COMPARISON OF HIGH-RESOLUTION MASS SPECTROMETRY WITH IMMUNOASSAY IN QUALITATIVE URINE DRUG SCREENING

By

PAIGE FISK

Bachelor of Science in Forensic Chemistry
University of Central Missouri
Warrensburg, Missouri
2016

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2019

COMPARISON OF HIGH-RESOLUTION MASS SPECTROMETRY WITH IMMUNOASSAY IN QUALITATIVE URINE DRUG SCREENING

Thesis Approved:

Dr. Jarrad Wagner

Thesis Adviser

Dr. Ronald Thrasher

Dr. Philip Kemp

ACKNOWLEDGEMENTS

I would like to thank all of those who provided support throughout this journey. To Dr. Wagner for always being ready to drive to campus and assist me in any way he could. To my committee, Dr. Thrasher and Dr. Kemp, for providing excellent advice on how to better myself and the research. I would also like to thank the faculty and staff at the School of Forensic Sciences for their support throughout these past two years.

Thank you to McCayla Lopez and Gina Fader for keeping me focused and becoming wonderful friends in the course of my time here in Tulsa. I would also like to thank Austin Ciesielski for being a good friend and mentor.

A huge thank you to my parents, Linda and Mike Fisk. Without you and your support, I would not have been able to make it to graduate school and pursue my dreams. I know you did not always understand my research but you listened and were genuinely interested to hear me talk about it. I love you dearly.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: PAIGE FISK

Date of Degree: MAY, 2019

Title of Study: COMPARISON OF HIGH-RESOLUTION MASS SPECTROMETRY WITH IMMUNOASSAY IN QUALITATIVE URINE DRUG SCREENING

Major Field: FORENSIC SCIENCES

Abstract: Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF) utilizes a high-resolution mass spectrometer and chromatography to identify drugs in urine, based on their exact masses and retention times. The current "gold standard" method of qualitative urine drug screening is the immunoassay, which uses antibodies developed against drugs or their metabolites to identify them. Since this antibody-based methodology may have significant cross-reactivities and loss of specificity associated with it, it has been proposed to replace it with LC-TOF. This research compares the detection of drugs in urine with immunoassay and LC-TOF, following the development and validation of a qualitative LC-TOF assay.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 3

2.1 Introduction 3
2.2 Urine Drug Testing in Clinical Setting 3
2.3 LC-TOFMS in Clinical Setting 8
2.4 Screening for Novel Substance 10
2.5 Conclusion 12

III. METHODOLOGY 14

3.1 Introduction 14
3.2 Materials 14
3.3 Solutions 15
3.3.1 Standards 15
3.3.2 Tune Mix 16
3.4 Sample Preparation 20
3.5 Instrument Parameters and Software 21
3.5.1 Liquid Chromatography 21
3.5.2 Time-of-Flight Mass Spectrometer 22
3.5.3 Software 24
3.6 Validation 24
3.6.1 Limit of Detection 24
3.6.2 Matrix Effects 25
3.6.3 Sample Stability 25
3.6.4 Process Sample Stability 26
3.6.5 Interference 26

Chapter Page

IV. FINDINGS 29

4.1 Validation 29
4.1.1 Limit of Detection 29
4.1.2 Matrix Effects 31
4.1.3 Sample Stability 32
4.1.4 Process Sample Stability 34
4.1.5 Interference 35
4.2 Correlation 37
4.3 Chromatogram 38
4.4 Statistics 39

V. CONCLUSION 41

5.1 Validation 41
5.2 Correlation 41
5.3 Liquid Chromatography Time-of-Flight Mass Spectrometry 42
5.4 Solutions 42
5.5 Conclusion 43

REFERENCES 44

LIST OF TABLES

Table Page

Table 1. Preparation of 5 mL of internal standard solution. Based off of the stock concentrations, a corresponding spike volume was added to reach a final concentration of 1µg/mL. 16
Table 2. Preparation of 2 mL of analyte stock solution used in making the QC calibrators. ..17
Table 3. Preparation and final concentrations of 1 mL of cutoff calibrator stock. 18
Table 4. Concentrations for both above and below cutoff QC calibrators...............................19
Table 5. Flexar UHPLC pump gradient profile of mobile phases A and B. 21
Table 6. Positive mode parameters for time-of-flight mass spectrometer. 22
Table 7. Optics parameters for positive mode for time-of-flight mass spectrometer. 22
Table 8. Analytes with corresponding formulas, exact masses, and retention times. 22
Table 9. Immunoassay cutoff concentrations 25
Table 10. Interference mix analytes used for validation. 26
Table 11. Drug analytes and their corresponding lower limit of detection concentrations. ..30
Table 12. Matrix effects percentages based on a perfect number of 100% suggesting no effects. 31
Table 13. Percent differences for each analyte and each storage condition over a period of 7 days. 32
Table 14. Process sample stability results showing percent difference from baseline sample 34
Table 15. Interference mix percent differences between both sample injections. Positive percentage suggests ion enhancement while negative percentage suggest ion suppression. ...36
Table 16. Unknown sample results for Immunoassay, LC-TOF, and LC-MS/MS analysis....37
Table 17. Total number of true positives, true negatives, false positives, and false negatives based on drug class for the time-of-flight and immunoassay. 40
Table 18. Sensitivity, specificity, PPV, NPV, and accuracy results of immunoasssay and time-of-flight 40

LIST OF FIGURES

Figure Page

Figure 1.Triple quadrupole mass spectrometer. (Taken from Ni J, Ouyang H, Aiello M, et al. Microdosing Assessment to Evaluate Pharmacokinetics and Drug Metabolism in Rats Using Liquid Chromatography-Tandem Mass Spectrometry. Pharm Res. 2008;25(7):1572-1582. doi:10.1007/s11095-008-9555-x)..8

Figure 2. Diagram of time-of-flight mass spectrometer mechanism. (Taken from http://what-when-how.com/proteomics/time-of-flight-mass-spectrometry-proteomics/ 9

Figure 3. Overall chromatogram for sample 16 in the correlation study. 39

Figure 4. Chromatogram displaying the individual analyte, morphine, for sample 16. 39

CHAPTER I

INTRODUCTION

The use of prescribed drugs to combat pain has become common in the United States. This pain management, unfortunately, has a high chance of turning into an addiction. Urine drug testing currently is the best way to determine compliance with the prescribed drug regimen from doctors. To perform urine drug testing, the urine sample must go through two stages: screening and confirmation.

Urine screening is commonly performed on an immunoassay instrument. In this research, the immunoassay instrument used is called EasyRA®. It is a clinical chemistry analyzer produced by Medica. Immunoassays work by using antibodies specific to drug classes and using them to interact with drugs present in urine.

Urine specimens that are screened with immunoassay are typically sent for confirmatory testing. Confirmation testing is used to definitively state there are certain substances present and at what concentration. Confirmation testing in this laboratory is done on a liquid chromatography-tandem mass spectrometer (LC-MS/MS) and utilizes a method clinically validated by the

laboratory. Samples analyzed by LC/TOF in this research were previously screened by immunoassay and quantitatively confirmed using LC-MS/MS.

The high-resolution mass spectrometer used was the AxION® 2 Time-of-Flight Mass Spectrometer (LC-TOF) from PerkinElmer. This instrument has the ability to calculate accurate mass measurements. Accurate mass is defined as the "experimentally determined mass of an ion to an appropriate degree of accuracy used to determine the elemental formula of the ion" (Brenton, 2010). In this instance, the LC-TOF can identify an analyte based on its mass carried out to four decimal places. This ability ensures the certainty that what has been identified is correct because of the specificity of the mass. However, the analyst must be aware that this technique does not take into account isobars, or analytes which have identical masses because they have identical formulas, such as Codeine and Hydrocodone.

The samples used in this research were also run on the LC-MS/MS to verify the working order and abilities of the LC-TOF. Because the LC-MS/MS has a validated method, it is considered the "gold standard" of quantitation. Therefore, the samples' results from each instrument will be compared to each other. If both instruments produce the same results, it can be said the LC-TOF is a viable instrument for urine drug screening.

Method validation was conducted on this instrument including matrix effects, interference, sample stability, and limit of detection. In addition, robustness, sensitivity, and specificity of the instrument were determined.

This research can be used to determine if the immunoassay method being used now is truly the best method or if there is something else on the market which can do the same job with more specificity, more sensitivity, and more cost and time efficiently. LC-TOF can also be used to identify newer drugs on the market because of its accurate mass screening and scanning abilities.

CHAPTER II

REVIEW OF LITERATURE

2.1 Introduction

Pain management is becoming increasingly prevalent in the United States. Chronic pain is found in a large portion of the adult population. Unfortunately, the drugs prescribed to combat this issue are at a high risk of causing addiction or drug abuse. Urine drug testing has become the best way to determine compliance or noncompliance with the prescribed drug regimen. It is often found that some of those who take pain medication also take illicit drugs as well. Some of these drugs are known as novel psychoactive substances and need to be identified and characterized to determine usage legalization issues. However, these drugs are difficult to identify. There are two main instruments currently being used to identify analytes in urine samples, gas chromatography mass spectrometry and liquid chromatography-tandem mass spectrometry. There has been an emergence of another instrument, liquid chromatography time-of-flight mass spectrometry, that may be superior to the previous instrumentation.

2.2 Urine Drug Testing in the Clinical Setting

Chronic pain is generally found in about 2% to 40% of the adult population (Cone, 2008). These patients are often maintained on one or multiple strong pain medications such as opioids

and benzodiazepines (Cone, 2008). This trend has increased over the past decade (Christo 2011). Unfortunately, these prescribed medications are at a high risk of addiction and abuse (Christo 2011). To help with combatting these developments, urine drug testing has become more often used in the medical field. Urine drug testing (UDT) has the ability to identify use of prescription drugs in order to track compliance. It can also identify non-prescribed or illicit drugs to help deter and/or treat possible abuse (Cone 2008, Christo, Heit 2004, Florete 2012). It has been found that many pain medication patients also self-administer illicit drugs. In a study completed with 10,922 pain patient specimens, 1195 (10.9%) were found to have various combinations of illicit drugs (Cone, 2008). UDT also helps provide proper drug therapy for individual patients (Christo). Christo *et al*. mentioned a study of almost a million pain patients who were urine drug tested. The results showed that 75% of patients were unlikely to be taking their pain medications with the prescribed regimen, 38% were found to have no detectable amount of prescribed medication in their system, 29% had non-prescribed medication in their system, 27% had concentrations lower than expected, 15% had concentrations higher than expected, and 11% had illicit drugs present (Christo 2011). Being able to identify these issues with UDT has allowed the doctors to know of these issues and to help the patients.

Positive results of prescribed drugs relay to the doctor and the patient that the patient is following the treatment plan (Heit, 2004). A positive result of a non-prescribed drug or illegal drug can help the doctor to identify, discuss, and treat the possibility of abuse or misuse (Heit, 2004). Detection, identification, and analysis of drug metabolites is essential in UDT because along with the parent drug, it indicates use of the drug (Plumb, 2003). Metabolites are also important because sometimes the parent drug concentration is below a detectable concentration or not present at all (Plumb, 2003).

Urine is used for many reasons instead of blood or serum. Collection is non-invasive, the specimen provides plenty of volume, drugs and their metabolites are usually present in higher

concentrations and for longer periods of time than in other specimens, the sample is easily preserved by refrigeration or freezing, and the testing is relatively cost effective (Heit, 2004, Lum, 2004). While urine is ideal for drug testing, it does have some disadvantages. Adulteration of the sample is possible if the individual is trying to avoid detection. Adulteration occurs when a patient adds to the sample, dilutes the sample, or uses another's urine as their own to avoid detection of drug use. Collection observation is not usually performed making adulteration or replacement of urine an issue. Urine also varies in dilution which can make false-negative results a possibility (ARUP, 2018).

Historically, there have been multiple methods utilized for UDT. Urine must first be screened to determine what drug classes may be present in the urine. This is typically carried out by immunoassay testing which are designed to find the classes present or absent in the specimen (Heit, 2004). Immunoassay tests use antibodies to identify and measure chemical substances, in this case, drugs. The antibodies, which are the body's response to a foreign substance, are created by animals that have the drug of interest injected into them. Antibodies that specifically bind to the drug are produced in a serum which is then collected. Isotopically labeled drugs are mixed into the serum and compete with unlabeled drug for the antibody binding sites. These tests work by comparing the amount of an isotopically labeled drug bound to the antibody against a reference standard that contains a known concentration. A higher concentration of drug in the tested sample will displace a large amount of the labeled drug while a lower concentration would displace less. This methodology is applied to unknown samples for identification.

A drawback of immunoassay is the low specificity and cross reactivity. Specificity refers to the ability of an assay to correctly identify the drug of interest. Cross reactivity refers to the response of the assay to substances other than what it was designed to respond to. The antibody will not bind with dissimilar structures to the specific drug, but will bind to structures similar to the drug (Levine, 2015). For example, the antibody assay for amphetamine will have the ability to

bind with methamphetamine, phentermine, and MDMA. Immunoassay also has the possibility to miss adulterated samples from those who are trying to hide what is in their urine samples.

Immunoassay tests identify drug classes instead of individual drugs because of the cross reactivity and specificity. These classes may include: amphetamines, barbiturates, benzodiazepines, cannabinoids, carisoprodol, cocaine, ecstasy, fentanyl, meperidine, methadone, opiates, oxycodone, and propoxyphene (Cone, 2008). After screening, the specimens that were found to be positive for one or more drug classes must go through confirmation testing (Cone, 2008, Heit, 2004). Confirmation testing is a secondary test by a different chemical method to positively identify an analyte (Lum, 2004). It is used to determine what specific drug caused the screen to be positive for a certain drug class and at what concentration that drug is in the urine (Heit, 2004). This type of testing is usually carried out by gas chromatography mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS/MS).

In previous years, GC-MS has been considered the “gold-standard” for drug analysis because it provides the most accurate results (Lum, 2004). The GC-MS volatizes a liquid sample which then travels through a column to separate the components. The time at which the components exit the column is known as the retention time and is used in identification of the analyte. Those components are then analyzed in the mass spectrometer to determine what components are present and at what concentrations (Skoog, 2007). GC-MS software contains a database that compares the results to already confidently identified analytes for further confirmation. A limitation to this instrumentation for drug analyses is that GC-MS can tend to give false-negatives (Pelander, 2003). A false-negative is possible if the drug is susceptible to thermal decomposition due to the extensively high temperatures produced in the instrument (Fessessework, 2013). GC-MS is still rather popular and is used today in many laboratories.

More recently, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrumentation has been used for drug analysis. The LC-MS/MS uses liquid chromatography for separation. This is accomplished by using a solid phase column. When the solution is sent through the column, the ions will have some degree of affinity to it and stick to the column. To release these ions, aqueous and organic mobile phases are sent through the column to release the ions. The greater the affinity of the ion, the greater the percentage of the corresponding mobile phase is needed. The time the ion is released and comes off of the column is referred to as the retention time (Skoog, 2007).

These components are then sent through a triple quadrupole mass spectrometer. A quadrupole mass spectrometer contains four rods that only allow an analyte with a single m/z to pass through. A triple quadrupole contains three of these sets of rods referred to as Q1, Q2, and Q3. The triple quadrupole selects parent ions of a specific m/z in the Q1 which are then sent to the Q2. The Q2 applies a voltage and a collision gas to cause ion collision and fragmentation of the parent ions. These fragmentations, or product ions, are sent on to the Q3 to select the fragmented ions desired. The product ions are sent to the detector to be identified and quantified (Skoog, 2007). Figure 1 shows an example setup of a triple quadrupole mass spectrometer. The multiple quadrupoles are why the instrument is referred to as “tandem.” LC-MS/MS is sensitive, specific, and can be programmed to detect an abundance of drugs. Unfortunately, one must instruct the instrument to look for specific ion transitions and fragmentation. An LC-MS/MS will find what it is told to scan for and disregard the rest of the ions (Fessessework, 2013). LC-MS/MS is utilized in many laboratories. While this instrumentation is commonly used for confirmatory testing, it can be used a general screening tool. However, LC-MS/MS has the disadvantage of the restricted number of target compounds that can be included in an assay as well as the requirement of method development and revalidation of existing analytes after a new one has been introduced (Partridge, 2018).

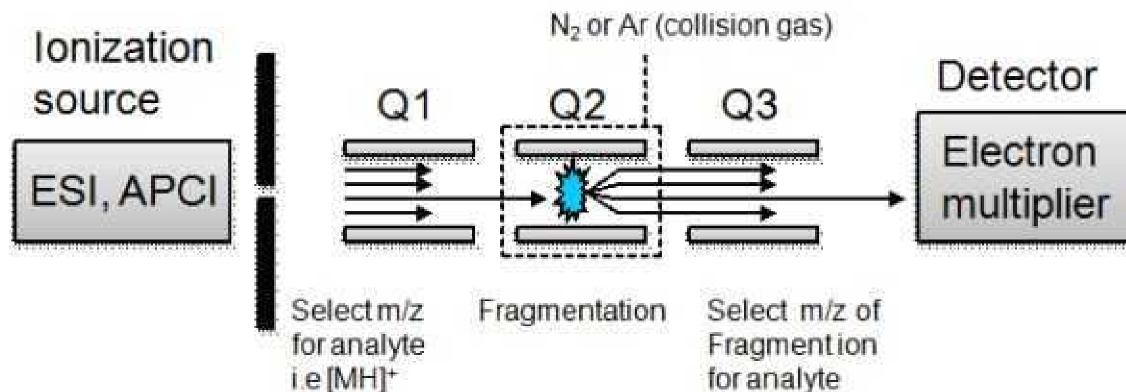

Figure 1. Triple quadrupole mass spectrometer. (Taken from Ni J, Ouyang H, Aiello M, et al. Microdosing Assessment to Evaluate Pharmacokinetics and Drug Metabolism in Rats Using Liquid Chromatography-Tandem Mass Spectrometry. Pharm Res. 2008;25(7):1572-1582. doi:10.1007/s11095-008-9555-x)

2.3 LC-TOFMS in the Clinical Setting

While GC-MS and LC-MS/MS are still being used regularly in clinical laboratories, there are newer, more sensitive instruments on the market. One of these instruments, the liquid chromatography time-of-flight mass spectrometer (LC-TOF) is becoming increasingly popular based on multiple published methods (Fessessework, 2013). LC-TOF uses the same principles as LC-MS/MS but uses the amount of time each molecule takes to reach the detector to identify the analyte (Skoog, 2007). After separation of the ions through liquid chromatography, the ions have a voltage applied to them and are sent through a drift tube which is under vacuum. The ions travel through the drift tube until they reach a reflectron which reflects the ions back down the tube towards the origin of the voltage and the detector. The ions will reach the detector based on the amount of time it takes the ion to reach it. Heavier ions travel slower than lighter ions and therefore reach the detector at a later time than the lighter ions. **Error! Reference source not found.** displays a diagram of this process. LC-TOF uses this time along with the retention time found in the chromatography to positively identify analytes.

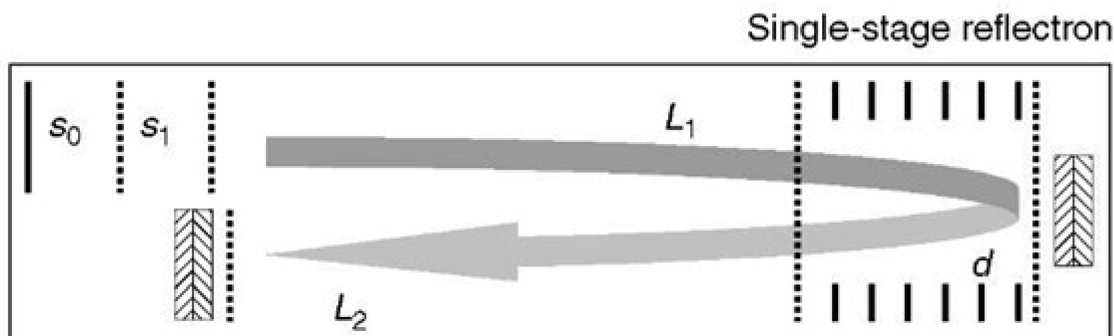

Figure 2. Diagram of time-of-flight mass spectrometer mechanism. (Taken from http://what-when-how.com/proteomics/time-of-flight-mass-spectrometry-proteomics/

LC-TOF is known for its accurate mass capacity (Ojanpera, 2006). Accurate mass is defined as "the experimentally determined mass of an ion measured to an appropriate degree of accuracy and precision used to determine, or limit the possibilities for, the elemental formula of the ion" (Brenton, 2010). The accurate mass capacity of the LC-TOF includes its ability to identify an analyte based on its mass of up to four decimal places ensuring confidence in the identification of the analyte. It also has high resolving power which is used to separate peaks, assign the peak centroid, and reduce ambiguity (Ojanpera, 2012). An LC-TOF not only has the ability to identify known analytes used in conjunction with reference standards, but also has the ability to identify unknown analytes without reference standards by being used as a screening instrument (Pelander, 2003). LC-TOF utilizes a calibration solution that contains analytes for lock masses. Lock masses are used to calibrate the instrument before and during use. The constant flow of the lock mass components in the calibration solution allows for real-time recalibration of mass accuracy by correcting any sources of measurement error that may be encountered (Chindarkar, 2015). This internal calibration during analysis to also assures no peak drift (Ojanpera, 2012). LC-TOF has greater selectivity, sensitivity, and speed when compared to GC-MS and LC-MS/MS (Ojanpera, 2012).

In a study done by Fessessework *et al*., a screening method for drugs and metabolites was validated using an LC-TOF (Fessessework, 2013). In the publication, it discusses the difficulties with new drugs that are available. Current methods are not easily adaptable and it takes a lot of work to constantly and consistently develop a new method for each new drug that comes onto the market. It was determined that an LC-TOF can screen and identify a multitude of drugs and their metabolites on demand without having to create or add to a working method. Also, adding a new analyte to a method does not require re-validation of current drugs when new ones are added like with LC-MS/MS (Partidge, 2018). This, as Fessessework stated, can lead to faster confirmations and turnaround times. This instrument can conduct an all-scan at all times to identify target knowns as well as retrospective searching for compounds that are not yet sought after. Screening with an LC-TOF allows for selective, rapid, and specific determination of target compounds and those unknown (Fessessework, 2013).

In other studies, urine drug testing results from an LC-TOF were compared to those of a GC-MS and an LC-MS/MS. Identification with the LC-TOF was based on accurate mass and retention time (Saleh, 2012). It was found that the LC-TOF had a higher number of compounds identified compared to the GC-MS along with no false negatives except for caffeine (Pelander, 2003). When compared to the LC-MS/MS, it had a lower false positive and false negative rate (Saleh, 2012). Both studies found the LC-TOF to be an excellent instrument in the clinical setting. A conductor of one of these studies, A. Saleh, agreed with Fessessework *et al*. in that a new screening technique for new drugs on the market is necessary and the LC-TOF is a good candidate for this job (Saleh, 2012).

2.4 Screening for Novel Substances

There is an array of drugs that urine pain management samples are analyzed for. These tend to be the drugs included in the drug classes mentioned before for urine screening. However,

recently there has been a demand for the screening of novel psychoactive substances, or NPS. These substances are newly created drugs or currently used drugs being used in new or "novel" ways (Orsolini, 2016). NPS are sold as legal highs, bath salts, plant food, and air fresheners that are disclaimed as "not for human consumption" to avoid legislation (Orsolini, 2016). They can be purchased online or in head shops that sell smoking accessories (Orsolini, 2016). As of 2016, it was determined that synthetic cathinones and cannabinoids as well as psychedelics and phenethylamines account for the largest number of NPS being used (Orsolini, 2016). There is now an "online drug culture" that has increased the availability of NPS (Orsolini, 2016). With the rapid development of new NPS, a urine screening method needs to be implemented to determine the use of them.

Because NPS are so quickly created, sold, and retired, there is no time for current methods to be modified or created to adapt to these substances. Quantification methods may not be required to be developed due to the fact that once compounds become regulated, new and sometimes more potent analogues are developed in order to avoid the law and increase psychoactive effects (Zawilska, 2015). This is when the LC-TOF becomes superior to previous methods. It is already known that the LC-TOF has the capability of identifying known and unknown analytes (Fessessework, 2013). It provides accurate mass and retention times of complex samples. The LC-TOF can operate at a constant all-scan which can identify the known analytes as well as the unknowns (Fessessework, 2013). It can complete these scans at practically an unlimited mass range meaning it would be able to characterize analytes without having to know the mass (Skoog, 2007). Due to its abilities, there have been screening methods created already on the LC-TOF for the everyday drugs looked for in urine analysis (Fessessewoek, 2013). However, there is need of a method that can accurately identify and potentially quantify NPS.

There are some NPS that are currently on many laboratories' analyte lists. These include 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxy-N-ethylamphetamine (MDEA)

also known as Eve, 3,4-Methylenedioxymethamphetamine (MDMA) also known as ecstasy, phencyclidine (PCP), fentanyl, norfentanyl, acetyl fentanyl, and acetyl norfentanyl along with other fentanyl analogues. Other drugs that may not be as common include analogues for phenethylamine, ketamine, and cathinones (Zawilska, 2015). Drugs such as these are likely being quantified in laboratories because they have been around for many years and are still prevalent today, an attribute that most NPS do not have. LC-TOF is able to conduct quantification methods as well as screenings, making it a versatile instrument. It is also possible to run a quantification method with a screening method behind it and constantly be looking for substances that the operator is unaware of. LC-TOF allows for retrospective data analyses and reprocessing of data without re-extraction or repeated instrumental analysis (Fessessework, 2013, Pasin, 2015). If an NPS is discovered as being used, previous data can be reprocessed and looked at retrospectively to determine if previous samples contain the NPS of concern which can help identify trends and around what time the drug began to be used in the area.

2.5 Conclusion

Liquid chromatography time-of-flight mass spectrometry is a good choice for an instrument for urine drug analysis and urine drug screening. LC-TOF has greater specificity and sensitivity as well as not having the issue of cross reactivity as is seen in immunoassay. It has multiple abilities that make it superior to immunoassay testing, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry for the same application. Few clinical laboratories presently have a liquid-chromatography time-of-flight mass spectrometer, making development of a screening and possible quantification method a fairly new idea. With the spread of usage of LC-TOF, methods will be needed. Also, with the rapid emergence of new novel psychoactive substances, a screening and potential quantifying method will be beneficial to the clinical setting for tentative identification and retrospective analysis of samples possibly containing these substances. These drugs have the possibility to be quickly identified and

characterized by LC-TOF even without drug standards. The ability of one instrument to screen and quantify both pain management drugs and NPS will save money and time.

CHAPTER III

METHODOLOGY

3.1 Introduction

The purpose of this research was to determine which screening method is superior for urine drug analysis. These two techniques compared were immunoassay and Liquid Chromatography-Time-of-Flight-Mass Spectrometry (LC-TOF). The first step of this research was to create and validate a qualitative method on the LC-TOF. Anonymized results from immunoassay were then compared to LC-TOF results of the same sample. These results were compared to a validated method on LC-MS/MS to determine reliability of the LC-TOF results.

3.2 Materials

The following drug standards were purchased from Cerilliant (Cerilliant Corporation, Round Rock, TX) at a concentration of 1 mg/mL in methanol: 6-monoacetylmorphine, 6-monoacetylmorphine-D6, 7-Aminoclonazepam, 7-Aminoclonazepam-D4, Acetyl fentanyl, Acetyl norfentanyl, α-Hydroxyalprazolam, α-Hydroxyalprazolam-D5, Alprazolam, Alprazolam-D5, Amphetamine, Benzoylecgonine, Benzoylecgonine-D8, Buprenorphine, Buprenorphine-D4, Carisoprodol, Carisoprodol-D7, Clonazepam, Clonazepam-D4, Cocaine, Cocaine-D3, Codeine, Codeine-D6, Dextromethorphan, Diazepam, Diazepam-D5, Dihydrocodeine, Dihydrocodeine-D6, Fentanyl, Flunitrazepam, Flurazepam, Heroin, Heroin-D9, Hydrocodone, Hydrocodone-D6,

Hydromorphone, Hydromorphone-D6, Lorazepam, Lorazepam-D4, MDA, MDA-D5, MDEA, MDEA-D6, MDMA, MDMA-D5, Meperidine, Meperidine-D4, Meprobamate, Meprobamate-D7, Methadone, Methadone-D9, Methamphetamine, Methamphetamine-D11, Midazolam, Morphine, Morphine-D6, Naloxone, Naltrexone, Nitrazepam, Norbuprenorphine-D3, Nordiazepam, Nordiazepam-D5, Norfentanyl, Norfentanyl-D5, Normeperidine-D4, Norpropoxyphene, Norproxyphene-D5, Oxazepam, Oxazepam-D5, Oxycodone, Oxycodone-D6, Oxymorphone, Oxymorphone, Oxymorphone-D3, Phenazepam, Phencyclidine (PCP), PCP-D5, Phentermine, Phentermine-D5, Propoxyphene, Propoxyphene-D11, Temazepam, Temazepam-D5, THCA, THCA-D3, and Tramadol. The following drug standards were purchased from Cerilliant (Cerilliant Corporation, Round Rock, TX) at a concentration of 100 µg/mL in methanol: Amphetamine-D11, Cyclobenzaprine-D3, Dextromethorphan-D3, EDDP, EDDP-D3, Fentanyl-D5, Flunitrazepam-D7, Midazolam-D4, Naloxone-D5, Naltrexone-D3, Nitrazepam-D5, Norbuprenorphine, Normeperidine, Phenazepam-D4, Sufentanil, Sufentanil-D5, and Tramadol-D3. Acetyl fentanyl-D5 and Acetyl norfentanyl-D5 were purchased from Cayman Chemical (Cayman Chemical, Ann Arbor, Michigan).

Methanol was purchased from Fisher Scientific (Fisher Scientific, Waltham, MA). Formic acid was purchased from EDM (EDM Millipore Corporation, Billerica, MA). HPLC grade water was collected from a Barnstead Nanopure water system (Thermo Scientific, Waltham, MA). Ammonium formate was purchased from Alfa Aesar (Alfa Aesar, Ward Hill, MA). ESI Tuning Mix was purchased from Agilent (Agilent Technologies, Santa Clara, CA), Acetonitrile was purchased Fisher Scientific (Fisher Scientific, Waltham, MA). Drug-free urine was purchased from UTAK (UTAK Laboratories Inc., Valencia, CA). IMCSzyme and rapid hydrolysis buffer were purchased from IMCS (IMCS, Irmo, SC).

3.3 Solution Preparation

3.3.1 Standards

Internal standard was prepared by diluting the deuterated drug standards in methanol to a concentration of 1 μg/mL. This process is shown in Table 1. A drug stock solution was created by spiking methanol with certified drug standards. Table 2 shows the preparation and final concentrations. Analytes listed in Tables 1 and 2 that are not listed in section 3.2 were used in a separate method and validation but are not analyzed in this method. A single calibrator for cutoff concentrations was made by adding each drug standard, bringing to volume with methanol and then diluting by a factor of 1:100 with urine. Table 3 displays this stock preparation. A quality control (QC) calibrator at concentrations above the cutoff was made by diluting the drug stock solution by a factor of 1:100 with urine. A QC calibrator at concentrations below the cutoff was made by diluting the high concentration QC by a factor of 1:10 with urine. Table 4 displays the concentrations of these calibrators for each analyte. The calibrator and QC calibrators were stored in a freezer at -19℃ with a range of -10℃ to -25℃.

3.3.2 Tune Mix

A calibration solution, or tune mix, was made to calibrate the instrument before each use and identify the lock masses needed for the instrument. Tune mix was made by adding 50 mL of ESI Tuning Mix from Agilent Technologies, 75 mL acetonitrile, 2 mL of a 500 μg/mL caffeine in 50:50 methanol to water solution, and 250 μL Formic acid in a 250 mL volumetric flask. The solution was brought to volume with methanol.

Table 1. Preparation of 5 mL of internal standard solution. Based off of the stock concentrations, a corresponding spike volume was added to reach a final concentration of 1μg/mL.

Internal Standard	Spike Volume (μL)	Final Concentration (μg/mL)
6-monoacetylmorphine (6-MAM)-D6	5	1
7-Aminoclonazepam-D4	5	1

Internal Standard	Spike Volume (µL)	Final Concentration (µg/mL)
a-Hydroxyalprazolam-D5	5	1
Alprazolam-D5	5	1
Amphetamine-D11	50	1
Benzoylecgonine-D8	5	1
Buprenorphine-D4	5	1
Clonazepam-D4	5	1
Cocaine-D3	5	1
Codeine-D6	5	1
Diazepam-D5	5	1
Dihydrocodeine-D6	5	1
EDDP-D3	50	1
Fentanyl-D5	50	1
Heroin-D9	5	1
Hydrocodone-D6	5	1
Hydromorphone-D6	5	1
MDEA-D6	5	1
MDMA-D5	5	1
Methadone-D9	5	1
Methamphetamine-D11	5	1
Morphine-D6	5	1
Norbuprenorphine-D3	5	1
Norfentanyl-D5	5	1
Oxycodone-D6	5	1
PCP-D5	5	1
THCA-D3	5	1
Tramadol-13C-D3	50	1
Total Spike Volume	320	
Total Methanol Volume	4680	
Total Volume	5000	

Table 2. Preparation of 2 mL of analyte stock solution used in making the QC calibrators.

Analyte	Spike Volume (µL)	Final Concentration (ng/mL)
6-monoacetylmorphine (6-MAM)	4	2000
7-aminoclonazepam	20	10000
a-Hydroxyalprazolam	8	4000

Analyte	Spike Volume (µL)	Final Concentration (ng/mL)
Alprazolam	8	4000
Amphetamine	8	4000
Benzoylecgonine	20	10000
Buprenorphine	4	2000
Clonazepam	8	4000
Cocaine	8	4000
Codeine	20	10000
Diazepam	8	4000
Dihydrocodeine	4	2000
EDDP	80	4000
Fentanyl	2	1000
Heroin	20	10000
Hydrocodone	20	10000
Hydromorphone	8	4000
MDEA	8	4000
MDMA	8	4000
Methadone	20	10000
Methamphetamine	20	10000
Morphine	8	4000
Norbuprenorphine	40	2000
Norfentanyl	2	1000
Oxycodone	8	4000
Phencyclidine (PCP)	4	2000
Tramadol	20	10000
Δ9 THC-COOH	60	30000
Total Spike Volume	456	
Total Methanol Volume	1544	
Total Volume	2000	

Table 3. Preparation and final concentrations of 1 mL of cutoff calibrator stock.

Analyte	Spike Volume (µL)	Final Concentration (ng/mL)
6-monoacetylmorphine (6-MAM)	1	1000
7-aminoclonazepam	5	5000
a-Hydroxyalprazolam	5	5000
Alprazolam	5	5000
Amphetamine	5	5000
Benzoylecgonine	5	5000

Analyte	Spike Volume (µL)	Final Concentration (ng/mL)
Buprenorphine	1	1000
Clonazepam	5	5000
Cocaine	5	5000
Codeine	5	5000
Diazepam	5	5000
Dihydrocodeine	5	5000
EDDP	50	5000
Fentanyl	0.5	500
Heroin	5	5000
Hydrocodone	5	5000
Hydromorphone	5	5000
MDEA	5	5000
MDMA	2	2000
Methadone	5	5000
Methamphetamine	5	5000
Morphine	5	5000
Norbuprenorphine	10	1000
Norfentanyl	0.5	500
Oxycodone	5	5000
Phencyclidine (PCP)	2.5	2500
Tramadol	15	15000
Δ9 THC-COOH	15	15000
Total Spike Volume	187.5	
Total Methanol Volume	812.5	
Total Volume	1000	

Table 4. Concentrations for both above and below cutoff QC calibrators.

Analyte	Conc. QC Above (ng/mL)	Conc. QC Below (ng/mL)
6-MAM	20	4
7-Aminoclonazepam	100	20
Alprazolam	100	20
Amphetamine	100	20
Benzoylecgonine	200	40
Buprenorphine	40	8
Clonazepam	100	20

Analyte	Conc. QC Above (ng/mL)	Conc. QC Below (ng/mL)
Cocaine	100	20
Codeine	200	40
Diazepam	100	20
Dihydrocodeine	100	20
EDDP	100	20
Fentanyl	20	4
Heroin	100	20
Hydrocodone	200	40
Hydromorphone	100	20
MDEA	100	20
MDMA	200	40
Methadone	100	20
Methamphetamine	200	40
Morphine	100	20
Norbuprenorphine	40	8
Norfentanyl	20	4
Oxycodone	100	20
PCP	100	20
THCA	600	120
Tramadol	300	60
α-Hydroxyalprazolam	100	20

3.4 Sample Preparation

Twenty urine specimens previously analyzed OSU-CLS with a validated clinical LC-MS/MS method were used in this research. All calibrators and unknown samples were extracted using the following "Dilute and Shoot" method. One hundred µL of calibrator or sample was added to polypropylene Eppendorf tubes. Following, 10 µL of IMCSzyme was added along with 50 µL of rapid hydrolysis buffer and 40 µL internal standard. IMCSzyme is the β-glucuronidase used to cleave the glucuronide bond from the drug and the buffer is used to keep the sample at the optimal pH for the enzyme activity. The Eppendorf tubes were vortexed for 10 seconds and incubated at 60℃ for 30 minutes. The Eppendorf tubes were then taken out of the incubator, and 50 µL of sample diluent was added. They were then vortexed for 10 seconds and centrifuged at

13000 RPM for 10 minutes. After centrifuging, 200 µL of supernatant was transferred to a vial insert. The insert was placed in a 2 mL vial and capped.

3.5 Instrument Parameters and Software

3.5.1 Liquid Chromatography

The liquid chromatography portion of the instrument was the Flexar UHPLC pump system provided by PerkinElmer® for this research. The column was a Restek Raptor™ Biphenyl 2.7 µm. The column was 100x2.1mm in size. This column utilized the EXP® connect holder and EXP® guard cartridge from Restek. This column was used to achieve adequate analyte separation. The autosampler was kept at a temperature of 20□ with a tolerance of +/- 2□. The injection volume was 15 µL. Mobile phase A used with the pump was 2mM Ammonium formate and 0.1% Formic acid in HPLC grade water. Mobile phase B used was 2mM Ammonium formate and 0.1% Formic acid in HPLC grade methanol. The UHPLC pump gradient was designed to separate the analytes efficiently as well as coeluting analytes. Total pump run time was 6.5 minutes. After each run, there was a 3-minute equilibration time to return to starting parameters. Table 5 displays the pump gradient.

Table 5. Flexar UHPLC pump gradient profile of mobile phases A and B.

Gradient Profile				
Step	Minutes	mL/min	%A	%B
0	1.0	0.45	98.0	2.0
1	0.5	0.45	98.0	2.0
2	0.5	0.45	65.0	35.0
3	1.0	0.45	65.0	35.0
4	3.0	0.45	5.0	95.0
5	1.0	0.45	5.0	95.0
6	0.1	0.45	98.0	2.0
7	0.4	0.45	98.0	2.0

3.5.2 Time-of-Flight Mass Spectrometer

The AxION® 2 Time-of-Flight Mass Spectrometer was provided by PerkinElmer® for this research. Each batch run used the same parameters. Table 6 displays the mass spectrometer parameters for positive mode. The calibration solution in the calibration vial was used to calibrate the instrument before each use and find the lock masses used for the instrument. Table 7 displays the optics parameters within the source for each run.

Table 6. Positive mode parameters for time-of-flight mass spectrometer.

Positive Mode			
Cylinder	-3500 V	Dry Gas Flow	15 L/min
Endplate	-4800 V	Dry Gas Heat	350□
Capillary Entrance	-5800 V	Right Nebulizer Gas	80 PSI
Endplate Heater	Medium	Left Nebulizer Gas	40 PSI
Diverter	Waste	Calibration Vial	Left

Table 7. Optics parameters for positive mode for time-of-flight mass spectrometer.

Optics			
Capillary Exit	140 V	RF Voltage	500 V
Skimmer	25	Offset Voltage	14.4 V
DAU	4000 V		

A time-of-flight mass spectrometer utilizes an exact mass feature up to four decimal places. These masses are used to identify the analytes being tested in the instrument and find the correct retention times of each analyte. Table 8 contains the list of analytes with corresponding formulas, exact masses, and retention times.

Table 8. Analytes with corresponding formulas, exact masses, and retention times.

Analyte	Formula	m/z [M+H]+	Retention Time
6-monoacetylmorphine (6-MAM)	$C_{19}H_{21}NO_4$	328.1543	2.875

Analyte	Formula	m/z [M+H]+	Retention Time
6-monoacetylmorphine (6-MAM)-D6	C19H15NO4D6	334.1920	2.863
7-aminoclonazepam	C15H12ClN3O	286.0742	3.661
7-Aminoclonazepam-D4	C15H8ClN3OD4	290.0993	3.644
a-Hydroxyalprazolam	C17H13ClN4O	325.0850	4.779
a-Hydroxyalprazolam-D5	C17H8ClN4OD5	330.1164	4.767
Alprazolam	C17H13ClN4	309.0902	5.03
Alprazolam-D5	C17H8ClN4D5	314.1215	5.015
Amphetamine	C9H13N	136.1121	2.318
Amphetamine-D11	C9H2ND11	147.1811	2.241
Benzoylecgonine	C16H19NO4	290.1387	3.488
Benzoylecgonine-D8	C16H11NO4D8	298.1889	3.458
Buprenorphine	C29H41NO4	468.3108	4.11
Buprenorphine-D4	C29H37NO4D4	472.3359	4.102
Clonazepam	C15H10ClN3O3	316.0483	4.656
Clonazepam-D4	C15H6ClN3O3D4	320.0735	4.643
Cocaine	C17H21NO4	304.1543	3.61
Cocaine-D3	C17H18NO4D3	307.1732	3.606
Codeine	C18H21NO3	300.1594	2.845
Codeine-D6	C18H15NO3D6	306.1971	2.826
Diazepam	C16H13ClN2O	285.0789	5.203
Diazepam-D5	C16H8ClN2OD5	290.1103	5.19
Dihydrocodeine	C18H23NO3	302.1751	2.812
Dihydrocodeine-D6	C18H17NO3D6	308.2127	2.794
EDDP	C20H23N	278.1982	4.367
EDDP-D3	C20H21ND3	282.2170	4.3620
Fentanyl	C22H28N2O	337.2274	4.093
Fentanyl-D5	C22H23N2OD5	342.2588	4.081
Heroin	C21H23NO5	370.1649	3.535
Heroin-D9	C21H14NO5D9	379.2214	3.516
Hydrocodone	C18H21NO3	300.1594	3.005
Hydrocodone-D6	C18H15NO3D6	306.1971	2.99
Hydromorphone	C17H19NO3	286.1438	2.4
Hydromorphone-D6	C17H13NO3D6	292.1814	2.374
MDEA	C12H17NO2	208.1332	3.128
MDEA-D6	C12H11NO2D6	214.1709	3.116
MDMA	C11H15NO2	194.1176	2.919
MDMA-D5	C11H9NO2D5	198.1411	2.91
Methadone	C21H27NO	310.2165	4.584

Analyte	Formula	m/z [M+H]+	Retention Time
Methadone-D9	C21H18NOD9	319.2730	4.571
Methamphetamine	C10H15N	150.1277	2.647
Methamphetamine-D11	C10H4ND11	161.1968	2.59
Morphine	C17H19NO3	286.1438	2.142
Morphine-D6	C17H13NO3D6	292.1814	2.12
Norbuprenorphine	C25H35NO4	414.2639	3.785
Norbuprenorphine-D3	C25H32NO4D3	417.2827	3.779
Nordiazepam-D5	C15H6ClN2OD5	276.0947	4.825
Norfentanyl	C14H20N2O	233.1648	3.276
Norfentanyl-D5	C14H15N2OD5	238.1962	3.258
Oxycodone	C18H21NO4	316.1543	2.947
Oxycodone-D6	C18H15NO4D6	322.1920	2.931
PCP-D5	C17H20ND5	249.2374	4.155
Phencyclidine (PCP)	C17H25N	244.2060	4.171
THCA-D3	C22H27O4D3	348.2270	5.39
Tramadol HCl	C16H25NO2	264.1958	3.362
Tramadol-13C-D3	C15H22NO2(13)CD3	268.2180	3.349
Δ9 THC-COOH	C22H30O4	345.2128	5.395

3.5.3 Software

The software used in conjunction with the time-of-flight was Chromera® version 3.4.4. Chromera® is licensed to PerkinElmer®.

3.6 Validation

A qualitative method validation was performed to ensure the viability of the method. Included in the validation was limit of detection, matrix effects, sample stability, process sample stability, and interference.

3.6.1 Limit of Detection

Limits of detection are the lowest and highest concentrations of analytes that the instrument can accurately see and identify. Lower limits of detection (LLOD) are the lowest

concentrations the instrument can identify. In this research, the LLOD were determined from the analyte cutoffs for the EasyRA® as well as relevant concentrations in the pain management community. The cutoffs for the calibrators (not including the cross-reactive analytes) for the EasyRA® can be seen in Table 9.

Table 9. Immunoassay cutoff concentrations

Analyte	IA LLOD Concentration (ng/mL)
EDDP	100
Benzoylecgonine	100
Oxycodone	100
Δ9 THC-COOH	500
Buprenorphine	5
Methamphetamine	500

3.6.2 Matrix Effects

Matrix effects studies are conducted to discover if there are any unwanted effects caused by the urine matrix. Ten urine samples were spiked as the high concentration QC by a dilution of 1:100 of the stock calibrator with urine. The urine samples from different sources were extracted and prepared as described above. The peak areas of each sample for each analyte were compared to a blank urine sample. The comparisons were given a percentage to determine effects. If the percentage was above 100, there may have been ion enhancement. If the percentage was below 100, there may have been ion suppression. (Partridge et al.)

3.6.3 Sample Stability

Sample stability is run to determine the effects of storage on each analyte. Fifteen samples are prepared and left in 3 different storage conditions. The samples were then run in sets of 3, 1 of each storage condition, over a period of 7 days. Five samples were left sitting on the

laboratory bench, 5 were stored in the freezer, and 5 were stored in the refrigerator. The peak areas of each analyte for each sample was recorded and compared to determine which storage condition had the least amount of effects (i.e.: analyte deterioration) on the sample.

3.6.4 Process Sample Stability

Process sample stability is used to determine sample stability within the instrument on the autosampler. It consists of running an individual sample on the instrument over a period of 3 days. The first injection is considered the "baseline" and run on the first day. The second run is done at 24 hours from the time of the first injection. The third run is done at 48 hours from the time of the first injection.

3.6.5 Interference

Interference is used to determine if any other analytes in a sample are going to interfere with the analyte of concern. Two mixes were made which each contain common analytes seen within samples. Interference uses 6 samples, 4 urine and 2 water. All samples are spiked as the high QC calibrator. Because the QC is made from a stock of multiple analytes, the analytes not being used in this method are also considered to be interference drugs. Two of the urine samples have one of the interference mixes added to them while the other two have the second mix added. The water samples do not have the mix added. The samples are then extracted as usual. The analyte areas are compared to determine if interference is present. Table 10 shows the list of drugs in each mix solution.

Table 10. Interference mix analytes used for validation.

Interference Mix 1	**Interference Mix 2**
(-)-Nicotine	Ibuprofen
Acetaminophen	Naproxen
Acetyl fentanyl	Acetyl fentanyl

Interference Mix 1	Interference Mix 2
Acetyl Fentanyl-D5	Acetyl Fentanyl-D5
Acetyl norfentanyl	Acetyl norfentanyl
Acetyl Norfentanyl-D5	Acetyl Norfentanyl-D5
Caffeine	R,R(-)-Pseudoephedrine
Carisoprodol	Carisoprodol
Carisoprodol-D7	Carisoprodol-D7
Cyclobenzaprine	Cyclobenzaprine
Cyclobenzaprine-D3	Cyclobenzaprine-D3
Dextromethorphan	Dextromethorphan
Dextromethorphan-D3	Dextromethorphan-D3
Flunitrazepam	Flunitrazepam
Flunitrazepam-D7	Flunitrazepam-D7
Flurazepam	Flurazepam
Lorazepam	Lorazepam
Lorazepam-D4	Lorazepam-D4
MDA	MDA
MDA-D5	MDA-D5
Meperidine	Meperidine
Meperidine-D4	Meperidine-D4
Meprobamate	Meprobamate
Meprobamate-D7	Meprobamate-D7
Midazolam	Midazolam
Midazolam-D4	Midazolam-D4
Naloxone	Naloxone
Naloxone-D5	Naloxone-D5
Naltrexone	Naltrexone
Naltrexone-D3	Naltrexone-D3
Nitrazepam	Nitrazepam
Nitrazepam-D5	Nitrazepam-D5
Nordiazepam	Nordiazepam
Nordiazepam-D5	Nordiazepam-D5
Normeperidine	Normeperidine
Normeperidine-D4	Normeperidine-D4
Norpropoxyphene	Norpropoxyphene
Norpropoxyphene-D5	Norpropoxyphene-D5
Oxazepam	Oxazepam
Oxazepam-D5	Oxazepam-D5
Oxymorphone	Oxymorphone
Oxymorphone-D3	Oxymorphone-D3
Phenazepam	Phenazepam

Interference Mix 1	Interference Mix 2
Phenazepam-D4	Phenazepam-D4
Phentermine	Phentermine
Phentermine-D5	Phentermine-D5
Propoxyphene	Propoxyphene
Propoxyphene-D11	Propoxyphene-D11
Sufentanil	Sufentanil
Sufentanil-D5	Sufentanil-D5
Temazepam	Temazepam
Temazepam-D5	Temazepam-D5

CHAPTER IV

RESULTS

4.1 Validation

4.1.1 Limit of Detection

The lower limits of detection (LLOD) were determined from the immunoassay cutoff concentrations and significant clinical pain management concentrations used in the OSU-CLS clinical method. The cutoffs were intended to be lower than immunoassay to avoid not being able to identify samples with a much lower concentration. Fentanyl and Norfentanyl had a LLOD of 5 ng/mL. The following drug analytes had a concentration of 10 ng/mL: 6-MAM, Buprenorphine, and Norbuprenorphine. MDMA had a concentration of 20 ng/mL and PCP had a concentration of 25 ng/mL. The following drug analytes had a concentration of 50 ng/mL: 7-Aminoclonazepam, α-Hydroxyalprazolam, Alprazolam, Amphetamine, Benzoylecgonine, Clonazepam, Cocaine, Codeine, Diazepam, Dihydrocodeine, EDDP, Heroin, Hydrocodone, Hydromorphone, MDEA, Methadone, Methamphetamine, Morphine, and Oxycodone. Tramadol and THCA had a LLOD concentration of 150 ng/mL. Table 11 displays the LLODs for the analytes used in this research.

Table 11. Drug analytes and their corresponding lower limit of detection concentrations.

Analyte	LLOD Concentration (ng/mL)
6-monoacetylmorphine (6-MAM)	10
7-aminoclonazepam	50
a-Hydroxyalprazolam	50
Alprazolam	50
Amphetamine	50
Benzoylecgonine	50
Buprenorphine	10
Clonazepam	50
Cocaine	50
Codeine	50
Diazepam	50
Dihydrocodeine	50
EDDP	50
Fentanyl	5
Heroin	50
Hydrocodone	50
Hydromorphone	50
MDEA	50
MDMA	20
Methadone	50
Methamphetamine	50
Morphine	50
Norbuprenorphine	10
Norfentanyl	5
Oxycodone	50
Phencyclidine (PCP)	25
Tramadol	150
Δ9 THC-COOH	150

4.1.2 Matrix Effects

The analytes along with their matrix effects results can be seen in Table 12. A percentage above 100% suggests ion enhancement due to the matrix while a percentage below 100% suggests ion suppression due to the matrix.

Table 12. Matrix effects percentages based on a perfect number of 100% suggesting no effects.

Analyte	Matrix Effects Percent (%)
6-monoacetylmorphine (6-MAM)	84.14
7-aminoclonazepam	96.75
a-Hydroxyalprazolam	130.35
Alprazolam	110.53
Amphetamine	307.54
Benzoylecgonine	116.26
Buprenorphine	98.31
Clonazepam	92.35
Cocaine	89.70
Codeine	109.12
Diazepam	104.33
Dihydrocodeine	81.06
EDDP	143.90
Fentanyl	98.00
Heroin	101.32
Hydrocodone	90.93
Hydromorphone	112.17
MDEA	89.26
MDMA	61.37
Methadone	83.55
Methamphetamine	87.02
Morphine	41.61
Norbuprenorphine	88.50
Norfentanyl	95.93
Oxycodone	111.13
Phencyclidine (PCP)	113.06
Tramadol	100.62
Δ9 THC-COOH	89.14

4.1.3 Sample Stability

The results of sample stability should show an increase of percentage difference due to the peak areas decreasing over time in relation to the "Day 0" peak for each analyte. This expected increase is due to the degradation of the analytes during storage. Results show that this research is not consistent with the expected trend. Because the percentages should have been in a consistent increase for the entire duration due to consistent degradation over time, it is hypothesized that the anomaly in the process was due to an improvement of the instrument's performance after a period of non-use. These percent differences can be seen in Table 13.

Table 13. Percent differences for each analyte and each storage condition over a period of 7 days.

		Percent Difference from Day 0 Area				
Analyte	**Storage**	Day 2	Day 3	Day 5	Day 6	Day 7
6-MAM	Freezer	-8.67	-10.19	-5.60	5.09	-2.68
	Refrigerator	-5.61	-9.61	-43.43	-3.60	1.20
	Bench	13.69	-21.30	-19.4	-16.77	-14.32
7 amino	Freezer	-14.25	-19.62	-15.11	-3.46	-1.22
	Refrigerator	-10.97	-16.90	-14.84	-6.71	-3.45
	Bench	-2.60	-10.53	-1.01	9.48	16.97
a-Hydroxyalprazolam	Freezer	-23.75	-28.43	-23.92	-15.88	-11.67
	Refrigerator	-19.25	-23.93	-24.69	-10.22	-11.22
	Bench	-15.32	-26.06	-24.14	-15.27	-8.65
Alprazolam	Freezer	-15.08	-22.02	-22.31	-8.59	-11.77
	Refrigerator	-9.93	-17.54	-17.36	-12.54	-10.97
	Bench	-15.99	-22.88	19.71	-12.70	-8.85
Amphetamine	Freezer	-11.04	-12.27	-6.49	-1.07	2.92
	Refrigerator	-11.60	-8.78	-6.65	4.98	11.77
	Bench	-1.10	-14.27	-4.68	3.44	15.12
Benzoylecgonine	Freezer	-14.57	-22.61	-17.62	-9.04	-6.31
	Refrigerator	-11.28	-15.91	-13.99	-6.33	-4.66
	Bench	-6.23	-19.35	-14.62	-7.27	-2.05
Buprenorphine	Freezer	-28.64	-29.44	-25.44	-20.69	-19.35
	Refrigerator	-24.23	-26.03	-25.72	-19.77	-16.90
	Bench	-26.55	-26.70	-25.06	-19.14	-18.03

Analyte	Storage	Percent Difference from Day 0 Area				
		Day 2	Day 3	Day 5	Day 6	Day 7
Clonazepam	Freezer	-19.34	-21.14	-19.96	-10.00	-11.01
	Refrigerator	-14.59	-19.07	-19.71	-11.59	-7.71
	Bench	-18.90	-13.84	-18.27	-5.75	-11.38
Cocaine	Freezer	-21.68	-29.18	-23.87	-16.67	-10.13
	Refrigerator	-18.50	-26.45	-25.71	-16.22	-8.84
	Bench	-17.01	-24.54	-19.09	-9.22	-3.38
Codeine	Freezer	-14.00	-19.80	-19.42	-6.28	-8.76
	Refrigerator	-9.03	-15.97	-15.40	-8.63	-6.65
	Bench	-10.76	-16.59	-17.12	-7.51	-3.52
Diazepam	Freezer	-16.16	-23.63	-20.43	-9.26	-7.82
	Refrigerator	-11.81	-17.75	-18.42	-8.00	-6.01
	Bench	-7.51	-21.09	-13.70	-7.17	-3.69
Dihydrocodiene	Freezer	-19.87	-19.48	-19.93	-11.64	-9.2
	Refrigerator	-14.09	-22.33	-20.19	-6.59	-7.44
	Bench	-11.38	-21.19	-17.74	-8.46	-5.57
EDDP	Freezer	-18.32	-23.54	-20.39	-10.58	-8.80
	Refrigerator	-11.99	-20.53	-20.38	-12.22	-8.35
	Bench	-12.78	-21.78	-18.53	-11.74	-5.85
Fentanyl	Freezer	-18.11	-24.47	-20.87	-8.81	-7.27
	Refrigerator	-14.65	-19.27	-20.27	-13.30	-6.10
	Bench	-18.41	-22.27	-18.29	-8.89	-4.03
Heroin	Freezer	-28.32	-35.05	-30.73	-21.67	-20.61
	Refrigerator	-22.55	-32.83	-28.81	-20.59	-15.90
	Bench	-17.81	-23.78	-16.49	-4.83	-0.01
Hydrocodone	Freezer	-14.33	-19.88	-19.50	-6.59	-8.90
	Refrigerator	-9.56	-14.16	-14.68	-7.88	-5.50
	Bench	-10.33	-15.08	-16.08	-6.86	-4.35
Hydromorphone	Freezer	-16.91	-24.71	31.84	-11.27	-12.2
	Refrigerator	-14.59	-23.56	-17.95	-10.04	-4.97
	Bench	-12.93	-19.38	-15.61	-8.59	-6.16
MDEA	Freezer	-18.81	-24.82	-18.41	-12.72	-7.49
	Refrigerator	-11.91	-17.74	-22.08	-10.56	-4.81
	Bench	-8.89	-22.53	-19.94	-9.77	-3.30
MDMA	Freezer	-14.51	-19.48	-15.73	-10.33	-7.08
	Refrigerator	-11.64	-15.97	-15.28	-5.02	-0.82
	Bench	-8.83	-17.97	-12.79	-1.81	-1.71
Methadone	Freezer	-20.69	-29.44	-26.53	-16.65	-5.18
	Refrigerator	-17.46	-26.75	-26.40	-14.23	-9.52
	Bench	-14.09	-26.52	-23.89	-16.18	-3.76
Methamphetamine	Freezer	-13.45	-20.22	-15.43	-3.41	-1.63
	Refrigerator	-3.51	-16.37	-15.89	-0.03	-1.24
	Bench	-2.90	-17.40	-12.69	-1.28	3.29

		Percent Difference from Day 0 Area				
Analyte	**Storage**	Day 2	Day 3	Day 5	Day 6	Day 7
Morphine	Freezer	-16.12	-24.02	-18.20	-9.95	-12.89
	Refrigerator	-15.80	-21.77	-16.96	-11.19	-7.41
	Bench	-12.16	-18.74	-16.96	-7.07	-4.82
Norbuprenorphine	Freezer	-25.02	-25.78	-23.36	-17.47	-18.65
	Refrigerator	-22.02	-24.26	-23.73	-15.59	-12.46
	Bench	-19.37	-23.60	-20.59	-17.53	-14.16
Norfentanyl	Freezer	-12.93	-18.39	-16.76	-10.04	-2.06
	Refrigerator	-6.84	-15.05	-15.89	-0.21	1.80
	Bench	4.07	-19.53	-14.99	-3.38	6.71
Oxycodone	Freezer	-19.44	-20.40	-21.86	-9.64	-10.01
	Refrigerator	-12.44	-18.60	-18.77	-8.88	-9.70
	Bench	-14.30	-21.11	-18.42	-8.34	-4.54
Phencyclidine (PCP)	Freezer	-19.19	-25.69	-23.64	-12.56	-7.51
	Refrigerator	-11.95	-22.38	-22.06	-12.79	-12.35
	Bench	-10.75	-24.81	-18.68	-12.17	-5.18
Tramadol	Freezer	-18.17	-23.18	-23.61	-7.41	-5.53
	Refrigerator	-10.20	-20.57	-21.64	-9.33	-5.00
	Bench	-11.79	-23.19	-19.91	-8.58	2.61
Δ9 THC-COOH	Freezer	-16.04	-23.15	-19.83	-8.66	-16.41
	Refrigerator	-4.48	-24.86	-16.68	-12.32	-7.65
	Bench	-17.13	-19.88	-18.62	-13.60	-11.80

4.1.4. Process Sample Stability

The results of process sample stability should show an increase of percentage difference due to the peak areas decreasing over time in relation to the baseline peak for each analyte. The results from the single sample run on the instrument over a period of 3 days are seen in Table 14. The entries labeled "N/A" refer to a lack of peak identification by the TOF.

Table 14. Process sample stability results showing percent difference from baseline sample

	Percent Difference from Baseline	
Analyte	**24 hrs**	**48 hrs**
6-monoacetylmorphine (6-MAM)	-25.60%	-25.16%
7-aminoclonazepam	-1.71%	-2.51%
a-Hydroxyalprazolam	-2.24%	-0.22%

	Percent Difference from Baseline	
Analyte	**24 hrs**	**48 hrs**
Alprazolam	-0.46%	1.18%
Amphetamine	-4.36%	1.52%
Benzoylecgonine	-3.43%	-3.06%
Buprenorphine	-0.58%	-0.35%
Clonazepam	-4.21%	-4.41%
Cocaine	-3.15%	-0.76%
Codeine	1.77%	5.23%
Diazepam	1.04%	1.60%
Dihydrocodeine	2.50%	2.74%
EDDP	-3.30%	-10.88%
Fentanyl	-3.50%	-2.75%
Heroin	0.98%	-2.14%
Hydrocodone	0.65%	1.62%
Hydromorphone	10.16%	0.49%
MDEA	0.76%	-0.43%
MDMA	6.50%	N/A
Methadone	0.28%	-0.87%
Methamphetamine	-0.10%	-1.85%
Morphine	-0.18%	-3.79%
Norbuprenorphine	N/A	N/A
Norfentanyl	-8.59%	50.00%
Oxycodone	2.10%	2.81%
Phencyclidine (PCP)	-3.30%	-0.51%
Tramadol	0.82%	-1.29%
Δ9 THC-COOH	50.00%	50.00%

4.1.5 Interference

The first mix used in interference contained (-)-Nicotine, Acetaminophen, and Caffeine. The second mix contained Ibuprofen, Naproxen, and R,R(-)-Pseudoephedrine. Table 15 displays the percent difference of area between the two injections of each mix solution. A positive percentage suggests the analyte would still be present in the sample but the interference mix is causing an enhancement. A negative percentage suggests the analyte would still be present in the sample but the interference mix is causing a suppression.

Table 15. Interference mix percent differences between both sample injections. Positive percentage suggests ion enhancement while negative percentage suggest ion suppression.

Analyte	Mix 1 Percent Difference (%)	Mix 2 Percent Difference (%)
6-monoacetylmorphine (6-MAM)	21.85	9.23
7-aminoclonazepam	8.20	-3.15
a-Hydroxyalprazolam	7.56	-9.34
Alprazolam	17.42	11.92
Amphetamine	31.16	16.01
Benzoylecgonine	9.05	-2.64
Buprenorphine	0.98	-12.14
Clonazepam	28.72	9.23
Cocaine	3.37	-2.04
Codeine	10.52	7.92
Diazepam	-0.81	-5.49
Dihydrocodeine	3.27	7.95
EDDP	6.56	2.60
Fentanyl	24.65	3.45
Heroin	8.99	-0.33
Hydrocodone	15.22	9.80
Hydromorphone	6.81	-29.25
MDEA	13.49	7.07
MDMA	13.04	16.12
Methadone	2.82	-4.89
Methamphetamine	4.50	-0.64
Morphine	12.54	-3.57
Norbuprenorphine	27.08	9.55
Norfentanyl	12.10	-39.76
Oxycodone	25.85	17.28
Phencyclidine (PCP)	-9.14	-18.88
Tramadol	0.70	8.87
Δ9 THC-COOH	20.86	10.01

4.2 Correlation

For analysis of unknown samples, 20 samples previously screened with immunoassay and confirmed with quantitative LC-MS/MS were extracted using the previously described "Dilute and Shoot" method. All 20 samples were also run on a liquid chromatography-tandem mass spectrometer with a clinically validated confirmation method to determine if the time-of-flight was providing valid results. Table 16 displays the results for comparison.

Table 16. Unknown sample results for Immunoassay, LC-TOF, and LC-MS/MS analysis.

Sample	Immunoassay Result	TOF Result	LC-MS/MS Result
1	Negative	Negative	Negative
2	Negative	Oxycodone, Tramadol	Tramadol
3	Negative	Oxycodone, Tramadol	Tramadol
4	Opiate	Hydrocodone, Dihydrocodeine	Hydrocodone
5	Opiate	Oxycodone	Oxycodone
6	Negative	Oxycodone, Tramadol	Tramadol
7	Negative	Oxycodone	Oxycodone
8	Opiate	Oxycodone, Dihydrocodeine	Oxycodone
9	Negative	Oxycodone	Oxycodone
10	Opiate, Methamphetamine	Amphetamine, Dihydrocodeine, Hydrocodone	Amphetamine, Hydrocodone

Sample	Immunoassay Result	TOF Result	LC-MS/MS Result
11	Negative	Tramadol	Tramadol
12	Opiate	Dihydrocodeine, Oxycodone	Oxycodone
13	Opiate	Dihydrocodeine, Hydrocodone	Hydrocodone
14	Negative	Negative	Negative
15	Negative	Oxycodone, Tramadol	Tramadol
16	Opiate	Morphine	Morphine
17	Opiate	Hydrocodone	Hydrocodone
18	Negative	Tramadol	Tramadol
19	Opiate	a-Hydroxyalprazolam, Dihydrocodeine, Hydrocodone	a-Hydroxyalprazolam, Hydrocodone, Hydromorphone
20	Opiate	Hydrocodone, Hydromorphone	Hydrocodone, Hydromorphone,

4.3 Chromatograms

Example chromatograms are shown below in Figures 3 and 4. Figure 3 displays an entire chromatogram for sample 16. Figure 4 displays an individual analyte, Morphine.

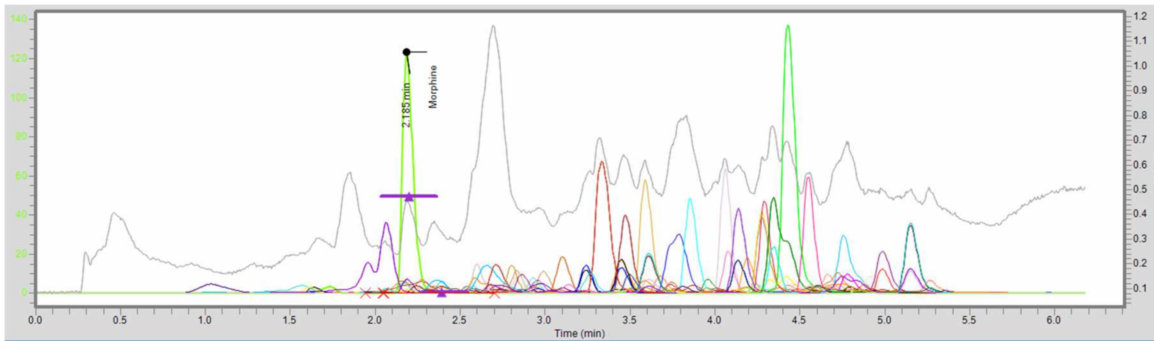

Figure 3. Overall chromatogram for sample 16 in the correlation study.

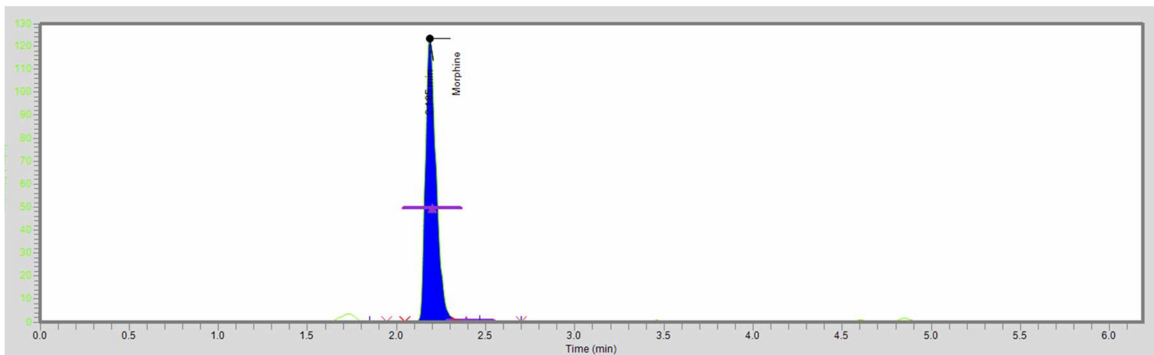

Figure 4. Chromatogram displaying the individual analyte, morphine, for sample 16.

4.4 Statistics

The number of true positives, true negatives, false positives, and false negatives were determined for both immunoassay results and time-of-flight results for each drug class A true positive (TP) is a result that detects the presence of a drug that is truly present. A true negative (TN) is a result that does not detect a drug when a drug is not present. A false positive (FP) is a result that detects a drug when the drug is not present. A false negative (FN) is a result that does not detect a drug when a drug is present.

The performance of each instrument, EasyRA® and AxION® 2, was done by evaluating the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. Sensitivity refers to the number of TP as a percent of all positives. Specificity refers to the number of TN as a percent of all negatives. Positive predictive value (PPV) is the number of samples with positive results that are TP expressed as a percentage. Negative predictive value (NPV) is the number of samples with negative results that are TN expressed as a percentage. Accuracy is the number of all correctly identified results, classified in a percentage. Table 17 shows the total number of TP, TN, FP, and FN for both the time-of-flight instrument and the immunoassay instrument. Sensitivity, specificity, PPV, NPV, and accuracy results can be seen in Table 18. False negatives for immunoassay include Tramadol and benzodiazepine data, which were present in the specimens but not tested for in the immunoassay panels.

Table 17. Total number of true positives, true negatives, false positives, and false negatives based on drug class for the time-of-flight and immunoassay.

	TP	TN	FP	FN
TOF	20	156	4	1
IA	11	140	0	9

Table 18. Sensitivity, specificity, PPV, NPV, and accuracy results of immunoasssay and time-of-flight

TOF		IA	
sensitivity	0.95	sensitivity	0.55
specificity	0.98	specificity	1.00
PPV	0.83	PPV	1.00
NPV	0.99	NPV	0.94
accuracy	0.97	accuracy	0.94

CHAPTER V

DISCUSSIONS AND CONCLUSIONS

5.1 Validation

The validation of this method was conducted under the standard practices for qualitative method validation created by the Scientific Working Group for Forensic Toxicology (SWGTOX). Most of the results were within recommended guidelines. Those that were not include matrix effects for α-Hydroxyalprazolam (130.35%), Amphetamine (307.54%), EDDP (143.90%), MDMA (61.40%), and Morphine (41.61%). The recommended percentage for matrix effects is +/- 30%. For sample stability, the inconsistencies of percentages in the analysis were hypothesized to be due to an improvement of the instrument's performance over the first few days of use after a period of non-use. All samples appeared to be stable for up to seven days in all evaluated storage conditions.

5.2 Correlation

When the time-of-flight instrument and the immunoassay instrument were compared to the liquid chromatography-tandem mass spectrometer at OSU-CLS, multiple false and true results were found. Based on the drug classes, the EasyRA® produced no false positive results, nine false negative results, eleven true positive results, and 140 true negative results for the drug class reference standards used. The AxION® 2 produced four false positive results, one false negative

result, twenty true positive results, and 156 true negative results. For the LC-TOF, sensitivity was calculated to be 95%, specificity was 98%, PPV was 83%, NPV was 99%, and accuracy was 97%. For the immunoassay, sensitivity was 55%, specificity was 100%, PPV was 100%, NPV was 94%, and accuracy was 94%. The two instruments were found to be statistically different based on a categorical statistical analysis performed in Microsoft® Office Excel.

5.3 Liquid Chromatography Time-of-Flight Mass Spectrometer

This research developed and validated a pain panel screening method on a liquid chromatography time-of-flight mass spectrometer. The method was applied to twenty unknown urine samples which were then compared to a liquid chromatography-tandem mass spectrometer to further verify the results found on the LC-TOF. LC-TOF was determined to have a higher sensitivity and overall accuracy than immunoassay. It was able to identify certain drugs present in a sample as opposed to a drug class as found with the immunoassay instrument. It was also determined to have a lower false negative rate than immunoassay.

5.4 Solutions

Immunoassay instruments use certain calibrators to identify drug classes. These calibrators are specific and if a calibrator is not being used for a certain drug, the screen will produce a negative result. The immunoassay used in this study did not use calibrators specific to Tramadol. Each Tramadol positive found in the LC-TOF screen had a negative screen for the immunoassay. Not having every possible calibrator could lead to missed analytes. Having these calibrators and having to constantly purchase and replace them leads to a costly investment of the immunoassay instrument. LC-TOF does not require these stock calibrators but laboratory-made QC calibrators of a low and high concentration, a calibrator of a LLOD concentration, mobile phase, and needle rinse for the instrument. Certified drug standards need to be purchased;

however, a single vial of one milliliter can last for an extended period of time and has a shelf life of multiple years upon purchase.

5.5 Conclusions

It was determined that the LC-TOF is more sensitive than immunoassay, and it was possible to design the LC-TOF qualitative screen to look for many specific compounds that would require quite a few immunoassay panels. While the positive predictive value was not ideal at only 83% (due to false positives), it seemed that this was better than the false negatives associated with immunoassay. In terms of costs, the TOF instrument has a higher initial cost and maintenance, but the reagents are much less than immunoassay. So, while it was felt that LC-TOF performed better than immunoassay, laboratories will need to determine which screening method is best suited for their needs.

In the future, a quantitative method can be developed and validated to complement the qualitative method of the LC-TOF. As of now, LC-TOF is not typically used for quantitation. It is not considered the ideal instrument for that application due to its difficulties of accurate concentrations and the possibility of saturation of the detector of the high concentration calibrators. An extended drug panel could be created to further identify drugs present within samples. Some of these drugs could include novel psychoactive substances such as fentanyl analogs, spice compounds, and cathinones, also known as bath salts.

In summary, LC-TOF could be an acceptable alternative to immunoassay screening. It is more sensitive, can identify novel drugs, and provide greater confidence in screening results.

REFERENCES

1. Cone EJ, Caplan, YH. Urine Drug Testing of Chronic Pain Patients: Licit and Illicit Drug Patterns. Journal of Analytical Toxicology. 2008;32(8):530-543. doi:https://doi.org/10.1093/jat/32.8.530

2. Christo PJ, Manchikanti L, Ruan X, et al. Urine Drug Testing In Chronic Pain. Pain Physician. 2011:123-143.

3. Heit HA, Gourlay DL. Urine Drug Testing in Pain Medicine. Journal of Pain and Symptom Management. 2004;27(3):260-267. doi:https://doi.org/10.1016/j.jpainsymman.2003.07.008

4. Florete Jr. OG. Urine Drug Testing in Pain Management. Practical Pain Management. https://www.practicalpainmanagement.com/treatments/pharmacological/opioids/urinary-drug-testing-pain-management?page=0,1. Published January 5, 2012.

5. Plumb RS, Stumpf CL, Granger JH, Castro-Perez J, Haselden JN, Dear GJ. Use of liquid chromatography/time□of□flight mass spectrometry and multivariate statistical analysis shows promise for the detection of drug metabolites in biological fluids. Rapid Communications on Mass Spectrometry. 2003;17(23):2632-2638. doi:10.1002/rcm.1250

6. Lum G, Mushlin B. Urine Drug Testing: Approaches to Screening and Confirmation Testing. Lab Med. 2004;35(6):368-373. doi:10.1309/QHJCKA4235EGPEGF

7. Pain and Addiction Management-Drug Testing. ARUP Consult. https://arupconsult.com/content/pain-and-addiction-management. Published June 2018.

8. Levine B. Immunoassay. In: *Principles of Forensic Toxicology*. 4th ed. United States: AACC Press; 2015:149-169.

9. Skoog D. Principles of Instrumental Analysis. In: 6th ed. Cengage Learning; 2007.

10. Pelander A, Ojanperä I, Laks S, Rasanen I, Vuori E. Toxicological Screening with Formula-Based Metabolite Identification by Liquid Chromatography/Time-of-Flight Mass Spectrometry. Anal Chem. 2003;75(21):5710-5718. doi:10.1021/ac030162o

11. Fessessework G, Shahreza S, Walterscheid JP, et al. Validation of LC–TOF-MS Screening for Drugs, Metabolites, and Collateral Compounds in Forensic Toxicology Specimens. Journal of Analytical Toxicology. 2013;37(1):17-24. doi:https://doi.org/10.1093/jat/bks084

12. Electronic Code of Federal Regulations. e-CFR CLIA; 2018. https://www.ecfr.gov/cgi-bin/textidx?SID=1248e3189da5e5f936e55315402bc38b&node=pt42.5.493&rgn=div5#se42.5.493_1845.

13. Ojanpera S. Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time□of□flight mass spectrometry. Rapid Communications on Mass Spectrometry. 2006;20(7):1161-1167.

14. Brenton AG, Godfrey AR. "Accurate Mass Measurement: Terminology and Treatment of Data." *American Society for Mass Spectrometry* 21 (June 19, 2010): 1821–35. https://doi.org/doi:10.1016/j.jasms.2010.06.006.

15. Ojanpera I, Kolmonen M, Pelander A. Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control. *Analytical and Bioanalytical Chemistry*. 2012;403(5):1203-1220. doi:https://doi.org/10.1007/s00216-012-5726-z

16. Chindarkar, Nandishkor S. Comparison of Different Time of Flight-Mass Spectrometry Modes for Small Molecule Quantitative Analysis. *JAT*. 2015;39(9):675-685.

17. Partridge E, Trobianni S, Stockham P, Scott T, and Kostakis C. “A Validated Method for the Screening of 320 Forensically Significant Compounds in Blood by LC/QTOF, with Simultaneous Quantification of Selected Compounds.” *Journal of Analytical Toxicology*, 2018, 1–12. https://doi.org/doi: 10.1093/jat/bkx108.

18. Saleh A, Stephanson NN, Granelli I, Villén T, Beck O. Evaluation of a direct high-capacity target screening approach for urine drug testing using liquid chromatography–time-of-flight mass spectrometry. *Journal of Chromatography B*. 2012;909:6-13. doi:10.1016/j.jchromb.2012.10.006

19. Orsolini L, Papanti D, Vecchiotti R, Valchera A, Corkery J, Schifano F. Novel psychoactive substances. *European Psychiatry*. 2016;33:S59-S60. doi:10.1016/j.eurpsy.2016.01.945

20. Zawilska JB, Andrzejczak D. Next generation of novel psychoactive substances on the horizon – A complex problem to face. *Drug and Alcohol Dependence*. 2015;157:1-17.

21. Pasin D, and Bidney S. “Analysis of New Designer Drugs in Post-Mortem Blood Using High-Resolution Mass Spectrometry.” *Journal of Analytical Toxicology* 39 (2015): 163–71. https://doi.org/doi:10.1093/jat/bku144.

VITA

Paige Elaine Fisk

Candidate for the Degree of

Master of Science

Thesis: COMPARISON OF HIGH-RESOLUTION MASS SPECTROMETRY WITH IMMUNOASSAY IN QUALITATIVE URINE DRUG SCREENING

Major Field: Forensic Science

Biographical:

Education:

Completed the requirements for the Master of Science in Forensic Sciences with specialization in Forensic Toxicology at Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma in May, 2019.

Completed the requirements for the Bachelor of Science in Forensic Chemistry at University of Central Missouri, Warrensburg, Missouri in December 2016.

Experience:

Lab Technician at OSU-Clinical Laboratory Services
August 2017 – Present

Chemistry Department Stockroom Employee at University of Central Missouri
August 2014 – July 2017

Professional Memberships:

Student Member of Society of Forensic Toxicologists (SOFT)
January 2018 – Present

Student Member of American Chemical Society (ACS) at University of Central Missouri
August 2014 – December 2016