DEVELOPMENT OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) METHODS FOR EXPLORATION OF CURRENCY CONTAMINATION WITH CONTROLLED SUBSTANCES

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NOMENCLATURE

6-AM	6-acetylmorphine
ACS	American Chemical Society
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
BEP	Bureau of Engraving and Printing
carboxy-THC	11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
CBD	cannabidiol
CBN	cannabinol
CE	capillary electrophoresis
d-	dextro
DC	direct current
DESI	desorption electrospray ionization
DESI-MS	desorption electrospray ionization mass spectrometry
DI	desorption ionization
ECL	electrochemiluminescence
EDDP	2-ethylidine-1,5dimethyl-3,3-diphenylpyrrolidine perchlorate
ESI	electrospray ionization
FDA	Food and Drug Administration

FT	Fourier transform
GC	gas chromatography
GC-MS	gas chromatography mass spectrometry
GC-NPD	gas chromatography with nitrogen-phosphorous detection
GLORIA	gold-labeled, optically read, rapid immunoassay
HPLC	high performance liquid chromatography
IAEA	International Atomic Energy Agency
ID1	identification ratio 1
ID2	identification ratio 2
IMS	ion-mobility spectrometry
l-	levo
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LLOQ	lower limit of quantitation
LOD	limit of detection
LOQ	limit of quantitation
LSD	lysergic acid diethylamide
m/z	mass-charge ratio

MDMA	3,4-methylenedioxymethamphetamine
MDP2P	1-(3,4-methylenedioxyphenyl)-2-propanone
Meth	methamphetamine
mg	milligrams
ml	milliliters
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
ng	nanogram
OH-THC	11-hydroxy-Δ^9-tetrahydrocannabinol
OSU	Oklahoma State University
PCP	phencyclidine
Q1	first quadrupole
Q2	second quadrupole
Q3	third quadrupole
Quant Ratio	quantitation ratio
r^2	r squared value, correlation coefficient
RF	radiofrequency
SIM	selected ion monitoring

SPE	solid-phase extraction
TD	thermal desorption
TD-MS/MS	thermal desorption tandem mass spectrometry
THC	Δ^9-tetrahydrocannabinol
TOF	time of flight
U.S.	United States
ULOQ	upper limit of quantitation
UNODC	United Nations Office on Drugs and Crime
μg	microgram
μl	microliters

Chapter I. Introduction

Drug use is a growing problem worldwide, with an estimated 172 to 250 million people worldwide using a controlled substance at least once in 2007 (Sandeep, et al., 2009). Due to the high number of drug users, several studies have looked at currency contamination with different drugs of abuse, especially cocaine, to establish a link between currency suspected in drug trafficking and currency in the general circulation. More importantly, due to the general contamination of currency with cocaine, a United States Court of Appeals ruled in 1994 that a drug dog hit on currency was not sufficient probable cause to confiscate the currency as having been involved in cocaine trafficking (Jenkins, 2001). Therefore, there is considerable interest in determining the current extent of contamination, and if there is a value above which the currency can be said to have had direct involvement with cocaine abuse or trafficking.

Contamination of currency has been proposed through several different mechanisms. These mechanisms include contact with the drug, contaminated hands, objects or surfaces, use of currency for snorting, and rollers in counting machines found in banks and post offices (Jenkins, 2001). Inks on currency provide a sticky surface where the controlled substances adhere, allowing for the eventual detection of contaminated currency. Drugs may also become physically trapped between fibers,

which spread apart over time, or even chemically bind to the cotton linen fibers (Sleeman, Burton, Carter, Roberts, & Hulmston, 2000).

Several methods have been used to detect drugs on currency, including different extraction methods as well as several different analytical methods. Extraction methods include vacuum sampling, thermal desorption, and solvent extraction. Analytical methods include immunoassays, thermal desorption tandem mass spectrometry (TD-MS/MS), ion-mobility spectrometry (IMS), gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE) (Armenta & de la Guardia, 2008). Desorption electrospray ionization mass spectrometry (DESI-MS) (Keil, et al., 2007) and Raman spectroscopy (Frederick, Pertaub, & Kam, 2004) have also been used.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is an analytical technique used in many different aspects of forensic science wherein the physical separation capabilities of the LC are combined with the ability of a tandem MS to ionize and identify ions based on their mass-charge ratio (*m/z*) (Skoog, Holler, & Crouch, 2007). Liquid chromatography is used to separate compounds in a sample chromatographically before they enter the ion source and mass spectrometer (Levine, 2006). The mass spectrometer consists of three main components: ionization, ion separation, and ion detection (P.J. Taylor, 2006). Optimization of these components is critical to the success of the method. LC-MS/MS is able to provide precise, accurate, sensitive, and selective results.

In this research, an LC-MS/MS method was initially developed to quantify cocaine contamination on currency based on prior work by Jourdan and Donnelly. The Oklahoma State University (OSU) LC-MS/MS method was then adapted to quantify codeine, heroin, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, and morphine. For the study, currency was collected from 35 cities throughout the United States (2,100 bills) and from 32 foreign cities (243 bills) for a combined total of 2,343 bills tested. Vacuum sampling was used to extract analytes from the bills prior to analysis by LC-MS/MS. Results from this quantitative study were combined with previous data collected by Jourdan and Donnelly, who only looked at cocaine contamination on currency. When combined, a total of 4,176 U.S. bills were examined for cocaine contamination.

Chapter II. Review of Literature

2.1. Currency Contamination

Use of controlled substances is a growing problem worldwide. According to the United Nations Office on Drugs and Crime (UNODC) 2009 World Drug Report, an estimated 172 to 250 million people worldwide used a controlled substance at least once in 2007. Between 15 and 21 million people used opiates, 16-21 million used cocaine, 143-190 million used cannabis, and 16-51 million used amphetamine-type stimulants (Sandeep, et al., 2009). These numbers include both casual users, who may have used drugs once the entire year, as well as heavy drug users. In 2000, Americans spent an estimated $65 billion on illicit controlled substances. Approximately $36 billion was spent on cocaine, $11 billion on marijuana, $10 billion on heroin, $5.4 billion on methamphetamine, and $2.4 billion on other controlled substances (Spiess, 2003). Due to the growing trend in the use of controlled substances, contamination of currency with these substances, especially cocaine, has been a topic of study in recent years.

Contamination of currency can occur through several different mechanisms. Contact with the drug itself, contaminated hands handling currency, contaminated objects or surfaces coming in contact with currency, use of currency to snort drugs, and counting

machines found in banks and post offices have all been proposed as methods in which currency becomes contaminated (Jenkins, 2001). Counting machines become contaminated with drugs as tainted currency passes through the rollers. The contaminated rollers then transfer drugs to subsequent bills that pass through the machine, thus contaminating a large portion of the bills in circulation.

There is an important implication that has followed the contamination of currency with cocaine. In 1994, a U.S. Court of Appeals ruled that detection of a drug on currency by a drug dog was not sufficient probable cause to confiscate the currency due to suspicion of its having been involved in cocaine trafficking (Jenkins, 2001). Therefore, there is considerable interest in determining the current extent of contamination, and if there is a threshold quantity of drug, above which the currency can be said to have had direct involvement with cocaine abuse or trafficking. In order to accomplish this, the general level of currency contamination must be known.

There have been several proposed mechanisms of how controlled substances become retained on currency. One idea is that the ink on the currency never fully dries. This provides a sticky surface for the controlled substances, as well as human oils, dirt and grime, to adhere to. Another idea is that the currency fibers spread apart over time, allowing small particles to become trapped within the fibers. It has also been postulated that the fibers, made of cotton linen, may bind chemically to the controlled substances (Sleeman, et al., 2000). All of these mechanisms would allow controlled substance

residues to adhere to currency for significant periods of time, even with everyday use of the bill.

The average life span of currency might play a factor in the differences in the percent contamination seen throughout the studies. For U.S. currency, the average life span of a $1 bill is 21 months, a $5 bill is 16 months, a $10 bill is 18 months, a $20 bill is 24 months, a $50 bill is 55 months, and a $100 bill is 89 months ("Frequently Asked Questions: Currency: Notes and Coins," 2010). The Bureau of Engraving and Printing (BEP) produces approximately 38 million bills a day, valued at approximately $750 million ("FAQs: Currency: Production & Circulation," n.d.). At any given time, more than $400 billion of US paper currency is in circulation (Furton, Hsu, Luo, Alvarez, & Lagos, 1997). Changes in drug consumption over the last several years could have caused highly contaminated bills to be removed from circulation, thus reducing the amount of contamination observed. Studies should be carried out on a regular basis to account for this turnover of paper currency as well as the variability of contamination as a function of time.

Cocaine is the most frequently encountered controlled substance on currency. This is due to the common practice of insufflation or snorting of the drug through the nose, which often involves the use of paper currency. Cocaine is also easier to isolate from currency than other controlled substances. The small size of cocaine crystals allow it to more readily adhere to currency unlike heroin and amphetamines whose crystals are much larger than cocaine (Carter, Sleeman, & Parry, 2003). Cannabis generally appears

as either dried leaves or resin, which can be deposited on currency, but are not often retained. Most other controlled substances (MDMA, morphine, etc.) are commonly found in tablet or pill form and are less likely to yield particulate matter that will adhere to currency (Sleeman, et al., 2000). Heroin and THC are readily hydrolyzed, which may be why they are difficult to detect (Carter, et al., 2003). Since cocaine seems to have the best ability to adhere to currency, it is the most often examined controlled substance.

There were scarcely any references in the literature on analytical procedures for the detection of controlled substances, specifically cocaine, on currency prior to 1994. As mentioned briefly earlier, these studies began in response to a 1994 decision by the Ninth Circuit Court of Appeals in U.S. v. U.S. Currency (Alexander) 39 F.3d 1039. The court acknowledged wide-spread contamination of currency by cocaine, and therefore said a drug-dog hit on a large sum of money was not longer sufficient probable cause to seize the money as being part of drug trafficking (Jenkins, 2001). The courts now want proof that the banknotes being seized are significantly different in terms of drug contamination from those found in general circulation in the region in which the bills were seized. It is not enough just to say qualitatively that a drug is present, rather it is important to know quantitatively how much is present. Even then, the amount will need to be compared with other bills to determine the significance of the quantity.

2.1.1. Extraction Methods

Several different methods have been used to extract controlled substances from currency and analyze samples. These methods include vacuum sampling, direct thermal

desorption (TD), solvent extraction, and solvent extraction coupled with solid-phase extraction (SPE) (Armenta & de la Guardia, 2008).

2.1.1.1. Vacuum Sampling

In vacuum sampling, bills are vacuumed with a portable vacuum cleaner containing a filter at the end of the hose to trap the analyte. Since the air initially passes through the filter before entering into the vacuum cleaner itself, it is assumed that any compounds that are identified on the filter have come from the bill or other object being examined (Sleeman, et al., 2000). Vacuuming is generally considered to be semi-quantitative method due to the low efficiency of removing the analyte from the sample and is often considered unsuitable for analyzing individual bills (Armenta & de la Guardia, 2008). Because of this, multiple bills, generally ten or more, make up each vacuum filter sample. Even though it will not extract all of the drug present on a bill, vacuum sampling is popular because it allows for on-site sampling and can positively identify if a drug is present on a set of bills.

2.1.1.2. Thermal Desorption

Direct thermal desorption (TD) is a method which utilizes heat to increase the volatility of contaminants so that they can be removed from the matrix, which in this case is the bill itself. Individual bills are inserted between two heat blocks (285°C) attached to the front of a mass spectrometer to thermally desorb any sufficiently volatile material from the bill. The bills are held between the blocks for approximately one second to allow sufficient desorption of any drug from the currency. The signal is then allowed to

return to baseline before the next bill is inserted, allowing for analysis of 50 bills in a four-minute period (Dixon, Brereton, Carter, & Sleeman, 2006). Direct TD allows for individual bills to be analyzed rather than analyzing multiple bills to generate a sample. This method is approximately 1,000 times more sensitive than vacuum sampling, but the disadvantage to this method is that it destroys the analyte and the bill cannot be retested (Sleeman, et al., 2000). However, analyzing only a small portion of the bill could resolve this problem.

2.1.1.3. Solvent Extraction

Solvent extraction of controlled substances from currency can be performed using organic solvents or dilute acids. The organic solvents and dilute acids are used to remove the drugs from the currency, putting them into a liquid solution that can be analyzed. Solvents such as chloroform, methanol, acetonitrile, ethanol, and others have been used to extract controlled substances from bills (Armenta & de la Guardia, 2008). Using this extraction method, a bill is placed in an extraction solvent and vortexed for several minutes to remove all residues from the bill. The liquid is then analyzed using solid phase extraction (SPE) followed by gas chromatography mass spectrometry (GC-MS) (Jenkins, 2001) or centrifuged to isolate the upper layer. The upper layer is then evaporated to dryness and reconstituted in ethyl acetate for analysis by GC-MS (Armenta & de la Guardia, 2008).

2.1.1.3.1. Solid Phase Extraction

As mentioned previously, solid-phase extraction (SPE) can be combined with solvent extraction to remove co-extracted compounds, such as inks, oils, fats, greases, and cosmetics commonly found on currency, from the samples being analyzed. Although SPE is a very useful method for sample clean-up and is extremely beneficial when analyzing biological samples, it is basically unnecessary for the analysis of contaminated currency (Armenta & de la Guardia, 2008) since the solvent can be evaporated to concentrate the sample.

2.1.2. Analytical Methods

Several analytical methods are published in the literature for the detection and quantitation of controlled substances on currency. Some analytical methods are able to directly analyze currency or solvent extracts, including immunoassays, thermal desorption tandem mass spectrometry (TD-MS/MS), and ion-mobility spectrometry (IMS). Other methods use separation techniques to analyze currency, including gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE) (Armenta & de la Guardia, 2008). Desorption electrospray ionization (DESI) is another method that has been used to directly analyze currency (Keil, et al., 2007). Raman spectroscopy can also be used in controlled substance contamination (Frederick, et al., 2004).

2.1.2.1. Immunoassay

Immunoassays have been used to detect controlled substances on currency. An example of this type of test is the gold-labeled, optically read, rapid immunoassay (GLORIA). This method uses disposable drug wipes, based on an immunochemical detection process that uses antigen gold conjugates, to detect opiates, cannabis, cocaine, and amphetamines on currency. The test takes three minutes and can detect nanogram quantities of the analyte. A positive test results in a pink coloration, which is stable for several months after analysis (Sleeman, et al., 2000). As with any immunoassay, selectivity is an issue as there can be unintended cross-reactivity of other analytes with the antibody. It is generally only as a preliminary screen or as a secondary method to support findings from a previously used method.

2.1.2.2. Thermal Desorption Tandem Mass Spectrometry

Thermal desorption tandem mass spectrometry (TD-MS/MS) is a common method used in currency contamination analysis due to simple sample preparation. Filters from vacuum sampling or the bill itself can be used with TD-MS/MS. Tandem mass spectrometry is described in more detail in section 2.3.2.

2.1.2.3. Ion Mobility Spectrometry

Ion-mobility spectrometry (IMS) can be used to detect controlled substances on currency by collecting a filter sample, heating it to vaporization, and ionize it with electrons emitted by a ^{63}Ni source. The ions produced drift through an electrical field as they make their way to the detector, which identifies the substances based on their drift

time, or amount of time it took the ions to reach the detector. Large numbers of bills can be analyzed using samples obtained through vacuum sampling. Individual bills can also be sampled by collecting a surface swab and inserting it into the vaporizer unit or by inserting the bill directly. IMS is capable of detecting nanogram amounts of controlled substances on currency and is ideal for use in the field. Drawbacks to this method are that drift time in IMS is not as specific as other mass spectrometry procedures making identification difficult, the desorption procedure is not completely effective and it is difficult to obtain precise quantitative results (Armenta & de la Guardia, 2008).

2.1.2.4. Gas Chromatography with Mass Spectrometry

Gas chromatography mass spectrometry (GC-MS) is the most frequently used method for the detection of controlled substances on currency. Gas chromatography can also be combined with nitrogen-phosphorous detection (GC-NPD) and tandem mass spectrometry (GC-MS/MS). Electron impact mass detectors are most commonly used with GC for the detection of controlled substances on currency, but positive chemical ionization mass detectors have also been proposed for use as well. GC-MS and GC-MS/MS are primarily used in currency contamination studies due to the high sensitivity, selectivity, reliability, and quantitative recovery they provide. They also allow for detection at extremely low levels of contaminants. GC-MS/MS can provide a 0.15 ng/bill limit of detection. Drawbacks to any method using GC is that time for sample preparation and analysis is required (Armenta & de la Guardia, 2008).

2.1.2.5. Liquid Chromatography with Mass Spectrometry

Liquid chromatography mass spectrometry (LC-MS) is not as commonly used in currency contamination studies, although it offers several advantages over GC-MS. Extraction procedures used for LC-MS are typically less extensive than for GC-MS, derivatization is not required which saves both time and money, and the LC is able to handle compounds that are not stable at high temperatures and are not well resolved by GC-MS (Levine, 2006). The first use of liquid chromatography mass spectrometry (LC-MS) for the quantification of controlled substances on currency was reported by Jourdan and Donnelly in 1995 in their effort to quantify cocaine on currency (Armenta & de la Guardia, 2008). Liquid chromatography tandem mass spectrometry (LC-MS/MS) can also be used and will be discussed in greater detail in section 2.3.2.

2.1.2.6. Capillary Electrophoresis

Capillary electrophoresis (CE) with electrochemiluminescence (ECL) detection has been used to detect cocaine and heroin on Chinese currency. CE lacks the specificity that MS-based methodologies provide (Armenta & de la Guardia, 2008) since the identification is based solely on migration time through the capillary, which is analogous to retention time in chromatography.

2.1.2.7. Desorption Electrospray Ionization (DESI) with Mass Spectrometry

Desorption electrospray ionization (DESI) has been used in combination with miniature hand-held mass spectrometers to detect controlled substances on currency

(Keil, et al., 2007). DESI is a combination of electrospray ionization (ESI) and desorption ionization (DI) methods. In this method, ionized water molecules are directed onto a surface for analysis. The ionized water molecules desorb and ionize surface molecules, like controlled substances, and bring them into the mass spectrometer down electromagnetic field gradients.

2.1.2.8. Raman Spectroscopy

Raman spectroscopy allows for the non-invasive analysis of controlled substance crystals on currency. It is a useful method because no sample pre-treatment is necessary. While it is non-destructive, it is not quantitative and will only detect surface contamination since it depends on wavelength changes in light that interacts with analytes. Raman spectroscopy is not as sensitive as mass spectrometric methods, and only highly contaminated bills will be detected. Fourier transform (FT) Raman spectroscopy with an infrared light source has been used to examine amphetamine, cocaine, and heroin, codeine, and morphine. It has also been used to differentiate between methamphetamine and amphetamine. A study performed by Frederick, Pertaub, and Kam (2004) showed that it is possible to identify single crystals in a heterogeneous mixture with dispersive Raman spectroscopy interfaced through a microscope.

2.1.3. Cocaine Contamination Studies

Several studies have examined the presence of cocaine on currency. The following are a few of the more notable studies done in this field. In 1989, Hearn analyzed 135 banknotes from banks in 12 cities across the United States. He found 97%

of the bills were contaminated with cocaine with an average of 7.3 μg per bill (Jenkins, 2001).

In 1995, Jourdan and Donnelly used ion mobility spectrometry, in combination with vacuum sampling, to screen ten bill aliquots from ten areas across the United States for cocaine contamination. If the screen indicated cocaine, a second vacuum sample was taken of the bills. Cocaine was able to be detected in nanogram amounts, but was not detected on bills from all areas. They determined the upper limit of background cocaine contamination on currency to be 13 ng since more than 95% of the bills in the study had cocaine levels less than that amount. Jourdan and Donnelly also examined bills from 40 cases. They found that more than 65% of the case submissions contained cocaine at levels less than 30 ng per bill and concluded that any bill containing at least 100 ng of cocaine was statistically different from background levels seen on currency in general circulation (Jenkins, 2001).

Oyler, Darwin, and Cone (1996) examined 136 $1 bills from several cities throughout the United States for the presence of cocaine using methanol solvent extraction, SPE, and GC-MS. They found that 79% of the bills analyzed were contaminated with cocaine in amounts greater than 0.1 μg and 54% were contaminated greater than 1.0 μg. The highest level of cocaine they detected on a $1 bill was 1327 μg.

Esteve-Turrillas *et al.* (2005) analyzed 16 euro banknotes for the presence of cocaine. A solvent extraction was performed using methanol and a GC-MS/MS was used for analysis. Results showed that all 16 banknotes were contaminated with cocaine at

levels from 1.25-889 μg. Another study of 45 euro banknotes was performed by Bones (2007) using a technique involving chromatography and mass spectrometry. He found 100% of the banknotes were positive for cocaine. 62% were contaminated with cocaine at levels greater than 2 ng/bill and 5% were contaminated at levels greater than 200 ng/bill.

Di Donato, Santos Martin, and De Martinis (2007) examined 46 Brazilian banknotes from nine cities. Cocaine was extracted from the banknotes using deionized water. Ethyl acetate was added to the aqueous phase before the tubes containing the solution were centrifuged and the organic layer was removed. The organic layer was evaporated to dryness with N_2 and reconstituted with methanol. Analysis was performed on a GC-MS. Results showed 93% were positive for cocaine with a concentration range of 2.38-275.10 μg/bill.

2.1.4. Illicit Drug Contamination Studies

Studies have also looked at controlled substances other than cocaine on currency. Jenkins (2001) analyzed ten randomly collected $1 bills from five cities (50 total) for cocaine, heroin, 6-acetylmorphine (6-AM), morphine, codeine, methamphetamine, amphetamine, and phencyclidine (PCP) using solvent extraction with acetonitrile, SPE, and analysis by GC-MS. She reported 92% of the bills were positive for cocaine with a concentration range of 0.01-922.72 μg/bill. Heroin was detected on seven bills with a concentration range of 0.03-168.50 μg/bill. 6-AM was detected on three bills; methamphetamine was detected on three bills; amphetamine was detected on one bill;

and PCP was detected on two bills in amounts of 0.78 and 1.87 μg/bill. Codeine was not detected on any of the bills analyzed.

Carter, Sleeman, and Parry (2003) used bundles of paper, similar to sterling banknotes used in the British Islands, to examine cocaine, heroin, tetrahydrocannabinol (THC) and 3,4-methylenedioxymethylamphetamine (MDMA) contamination from the counting process, both by counting machines and by hand using TD-MS/MS. They were unable to detect heroin, THC, or MDMA, but were able to detect nanogram amounts of cocaine on the paper.

Lavins, Lavins, and Jenkins (2004) examined cannabis contamination of U.S. and foreign currency using solvent extraction with acetonitrile and analysis by GC-MSD. They analyzed 125 $1 U.S. banknotes and found THC present on 1.6% of the bills, cannabinol (CBN) on 10.31%, and cannabidiol (CBD) on 1.6%. 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-THC) and 11-hydroxy-Δ^9-tetrahydrocannabinol (OH-THC) were not detected. 40 foreign currency banknotes were examined. THC and CBN were present on 22.5% of the notes, but CBD, carboxy-THC, and OH-THC were not detected.

Ebejer, Brereton, Carter, Ollerton, and Sleeman (2005) examined diacetylmorphine, the major active component of heroin, on sterling banknotes using TD-MS/MS. They found that 2-3% of banknotes in circulation were contaminated with heroin.

Dixon, Brereton, Carter, and Sleeman (2006) examined 7,157 sterling banknotes as background and 4,826 case study banknotes for the presence of cocaine, heroin, tetrahydrocannabinol (THC) and 3,4-methylenedioxymethylamphetamine (MDMA) using TD-MS/MS. They determined it was possible to effectively discriminate between background and case study banknotes. Background samples were correctly classified 96.8% of the time, while case study samples were correctly classified 89.37% of the time.

Xu, Gao, Wei, Du, and Wang (2006) examined 100 Chinese banknotes exposed to cocaine and heroin using capillary electrophoresis with an electrochemiluminescence detection system. All banknotes were soaked together in acetic acid to dissolve any drugs present on the note prior to analysis. By using this method, they were able to determine whether the banknotes were contaminated with illicit drugs as well as avoid fluorescence disturbance from the notes and did not cause damage to the bills.

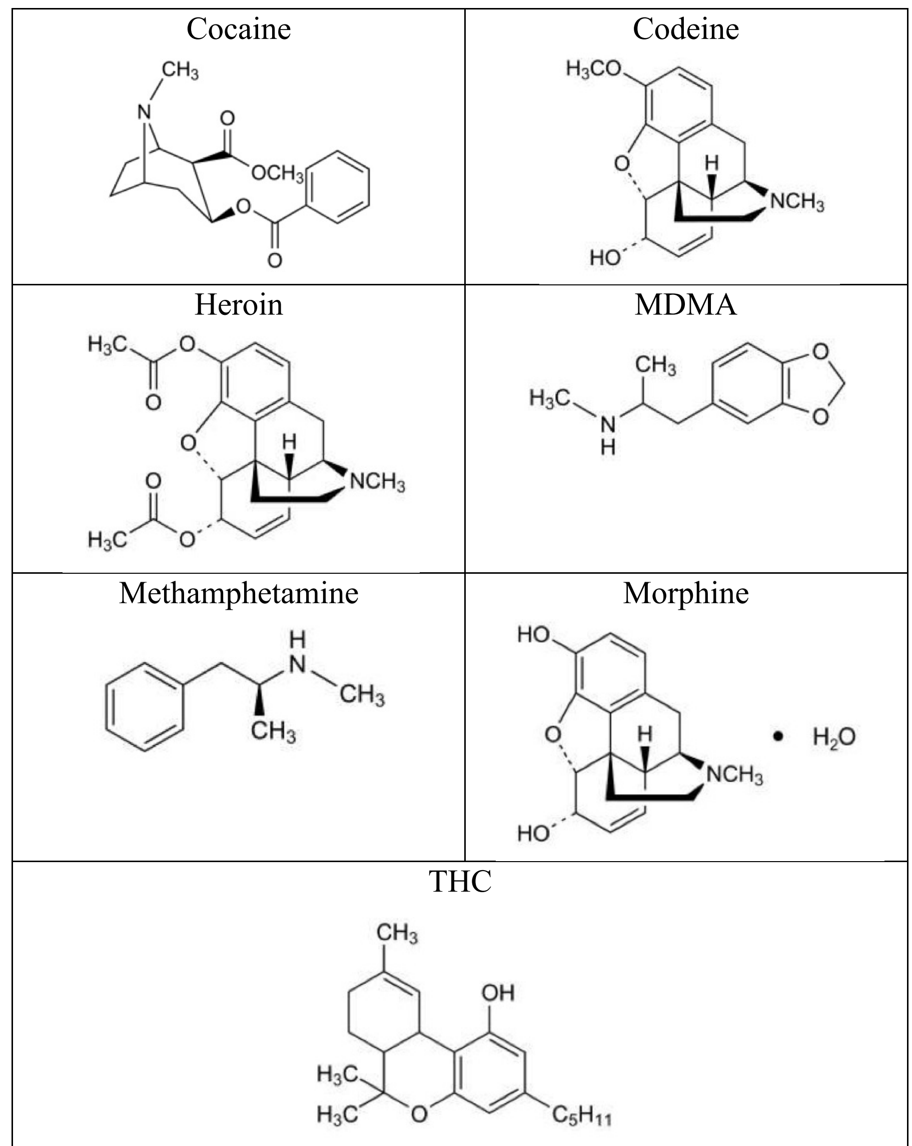
Ebejer, Lloyd, Brereton, Carter, and Sleeman (2007) analyzed 800 sterling banknotes from diverse locations within the United Kingdom for the presence of cocaine, diamorphine, THC, and MDMA using TD-MS/MS. They detected contamination on the bills and proposed the presence of drugs might relate to whether the source of the currency was rural or urban, in the north or south portion of the United Kingdom, or was a port of entry. The authors also considered the socioeconomic class and proportion of the drug offenders in the area, as well as the denomination of the banknotes as possible influential factors. They investigated these factors and found that social, economic, and criminal activity did not have a significant influence on currency contamination.

Bones, Macka, and Paull (2007) analyzed 45 Irish euro banknotes for 16 illicit drugs (morphine, amphetamine, MDMA, benzoylecgonine, ketamine, heroin, cocaine, cocaethylene, lysergic acid diethylamide (LSD), 2-ethylidine-1,5dimethyl-3,3-diphenylpyrroldine perchlorate (EDDP), papaverine, methadone, fluoxetine, temazepam, diazepam, and THC) using methanol extraction followed by LC-MS/MS. The authors compared two different HPLC column types in their study and cocaine was detected on all 45 notes sampled. In some instances benzoylecgonine, the breakdown product of cocaine, was also detected. Heroin was found on 3 of the 45 bills.

2.2. Specific Drugs

Seven drugs (cocaine, codeine, heroin, MDMA, methamphetamine, morphine, and THC) were analyzed in this study. Their chemical structures can be seen in Table 1.

Table 1. Chemical Structures of the Seven Drugs Analyzed in Study
(Moffat, Osselton, Widdop, & Galichet, 2004)



2.2.1. Cocaine

Cocaine is a naturally occurring alkaloid found in the leaves of *Erythroxylon coca*, a plant found in the northern South American Andes, India, Africa, and Indonesia (Isenschmid, 2002). Cocaine is one of the oldest known drugs and is an extremely

addictive central nervous system stimulant. Cocaine can be found in two forms: cocaine hydrochloride and crack cocaine. Cocaine hydrochloride, the salt form, is a colorless or white crystal that is generally administered by nasal insufflation or injection. Crack, the free-base form, is a white crystal that is usually smoked (Levine, 2006). The chemical structure of cocaine is shown in Table 1.

Cocaine is one of the most commonly abused controlled substances in the world. There are an estimated 16-21 million users worldwide, with almost 6 million people reported using cocaine on a regular basis in the United States alone. North America has the largest cocaine market, followed by West and Central Europe and South America (Sandeep, et al., 2009). Approximately 65% of all cocaine entering into the United States crosses the U.S./Mexico border ("Drug Trafficking in the United States," n.d.). Despite being the largest market, a significant decline of trafficking into North America has been seen in recent years. This decline has been reflected in rising prices and falling purity levels. North America has also seen significant declines in cocaine usage, especially in the United States. This is in contrast to usage in Western Europe where usage has stabilized and South Africa where usage has increased (Sandeep, et al., 2009). Wholesale cocaine prices range from $12,000 to $35,000 per kilogram, having an average purity of 73%. Rock cocaine, derived from cocaine hydrochloride, ranges from $3 to $50 a rock ("Drug Trafficking in the United States," n.d.).

2.2.2. Codeine

Codeine is a narcotic analgesic derived from opium. It is produced by methylation of morphine, which is found in the plant *Papaver somniferum* (Baselt, 2008). Like other narcotic analgesics, codeine is a weak base and is used in pain relief. Because of its relatively low potency, codeine is used in proprietary preparations as well as in over-the-counter medications. Codeine is commonly found in preparation with non-narcotic analgesics, such as acetaminophen and aspirin, antihistamines, and other drugs (Baselt, 2008). It is available in capsules, tablets, and syrups as the phosphate or sulfate salt. The chemical structure of codeine is shown in Table 1.

2.2.3. Heroin

Heroin, also known as diamorphine or diacetylmorphine, is a narcotic analgesic produced from the acetylation of morphine, a naturally occurring opioid found in the plant *Papaver somniferum* (Moffat, et al., 2004). Heroin's potency is 2-3 times greater than morphine itself and is able to cross the blood-brain barrier more easily due to the presence of two acetyl groups, which make it lipid-soluble (Levine, 2006). Peak plasma concentrations occur within minutes after administration. The extended duration of effects occur due to active metabolites. Heroin has no recognized medical use and is thus referred to as a Schedule I drug in the United States. Some countries, including Canada and the United Kingdom, allow its use for chronic pain management in terminally ill patients (Levine, 2006). Heroin is generally seen as a white or brown powder and is

generally administered through injection or insufflation (Moffat, et al., 2004) (Baselt, 2008). The chemical structure of heroin is shown in Table 1.

An estimated 15-21 million people use opiates, including heroin, each year. More than half of the world's opiate users are thought to live in Asia. Southwest Asian countries reported more than two-thirds of all opiate seizures in 2007. Europe accounted for the second largest number of opiate seizures (Sandeep, et al., 2009). Heroin available in the United States is produced in South America (Colombia), Southeast Asia (Burma), Mexico, and Southwest Asia/Middle East (Afghanistan). Over the last several decades, the U.S. has shifted from a market dominated by Southeast Asian heroin to a dominance by South American heroin. In 2000, wholesale South American heroin ranged from $50,000 to $200,000 a kilogram. Southeast and Southwest Asian heroin ranged from $40,000 to $190,000 per kilogram. Mexican heroin had the lowest price range, from $13,000 to $175,000 per kilogram. The average purity of heroin in 2000 was 36.8% ("Drug Trafficking in the United States," n.d.).

2.2.4. MDMA

3,4-Methylenedioxymethamphetamine, commonly known as MDMA or Ecstasy, is a phenethylamine designer drug. It was first synthesized in 1914 as a derivative of methamphetamine (Baselt, 2008). MDMA can be synthesized by an amine displacement method involving safrole or by using the intermediate 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP2P) with isosafrole or nitrostyrene (Moffat, et al., 2004). It was used legally until 1985 for therapeutic use and as an adjunct to psychotherapy. MDMA's

widespread use as a recreational drug because of its hallucinogenic and psychoactive properties led it to become classified as a Schedule I drug. MDMA produces acute effects of empathy, euphoria, excitement, and cognitive and psychomotor impairments (Levine, 2006). MDMA is generally sold in tablet form and taken orally. Tablets range in weight from 150 to 350 mg, containing between 70 to 120 mg of MDMA ("Drug Trafficking in the United States," n.d.). The chemical structure of MDMA is shown in Table 1.

An estimated 12-24 million people worldwide use ecstasy-group drugs each year. An estimated 72-137 metric tons of ecstasy-group drugs were manufactured in 2007 (Sandeep, et al., 2009). The street value of MDMA can be as high as $40 a tablet. The vast majority of MDMA consumed in the United States is produced in Europe for as little as 25 to 50 cents a tablet ("Drug Trafficking in the United States," n.d.).

2.2.5. Methamphetamine

Methamphetamine exists in the dextro (*d*-) and levo (*l*-) isomeric forms, with the levo form producing little to no physiological effects. *l*-methamphetamine is used as a decongestant in non-prescription inhalers while *d*-methamphetamine is classified as a Schedule II drug and is what most people are referring to when they mention methamphetamine (Logan, 2002). Methamphetamine acts as a central nervous system stimulant and is available in tablet form (Baselt, 2008), although most illicit methamphetamine is in the form of a crystalline powder that is the hydrochloride salt. The chemical structure of methamphetamine is shown in Table 1.

An estimated 16-51 million people use amphetamine-group substances worldwide each year. An estimated 230-640 metric tons of amphetamine-group drugs were manufactured in 2007 (Sandeep, et al., 2009). Prices of methamphetamine vary throughout the United States. In areas such as California and Texas, prices are around $3,500 per pound, but can be up to $21,000 per pound in southeastern and northeastern parts of the country. Retail prices range from $400 to $3,000 per ounce with a purity of about 40.1% ("Drug Trafficking in the United States," n.d.).

2.2.6. Morphine

Morphine, a narcotic analgesic, has been available for thousands of years and is used in the treatment of moderate to severe pain, most commonly through subcutaneous, intramuscular, intravenous, epidural, or intrathecal injection. It was the first active alkaloid extracted from the opium poppy plant in 1803 (Baselt, 2008).

In 2007, the number of morphine seizures declined by 41% due to the low number of seizures reported in Pakistan, the country with the world's largest morphine seizures. Iran and Afghanistan also have a high number of reported morphine seizures (Sandeep, et al., 2009). The chemical structure of morphine is shown in Table 1.

2.2.7. THC

THC, short for Δ^9-tetrahydrocannabinol (Δ^9-THC), is the main psychoactive substance in the *Cannabis* plant. THC produces the "high" associated with marijuana use.

An estimated 143 to 190 million people used cannabis at least once in 2007, with the highest levels of use in North America and Western Europe (Sandeep, et al., 2009). It has been estimated that one-third of the U.S. population has used marijuana at some point in their lives. Marijuana prices have remained relatively stable over the past few years, ranging from $400 to $1,000 per pound ("Drug Trafficking in the United States," n.d.). The chemical structure of THC is shown in Table 1.

2.3. Liquid Chromatography with Tandem Mass Spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combines the physical separation capabilities of a LC with the ability of a tandem MS to ionize and identify ions based on their mass-charge ratio (m/z) (Skoog, et al., 2007). This differs from single quadrupole mass spectrometry in that the mass spectrometer is able to separate mixtures, making chromatography less critical for accurate identification and quantitation.

2.3.1. Liquid Chromatography

Liquid chromatography separates compounds in a sample chromatographically before they enter the ion source and mass spectrometer. Techniques used to chemically modify a compound into a product with a similar structure that is compatible with an analysis method, known as derivatization, are not often used in LC procedures, making it a useful tool for sample analysis (Levine, 2006).

Samples are injected into the LC and are carried through a column using a liquid mobile phase, which is generally a mixture of water and organic solvents. The LC is able to separate a wide variety of compounds, including non-volatile, high molecular weight, highly polar, and thermally fragile compounds (Wood, et al., 2006).

2.3.2. Mass Spectrometry

Mass spectrometers (schematic provided in Figure 1) work by ionizing molecules and then separating, sorting and detecting/identifying the ions produced based on their mass-charge ratio (*m/z*). The three main components of this process, ionization, ion separation, and ion detection, are critical to the success of the method. Ionization and ion separation must be optimized during the development of the method, which can be achieved through direct infusion of the analytes into the mass spectrometer (P.J. Taylor, 2006).

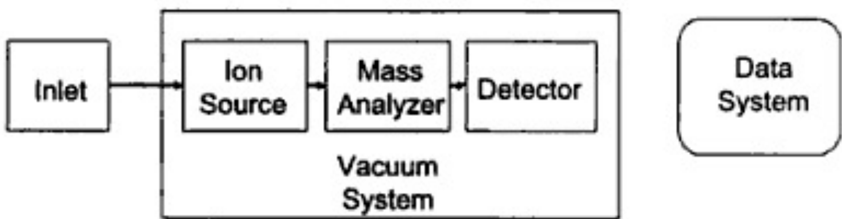


Figure 1. Schematic of a Mass Spectrometer
Basic components of a mass spectrometer: ionization source, mass analyzer, and detector (Levine, 2006)

2.3.2.1. Ionization

There are two main types of ionization in LC-MS: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both ESI and APCI are in the atmospheric pressure ionization (API) family of sources, meaning ions are created at atmospheric pressure in both techniques (Politi, Groppi, & Polettini, 2006).

ESI (see Figure 2 for schematic) was the first API ionization source to be developed and is the most commonly used technique in MS. It can be used to ionize a wide variety of analytes, from small drugs to large macromolecules, but is inefficient at ionizing some non-polar molecules. ESI requires the analyte to exist in solution as an ion (Politi, et al., 2006). This method is used to get solution phase ions into the gas phase so they can be broken down and analyzed by the mass spectrometer.

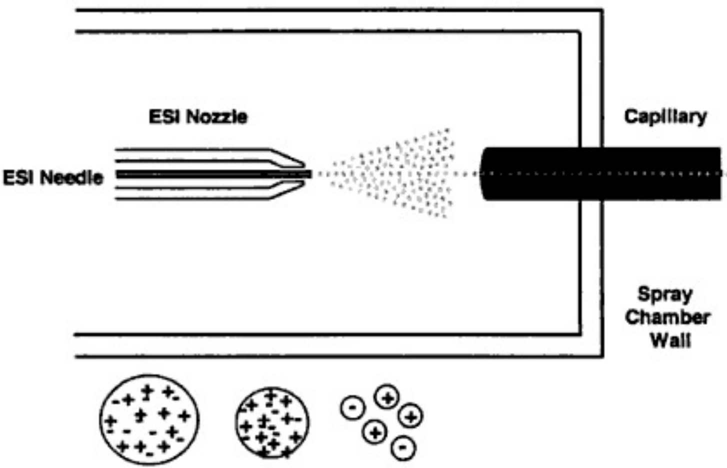


Figure 2. Schematic of an Electrospray Ionization Source
An analyte is forced through a charged capillary, which nebulizes the sample, resulting in ions that can be analyzed by a mass spectrometer (Levine, 2006).

The ESI source applies a voltage to a capillary tube that is surrounded by nebulizing or sheath gas, which causes the liquid in the capillary to be nebulized into a fine spray of highly charged droplets that enter into the vacuum region of the mass spectrometer. Depending on the voltage polarity, the nebulized droplets will be positively or negatively charged. Due to solvent evaporation, which can be increased by additional heat in the source, the droplets shrink, which causes the charge concentration in the droplets to increase. This eventually leads to repulsive forces between charges to exceed cohesive forces of the droplet, causing the ions to be ejected into the gas phase. The ions then pass into the mass analyzer (Politi, et al., 2006).

ESI can be operated in positive or negative mode. Positive mode is generally used for basic drugs that form a stable HCl salt. $[M+H]^+$ is the primary ion formed. $[M+nH]^{n+}$ and $[M+Na^+]^+$ can also be formed. Negative mode is generally used for acidic drugs that form stable Na salts. $[M-H]^-$, $[M-nH]^{n-}$, and $[M+I^-]^-$ ions may be formed ("Why LC/MS/MS?: Background and Theory of Electrospray and Tandem Mass Spectrometry," n.d.).

A drying gas, generally nitrogen, and a heating device are often used with an ESI source to assist in droplet formation and solvent evaporation. Flow rates can be adjusted to obtain the best ion separation.

The APCI source (schematic provided in Figure 3) uses a capillary tube and coaxial flow of nitrogen to nebulize liquid into a heated chamber (400-500°C), where the

solvent and analyte are evaporated. Ionization occurs in the gas phase rather than in solution (Politi, et al., 2006).

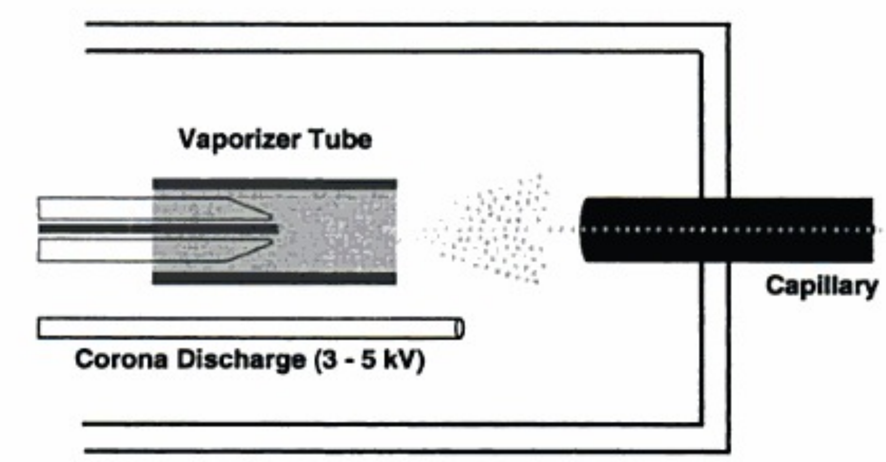


Figure 3. Schematic of an Atmospheric Pressure Chemical Ionization Source
An analyte is forced through a capillary tube where it is then heated and nebulization occurs. The nebulized liquid is then subjected to a corona discharge needle, which creates ions that can be analyzed by the mass spectrometer (Levine, 2006).

The gas phase solvent molecules are ionized by electrons given off by a corona discharge electrode (2-5 kV), which is placed near the tip of the capillary. The solvent ions transfer charges to analyte ions through the process of chemical ionization. The ions then pass into the mass analyzer (Politi, et al., 2006).

All ionization sources require optimization of gas flows and source temperature. The nebulizer gas flows that facilitate droplet formation can be modified to enhance droplet formation. Some sources also use a heated gas to desolvate the ions, and the gas

flow and temperature can be modified to increase sensitivity. ("Why LC/MS/MS?: Background and Theory of Electrospray and Tandem Mass Spectrometry," n.d.).

2.3.2.2. Ion Separation

Ion separation occurs in the mass analyzer. There are several types of mass analyzers including: quadrupole, ion trap, linear ion trap, and time of flight. The quadrupole mass spectrometer is the most commonly used mass analyzer. A quadrupole consists of four parallel rods, or poles, arranged in a square formation. An electrical charge, either direct current (DC) or radiofrequency (RF), is applied to each rod, with adjacent rods having opposite charges. This generates an electromagnetic field. The electromagnetic field acts as a filter and determines which ions can pass through to the detector based on a set m/z, or mass to charge ratio. Quadrupole mass analyzers can be operated in scan mode or selected ion monitoring (SIM) mode. Scan mode allows for the monitoring of a complete set, or range, of masses whereas only a few masses are monitored in SIM mode. SIM mode is much more sensitive than scan mode, focusing on specific ions (Politi, et al., 2006).

Several quadrupoles may be linked together, referred to as a triple quadrupole or as tandem mass spectrometry (Figure 4), to analyze ions.

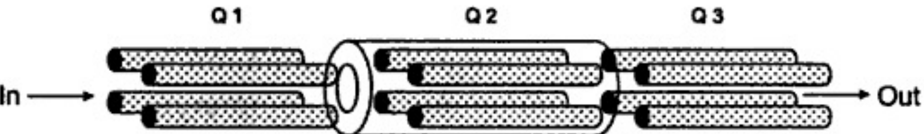


Figure 4. Schematic of a Triple Quadrupole Mass Spectrometer
Q1 and Q3 function as mass filters, while Q2 functions as a collision cell where precursor ions collide and are broken up into product ions (Levine, 2006).

The first quadrupole (Q1) is generally used to filter out any ions that do not correspond to the *m*/*z* of the ion of interest. The ion of interest, known as the precursor or parent ion, then passes into the second quadrupole (Q2) where fragmentation occurs due to the presence of collision gas. The ions formed from fragmentation of precursor ions, known as product or daughter ions, pass into the third quadrupole (Q3) where certain ions are filtered through to the detector (Levine, 2006).

A triple quadrupole instrument can be operated in several different ways. It can be set up to resemble a single quadrupole instrument by allowing either Q1 or Q3 to act as a filter while the other quadrupole is passive. It can also be set to perform a product ion scan, where a precursor ion is selected in Q1 and the product ions produced in Q2 are scanned in Q3, producing a product ion spectrum. Several other methods can also be performed with a triple quadrupole instrument (Politi, et al., 2006).

Ion trap mass analyzers consist of a circular ring of electrodes and two end cap electrodes. Ions are trapped in the in the ring by applying RF voltage to the electrodes. The voltage of the electrical field is amplified to cause destabilization and subsequent

ejection of selected ions to the detector. Continually increasing the voltage of the electrical field causes destabilization of ions with increasing *m*/*z* values until all ions within the selected range have been ejected to the detector (Levine, 2006).

Linear ion trap mass analyzers consist of a linear quadrupole mass filter that is operated using only RF voltages. It allows the third quadrupole in a tandem mass spectrometry setup to function as a trap and increase the sensitivity and specificity of the analysis at low concentrations.

Time of flight (TOF) mass analyzers use an electric field to separate ions based on the time it takes for them to reach the detector. The higher the *m*/*z* ratio, the longer it will take to travel to the detector. TOF only operates in scan mode, but it doesn't use a filter to control the masses traveling; rather, it has a gate and will provide the masses of all the ions based on the time to traverse a specific distance in the MS (Politi, et al., 2006).

2.3.2.3. Ion Detection

The detector is the portion of the mass spectrometer that converts ions separated by the mass analyzer into a measurable electronic signal. Generally, an electron multiplier device is used for detection of ions. In the electron multiplier, ions hit the surface of a dynode electrode and are converted to electrons. The detector records the induced current of the emitted electrons. The signal can be amplified by the use of a series of dynodes, which multiply the electrons produced from the previous dynode. A horn-shaped continuous dynode may also be used to amplify electrons. In this method, electrons repeatedly collide with the internal surface of the detector (Politi, et al., 2006).

The signal from the detector is then provided to the controller, generally a computer, so that the mass to charge ratio of the ion detected can be determined based on the time of flight or quadrupole settings at the time of detection.

2.4. Method Development

There are several steps involved in successfully developing a quantitative LC-MS/MS method, which requires the optimization of various parameters. The first step is to define the problem. This involves determining whether metabolites will be examined along with the parent compound, the matrix that will be analyzed, sample limitations, linear range, lower limit of quantitation, as well as other factors associated with analyzing the problem. Once the problem is defined, a literature search for physiochemical properties of the analytes, chemically related compounds, mass spectrometric methods, LC conditions, sample preparation, etc. can be performed. A literature search should also be performed to select a suitable internal standard to use in analysis. There are three types of internal standards that can be used in LC-MS methods: the analyte labeled with several stable isotopes (^{18}O, ^{15}N, ^{13}C, or ^{2}H or D), a structural analogue of the analyte, or any other chemical. Labeled internal standards are most often used because they are chemically identical to the analyte (P.J. Taylor, 2006).

Once the problem has been defined, mass spectrometer conditions must be selected and optimized. The proper ionization mode (ESI or APCI) must be selected to transfer the compound or compounds of interest from the liquid mobile phase to the gas phase for analysis.

Choosing what fragment ions to examine and optimizing the collision energy is also necessary. Collision energies can be optimized by infusing the compound or compounds of interest and monitoring their mass transitions (P.J. Taylor, 2006).

After the mass spectrometer conditions have been optimized for the ions of interest, the source conditions are then modified in order to increase sensitivity. This includes optimization of the ionization parameters, ionization source voltage, gas flows, and temperature.

After the mass spectrometer conditions have been set, chromatography needs to be examined. The proper column type needs to be selected to obtain the highest degree of sensitivity and selectivity for the assay through optimization of the mobile phase and flow rates (P.J. Taylor, 2006).

The next step is sample preparation. Sample preparation is the process of getting the sample into a form that can be analyzed by the instrument, while retaining as much of the analyte as possible. Extraction methods that can be used include sample dilution and protein precipitation, liquid-liquid extraction, solid phase extraction, and two-dimensional chromatography (P.J. Taylor, 2006).

Once the method has been developed, method validation is required to confirm that that the method accurate, precise, selective, and sensitive.

2.5. Method Validation

Once a LC-MS/MS method has been developed, several tests must be performed to establish that the method can be used for its intended purpose and is able to measure what it is intended to measure. These tests are collectively known as method validation (Zhou, Song, Tang, & Naidong, 2005). The main parameters evaluated in method validation are: accuracy, precision, selectivity, sensitivity, reproducibility, and stability (US Food and Drug Administration, 2001). These factors are essential in order to analyze an analyte over a specified range and thus determine the reliability of the method.

2.5.1. Accuracy

The first parameter evaluated in method validation is accuracy. Accuracy, sometimes referred to as trueness, is the degree of closeness between the experimental sample value and a known value. Analyzing a reference sample of a known concentration and comparing the calculated value to the calculated value of the experimental sample is one approach to determining the accuracy of a method. Another approach to determine accuracy is comparing results from the newly developed method to those from an existing validated method (Shabir, 2003). The Food and Drug Administration (FDA) states that the experimental sample value should be within 15% of the known value except at the lower limit of quantitation (LLOQ), where it should be within 20% (US Food and Drug Administration, 2001). The LLOQ is the lowest concentration of an analyte that can be quantitatively determined from background noise.

Accuracy is the most critical aspect in method validation and should be evaluated in any method development.

2.5.2. Precision

The next parameter in method validation is precision. Precision is the closeness of the quantitative values obtained from repeated measurements of the same sample at the same concentration. In general, a minimum of three concentration levels (low, medium, and high) should be run in triplicate to determine precision (Araujo, 2009). Although only three concentrations are required, a five-point standard curve, comprising five different concentrations, is generally recommended (Stockl, D'Hondt, & Thienpont, 2009). Precision should be within 15% of the coefficient of variation (CV) for a valid method except for the LLOQ where is should be within 20% of the CV (US Food and Drug Administration, 2001). Determining precision is critical to establish reproducibility for method validation. Precision can be determined for tests conducted on the same day (intraday), as well as test conducted on different days (interday).

2.5.3. Selectivity

The third parameter in method validation is selectivity. Selectivity is the ability to detect an analyte in a matrix without interference from other components. It is determined by analyzing blank samples for interference. Specificity is ultimate selectivity, with no interferences from other components in the matrix occurring. Selectivity is a graded term unlike specificity, which is an exact term. Selectivity can be described as good, bad, high, low, etc. When the term specificity is used, it always refers

to a method that is 100% selective, or in other words has 0% interferences (Araujo, 2009). Selectivity is tested by determining matrix effects.

2.5.3.1. Matrix Effects

Testing for matrix effects should be done to investigate potential factors that could cause unwanted modification to the quantitated values should be examined. Matrix effects, or the alterations of ionization efficiency by the presence of coeluting substances, must be assessed when validating any LC-MS/MS method (P. J. Taylor, 2005). If matrix effects are not addressed, the accuracy, precision, selectivity, and sensitivity of data collected may be significantly affected. Although the exact mechanism of this effect is unknown, matrix effects are believed to be caused by competition between the analyte and a coeluting component in the matrix, which is undetected (P. J. Taylor, 2005). Matrix effects can lead to ion suppression or ion enhancement. Ion suppression is a decrease in formation of the analyte ions present whereas ion enhancement is an increase in analyte ion formation. Both of these will cause inaccurate quantitation of the analyte.

Matrix effect can be assessed by two methods: postextraction addition or postcolumn infusion. The postextraction addition method (Figure 5) compares samples with the analyte added postextraction to pure samples prepared in the mobile phase.

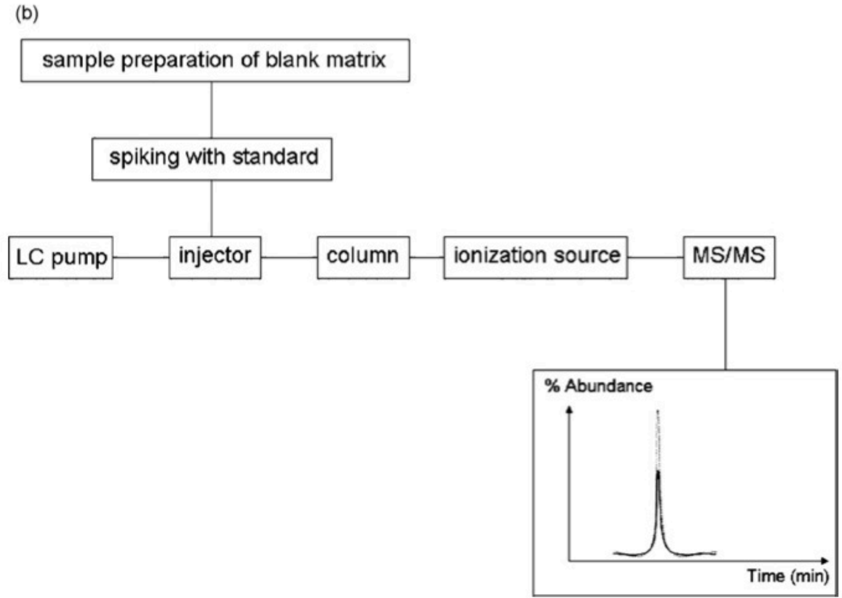


Figure 5. Schematic of Postextraction Addition Method
Samples with the analyte of interest added postextraction is compared to pure samples prepared in mobile phase (Van Eeckhaut, Lanckmans, Sarre, Smolders, & Michotte, 2009).

Taking the difference between the response of the postextraction sample and the pure sample and dividing by the response of the pure sample assesses the extent of any matrix effect occurring (P. J. Taylor, 2005). The postextraction addition method only evaluates matrix effect at the point of elution of the analyte of interest. Postcolumn infusion (Figure 6), on the other hand, is a much more robust technique for determining matrix effect and allows for analyte response to be examined over the entire run.

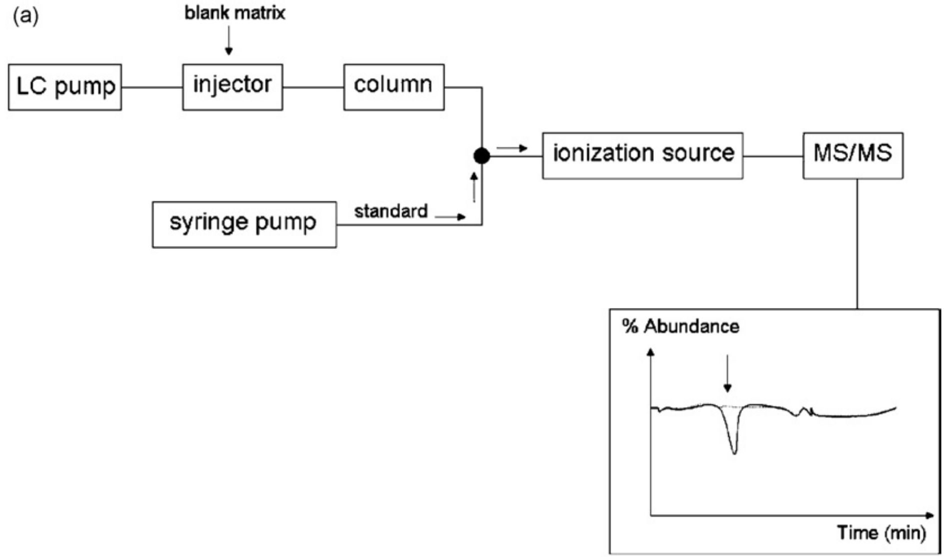


Figure 6. Schematic of Postcolumn Infusion Method
An infusion pump is used to deliver a constant flow of analyte into the HPLC eluent after the column, but before the mass spectrometer. A blank sample is then injected to determine matrix effects (Van Eeckhaut, et al., 2009).

Using this approach, a syringe pump and HPLC system are simultaneously coupled to the mass spectrometer. During postcolumn infusion, the analyte is infused using the syringe pump into the constant flow of eluent after the chromatographic column and before the mass spectrometer ionization source (P. J. Taylor, 2005). The HPLC delivers the sample blank, which allows for the determination of matrix effects over the entire chromatographic run.

Modifications of the sample extraction or improved chromatographic separation are techniques that can be used to minimize or eliminate matrix effect. Matrix effects are compound dependent, with the most polar compounds having the largest ion suppression and the least polar compounds affecting matrix effects to a lesser extent. Liquid-liquid

extraction and solid phase extraction procedures produce less matrix effects as compared to a “dilute and shoot” or protein precipitation method of sample preparation (P. J. Taylor, 2005).

Adjusting the chromatographic separation can also reduce matrix effects. Matrix effects are most often observed in the solvent front of a run. Thus, by modifying the chromatographic separation to retain the analytes for a longer period of time, matrix effects can be reduced. Another factor influencing matrix effect is the type of ionization used. Several studies have shown that electrospray ionization is more prone to matrix effects than atmospheric pressure chemical ionization (P. J. Taylor, 2005).

2.5.4. Sensitivity

The next parameter is sensitivity, which describes the smallest quantity that the method can detect (limit of detection (LOD)) or quantitated (limit of quantitation, LOQ). Sometimes the LOQ is written as the lower LOQ, or LLOQ. Sensitivity refers to the slope of the standard curve, or the change in response of a measuring instrument over the corresponding change in the stimulus (Taverniers, De Loose, & Van Bocktaele, 2004). A method is deemed sensitive if a small change in the concentration of the analyte results in a detectable change in the measured signal (Taverniers, et al., 2004).

2.5.5. Reproducibility and Stability

The fifth parameter in method validation is reproducibility. Reproducibility is the ability to replicate results over a period of time. It is the precision of the method after changing one or more of the global factors over a short or extended period of time

(Araujo, 2009). Reproducibility also relates to the precision of results obtained between multiple laboratories using the method.

The last parameter is stability. Stability is the ability to maintain the analyte as stored in the matrix over a given period of time. It is important to know under what conditions a sample should be stored to prevent degradation.

2.5.6. Linearity

Linearity should also be evaluated when validating a method. It is the straight-line relationship between the experimental response value and the analytical concentration (Araujo, 2009). Based on this relationship, known as a standard curve, a correlation coefficient, or r^2 value, can be determined. If a run has perfect linearity, the r squared value will equal 1. The standard curve should be reproducible from day to day.

2.5.7. Carryover

Carryover is the contamination of new samples by residual analyte from a previously run sample. To test for carryover, a blank sample, containing no analyte, is injected into the instrument following an injection of a sample containing the upper limit of quantitation (ULOQ) concentration sample (Clouser-Roche, Johnson, Fast, & Tang, 2008). The ULOQ is the highest concentration of analyte in a sample that can quantitatively be determined with accuracy and precision. When a peak can be seen in a blank, an analyte being retained from a previous injection or injections and carryover is occurring. As a rule, if a peak is seen in the blank sample, it should have an area less than 20% of the lower limit of quantitation (LLOQ) in order for the run not to be

considered a failure (Clouser-Roche, et al., 2008). The ULOQ and LLOQ are different from the limit of detection (LOD), which is the lowest concentration of an analyte that can be detected in a sample, but cannot be quantitated (Shabir, 2003).

Validating a new method to be used in lab is critical to ensure reliability of results. Although issues such as matrix effect and carryover can occur, it is important to minimize these problems to obtain the most accurate results possible. By following the steps outlined to create and validate a new method, high quality data can be achieved.

Chapter III. Methodology

3.1. Instrumentation

All samples were analyzed with a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a system controller, CBM-20A, a solvent delivery unit, LC-20AD, an auto-sampler, SIL-20AC, and a column oven, CTO-20AC. A Restek Allure Pentafluorophenyl (PFP) Propyl 5µm 50x2.1mm column was used for LC separation (Restek, Bellefonte, PA).

The Shimadzu HPLC system was attached to an Applied Biosystems 4000 Q-Trap LC-MS/MS System (Applied Biosystems, Foster City, CA). The mass spectrometer was equipped with a Turbo V™ electrospray ionization source, a Harvard Apparatus syringe pump (Holliston, MA) (Figure 7) and a NitroGen N300DR nitrogen generator as the source of instrument gases (Peak Scientific Instruments Ltd, Paisley, United Kingdom). Analyst® 1.5 Software was used to control the instrument and for data analysis.

Figure 7. LC-MS/MS Used in Study
Shimadzu HPLC system attached to an Applied Biosystems 4000 Q-Trap LC-MS/MS System in the Oklahoma State University laboratory.

3.2. Materials

Methanol (VWR International, West Chester, PA) and 98% formic acid (EMD Chemicals, Gibbstown, NJ) were both ACS grade; acetonitrile (OmniSolv, EM Science, Gibbstown, NJ) was HPLC grade. Ammonium formate, 99%, was obtained from Alfa Aesar (Alfa Aesar, Ward Hill, MA). Drug standards (cocaine, cocaine-D3, codeine, heroin, (±)-MDMA, (±)-methamphetamine, morphine, and (-)-delta9 THC) were purchased from Cerilliant (Cerilliant Corporation, Round Rock, TX).

Table 2. Cerilliant Drug Standards Used in Study

	Concentration	Solvent
Cocaine-D3 (internal standard)	100 µg/ml	1 ml Acetone
Cocaine	1.0 mg/ml	1 ml Acetone
Codeine	1.0 mg/ml	1 ml Methanol
Heroin	1.0 mg/ml	1 ml Acetone
(±)-MDMA	1.0 mg/ml	1 ml Methanol
(±)-Methamphetamine	1.0 mg/ml	1 ml Methanol
Morphine	1.0 mg/ml	1 ml Methanol
(-)-delta9 THC	1.0 mg/ml	1 ml Methanol

3.3. Preparation of Standards

Cocaine standards were prepared at five concentrations: 100, 20, 10, 2, and 0.2 ng/ml using Cerilliant drug standards diluted in methanol. Internal standards of cocaine-D3 were prepared at 5 and 10 ng/ml, also using Cerilliant drug standards and methanol.

Multi-drug standards containing cocaine, codeine, heroin, MDMA, methamphetamine, morphine, and THC were prepared at six concentrations: 100, 50, 20, 10, 2, and 0.2 ng/ml using Cerilliant drug standards diluted with methanol. Internal standards of cocaine-D3 were prepared at 5 and 10 ng/ml, also using Cerilliant drug standards and methanol.

3.4. Method Development

3.4.1. Cocaine Method

A 50 ng/ml cocaine solution was used to optimize the mass spectrometer. The cocaine standard was infused with a syringe pump, at a flow rate of 40 µl/min, directly into the mass spectrometer through a Turbo V™ source in electrospray configuration.

A single quadrupole scan, performed in the first quadrupole mass analyzer, was performed to determine the presence of cocaine at a mass-charge ratio (*m*/*z*) of 304.1.

A product ion scan was then performed to analyze all products of the cocaine precursor ion of 304.1 *m/z*. The first quadrupole was fixed at 304.1 *m*/*z* while the third quadrupole was set to scan for products created in the collision cell (Q2). While multiple product ions were identified, three product ions (182.1, 82.1, and 77) were selected for inclusion in the assay based on sensitivity and selectivity.

Parameters that affect the progression of each of the monitored ions representing cocaine through the mass spectrometer were then optimized to increase sensitivity.

3.4.1.1. LC Parameters

An aqueous mobile phase (Eluent A: 0.2% Formic, 0.2 % Ammonium formate in water) as well as an organic mobile phase (Eluent B: 0.2% Formic, 0.2 % Ammonium formate in acetonitrile) were used to carry the sample through the HPLC column. The organic mobile phase (Eluent B) increased as a gradient from 10% to 50% over the first

four minutes of the run and then returned to 10% 50 seconds later for the rest of the 6 minute run. The sample was injected at a volume of 10 μl with a total flow rate of 0.5 ml/min.

3.4.1.2. Source Parameters

The source parameters were as follows:

- Curtain Gas: 10.0 psi
- Gas 1: 40.0 psi
- Gas 2: 70.0 psi
- Temperature: 500.0°C
- Entrance Potential: 10 volts
- Ionspray Voltage: 4000 volts

3.4.1.3. MS Parameters

The mass spectrometer was run in positive mode. Multiple reaction monitoring (MRM) was used to allow multiple user defined ion fragments to be monitored. MRM parameters can be seen in Table 3.

Table 3. Cocaine MS Parameters

Name	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
Cocaine	304.1	182.1	51.0	29.0	44.0
	304.1	82.1	51.0	71.0	12.0
	304.1	77.0	51.0	73.0	0.0
Cocaine-D3	307.0	185.2	60.0	29.0	34.0

Calibrators used in the runs were prepared by mixing 0.5 ml of the appropriate concentration of cocaine standard with 0.5 ml of 10 ng/ml cocaine-D3 in an injection vial. Mixing the cocaine standard and internal standard dilutes the

final concentrations by half, making the final calibrator concentrations 50, 25, 10, 5, 1, and 0.1 ng/ml cocaine with 5 ng/ml cocaine-D3. Blank calibrators were prepared by mixing 0.5 ml cocaine-D3 with 0.5 ml methanol. The Analyst® software was used to generate best fit lines of the data and determine quantitative values of the unknown samples.

3.4.2. Multi Drug Method

The multi drug method was set up just like the cocaine method, except seven drugs (cocaine, codeine, heroin, MDMA, methamphetamine, morphine, and THC) were analyzed simultaneously. A 50 ng/ml multi drug standard was infused at a flow rate of 50 µl/min directly into the mass spectrometer through a Turbo V™ source in the electrospray configuration.

A single quadrupole scan, performed in first quadrupole mass analyzer, was performed to determine the *m*/*z* of cocaine (304.1), codeine (300.0), heroin (370.0), MDMA (194.0), methamphetamine (150.0), morphine (286.0) and THC (315.0).

A product ion scan was then performed to analyze all products of the seven drugs. The first quadrupole was fixed to scan at *m*/*z* 304.1, 300.0, 370.0, 194.0, 150.0, 286.0, and 315.0 while the third quadrupole was set to scan for products created by the collision cell over the mass ranges. Three product ions for each drug were monitored: cocaine (182.1, 82.1, and 77), codeine (165.0, 152.0, and 115.0), heroin (165.0, 58.0, and 43.0), MDMA (163.0, 105.0, and 77.0), methamphetamine (119.0, 91.0, and 65.0), morphine (165.0, 152.0, and 115.0), and THC (193.0, 123.0, and 77.0).

A quantitation method was created in Analyst® to measure the amount of each drug found in the samples. The weighting of the linear regression was changed to get the most accurate calculated concentrations.

3.4.2.1. LC Parameters

The aquatic and organic mobile phases were the same as used in the cocaine method. The organic mobile phase increased as a gradient from 10% to 90% over the entire 11 minute run. The sample was injected at a volume of 20 μl with a total flow rate of 0.5 ml/min.

3.4.2.2. Source Parameters

Source parameters for the multi drug method were the same as for the cocaine method.

3.4.2.3. MS Parameters

The mass spectrometer was run in positive mode. Multiple reaction monitoring (MRM) was used to allow multiple user defined ion fragments ions to be monitored. MRM parameters can be seen in Table 4.

Table 4. Multi Drug MS Parameters

Name	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
Cocaine	304.1	182.1	51.0	29.0	44.0
	304.1	82.1	51.0	71.0	12.0
	304.1	77.0	51.0	73.0	0.0
Cocaine-D3	307.0	185.2	60.0	29.0	34.0
Codeine	300.0	165.0	91.0	51.0	8.0
	300.0	152.0	91.0	85.0	22.0
	300.0	115.0	91.0	101.0	8.0
Heroin	370.0	165.0	106.0	69.0	28.0
	370.0	58.0	106.0	57.0	8.0
	370.0	43.0	106.0	115.0	4.0
MDMA	194.0	163.0	56.0	19.0	10.0
	194.0	105.0	56.0	33.0	16.0
	194.0	77.0	56.0	59.0	10.0
Meth	150.0	119.0	56.0	15.0	4.0
	150.0	91.0	56.0	25.0	14.0
	150.0	65.0	56.0	57.0	8.0
Morphine	286.0	165.0	96.0	59.0	12.0
	286.0	152.0	96.0	81.0	22.0
	286.0	115.0	96.0	89.0	4.0
THC	315.0	193.0	66.0	33.0	34.0
	315.0	123.0	66.0	43.0	6.0
	315.0	77.0	66.0	87.0	8.0

Calibrators used in the runs were prepared by mixing 0.5 ml of the appropriate concentration of multi drug standard with 0.5 ml of 10 ng/ml cocaine-D3 in an injection vial. Mixing the multi drug standard and internal standard dilutes the final concentrations by half, making the final calibrator concentrations 50, 25, 10, 5, 1, and 0.1 ng/ml cocaine with 5 ng/ml cocaine-D3. Blank calibrators were prepared by mixing 0.5 ml cocaine-D3 with 0.5 ml methanol.

3.5. Sample Collection

Dr. Thomas Jourdan, formerly of the FBI, supplied the currency samples analyzed in this study. He used an FBI procedure to collect samples from 35 domestic and 32 foreign cities.

Figure 8. Filter Samples from Kenai, Alaska
7 tests tubes ($1, 5, 10, 20, 50, 100, filter blank) collected from Kenai, Alaska

For the domestic samples, one thousand, eight hundred and sixty dollars ($1,860: ten bills of each denomination: $1, 5, 10, 20, 50, 100) were obtained from a bank in each city. A vacuum sampling of one side of the ten bills from each denomination was taken by placing a filter at the end of a portable vacuum cleaner hose, allowing any drug particles on the bills to be trapped on the filter. A Barringer Ionscan® IMS system was

used to screen the filters for the presence of drug residues (Barringer Technologies Inc., Warren, NJ). If the Ionscan indicated drug residues, a second vacuum sample was taken from the other side of the original ten bills. After vacuuming, each filter was placed into an individual test tube.

The same process was used for the collection of foreign samples, although the number of bills differed at each location.

3.6. Preparation of Samples

Filter samples were prepared by adding 1 ml of 5 ng/ml cocaine-D3 in methanol to each test tube containing a filter. The test tubes were recapped and each test tube was vortexed for 10 seconds. The solution was pipetted out of the test tube and transferred to an injection vial for LC-MS/MS analysis.

3.7. Analytical Procedure

All runs were performed in duplicate. Each run consisted of the set of calibrators from high to low, followed by filter sample denominations from low to high denomination ($1, 5, 10, 20, 50, 100, filter blank (if applicable)), blank, filter sample denominations from high to low, and calibrators from low to high. Samples were run in this fashion in order to identify the presence of carryover. All peaks were reviewed for accurate integration.

3.8. Method Validation

3.8.1. Precision

Precision was examined by looking at intraday and interday variability, or the variability of calibrators run on the same day and the variability of calibrators run on different days, respectively. Since all days did not have the same number of replicate analyses, a weighting method was used to correctly represent the variability observed over the course of a day. Intraday variability was calculated for each calibrator concentration by taking the standard error of the mean of each day when multiple samples were run at the same level, then dividing by the mean of the quantitative ratio for that level, and multiplying that number by the number of samples run that day. These daily intraday variabilities were then pooled by adding those days together and dividing by the total number of samples pooled. This could be represented for one calibrator level by the formula:

$$Variability_{intraday}=((n_{day1}*(SEM_{day1}/Mean_{day1}))+(\ (n_{day2}*(SEM_{day2}/Mean_{day2}))\ldots)/n_{pooled}$$

Interday variability was calculated for each calibrator concentration by using the mean, standard error of the mean, and number of calibrators for each different day, then determining the variability in the daily numbers using column statistics in GraphPad Prism®.

3.8.2. Accuracy

The accuracy of each calibrator concentration was calculated by determining the percent error. This was calculated by taking the calculated calibrator concentration, subtracting the accepted concentration value, dividing by the accepted concentration value, and multiplying by 100.

3.8.3. Sensitivity

The LOD and LOQ were calculated by selecting three randomly chosen runs. Quantitation ratios were calculated for the lower end calibrators, 0.1-10 ng/ml, by dividing the analyte peak area of the Q3 ion used for quantitation by the peak area of the internal standard. The entire calibration set was not used because it would lead to an overestimation of LOD and LOQ values. The quantitation ratio values from each run were plotted in a graph to obtain the y-intercept and slope for each run. The y-intercept and slope values were then used to calculate the LOD and LOQ. The standard deviation of the three y-intercepts was calculated along with the mean of the three slopes. The LOD equals (3.3*SD)/mean and the LOQ equals (10*SD)/mean (*ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Methodology*, 1996).

3.8.4. Selectivity

A multi drug standard, containing all seven drugs, was prepared by mixing 100 µl of each drug standard (1 µg/ml) with 100 µl of cocaine-D3 (10 ng/ml). A 3-way valve was used to allow infusion from both the LC and the syringe pump into the MS/MS. An injection vial of methanol was placed in the LC to use as a background comparison. A

syringe was filled with multi drug standard and was connected to a capillary tube attached to the MS/MS. The methanol sample was run with the same Multi Drug MRM acquisition method that was used to run the currency samples. The multi drug standard was infused at an injection flow rate of 50 µl/min. A currency blank from Kenai, Alaska was then run through the LC using the same process as the methanol blank. The multi drug standard was again infused with an injection flow rate of 50 µl/min. The chromatograms were reviewed for suppressions and enhancements.

3.9. Quantitation of Currency Contamination

Separate ID ratios (designated as ID1 and ID2) of the calibrators were used to confirm the calculated concentrations from the bills. ID1 was calculated by taking the analyte peak area of the second largest Q3 product ion (e.g. cocaine 82) and dividing it by the analyte peak area of the largest Q3 product ion (e.g. cocaine 182). ID2 was calculated by taking the analyte peak area of the third largest Q3 product ion (e.g. cocaine 77) and dividing it by the analyte peak area of the largest Q3 product ion (e.g. cocaine 182). The average ID1 and ID2 ratio using all the calibrators was calculated and a 20% upper and lower range was determined from that number. For the calculated concentration from a bill to be confirmed, the ID1 and ID2 ratio must be within the calculated range.

3.10. Statistical Analysis

All statistical analyses were performed using GraphPad Prism® Version 5.0 (GraphPad Software, San Diego, CA) and Microsoft Excel® 2007 (Microsoft Corporation, Redmond, WA).

Chapter IV. Results

4.1. Precision

4.1.1. Cocaine Method

Intraday and interday variability for the 50, 10, 5, 1, and 0.1 ng/ml cocaine calibrators was calculated and these values can be seen in Table 5.

Table 5. Intra/Interday Variability of Cocaine Calibrators

Domestic		50	10	5	1	0.1
Cocaine	Intraday	1.38%	0.80%	0.86%	5.25%	2.78%
	Interday	1.08%	3.09%	1.10%	4.05%	1.54%

4.1.2. Multi Drug Method

Intraday and interday variability for the 50, 25, 10, 5, 1, and 0.1 ng/ml calibrator of each of the six drugs was examined. These values can be seen in Table 6.

Table 6. Intra/Interday Variability of Multi Drug Calibrators

Domestic		50	25	10	5	1	0.1
Cocaine	Intraday	0.58%	0.49%	0.53%	0.57%	1.09%	2.09%
	Interday	1.12%	0.82%	1.70%	2.51%	4.54%	9.38%
Codeine	Intraday	2.19%	1.27%	1.40%	1.58%	1.50%	5.23%
	Interday	2.83%	3.92%	7.11%	5.76%	7.21%	9.44%
Heroin	Intraday	2.70%	1.47%	1.68%	1.76%	1.88%	3.85%
	Interday	5.00%	2.71%	7.57%	4.07%	5.61%	63.13%
MDMA	Intraday	1.53%	1.07%	1.26%	1.32%	1.32%	2.36%
	Interday	2.06%	3.32%	7.81%	4.40%	5.92%	12.23%
Meth	Intraday	1.58%	0.76%	0.91%	0.86%	0.99%	2.16%
	Interday	2.20%	1.71%	3.80%	2.91%	4.03%	57.83%
Morphine	Intraday	2.80%	1.79%	1.73%	1.88%	2.17%	4.44%
	Interday	9.60%	10.10%	4.43%	12.61%	13.90%	64.98%

4.2. Accuracy

4.2.1. Cocaine Method

Accuracy of the 50, 10, 5, 1, and 0.1 ng/ml cocaine calibrators is shown in Table 7.

Table 7. Accuracy of Cocaine Calibrators

Calibrator (ng/ml)	%Error
50	0.38%
10	19.40%
5	14.84%
1	20.90%
0.1	20.40%

4.2.2. Multi Drug Method

Accuracy of the 50, 25, 10, 5, 1, and 0.1 ng/ml calibrator of each of the six drugs was examined and the results are shown in Table 8.

Table 8. Accuracy Multi Drug Calibrators

Drug	%Error					
	50	25	10	5	1	0.1
Cocaine	1.36%	8.96%	3.80%	4.30%	9.45%	5.30%
Codeine	0.14%	0.76%	2.96%	4.68%	15.80%	295.30%
Heroin	0.82%	1.32%	1.83%	4.24%	1.90%	109.70%
MDMA	0.26%	0.40%	2.47%	5.26%	1.82%	51.10%
Meth	0.32%	0.80%	2.25%	5.04%	1.89%	64.70%
Morphine	0.02%	1.88%	2.68%	5.46%	2.60%	155.00%

4.3. Sensitivity

4.3.1. Cocaine Method

Table 9 shows the low end calibrators that were calculated to determine sensitivity of the method.

Table 9. Quant Ratios Used to Calculate LOD/LOQ for Cocaine Method

Quant Ratios	7/26 (1)	8/3 (5)	8/10 (2)
10	1.3396	1.3390	1.3931
5	0.6477	0.6321	0.6637
1	0.1370	0.1373	0.1447
0.1	0.0140	0.0135	0.0138

The quantitation ratios were plotted on a graph (Figure 9) and the mean and standard deviation (

Table 10) were calculated to determine LOD and LOQ.

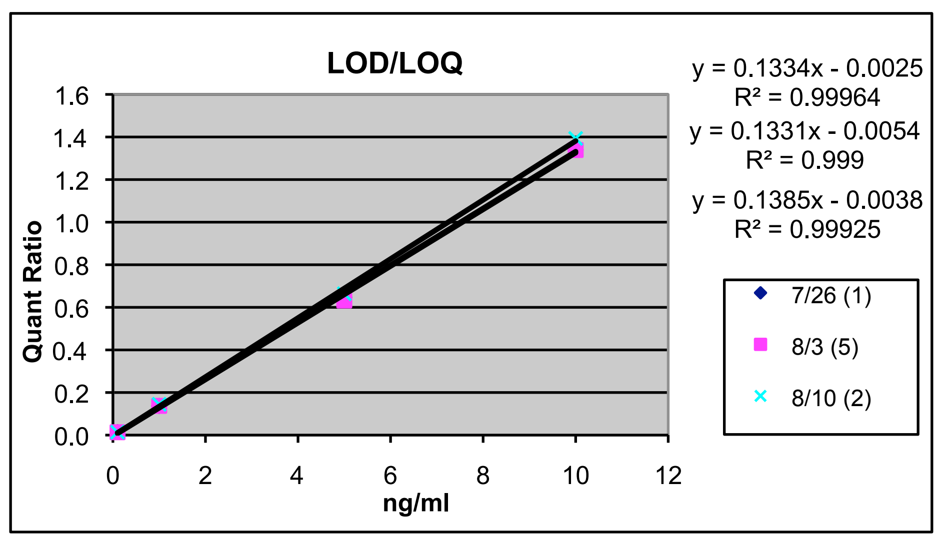


Figure 9. Cocaine LOD/LOQ Graph
Quant ratios of the low end calibrators (0.1-10 ng/ml) of three randomly chosen runs were plotted to obtain y-intercepts and slopes for LOD/LOQ calculations.

Table 10. Y-Intercepts and Slopes Used to Calculate LOD/LOQ for Cocaine Method

	Y-intercept	Slope	
7/26 (1)	-0.0025	0.1334	
8/3 (5)	-0.0054	0.1331	
8/10 (2)	-0.0038	0.1385	
Std Dev	0.0015	0.1350	Mean
LOD (ng/ml)	0.0355		
LOQ (ng/ml)	0.1076		

As mentioned earlier, each filter sample consists of ten bills, so to determine the LOD and LOQ on an individual bill the calculated value has to be divided by ten. The LOD was calculated at 0.0355 ng/ml, or .0036 ng/bill. The LOQ was calculated at 0.1076 ng/ml, or 0.0108 ng/bill.

4.3.2. Multi Drug Method

LOD and LOQ values for the multi drug method were calculated in the same manner as for the cocaine method. Using this method, the LOD for cocaine was calculated at 0.2191 ng/ml, with an LOQ of 0.6640 ng/ml. Codeine had an LOD of 0.2449 ng/ml, with an LOQ of 0.7421 ng/ml. Heroin had an LOD of 0.2452 ng/ml, with an LOQ of 0.7430 ng/ml. MDMA had an LOD of 0.2407 ng/ml, with an LOQ of 0.7295 ng/ml. Methamphetamine had an LOD of 0.2109 ng/ml, with an LOQ of 0.6390 ng/ml. Morphine had an LOD of 0.0885 ng/ml, with an LOQ of 0.2683 ng/ml. These values, presented in ng/bill, are shown in Table 11.

Table 11. LOD/LOQ Values Calculated for the Multi Drug Method (ng/bill)

	Cocaine	Codeine	Heroin	MDMA	Meth	Morphine
LOD	0.0219	0.0245	0.0245	0.0241	0.0211	0.0089
LOQ	0.0664	0.0742	0.0743	0.0730	0.0639	0.0268

4.4. Selectivity

Matrix effects were examined using the postcolumn infusion method. A methanol blank as well as a filter blank was used to determine matrix effects. Each blank was run three times. Chromatograms of the runs were examined for suppressions and enhancements of the signal.

4.4.1. Cocaine Method

Matrix effects for the cocaine method were not performed.

4.4.2. Multi Drug Method

The retention times of the peaks associated with suppression or enhancement of signal during sample analysis can be seen in Table 12. A lack of overlap between the analyte retention times and suppression or enhancement retention times indicates that matrix effects will not affect quantitation. The chromatogram of the matrix effects study including all seven drugs, as well as the internal standard are shown in Figure 10.

Table 12. Peak Retention Times Seen During Matrix Effects Test

Drug	RT (in min)	Sample	Retention Time (min) of Suppression	Retention Time (min) of Enhancement
Cocaine	**5.1**	**Methanol**	0.4*, 3.7, 3.9, 4.4	0.4, 0.5
		Currency Blank	0.4, 0.5, 3.7, 3.9, 4.4, 10.3	0.4, 0.5, 8.3*
Codeine	**2.5**	**Methanol**	0.4, 3.7, 3.9, 4.4	0.5, 6.8*
		Currency Blank	0.4, 0.5, 3.7, 3.8, 4.4, 10.3	0.6, 10.5*
Heroin	**4.5**	**Methanol**	0.4*, 3.7, 3.9, 4.4	0.4*, 0.5*, 10.5*
		Currency Blank	0.5, 3.7, 3.8, 4.4	0.6, 10.3
MDMA	**3.4**	**Methanol**	0.4, 3.7, 3.8, 4.4	0.5, 6.7, 8.3*, 10.5*
		Currency Blank	0.4, 3.7, 3.8, 4.4	0.5, 6.7*, 8.3*, 10.2
Meth	**3.1**	**Methanol**	0.4, 3.7, 3.8, 4.4	0.5, 6.7, 8.3*, 10.5*
		Currency Blank	0.3*, 0.4, 3.7, 3.8, 4.4, 10.2	0.5, 6.7*
Morphine	**0.9**	**Methanol**	0.4, 3.7, 3.9, 4.4, 8.3	0.5, 6.8
		Currency Blank	0.4, 3.7, 3.8, 4.4, 10.2	0.6, 6.7
THC	**8.9**	**Methanol**	0.5*, 4.4, 8.3	0.4
		Currency Blank	0.5, 4.4, 10.3	0.4

*Represents peaks not seen in all 3 runs

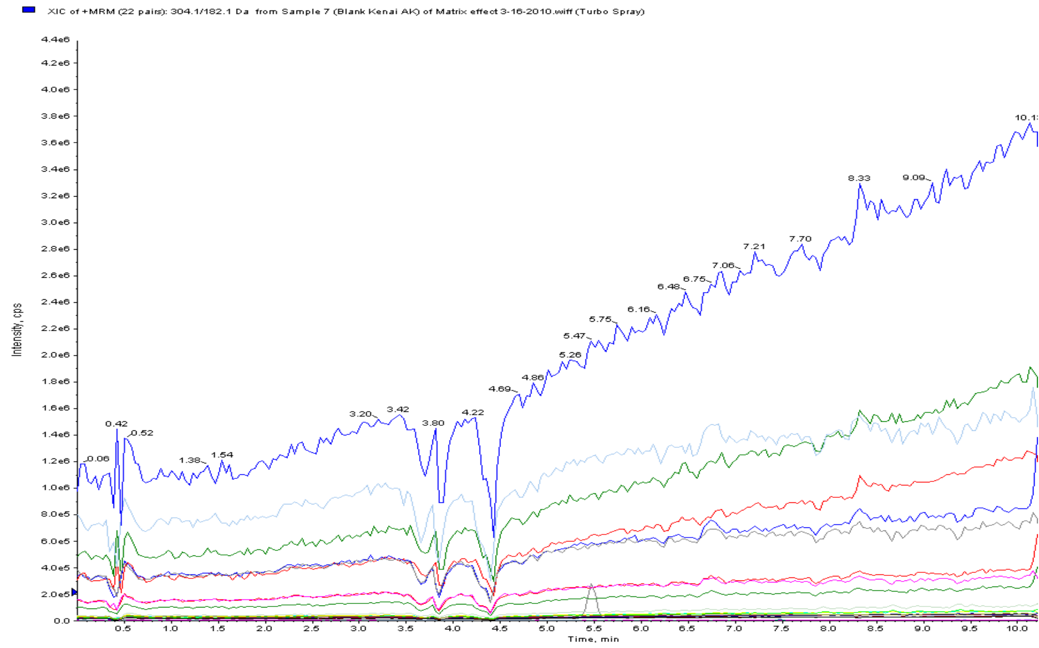


Figure 10. Multi Drug Matrix Effects (All 7 Drugs)
Chromatogram of matrix effects seen using the postcolumn infusion method. Internal standard peak can be seen at approximately 5.5 min.

4.5. Contamination on Currency

4.5.1. Cocaine Method

Specific parameters of the quantitation method used to detect cocaine on currency are summarized in Table 13.

Table 13. Cocaine Quantitation Method

Drug	Fit	Weighting	Bunching Factor	Number of Smooths	Expected Retention Time (min)
Cocaine	Linear Through 0	none	2	2	5.389

As seen in Table 14, 350 bills of each denomination were sampled in this study, for a total of 2,100 bills. The $10 bills had the highest average contamination per bill,

1.0408 ng/bill, while the $20 bills had the lowest average contamination per bill, 0.2865 ng/bill. The $1, 5, 50, and 100 averaged 0.4995, 0.8813, 0.6081, and 0.3555 ng/bill respectively. The average overall contamination of cocaine on currency is 0.6120 ng/bill. ID ratios were used to confirm that the calculated concentrations were accurate.

Table 14. Cocaine Domestic Contamination as a Function of Currency Denomination

Denomination	Average Contamination in # ng/bill	# Bills Sampled
$1	0.4995	350
$5	0.8813	350
$10	1.0408	350
$20	0.2865	350
$50	0.6081	350
$100	0.3555	350
Average:	0.6120	2100

Table 15 shows the cocaine contamination on foreign currency. A total of 243 foreign bills were sampled. The sample from Vienna, Austria was taken from the International Atomic Energy Agency (IAEA) building at the Vienna International Centre. It was U.S. currency, but since it was collected outside of the U.S. it was considered foreign currency in this study. It has the highest contamination of any of the foreign currency samples, with 210.00 ng/bill. The sample from Ottawa, Canada is the only other sample to have over 1.0 ng/bill, with 2.4571 ng/bill. All other foreign

currency samples had less than 0.3 ng/bill of cocaine. The sample collected in 2007 from Singapore had the least amount of cocaine contamination, with 0.0139 ng/bill.

Table 15. Cocaine Foreign Currency Contamination

City, Country	# bills	ng/bill
Vienna, Austria	60	210.00
Prague, Czech Republic '05	4	0.0232
Aix en Provence, France '04	3	0.0285
Vienna, Austria '00	4	0.0299
Puerto Vallarta, Mexico '01	4	0.0341
Islamabad, Pakistan '07	7	0.0275
Rio de Janeiro, Brazil '07	4	0.1295
Beijing, China '07	15	0.0170
Mumbai, India '07	7	0.0273
Singapore, Singapore '09	11	0.0208
Pretoria, South Africa '07	7	0.0359
Pusan, South Korea '08	3	0.0342
Ottawa, Canada '09	7	2.4571
Singapore, Singapore '07	8	0.0139
Tel Aviv, Israel '98	5	0.0489
Lake Como, Italy '97	6	0.0457
Moscow, Russia '96	4	0.0227
Mumbai, India '09	8	0.0335
Lockerbie, Scotland '91	7	0.1657
Kuwait '04	3	0.0323
Jordan '09	5	0.0348
Iraq '04	7	0.0836
China '98	6	0.0218
Croatia '92	5	0.0524
Slovenia '92	5	0.0337
Netherlands '92	3	0.1242
Ukraine '05	4	0.0423
Finland '94	3	0.0755
Italy '99	5	0.2290
USSR '91	3	0.0342
Russia '94	12	0.0156
Hungary '03	8	0.0403

4.5.2. Multi Drug Method

A representative chromatogram of a calibrator can be seen in Figure 11. Figure 12 is a representative chromatogram of an actual $1 filter sample from Portland, Oregon.

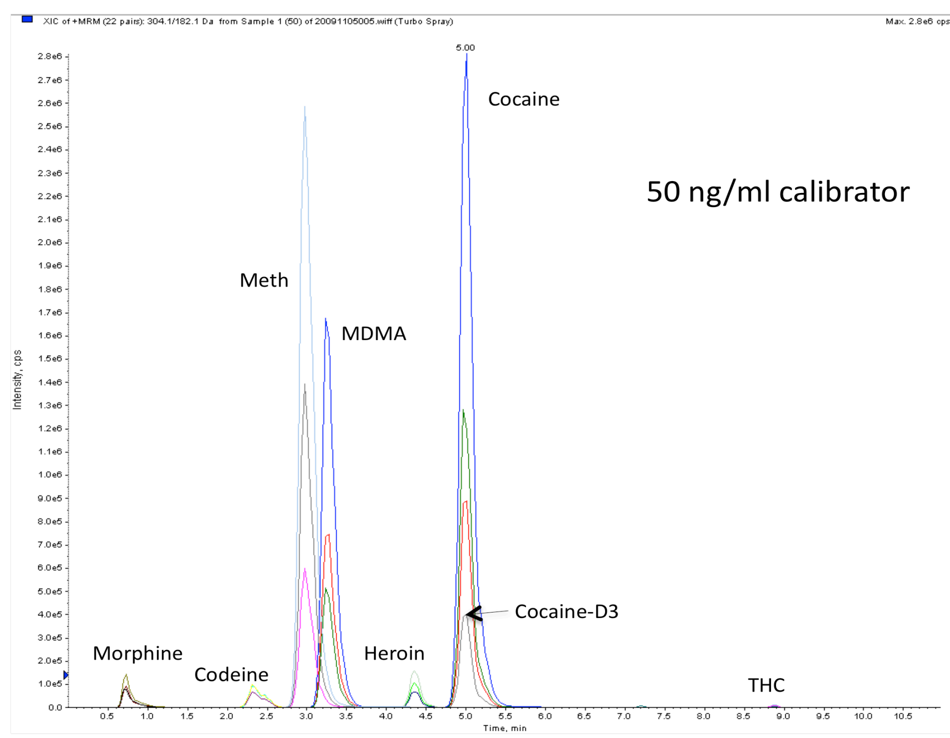


Figure 11. Chromatogram of the Seven Drugs Examined
Chromatogram of a 50 ng/ml multi drug calibrator showing the seven drugs examined in this study (cocaine, codeine, heroin, MDMA, methamphetamine, morphine, and THC) as well as the internal standard (cocaine-D3).

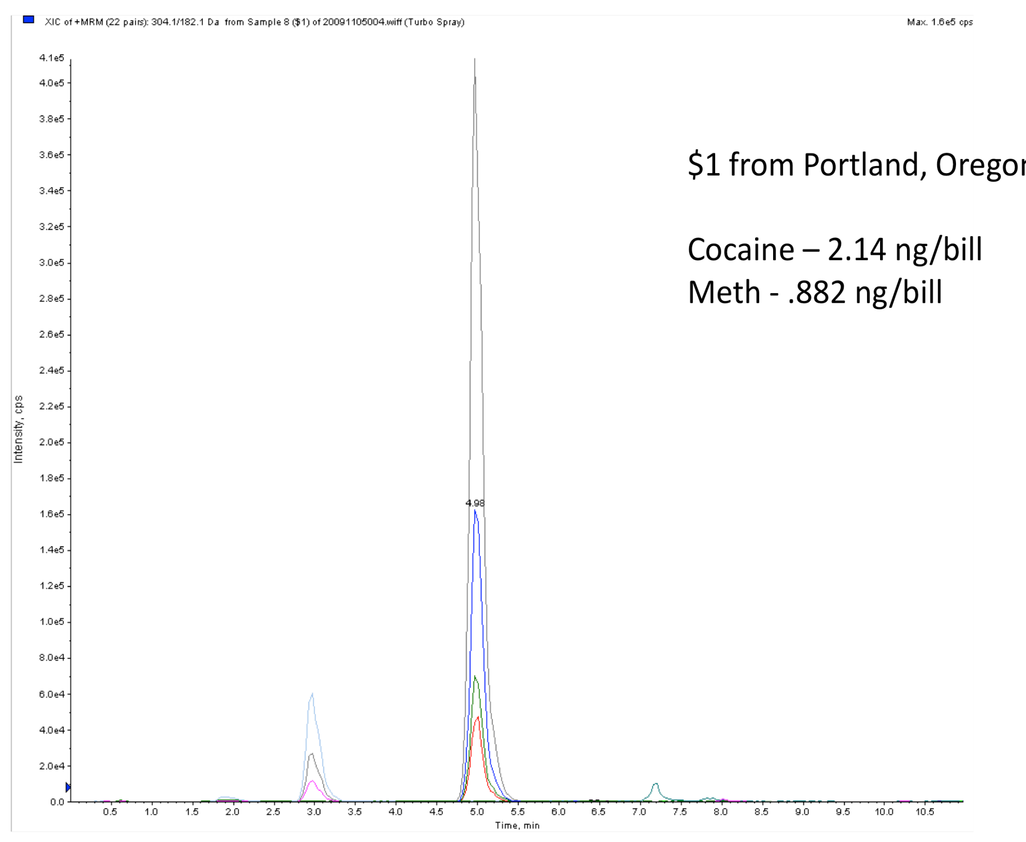


Figure 12. Chromatogram of $1 Filter Sample from Portland, Oregon
Cocaine was detected at 2.14 ng/bill and methamphetamine was detected at 0.882 ng/bill. No other drugs were detected.

Results of screening U.S. currency using the multi drug quantitation method can be seen in Table 16.

Table 16. Multi Drug Quantitation Method

Drug	Fit	Weighting	Bunching Factor	Number of Smooths	Expected Retention Time (min)
Cocaine	Power	ln y	2	2	5.142
Codeine	Quadratic	ln x	2	2	2.507
Heroin	Quadratic	ln y	2	2	4.512
MDMA	Quadratic	ln y	2	2	3.430
Methamphetamine	Quadratic	ln y	2	2	3.131
Morphine	Quadratic	ln y	2	2	0.911
THC	Quadratic	ln y	2	2	8.973

Table 17 shows the number of bills contaminated with each of the five drugs examined as well as the percentage of overall currency contamination with each drug. The percent contamination was calculated by taking the number of contaminated samples of each drug and dividing by the total number of samples (35 cities and 6 currency denominations equals 210 total samples for each drug). Cocaine was detected on 171 samples (81.43%); codeine was detected on 1 sample (0.48%); heroin was detected on 3 samples (1.43%); MDMA was detected on 4 samples (1.90%); methamphetamine was detected on 9 samples (4.29%); and morphine was detected on 7 samples (3.34%).

Table 17. Multi Drug Domestic Percentage of Contaminated Bills

Drug	# Contaminated Samples (n = 210)						% Contamination
	$1	$5	$10	$20	$50	$100	
Cocaine	33	31	31	25	29	22	81.43%
Codeine	-	-	-	1	-	-	0.48%
Heroin	-	2	1	-	-	-	1.43%
MDMA	-	1	2	1	-	-	1.90%
Meth	1	4	-	3	-	1	4.29%
Morphine	-	2	3	0	1	1	3.34%

The amount of drug found on each denomination of bills is shown in Table 18. Cocaine had the highest average contamination with 0.6207 ng/bill, followed by heroin with 0.4040 ng/bill, MDMA with 0.1972 ng/bill, codeine with 0.1435 ng/bill, morphine with 0.1006 ng/bill, and methamphetamine with 0.0961 ng/bill.

Table 18. Multi Drug Domestic Contamination as a Function of Currency Denomination

Drug	Contamination (ng/bill)						Average Contamination (ng/bill)
	$1	$5	$10	$20	$50	$100	
Cocaine	0.4392	0.9060	0.8476	0.3483	0.6530	0.5302	0.6207
Codeine	-	-	-	0.1435	-	-	0.1435
Heroin	-	0.2645	0.5435	-	-	-	0.4040
MDMA	-	0.1020	0.2465	0.2430	-	-	0.1972
Meth	0.0846	0.1164	-	0.0911	-	0.0924	0.0961
Morphine	-	0.1400	0.0545	-	0.0314	0.2770	0.1006

Cocaine was found on currency samples from Austria, Canada, Scotland, and Italy. The amounts in ng/bill can be seen in Table 19.

Table 19. Multi Drug Foreign Currency Contamination

City, Country	# bills	ng/bill
Vienna, Austria	60	90.18*
Ottawa, Canada '09	7	2.393
Lockerbie, Scotland '91	7	0.1538
Italy '99	5	0.2111

* ID1 and ID2 ratios out of range

Codeine, heroin, MDMA, methamphetamine, and morphine were not detected on any of the foreign currency samples.

Chapter V. Discussion

5.1. Contamination

The initial goal of this study was to develop an LC-MS/MS method that would enable the detection of seven illicit drugs on currency. Of the seven drugs (cocaine, codeine, heroin, MDMA, methamphetamine, morphine, and THC), six were reliably detected and quantified with the method developed (Figure 11). THC was not included in the final results because of chromatography and mass spectrometry issues while measuring the other drugs. THC was not ionizing well in positive mode and therefore sensitivity was an issue. It would be difficult to maintain method integrity if the combination of positive and negative ionization were attempted in the same LC-MS/MS analysis

5.2. Internal Standard

Cocaine-D3 was used as the internal standard for both the cocaine and multi drug method. Filter samples were extracted using cocaine-D3, since we were initially looking for cocaine only. We eventually decided to expand the method to look at the seven drugs previously mentioned. Since the filter samples had already been extracted using cocaine-

D3, it was the only internal standard used for the multi drug method since deuterated versions of the other drugs could not be added after the fact. It would have been better to include a deuterated version of each of the seven drugs, rather than just cocaine, but having one internal standard was sufficient.

5.3. Validation of the Methods

5.3.1. Precision

Intraday and interday variability was examined for each calibrator concentration to determine the precision of the method. For the cocaine method, both intraday and interday variability was less than 6%, which is below the generally accepted 15% variability reported by the FDA (US Food and Drug Administration, 2001). When looking at the precision of the multi drug method, variability of the 50 down to the 1 ng/ml calibrators were all less than 15%, with the highest being interday variability of morphine (13.90%). The variability of the 0.1 ng/ml calibrator was not under the accepted 20% allowed at the LOQ for all of the drugs. Interday variability for heroin (63.13%), methamphetamine (57.83%), and morphine (64.98%) were above the accepted 20% due to the fact that 0.1 ng/ml is below the LOQ of each of these drugs. All other variability calculations for the 0.1 ng/ml calibrator were under 20%.

5.3.2. Accuracy

Accuracy for the cocaine method was sufficient in that calculated calibrator values were all within 21% of their known values. The high end calibrators (50, 10, and

5 ng/ml) did not deviate by more than 20%, which is above the recommended 15% deviation. The low end calibrators (1 and 0.1 ng/ml) did not deviate by more than 21%, which is greater than the 20% deviation recommended by the FDA at the LOQ. Accuracy of the high end multi drug calibrators (50, 25, and 10 ng/ml) did not deviate by more than 9%. The lower end calibrators (5, 1, and 0.1 ng/ml) deviated more than that, which is expected as analyte levels approach the LOD. The 5 and 1 ng/ml calibrators did not deviate by more than 16%, but the 0.1 ng/ml calibrators deviated by up to 296%. Since the LOD of all the drugs was over 0.2 ng/ml, these findings are not surprising. Better accuracy is seen with the cocaine method because the LOD was much lower at 0.04 ng/ml.

5.3.3. Sensitivity

The LOD and LOQ were calculated for both the cocaine method and the multi drug method to determine sensitivity. The cocaine method was found to be more sensitive than the multi drug method. The cocaine method had a LOD of 0.0355 ng/ml and a LOQ of 0.1076 ng/ml while the multi drug method had a LOD of 0.2191 ng/ml and a LOQ of 0.6640 ng/ml. This difference can be attributed to the runs chosen to perform the calculations. Since the runs were randomly chosen, the standard deviation of the y-intercept and the mean of the slope varied more in multi drug method than it did in the cocaine method. More sensitivity can also be achieved when looking for one drug rather than seven due to the ability to optimize the parameters for one precursor ion and three product ions rather than seven precursor ions and twenty one product ions.

5.3.4. Selectivity

Matrix effects were examined to determine the selectivity of the method. It is important to look at matrix effects to determine if the instrument's response is due to the analyte or interference.

5.3.4.1. Matrix Effects

Matrix effects were examined using the postcolumn infusion method. The baseline intensity increases throughout the run, as can be seen in Figure 10, due to the organic mobile phase being increased throughout the entirety of the run. Multiple suppressions and enhancements were seen throughout the run, but they did not occur at the retention times of any of the seven drugs examined in this study (see Table 12). Therefore, matrix effects were not a factor, meaning the method is selective.

5.4. Combined Study

Results from this study were combined with samples previously collected by Jourdan between 1993 and 2002 (Table 20). Results from 27 previously analyzed locations were combined with the data from the 35 cities analyzed in this study for a total of 4,176 bills.

Table 20. Contamination as a Function of Currency Denomination (Combined Study)

Denomination	Average Contamination in # ng/bill	# Bills Sampled
$1	2.18	630
$5	3.74	628
$10	3.23	678
$20	1.67	870
$50	2.35	690
$100	1.78	680
Average:	2.44	4176

The average contamination seen per bill when the studies were combined varied greatly from the results seen in this study. An average of 0.6120 ng/bill was seen in the 2,100 bills analyzed for this study. When combined with the additional 2,076 bills previously sampled, the average rose to 2.44 ng/bill. The average of the combined study is so high because the average contamination of each denomination is higher than those seen in this study. The average contamination for the $1 in this study was 0.4995 ng/bill as compared to 2.18 ng/bill in the combined study; $5 was 0.8813 ng/bill compared to 3.74; $10 was 1.0408 ng/bill compared to 3.23 ng/bill; $20 was 0.2865 ng/bill compared to 1.67 ng/bill; $50 was 0.6081 ng/bill compared to 2.35 ng/bill; and $100 was 0.3555 ng/bill compared to 1.78 ng/bill. Both studies showed the $5 and $10 having the highest average contamination, but this study showed that the $10 had the highest average contamination while the combined study showed the $5 had the highest average contamination.

Significance tests were performed on the data from this study as well as Jourdan's study to determine if the averages between denominations were significantly different. One way ANOVA with Tukey's Multiple Comparison Test was used to compare denomination averages from the OSU study with that of the prior Jourdan study, and when the denominations were compared one on one (e.g. OSU $1 vs. Jourdan $1), the only significant difference at $p < 0.05$ was seen with the $5 bill (OSU: 0.8813 ng/bill vs. Jourdan: 7.3080 ng/bill). In addition, the same Tukey post-test demonstrated there was no significant difference between denominations within the OSU study and within the prior Jourdan study (e.g. OSU $1 vs. OSU $5). Using an unpaired Student's t-test, the average contamination from the OSU study (0.6120 ng/bill, n=35) was found to be significantly less than that of the prior Jourdan study (3.633 ng/bill, n=55) at a $p < 0.0005$.

Figure 13 shows the overall background currency contamination by cocaine on the bills analyzed in this combined study.

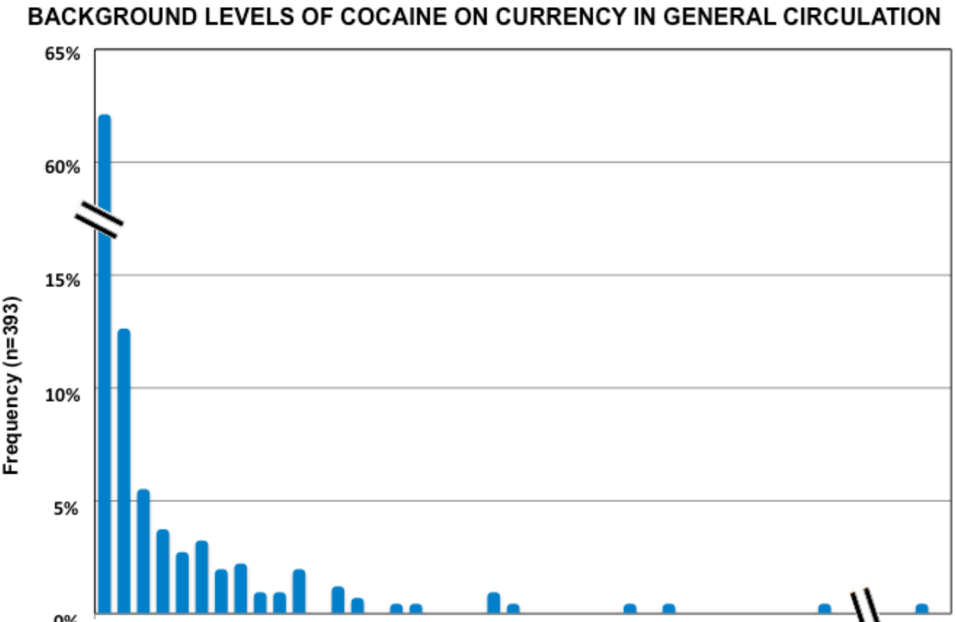


Figure 13. Overall Background Level of Cocaine on Currency (Combined Study)
Graph showing cocaine contamination as a percent of the total contamination seen (ex: cocaine contamination less than 1 ng/bill makes up 60% of the total contaminated bills)

There are several possible reasons why Jourdan found higher average cocaine contamination on currency than this study did. One reason could be the years in which the bills were collected. The samples analyzed by Jourdan were collected between 1993 and 2002. The samples for this study were collected between 2003 and 2009. As mentioned earlier, a significant decline in cocaine usage in the United States has been seen in recent years. It is possible that this decrease in usage has caused a decrease in the

amount of contamination seen on currency. Another reason could be the cities in which the bills were collected. The samples Jourdan analyzed were mostly collected from the east and west coasts. Very few samples were collected from the middle of the country. The samples analyzed in this study were more centrally located. Different regions of the country have been found to have different drug usage, which could account of the different contamination of currency seen. A final possible cause could be the amount of time the filters sat in the test tubes before being analyzed. Samples for this study were collected as early as 2003, but were not analyzed until late 2009. It is possible that the drug on the filters became degraded over time, leading to a lower calculated average contamination.

5.5. Significance

Not many studies have looked at controlled substance contamination on currency. There was no literature on the subject prior to 1987. As shown in Figure 14, interest in the study of currency contamination did not begin until 1994. Since then the number of studies on the topic has grown significantly with 1.8 papers per year from 1994-2003 and 4.5 papers a year from 2005-2007. The insert in Figure 14 shows the distribution of different techniques used in reports to detect cocaine contamination on currency. As of 2007, gas chromatography and thermal desorption methods have been used in the vast majority of currency contamination studies (Armenta & de la Guardia, 2008).

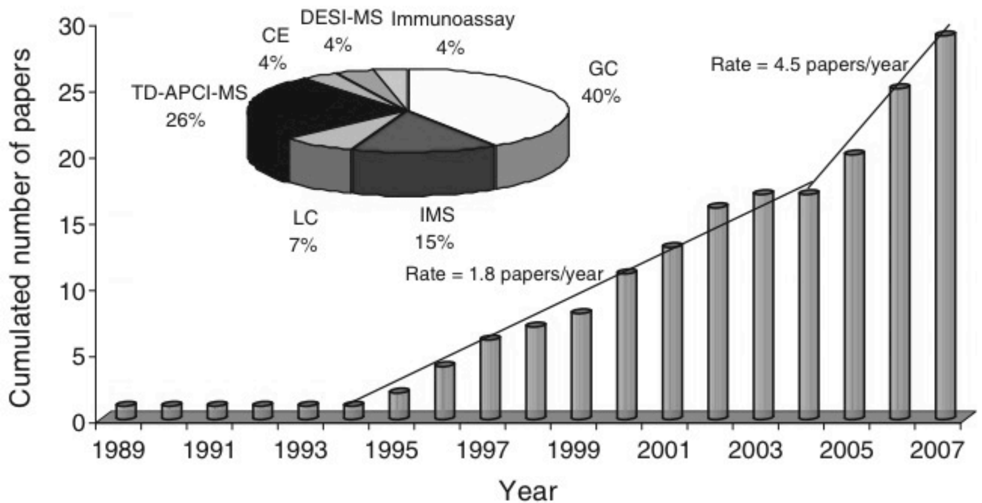


Figure 14. Published Literature About Cocaine on Currency Through 2007 (Adapted from Figure 1 of Armenta & de la Guardia, 2008)
Inset: Distribution of the different techniques used to detect cocaine. GC – gas chromatography; IMS – ion mobility spectrometry; LC – liquid chromatography; TD-APCI-MS – thermal desorption-atmospheric pressure chemical ionization-mass spectrometry; CE – capillary electrophoresis; DESI-MS – desorption electrospray ionization-mass spectrometry (Armenta & de la Guardia, 2008)

5.5.1. Other LC-MS/MS Studies

Scarcely any studies have used liquid chromatography tandem mass spectrometry (LC-MS/MS) to examine currency contamination with controlled substances. In 2007, the first LC-MS/MS procedure was developed, optimized, and validated to determine currency contamination on banknotes, which allowed for improved sensitivity over previous methods (Bones, et al., 2007). The authors used methanol extraction rather than the vacuum sampling method used in this study, hereinafter referred to as the Bones

study. Our method detection limit was about 4 picograms per bill for cocaine, which is comparable with the Bones study. However, this detection limit assumes that the filters had all of the cocaine transferred from the bill, which is not likely to be true with our vacuum sampling method. Solvent extraction is better at removing analytes from the bill as compared to vacuum sampling, which provides low efficiency of removing the analyte from the sample. Both studies used electrospray ionization. Bones and colleagues chose to look at only one product ion, while the OSU approach examined three. Using three product ions provides more quality assurance that the ion pairs being observed are representative of the drugs being examined in this study. The Bones study had a run time was 30 minutes per injection for a full screen of all 16 drugs they examined in their study. The current OSU study had an 11 minute run time to obtain a full screen of the 7 drugs chosen for examination.

5.6. Comparison to Other Studies

This study looked at 2,100 bills and when combined with Jourdan's previous work totaled 4,176 bills. This is more than most other studies conducted. Early studies by Jordan and Donnelly, as well as studies by Jenkins (2001), Esteve-Turrillas *et al.* (2005), Di Donato *et al.* (2007), Xu *et al.* (2006), and Bones (2008) all sampled 100 bills or less. Hearn (1998), Oyler *et al.* (1996), Lavins *et al.* (2004), and Ebejer *et al.* (2007) all sampled less than 1,000 bills. Dixon *et al.* (2006) sampled over 7,000 bills in a background study and close to 5,000 in a case study.

The amount of cocaine detected on the bills in this study was also less than what was reported in many of the previous studies. Using the cocaine method developed in this study, 100% of the bills tested were contaminated with cocaine. In the combined study, 97% of the bills were contaminated with cocaine. In the Hearn study, 97% of the bills tested were contaminated with an average of 7.3 µg per bill (Jenkins, 2001); Jourdan and Donnelly reported 95% cocaine contamination at an average of 13 ng per bill (Jenkins, 2001); Oyler *et al.* (1996) reported 79% of currency had an average contamination of 0.1 µg per bill and 54% had a contamination of over 1.0 µg per bill; Esteve-Turrillas *et al.* (2005) found cocaine contamination of bills ranging from 1.25-889 µg per bill; Di Donato *et al.* (2007) found an average contamination of 93% with contamination ranging from 2.38-275.10 µg per bill; Jenkins (2001) reported 92% contamination with a range of 0.01-922.72 µg per bill; and Bones (2007) found 100% cocaine contamination with 62% over 2 ng per bill and 5% over 200 ng per bill.

The amount of other controlled substances detected also differs from what has been reported in other studies. This study found 0.48% codeine contamination, 1.43% heroin contamination, 1.90% MDMA contamination, and 4.29% methamphetamine contamination. Jenkins (2001) reported 14% heroin contamination ranging from 0.03-168.50 µg per bill, morphine and methamphetamine were each found on 6% of bills, and codeine was not detected; Ebejer (2005) reported heroin contamination on 2-3% of bills in circulation. Bones (2007) reported 7% heroin contamination and was unable to detect

morphine, amphetamine, MDMA, ketamine, cocaethylene, LSD, EDDP, papaverine, methadone, fluoxetine, temazepam, diazepam, and Δ^9-THC.

This method does not destroy currency like some other methods. Although vacuum sampling is only considered semi-quantitative and provides a low efficiency of removing the analyte from the sample, it is useful in that it does not destroy the currency and can be used in the field. Thermal desorption destroys the analyte and the bill, or that portion of the bill tested. Several solvents, including acetonitrile and chloroform, have been found to cause damage to the security band and holographic marks of euro notes, rendering them useless after testing as well. Methanol has been found not to cause destruction to bills (Esteve-Turrillas, et al., 2005). Water has also been used as a non-destructive method to extract drugs from currency. It preferably extracts hydrophilic chemicals, such as cocaine salt, but not hydrophobic compounds (Zuo, Zhang, Wu, Rego, & Fritz, 2008).

5.7. Future Work

Future work that could be done in relation to this study would be to obtain "real" blanks from the Federal Reserve before they are run through counting machines. It is important to see if a true blank bill, which has not been put through a currency counter, will show any compound or material that may interfere with drug detection.

Another study that could be done would be to spike some blank bills that have not been put through a counting machine and use the vacuum sampling extraction method to

quantitate recovery. This experiment would give provide an idea of how much of the total drug on the bill we are actually extracting using the vacuum sampling method. Bills could be spiked and other extraction methods could be used, such as thermal desorption or solvent studies. In order to determine the likelihood that a currency sample has been involved in drug trafficking, more background measurements will need to be obtained.

5.8. Conclusions

This study yielded results that were consistent with prior studies in the field of currency contamination with drugs. The additional analysis of drugs other than cocaine, with a large number of bills sampled, both foreign and domestic, allows this work to stand out among other studies. In order to determine the likelihood that a currency sample has been involved in drug trafficking, more background measurements will need to be obtained.

Chapter VI. References

Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *J Chromatogr B, 877*(23), 2224-2234.

Armenta, S., & de la Guardia, M. (2008). Analytical methods to determine cocaine contamination of banknotes from around the world. [Article]. *Trac-Trends in Analytical Chemistry, 27*(4), 344-351.

Baselt, R. (2008). *Disposition of Toxic Drugs and Chemicals in Man* (8th ed.): Biomedical Publications.

Bones, J. (2007). Cocaine contaminates circulating currency. [News Item]. *Trac-Trends in Analytical Chemistry, 26*(3), III-IV.

Bones, J., Macka, M., & Paull, B. (2007). Evaluation of monolighic and sub 2 um particle packed columns for the rapid screening for illicit drugs - application to the determination of drug contamination on Irish euro banknotes. *The Analyst, 132*, 208-217.

Carter, J. F., Sleeman, R., & Parry, J. (2003). The distribution of controlled drugs on banknotes via counting machines. [Article]. *Forensic Science International, 132*(2), 106-112.

Clouser-Roche, A., Johnson, K., Fast, D., & Tang, D. (2008). Beyond pass/fail: A procedure for evaluating the effect of carryover in bioanalytical LC/MS/MS methods. *Journal of Pharmaceutical and Biomedical Analysis, 47*, 146-155.

Di Donato, E., Santos Martin, C. C., & De Martinis, B. S. (2007). Determination of cocaine in Brazilian paper currency by capillary gas chromatography/mass spectrometry. [Article]. *Quimica Nova, 30*(8), 1966-1967.

Dixon, S. J., Brereton, R. G., Carter, J. F., & Sleeman, R. (2006). Determination of cocaine contamination on banknotes using tandem mass spectrometry and pattern recognition. [doi: DOI: 10.1016/j.aca.2005.11.082]. *Analytica Chimica Acta, 559*(1), 54-63.

Drug Trafficking in the United States. (n.d.). *U.S. Drug Enforcement Administration* Retrieved June 3, 2010, from http://www.justice.gov/dea/pubs/state_factsheets.html

Ebejer, K. A., Brereton, R. G., Carter, J. F., Ollerton, S. L., & Sleeman, R. (2005). Rapid comparison of diacetylmorphine on banknotes by tandem mass spectrometry. [Article]. *Rapid Communications in Mass Spectrometry, 19*(15), 2137-2143.

Ebejer, K. A., Lloyd, G. R., Brereton, R. G., Carter, J. F., & Sleeman, R. (2007). Factors influencing the contamination of UK banknotes with drugs of abuse. [Article]. *Forensic Science International, 171*(2-3), 165-170.

Esteve-Turrillas, F. A., Armenta, S., Moros, J., Garrigues, S., Pastor, A., & de la Guardia, M. (2005). Validated, non-destructive and environmentally friendly determination of cocaine in euro bank notes. [Article]. *Journal of Chromatography A, 1065*(2), 321-325.

FAQs: Currency: Production & Circulation. (n.d.). *United States Department of the Treasury* Retrieved June 3, 2010, from http://www.ustreas.gov/education/faq/currency/production.shtml

Frederick, K. A., Pertaub, R., & Kam, N. W. S. (2004). Identification of individual drug crystals on paper currency using Raman microspectroscopy. [Article]. *Spectroscopy Letters, 37*(3), 301-310.

Frequently Asked Questions: Currency: Notes and Coins. (2010, April 26, 2010). *The Federal Reserve Board* Retrieved June 3, 2010, from http://www.federalreserve.gov/generalinfo/faq/faqcur.htm

Furton, K. G., Hsu, Y.-L., Luo, T.-Y., Alvarez, N., & Lagos, P. (1997). *Novel sample preparation methods and field testing procedures used to determine the chemical basis of cocaine detection by canines*.

ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Methodology. (1996). Paper presented at the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Isenschmid, D. S. (2002). Cocaine - Effects on Human Performance and Behavior. *Forensic Science Review, 14*(1), 62-100.

Jenkins, A. J. (2001). Drug contamination of US paper currency. [Article]. *Forensic Science International, 121*(3), 189-193.

Keil, A., Talaty, N., Janfelt, C., Noll, R. J., Gao, L., Ouyang, Z., et al. (2007). Ambient mass spectrometry with a handheld mass spectrometer at high pressure. *Analytical Chemistry, 79*(20), 7734-7739.

Lavins, E. S., Lavins, B. D., & Jenkins, A. J. (2004). Cannabis (marijuana) contamination of United States and foreign paper currency. [Article]. *Journal of Analytical Toxicology, 28*(6), 439-442.

Levine, B. (2006). *Principles of Forensic Toxicology* (2nd Edition ed.). Washington D.C.: AACC Press.

Logan, B. K. (2002). Methamphetamine - Effects on Human Performance and Behavior. *Forensic Science Review, 14*(1), 134.

Moffat, A. C., Osselton, M. D., Widdop, B., & Galichet, L. Y. (2004). *Clarke's Analysis of Drugs and Poisons* (3rd ed.): Pharmaceutical Press.

Oyler, J., Darwin, W. D., & Cone, E. J. (1996). Cocaine contamination of United States paper currency. [Article]. *Journal of Analytical Toxicology, 20*(4), 213-216.

Politi, L., Groppi, A., & Polettini, A. (2006). Ionisation, ion separation, and ion detection in LC-MS. In A. Polettini (Ed.), *Applications of LC-MS in Toxicology* (pp. 1-22): Pharmaceutical Press.

Sandeep, C., Bussink, C., Degenhardt, L., Johansen, R., Kazanxhiu, L., Korenblik, A., et al. (2009). *World Drug Report 2009* (Report): United Nations Office on Drugs and Crime.

Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A, 987*(1-2), 57-66.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2007). *Principles of Instrumental Analysis Sixth Edition* (6th ed.): Thomson.

Sleeman, R., Burton, R., Carter, J., Roberts, D., & Hulmston, P. (2000). Drugs on money. [Article]. *Analytical Chemistry, 72*(11), 397A-403A.

Spiess, M. (2003). *Drug Data Summary*.

Stockl, D., D'Hondt, H., & Thienpont, L. M. (2009). Method validation across the disciplines-Critical investigation of major validation criteria and associated experimental protocols. *Journal of Chromatography B, 887*, 2180-2190.

Taverniers, I., De Loose, M., & Van Bocktaele, E. (2004). Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry, 23*(8), 535-552.

Taylor, P. J. (2005). Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clinical Biochemistry, 38*, 328-334.

Taylor, P. J. (2006). Method Development and Optimisation of LC-MS. In A. Polettini (Ed.), *Applications of LC-MS in Toxicology* (pp. 23-42): Pharmaceutical Press.

US Food and Drug Administration. (2001). *Guidance for Industry: Bioanalytical Method Validation*. Retrieved from http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf.

Van Eeckhaut, A., Lanckmans, K., Sarre, S., Smolders, I., & Michotte, Y. (2009). Validation of bioanalytical LC-MS/MS assays: Evaluation of matrix effects. [doi: DOI: 10.1016/j.jchromb.2009.01.003]. *Journal of Chromatography B, 877*(23), 2198-2207.

Why LC/MS/MS?: Background and Theory of Electrospray and Tandem Mass Spectrometry. (n.d.). National Institute of Justice.

Wood, M., Laloup, M., Samyn, N., del Mar Ramirez Fernandez, M., de Brujin, E. A., Maes, R. A. A., et al. (2006). Recent applications of liquid chromatography-mass spectrometry in forensic science. *Journal of Chromatography A, 1130*(1), 3-15.

Xu, Y. H., Gao, Y., Wei, H., Du, Y., & Wang, E. K. (2006). Field-amplified sample stacking capillary electrophoresis with electrochemiluminescence applied to the determination of illicit drugs on banknotes. [Article]. *Journal of Chromatography A, 1115*(1-2), 260-266.

Zhou, S., Song, Q., Tang, Y., & Naidong, W. (2005). Critical Review of Development, Validation, and Transfer for High Throughput Bioanalytical LC-MS/MS Methods. *Current Pharmaceutical Analysis, 1*, 3-14.

Zuo, Y. G., Zhang, K., Wu, J. P., Rego, C., & Fritz, J. (2008). An accurate and nondestructive GC method for determination of cocaine on US paper currency. [Article]. *Journal of Separation Science, 31*(13), 2444-2450.

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Master of Science

Thesis: DEVELOPMENT OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) METHODS FOR EXPLORATION OF CURRENCY CONTAMINATION WITH CONTROLLED SUBSTANCES

Major Field: Forensic Sciences

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Title of Study: DEVELOPMENT OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) METHODS FOR EXPLORATION OF CURRENCY CONTAMINATION WITH CONTROLLED SUBSTANCES

Pages in Study: 88 Candidate for the Degree of Master of Science

Major Field: Forensic Sciences

Scope and Method of Study: The purpose of this research was to develop and validate a method for the quantitation of controlled substances on currency using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The LC-MS/MS instrument was optimized for the quantitation of cocaine, codeine, heroin, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, and morphine in methanol by adjusting instrument parameters in the ion path and the source. Validation was performed to determine precision, accuracy, sensitivity and selectivity of the analytical method. Filters to be analyzed were obtained through vacuum extraction of sets of ten bills of each of the following denominations: $1, $5, $10, $20, $50, and $100. The filters were extracted in methanol containing deuterated internal standard and the concentrations of controlled substances on the filter were determined through LC-MS/MS. Domestic and foreign currencies were analyzed in this study.

Findings and Conclusions: The LC-MS/MS method was successfully developed to quantitate cocaine, codeine, heroin, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, and morphine and was subsequently validated. The method utilized a gradient LC method with electrospray ionization and three ions per analyte in multiple reaction monitoring (MRM) mode. The limit of quantitation for cocaine was 0.0664 ng/bill, codeine was 0.0742 ng/bill, heroin was 0.0743 ng/bill, 3,4-methylenedioxymethamphetamine (MDMA) was 0.0730 ng/bill, methamphetamine was 0.0639 ng/bill, and morphine was 0.0268 ng/bill. There was a total of 2,100 domestic bills sampled and contamination percentage was 81.43% for cocaine, 0.48% for codeine, 1.43% for heroin, 1.90% for 3,4-methylenedioxymethamphetamine (MDMA), 4.29% for methamphetamine, and 3.34% for morphine. Cocaine was detected from 4 of 32 foreign currency locations and no other controlled substances were detected.

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