</td>
<td>100</td>
<td>18.2797</td>
<td>14.8512</td>
<td>5470.549</td>
<td>6733.463</td>
<td>6102.006</td>
</tr>
<tr>
<td>E 5%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E 10%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E 25%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E 50%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D 5%</td>
<td>0</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D 10%</td>
<td>0</td>
<td>65.036</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D 25%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D 50%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Benzodiazepine Results

Samples obtained in the latter portion of the DART study did not dictate the use of benzodiazepines and therefore samples used for the evaluation of ELISA and test strips were reevaluated using the SPE-it/DART method. Sample #2 was evaluated in both positive and negative ionization mode (Figure 14) with the addition of spiked internal standard to the cut-off level of 200 ng/ml (Table 1). In both ionization modes neither target peak, 145.1732 [M+H]^+ or 285.7402 [M+H]^+ were present. Other disclosed drugs for sample 2 were examined for presence on the mass spectrum, but there was no identification. Remaining samples were also evaluated in both positive and negative ionization mode, but none presented peaks indicating the target drug classification or internal standard.

DART-HR/MS Results

Figure 14. DART-HR/MS Benzodiazepine Sample #2 Positive and Negative Mode Comparison
CHAPTER 8: CONCLUSION

Drug screenings are widely used to evaluate individuals who hold employment status or are in other positions such as court proceedings. The current screening techniques used include the use of an immunoassay and specimen integrity test. However, the addition of products in vitro to a sampling medium of urine, can alter the mechanisms of analyte detection producing a false-negative result. Therefore, the purpose of this study was to evaluate adulterant effects on real-world samples with metabolized drugs of abuse on current screening techniques, ELISA and adulterant test strips (AC6 and In7), versus a new method using DART. ELISA has an antigen-antibody binding system that can be manipulated when certain products such as bleach, Drano®, and eye drops. Therefore, the hypothesis of this study was that in the development of a DART method for urinalysis, peaks exhibiting the drug of interest and/or the presence of adulterant products will appear on the mass spectrum. Additionally, the DART method would provide semi-quantitative analysis of concentration values between the unadulterated samples and adulterated samples at their various concentrations.

Comparison of screening techniques

In the ELISA and adulterant test strip method, certain adulterants were able to decrease the detectable metabolite concentrations to below the cut-off levels at low adulterant concentration levels. For example, at 5 % bleach and Drano® produced a false negative result on ELISA for the THC and cocaine assays. Additionally, for a majority of the immunoassays, eye drops at the 50% level decreased the concentration of target metabolites to below the cut off levels but remained undetected on adulterant test strips.
While eye drops exhibited a color response at 50% on In7, the color change was slight and highly subjective to analyst interpretation.

The only target drug that could be evaluated using the SPE-it/DART method was amphetamines; the results revealed the ability to detect drug metabolites at higher concentrations of adulterant and/or the identification of peaks exhibiting the addition of masking products. Additionally, the concentration of dextroamphetamine remained relatively consistent throughout the various concentrations of certain adulterants (i.e. vinegar and sodium nitrite). This work shows promising for the use of DART as a screening technique because of the ability to identify target components and outlying protonated peaks. However, a result exemplified by the evaluation of the cocaine sample was the masking of the target drug when additional drugs are present within the sample. The only counter to this would be that the presence of any drug in this screening technique would indicate further testing by confirmatory methods. Future work is currently being conducted to optimize the protocol parameters for the other drugs of interest. This including the purchase of C18 SPE-it fibers to determine if this would have higher binding affinity for THC, cocaine and benzodiazepines.
REFERENCES