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Comparing the Quantitation of Opiates from Possible Drug Overdose Cases Using Results of Blood Analysis and Liver Analysis

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by

Lee Ann Garozzo

An Abstract of a Thesis
in
Forensic Science

Submitted in Partial Fulfillment
of the Requirements
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State University of New York
College at Buffalo
Department of Chemistry

ABSTRACT OF THESIS

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Currently the quantitation of opiates at the Erie County Medical Examiner's Office Toxicology Laboratory is conducted through whole blood analysis. The objective of this thesis project was to determine if the analysis of opiates could be conducted through liver analysis, and if the analysis of opiates would provide a more accurate quantitation compared to the blood analysis. The quantitation of opiates was conducted from the livers of sixty-four possible overdose cases that were brought into the Erie County Medical Examiner's Office between 2013 and 2015. Results showed that the opiate drugs could successfully be quantitated using the liver analysis. Generally the data showed there to be quantitatively more drugs measured in the liver in comparison to the blood. The liver is the primary site for the metabolism of toxins in the body, and results conclude that opiates are present, generally, in higher levels in the liver than in the blood.

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Chapter I

Introduction

Sixty four cases from the Erie County Medical Examiner's Office were chosen for this thesis study based on an initial suspicion of opiate overdose. If the case tested positive for opiates during the immunoassay screening process, a detailed blood analysis is conducted to determine the quantitation of opiates present in that individual at the time of autopsy. A liver analysis was conducted on these sixty four cases in order to determine if the liver quantitation provides more accurate opiate concentration than measured in the blood.

Every death that gets reported to the Medical Examiner's Office (e.g. accidents, suicides, homicides, etc.) receives a toxicology screen that consists of a blood-alcohol test and three presumptive tests for drugs; an oxycodone/oxymorphone direct enzyme-linked immunosorbent assay (ELISA) blood kit test, a basic drug screen from the gas chromatograph mass spectrometer (GC/MS), and an acid neutral screen from the liquid chromatograph dual mass spectrometer (LC/MS/MS) (Erie County Department of Health, 2015).

Analysis of volatiles, which includes the quantitation of ethanol, methanol, isopropanol and acetone, is done in each case. The screening method uses a headspace gas chromatograph to analyze these volatiles and semi-volatile organics which may be present in the samples. The immunoanalysis oxycodone direct ELISA kit checks for the

possible presence of amphetamine, benzodiazepines, buprenorphine, cannabinoids, carisoprodol, cocaine metabolite, fentanyl, methadone, opiates, oxycodone, sympathomimetics and zolpidem.

The basic drug screen conducted through the GC/MS detects about 40 basic drugs with a pH > 7, and the acid neutral drug screen detects acidic and neutral drugs (pH ≤ 7). The Erie County Medical Examiner's Office Toxicology Lab has been working toward incorporating a Xevo G2-XS Time of Flight (Tof) instrument by Waters technology to identify and confirm compounds in samples (Waters, 2016). The instrument has about 3,000 compounds in its database that it is capable of identifying. When the instrument is successfully in analyzing samples the Toxicology Lab hopes to replace both the basic, acid, and neutral screening tests with the Xevo G2-XS Tof (Private conversation with C. Corcoran, Toxicologist, Erie County Medical Examiner's Office, March 16, 2016).

Any positive tests that result from these presumptive tests must go through confirmatory and qualitative analysis. Results from the presumptive drug screens will determine which samples will be tested for further confirmation and quantitation. If the quantitation test is negative, then the result of those drugs are "non-detected," and if the quantitation results are over the limit of detection then the quantitation analysis confirms the presence and concentration of those drugs (Private conversation with C. Corcoran, Toxicologist, Erie County Medical Examiner's Office, March 16, 2016).

Currently, the quantitation of opiates in the blood is conducted through LC/MS/MS. Specifications of the extraction procedure and the LC/MS/MS are listed in Appendix II. The process is designed to quantitate the opiate analytes in whole blood

(plasma and serum). The opiate drugs currently tested in the Erie County Toxicology lab include morphine, morphine-3-glucuronide, morphine-6-glucuronide, 6-monoacetylmorphine (6-MAM), hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, fentanyl, norfentanyl, buprenorphine, norbuprenorphine, also naloxone.

Many opiate drugs are commonly used as pain medication. Opiate drugs are generally called “opiates” because they can be derived from opium or from chemical derivatives (International Narcotics Control Board, 2007). “Opioid” is a general term for natural and synthetic drugs similar to morphine but not necessarily similar in chemical structure (International Narcotics Control Board, 2007).

According to Figure 1 the consumption of opioids, opiates and synthetic opioids has significantly increased from 1994-2013, simultaneously there has been an increase in the abuse of prescriptions drugs which have related to some overdose deaths in those countries of high consumption (International Narcotics Control Board, 2014).

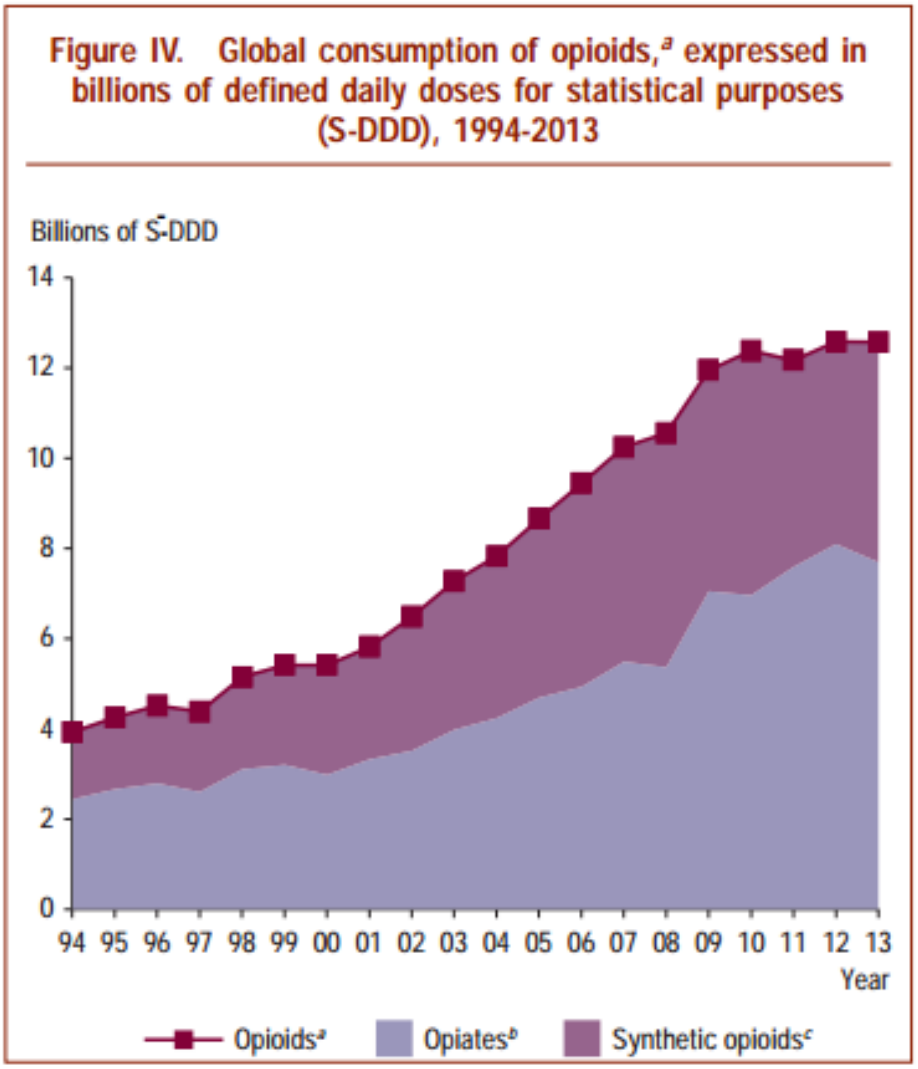

Figure 1. Displays the daily doses of opioid consumption calculated from countries around the world calculated from the International Narcotics Control Board for 2013.

[a] Opioids: opiates and synthetic opioids
[b]Opiates: Including buprenorphine, an opiate controlled under the Convention on Psychotropic Substances of 1971
[c]Synthetic Opioids: Including pentazocine, a synthetic opioid controlled under the Convention on Psychotropic Substances of 1971
(International Narcotics Control Board, 2014)

Morphine and codeine are natural alkaloids which can be extracted from the raw materials of the poppy plant, *Papaver somniferum*; these raw materials consist of opium and poppy straw (International Narcotics Control Board, 2007). Morphine acts on the central nervous system to decrease the feeling of pain.

Heroin, also known as 3, 6-diacetylmorphine, or "diamorphine," is a derivative of morphine formed through acetylation. Heroin was derivatized from morphine in the hope of producing a drug with reduced the physical dependence compared to morphine, and with increased analgesic effects (Bedford, 1991). Although the analgesic effects were much stronger than morphine, the physical dependence from using the drug was increased much more as well (Bedford, 1991). The highest number of deaths are from opiate substance abuse according to a study conducted in 2013 (GBD 2013 Mortality and Causes of Death Collaborators, 2014). Figure 2 displays the most dangerous drugs which are often abused (Nutt, 2007).

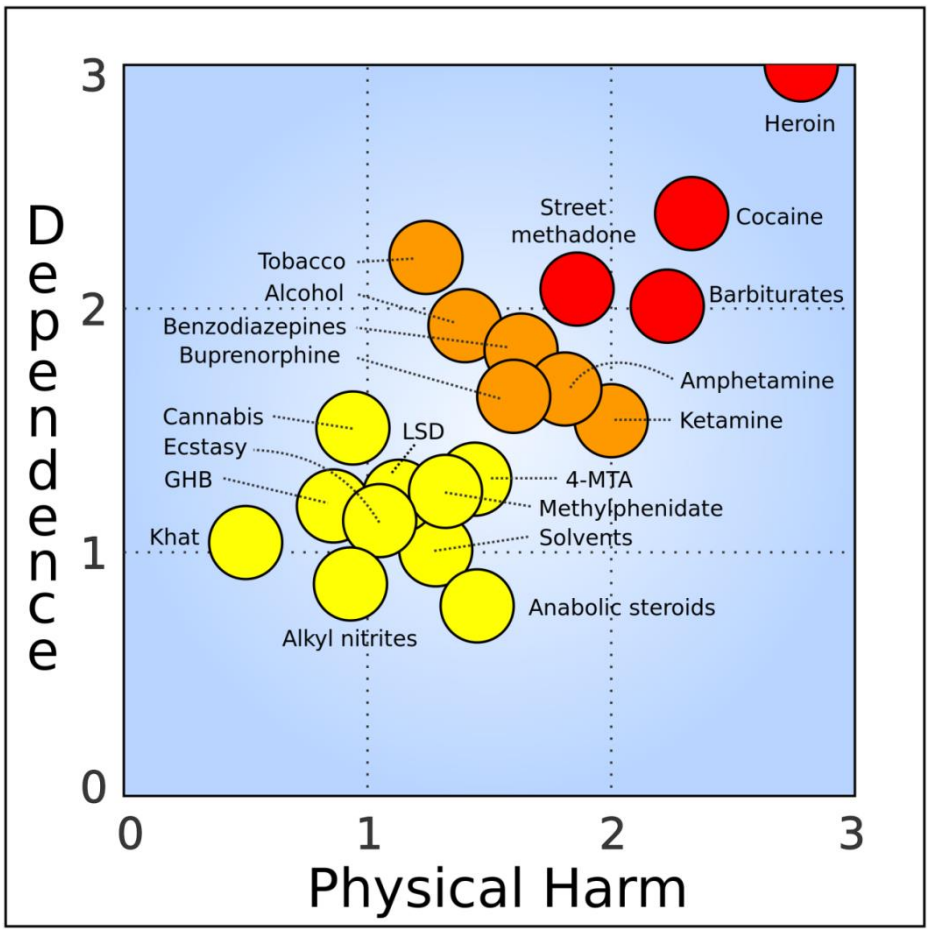

Figure 2. The comparison of dangerously addictive various drugs are generated from information by medical psychiatrists who specialize in addiction treatment (Nutt, 2007).

Codeine is an opiate used to treat pain, diarrhea and for use as a cough suppressant. Although it is a natural alkaloid most of the time it is created through a semi-synthetic process using morphine (International Narcotics Control Board, 2007). In 2010-2013 it was labeled as one of the most commonly used narcotic drugs among the world through dosage and the number of countries it was used in (International Narcotics Control Board, 2014).

Codeine is usually ingested and is converted into morphine by the cytochrome P450 enzyme CYP2D6 in the liver by O- and N-demethylation (Srinivasan, 1997). Some

medications are inhibitors of this enzyme, such as Paxil, Prozac, Benadryl, and can block the conversion of codeine to morphine while other drugs if present in the body simultaneously, may speed up the conversion of codeine into morphine (Srinivasan, 1997). Codeine and its metabolites are mostly excreted from the body through the kidneys (DataPharm, 2015).

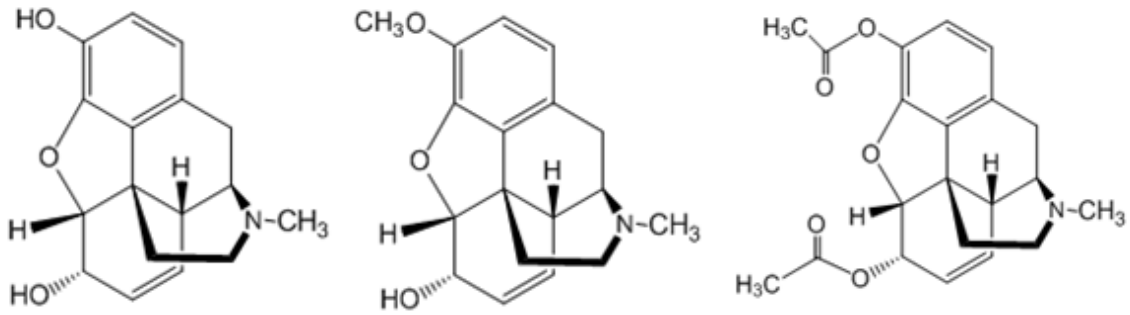

Figure 3. The chemical structure of morphine, codeine, and heroin, respectively (Bedford, 1991).

Diamorphine ("heroin") is a Schedule 1 drug in the U.S., in order to possess it legally one must have a license provided by the Drug Enforcement Administration (U.S. Department of Justice Drug Enforcement Administration, 2015a). The punishable sentence of possessing over 100 grams of diamorphine, or a mixture of diamorphine could be more than 5 years federal imprisonment.

According to the most recent statistics, India is the major manufacturer of licitly produced of opium. The bulk of opium exported to other countries is used for the extraction of alkaloids while some countries use prepared opium for medicinal purposes such as treating diarrhea and coughs. Although India is a major exporter of licitly

produced opium, the U.S. acquires most of its heroin illegally from Mexico and Colombia through Mexican criminal cartels (International Narcotics Control Board, 2014; Yucatan Times, 2014).

Diamorphine can be administered in the body orally, intravenously, by smoking, snorting or as a suppository. Administering the drug orally is the least popular method since the diamorphine becomes completely metabolized into morphine through first-pass metabolism in the liver and gut wall, greatly reducing the bioavailability of the drug and the physical effects (Rowland, 1972).

Injecting heroin in the body is the most popular method of use and is the fastest route of administration. When administered in the body intravenously, heroin is metabolized in the blood immediately into 6-monoacetyl morphine, also called 6-MAM (Baselt, 2011; Bedford, 1991). Diamorphine's first metabolite, 6-MAM can be found more concentrated in the skeletal muscle, kidney, lung, liver and spleen before showing up in the blood (DataPharm, 2013). However, in an overdose death, neither 6MAM or heroin can be detected in post-mortem tissue unless the heroin was administered in the body very close to the time of death (Bedford, 1991). The half-life of morphine is 2-3 hours and the half-life of diamorphine is 2-6 minutes (DataPharm, 2013). This is one reason why 6-MAM was difficult to quantitate during this project. Once the 6-MAM crosses the blood-brain barrier, which separates circulation blood from brain extracellular fluid in the central nervous system so it can react with opiate receptors, it gets metabolized into morphine through hydrolysis (Baselt, 2011). Then morphine is carried to the liver where it is broken down through first-pass metabolism. About 40-50% of the morphine from the liver makes it to the central nervous system and during metabolism,

morphine forms conjugates with glucuronic acid by the phase II metabolism enzyme UDP-glucuronosyl transferase-2B7, forming about 60% morphine-3-glucuronide and about 6-10% morphine-6-glucuronide leading to the formation of morphine-3,6-diglucuronide as shown in Figure 4 (Bedford, 1991; DataPharm, 2013; Mandal, 2013).

Morphine can also be metabolized into, normorphine, codeine and hydromorphone while in the body, but in much smaller concentrations; shown in Figure 5 (Mandal, 2013). The majority of what started as heroin is excreted from the kidneys as morphine and its glucuronide metabolites in urine (DataPharm, 2013; Mandal, 2013). About 7-10% of the drug is eliminated through the liver, gallbladder and bile ducts, while about 35% of the morphine component remains bound to human plasma proteins (DataPharm, 2013). If death occurs shortly after administering the drug, the concentration of morphine and its metabolites in the liver may be low; however, if levels in the liver and bile are higher than the blood concentration it's possible that the individual had a prolonged survival time (Bedford, 1991).

Morphine can also be administered in the form of tablets or capsules, rectally, intravenously, subcutaneously, inhaled, or snorted in powder form. Morphine itself is a highly addictive opioid often used in the medical field for surgery and pain relief. Once administered in the body, morphine is absorbed into the bloodstream and is metabolized as previously stated: morphine is primarily broken down in the liver and glucuronidated by the phase II metabolism enzyme to form its metabolites, and some may get stored in fat, where it may be detected after a long period of time and sometimes until death. The length of time these drugs remain in fat cells depends on different characteristics of that individual; such as, age, mass, health, and rate of metabolism. The elimination half-life

for morphine is about 120 minutes, and then it's usually excreted in through the urine in the form of a metabolite (Mandal, 2013).

Drug concentrations recorded from the autopsy may not reflect the accurate drug concentrations from that individual at the time of death due to postmortem redistribution (PMR). PMR is the distribution of drugs to the surrounding tissues after death and may occur through a number of mechanisms such as diffusion through blood vessels or through diffusion of organ cavities to surrounding organs. Many of the effects of PMR are still unknown, and different drugs react differently; but in many cases, drug concentration, volume of drug distribution and the temperature of the corpse may influence the mechanism of PMR (Pélissier-Alicot, 2003). Many cases have shown drugs in the stomach and gastrointestinal tract to diffuse to the lower lobe of the left lung, the left kidney and the left posterior section of the liver; therefore doctors tend to sample the right sides of organs during an autopsy since they may be less prone to PMR. According to the Doctors at the Erie County Medical Examiner's Office, all the liver samples collected from autopsy; including all the samples used in this project, are obtained from the right lobe of the liver unless physical effects prevent the occurrence of this procedure. Drugs that may be present in the liver, such as opiates, may often become redistributed through the hepatic veins to right chambers of the heart and its surrounding blood vessels, which may cause the drug concentration to decrease in the hepatic lobes which may contribute to the difference in drug concentration in the different sampling sites (Pélissier-Alicot, 2003).

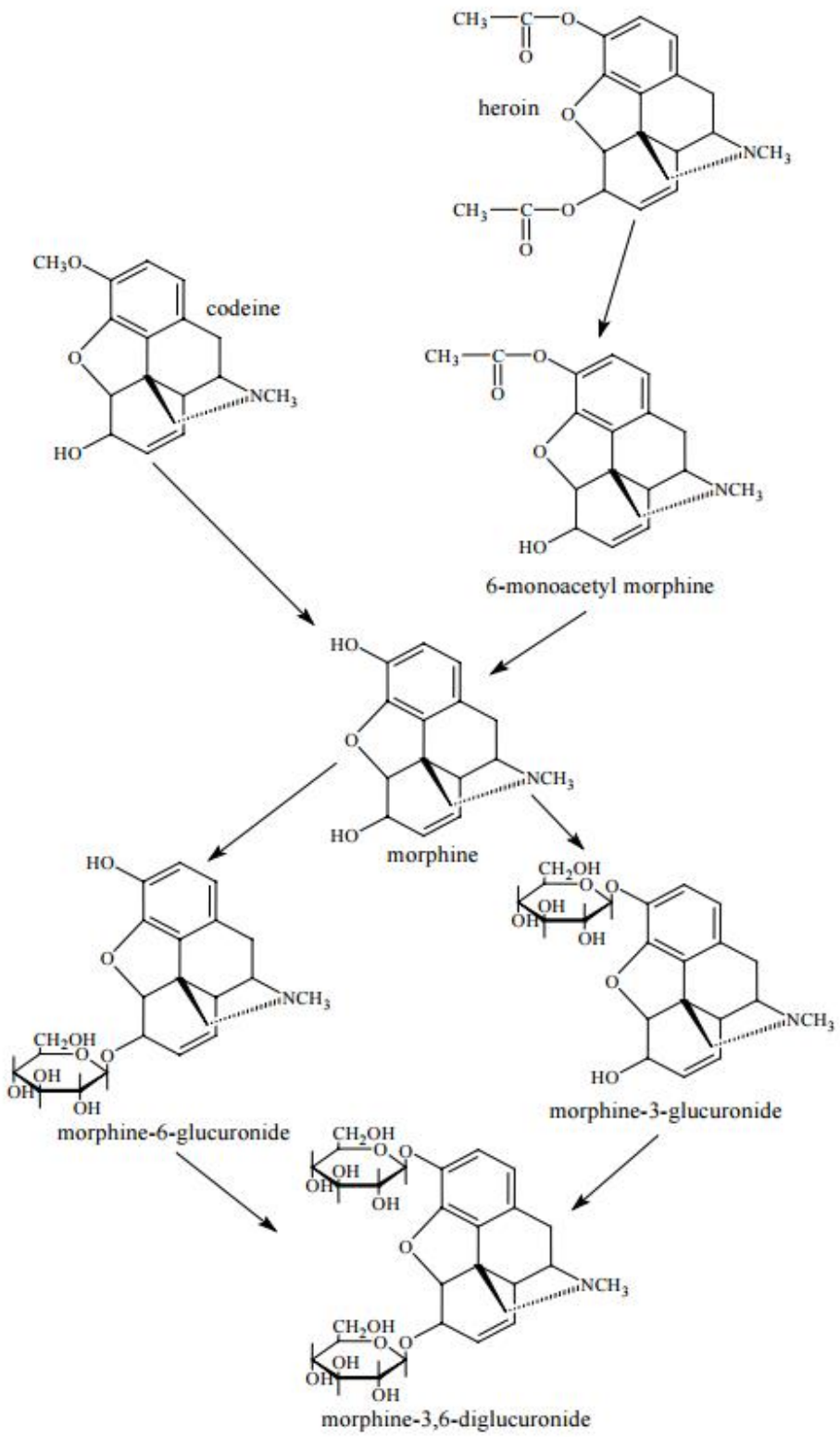

Figure 4. The metabolic pathway of codeine and heroin opiates (Bedford, 1991)

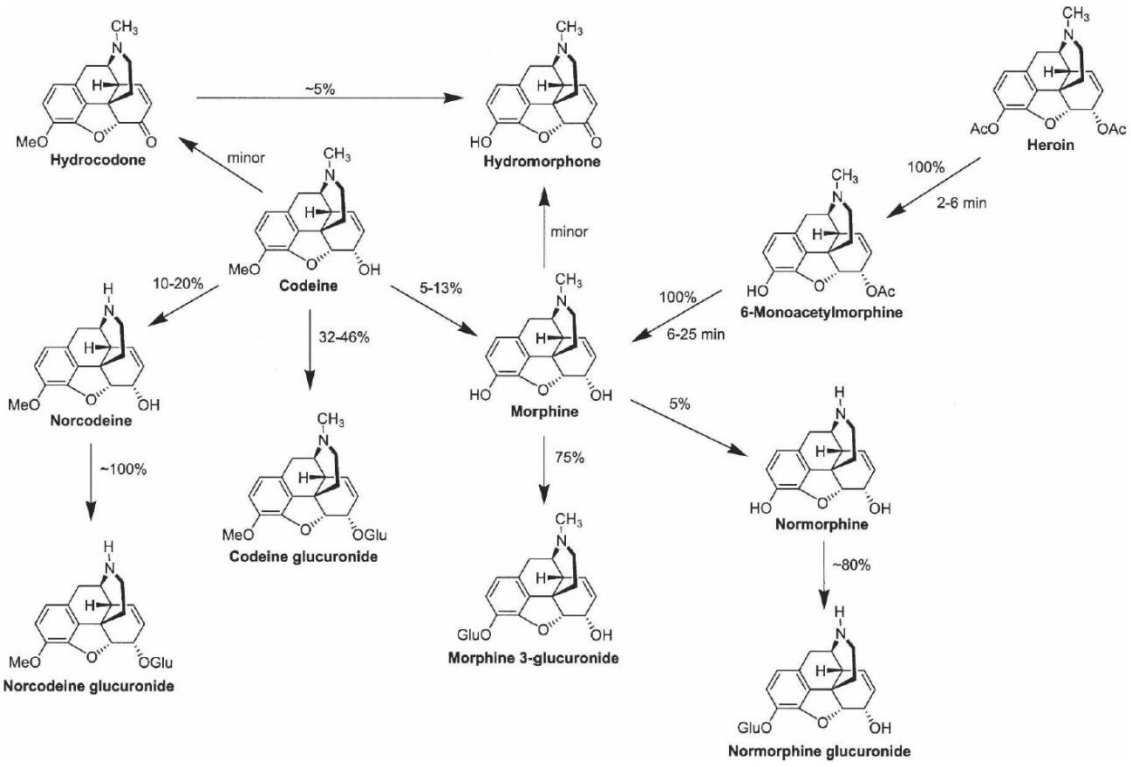

Figure 5. Metabolic pathways for morphine, codeine and heroin (Reisfield, 2007).

Morphine, oxymorphone, oxycodone, fentanyl, and hydromorphone are all schedule II controlled opioid agonists due to their high risk for abuse and increased risk of respiratory depression which may result in death (Drugs.com, 2014).

Hydromorphone is a derivative of morphine, containing a ketone formed through the hydrogenation. Both structures are shown in Figure 6. Hydromorphone is another analgesic drug that is commonly administered orally and gets absorbed in the gastrointestinal tract shortly after ingestion. It undergoes first-pass metabolism where it binds to opioid receptors in the brain and spinal cord (Drugs.com, 2014). About 8-19% of the hydromorphone is bound to plasma proteins, but most is metabolized in the liver through the process of glucuronidation (Drugs.com, 2014). About 95% of the drug is

metabolized into hydromorphone-3-glucuronide, some gets metabolized into 6-hydroxy reduction metabolites, and a small amount is unchanged hydromorphone before being excreted in the urine (Drugs.com, 2014).

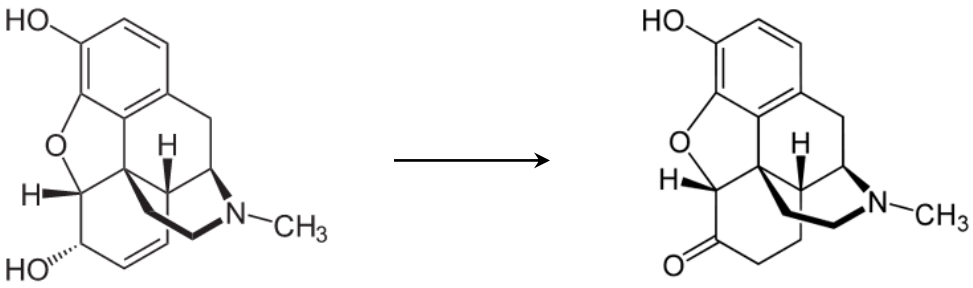

Figure 6. Morphine can become synthesized into hydromorphone.

Hydrocodone can be semi-synthesized from codeine as shown in Figure 7. As previously stated, codeine is generally obtained from the poppy plant through an extraction process. The opioid pain medication hydrocodone is a schedule II substance in the U.S. and is generally prescribed to be taken orally. Some studies discovered hydrocodone to be stronger than codeine at binding to opioid receptors, but only one-tenth as potent as morphine; although, it has been measured that hydrocodone to be about half as strong as morphine in its analgesic properties (Davis, 2005). In the liver, the hepatic cytochrome P450 enzyme CYP2D6 metabolizes hydrocodone into hydromorphone, and CYP3A4 converts hydromorphone into its other major metabolite, norhydrocodone through catalyzed oxidation as shown in Figure 8 (Vuilleumier, 2012). hydrocodone is metabolized into other minor metabolites as well, and 40% of hydrocodone is metabolized through non-cytochrome-catalyzed reaction (Vuilleumier, 2012). Another study showed that after individuals ingested hydrocodone, the majority of

it was excreted through urine as the norhydrocodone metabolite compared to the concentration excreted as unchanged hydrocodone, and even less was excreted as the hydromorphone metabolite (Valtier, 2012).

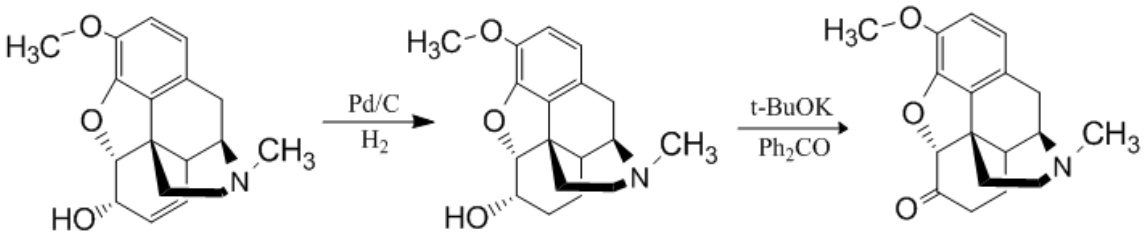

Figure 7. Hydrocodone is a semi-synthetic opioid derived from codeine.

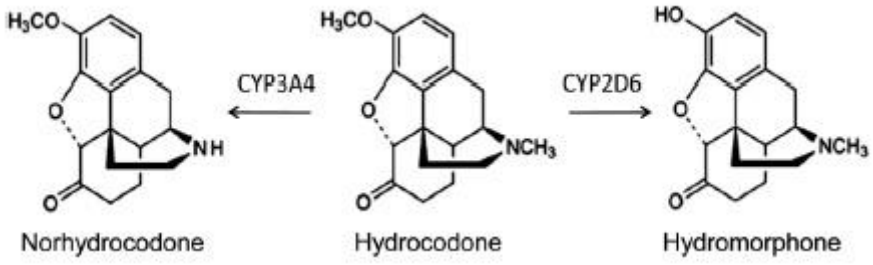

Figure 8. Hydrocodone and its metabolites; hydromorphone, and norhydrocodone, formed from the hepatic cytochrome P450 enzyme.

Oxymorphone is produced commercially from the chemical, thebaine, which is acquired from the poppy plant. Oxymorphone, as well as hydromorphone, hydrocodone, oxycodone and actelymorphine, can be prepared from solutions of codeine, morphine or dionine by using palladium or platinum catalyst shown in Figure 9 (German Patent, 1936). This method is much easier than the alternative method of using hydrogen gas to form the target compound. Oxymorphone can be administered by parenteral injection, orally, or as a suppository and has known to be more potent than morphine in analgesic

effects as it has a higher affinity for μ-opioid receptors than morphine does (Prommer, 2006). Oxymorphone's bioavailability is about 10% if taken orally and about 20-40% of the compound binds to protein (Prommer, 2006). Oxymorphone is metabolized in the liver through a hepatic metabolism mechanism (Prommer, 2006). The drug concentration is greatly reduced before it reaches systemic circulation, and forms conjugates with glucuronic acid which converts it to oxymorphone 3-glucuronide and 6-OH-oxymorphone before being excreted in the urine (Prommer, 2006).

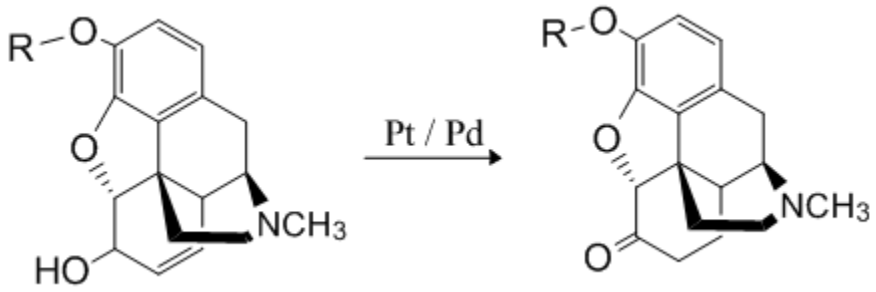

Figure 9. Procedure for the production of dihydromorphinones acquired from a German Patent in 1936 (German Patent, 1936).

Oxycodone is another semi-synthesized opioid from thebaine shown in Figure 10 (MedChemPedia, 2015). Oxycodone can be administered orally, rectally, or via parenteral or intranasal administration; however, in the United States, oxycodone is medically approved for oral administration only due to its high abuse potential when administered through other methods (Stoops, 2010). *OxyContin* is a common name brand of oxycodone that supplies the drug in a controlled-release tablet form (Purdue Pharma LP, 2016). When administered orally or rectally, the bioavailability of oxycodone is

about 60-87%, and intranasal bioavailability is about 46% (Analgesic Expert Group, 2007).

As most opioids, oxycodone acts on the μ-opioid receptors, a class of opioid receptors that has a high affinity for peptides involved in regulating the sensory nervous system's response to potentially harmful stimuli, and produces effects that are typical of μ-opioid agonists such as euphoria, increased relaxation, and anti-anxiety (Purdue Pharma LP, 2016).

Oxycodone is metabolized in the liver by the cytochrome P450 enzyme CYP2D6, which is one of the primary enzymes involved in the metabolism of xenobiotics in the body. From there, oxycodone is metabolized into a major metabolite; oxymorphone, which in turn, is more potent opioid agonist with stronger/higher binding affinity to μ-opioid receptors compared to oxycodone (Purdue Pharma LP, 2016; Wang, 2009). Although oxymorphone has more analgesic properties, when transformed from the initial state of oxycodone, it has only shown to be present in small concentrations in the plasma and usually gets metabolized further into oxymorphone 3-glucuronide and noroxymorphone (Purdue Pharma LP, 2016).

People taking CYP2D6 inhibitors or have slow metabolism are at risk of toxicity if not careful administering oxycodone (Purdue Pharma LP, 2016). Other metabolites produced from hepatic mechanisms of oxycodone include: noroxycodone, α- and ß-oxycodol, noroxycodol and oxymorphol (Purdue Pharma LP, 2016). Finally, oxycodone and its metabolites are excreted from the body via the kidney. Free and conjugated oxycodone was measured in the urine to be about 8.9%, free noroxycodone was 23%,

free oxymorphone was less than 1%, conjugated oxymorphone was 10%, free and conjugated noroxymorphone was measured to be about 14% in the urine (Purdue Pharma LP, 2016).

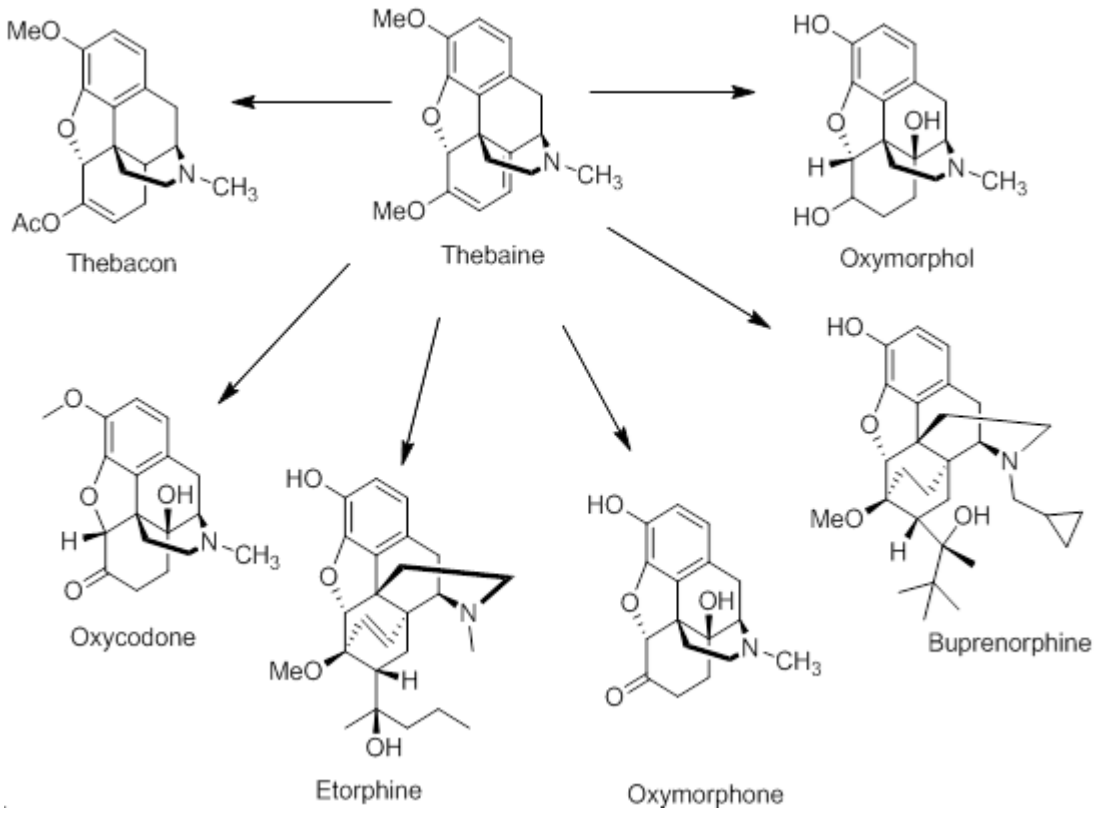

Figure 10. Synthesis of thebaine (MedChemPedia, 2015).

Fentanyl is also used for pain relief as it is a strong μ-opioid agonist and a very potent opioid analgesic with rapid onset of effects. Fentanyl is about 100 times more potent than morphine and generally only used in small doses, for example 100 micrograms of fentanyl is equivalent to about 10 milligrams of morphine. Since the

1990s, fentanyl patches have been prescribed to patients for a controlled release of the drug into the body (Current, 2007; International Narcotics Control Board, 2007).

Fentanyl is available for administration into the body in various forms: intravenously, via lozenges, buccal tablets, which are meant to be held between the cheek and gum and dissolve slowly through the oral mucosa; nasal spray, inhaled spray, and as active transdermal patches. Fentanyl has high lipophilicity and low water solubility which allows it to penetrate the central nervous system more easily via the brain, spinal cord and other tissues. When administered trans dermally (via dermal patch), fentanyl has a distribution time of about 6 minutes with a 60-minute calculated redistribution time, and the half-life elimination is about 16 hours (Mayes, 2006). When administered intramuscular drug effects start within 7-8 minutes, and trans mucosal (via buccal tablet) drug effects start within 5-15 minutes. A trans mucosal administration has a 50% bioavailability of the total concentration and a transdermal patch will continue to release a concentration over the course a few days, depending on the dosage (PremierTox Laboratory, 2015).

Shortly after administered trans dermally, the drug will move into loose connective tissues and skeletal muscles before it gets discharged into the bloodstream. When fentanyl is administered intravenously, drug effects are nearly immediate, and the drug concentration may be increased in the kidneys, lungs spleen, heart and possibly the brain (Mayes, 2006). Change in pH has shown to vary the distribution of fentanyl between the central nervous system and plasma; its binding capacity in the plasma proteins is generally 80-86%. Fentanyl has a high first-pass clearance effect during the hepatic metabolism. The primary metabolism of fentanyl occurs in the liver by N-

dealkylation via the CYP3A4 enzyme where it becomes fentanyl's primary metabolite, norfentanyl, shown in Figure 11.

According to the pharmacology research conducted by Mayes in 2006, norfentanyl and other metabolites that are formed have minimal pharmacological activity in the body and become excreted through the urine and bile. Following a trans mucosal dose, about 0.4-6% fentanyl and 26-55% norfentanyl gets excreted from the body shortly after administration (PremierTox Laboratory, 2015). Following an intravenous administration of fentanyl, about 75% of the drug is excreted in the urine with about 10% unchanged fentanyl, and about 9% may be excreted from the bile as metabolites of fentanyl (Mayes, 2006).

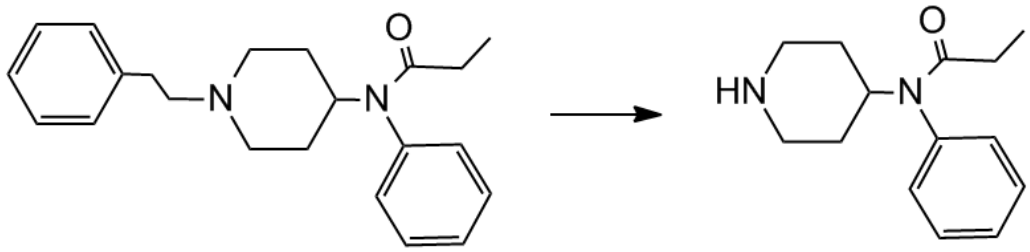

Figure 11. Fentanyl and its metabolism into norfentanyl (PremierTox Laboratory, 2015).

Buprenorphine is also a semisynthetic opioid derivative of thebaine, shown in Figure 10. Buprenorphine is about 20-40 times more potent than morphine and is considered a Schedule III drug in the U.S. because its potential for abuse is less than schedule I and II (PremierTox Laboratory, 2015; U.S. Department of Justice Drug Enforcement Administration, 2015b). Buprenorphine is an analgesic with mixed agonist and antagonist properties sometimes used for substitution treatment of opioid dependence

(International Narcotics Control Board, 2007; PremierTox Laboratory, 2015). It was used for that purpose in more than 40 countries in 2013 (International Narcotics Control Board, 2007).

According to information from PremierTox laboratory in 2015, buprenorphine can be administered orally, via transdermal patch or parenteral injection. Sublingual administration has a bioavailability of about 51%, and a buccal film has a bioavailability of about 28%. Buccal films may appear as a rectangular film that's manufactured to adhere upon contact with the moist buccal mucosa in order for the drug to be absorbed orally in the inner cheeks and lips. According to laboratory findings, after a 2 mg sublingual dose, the highest plasma concentration was reached after 1.3 hours, and the drug had a half-life of about 18-49 hours. A similarly studied intravenous dose of 0.3 mg buprenorphine reached its highest concentration in the plasma after 2 minutes, and it had a half-life of 2-4 hours (PremierTox Laboratory, 2015).

Buprenorphine, like most of the other opioids, is metabolized in the liver by cytochrome P450 enzyme CYP3A4 where it's converted into norbuprenorphine by N-dealkylation (PremierTox Laboratory, 2015). Figure 12 shows the N-dealkylation of buprenorphine into norbuprenorphine.

Norbuprenorphine is the major metabolite of buprenorphine and it's a full agonist of μ, δ, and κ opioid receptors which help induce analgesic properties (Brown, 2012). As previously stated, μ-opioid receptors are a class of opioid receptors that has a high affinity for peptides involved in regulating the sensory nervous system's response to potentially harmful stimuli. The δ-opioid receptors are 7-transmembrane G protein-

coupled receptors that bind the guanine ("G") nucleotides guanine diphosphate (GDP) and guanine triphosphate (GTP); and when activated, produce analgesic effects, although there is still much unknown about the details of how exactly the δ receptor responds this way (Varga, 2004). The κ opioid receptors help bind opioid-like compounds in the brain which helps produce the effects of these compounds. Such effects include the alteration of pain perception, mood, and motor control. Norbuprenorphine tends to have more effects of respiratory depression, and less effectiveness of blocking pain sensory neurons. It has been suggested that norbuprenorphine's effects may be due in part to its high affinity for the P-glycoprotein substrate (permeability glycoprotein), a protein in the cell membrane that pumps foreign substances or toxins out of cells, as well its lack of blood-brain-barrier penetration (Brown, 2012).

After about 6-11 days, about 95% of a dose of buprenorphine gets excreted from the body from the bile and kidneys. About 68% is excreted in the feces, and about 27% gets excreted in the urine; about 9.4% of the drug is excreted as buprenorphine glucuronide, and about 11% gets excreted as norbuprenorphine glucuronide (PremierTox Laboratory, 2015).

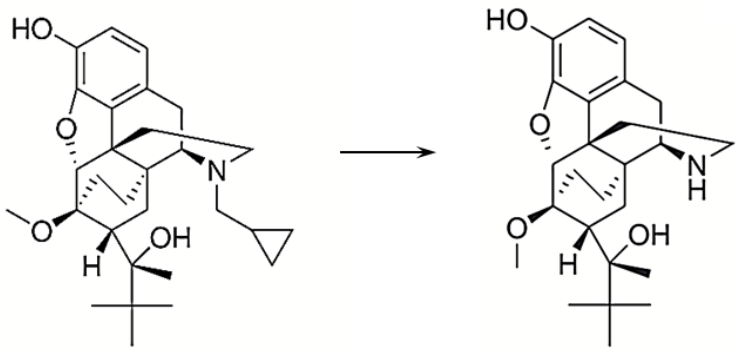

Figure 12. Synthesis of buprenorphine into norbuprenorphine.

Opioids tend to be highly addictive substances, and can have additional side effects such as constipation, nausea, vomiting, dizziness, dry mouth and drowsiness. Most times these drugs can be dangerous if they are combined with other drugs or alcohol. The use of opioids can lead to chemical dependence and withdrawal.

Many times post mortem interpretation of opiates is complicated by the drug tolerance that is built up by the individuals that sometimes these addicts are capable of surviving up to ten times the normal lethal dose (Bedford, 1991). Known toxicity levels are displayed in Table 1.

Table 1. Toxicity levels in opioids (Baselt, 2011).

	Toxic levels (mg/L)					**Half-life (t ½)**
	Blood	**Liver**	**Kidney**	**Urine**	**Bile**	
Morphine	0.2-2.3	0.4-18		14-81		1.3 – 6.7 Hours
average	*0.7*	*3*		*52*		
Heroin	0.38	0.35	0.7	0.49		2 – 6 Minutes
6-Monoacetylmorphine	0.01-3					6 – 25 Minutes
average	*0.43*					
Hydromorphone	0.02-1.2	0.07-7.7	0.1-5.2	1-29	1-20	3 – 9 Hours (Intravenous or normal release) 10 – 22 Hours (Extended release)
average	*0.3*	*1.4*	*1.2*	*8.6*	*9.2*	
Oxymorphone	0.03-0.12					4 – 12 Hours
Codeine	1.0-8.8	0.6-45	2.3-36	29-229	5.0-43	1.2 – 3.9 Hours
average	*2.8*	*6.8*	*12*	*104*	*18*	
Hydrocodone	0.12-3.6					3.4 – 8.8 Hours
average	*0.53*					
Oxycodone	0.1-8.0					3 – 6 Hours
average	*1.2*					
Fentanyl	0.03-0.28	0.059-0.78	0.061-0.42	0.05-0.93		3 – 12 Hours
average	*0.083*	*0.37*	*0.18*	*0.28*		
Buprenorphine	0.011-0.29	0.04-2.73	0.081-1.38	0.04-10.33		2 – 4 Hours (Parenteral) 18 – 49 Hours (Sublingual)
average	*0.084*	*0.8*	*0.35*	*1.72*		

Chapter II

Objective

The quantitation of opiates in the blood is conducted through whole blood analysis at the at the Erie County Medical Examiner's Office Toxicology Laboratory. The objective of this thesis project was to find out if the analysis of opiates could be conducted using the liver from case samples, and if the analysis of these components could provide more accurate quantitation of those drugs than in the blood analysis. If the analysis proves more successful compared to quantitation from blood analysis, it is possible the Erie County Toxicology Laboratory would consider using livers from case samples for future opioid quantitation.

Chapter III

Methods & Results

3.1 Prepare Liver Dilutions and Standards

The first part of this project involved preparing the liver dilution samples and controls. All the livers used in this project were stored in a freezer unit kept at -21°C. The case livers were collected at time of autopsy then also stored in the freezer. A 1:3 liver homogenate was created using the case liver measured by taking five grams of liver, adding it to ten milliliters deionized water and using the homogenizer to slurry the mixture (Appendix I). Using the 1:3 homogenate we created multiple dilution samples (1:6, 1:9, 1:15, 1:30 and 1:60) using the liver matrix and whole sheep blood. Whole sheep blood was used in this project because it is more readily available than human blood and provides a similar biological matrix. Each sample was one milliliter in volume. The 1:3 dilution was used as is; a half a milliliter 1:3 homogenate and half a milliliter whole blood was combined to make the 1:6 dilution; 0.33 mL combined with 0.67 mL whole blood to make 1:9 dilution. To make the 1:15 dilution, two milliliters of 1:3 homogenate was added to a ten volumetric flask then filled with whole blood. The 1:30 dilution was then made by taking half a milliliter of 1:15 dilution and adding half a milliliter of whole blood. Finally 0.25 mL of 1:15 dilution was combined with 0.75 mL whole blood to make the 1:60 liver dilution.

For the first extraction conducted, we used a case liver from the morgue that was negative for opiates and fortified it with known concentration of opiates in order to prove that the liver matrix would not affect the opiate concentration during analysis. After the

liver dilutions were prepared as one milliliter samples, known concentrations of opiates were added to the samples using the working stock solutions “A,” “B,” and “C.” The concentration values for working stock solutions A, B & C have been listed in Table 2 of the Reagents & Materials section in Appendix II. The working stock solutions used in this procedure are the same used for routine opiate extraction/analysis of blood samples at the Erie County Toxicology Lab.

After the third extraction performed for the extraction of opiates in the case livers, beef liver was used to make up the liver control samples, as it was more readily available and provided similar results to human liver. The beef liver used to make the liver dilutions was also stored in the freezer below -21°C. Once the procedure was established to further analyze all the case samples; low, medium, and high controls of a 1:9 beef liver dilution were always used as well as a negative 1:9 liver control sample. The liver 1:9 dilutions were prepared first, then specific opiate concentrations listed in Table 22 of Appendix III were added to the liver samples to create the low, medium and high liver controls. In addition to the liver control samples, all of the case livers were prepared using 1:9 dilutions.

3.2 Prepare Blood Standards

Standard solutions #1-7 were prepared using whole sheep blood and working stock solutions “A,” “B,” and “C” (Appendix II) which consisted of known opiate concentrations. All the whole blood used to make samples was sheep blood acquired from Hemostat Laboratories. In addition to standard blood samples #1-7, the calibration samples included one sample of whole blood used as a negative control, and pre-prepared

low-positive and high-positive controls of the blood spiked with known amounts of opiates (Table 20 in Appendix III).

An internal standard, containing the deuterated form of all the opiate compounds was added to all the samples including the blood standards and the liver case samples. The concentrations of all the components in the internal standard are listed in Appendix II. Deuterated samples are structurally identical to the compounds of interest, but have stable labeled isotopes; usually deuterium, carbon or nitrogen atoms. These isotopic differences allow the internal standard to account for differences which may occur in the extraction process and during instrumental analysis as well as provide a measure of control in the mass spectroscopy ionization variability and physical properties of the compounds of interest. Deuterated internal standards should have the same extraction recovery, should co-elute with the compound that's to be quantified, and should have the same chromatographic retention time (AptoChem, 2008).

3.3 Extraction Procedure

Once the blood standard, liver standard, calibrators and case samples were prepared, they were ready for the extraction procedure. The samples were mixed with a vortex for about thirty seconds, placed in an ultrasonicator bath for ten minutes, and then followed by a centrifugation at 3600 revolutions per minute for ten minutes. The solid phase extraction (SPE) tubes were preconditioned using methanol, deionized water, and ammonium carbonate according to the procedure in Appendix II. Once the SPE tubes were ready, the blood and liver samples were added to the tubes in order to separate the compounds of interest. The samples were washed with 0.01 molar ammonium carbonate buffer then methanol was added to elute the opiate components into new pre-labeled

culture tubes. The methanol was evaporated at fifty degrees Celsius and replaced with reconstitution solvent. The reconstitution solvent was made up of 95% mobile phase "A" (ten millimolar ammonium formate) and 5% of mobile phase "B" (acetonitrile). The samples were again, vortexed for twenty seconds, then centrifuged at the same speed for about three minutes before placing them in the LC/MS/MS instrument.

3.4 Quantitating Results

The specifications of the LC/MS/MS are listed in Appendix II. The chromatograph spectrums had the capability to display all the components in the sample, or isolate individual components a sample on the spectrum. For the LC/MS/MS quantitation conducted in this experiment, two transitions for each component were always measured. A transition is the measurement from the analysis of the parent mass of the compound during the MS/MS fragmentation, and then monitoring the specific single fragment ion. The results are measured in relative intensity vs. retention time.

3.5 012915 Extraction & Results

Our first extraction "012915" was performed on all the twenty-one samples, listed in Tables 1 & 2 of Appendix III which included the calibrators, the liver dilutions and their duplicates. The opioid extraction procedure is listed in Appendix II. Immediately following the extraction procedure, all the samples were analyzed using LC/MS/MS.

Known amounts of opiate drugs were added to the human liver samples (displayed in Table 2 of Appendix III), and different concentrations of these drugs were present after analysis. Results of this analysis show that 6-monoacetylmorphine (6-MAM) was not present after analysis (Table 2, Appendix III). It may be possible that coexisting enzymes were present in the sample and may have continued to metabolize the

6-monoaetylmorphine component in vitro such as Moriya discovered with cholinesterase activity in the blood while studying other drug components from a cadaver three weeks after its storage at room temperature (Moriya, 2005). The results showed the internal standard of the 6-MAM to have been metabolized; therefore the 6-MAM component was unable to be measured in the liver samples. Observations from using human liver as the matrix showed that the more diluted the liver was, the resulting concentration of opiates were much lower, and closer in comparison to the concentration values measured from the blood analysis. For example, morphine had a higher concentration in the [less dilute] 1:6 liver (104.95 mcg/L), and had a much lower concentration in [more dilute] 1:30 liver dilution (24.05 mcg/L); listed in Table 2, Appendix III.

Figure 13 is a representation of the 6-MAM in a typical analysis. The top chromatogram of Figure 13 is of all the components in the low positive control sample from the 012915 extraction. The middle chromatogram is 6-MAM that's present in the sample, and the bottom chromatogram is the deuterated 6-monoacetylmorphine from the internal standard that was added to the sample for quantitative analysis of the sample.

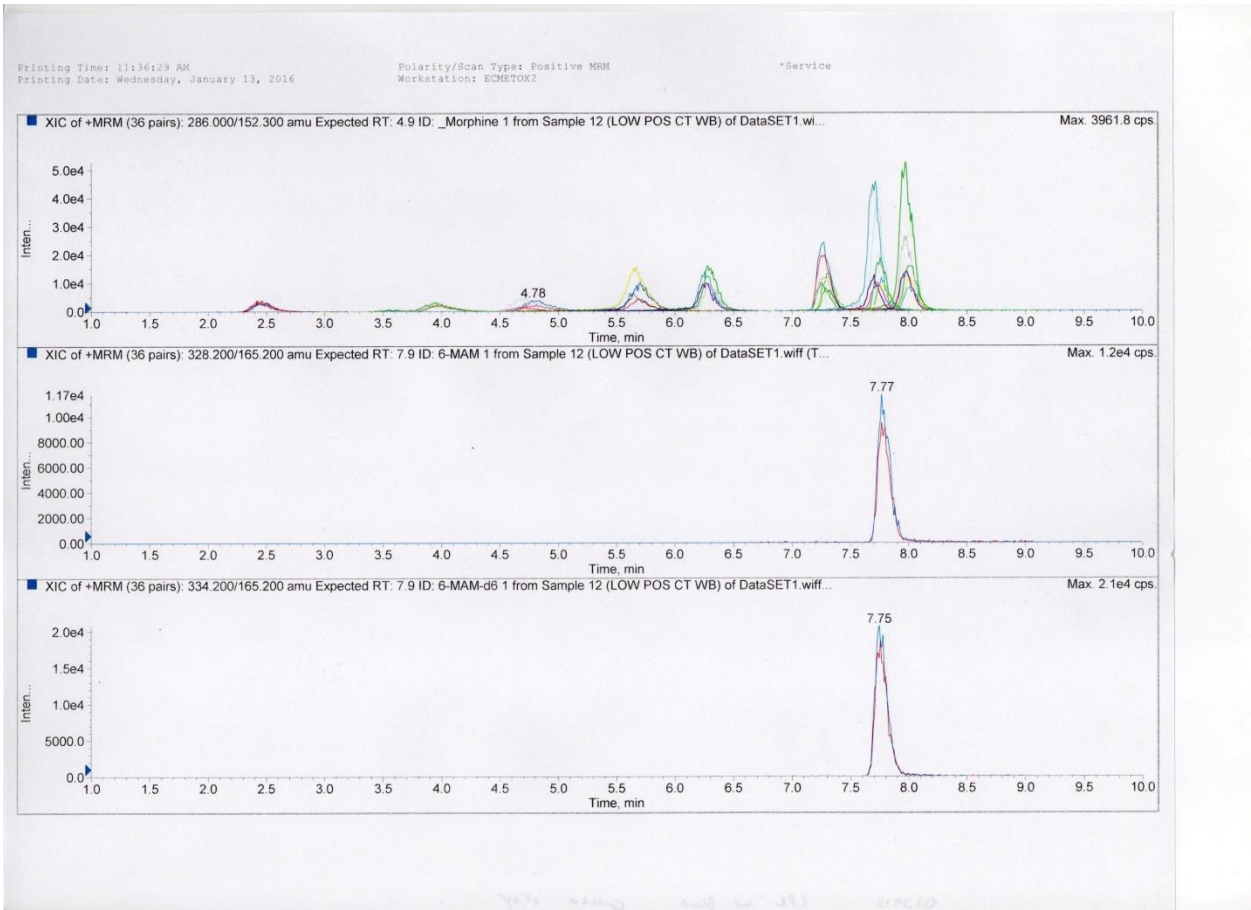

Figure 13. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the low positive control sample from the 012915 extraction. The top line displays all the components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

Figure 14. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the negative control 1:3 liver sample from the 012915 extraction. The top line displays all the components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

Figure 14 is the chromatogram for the negative (1:3) liver sample for the 012915 extraction. The 6-MAM chromatogram has been singled out for that sample; however its normal peaks do not show up in the analysis. The top chromatogram of Figure 14 is of all the components in the negative (1:3) liver sample from the 012915 extraction. The middle chromatogram displays no measured peaks for 6-MAM sample, and the bottom chromatogram should show the presence of deuterated 6-monoacetylmorphine from the

internal standard that was added to the sample for quantitative analysis of the sample, but also has no visible reference peaks.

Finally Figure 15 from the 012915 extraction shows the chromatogram for the liver 1:30 dilution sample. The 6-MAM cannot be quantitated because no qualitative peaks showed up in the liver sample representing the compound of interest. Also no internal standard reference peaks showed up. It's possible this occurred from some interference between 6-MAM and the liver matrix, or the possibility of the presence of coexisting enzymes in the sample (Moriya, 2005).

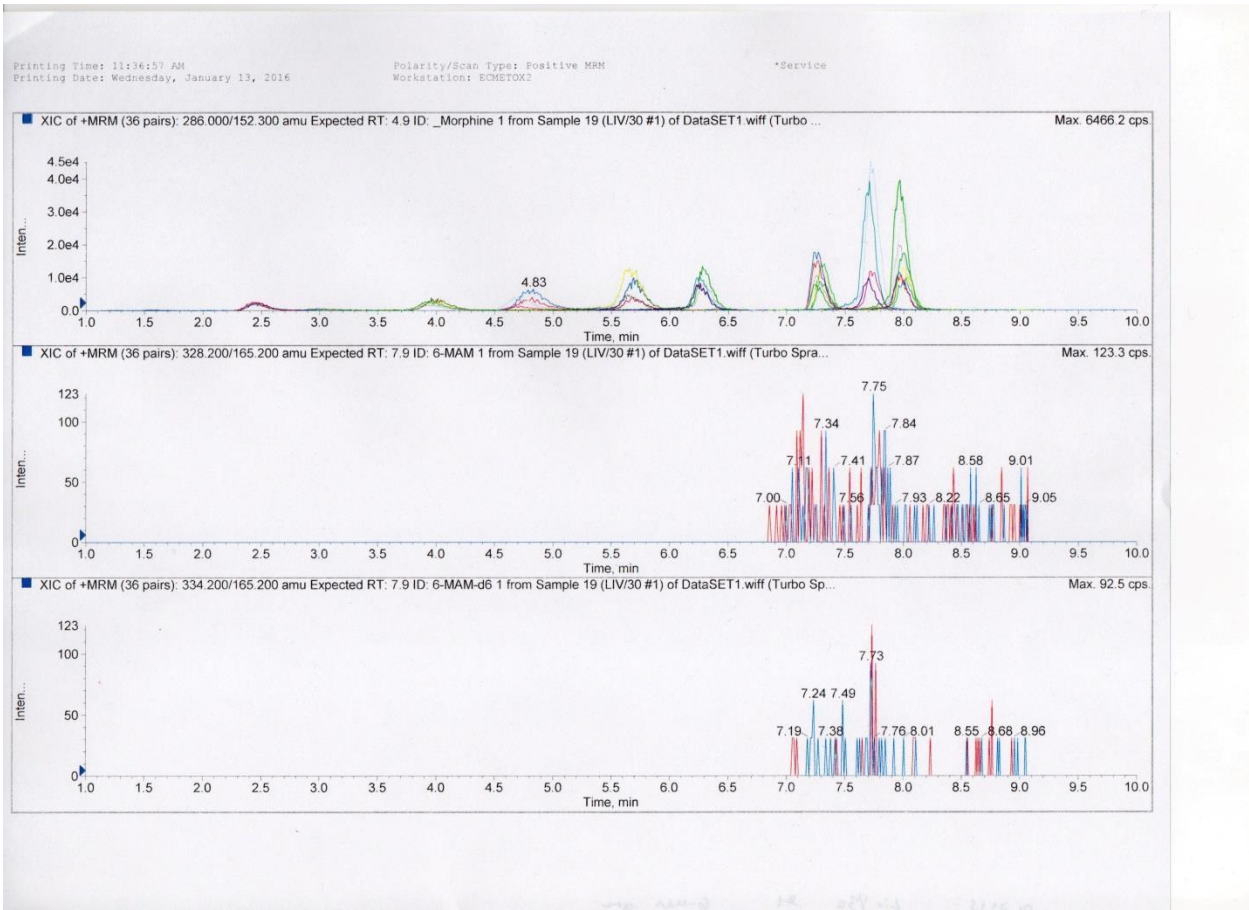

Figure 15. LC-MS/MS chromatographic peaks for qualitative analysis of 6-MAM in the liver 1:30 dilution (#1) sample from the 012915 extraction. The top line is the qualititative analysis of components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

Tabulated results for all of the 012915 extraction can be viewed in Tables 1 & 2 of Appendix III. For the instances where there is no value recorded, it is due to the fact that the internal standard was at a level too low to be detected during analysis, and could no longer be referenced to calculate the amount of that component in the sample.

3.6 021215 Extraction & Results

In our second extraction “021215,” we performed extraction and analysis using the liver from Case #1. We made 1/3 homogenate with the liver matrix from that case, then diluted to have 1/6, 1/9, and 1/15 liver matrix dilutions. The 1/3 dilution was too concentrated and outside the range of our upper calibration limit.

Results from the analysis of Case #1 showed that the 3 liver matrix dilutions did work. Values were seen with 1/6, 1/9 and 1/15 dilutions. Morphine was averaged about 14 times higher in the liver dilutions than the blood, although oxycodone average about 10 times higher in the liver than in the blood measurements (Table 4, Appendix III). Once again, 6-MAM was unable to be analyzed qualitatively or quantitatively for Case #1 and Case #1 (duplicate) because there were no peaks present from the compound or the internal standard. We proposed the possibility that the liver matrix may be consuming the 6-MAM.

All the dilutions worked well for detecting the analytes in extraction 021215 especially dilution 1:15; however, we decided to go with the 1:9 dilution for all further extractions. The accuracy percentage for morphine was closest to 100% in the 1:9 dilutions, and we decided this dilution may be appropriate for a wider quantitation range. 1:9 was also simpler to prepare since we used 1:3 homogenate to prepare the dilutions (Tables 3 & 4, Appendix III).

3.7 021315 Extraction & Results

Our next step was to see if a cow liver matrix is compatible with internal standards and to reanalyze Case #1 in order to compare results to the calf liver calibrators. Samples for this analysis 021316 are listed in Tables 5 & 6, Appendix III, and

consisted of calibration standards in whole blood matrix including a negative control of whole (sheep) blood, calibration standards in 1:9 dilution calf liver and a negative control 1:9 calf liver. The extraction procedure was performed, and the samples were run through LC MS MS for analysis of the opiates.

Calf liver results showed more consistent results than the fortified human liver, with more of the analytes within 20% accuracy (Tables 6 & 7, Appendix III). In the 1:9 calf liver dilutions, all the opiates were within 80-120% accuracy except morphine and 6-MAM. At 10 mcg/L, morphine was high around (134.5%) accuracy, at 100 mcg/L morphine was at (152.5%) accuracy, and at 200 mcg/L for the initial concentration, morphine was high (182.5%). In all 3 cases, 6-MAM internal standard was gone; therefore the concentration was unable to be measured as shown in Figures 16-18. Figure 19 also shows that the deuterated form of 6-MAM disappeared in the results for the Negative (1:9) calf liver sample which also had internal standard added. All the whole [sheep] blood controls performed as expected and 6-MAM was present in the blood results as shown in Table 5, Appendix III, as well as in the negative blood control in Figure 20.

Figures 21 and 22 display the quantitative analysis of morphine present in the HPC liver (1:9) sample and in the standard 3 sample, and showed morphine to be within 80-120% accuracy for both of those samples.

Since the results were satisfactory, we decided to use the calf liver as our calibration liver matrix. The calf liver was easier to access, and there was no pre-screening for opiates necessary before preparing the calf liver for the samples.

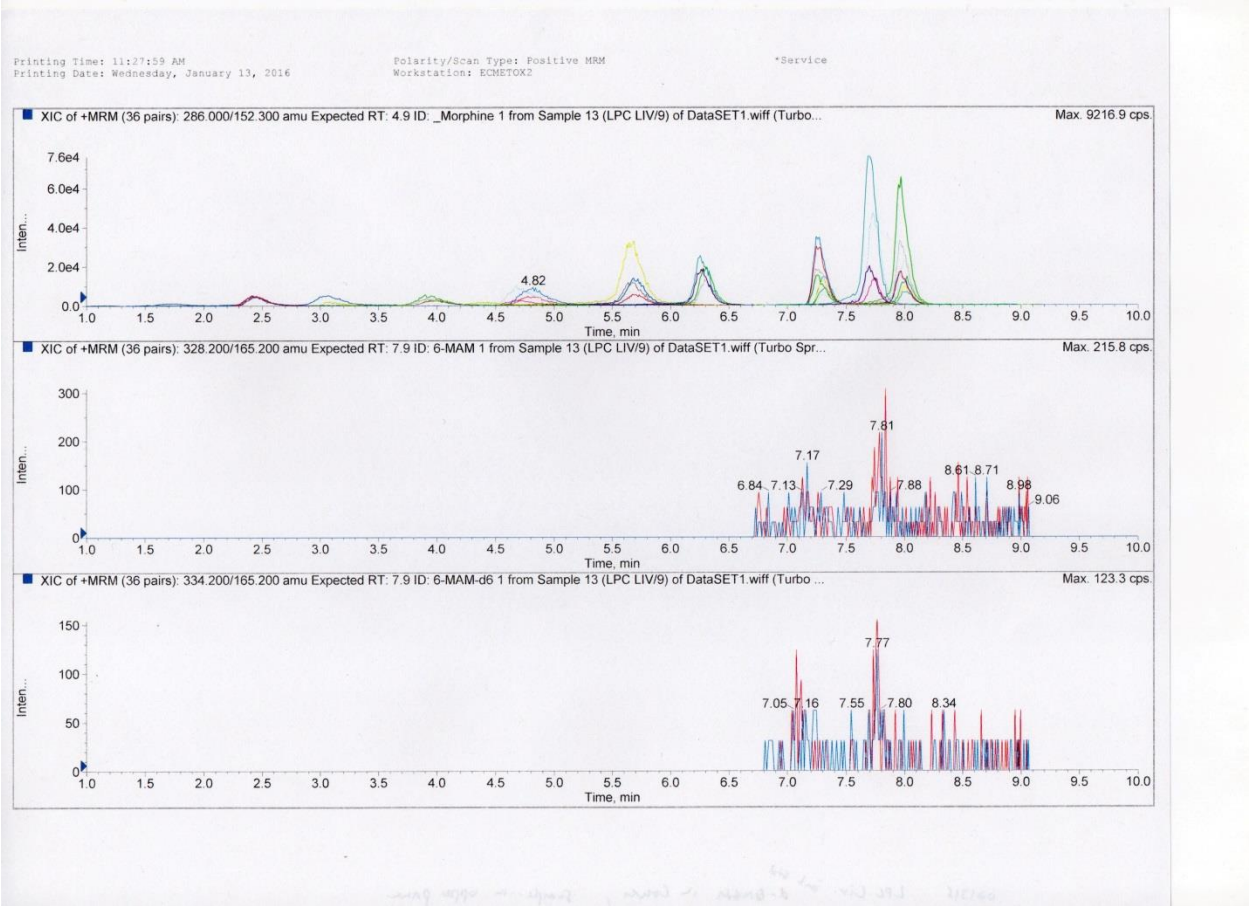

Figure 16. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the low positive control (1:9) calf liver sample from the 021315 extraction. The top line is the qualitative analysis of components in the LPC 1/9 calf liver sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

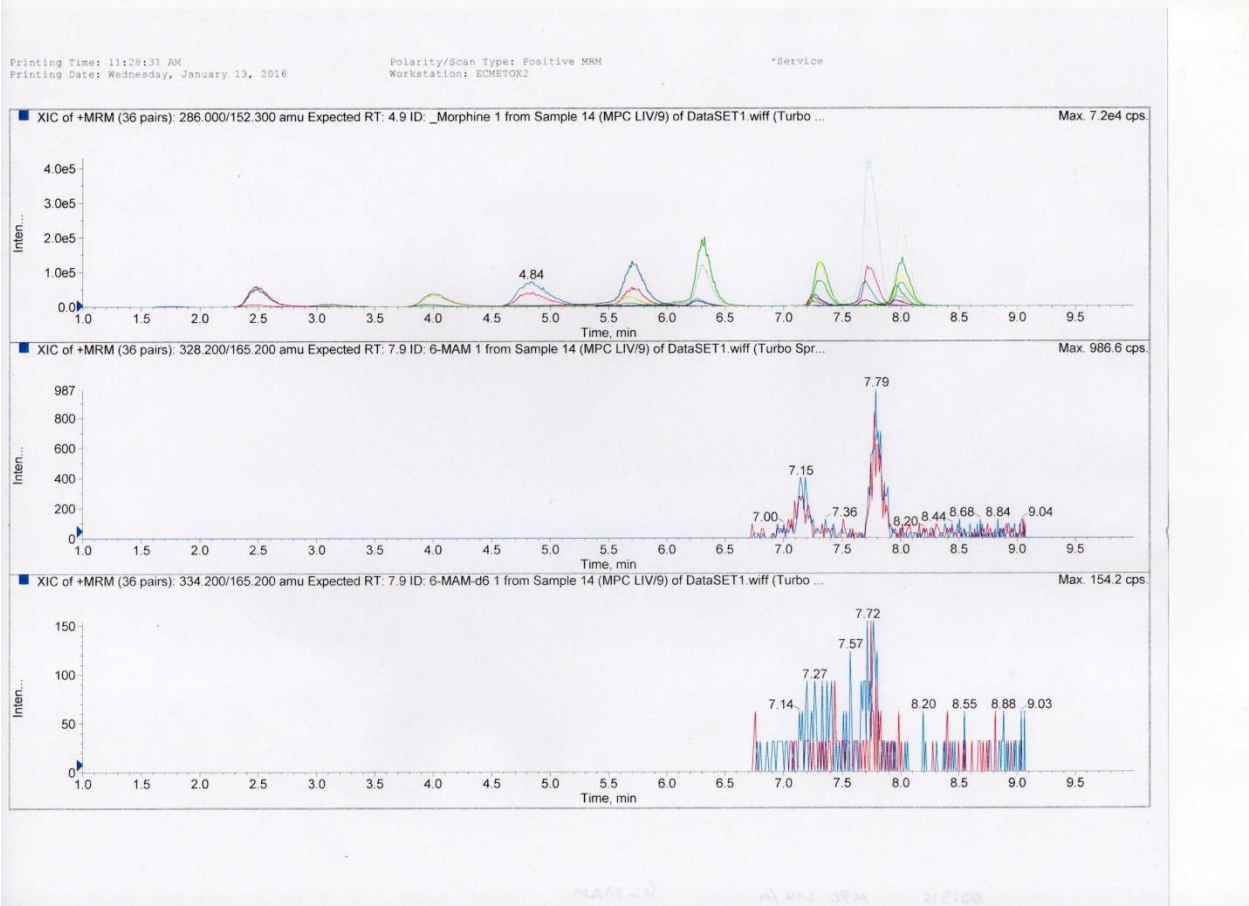

Figure 17. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the medium positive control (1:9) calf liver sample from the 021315 extraction. The top line is the qualitative analysis of components in the MPC 1/9 calf liver sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

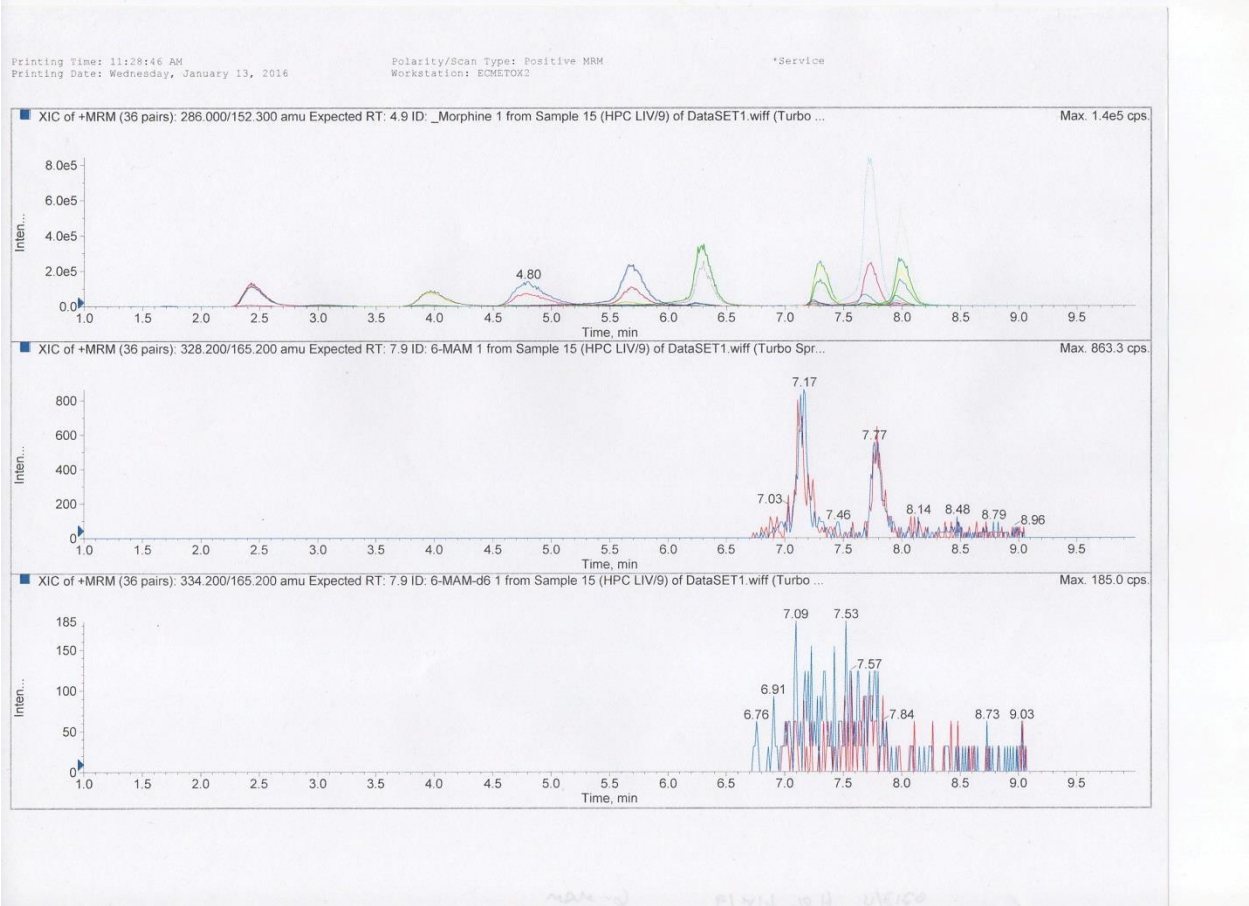

Figure 18. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the high positive control (1:9) calf liver sample from the 021315 extraction. The top line is all of the components in the HPC 1/9 calf liver sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

Figure 19. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the negative control (1:9) calf liver sample from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

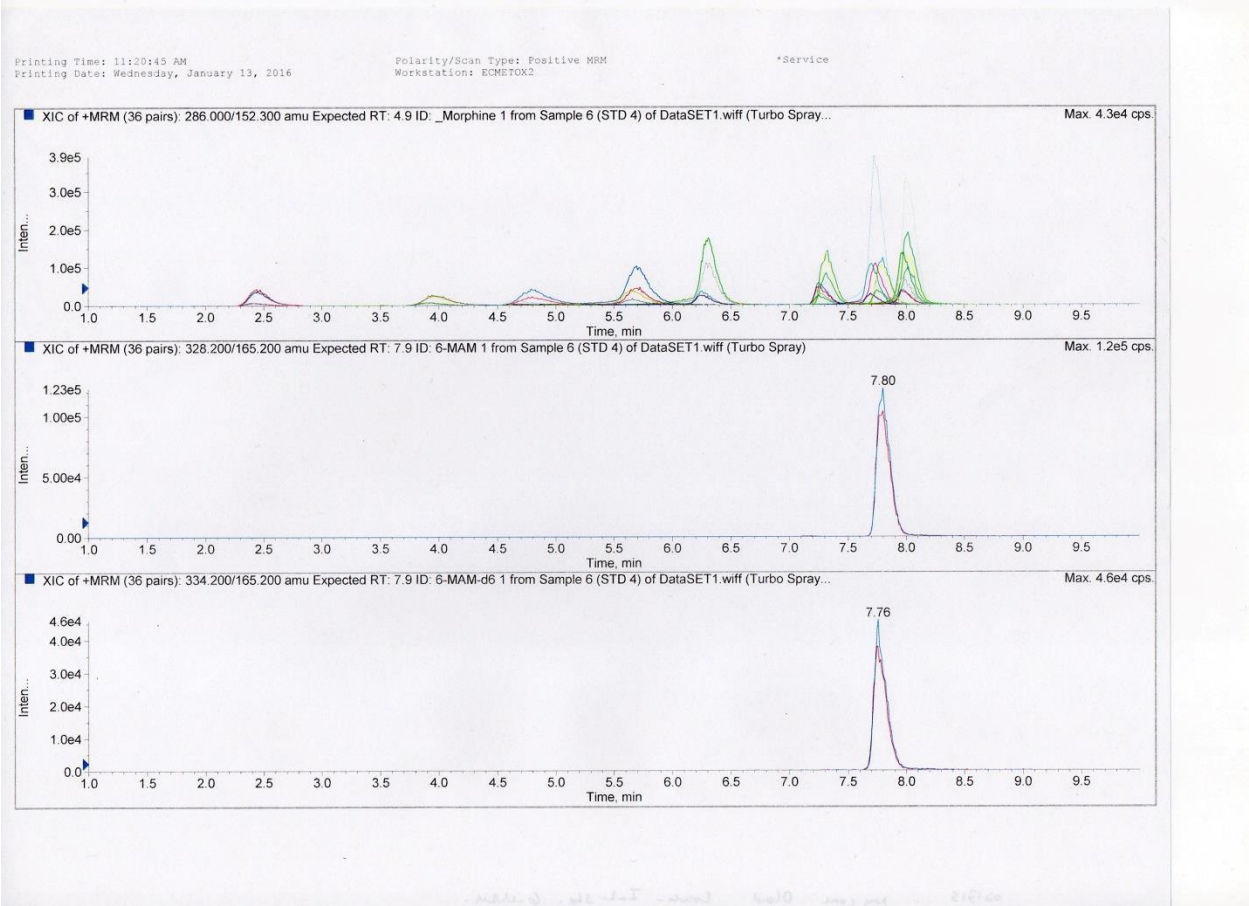

Figure 20. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the negative sheep blood control sample from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

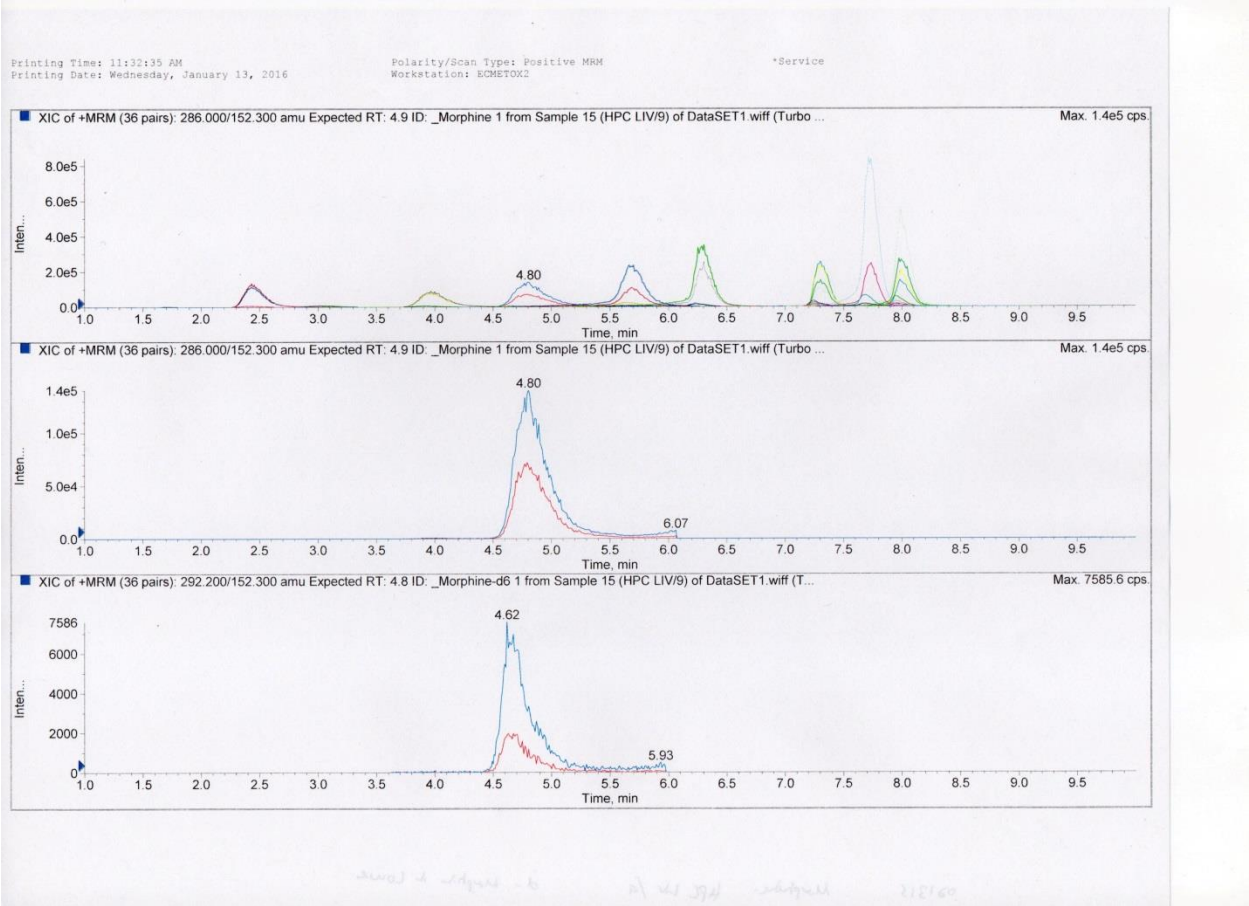

Figure 21. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine in the high positive liver (1:9) control sample from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine in the sample, and the bottom line represents the morphine internal standard that was added to the sample. The red and blue lines represent two transitions.

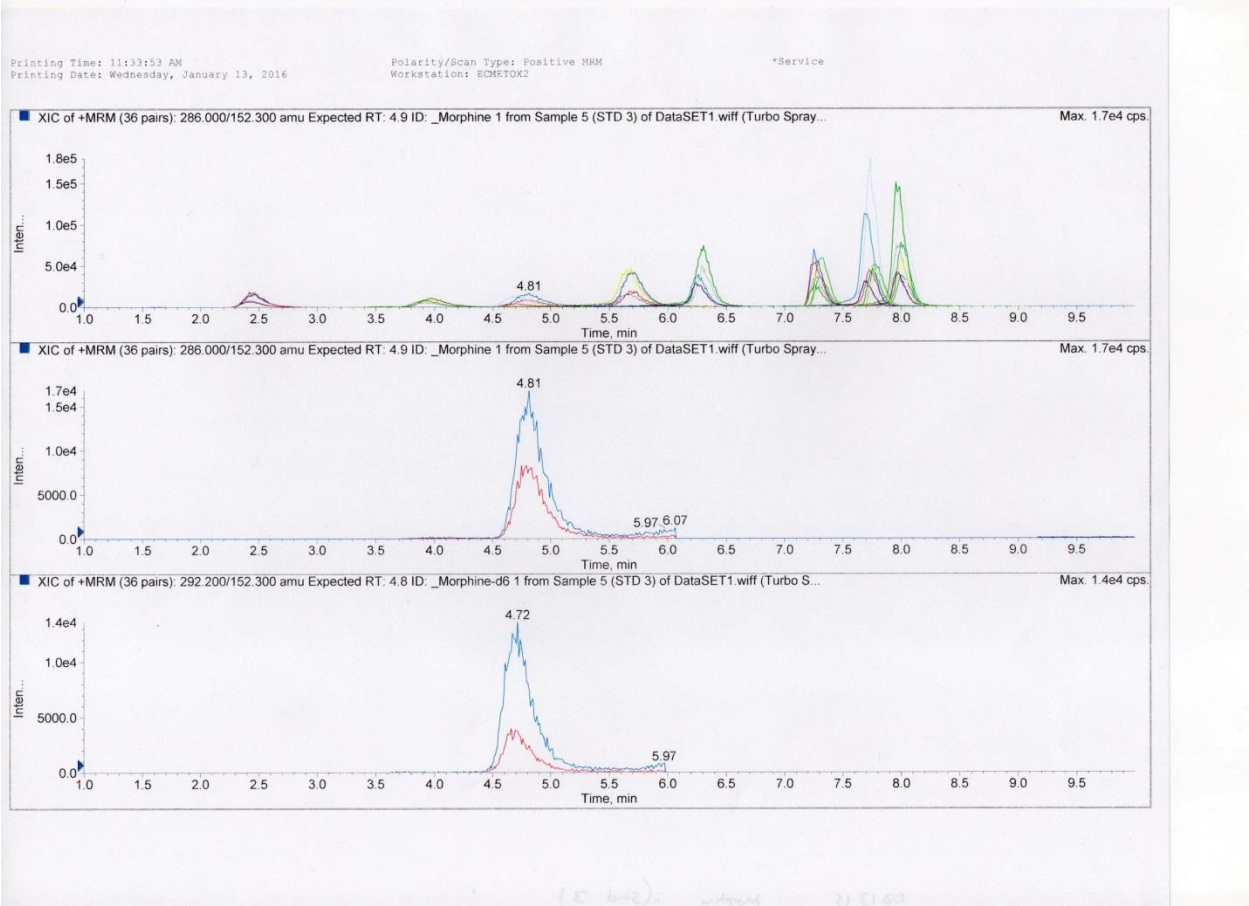

Figure 22. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine in the standard #3 control sample from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine in the sample, and the bottom line represents the morphine internal standard that was added to the sample. The red and blue lines represent two transitions.

Table 7 in Appendix III shows the comparison of liver extraction and blood extraction analysis for Case #1 and its duplicate from extraction 021315. Morphine results were measured at 253.8 mcg/L and 243.9 mcg/L respectively. The toxicology results of blood analysis were 18 mcg/L. The morphine measurements in the liver calculate to be about 14 times higher in the liver than in the blood which is comparable to extraction 021215 results (Table 4, Appendix III); however, the results this procedure displayed more precision. Both results of morphine from initial and duplicate samples

were very close results (253.8 and 243.9 mcg/L). For Case #1 and its duplicate, 6-MAM showed up in the internal standard and was able to be quantitated in the sample (Case #1 shown in Figure 23), although values were very small at <1 mcg/L. Also there were no recorded blood values for the 6-MAM component. Fentanyl results measured to be 37.440 and 36.315 mcg/L respectively. The toxicology results of blood analysis was 16.6 mcg/L. Therefore the liver results showed to be 2.22 times higher in the liver than in the blood. Quantitation results for 6-MAM, morphine, morphine-3-glucuronide, morphine-6-glucuronide, fentanyl, and norfentanyl in Case #1 are shown in Figures 22-28.

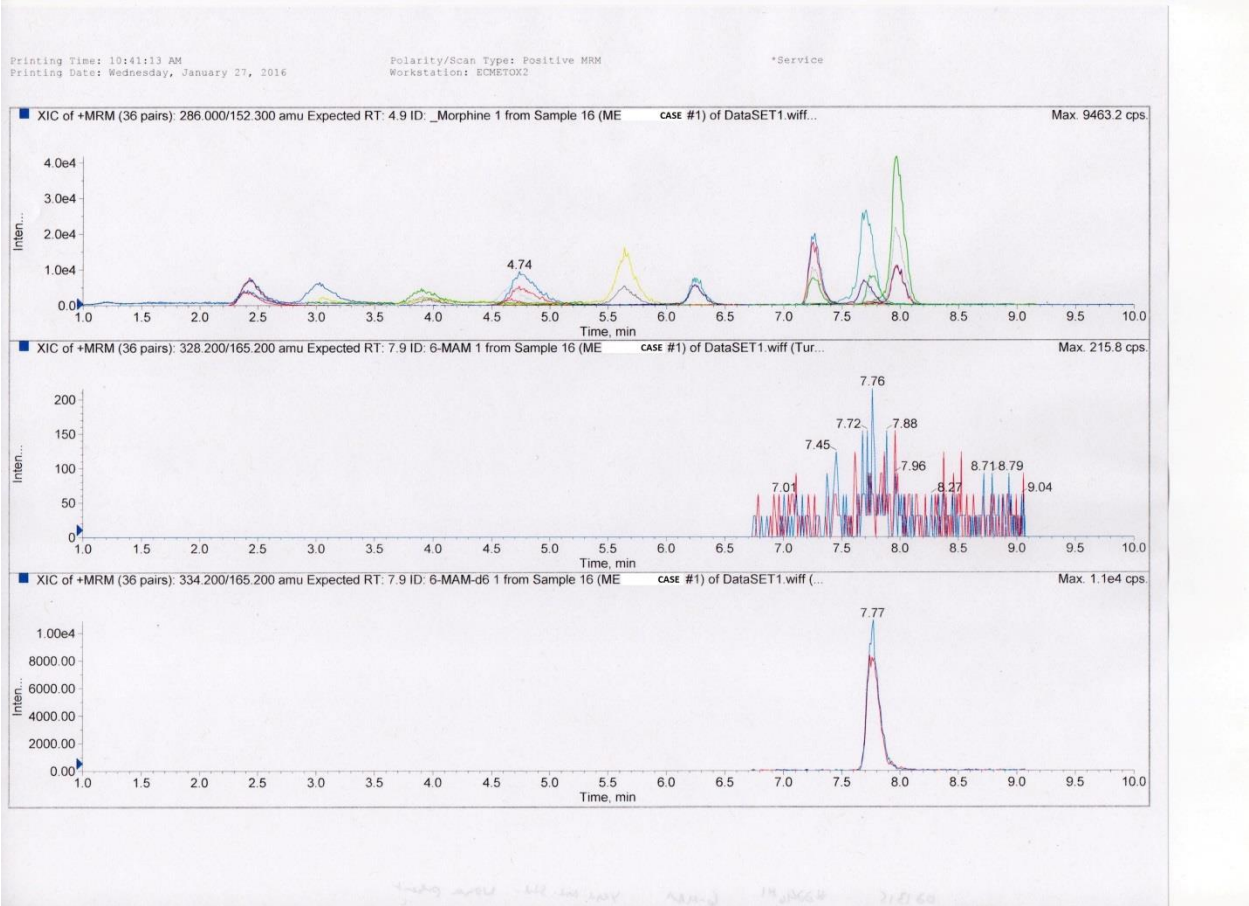

Figure 23. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays 6-MAM present in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

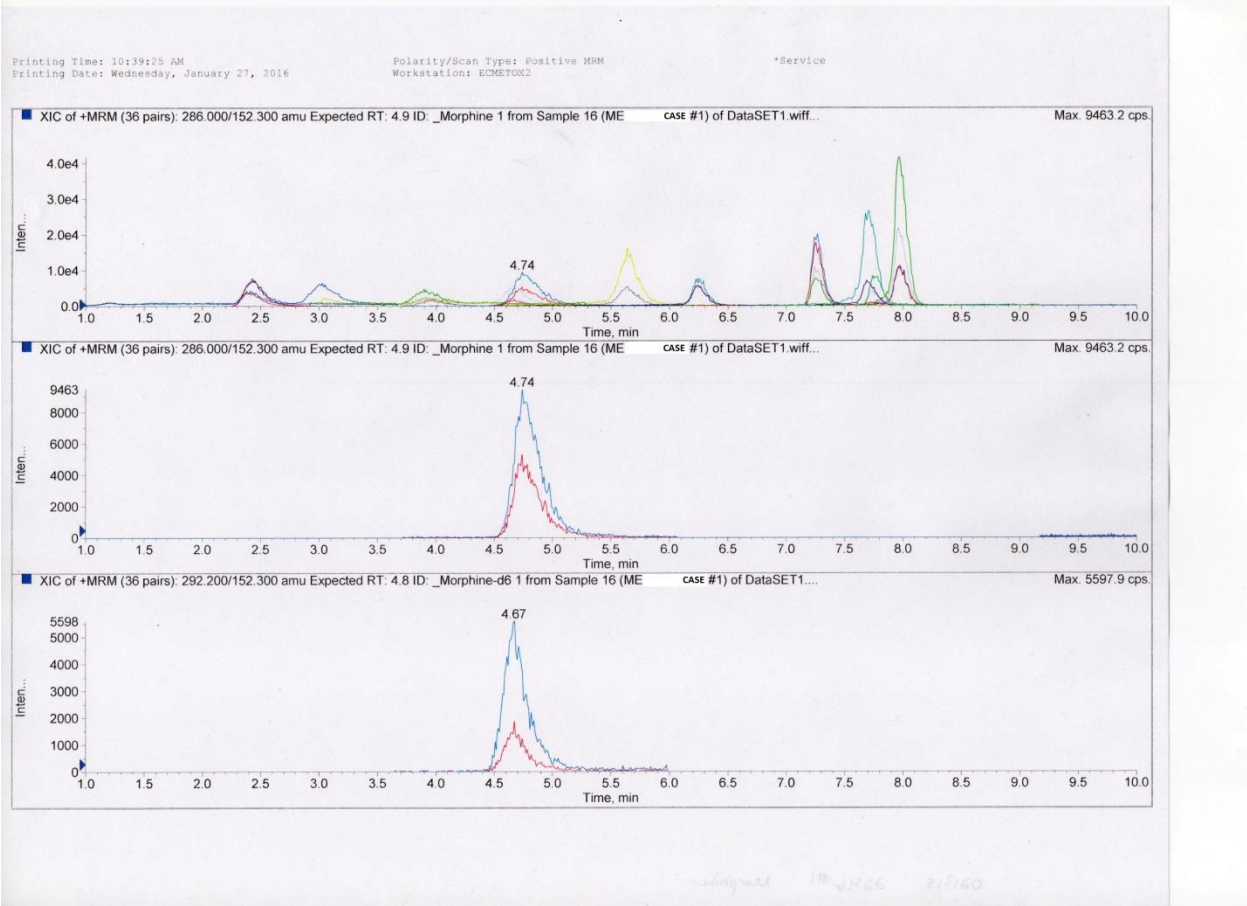

Figure 24. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine present in the sample, and the bottom line represents the morphine internal standard that was added to the sample. The red and blue lines represent two transitions.

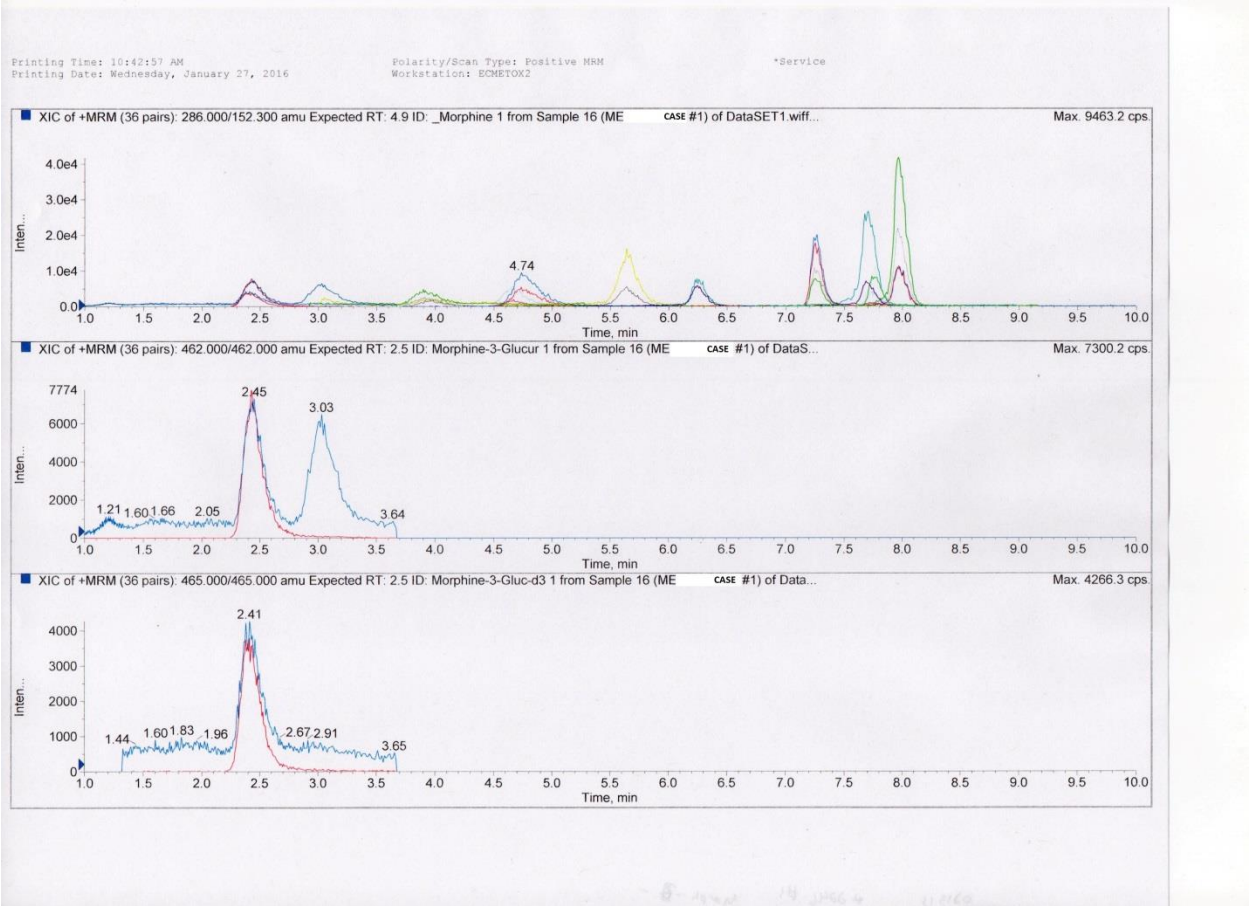

Figure 25. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine-3-glucuronide in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine-3-glucuronide present in the sample, and the bottom line represents the morphine-3-glucuronide internal standard that was added to the sample. The red and blue lines represent two transitions.

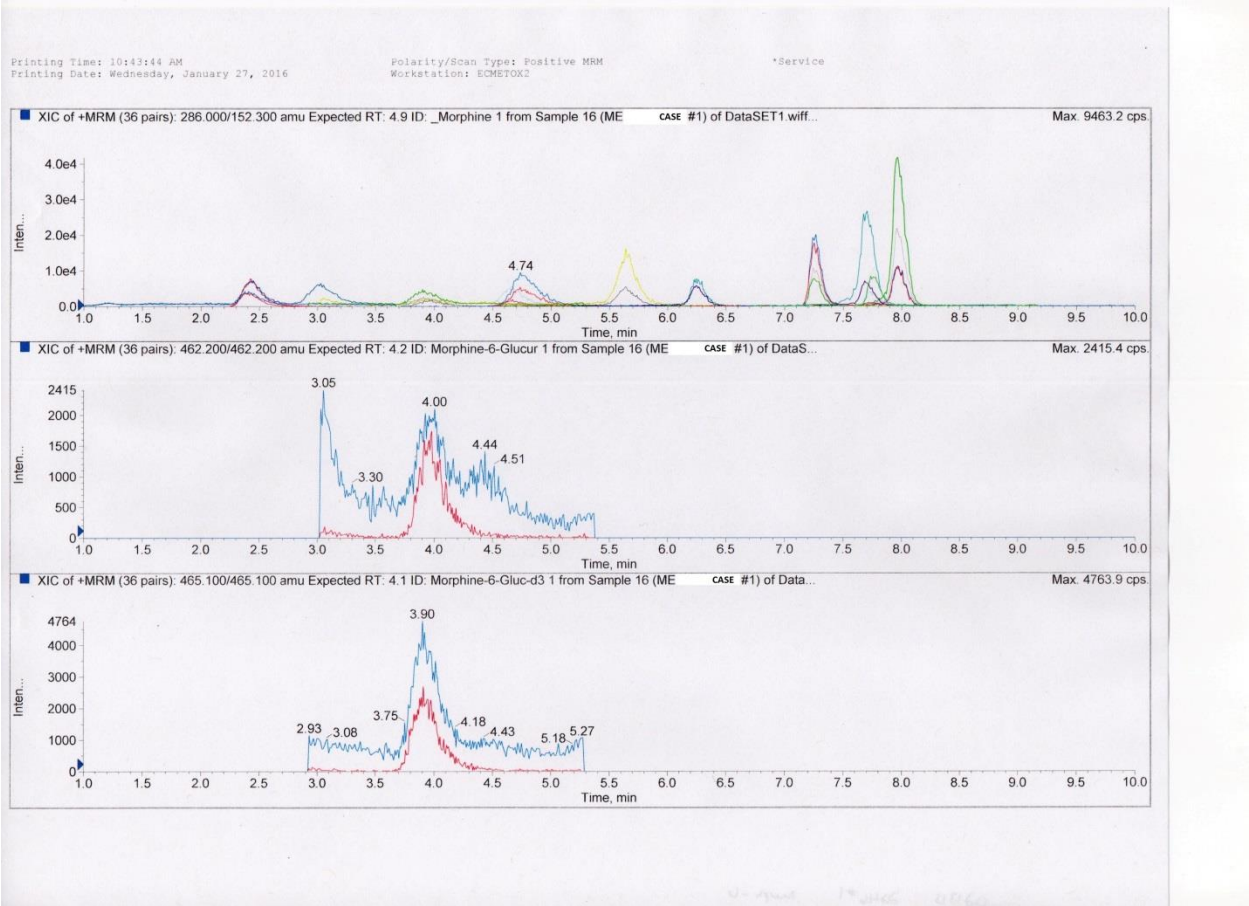

Figure 26. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine-6-glucuronide in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine-6-glucuronide present in the sample, and the bottom line represents the morphine-6-glucuronide internal standard that was added to the sample. The red and blue lines represent two transitions.

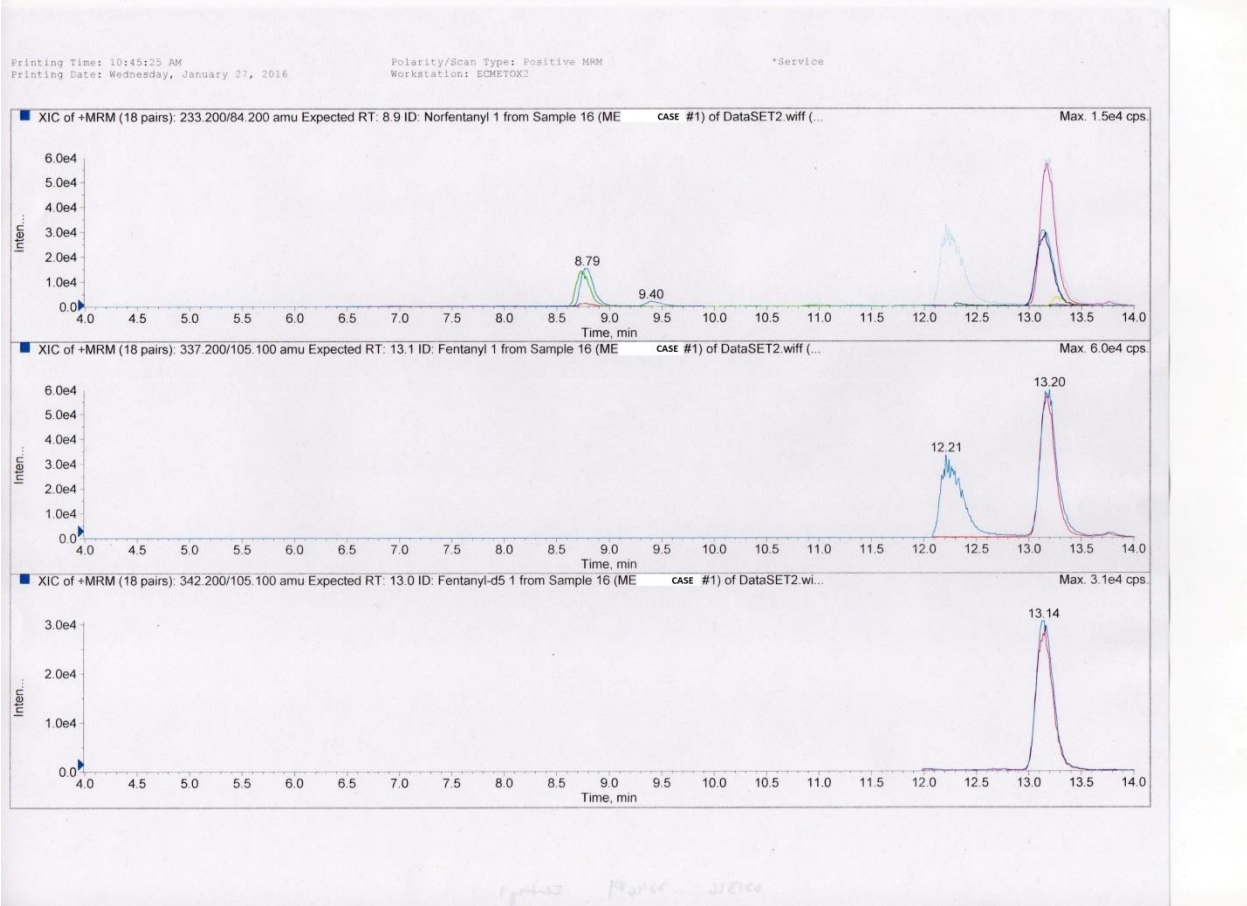

Figure 27. LC-MS/MS chromatographic peaks for the qualitative analysis of fentanyl in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays fentanyl present in the sample, and the bottom line represents the fentanyl internal standard that was added to the sample. The red and blue lines represent two transitions.

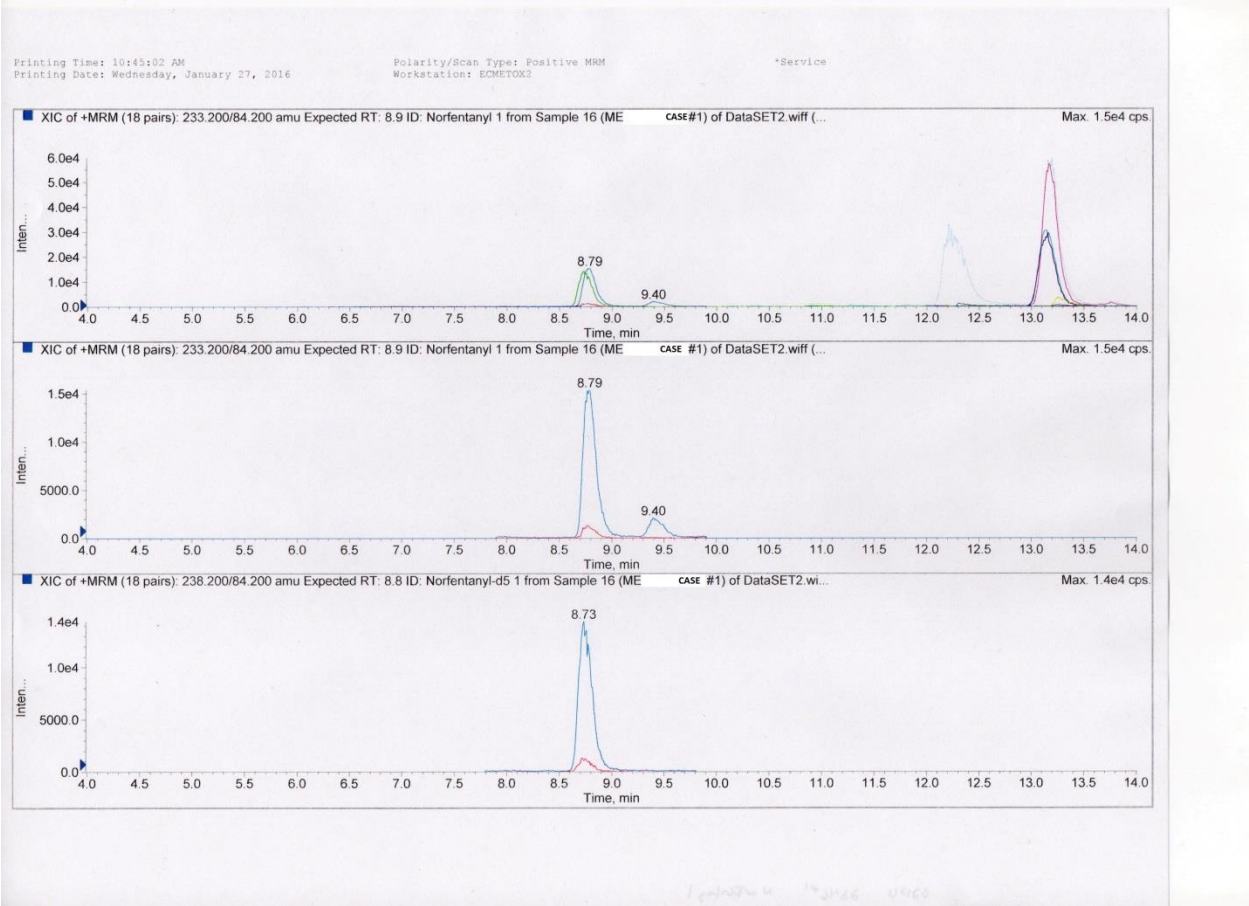

Figure 28. LC-MS/MS chromatographic peaks for the qualitative analysis of norfentanyl in the Case #1 from the 021315 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays norfentanyl present in the sample, and the bottom line represents the norfentanyl internal standard that was added to the sample. The red and blue lines represent two transitions.

3.8 6-MAM Conversion Test; Extraction 021916

Based on the consistent results elevated morphine measurements and missing 6-MAM concentration, we performed a 6-MAM conversion test using beef liver matrix (1/9 dilution) in order to see if morphine was produced after the addition of 6-MAM. No internal standard was added which contains all of the deuterated opiate components. Morphine and deuterated morphine-D6 were used as control samples and drug dilution

samples were spiked with known amounts of 6-MAM, 6-MAM d6, morphine and morphine d6. Table 2 displays the four samples used in this extraction.

Table 2. Four samples used for the 6-MAM conversion test (extraction 021916) all containing 1 gram calf liver 1:9 dilution.

Sample #	Drugs	Concentration (mcg/ml)
1	Negative Control	0
2	6-MAM	100
3	6-MAM d6	100
4	Morphine + Morphine d6	100 + 100

The same extraction and analysis procedure was conducted using these samples and controls. LC MS MS results showed that the 6-MAM was possibly converted into morphine. The internal standards for morphine and 6-MAM both disappeared, and the Figures 29-31 show that the chromatographic peak for morphine was the only component present in the samples. Something occurred during analysis to cause the internal standard of 6-MAM to disappear. Based on these results we decided to exclude 6-monoacetlymorphine from the future liver analysis. 6-MAM is very difficult to trace from an autopsy case as it is typically the first metabolite to appear in the blood after heroin has been administered into the human body, but then quickly converts to morphine and other metabolites (Bedford, 1991).

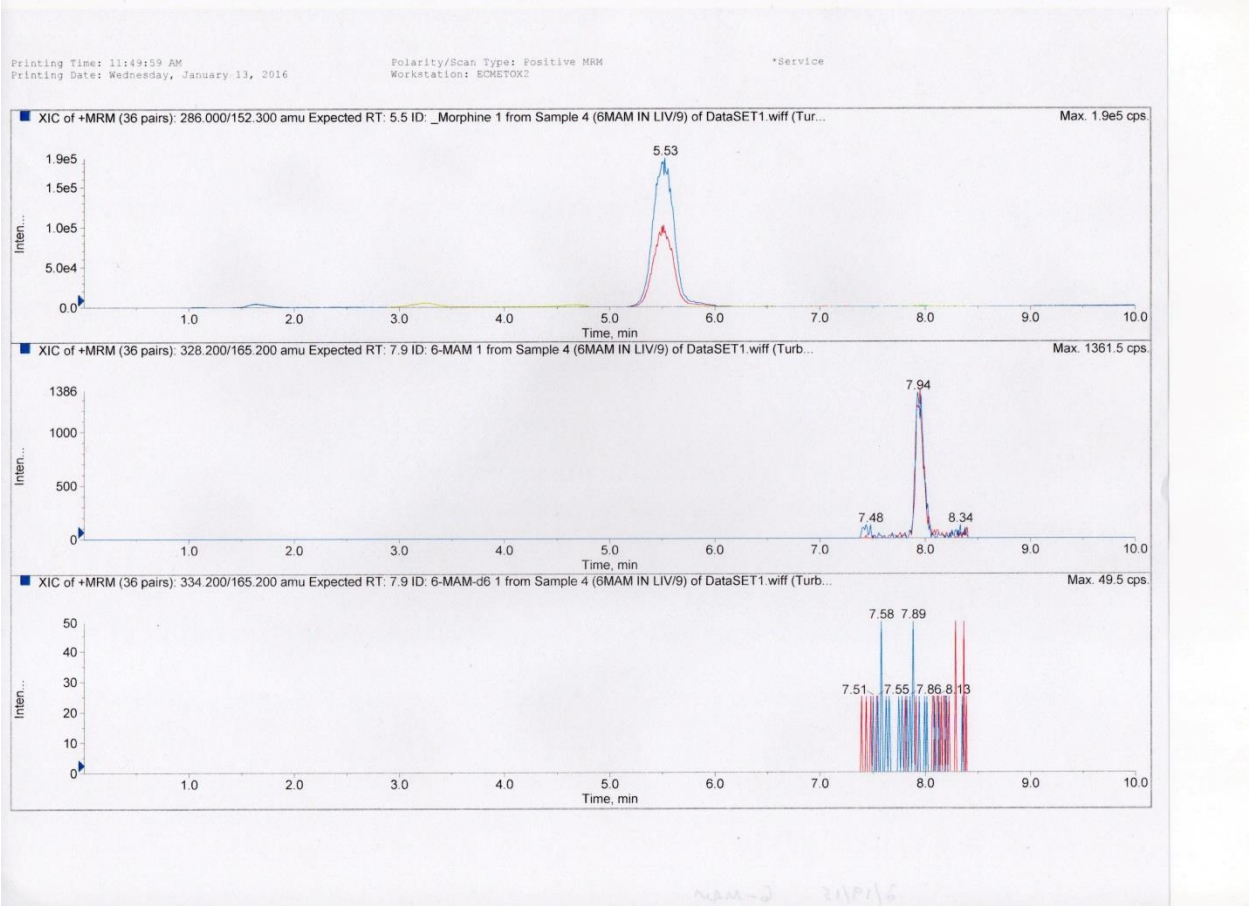

Figure 29. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM in the liver 1:9 dilution from the 021915 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays 6-MAM in the sample, and the bottom line represents the 6-MAM internal standard that was added to the sample. The red and blue lines represent two transitions.

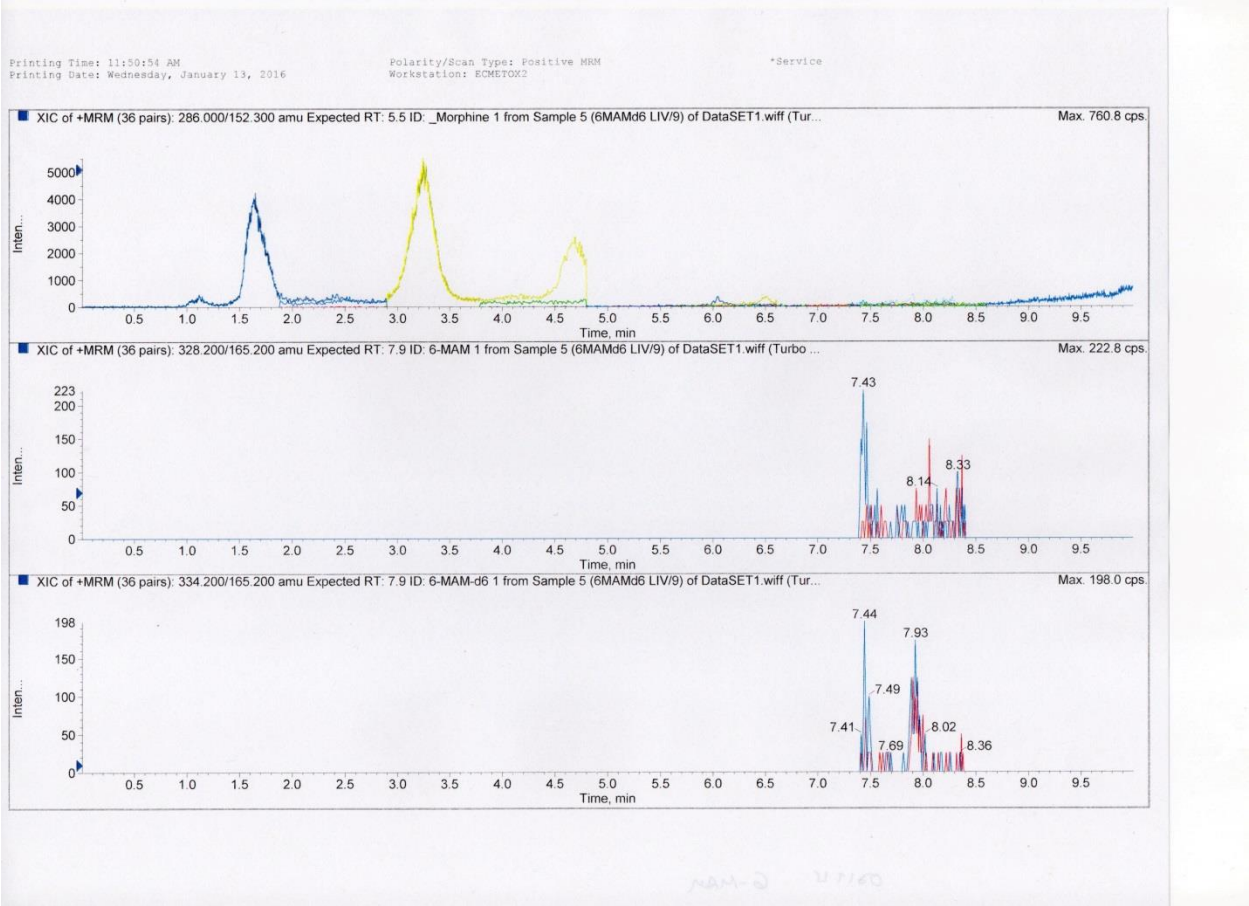

Figure 30. LC-MS/MS chromatographic peaks for the qualitative analysis of 6-MAM internal standard (6-MAM d6) in the liver 1:9 dilution from the 021915 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays 6-MAM d6 in the sample, and the bottom line represents the 6-MAM d6 internal standard that was added to the sample. The red and blue lines represent two transitions.

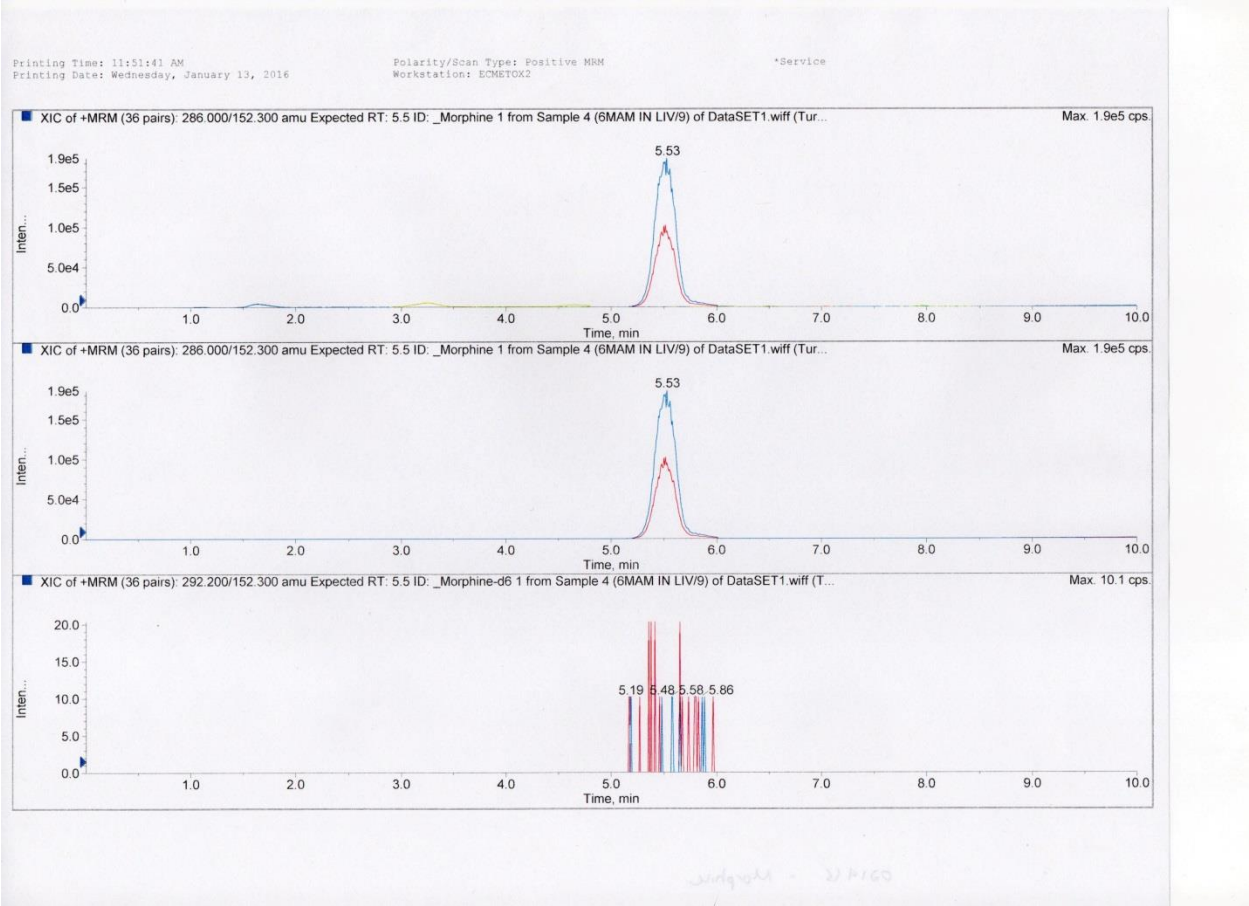

Figure 31. LC-MS/MS chromatographic peaks for the qualitative analysis of morphine in the liver 1:9 dilution from the 021915 extraction. The top line is the qualitative analysis of components in the sample, the middle line displays morphine in the sample, and the bottom line represents the morphine internal standard that was added to the sample. The red and blue lines represent two transitions.

3.9 030515 Extraction & Results

Once the procedure was established and proved practical, we created new working stock solutions for our calibrators without 6-MAM to prevent any possible disruptions in 6-MAM and morphine measurements (Tables 21 & 22, Appendix III). Once new calibrators were made, we proceeded to analyze sixty-four (64) cases through six extractions following the procedure in Appendix II. We continued to add the internal

standard to all of the samples. The internal standard contained the deuterated form of all the analytes, including 6-MAM (Internal standards section of Appendix II).

Results from this extraction of Case #1-10 can be viewed in Table 9, Appendix III. Morphine had concentrations for the blood and liver analysis in Case #1, #3, #4, and #9. In Case #1, alcohol and drugs other than opiates were present at time of analysis, and morphine was about 13 times higher in the liver than the blood. Case #3 showed the liver to be 1.4 times more concentrated in the liver than in the blood, and in Case #4 the liver was 2.9 times more concentrated than the blood. Cases #9 & #10 were decomposition cases, where blood was not available for sampling due to the extreme putrefaction of the organs. The specimen collected for the initial quantitation analysis was fluid which was collected from the right side of body cavity, termed "decomposition fluid" (Private conversation with Dr. Maloney, Associate Chief Medical Examiner, Erie County Medical Examiner's Office, March 30, 2016). In case #9, analysis showed that decomposition fluid was five times more concentrated with morphine than the liver. For Case #9 oxycodone and morphine-3-glucuronide resulted with higher concentration in the decomposition fluid than in the liver, this may have occurred due to the time that this individual's heart stopped pumping blood which allowed the blood to retain all the drugs which eventually turned into "decomposition fluid" and halted any additional drugs from traveling to the liver for further metabolic processing.

For many of the analytes that did not have a quantitative value from the blood analysis, there was a value for the liver analysis. Morphine-6-glucuronide in Case #2 was measured at 78.278 mcg/L in the liver; however, there was no value in the blood analysis. All the cases in this extraction with morphine present in the blood and liver analysis had a

concentration for morphine-3-glucuronide present as well. Morphine-3-glucuronide was about 1.5 to 3 times more concentrated in the liver than in the blood for cases #1, #3, #4 and #5. For case #1 and #3, morphine-6-glucuronide was analyzed to be 3 and 5 times more concentrated in the liver than in the blood, respectively. Case #4 was 24 times more concentrated with morphine-6-glucuronide in the liver than in the blood, and case #5 only had a concentration in the liver and not in the blood.

Codeine was another opiate that was present with a concentration in the liver for all the cases in this extraction, but only for cases #3 and #4 was there a concentration in the blood for comparison. In case #3 the codeine concentration was about the same in the liver and blood, and case #4 the liver was twice as concentrated as the blood.

Oxycodone had concentrations in the blood and the liver for Cases #2, #6, #7, and #8. These four cases were all females between 120 and 185 pounds that may have involved acute ingestion of oxycodone and in two cases possibly methadone. Case #2 had about twice the concentration of codeine in the liver than in the blood, Case #6 and #7 had similar concentrations in the liver and the blood, and case #8 had about 5 times the concentration of codeine in the liver than in the blood. Drugs other than opiates were present in all of the cases except Case #6, and a wide variety of drugs were found to be present for Case #8.

Case #10 was another decomposition case which resulted in 7900 mcg/L present in the decomposition fluid, and zero concentration in the liver. Oxymorphone, hydrocodone and hydromorphone were present in case #10 and those analytes all had a higher concentration in the liver than in the decomposition fluid. About 419 ug/L of

codeine was present in the liver quantitation and no codeine concentration was measured from the blood analysis (table 9 Appendix III). The case history suggests that a prescription bottle of oxycodone was ingested by the individual at time of death, and the death was ruled as an acute oxycodone and hydrocodone intoxication in Appendix IV.

Oxymorphone, hydromorphone, hydrocodone, and oxycodone can be prepared from solutions of codeine, so it's possible that codeine may have been present in analysis from the manufacturing process of these drugs if they were ingested (German Patent, 1936). Oxymorphone, oxycodone, codeine, hydrocodone and hydromorphone have all been subject to post mortem distribution (PMR) in past studies (Baselt, 2011). PMR may have altered the true concentration measurements of these drugs since this individual was considered decomposed before conducting the autopsy; allowing for a maximum time for post mortem redistribution of drugs in this individual's organs, and tissues, including the stomach, which may be a factor as to why a significant amount of oxycodone was measured in the blood (Moriya, 2005; Pélissier-Alicot, 2003).

Stomach contents are collected at the Erie County Medical Examiner's Office when there is a suspected intentional overdose case, and usually for the cases when the individual is an infant. When sampled, all of the gastric contents must be collected; however, the contents are only analyzed if the blood is not a satisfactory representative of the drug components present in the individual at the time of death. Since all the cases in this study, including case #10 had a significant concentration of drug components from the blood and decomposition fluid analyses, the stomach contents were not analyzed (Private conversation with Dr. Maloney, Associate Chief Medical Examiner, Erie County Medical Examiner's Office, March 30, 2016).

As previously stated; oxycodone and hydrocodone are metabolized in the liver by CYP2D6 into their major metabolites respectively, oxymorphone and hydromorphone (Purdue Pharma LP, 2016; Vuilleumier, 2012). In case #10, although there was a high concentration of oxycodone in the blood and none present in the liver, 281.25 µg/L oxymorphone was still present from the liver analysis. In comparison, 6 µg/L hydrocodone, and 10 µg/L of the metabolite hydromorphone were measured in the blood while 91.8 µg/L and 46.49 µg/L were measured in the liver respectively; the increased values most likely due to the hepatic metabolism characteristics of these drugs.

The highest measurement where there were values in both the liver and the blood in this extraction was for oxymorphone in Case #2. In this case the oxymorphone concentration was about 16 times higher in the liver than in the blood.

Buprenorphine had a detectable level in the liver for each case in this extraction. Case #10 had the highest concentration for buprenorphine which was measured at 343.512 mcg/L. Even at that high of a concentration there was no detection of buprenorphine measured from the blood analysis.

3.10 032015 Extraction & Results

Results from this extraction of Case #11-20 can be viewed in Table 11, Appendix III. Morphine was analyzed in Case #13, #19, and #20. All the cases had about 3-5 times higher concentration of morphine in the liver than the blood. Case #13's height and weight were previously mentioned at 65 inches, 124 pounds, while Case #19 was at 65.5 inches, 226 pounds, and Case #20 was 66.5 inches, 283 pounds. #19 and 20 had a known history of drug use, evidence of suspected drug overdose and other drugs present in the

body at time of initial analysis, and Case #13 and #19 had alcohol present in their toxicology screening analysis.

Case # 11, #17, and # 19 all had 30 mcg/L of hydrocodone from the blood analysis. For Case #11, hydrocodone measurements were about nine times higher in the liver than the blood. Case # 17 & # 19, measurements for hydrocodone were respectively, about five and three times higher in the liver than the blood. Alcohol was present in cases #11 & #19, and all three cases had a history of drug abuse, and other drugs present at the time of death. The only noted difference in the three cases is that Case #11 had CPR initiated; the decedent was intubated and given epinephrine, naloxone, calcium and sodium bicarbonate. It may be possible that the liver was functioning longer in Case #11 than the other overdose cases as an explanation for the higher concentration in the liver (about nine times higher than the blood) than the other two cases.

In this extraction, hydromorphone was measured in the liver analysis only for cases #11-14, and #17-20. Only case #12 had a concentration in the blood and liver, and the blood was about five times more concentrated with hydromorphone than the liver. Case #12 was a 56 year old male at 61 inches, 83 pounds with a known history of drug abuse and only opiates present in the body at time of analysis. In the case history it was stated that this individual never saw a doctor. Noting the case history, and the low weight of this individual, to explain the low concentration of hydromorphone in the liver, it may be possible the liver in this case#12 was not functioning as that of a healthy individual.

Fentanyl was compared between liver and blood analysis in Table 11, Appendix III in cases # 12, #13, #15, #16, and #18. Case #12 was higher in the blood analysis than

the liver analysis; in contrast, Case #18 was almost 27 times higher in the liver than the blood analysis. Both cases had only opiates present from the initial drug screening, but only Case #12 had a known history of drug abuse. As previously mentioned, Case #12 was 61 inches and 83 pounds, while Case #18 was almost twice that size at 73 inches, 173 pounds. Case #13 was 65 inches, 124 pounds, alcohol was present in the body and the fentanyl measured to be about 10 times more concentrated in the liver than the blood. Cases #15 and #16 both had other drugs present at the time of drug screening, and had about three times more fentanyl concentration in the liver than the blood. Case #15 was 72.5 inches, 191 pounds and Case #16 was 71 inches, 236 pounds with a known history of past drug abuse. Using height and weight measurements, one can calculate the mean mass ratio which is important in pharmacological investigations of determining appropriate dosage levels when prescribing drugs (Pharmacorama, 2006). Such calculations were performed to determine the dosage levels in select individuals at the time of death. Calculations for two cases were performed on two cases which are documented in Appendix V.

Norfentanyl was about the same liver: blood concentration ratio as fentanyl in case #12 and Case #16. In Case #12, fentanyl concentration was 1.3 times higher in blood than liver, norfentanyl concentration was about 1.5 times higher in blood than liver. In case #16, fentanyl concentration was 3.1 times higher in liver than blood, and norfentanyl concentration was about 3.7 times higher in liver than blood. in case #13, norfentanyl was half as concentrated as fentanyl in the liver than blood (fentanyl 10 times more concentrated in liver than blood, norfentanyl four times more concentrated in liver than blood). Case #15 norfentanyl was about five times more concentrated in liver than blood,

and finally Case #18 only had norfentanyl concentration detected in the liver analysis even though fentanyl had a measureable concentration in the blood and the liver analysis.

Buprenorphine and norbuprenorphine had measurements in the liver analysis for all the cases in this extraction (Case #11-20). The measured concentration for norbuprenorphine in the liver was higher than buprenorphine in each case.

3.11 032815 Extraction & Results

All the individuals in this set of cases were between 148 and 284 pounds. Results from this extraction of Case #21-29 can be viewed in Table 13, Appendix III. Case #24 had the highest concentration of morphine out of the study at almost 4000 (3982.5) mcg/L in the liver, about 12 times the concentration of the blood. The other Cases #21, 23, 26, 28 and 29 had the morphine concentration in the liver around 1.5 to 6 times the concentration from the blood. Out of these five cases, all but case #26 had drugs other than opiates present at the time of screening.

Morphine-3-glucuronide was also present in the liver analysis for all the cases in this extraction set. Case #26, and #29 the concentration was in the blood and liver analysis and about 3 and 1.4 times higher concentration in the liver than in the blood, respectively. Case #24 and Case #26 had morphine-6-glucuronide present in the blood and liver analysis and was about four and five times higher in the liver than in the blood, respectively.

Codeine was present in the liver analysis for all the cases #21-29 for this extraction. Only case #21 and #26 had a concentration for codeine in the blood and liver. Both times the concentration in the liver was about twice the concentration in the blood.

Oxycodone was only present in Case 24 of this extraction set, and was found to be almost three times more concentrated in the liver than in the blood.

In this set of case extractions, the lower the concentration of fentanyl present in the liver, the higher concentration difference between the liver and the blood. For Case # 22, 23, 26, and 29 the blood concentration was between 2.7-14.8 mcg/L and the liver concentration about 10-23 times higher in the liver. Case # 27 and 28 had fentanyl concentration of 37.8 and 35.8 mcg/L respectively in the blood analysis, and was only four to seven times more concentrated in the liver.

Norfentanyl had a concentration in the blood and liver in Case # 23, 26, 27 and 28. In all cases, the concentration was between 3.6 and 7.6 times higher in the liver than in the blood.

All the cases in this extraction (case #21-29) except Case #27 had norbuprenorphine present in the liver analysis. Buprenorphine was only present in the liver analysis for Case #26 and Case #28. Case #28 had a concentration for both buprenorphine and norbuprenorphine in the liver and blood; norbuprenorphine was about eight times higher concentration in the liver than blood and buprenorphine was about five times higher in the liver than blood.

3.12 041815 Extraction & Results

Four cases in this extraction set were positive for morphine in the blood and liver analysis. Case #31, #32, #34 and #38 had morphine concentration 13-40 mcg/L in the blood. Analysis of the liver showed morphine concentration to be about two to four times higher in the liver than the blood. The highest concentration was Case 38 with 40mcg/L

in the blood. This individual was 330 pounds with a known history of drug abuse, only opiates present at time of screening, and the liver analysis showed the morphine concentrations was 3.76 times higher in the liver than the blood.

Cases #32-38 had had a concentration of fentanyl in both the blood and liver. All but case #37 had a known history of drug abuse. Case #32 was the only female in this group of extractions, as well as the oldest individual; 47 years old at 176 pounds. Fentanyl was at five times higher concentration in the liver than the blood. The other cases #33-38 were males between the ages of 29 and 36 years old. Case #34 had the lowest concentration difference of fentanyl; about 8 times higher in the liver than the blood, while Case #37 had the biggest difference; close to 33 times higher in the liver than the blood. Case #37 had drugs other than opiates present in the body at time of screening, was 69.6 inches, and 222 pounds.

Norfentanyl, the metabolite of fentanyl was present in all the cases that had fentanyl for this extraction and at a much lesser concentration difference for all the cases except case #36. All the cases with norfentanyl were around half to a quarter of the concentration difference in the liver and blood for fentanyl, but Case #36 was about 11 times higher in the liver than the blood for fentanyl and norfentanyl (Table 15, Appendix III). Case #36 had the highest measured concentration of fentanyl in the liver out of all the cases in this extraction set at 410.4 mcg/L; also a known history of drug abuse and only opiates in the body at time of screening. As previously stated, fentanyl is primarily metabolized in the liver where it becomes norfentanyl. According to Mayes, once administered in the body, the distribution time for fentanyl is about six minutes, with a 60 minute redistribution time and elimination half-life of about 16 hours. About 75% gets

excreted as norfentanyl and about 10 percent is excreted as fentanyl through the urine and bile (Mayes, 2006).

In all the cases where buprenorphine and norbuprenorphine were measured for this extraction, had higher concentration for norbuprenorphine each time. In case #36, norbuprenorphine (11.97 mcg/L) was nearly eight times higher buprenorphine (1.566 mcg/L) in the liver concentration shown in Table 15, Appendix III.

3.13 060915 Extraction & Results

Results from this extraction of Case #39-52 can be viewed in Table 17, Appendix II. Case #40, #40, #42, #45, #46, #47, #51, #52 had morphine measurements in the liver and the blood. Case # 48 was the only case in the entire study that had a blood measurement that had no concentration measured in the liver. A concentration of 3 mcg/L of morphine was measured in the blood and no morphine was detected in the liver analysis. Morphine-3-glucuronide had a concentration in the blood and liver for this case, but the liver had lower levels than the blood (16 mcg/L in Blood, 0.6174 mcg/L in liver). Case #48 had a known history of drug abuse and drugs other than opiates were present in the body at time of analysis.

After a drug is administered orally, it gets absorbed by the digestive system and is subjected to hepatic metabolism usually carried to the liver through the portal vein before it reaches systemic circulation (Rowland, 1972). First pass metabolism greatly reduces the bioavailability, or initial dose of the unchanged the drug due to incomplete absorption during the first pass effect process. However; when a drug is administered

intravenously, its bioavailability is 100% because it avoids first-pass metabolism and reaches systemic circulation immediately through the bloodstream (Griffin, 2009).

Case #48 had a medical history of Hepatitis C, a history of heroin use, and evidence of syringes present at the scene of the death. Evidence of syringes found at the scene may suggest the decedent administered drugs intravenously, in which case the bioavailability of drugs in the decedent would be 100%, and would have reached systemic circulation before passing through the liver for detoxification. Based on evidence for Case #48 and knowledge drug metabolism; if the decedent administered these opiate drugs intravenously, it is possible the death was rapid and there was insufficient time for these drug components to be metabolized in the liver. These events may help explain why a morphine concentration was measured in blood and not liver.

Hepatitis C may cause inflammation of the liver, death of liver cells, scarring and cirrhosis of live tissue. It is also worth noting for this case, if the liver had failed to filter toxins due to the progression of Hepatitis C in this decedent, the toxins would build up in the bloodstream rather than the liver (Pietrangelo, 2016).

Throughout this study, for most of the cases, the liver was measured to have a higher concentration of the drugs than were present in the blood. For the other cases in this extraction set that compared morphine in the blood and liver, the concentration was between one and four times higher in the liver than the blood, except case #52 where the concentration of morphine was about eight times higher in the liver than blood.

For the cases in this extraction; morphine-3-glucuronide was present in the liver for all the cases where morphine was present blood and liver. Where there was a

concentration measured in the blood and liver for morphine-3-glucuronide, the concentration in the liver was between one and three times higher in the liver than in the blood with the exception of Case #48 and Case #51. In Case #48, morphine-3-glucuronide was higher in the blood than the liver, and Case #51 the concentration for morphine-3-glucuronide was about ten times higher in the liver than in the blood.

Morphine-3-glucuronide and morphine-6-glucuronide are metabolites of morphine. Morphine-3-glucuronide was mostly present whenever morphine was throughout this study; however, morphine-6-glucuronide was present less often than morphine-3-glucuronide. In this extraction morphine-6-glucuronide was present in half the cases where morphine was present in the blood measurements (Case #40, #46, #47, #51). In these cases the concentration of morphine-6-glucuronide was between one and a half, and three times higher in the liver than the blood, except Case #47 where it was about 10 times higher in the liver than the blood. Case #47 was a seventeen year old female with a known history of drug abuse and drugs other than opiates present in the body at time of screening. These characteristics may have affected the increase concentration in the liver compared to the blood.

Hydrocodone was analyzed in Case #40, #43, and #44 in this extraction and was found to be one, three, and four times higher concentration in the liver than the blood respectively.

Fentanyl concentration was compared in the blood and liver in case #40-44, #46-50, and #52. All these cases had a known history of drug abuse and all but case #41 had drugs other than opiate present in the body at time of analysis. Case #40, #47 and #48 had

the lowest concentration difference in the liver and blood about six and eight times higher in the liver than the blood. All the other cases were analyzed to have fentanyl around eleven to twenty three times higher in the liver than in the blood. There were no special correlations found to explain the extreme difference between the concentration differences measured in the liver and the blood.

Norfentanyl had a concentration present in the liver and blood for each of the cases where fentanyl was present in the liver and blood. Norfentanyl was also present in Case #39 at a higher concentration of nearly six times in the blood than in the liver. For Case #39, fentanyl was not measured in the blood and only present in the liver analysis (38.43 mcg/L). This case (#39) had a known history of drug abuse and like many of the other cases had other non-opiate drugs present in the body at time of screening. All the other cases where norfentanyl was analyzed in the blood and liver, there was at least 4 to 11 times higher concentration of norfentanyl in the liver than in the blood.

Buprenorphine and norbuprenorphine had measurements in the liver analysis for all the cases in this extraction (Case #39-52). The measured concentration for norbuprenorphine was higher than buprenorphine every time. Buprenorphine is metabolized in the liver by cytochrome P450 Enzyme CYP3A4 where it's converted into norbuprenorphine. A study performed by McMillin consisted of 1,946 urine samples in effort to display patterns of buprenorphine and its metabolites. The study showed 97.9% of the samples tested positive for norbuprenorphine, and 45.5% of the samples tested positive for buprenorphine (McMillin, 2012). These results support the idea that norbuprenorphine is more abundant than buprenorphine before becoming excreted, in

addition to supporting reasoning for the results of Cases #39-52 where the concentration of norbuprenorphine was higher than buprenorphine in the liver.

3.14 061015 Extraction & Results

Results from this final extraction of Cases #53-64 can be viewed in Table 19, Appendix II. Cases #53, #60, and #62-64 had morphine concentrations in the liver and blood analysis. All were between one and five times higher in the liver than in the blood except Case #60 which had a little over half as much morphine in the liver than in the blood (4 mcg/L in the blood, 2.421 mcg/L in the liver), and a similar concentration morphine-3-glucuronide (7 mcg/L in blood, 4.473 mcg/L in liver). Case #60 was a 22 year old male with a known history of drug abuse and only opiates in the body at time of analysis. According to the blood analysis, fentanyl and norfentanyl were the only other drugs present in this individual at time of analysis. Case #60 was 65 inches, 136 pounds, based on all of these known characteristics, it remains unknown how the morphine concentration and morphine-3-glucuronide concentration would be lower in the liver than in the blood.

Morphine-3-glucuronide was present in the liver and blood samples in all cases where morphine was present. Concentrations varied between liver and blood. Each time morphine-6-glucuronide was present in this extraction set; this metabolite was more concentrated in the liver than in the blood analysis compared to blood/liver concentration comparison of morphine-3-glucuronide. Case #62 had a morphine concentration twice as high in the liver than in the blood, but the metabolite morphine-3-glucuronide was 1.5 times higher in the blood than in the liver. For Case #62 the morphine-6-glucuronide metabolite of morphine was measured to be about the same in the blood and liver

analysis (160 and 166.5 mcg/L, respectively). Case #53 measured morphine-3-glucuronide to be close to four times in the liver than the blood, and morphine-6-glucuronide had a concentration in the liver, but not in the blood. Case #63 was measured to have 2.5 times the concentration of morphine-3-glucuronide in the liver than in the blood, and morphine-6-glucuronide was about 16 times higher in the liver than the blood. In Case #64, morphine-3-glucuronide was measured to be about ten times more concentrated in the liver than in the blood, and morphine-6-glucuronide was 29 times more concentrated in the liver than in the blood.

In this set of Cases #53-64, codeine had a concentration measurement in the liver for each; however, the blood analysis only detected codeine in three of these cases: #62, #63, and #64. For these three cases, the concentration of codeine was between one and three times more concentrated in the liver than in the blood.

Hydrocodone was only detected in Case # 59 for this extraction, and was found to be 3.6 times more concentrated in the liver than in the blood.

A concentration for fentanyl was detected in the liver analysis for all the cases in this set, and for the blood analysis, all but Case #62 had detected a fentanyl concentration. When comparable, the concentration of fentanyl was between two and fourteen times higher in the liver than in the blood.

Norfentanyl had a concentration present in both the liver and the blood for most of the cases (#53-58, #60, and #61). For all of those cases except #58 and #61, the amount of norfentanyl higher in the liver than the blood was half the times higher in liver than the blood for fentanyl. For example in Case #56, fentanyl was about ten times more

concentrated in the liver than the blood, and norfentanyl was about half, or five times more concentrated in the liver than in the blood. For the exceptions case #58 and case #61, the fentanyl was the same amount higher in the liver than in the blood as norfentanyl was. Case #58 was about five times more concentrated in the liver than the blood for fentanyl and norfentanyl, Case #61 was about three times more concentrated in the liver than in the blood for fentanyl and norfentanyl. Case #59, #62, #63, and #64 norfentanyl was only detected in the liver. It is likely that norfentanyl was at concentrations too low to be detected in the blood.

Norbuprenorphine had measurements in the liver analysis for all the cases in this extraction (Case #53-64). When buprenorphine had a concentration detected in the liver, the measured concentration for norbuprenorphine was higher than buprenorphine in all instances.

Chapter IV

Discussion

Results from the six opiate extractions (030515, 032015, 032815, 041815, 060915, and 061015) had no interference between the analytes and the liver matrices from the quantitative data. All the samples were spiked with an internal standard of the deuterated compounds of interest in order to detect any interference between the drug components and blood and liver matrix. Also, the internal standard is necessary in order to act as a measure of control during the extraction process, instrumental analysis and quantitation procedure.

The calibration samples included one sample of whole blood used as a negative control, seven standard solutions, pre-prepared low-positive and high-positive controls of the blood, and also high, medium and low (1:9) liver dilution controls, all which were spiked with known amounts of opiates and internal standard in order to monitor performance and interferences during analysis. The high and low blood controls were pre-prepared and had lot numbers recorded in case systemic errors occurred. The extraction columns also had lot numbers recorded in the event of any troubleshooting that may have occurred during the extraction procedure.

The tables in Appendix III where blood analysis is compared to liver analysis, many of the extraction results showed more analytes present in the liver than in the blood. In many instances there were values for opiates in the liver but not in the blood. Many times there were values for opiates in the liver analysis, but not the blood analysis. It is possible that in many cases, the opiates measured in the blood were below the limit of

detection, but just above the limit of detection in the 1:9 liver analyses. Despite these occurrences, there were no observed, predictable quantitative correlations between the opiates found in the liver compared to the blood analyses.

Figures 32 & 33 and Tables 3 & 4 show the percentage comparisons of drugs present in all 64 cases studied after blood analysis and liver analysis. Fentanyl was detected in about sixty-six (65.63) percent of total cases from quantitation of the opiates in the blood analysis. Results of the liver analysis showed norbuprenorphine to appear in close to ninety-five (95.31) percent of all the sixty-four cases analyzed, and fentanyl appeared in about eighty-eight (87.50) percent of the cases.

Figures 34-45 and Tables 5-16 display each of the 12 measured opiates and the cases in which they were present. The concentration of these opiates is charted when present in the liver and in the blood. Case #24 had the highest amount of morphine detected from the liver analysis at 3982.5 mcg/L when compared to the blood analysis. The concentration in the blood analysis for that case was 320 mcg/L. Case #19 had the highest amount of morphine measured from the blood quantitation, at 460 mcg/L, with only 1386 mcg/L measured from the liver analysis. Hydromorphone was not frequently found detected in the cases for this project only showing up in the blood and liver analysis for Cases #10, #12 and #25. Codeine showed up in the analyses for ten of the cases with the highest concentration at 40.41 mcg/L in the liver and 18 mcg/L in the blood for Case #19. The liver concentration for codeine was about two times higher than the blood concentration in that case. Case #25 had the highest concentration for hydrocodone in both the blood and the liver at 845.1 mcg/L and 420 mcg/L, respectively. Hydrocodone measured to be around two times higher in liver than in the blood for Case

#25. Oxymorphone was analyzed in five cases out of the total cases analyzed in the project. The highest concentration difference of this opiate was measured in Case #2 where oxmorphone was over sixteen times higher concentration in the liver than in the blood. Oxymorphone was measured at 83.205 mcg/L in the liver and at 5 mcg/L in the blood for Case #2. Case #2 had the highest amount of oxycodone detected from the liver analysis at 1318.5 mcg/L. The liver concentration was almost two times the concentration of the blood analysis for that case which was measured at 730 mcg/L. Morphine-3-glucuronide was measured in twenty-four cases. The highest concentration in the liver was measured at 1053 mcg/L, about ten times higher than the blood analysis measurement for Case #51. Morphine-6-glucuronide was measured in fourteen cases with the highest concentration from the liver analysis at 1633.5 mcg/L; about five times higher than the blood concentration measurement in Case #24. Case #64 had a morphine-6-glucuronide concentration at 377.1 mcg/L from the liver analysis, and at 13 mcg/L from the blood analysis, a concentration twenty-nine times higher in the liver than in the blood. Norfentanyl had a concentration measured in the blood for 57.81% of the cases, and a concentration in the liver for 87.50% of the cases. Case #55 had the highest concentration of norfentanyl which was measured at 97.65 mcg/L in the liver, while the concentration was 35.4 mcg/L in the blood. Fentanyl had a measurement in the blood for 65.63% of the cases analyzed, and a measurement in the liver for 87.50% of the cases. The highest concentrations for fentanyl were measured in the liver, measuring around 530.1-582.3 mcg/L, while the highest concentration measured in the blood was 112.2 mcg/L. Norbuprenorphine was measured in the liver for 95.31% of the cases, however, only six of the cases had measurements from the blood analysis for the ability to compare the two

concentrations. Case #55 had the highest norbuprenorphine concentration measured from the liver analysis was 50.895 mcg/L while the measurement in the blood was 6.6 mcg/L. Buprenorphine concentrations showed up in 75.00% of the cases from the liver analysis, and 9.38% of the cases from blood analysis. When both values were compared, Case #55 again, had the highest measurement for buprenorphine in the liver at 34.65 mcg/L, and 1.5 mcg/L in the blood.

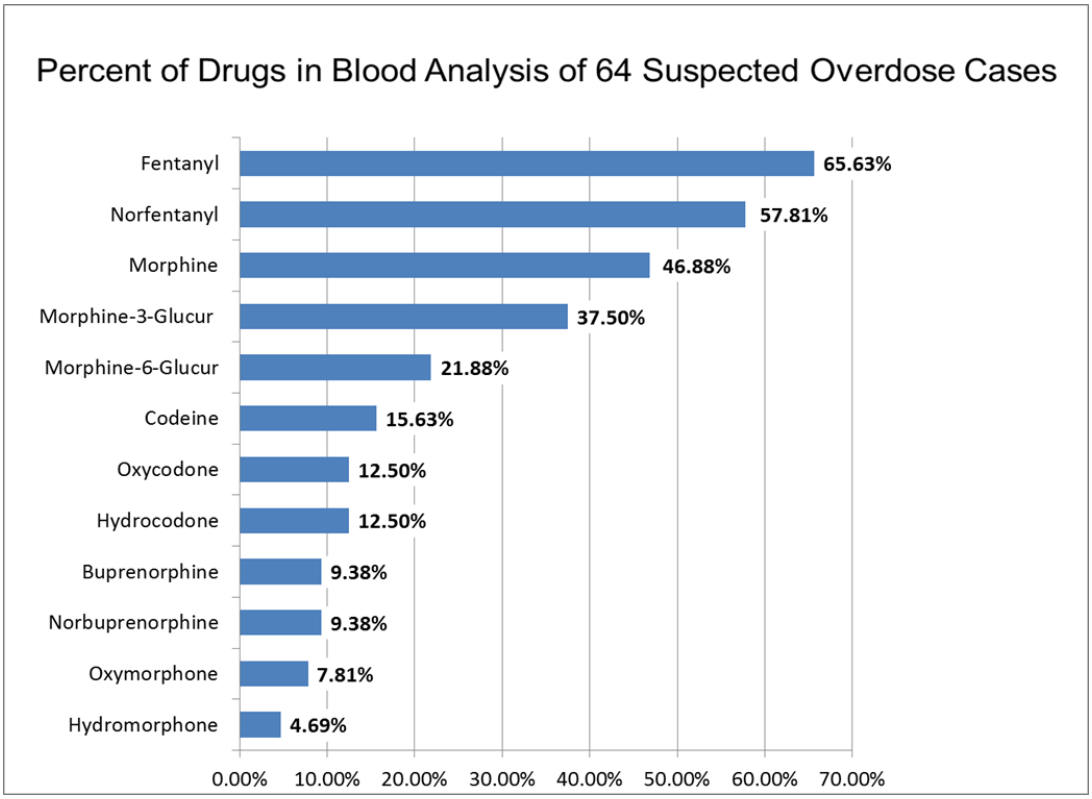

Figure 32. Percent of opiates present in 64 suspected overdose cases that were analyzed with blood samples.

Table 3. Tabulated values from Figure 32.

Drugs	Number of cases where drug was present in Blood	Percent of time drug was found in the 64 cases
Hydromorphone	3	4.69%
Oxymorphone	5	7.81%
Norbuprenorphine	6	9.38%
Buprenorphine	6	9.38%
Hydrocodone	8	12.50%
Oxycodone	8	12.50%
Codeine	10	15.63%
Morphine-6-Glucuronide	14	21.88%
Morphine-3-Glucuronide	24	37.50%
Morphine	30	46.88%
Norfentanyl	37	57.81%
Fentanyl	42	65.63%

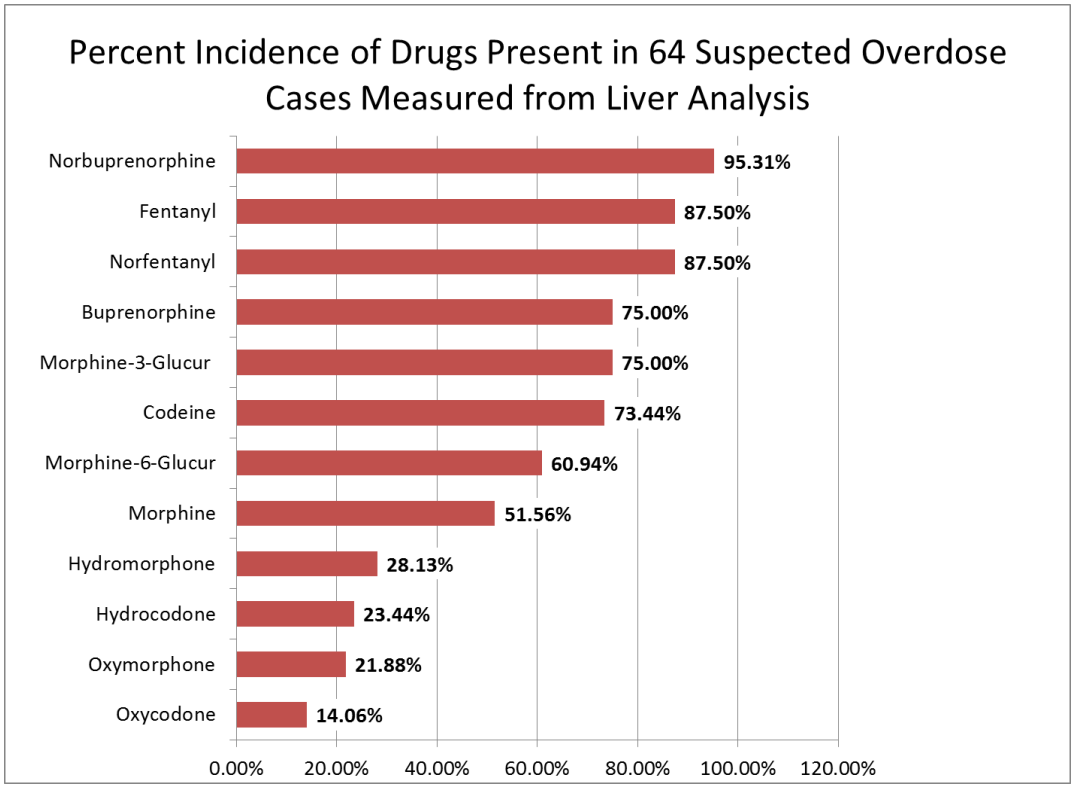

Figure 33. Percent incidence of drugs present in 64 suspected overdose cases measured from liver analysis.

Table 4. Tabulated values from Figure 33.

Drugs	Number of cases where drug was present in Liver Analysis	Percent of time drug was found in the 64 cases
Oxycodone	9	14.06%
Oxymorphone	14	21.88%
Hydrocodone	15	23.44%
Hydromorphone	18	28.13%
Morphine	33	51.56%
Morphine-6-Glucuronide	39	60.94%
Codeine	47	73.44%
Morphine-3-Glucuronide	48	75.00%
Buprenorphine	48	75.00%
Norfentanyl	56	87.50%
Fentanyl	56	87.50%
Norbuprenorphine	61	95.31%

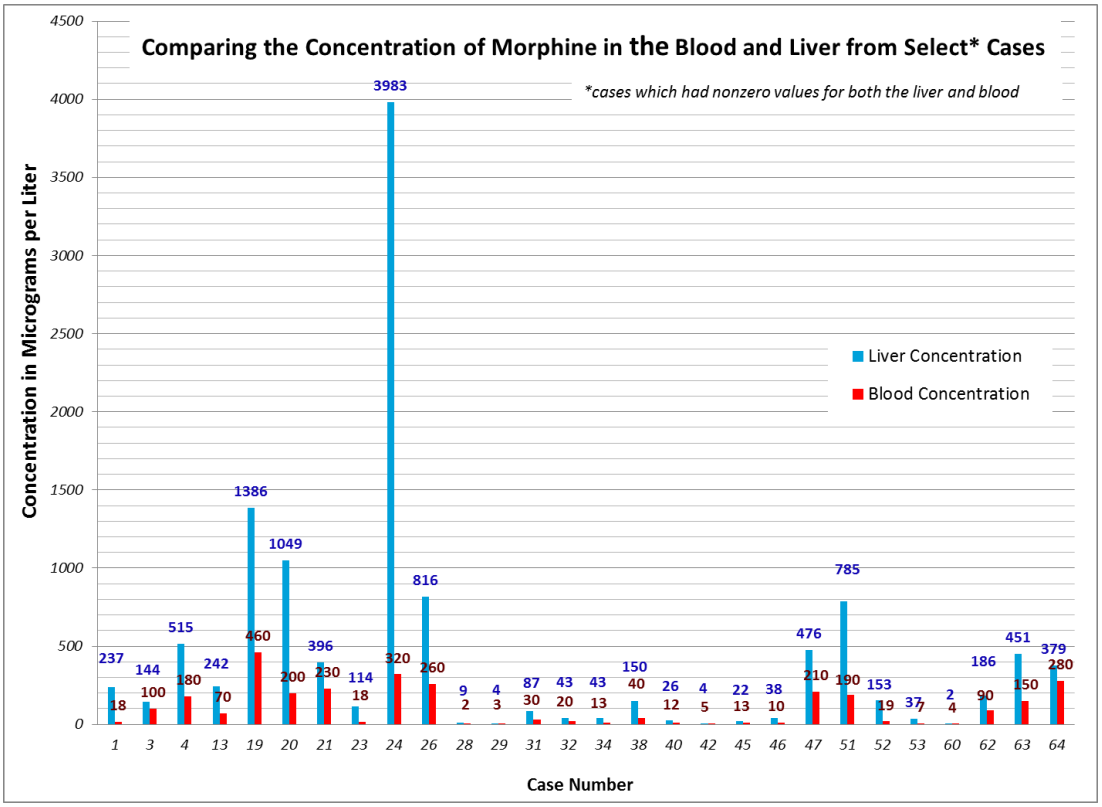

Figure 34. Comparison of the morphine concentration in the liver and the blood.

Table 5. Tabulated values comparing the concentration of morphine in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times higher in liver than blood
1	23	Male	67	139	18	237.15	13.175
3	24	Male	66	146	100	143.55	1.436
4	21	Female	62	147	180	515.25	2.863
13	28	Female	65	124	70	242.1	3.459
19	26	Male	65.5	226	460	1386	3.013
20	17	Male	66.5	283	200	1048.5	5.243
21	34	Male	77.5	228	230	396.45	1.724
23	28	Male	71	191	18	113.85	6.325
24	45	Female	64	159	320	3982.5	12.445
26	45	Male	73	231	260	815.85	3.138
28	42	Female	62	138	2	8.577	4.289
29	22	Male	75.5	284	3	4.374	1.458
31	50	Male	70	221	30	87.435	2.915
32	47	Female	62.5	176	20	42.66	2.133
34	32	Male	71.5	162	13	42.84	3.295
38	32	Male	69.5	330	40	150.3	3.758
40	35	Female	63	181	12	25.83	2.153
42	44	Male	72	217	5	3.564	0.713
45	48	Female	63	175	13	21.6	1.662
46	26	Female	64	116	10	38.43	3.843
47	17	Female	62	146	210	475.65	2.265
48	34	Male	72	168	3	0	0
51	31	Male	70.5	259	190	784.8	4.131
52	32	Male	74	163	19	152.55	8.029
53	36	Male	73	284	7	37.125	5.304
60	22	Male	65	136	4	2.421	0.605
62	28	Female	62	177	90	186.3	2.070
63	32	Male	68.5	170	150	450.9	3.006
64	40	Female	64	159	280	378.9	1.353

Table 5a. Tabulated values comparing the concentration of morphine analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
9	52	Female	65.5	240	1700	334.8	0.197

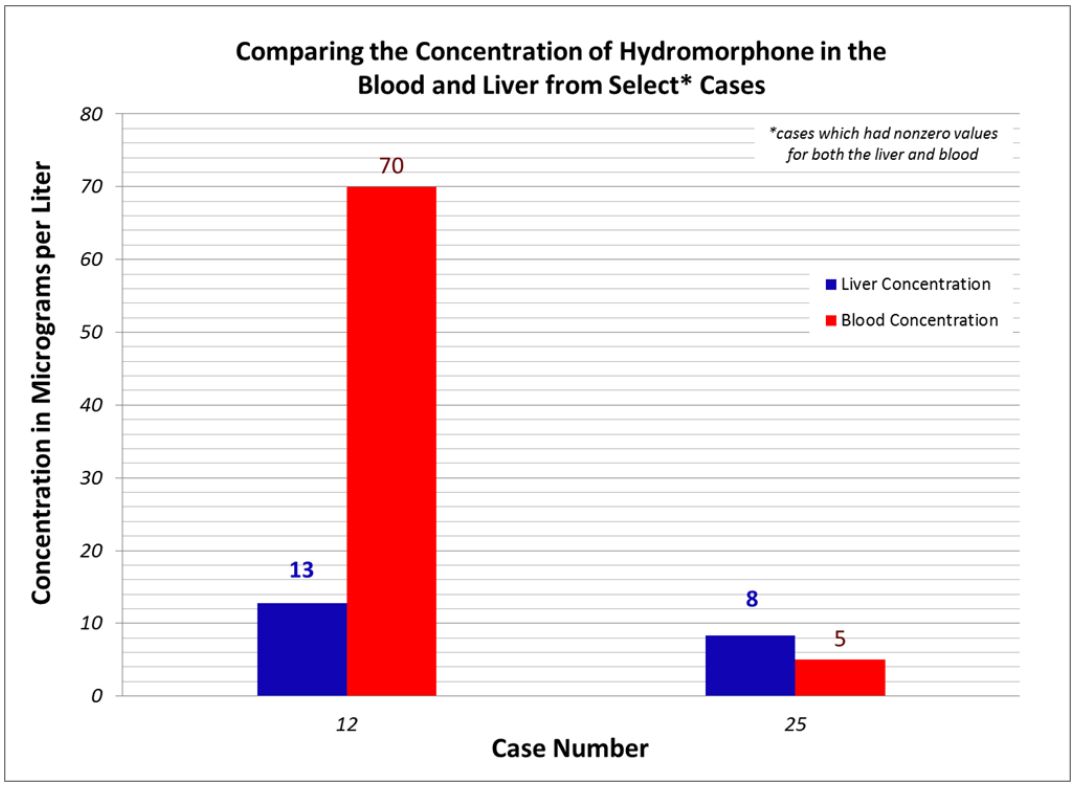

Figure 35. Comparison of the hydromorphone concentration in the liver and the blood.

Table 6. Tabulated values comparing the concentration of hydromorphone analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
12	56	Male	61	83	70	12.735	0.182
25	52	Male	72	148	5	8.307	1.661

Table 6a. Tabulated values comparing the concentration of hydromorphone analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
10	64	Female	65	99	10	46.485	4.649

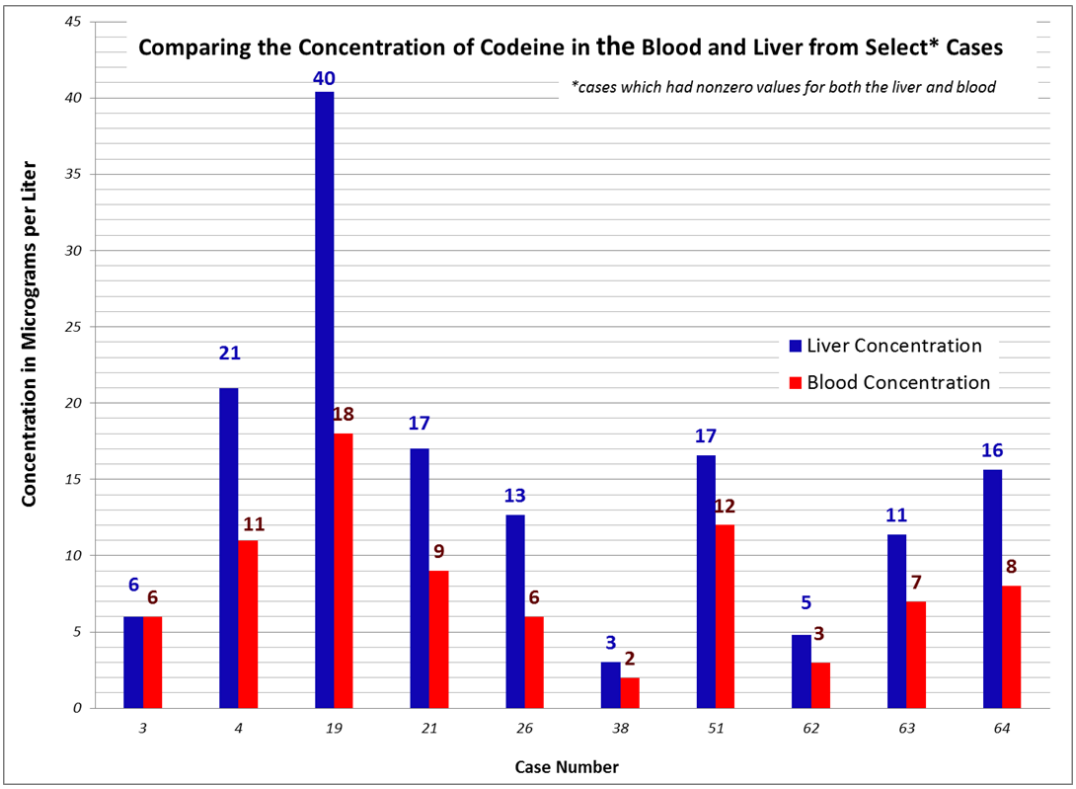

Figure 36. Comparison of the codeine concentration in the liver and the blood.

Table 7. Tabulated values comparing the concentration of codeine analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
3	24	Male	66	146	6	5.9895	0.998
4	21	Female	62	147	11	20.97	1.906
19	26	Male	65.5	226	18	40.41	2.245
21	34	Male	77.5	228	9	17.01	1.890
26	45	Male	73	231	6	12.69	2.115
38	32	Male	69.5	330	2	3.033	1.517
51	31	Male	70.5	259	12	16.56	1.380
62	28	Female	62	177	3	4.815	1.605
63	32	Male	68.5	170	7	11.385	1.626
64	40	Female	64	159	8	15.615	1.952

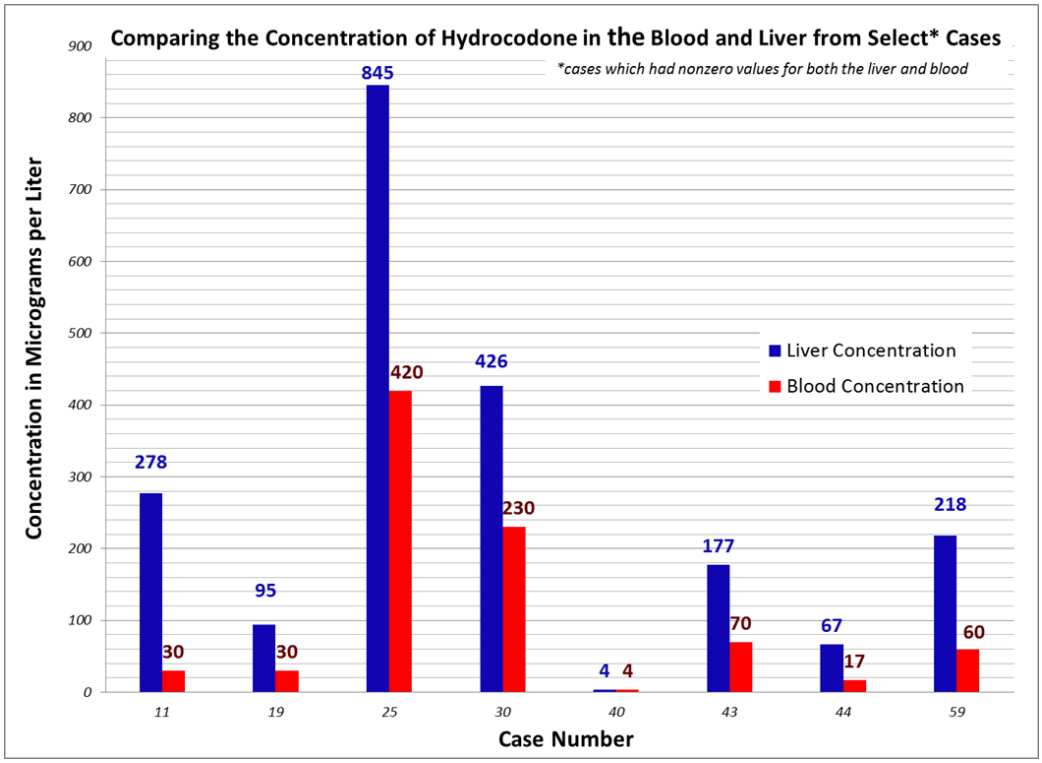

Figure 37. Comparison of the hydrocodone concentration in the liver and the blood.

Table 8. Tabulated values comparing the concentration of hydrocodone analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
11	75	Female	64	113	30	277.65	9.255
19	26	Male	65.5	226	30	94.5	3.150
25	52	Male	72	148	420	845.1	2.012
30	44	Female	62.5	148	230	426.15	1.853
40	35	Female	63	181	4	3.6135	0.903
43	33	Male	69	205	70	177.3	2.533
44	49	Male	72.5	212	17	66.51	3.912
59	37	Male	72	236	60	218.25	3.638

Table 8a. Tabulated values comparing the concentration of hydrocodone analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
10	64	Female	65	99	6	91.8	15.300

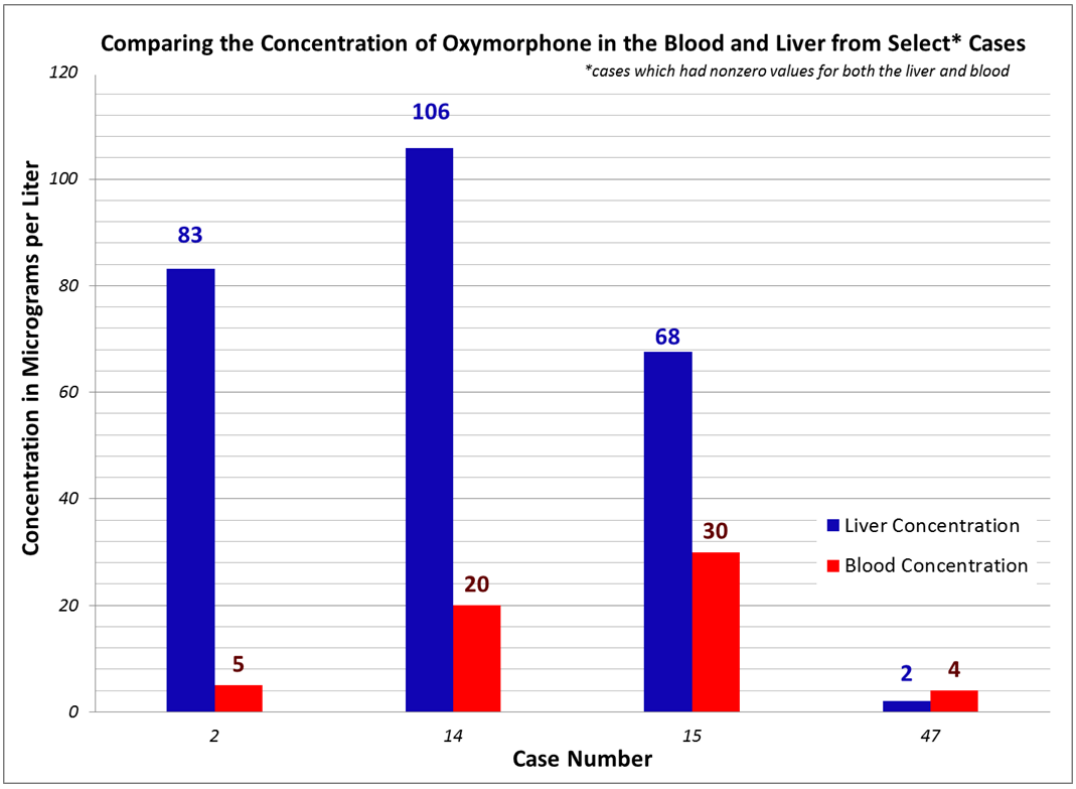

Figure 38. Comparison of the oxymorphone concentration in the liver and the blood.

Table 9. Tabulated values comparing the concentration of oxymorphone analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
2	36	Female	64	140	5	83.205	16.641
14	45	Male	71	237	20	105.75	5.288
15	24	Male	72.5	191	30	67.635	2.255
47	17	Female	62	146	4	2.088	0.522

Table 9a. Tabulated values comparing the concentration of oxymorphone analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
10	64	Female	65	99	210	281.25	1.339

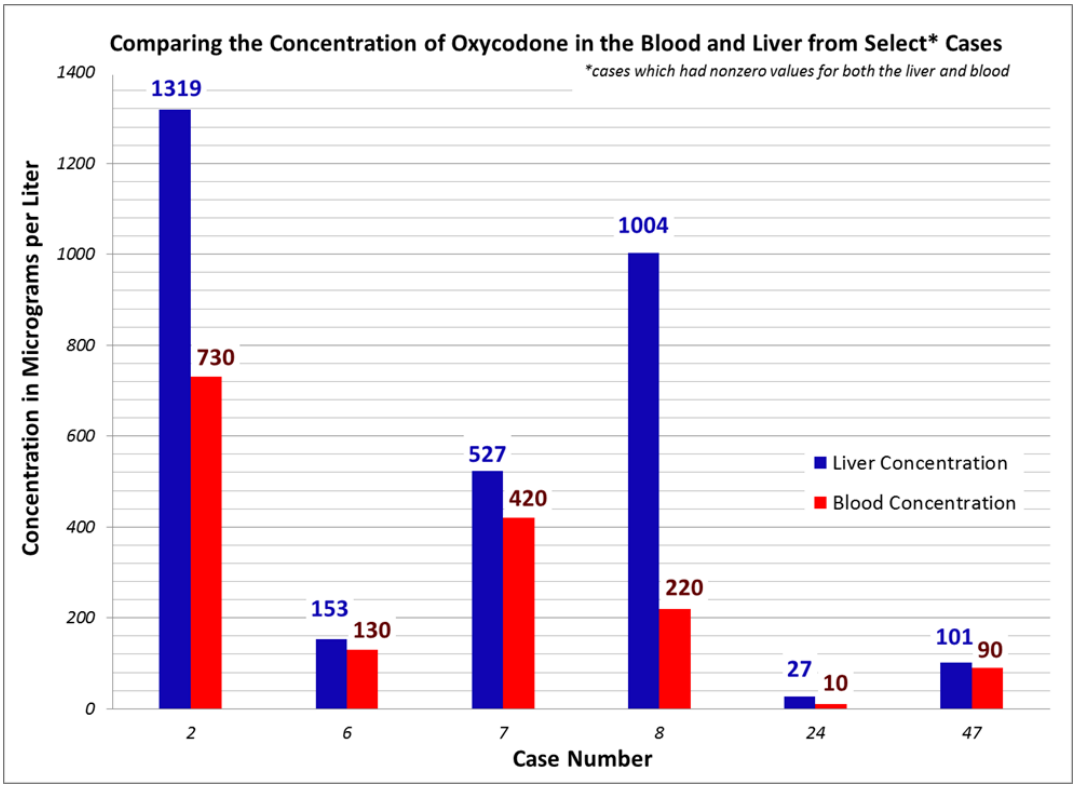

Figure 39. Comparison of the oxycodone concentration in the liver and the blood.

Table 10. Tabulated values comparing the concentration of oxycodone analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
2	36	Female	64	140	730	1318.5	1.806
6	46	Female	67	122	130	153	1.177
7	33	Female	59.5	183	420	527.4	1.256
8	53	Female	64	123	220	1003.5	4.561
24	45	Female	64	159	10	27.135	2.714
47	17	Female	62	146	90	101.25	1.125

Table 10a. Tabulated values comparing the concentration of oxycodone analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
9	52	Female	65.5	240	140	8.289	0.059
10	64	Female	65	99	7900	0	0.000

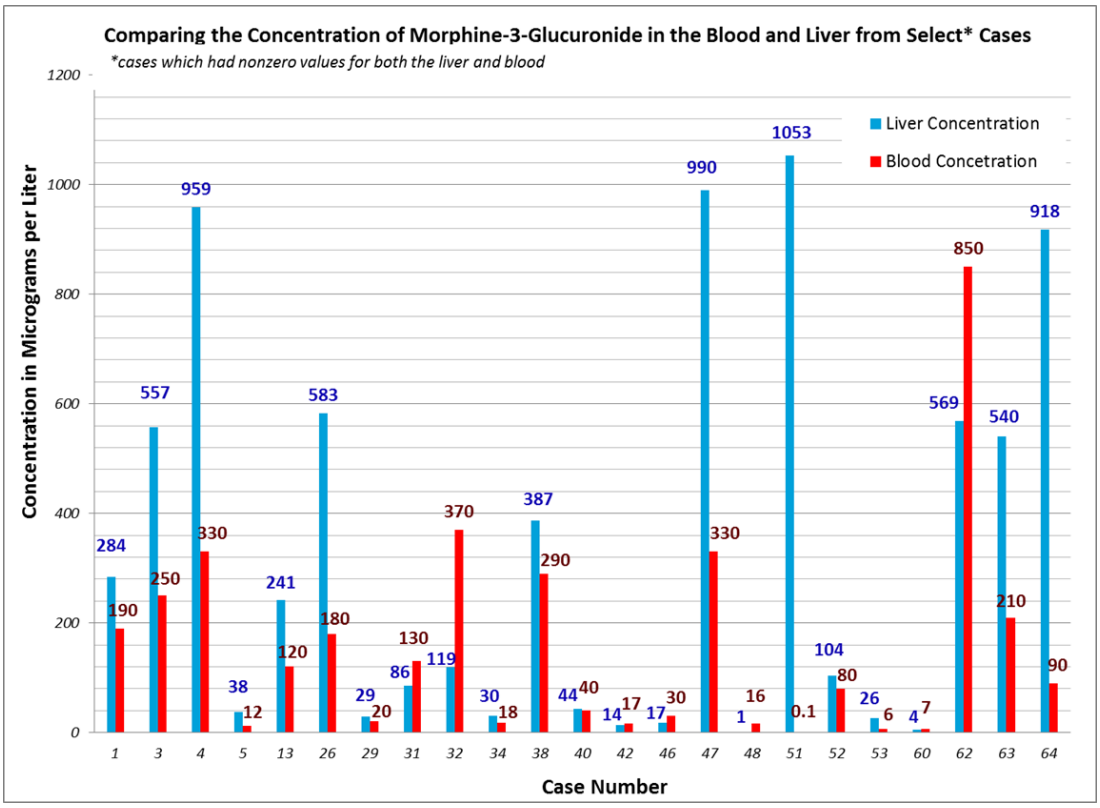

Figure 40. Comparison of the morphine-3-glucuronide concentration in the liver and the blood.

Table 11. Tabulated values comparing the concentration of morphine-3-glucuronide analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
1	23	Male	67	139	190	284.4	1.497
3	24	Male	66	146	250	557.1	2.228
4	21	Female	62	147	330	958.5	2.905
5	0	Female	10	1	12	37.98	3.165
13	28	Female	65	124	120	241.2	2.010
26	45	Male	73	231	180	583.2	3.240
29	22	Male	75.5	284	20	28.665	1.433
31	50	Male	70	221	130	85.59	0.658
32	47	Female	62.5	176	370	119.25	0.322
34	32	Male	71.5	162	18	29.925	1.663
38	32	Male	69.5	330	290	386.55	1.333
40	35	Female	63	181	40	43.65	1.091
42	44	Male	72	217	17	14.04	0.826
46	26	Female	64	116	30	17.1	0.570
47	17	Female	62	146	330	990	3.000
48	34	Male	72	168	16	0.6174	0.039
51	31	Male	70.5	259	>100	1053	>10.636
52	32	Male	74	163	80	103.5	1.294
53	36	Male	73	284	6	25.92	4.320
60	22	Male	65	136	7	4.473	0.639
62	28	Female	62	177	850	568.8	0.669
63	32	Male	68.5	170	210	540	2.571
64	40	Female	64	159	90	918	10.200

Table 11a. Tabulated values comparing the concentration of morphine-3-glucuronide analyzed in the decomposition fluid and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Concentration in Decomposition Fluid (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
9	52	Female	65.5	240	380	164.79	0.434

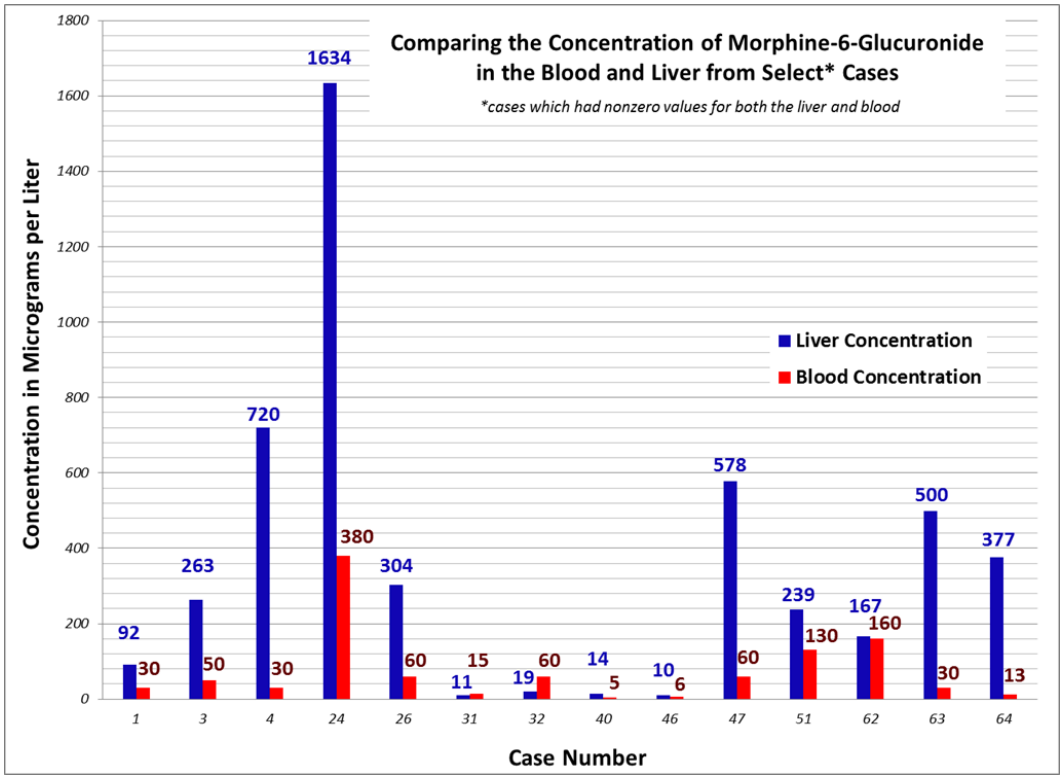

Figure 41. Comparison of the morphine-6-glucuronide concentration in the liver and the blood.

Table 12. Tabulated values comparing the concentration of morphine-6-glucuronide analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
1	23	Male	67	139	30	91.8	3.060
3	24	Male	66	146	50	262.8	5.256
4	21	Female	62	147	30	720.45	24.015
24	45	Female	64	159	380	1633.5	4.299
26	45	Male	73	231	60	303.75	5.063
31	50	Male	70	221	15	11.25	0.750
32	47	Female	62.5	176	60	19.44	0.324
40	35	Female	63	181	5	13.68	2.736
46	26	Female	64	116	6	9.9	1.650
47	17	Female	62	146	60	577.8	9.630
51	31	Male	70.5	259	130	238.5	1.835
62	28	Female	62	177	160	166.5	1.041
63	32	Male	68.5	170	30	499.5	16.650
64	40	Female	64	159	13	377.1	29.008

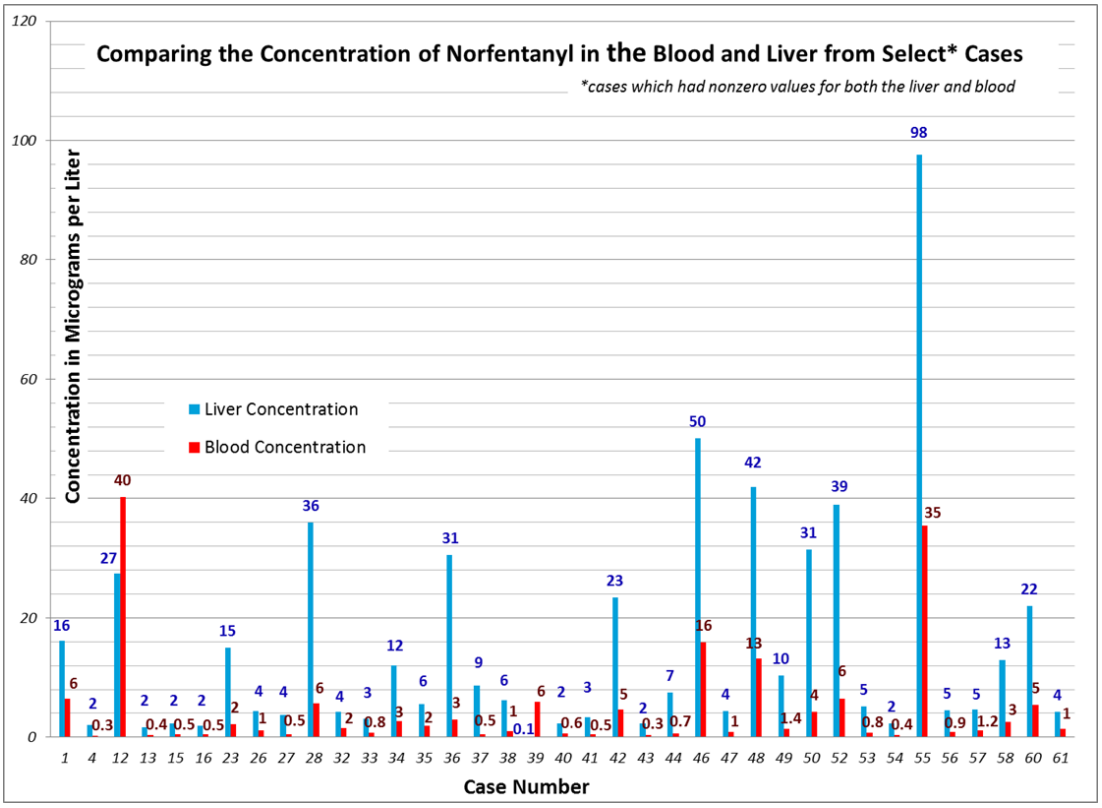

Figure 42. Comparison of the norfentanyl concentration in the liver and the blood.

Table 13. Tabulated values comparing the concentration of norfentanyl analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
1	23	Male	67	139	6.4	16.155	2.524
4	21	Female	62	147	0.27	2.0385	7.550
12	56	Male	61	83	40.2	27.495	0.684
13	28	Female	65	124	0.42	1.674	3.986
15	24	Male	72.5	191	0.45	2.295	5.100
16	36	Male	71	236	0.53	1.9485	3.676
23	28	Male	71	191	2.2	15.03	6.832
26	45	Male	73	231	1.2	4.3695	3.641
27	28	Female	65.5	188	0.49	3.7395	7.632
28	42	Female	62	138	5.7	35.955	6.308
32	47	Female	62.5	176	1.5	4.2705	2.847
33	29	Male	75	277	0.78	3.042	3.900
34	32	Male	71.5	162	2.7	12.015	4.450
35	29	Male	74.5	200	1.9	5.562	2.927
36	36	Male	67	164	2.9	30.555	10.536
37	33	Male	69.5	222	0.51	8.6175	16.897
38	32	Male	69.5	330	0.97	6.228	6.421
39	29	Female	64	153	6	0.106425	0.018
40	35	Female	63	181	0.62	2.2995	3.709
41	21	Male	70	319	0.51	3.3705	6.609
42	44	Male	72	217	4.6	23.445	5.097
43	33	Male	69	205	0.33	2.313	7.009
44	49	Male	72.5	212	0.67	7.452	11.122
46	26	Female	64	116	15.9	50.13	3.153
47	17	Female	62	146	0.92	4.4325	4.818
48	34	Male	72	168	13.2	41.895	3.174
49	35	Male	63.5	207	1.4	10.305	7.361
50	24	Male	72	164	4.2	31.41	7.479
52	32	Male	74	163	6.4	38.97	6.089
53	36	Male	73	284	0.77	5.1255	6.656
54	46	Male	67	175	0.35	2.322	6.634
55	35	Male	71	192	35.4	97.65	2.758
56	31	Male	73	273	0.92	4.5675	4.965
57	32	Male	69	208	1.2	4.635	3.863
58	42	Male	68	205	2.6	12.96	4.985
60	22	Male	65	136	5.4	22.05	4.083
61	40	Male	72	208	1.4	4.311	3.079

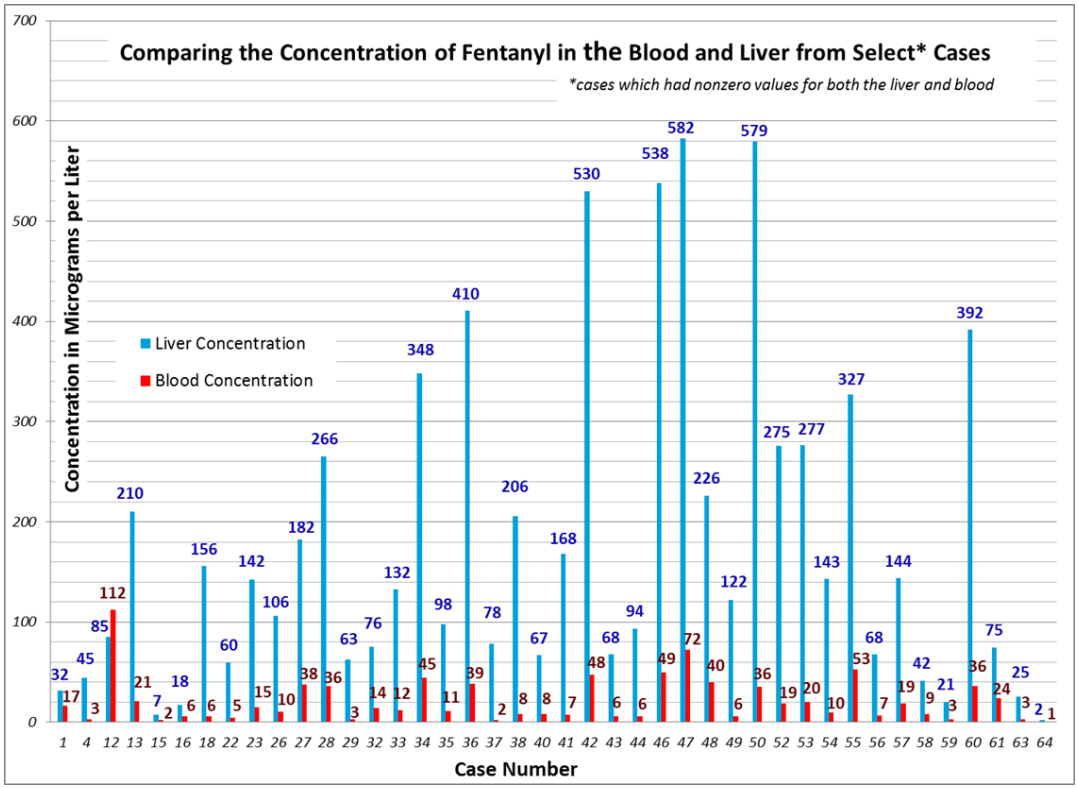

Figure 43. Comparison of the fentanyl concentration in the liver and the blood.

Table 14. Concentration values of fentanyl analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
1	23	Male	67	139	16.6	31.905	1.922
4	21	Female	62	147	3.1	44.595	14.385
12	56	Male	61	83	112.2	85.14	0.759
13	28	Female	65	124	21.2	210.15	9.913
15	24	Male	72.5	191	1.9	7.1865	3.782
16	36	Male	71	236	5.7	17.595	3.087
18	27	Male	70	173	5.8	155.7	26.845
22	47	Male	65	172	4.5	59.715	13.270
23	28	Male	71	191	14.8	142.2	9.608
26	45	Male	73	231	10.4	106.2	10.212
27	28	Female	65.5	188	37.8	182.25	4.821
28	42	Female	62	138	35.8	265.5	7.416
29	22	Male	75.5	284	2.7	62.55	23.167
32	47	Female	62.5	176	14.4	75.51	5.244
33	29	Male	75	277	12.1	132.3	10.934
34	32	Male	71.5	162	44.6	348.3	7.809

35	29	Male	74.5	200	11.2	97.65	8.719
36	36	Male	67	164	38.7	410.4	10.605
37	33	Male	69.5	222	2.4	78.39	32.663
38	32	Male	69.5	330	8.1	205.65	25.389
40	35	Female	63	181	8.1	66.96	8.267
41	21	Male	70	319	7.3	167.85	22.993
42	44	Male	72	217	47.6	530.1	11.137
43	33	Male	69	205	6.1	67.77	11.110
44	49	Male	72.5	212	6.2	93.6	15.097
46	26	Female	64	116	49.4	538.2	10.895
47	17	Female	62	146	72	582.3	8.088
48	34	Male	72	168	39.6	226.35	5.716
49	35	Male	63.5	207	6	121.95	20.325
50	24	Male	72	164	35.7	579.15	16.223
52	32	Male	74	163	18.7	275.4	14.727
53	36	Male	73	284	20.3	276.75	13.633
54	46	Male	67	175	10	143.1	14.310
55	35	Male	71	192	53	326.7	6.164
56	31	Male	73	273	6.9	67.68	9.809
57	32	Male	69	208	18.6	143.55	7.718
58	42	Male	68	205	8.6	41.76	4.856
59	37	Male	72	236	2.6	20.565	7.910
60	22	Male	65	136	35.9	391.5	10.905
61	40	Male	72	208	23.9	74.7	3.126
63	32	Male	68.5	170	2.7	25.38	9.400
64	40	Female	64	159	1	2.2545	2.255

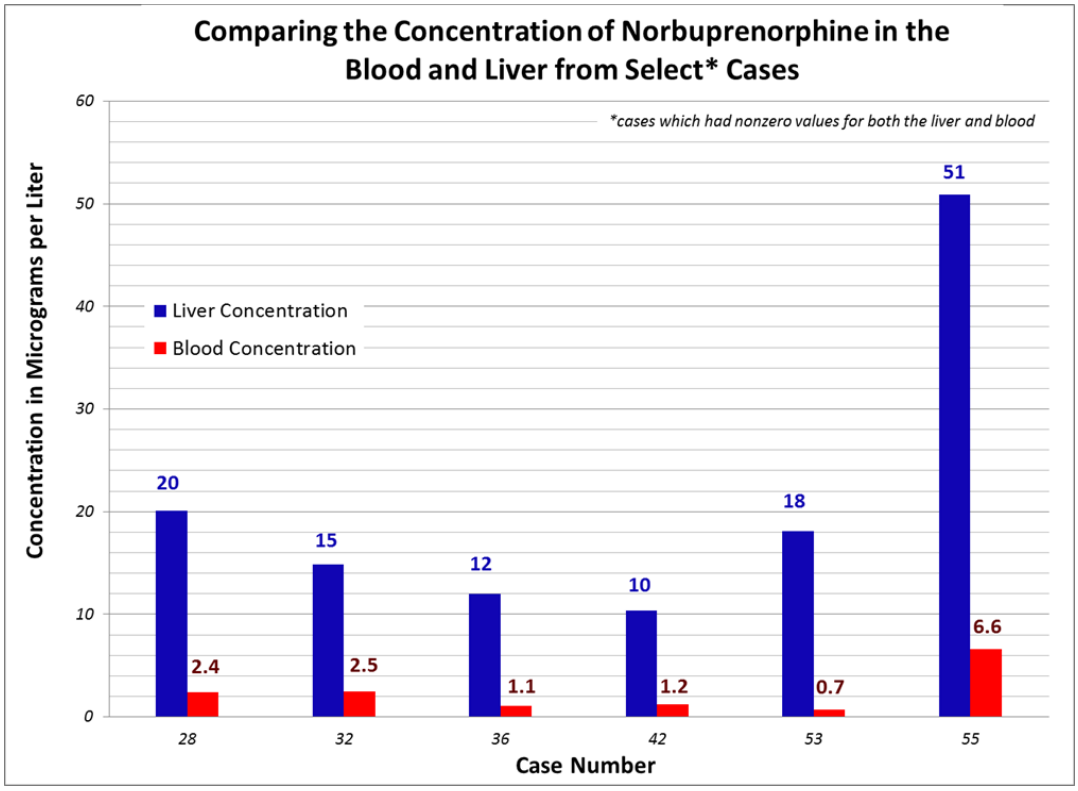

Figure 44. Comparison of the norbuprenorphine concentration in the liver and the blood.

Table 15. Tabulated values comparing the concentration of norbuprenorphine analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
28	42	Female	62	138	2.4	20.115	8.381
32	47	Female	62.5	176	2.5	14.85	5.940
36	36	Male	67	164	1.1	11.97	10.882
42	44	Male	72	217	1.2	10.35	8.625
53	36	Male	73	284	0.69	18.09	26.217
55	35	Male	71	192	6.6	50.895	7.711

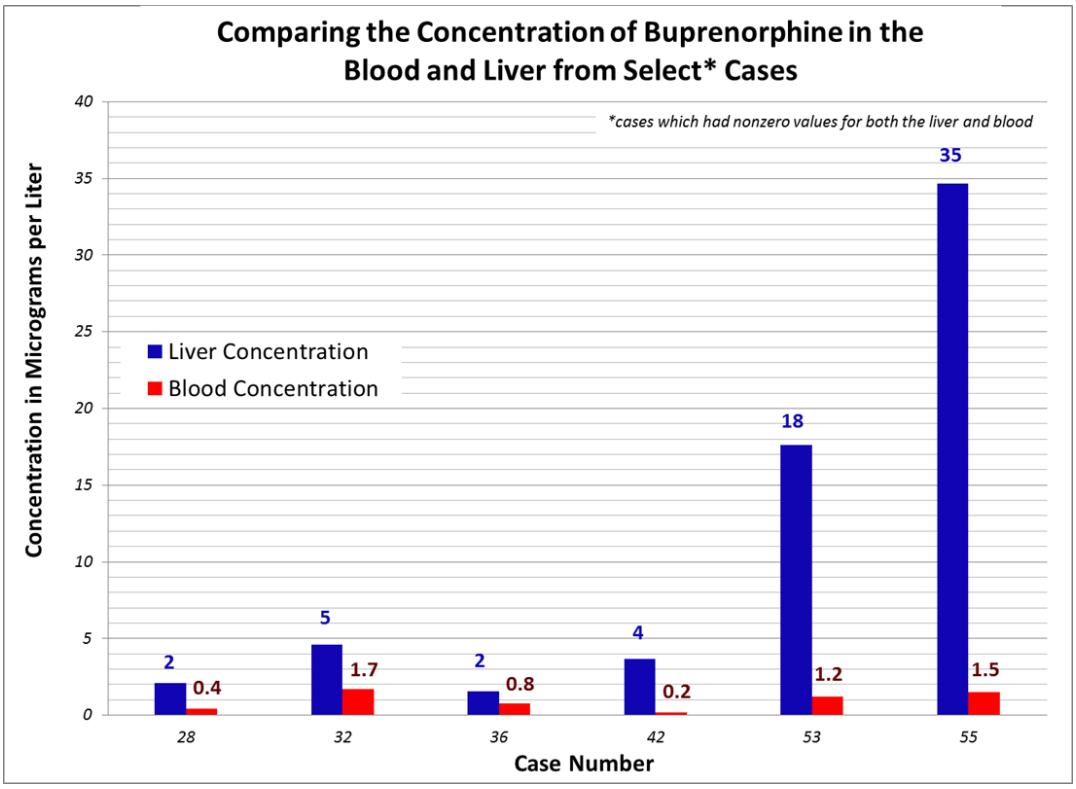

Figure 45. Comparison of the buprenorphine concentration in the liver and the blood.

Table 16. Tabulated values comparing the concentration of buprenorphine analyzed in the blood and liver.

Case #	Age	Gender	Height (Inches)	Weight (lbs.)	Blood Concentration (mcg/L)	Liver Concentration (mcg/L)	Times Higher in Liver than Blood
28	42	Female	62	138	0.44	2.1015	4.776
32	47	Female	62.5	176	1.7	4.581	2.695
36	36	Male	67	164	0.78	1.566	2.008
42	44	Male	72	217	0.2	3.6765	18.383
53	36	Male	73	284	1.2	17.595	14.663
55	35	Male	71	192	1.5	34.65	23.100

The decomposition cases were sometimes higher in the blood than the liver, as shown in Figure 47, and post mortem redistribution (PMR) may have been a factor as to why this occurred. PMR may affect drug concentrations in the stomach and upper gastrointestinal tract depending on the drug concentration present at time of expiration, the volume of content present, temperature of the corpse, and time between death and sampling. According to (Pélissier-Alicot, 2003), PMR has been shown to increase the longer time has lapsed between death and autopsy, and has been shown to slow when the body is stored at -4°C. In the gastrointestinal tract, PMR has been found to redistribute drugs to surrounding tissues such as cardiac chambers, thoracic vessels, and sometimes the left lung, liver and inferior vena cava. PMR from the liver has been shown to redistribute drugs to the hepatic veins, inferior vena cava, right cardiac chambers, pulmonary vessels and peripheral vessels containing deoxygenated blood (Pélissier-Alicot, 2003).

Both Cases #9 and #10 were in a state of decomposition at the time the autopsy was performed. Case #10 had oxycodone measured at 7900 mcg/L in the decomposition fluid while no oxycodone was detected from the liver analysis for that case. If oxycodone was mainly present in the stomach of the individual for Case #10, PMR may have caused oxycodone to diffuse to surrounding organ tissues and vessels before extreme putrefaction occurred. In the event that the Case #10 was exposed to environmental temperatures which may have influenced the effect of PMR on that individual, it is worth noting that Case #10 expired on a warmer climate date of 7/30/14. Case #9 had an expiration date of 1/22/14 although drug concentrations measured higher in the blood

than in the liver for that case. Whether or not climate had an effect on PMR of the decedents in these cases, temperature readings were not recorded for this study.

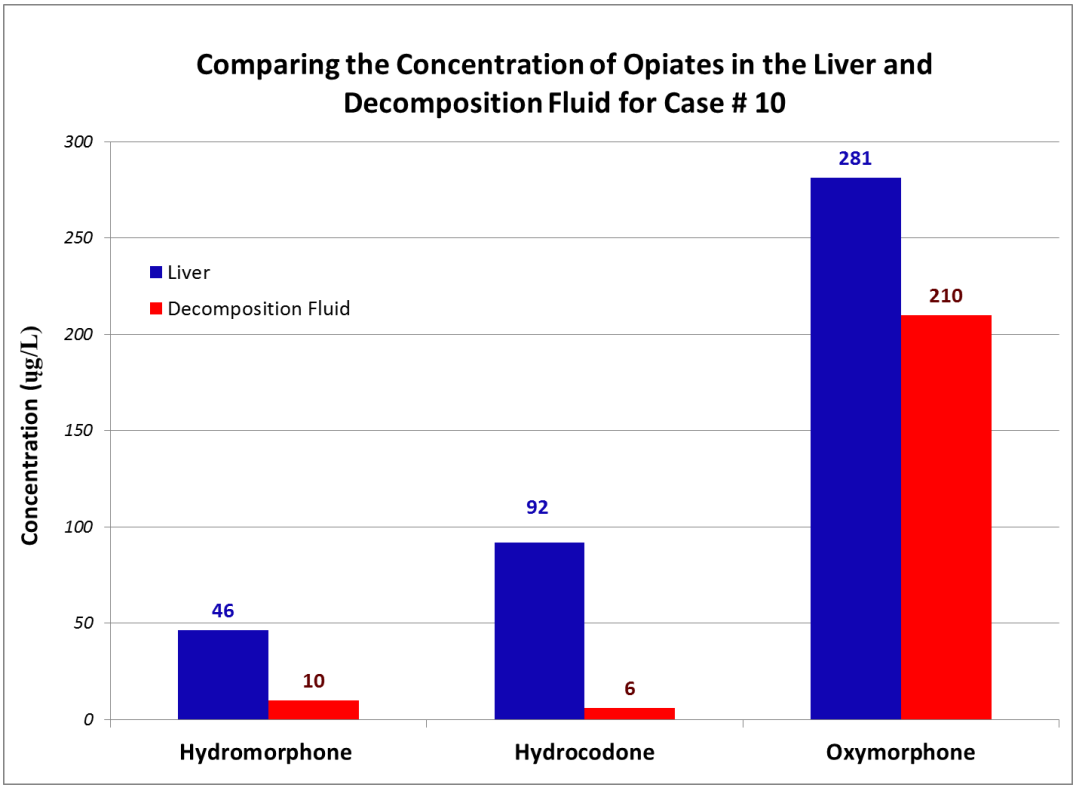

Figure 46. Hydromorphone, hydrocodone and oxymorphone concentrations in the liver compared to the concentrations in the decomposition fluid that was analyzed for Case #10. The biological samples collected were labeled as decomposition fluid instead of blood due to the decomposed state of the body at the time of autopsy.

Table 17. Tabulated values comparing the concentrations of hydromorphone, hydrocodone, oxymorphone, and oxycodone for Case #10.

Component	Case #	Liver Concentration	Decomposition Fluid	Units
Hydromorphone	10	46.485	10	mcg/L
Hydrocodone	10	91.8	6	mcg/L
Oxymorphone	10	281.25	210	mcg/L
Oxycodone	10	0	7900	mcg/L

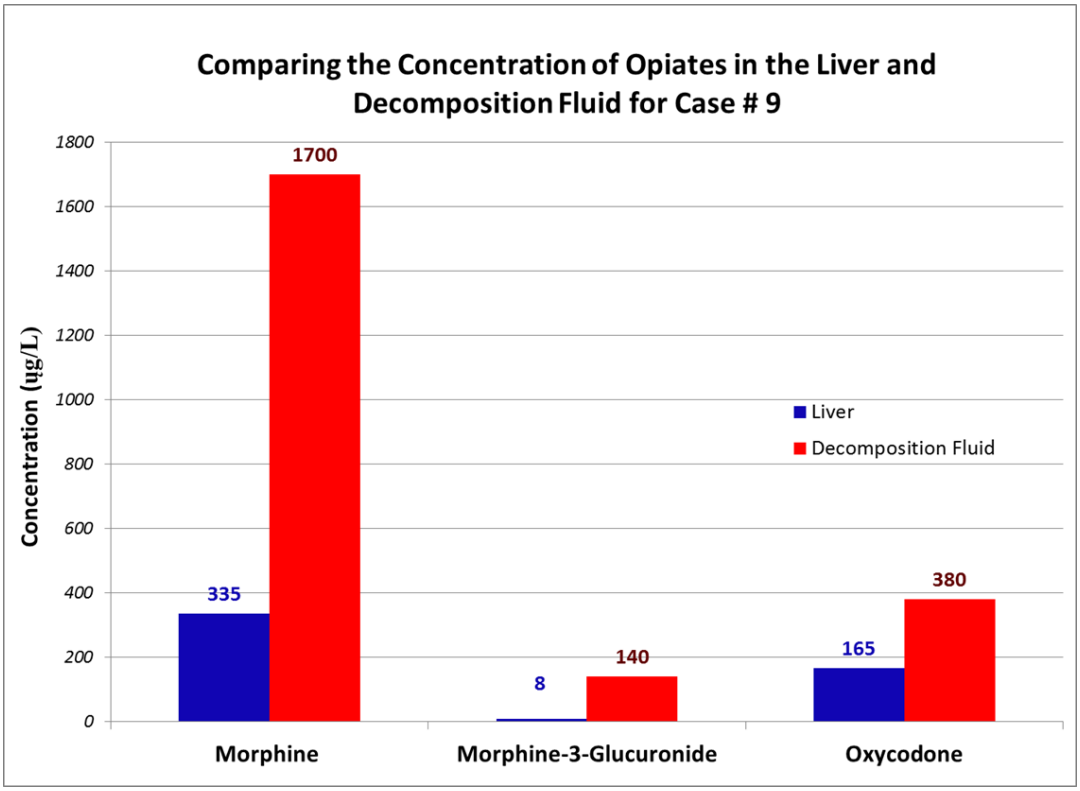

Figure 47. Morphine, morphine-3-glucuronide, and oxycodone concentrations in the liver compared to the concentrations in the decomposition fluid that was analyzed for Case #9. The biological samples collected were labeled as decomposition fluid instead of blood due to the decomposed state of the body at the time of autopsy.

Table 18. Tabulated values comparing the concentrations of morphine, morphine-3-glucuronide, and oxycodone for Case #9.

Component	Case #	Liver Concentration	Decomposition Fluid	Units
Morphine	9	334.8	1700	mcg/L
Morphine-3-Glucuronide	9	8.289	140	mcg/L
Oxycodone	9	164.79	380	mcg/L

Figures 48-59 are pie charts displaying the percentage for each of the 12 opiates present in liver, blood or both liver and blood for the 64 cases analyzed. Tables 19-30 are the number of cases where those select opiates were present after blood and liver analysis. Morphine was present in the liver and blood analysis for almost half (45.31%) of the total cases analyzed. Four cases had morphine concentrations measured in the liver only, while only one case (Case #48) had a morphine concentration in the blood and not in the liver. Hydromorphone concentrations were present in the liver only for almost a quarter (23.44%) of the total cases, while only five percent of the total cases at concentrations in the liver and the blood. Codeine measurements were recorded in the liver only for over half the cases (57.81%) and 15.63% of the cases had concentrations in the liver and the blood. Only 9.38% of the total cases had hydrocodone concentration measurements in the liver only, while 14.06% had concentrations in the liver and the blood. Oxymorphone's results were close to opposite that of hydrocodone; with 14.06% having concentration measurements in the liver only, and 7.81% of the cases having Oxymorphone concentration in the blood and the liver. Oxycodone measurements were present in the liver only for two cases, in the blood only for one case, and in the liver and the blood for seven cases. Almost a third of the cases (37.50%) had morphine-3-glucuronide measurements in the liver only; also the same percentage of cases (37.50%) had concentrations in the liver and the blood. Morphine-6-glucuronide had measurements in the liver only for 39.06% of the cases analyzed, and concentration measurements in the liver and blood for 21.88% of the cases. Over a quarter (29.69%) of the cases analyzed had concentrations of norfentanyl in the liver only, and over half (57.81%) of the cases had concentrations in the liver and the blood. Fentanyl was measured in the liver and the

blood for over half the cases (65.63%), and 21.88% of the cases had concentrations in the liver only. norbuprenorphine had concentration measurements in the liver only for 85.94% of the total cases analyzed, while 9.38% of the cases measured norbuprenorphine in the liver and in the blood. Lastly, buprenorphine had concentration measurements in over half the total cases (65.63%) in the liver only, while this component was measured in both the liver and the blood for 9.38% of the cases analyzed.

Figure 60 shows the total number of measurements recorded for the 12 drugs analyzed in the liver and blood for 64 cases in the form of a pie chart. Table 31 contains the number of measurements, while Figure 60 displays the percentage of these measurements. Less than one percent (0.26%) of the opiates was measured in the blood only for the sixty four total cases analyzed, while 25.00% had opiate measurements present in the liver and the blood, and 32.81% had opiate measurements in the liver only.

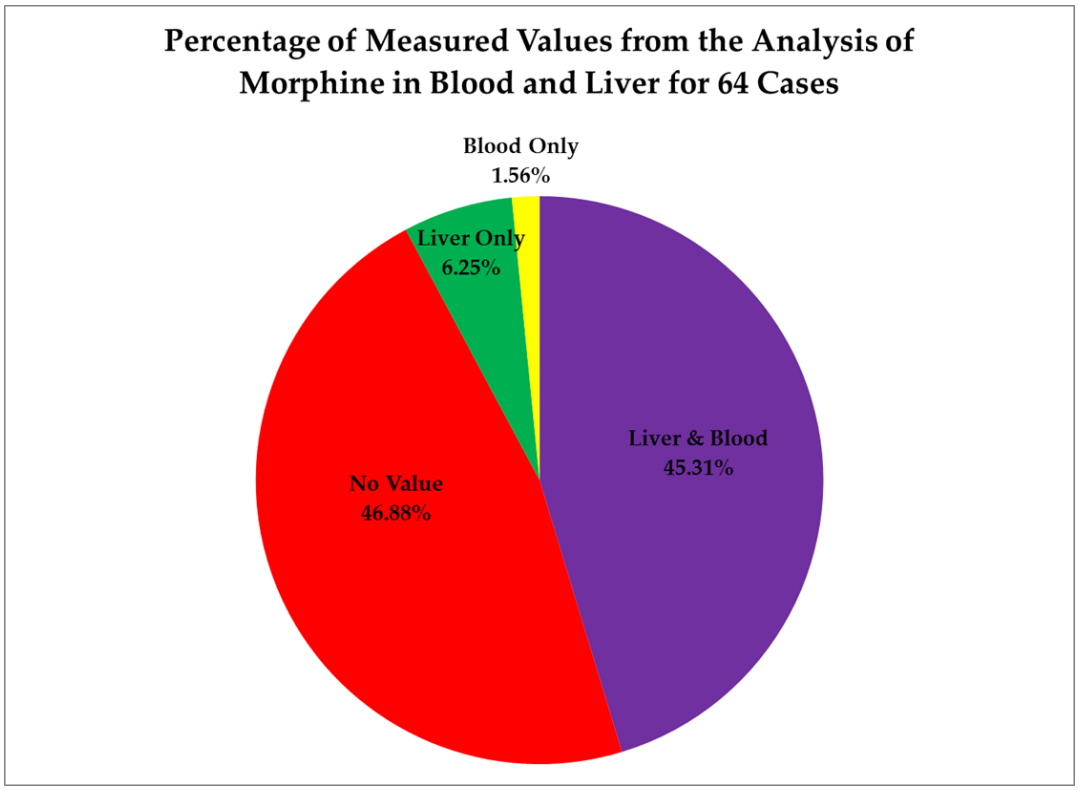

Figure 48. Comparison of morphine concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases that had no detectable morphine level.

Table 19. The number of cases that had a value for morphine in the liver and blood, in the liver only, in the blood only and the number of cases that had no detectable morphine level.

Morphine				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
29	30	4	1	64

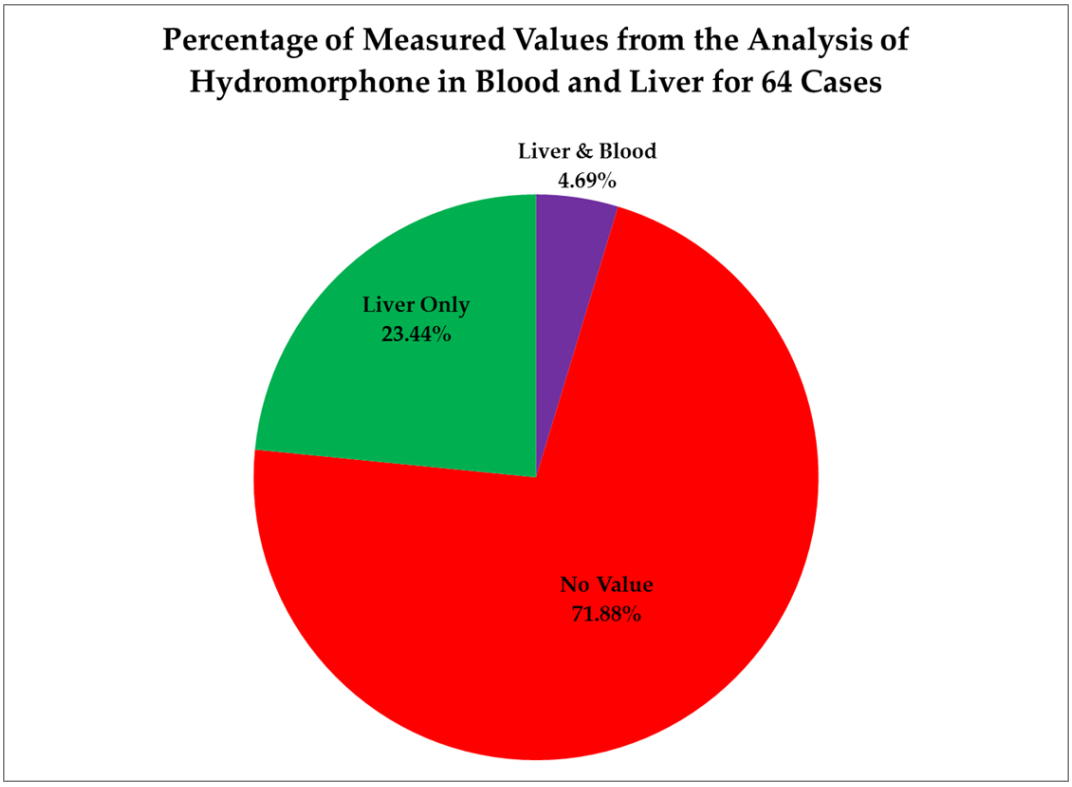

Figure 49. Comparison of hydromorphone concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases that had no detectable hydromorphone level.

Table 20. The number of cases that had a value for hydromorphone in the liver and blood, in the liver only, in the blood only and the number of cases that had no detectable hydromorphone level.

Hydromorphone				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
3	46	15	0	64

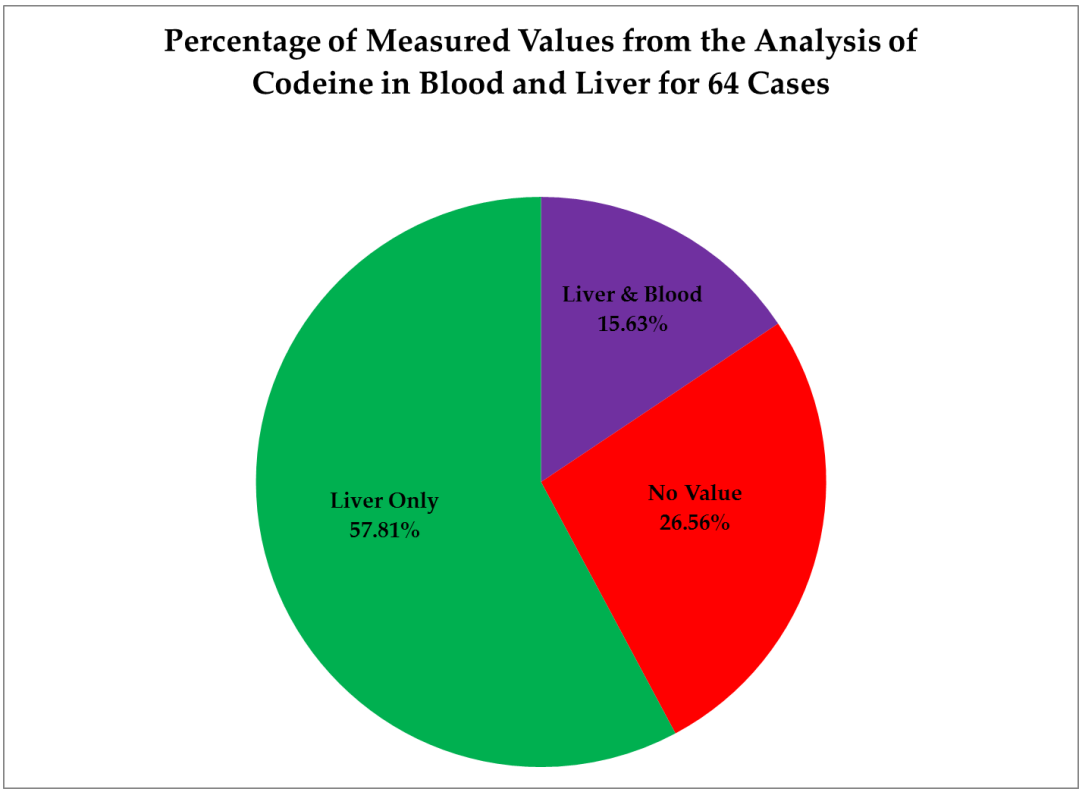

Figure 50. Comparison of codeine concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases that had no detectable codeine concentration.

Table 21. The number of cases that had a value for codeine in the liver and blood, in the liver only, in the blood only and the number of cases that had no detectable codeine level.

Codeine				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
10	17	37	0	64

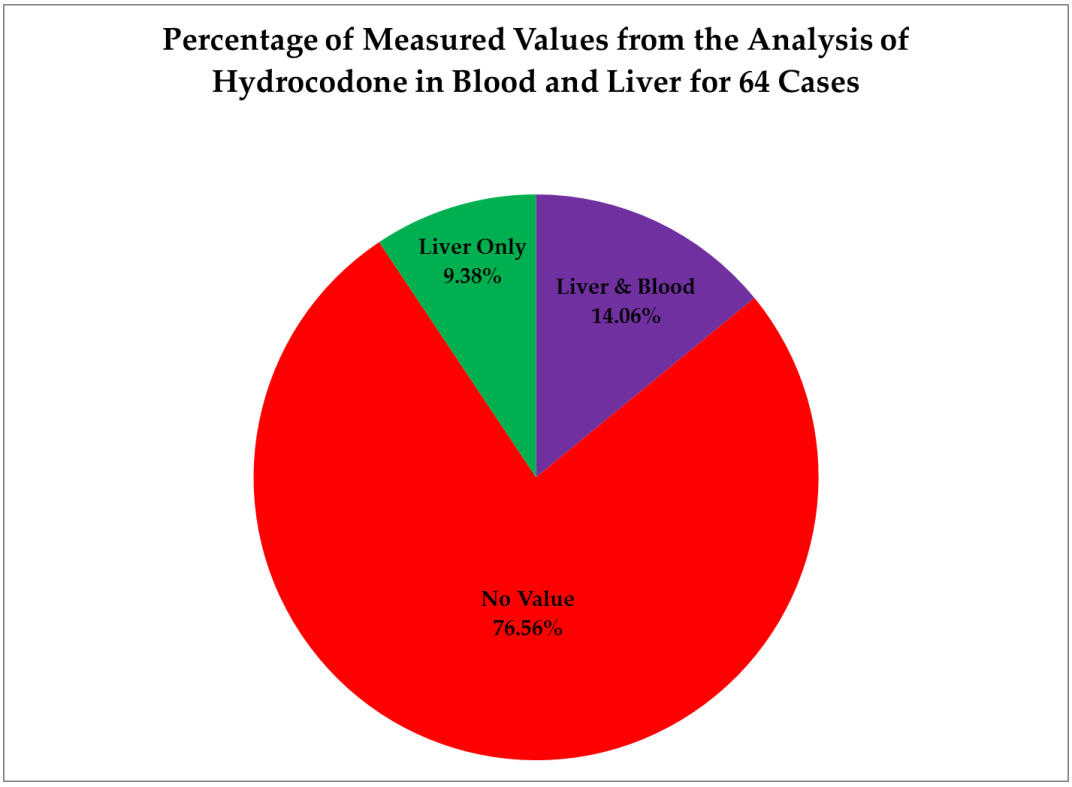

Figure 51. Comparison of hydrocodone concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases that had no detectable hydrocodone level.

Table 22. The number of cases that had a value for hydrocodone in the liver and blood, in the liver only, in the blood only and the number of cases that had no detectable hydrocodone level.

Hydrocodone				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
9	49	6	0	64

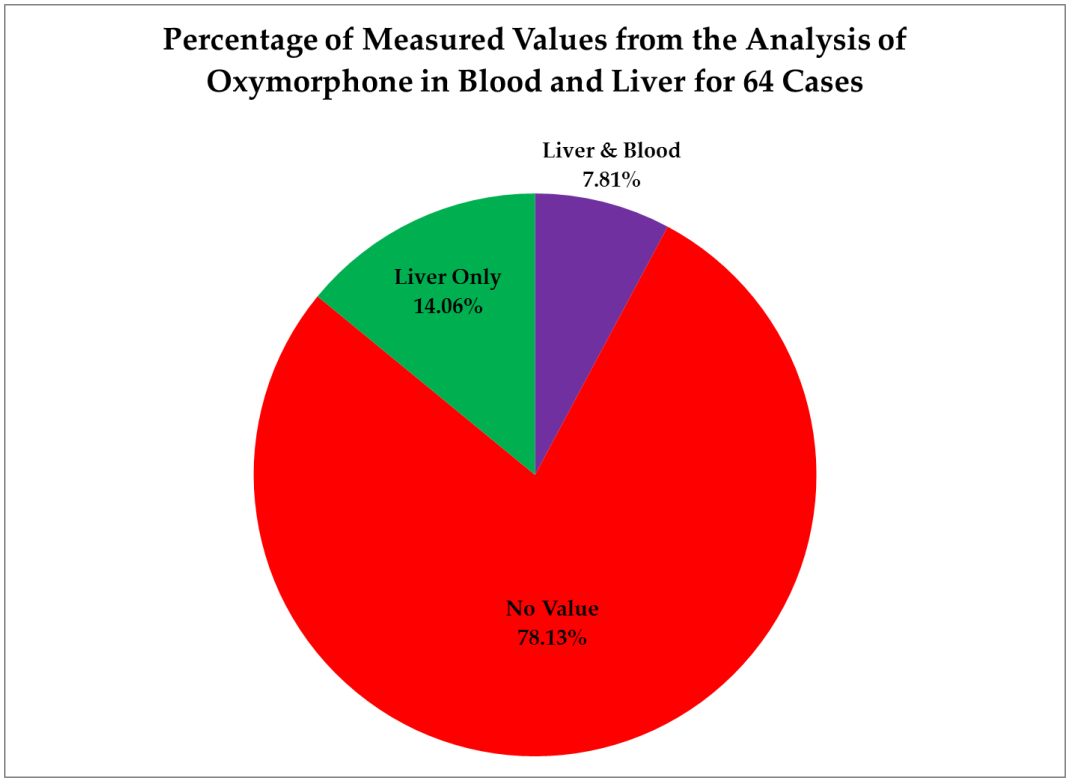

Figure 52. Comparison of oxymorphone concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases in which no oxymorphone was detected.

Table 23. The number of cases that had a value for oxymorphone in the liver and blood, in the liver only, in the blood only, and the number of cases in which oxymorphone was not detected.

Oxymorphone				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
5	50	9	0	64

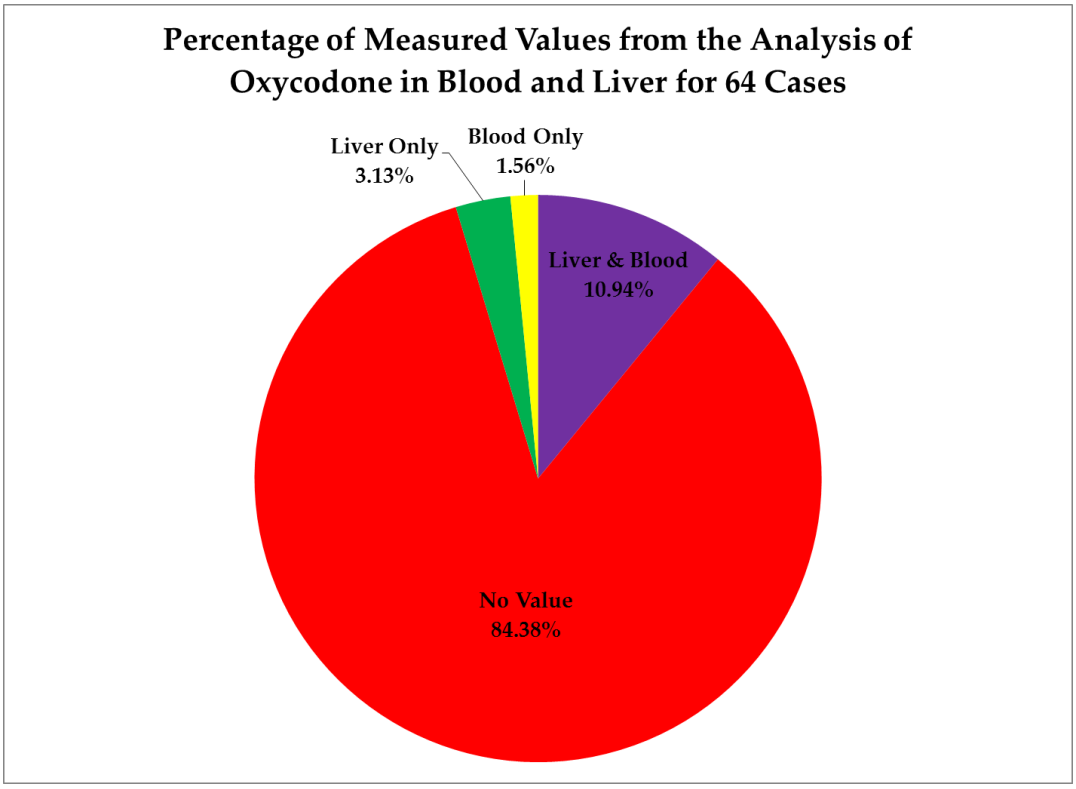

Figure 53. Comparison of oxycodone concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases in which no oxycodone was detected.

Table 24. The number of cases that had a value for oxycodone in the liver and blood, in the liver only, in the blood only, and the number of cases in which no oxycodone was detected.

Oxycodone				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
7	54	2	1	64

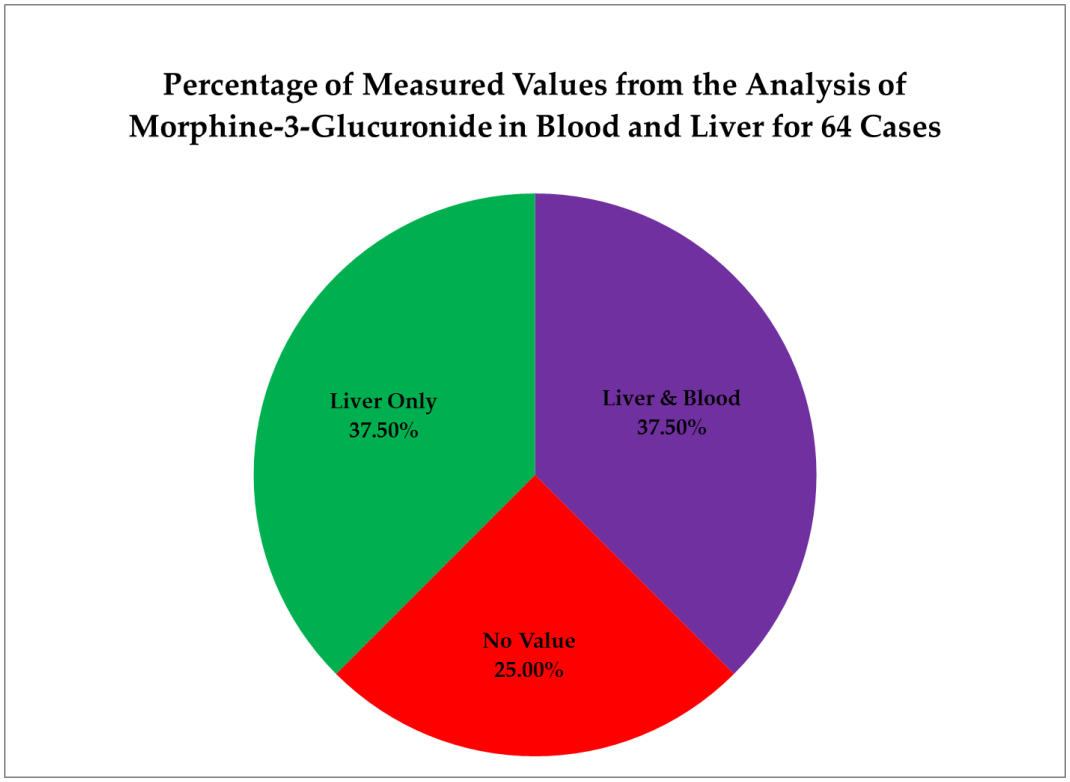

Figure 54. Comparison of morphine-3-glucuronide concentration measurements present in both the liver and the blood, in the liver only, in the blood only and the number of cases in which no morphine-3-glucuronide was detected.

Table 25. The number of cases that had a value for morphine-3-glucuronide in the liver and blood, in the liver only, in the blood only, and the number of cases in which no morphine-3-glucuronide was detected.

Morphine-3-Glucuronide				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
24	16	24	0	64

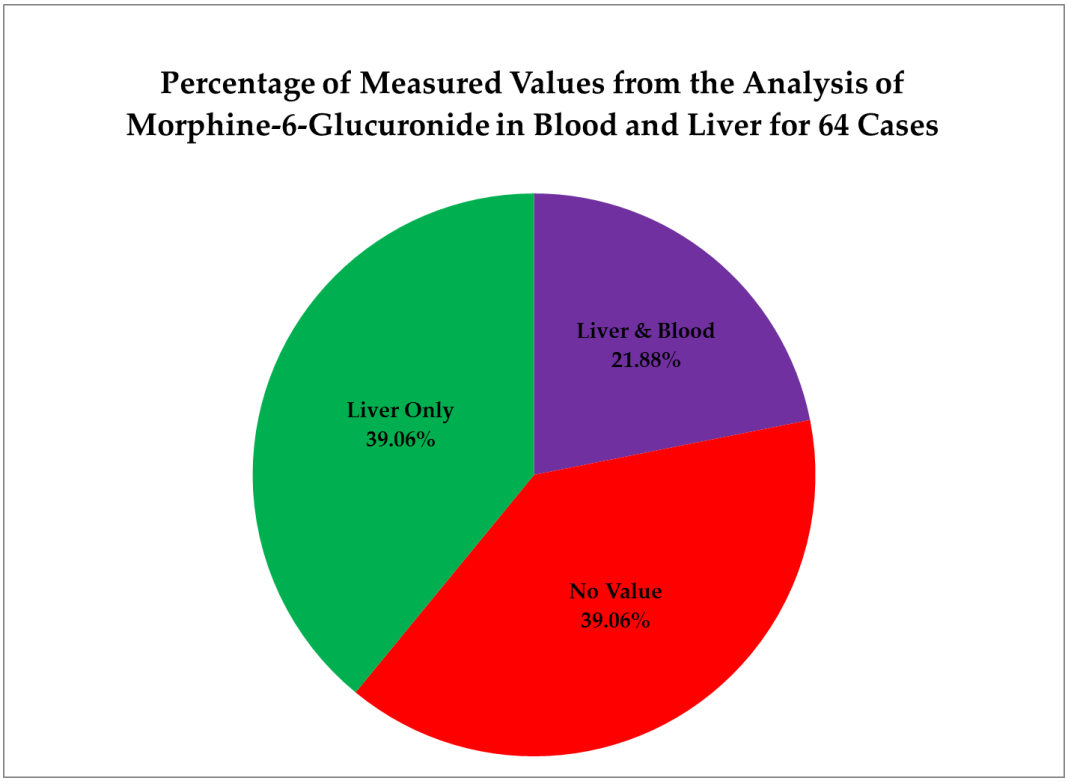

Figure 55. Comparison of morphine-6-glucuronide concentration measurements present in both the liver and the blood, in the liver only, in the blood only and those cases in which no morphine-6-glucuronide was detected.

Table 26. The number of cases that had a value for morphine-6-glucuronide in the liver and blood, in the liver only, in the blood only, and those cases in which no morphine-6-glucuronide was detected.

Morphine-6-Glucuronide				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
14	25	25	0	64

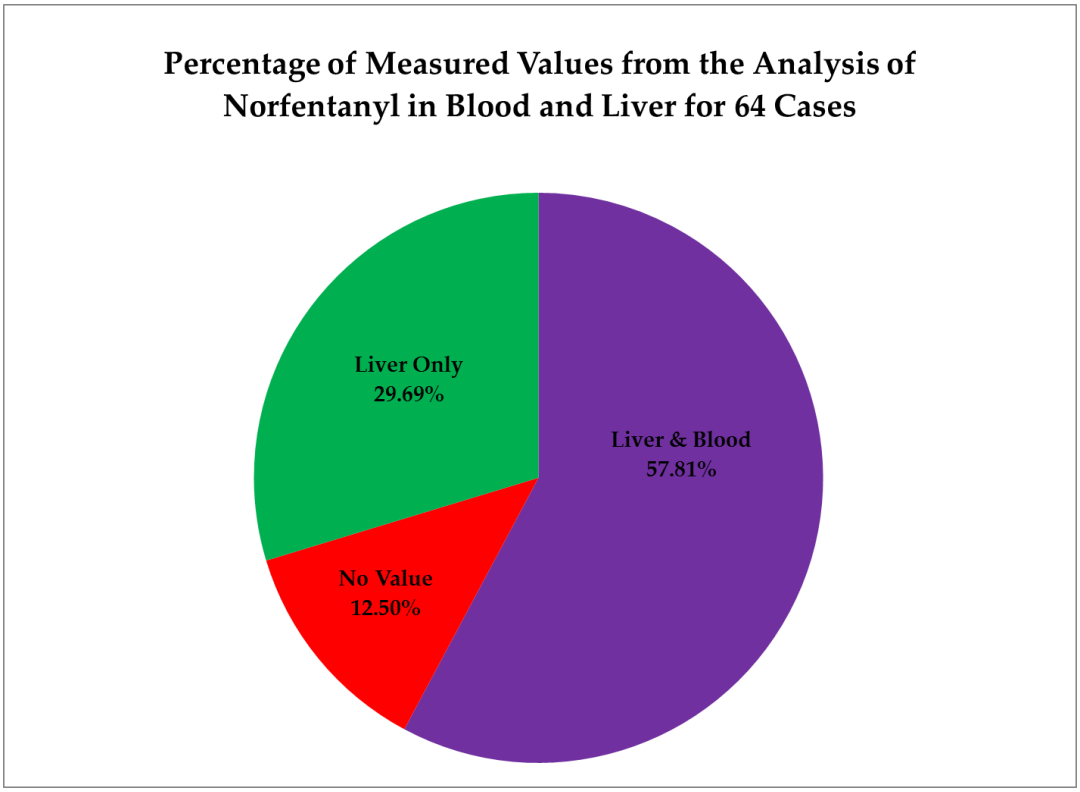

Figure 56. Comparison of norfentanyl concentration measurements present in both the liver and the blood, in the liver only, in the blood only and those cases in which no norfentanyl was detected.

Table 27. The number of cases that had a value for norfentanyl in the liver and blood, in the liver only, in the blood only, and those cases in which no norfentanyl was detected.

Norfentanyl				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
37	8	19	0	64

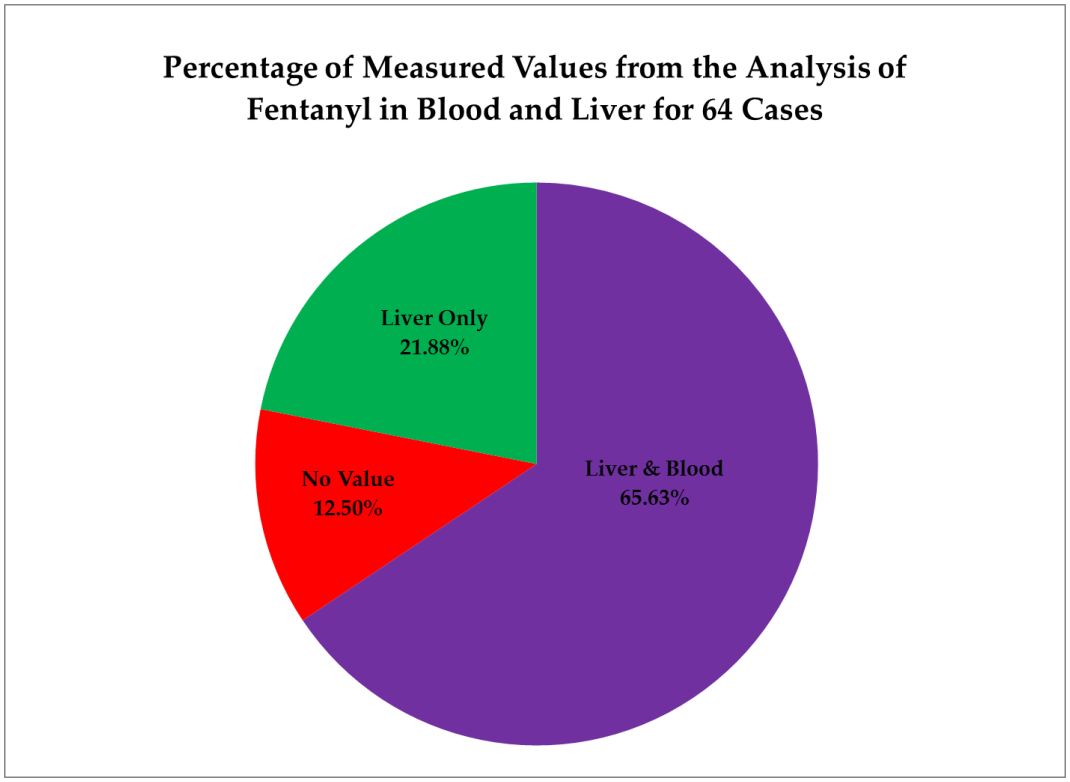

Figure 57. Comparison of fentanyl concentration measurements present in both the liver and the blood, in the liver only, in the blood only and those cases in which no fentanyl was detected.

Table 28. The number of cases that had a value for fentanyl in the liver and blood, in the liver only, in the blood only, and those cases in which no fentanyl was detected.

Fentanyl				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
42	8	14	0	64

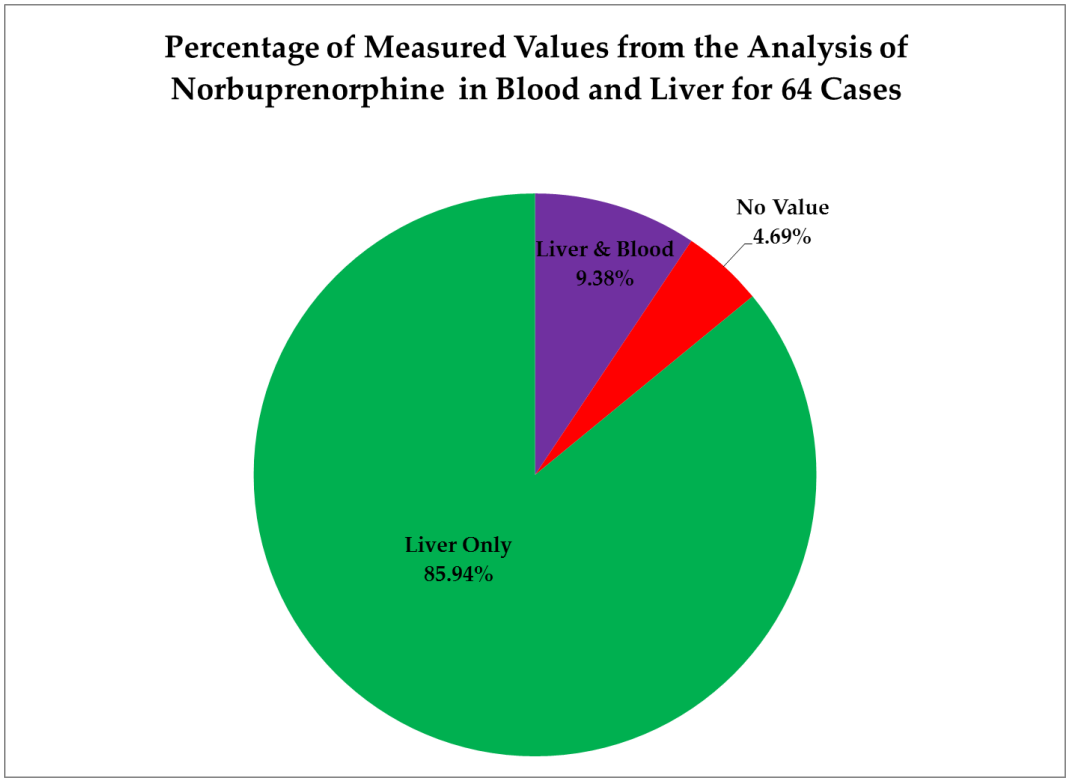

Figure 58. Comparison of norbuprenorphine concentration measurements present in both the liver and the blood, in the liver only, in the blood only and those cases in which no norbuprenorphine was detected.

Table 29. The number of cases that had a value for norbuprenorphine in the liver and blood, in the liver only, in the blood only, and those cases in which no norbuprenorphine was detected.

Norbuprenorphine				
Liver & Blood	No Value	Liver Only	Blood Only	Sum of Cases
6	3	55	0	64

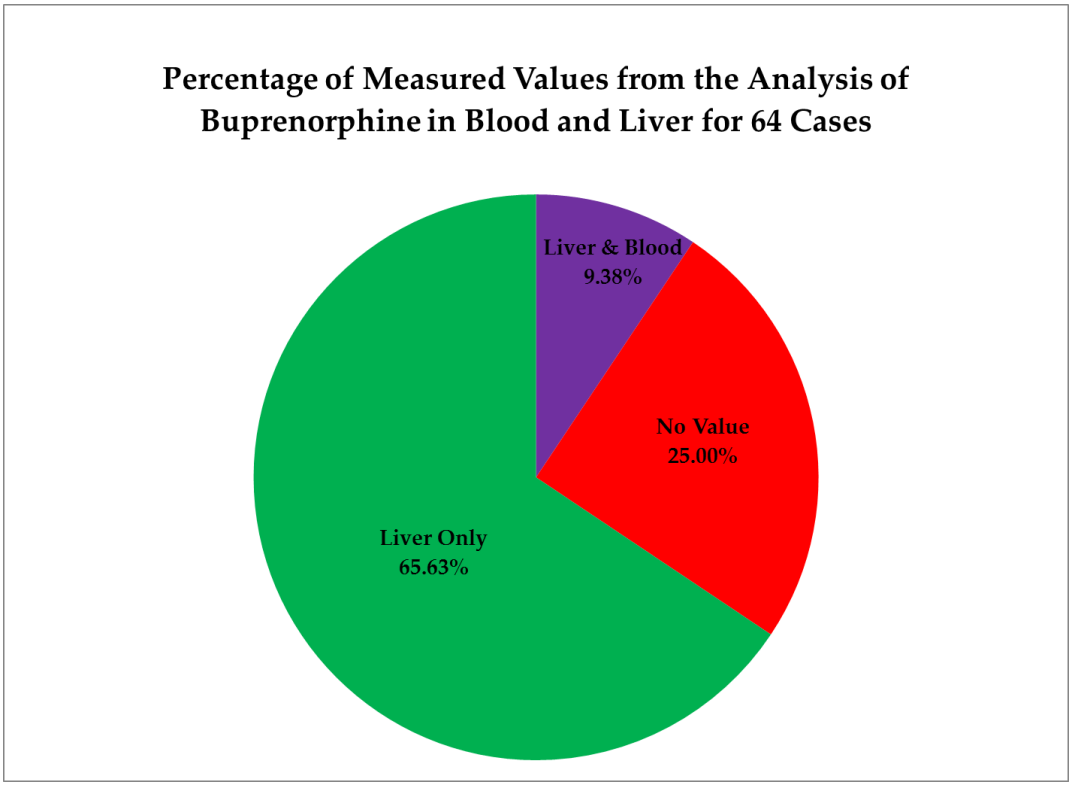

Figure 59. Comparison of buprenorphine concentration measurements present in both the liver and the blood, in the liver only, in the blood only and those cases in which no buprenorphine was detected.

Table 30. The number of cases that had a value for buprenorphine in the liver and blood, in the liver only, in the blood only, and those cases in which no buprenorphine was detected.

Buprenorphine				
Liver &	No Value	Liver Only	Blood Only	Sum of Cases
6	16	42	0	64

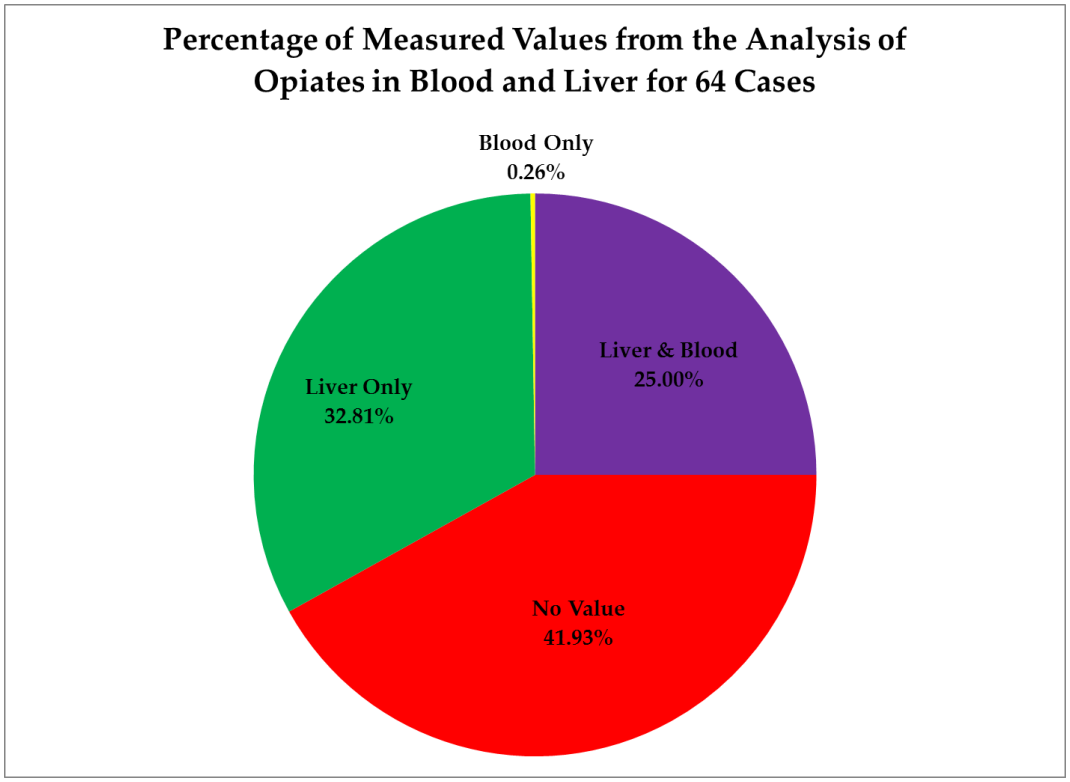

Figure 60. Comparison of total opiate concentration measurements of 12 different opiates (morphine, hydromorphone, codeine, hydrocodone, oxymorphone, oxycodone, morphine-3-glucuronide, morphine-6-glucuronide, norfentanyl, fentanyl, norbuprenorphine, and buprenorphine) present in both the liver and the blood, in the liver only, in the blood only and those cases in which none of the 12 opiates were detected.

Table 31. The number of cases that had a value for each of the opiates in the liver and blood, in the liver only, in the blood only, and those cases in which none of the 12 opiates were detected.

Total Opiates present in 64 Cases				
Liver & Blood	No Value	Liver Only	Blood Only	# of Measurements (12 drugs x 64 total cases)
192	322	252	2	768

Five categories were used to classify the data from the 64 cases chosen for analysis. The categories consisted of classification using the case history and toxicology analysis from each sample.

The five categories:

1.) Case had known history of drug abuse
2.) The case sample had only opiates (including fentanyl) present in toxicology analysis
3.) Opiates and alcohol were present in the case sample
4.) Opiate and other drugs (including marijuana) were present in toxicology analysis
5.) Unclassified category; the case did not fit any of the categories 1-4.

Figures 61-63 and Tables 32-34 display information based on these categories. Figure 61 and its corresponding Table 32 display the percentage of each category compared to the total 64 cases. Over seventy percent (70.31%) out of the total number of cases had a history of drug abuse, 73.44% of the cases had opiates present with other drugs, 31.25% of the cases had opiates and alcohol present, and 18.75% of the cases had opiates present only at the time of analysis. Figure 62 and its corresponding Table 33 show the percentages of how frequent opiates and other drugs appear in the toxicology analysis of 64 case samples. Almost half (46.88%) the number of cases sampled had opiates and other drugs present at the time of analysis. About a quarter (26.56%) of the cases had opiates, alcohol and other drugs present, 18.75% had opiates only, 4.69% had opiates and alcohol present, and 3.13% of the cases were unclassified. Figure 63 and Table 34 compared the cases that had a known drug abuse history with those cases that did not

have a drug abuse history and to those cases with an unknown history. Over half (70.31%) of the cases sampled had a known history of drug abuse.

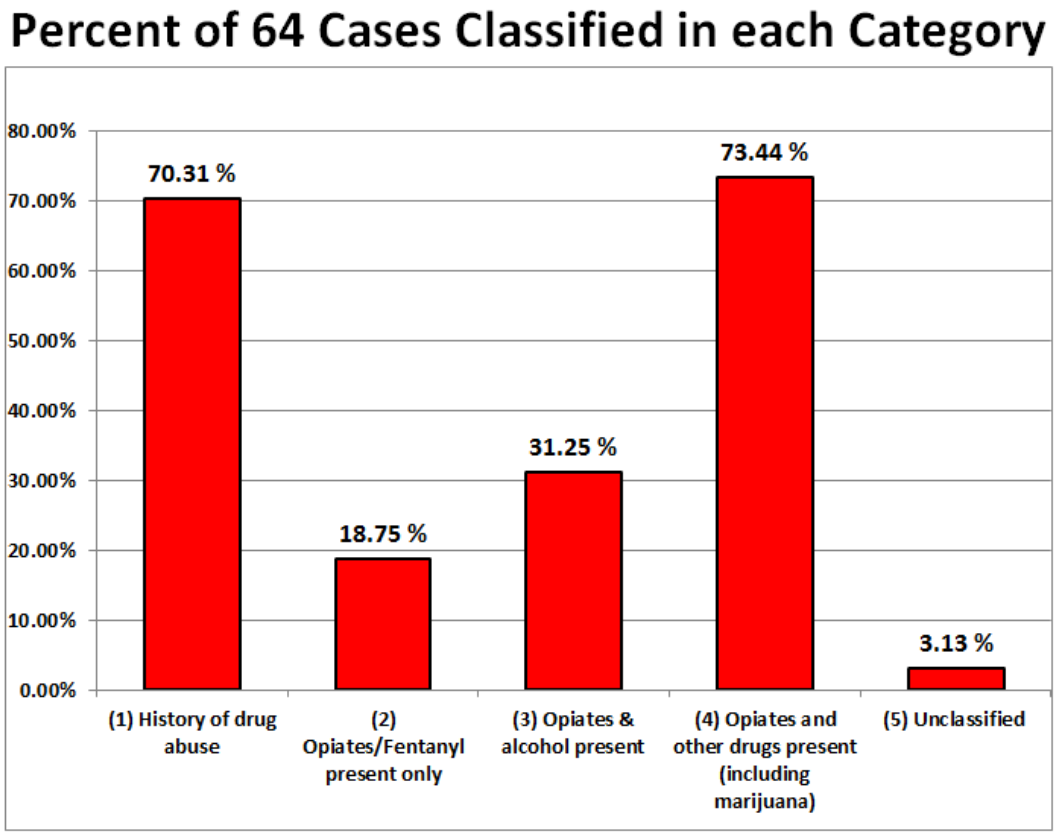

Figure 61. Percent of categories (1) – (5) out of the 64 total cases sampled.

Table 32. Tabulated values from Figure 61.

Categories	**Number of**	**% (of 64 cases)**
(1) History of drug abuse	45	70.31
(2) Opiates/Fentanyl present only	12	18.75
(3) Opiates & alcohol present	20	31.25
(4) Opiates and other drugs present (including	47	73.44
(5) Unclassified	2	3.13

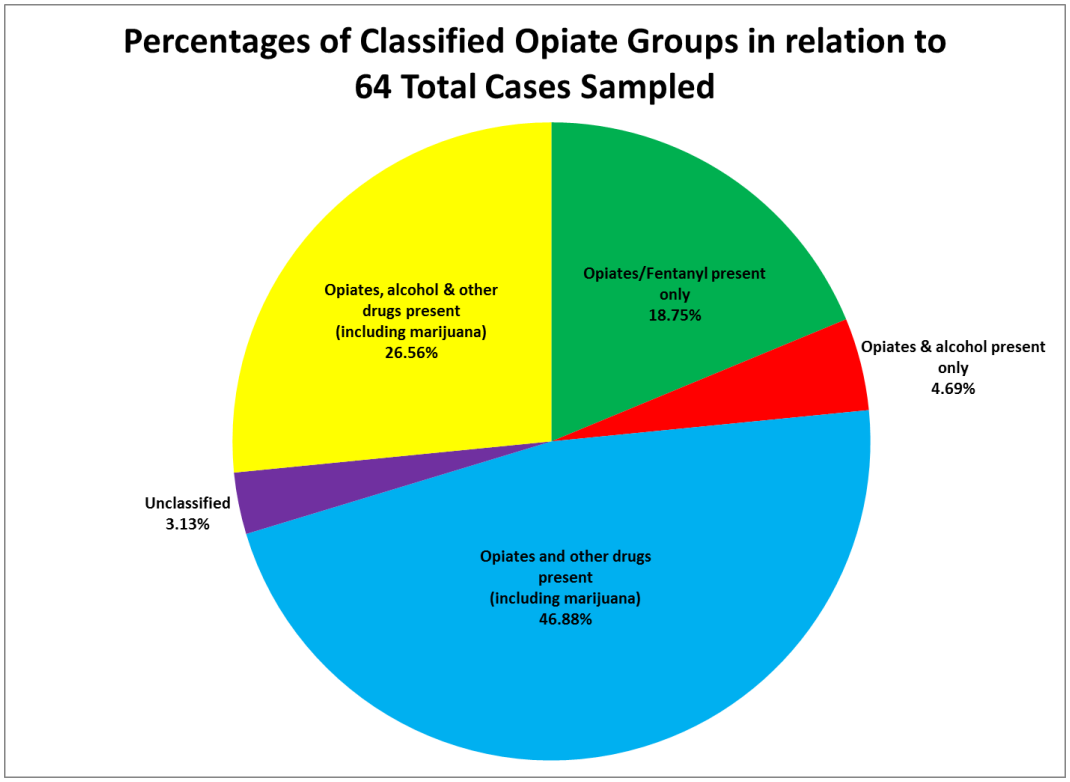

Figure 62. Drugs present in each category in relation to the 64 total cases.

Table 33. Tabulated values from Figure 62.

Category ID	Categories	Number of Cases	% (of 64 cases)
(2)	Opiates/Fentanyl present only	12	18.75%
(3)	Opiates & alcohol present only	3	4.69%
(4)	Opiates and other drugs present (including marijuana)	30	46.88%
(5)	Unclassified	2	3.13%
(3,4)	Opiates, alcohol & other drugs present (including marijuana)	17	26.56%

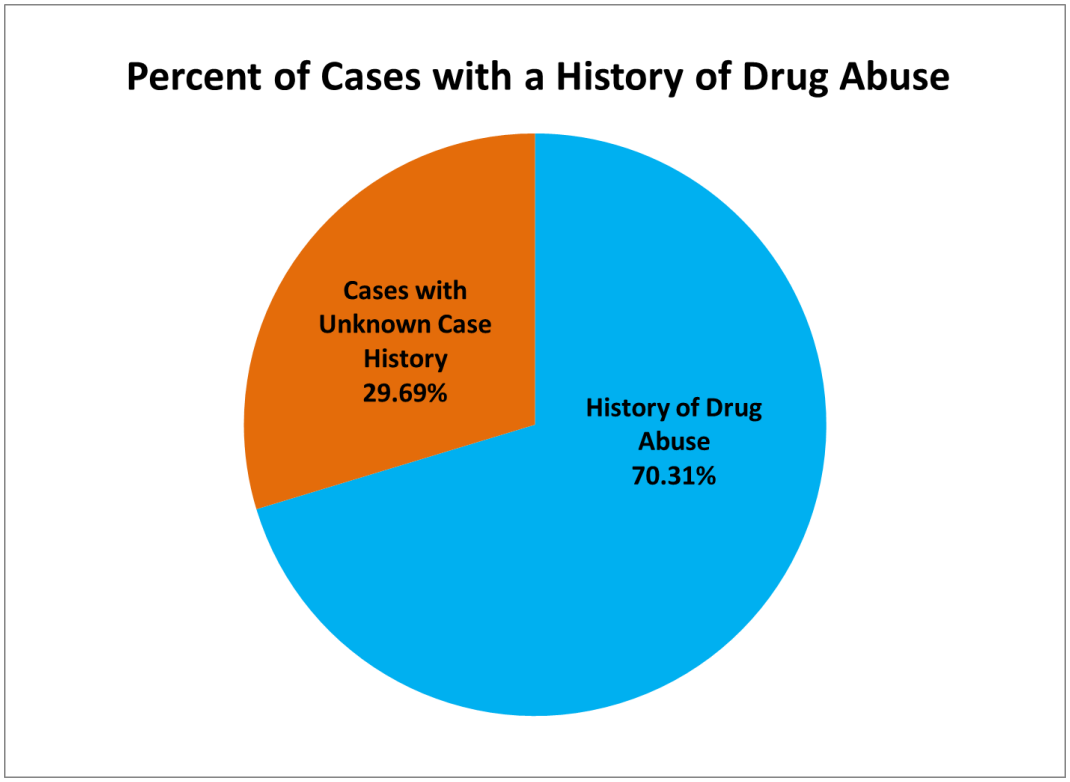

Figure 63. Percent of 64 total cases that had a known history of drug abuse.

Table 34. Tabulated values from Figure 63.

Category ID	Categories	Number of Cases	% (of 64 cases)
(1)	History of Drug Abuse	45	70.31%
	Cases with unknown drug-use history	19	29.69%

For the cases where opiates and alcohol are present, Figure 64 compares the Ethanol levels measured in the blood, urine, vitreous humor, bile, and decomposition fluid. Table 35 corresponds to Figure 64 and contains the measured ethanol values for the cases which alcohol was involved. Case #25 had the highest ethanol concentration which was measured in the blood (0.307 % w/v) and vitreous humor (0.371 % w/v), while Case #45 had the highest ethanol concentration measured in the urine (0.357 % w/v).

Methanol was present in the blood and vitreous humor of Case #31 at 0.336 and 0.352 % w/v respectively. Methanol most likely was present from embalming fluid, as this was the only case that had been embalmed prior to sampling. Embalming fluid is a mixture of chemicals used to preserve cadavers and may contain 5-30% methanol (Beck, 1966).

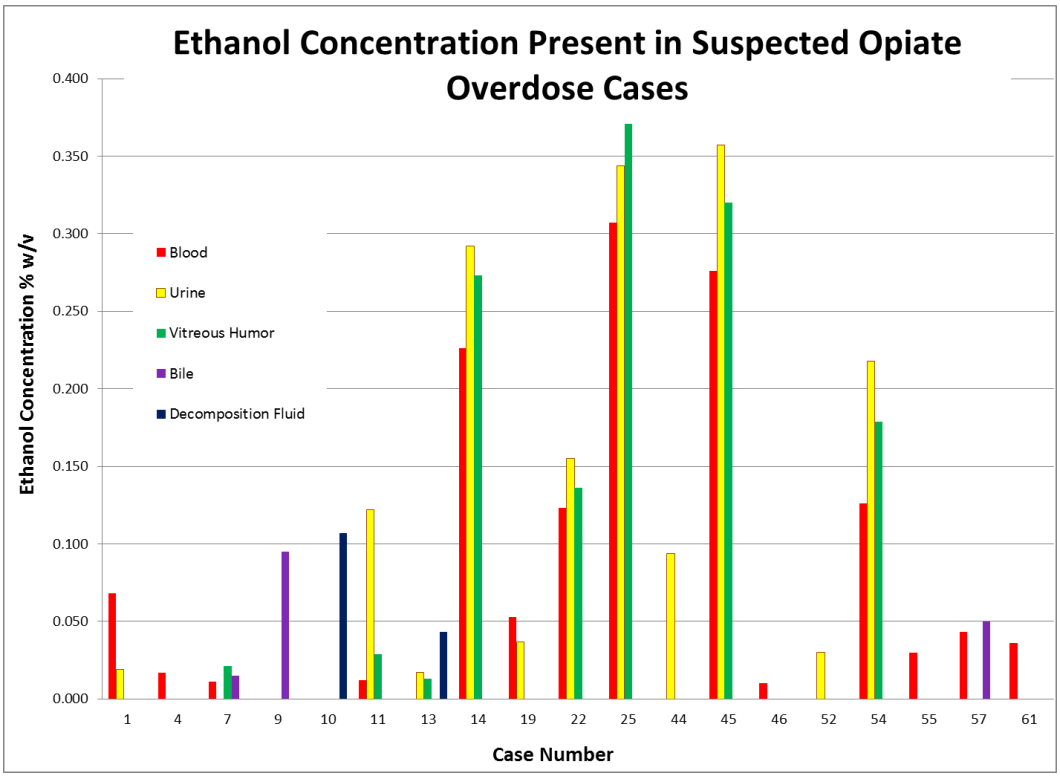

Figure 64. Ethanol concentration in blood, urine, vitreous humor, bile, and decomposition fluid where present during analysis of suspected overdose cases.

Table 35. Tabulated ethanol measurement data from Figure 64.

Case #	Blood (% w/v)	Urine (% w/v)	Vitreous Humor (% w/v)	Bile (% w/v)	Decomposition Fluid (% w/v)
1	0.068	0.019	-	-	-
4	0.017	-	-	-	-
7	0.011	-	0.021	0.015	-
9	-	-	-	0.095	-
10	-	-	-	-	0.107
11	0.012	0.122	0.029	-	-
13	-	0.017	0.013	-	0.043
14	0.226	0.292	0.273	-	-
19	0.053	0.037	-	-	-
22	0.123	0.155	0.136	-	-
25	0.307	0.344	0.371	-	-
44	-	0.094	-	-	-
45	0.276	0.357	0.320	-	-
46	0.010	-	-	-	-
52	-	0.030	-	-	-
54	0.126	0.218	0.179	-	-
55	0.030	-	-	-	-
57	0.043	-	-	0.050	-
61	0.036	-	-	-	-

Looking at age, gender, and ethnicity as factors to the concentration difference of opiates in the liver and blood, most of the suspected overdose deaths were white males with an age range of 30-34, shown in Figures 66-69 and Tables 37-40.

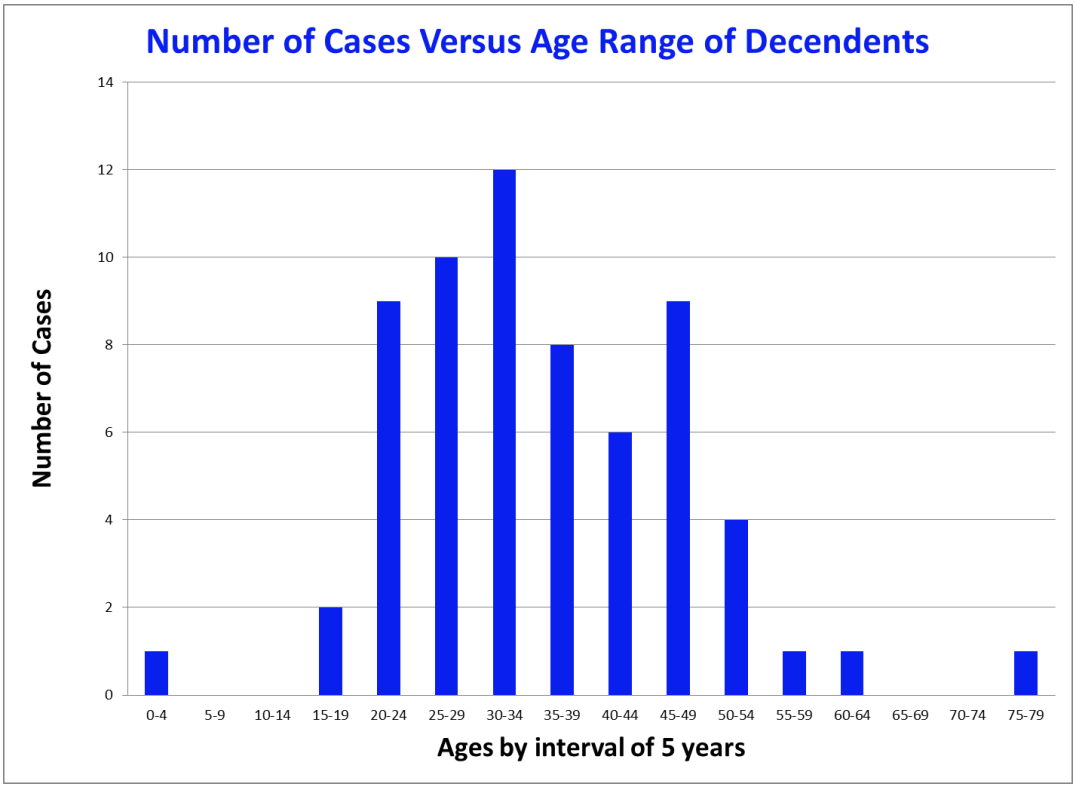

Figure 65. Number of cases versus the age of the individuals by intervals of 5 years.

Table 36. Tabulated values from Figure 65.

Age Range	Number of Cases
0-4	1
5-9	0
10-14	0
15-19	2
20-24	9
25-29	10
30-34	12
35-39	8
40-44	6
45-49	9
50-54	4
55-59	1
60-64	1
65-69	0
70-74	0
75-79	1

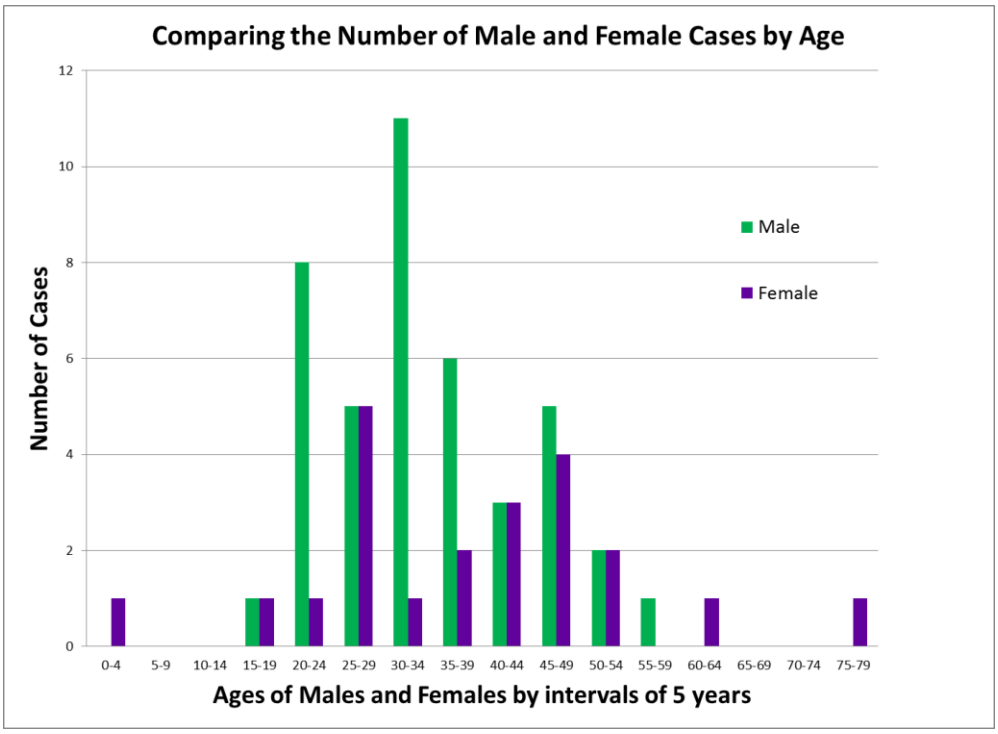

Figure 66. Comparing the number of male and female individuals by age (intervals of 5 years).

Table 37. Tabulated values from Figure 66.

	Number of Cases	
Age Range	**Male**	**Female**
0-4	0	1
5-9	0	0
10-14	0	0
15-19	1	1
20-24	8	1
25-29	5	5
30-34	11	1
35-39	6	2
40-44	3	3
45-49	5	4
50-54	2	2
55-59	1	0
60-64	0	1
65-69	0	0
70-74	0	0
75-79	0	1

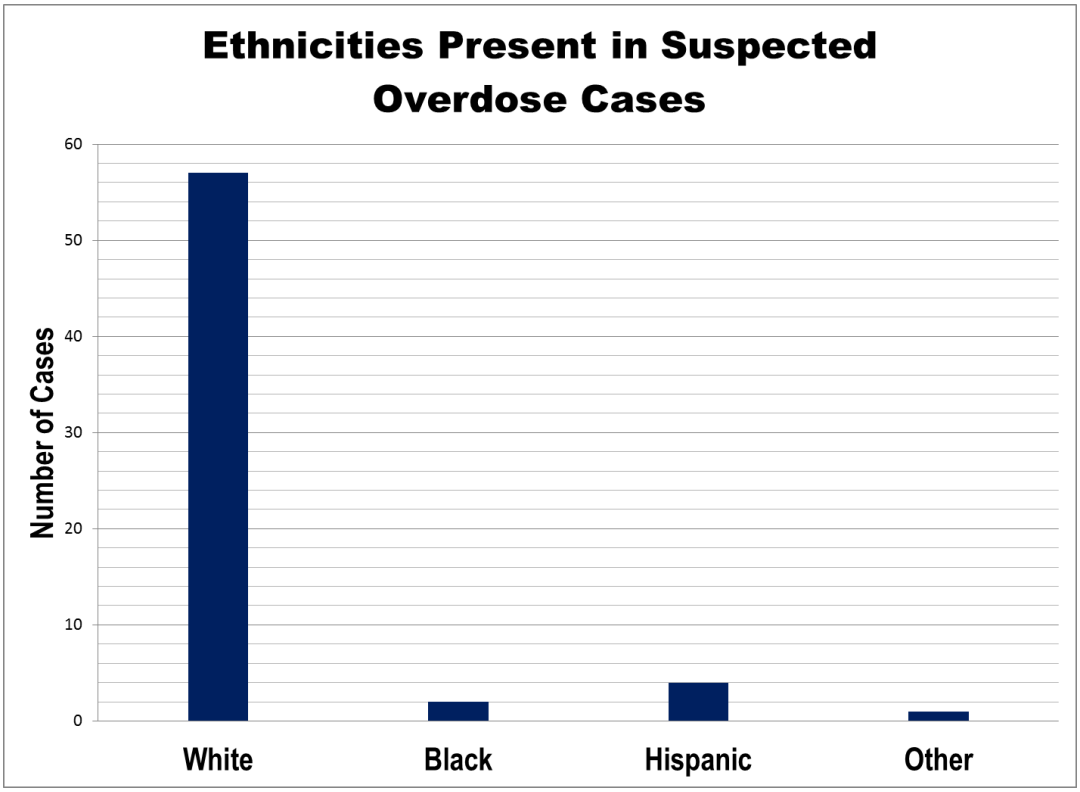

Figure 67. Number of cases according to the ethnicity of the individuals.

Table 38. Tabulated values from Figure 67 including number of cases for each ethnic group observed for the age of the individuals by intervals of five years, and the total number of cases for each ethnic group.

	Number of Cases			
Age Range	**White**	**Black**	**Hispanic**	**Other**
0-4	1	0	0	0
5-9	0	0	0	0
10-14	0	0	0	0
15-19	1	0	1	0
20-24	8	1	0	0
25-29	10	0	0	0
30-34	10	1	0	1
35-39	7	0	1	0
40-44	5	0	1	0
45-49	8	0	1	0
50-54	4	0	0	0
55-59	1	0	0	0
60-64	1	0	0	0
65-69	0	0	0	0
70-74	0	0	0	0
75-79	1	0	0	0
Total Number	**57**	**2**	**4**	**1**

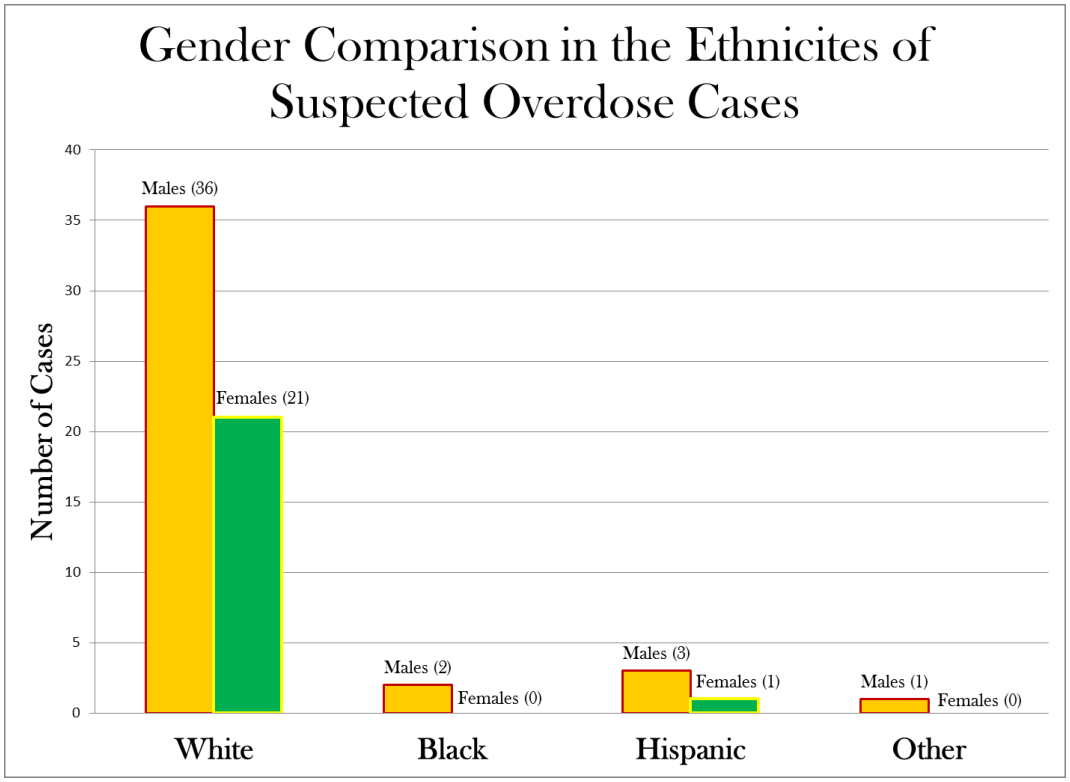

Figure 68. Comparing the number of males and females for each ethnicity observed in the 64 cases.

Table 39. Tabulated values from Figure 68.

	Number of Cases			
Gender	**White**	**Black**	**Hispanic**	**Other**
Male	36	2	3	1
Female	21	0	1	0

The analytical method of this project has been comparative to the standard practices incorporated the toxicology validation process. Screening has been conducted in order to find out which is the best liver dilution for the widest range of drug detection. Sheep blood, human liver and beef liver matrices have been successful during analysis and without interference, also frozen liver samples were stable specimen and successfully

quantitated. Blood and liver calibrators and controls were incorporated in every extraction which would fulfill requirements of qualitative confirmation and identification analysis, as well as quantitative analysis.

Bias analysis and interference studies could be further researched for this project. The 6-MAM disappeared from the chromatograph spectrum results after the liver samples were fortified with known concentrations, therefore was removed from the list of opiates analyzed in this thesis project. There was only one human liver that was screened negative for opiates that was used as a control before the project switched the liver controls to beef liver. However; even after the beef liver was used for control samples in the analysis, the beef liver samples were made up from only one cow's liver. More human and cow control livers need to be analyzed to conclude that those liver matrices were not the reason for interrupting the 6-MAM during the extraction analysis, also to rule out the possibility that there may have been coexisting enzyme interference from those control livers. What happened to the 6-MAM during the extraction procedure? Did all or some of it convert to morphine? Did only the deuterated forms of morphine and 6-MAM convert, or get deuterium cleaved off? Will newly developed opiates act similarly to 6-MAM during liver analysis? Many questions remain unanswered after completion of this project, and newly synthesized opiates continue to emerge from the market. Future research could deduce what happened to the 6-MAM after the extraction process was performed as well as continue to aid in the understanding of opiate drug detection in liver analysis.

Chapter V

Conclusion

Cases chosen for screening were suspected overdose cases. Some blood (usually collected from the femoral artery), and liver samples were collected for further analysis. Immunoassays were performed to screen for opiates; the cases that screened positive for opiates and fentanyl were then subjected to further analysis using solid phase extraction, and then analyzed with the LC/MS/MS and each opioid was quantitated.

From the data collected, it is very difficult to find correlations and reasons for why liver concentration was so variable and typically at higher levels than in blood for each of the cases analyzed. It's difficult to determine if non-opiate drugs or alcohol present in some cases had interference with liver metabolism and the opiate quantitation results from the liver analysis.

Opioid metabolism may be influenced by genetic factors including age, gender, and ethnicity; or medical issues such as impaired liver function. Past studies have shown morphine, codeine, fentanyl and oxymorphone to metabolize at a slower rate in older individuals. Related opioid metabolism studies found oxycodone concentrations to be 25% higher in women than in men after appropriate calculated dosages were administered to each individual based on body weight and known pharmacokinetics of these opioids (Smith, 2009).

Chinese individuals were found to produce less morphine from codeine and displayed reduced sensitivity to morphine compared to Caucasian individuals in a study

which was conducted to evaluate ethnic origin and pharmacodynamics effects of codeine and quinidine (Caraco Y., 1999).

Different characteristics may have played a role in the correlation of drug concentrations in the blood compared to the liver including physique, the degree of putrefaction of the individual, sampling site and the amount of time between death and sampling contributing to post mortem redistribution (PMR) (Moriya, 2005; Pélissier-Alicot, 2003). PMR may not be a significant factor in heroin fatalities based on a study of 40 heroin fatality cases that measured PMR in heart and femoral blood concentration ratios for morphine and morphine 3- and 6-glucuronides to be close to one; however, another study of 42 heroin fatalities was measured to have heart and femoral blood concentration ratios of 1.8 for morphine. In the cases of morphine, hydromorphone, oxycodone, oxymorphone, codeine, hydrocodone, and fentanyl, past studies have shown that PMR may have contributed to the difference in drug concentration in the different sampling sites (Baselt, 2011).

Opiates are broken down and processed by the liver. This process is conducted through phase one metabolism in the Cytochrome P450 pathway, by phase two metabolism of glucuronidation, or both phase one and phase two. Phase one metabolism involves the CYP3A4 and CYP2D6 enzymes. Both CYP3A4 and CYP2D6 are part of the Cytochrome P450 family of oxidizing enzymes, found in the intestines and liver, with the purpose of oxidizing xenobiotics in the body.

Fentanyl and oxycodone are primarily metabolized by the CYP3A4 enzyme as well as more than 50% of all drugs therefore these drugs may have a higher risk of drug-

drug interactions, and may affect how they are metabolized in the liver. The drugs which undergo phase two glucuronidation, such as morphine, oxymorphone, and hydromorphone, tend to have little involvement with the Cytochrome P450 pathway, and may have decreased risk of drug-drug interactions. However, the risk of pharmacodynamics drug-drug interactions still remains possible with all opioids when combining with other drugs, such as benzodiazepines, antihistamines, antagonistic drugs, or alcohol, and may result in toxicity (Smith, 2009).

The liver's function is to process blood as it circulates through the body, detoxifying harmful substances, such as drugs, and metabolizing nutrients. Enzymes in the liver help drive these chemical reactions and keep the liver functioning properly. Although opioids have rarely been found to cause liver injury in therapeutic doses, in cases of overdose, according to the U.S. National Library of Medicine, there have been cases of acute liver injury and toxicity. It may also be possible that liver injury is not directly related to the presence of opiates, but possibly the physical effects of opiate drugs, such as respiratory failure, cardiovascular collapse, shock and the absence of oxygen (U.S. National Library of Medicine, 2016). When death occurs, the liver cells containing these enzymes become damaged from lack of oxygen and nutrients, allowing drugs to diffuse outside the cells more easily (Moriya, 2005).

We have established it is possible to quantitate opiates using liver samples for analysis. In most cases opiates showed up more frequently in liver than in blood analysis for these sixty four cases. It is possible that active enzyme metabolism in the liver has accounted for lower levels in the liver than in the blood in some cases. Heroin (diamorphine) is one of the opiates that is not directly metabolized by the liver. Heroin is

deacetylated in whole blood to form 6-MAM shortly after intravenous administration (Baselt, 2011). From the blood, 6-MAM is metabolized into morphine. Morphine is then further broken down in the liver to form glucuronides and metabolites before becoming excreted. Most of the opiates analyzed in this project metabolize in the liver; codeine metabolizes into morphine, morphine into morphine-3-glucuronide and morphine-6-glucuronide, hydromorphone into hyromorphine-3-glucuronide, hydrocodone into hydromorphone, oxymorphone into oxymorphone-3-glucuronide, fentanyl into norfentanyl, buprenorphine into norbuprenorphine, as well as the metabolization of these drugs into all of their minor metabolites (Baselt, 2011).

In most situations, the concentration of opiates in the liver was higher than levels found in the blood. In some cases, although no opiates were detected in the blood, levels were detected in the liver. Therefore, with the exception Case #48, where morphine appeared in the blood analysis and not the liver analysis, I propose that the liver may be a better source for detection of opiates than the blood.

Glossary

Bioavailability (F_b): The fraction of an administered dose of unchanged drug that reaches systemic circulation, the part of cardiovascular system carrying oxygenated blood away from the heart to the body and returns deoxygenated blood back to the heart. When a drug is administered intravenously the bioavailability is 100%. Drug administration by any other route is less than or equal to 100%.

Decomposition Fluid: Accumulated fluid in the body cavity from the putrefaction of organs. Decomposition fluid is typically collected when there is no longer blood or anything of blood consistency available for collection due to decomposition and breakdown of body organs due to bacterial growth.

δ-opioid receptor: Also identified as DOR (delta opioid receptor); δ-opioid receptors are 7-transmembrane G protein-coupled receptors. They are "G" proteins because they bind the guanine nucleotides guanosine diphosphate (GDP) and guanosine triphosphate (GTP). When activated, these receptors produce analgesic effects, although there is still much unknown about the details of how exactly the δ receptor responds this way.

First pass effect: Also known as "first pass metabolism," an occurrence where the concentration of a drug is greatly reduced before it reaches the systemic circulation. Following oral administration of a drug, the drug gets absorbed by the digestive system and then is carried to the liver through the portal vein where it is subject to hepatic metabolism. This first pass metabolism in the liver greatly reduces the bioavailability of drugs due to incomplete absorption before the active drug travels to the rest of the circulatory system.

Half-life (t ½): The time taken for half the drug dose to be eliminated or metabolized. Half-life may be influenced by many factors including metabolic and genetic factors.

Hepatic metabolism: Chemical reactions that occur in an organism's liver essential for survival including protein synthesis, production of biochemical necessary for digestion, the metabolism of xenobiotics including the detoxifications of drugs and toxins.

HPC: High positive control for blood and liver listed in Appendix III, Tables 20 and 22.

κ-opioid receptor: Also identified as KOR (kappa opioid receptor); A protein and a type of opioid receptor which binds opioid-like compounds in the brain and consequently the physical effects of these compounds are produced.

Limit of Detection (LOD): The lowest quantity of a component that can be reliably distinguished from a "blank," or the absence of that component, within a stated confidence limit. The confidence limit for LOD is generally 1%.

Limit of Quantitation: The lowest concentration of an analyte that can be reliably measured by an analytical procedure where some predefined goals for bias and imprecision can be met.

Lipophilicity: Refers to the ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents such as hexane or toluene.

LPC: Low positive control for blood and liver listed in Appendix III, Tables 20 and 22.

μ-opioid receptor: Also identified as MOR (mu opioid receptor); belongs to a class of opioid receptors which have a high affinity for enkephalins and beta-endorphin, but a low affinity for dynorphins. Morphine is a μ-opioid receptor agonist.

Opiate: a term generally used to label drugs that were derived from opium or chemical derivatives of opium.

Opioid: A substance that acts on opioid receptors to produce morphine-like effects. They reduce the intensity of pain signals sensed in the brain and affect the areas of the brain responsible for controlling pain receptors and the stimuli responding to pain. This class of drugs is chemically similar to alkaloids found in opium poppies.

P-glycoprotein: A permeability glycoprotein also identified as *P-gp*. It is a protein in the cell membrane that pumps foreign substances or toxins out of cells. In liver cells it can pump toxins/drugs into bile ducts, in the proximal tubule of the kidney it can pump substances into urine ducts, and in the blood-brain barrier it can pump toxins back into capillaries.

Postmortem redistribution (PMR): Changes that may occur in the drug concentration of an individual after death. Antemortem drug concentrations present in an individual may not reflect those same concentration values in that individual postmortem.

Respiratory depression: Also known as *hypoventilation*; a decrease in the ability to exhale and inhale. Occurs when ventilation is unable to provide a successful gas exchange and may cause increased carbon dioxide concentration and respiratory acidosis.

Retention Time: The measured amount of time it takes a solute to travel through a chromatography column starting from the point at which the sample is injected until the time it is detected. Retention time includes the sum of the time spent in the stationary phase and the mobile phase.

6-MAM: 6-monoacetylmorphine

Transition (from Chromatogram): Measurement from analyzing the parent mass of the compound from the MS/MS fragmentation, and then monitoring the specific single fragment ion. The results are shown in relative intensity vs. retention time. For the LC/MS/MS quantitation conducted in this experiment, two transitions for each component were always measured.

Upper Limit of Linearity: The highest concentration at which a further increase in concentration or mass flow-rate will still give an observable increase in detector signal.

Volume of Distribution (V_d): The presumed volume needed to contain the total amount of an administered drug at the measured concentration that drug is observed in the blood plasma. V_d is a theoretical measurement calculated as a ratio of the drug dose present in an individual and that individual's blood plasma concentration.

A = amount of drug in the body

C = plasma concentration

$$Vd = \frac{A}{C}$$

References

Analgesic Expert Group. (2007). *Therapeutic Guidelines: Analgesic* (4 ed.). West Melbourne, Australia: Therapeutic Guidelines Limited.

AptoChem. (2008). Deuterated Internal Standards. Retrieved from http://www.aptochem.com/t-bioanalysis.aspx

Baselt, R. C. (2011). *Disposition of Toxic Drugs and Chemicals in Man* (Ninth Edition ed.). Foster City, CA: Biomedical Publications.

Baselt, R. C. (2014). *Disposition of Toxic Drugs and Chemicals in Man* (Tenth ed.). Foster City, CA: Biomedical Publications.

Beck, J. B. (1966). United States Patent No. US3293127 A. U. S. P. Office.

Bedford, K. (1991). *Opiate Chemistry And Metabolism*. University of Auckland.

Brown, S. M., Campbell, S. D., Crafford, A., Regina, K. J., Holtzman, M. J., & Kharasch, E. D. (2012). P-Glycoprotein Is a Major Determinant of Norbuprenorphine Brain Exposure and Antinociception. *The Journal of Pharmacology and Experimental Therapies, 343*(1), 53-61. doi:10.1124/jpet.112.193433

Caraco Y., S. J., Wood A. J. (1999). Impact of ethnic origin and quinidine coadministration on codeine's disposition and pharmacodynamic effects. *J Pharmacol Exp Ther, 290*(1), 413-422. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10381807

Current, J. D. (2007). Pharmacology for Anesthetists. *Anesthetics and Adjuvants*. http://pediapress.com/books/show/pharmacology-for-anesthetists-anesthetics/ Retrieved from http://pediapress.com/books/show/pharmacology-for-anesthetists-anesthetics/

DataPharm. (2013). Diamorphine Hydrochloride Injection 30 mg. Retrieved from http://www.medicines.org.uk/emc/medicine/28258/SPC/Diamorphine+Hydrochloride+Injection+30+mg/

DataPharm. (2015). Codeine Phosphate Tablets 30mg. Retrieved from http://www.medicines.org.uk/emc/medicine/23910#PHARMACOKINETIC_PROPS

Davis, M. P., Glare, P., & Hardy, J. (2005). *Opioids in Cancer Pain*. Oxford, UK: Oxford University Press.

Drugs.com. (2014). Hydromorphone Retrieved from http://www.drugs.com/pro/hydromorphone.html. Retrieved March 5, 2016 http://www.drugs.com/pro/hydromorphone.html

Erie County Department of Health. (2015). Medical Examiner's Office. Retrieved from http://www2.erie.gov/health/index.php?q=medical-examiner

GBD 2013 Mortality and Causes of Death Collaborators. (2014). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of

death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet, 385*(9963), 117-171. doi:10.1016/S0140-6736(14)61682-2

German Patent. (1936). Germany Patent No.: E. P. search.

Griffin, J. P. (2009). *The Textbook of Pharmaceutical Medicine* (Sixth ed.). Hoboken, New Jersey: Wiley-Blackwell.

International Narcotics Control Board. (2007). *Narcotic Drugs: Estimated World Statistics for 2008, Statistics for 2006*. New York, NY: United Nations Publication.

International Narcotics Control Board. (2014). *Narcotic Drugs: Estimated World Requirements for 2015, Statistics for 2013*. New York, NY: United Nations Publications.

Mandal, A. (2013). Morphine Pharmocokinetics. *News Medical Life Sciences & Medicine*. Retrieved from http://www.news-medical.net/health/Morphine-Pharmacokinetics.aspx

Mayes, S., Ferrone, M. (2006). Fentanyl HCl Patient-Controlled Iontophoretic Transdermal System for the Management of Acute Postoperative Pain. *The Annals of Pharmacotherapy, 40*(11), 2178-2186. Retrieved from http://www.medscape.com/viewarticle/549359_3

McMillin, G. A., Davis, R., Carlisle, H., Clark, C., Marin, S. J., Moody, D. E. (2012). Patterns of Free (Unconjugated) Buprenorphine, Norbuprenorphine, and Their

Glucuronides in Urine Using Liquid Chromatography–Tandem Mass Spectrometry. *Journal of Analytical Toxicology, 36*, 81-87. doi:10.1093/jat/bkr020

MedChemPedia. (2015). Natural Products Chemical Modification: Importance in Drug Discovery (Part-II). Retrieved from http://www.medchempedia.com/2015/03/natural-products-chemical-modification-importance-drug-discovery-part-II.html

Moriya, F. (2005). Pitfalls and cautions in analysis of drugs and poisons *Drugs and Poisons in Humans* (pp. 17-24). Heidelberg, Germany: Springer Berlin Heidelberg.

Nutt, D., King, L. A., Saulsbury, W., & Blakemore, C. (2007). Development of a rational scale to assess the harm of drugs of potential misuse. *The Lancet, 369*(9566), 1047-1053. doi:http://dx.doi.org/10.1016/S0140-6736(07)60464-4

Pélissier-Alicot, A. L., Gaulier, J.M., Champsaur, P., & Marquet, P. (2003). Mechanisms Underlying Postmortem Redistribution of Drugs: A Review. *Journal of Analytical Toxicology, 27*, 533-544. Retrieved from http://jat.oxfordjournals.org/content/27/8/533.long

Pharmacorama. (2006). Pharmacokinetics and dosage of drugs. Retrieved from http://www.pharmacorama.com/en/Sections/Pharmacokinetics-10.php

Pietrangelo, A. (2016). The Progression of Hepatitis C: What Are the Stages? Retrieved from http://www.healthline.com/health/hepatitis-c/stages-progression#2

PremierTox Laboratory (Producer). (2015, March 6, 2016). Buprenorphine. Retrieved from http://www.premiertox.com/buprenorphine

Prommer, E. (2006). Oxymorphone: a review. *Supportive Care in Cancer, 14*(2), 109-115. Retrieved from http://link.springer.com/article/10.1007%2Fs00520-005-0917-1

Purdue Pharma LP. (2016). OxyContin Retrieved from http://medlibrary.org/lib/rx/meds/oxycontin-10/. MedLibrary.org Retrieved March 6, 2016, from MedLibrary.org http://medlibrary.org/lib/rx/meds/oxycontin-10/

Reisfield, G. M., Salazar, E., & Bertholf, R. L. (2007). Review:

Rational Use and Interpretation of Urine Drug Testing in Chronic Opioid Therapy. *Annals of Clinical & Laboratory Science, 37*(4), 301-314. Retrieved from http://www.annclinlabsci.org/content/37/4/301.full.pdf

Rowland, M. (1972). Influence of Route of Administration on Drug Availability. *Journal of Pharmaceutical Sciences, 61*(1), 70-74. doi:http://dx.doi.org/10.1002/jps.2600610111

Smith, H. S. (2009). Opioid Metabolism. *Mayo Clin Proc., 84*(7), 613-624. doi:10.1016/S0025-6196(11)60750-7

Srinivasan, V., Wielbo, D., & Tebbett, I.R. (1997). Analgesic effects of codeine-6-glucuronide after intravenous administration. *European Journal of Pain, 1*(3), 185-190. doi:10.1016/S1090-3801(97)90103-8

Stoops, W. W., Hatton, K. W., Lofwall, M. R., Nuzzo, P. A., & Walsh, S. L. (2010). Intravenous Oxycodone, Hydrocodone and Morphine in Recreational Opioid Users: Abuse Potential and Relative Potencies. *Psychopharmacolcoy (Berl), 212*(2), 193-203. doi:10.1007/s00213-010-1942-4

Code of Federal Regulations, Part 1308-Schedules of Controlled Substances, 1308.11 Schedule I C.F.R. (2015a).

Code of Federal Regulations, Part 1308-Schedules of Controlled Substances, 1308.13 Schedule III C.F.R. (2015b).

U.S. National Library of Medicine. (2016). Opioids, Opioid Antagonists. Retrieved from http://livertox.nih.gov/Opioids.htm#reference

Valtier, S., Bebarta, V. S. (2012). Excretion profile of hydrocodone, hydromorphone and norhydrocodone in urine following single dose administration of hydrocodone to healthy volunteers. *Journal of Analytical Toxicology, 36*(7), 507-514. doi:10.1093/jat/bks058

Varga, E. V., Navratilova, E., Stropova, D., Jambrosic, J., Roeske, W. R., Yamamura, H. I. (2004). Agonist-specific regulation of the delta-opioid receptor. *Life Sci., 76*(6), 599-612. doi:10.1016/j.lfs.2004.07.020

Vuilleumier, P. H., Stamer, U. M. & Landau, R. (2012). Pharmacogenomic considerations in opioid analgesia. *Pharmacogenomics and Personalized Medicine, 5,* 73-87. doi:10.2147/PGPM.S23422

Wang, B., Yang, L. P., Zhang, X. Z., Huang, S.Q., Bartlam, M., Zhou, S. F. (2009). New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme. *Drug Metab. Rev., 41*(4), 573-643. doi:10.1080/03602530903118729

Waters. (2016). Xevo G2-XS Tof. Retrieved from http://www.waters.com/waters/en_US/Xevo-G2-XS-Tof/nav.htm?cid=134798183&locale=en_US

Yucatan Times. (2014). 50% of the Heroin consumed in the United States is produced in Mexico. *The Yucatan Times*. http://www.theyucatantimes.com/2014/11/50-of-the-heroin-consumed-in-the-united-states-is-produced-in-mexico/ Retrieved from http://www.theyucatantimes.com/2014/11/50-of-the-heroin-consumed-in-the-united-states-is-produced-in-mexico/

Appendix I

Procedures Part A

Create 1:3 homogenate solutions with liver matrix

1) Weigh 5 grams of liver and add 10 milliliters of deionized water into a 50mL centrifuge test tubes plastic sample specimen bottle container with screw top.

2) Use homogenizer *Polytron PT 10-35, Dispersing and Mixing Technology by Kinematica*, to make (1:3) homogenate solution: Blend together 5g Liver + 10 milliliters deionized water.

Appendix II

Procedures Part B

Opioid Quantitation by LC/MS/MS

(*Procedure from Erie County Toxicology Lab*)

First incorporated: 12/10/09

Last update: 10/24/14

I. Principle

1.) Whole blood, plasma, and serum specimens are extracted for narcotic analgesics and their metabolites, along with their deuterated counterparts as internal standards by diluting and isolating the analytes on a C18 solid phase extraction column. Elution with methanol allows for the recovery of all thirteen analytes in a single residue, which is subsequently evaporated, reconstituted, and subjected to an LC triple quadrapole, utilizing a MS/MS detection technique in the positive electrospray ionization mode.

II. Specimen

1) This analytical assay is designed to quantitate morphine, morphine-3-glucuronide, morphine-6-glucuronide, 6-monoacetylmorphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, fentanyl, norfentanyl, buprenorphine, and norbuprenorphine in whole blood, plasma, and serum.

III. Reagents and Materials

A. Standards

1.) Table 1 (Standard Preparation)

Name	Refer. Stock Cat. #	Conc. (mg/L)	Amt. to use	Vol. Flask size	Final Standard Conc.
Morphine	Cerilliant M-005	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Morphine-3-Glucuronide	Cerilliant M-031	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Morphine-6-Glucuronide	Cerilliant M-096	1,000	1.0 ml	10.0 ml	100 mg/L MeOH/H_2O
Hydromorphone	Cerilliant H-004	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Oxymorphone	Cerilliant O-004	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
6-MAM	Cerilliant A-009	1,000	1.0 ml	10.0 ml	100 mg/L in ACN
Codeine	Cerilliant C-006	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Hydrocodone	Cerilliant H-003	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Oxycodone	Cerilliant O-002	1,000	1.0 ml	10.0 ml	100 mg/L in MeOH
Fentanyl	Cerilliant F-002	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Norfentanyl	Cerilliant N-031	1,000	1.0 ml	100.0 ml	10 mg/L in MeOH
Buprenorphine	Cerilliant B-902	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Norbuprenorphine	Cerilliant N-045	100	1.0 ml	10.0 ml	10 mg/L in MeOH

Table 2 (Working Stock Preparation)

Name	Stock Standard Conc.	W.S."A" conc.	Stock amt.	W.S."B" conc.	W.S."C" conc.
			10 ml vol. flask	1/10 of W.S."A"	1/10 of W.S."B"
Morphine	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Morphine-3-Glucuronide	100 mg/L in MeOH	10.0 mg/L	1.0 ml	1.0 mg/L	0.1 mg/L
Morphine-6-Glucuronide	100 mg/L MeOH/H_2O	10.0 mg/L	1.0 ml	1.0 mg/L	0.1 mg/L
Hydromorphone	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Oxymorphone	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
6-MAM	100 mg/L in ACN	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Codeine	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Hydrocodone	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Oxycodone	100 mg/L in MeOH	5.0 mg/L	0.5 ml	0.5 mg/L	0.05 mg/L
Fentanyl	10 mg/L in MeOH	0.5 mg/L	0.5 ml	0.05 mg/L	0.005 mg/L
Norfentanyl	10 mg/L in MeOH	0.5 mg/L	0.5 ml	0.05 mg/L	0.005 mg/L
Buprenorphine	10 mg/L in MeOH	0.25 mg/L	0.25 ml	0.025 mg/L	0.0025 mg/L
Norbuprenorphine	10 mg/L in MeOH	0.25 mg/L	0.25 ml	0.025 mg/L	0.0025 mg/L

B. Internal Standards

1. Table 5 (Deuterated Internal Standard Preparation)

Name	Refer. Stock Cat. #	Conc. (mg/L)	Amt. to use	Vol. Flask size	Final Control Conc.
Morphine-d6	Cerilliant M-085	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Morphine-3-Glucuronide-d3	Cerilliant M-017	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Morphine-6-Glucuronide-d3	Cerilliant M-120	100	1.0 ml	10.0 ml	10 mg/L MeOH/H_2O
Hydromorphone-d6	Cerilliant H-049	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Oxymorphone-d3	Cerilliant O-003	100	1.0 ml	10.0 ml	10 mg/L in MeOH
6-MAM-d6	Cerilliant A-026	100	1.0 ml	10.0 ml	10 mg/L in ACN
Codeine-d6	Cerilliant C-040	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Hydrocodone-d6	Cerilliant H-047	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Oxycodone-d6	Cerilliant O-007	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Fentanyl-d5	Cerilliant F-001	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Norfentanyl-d5	Cerilliant N-030	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Buprenorphine-d4	Cerilliant B-901	100	1.0 ml	10.0 ml	10 mg/L in MeOH
Norbuprenorphine-d3	Cerilliant N-920	100	1.0 ml	10.0 ml	10 mg/L in MeOH

2. Combination Internal Standard

1) Add to a 100 ml volumetric flask 2.0 ml of morphine-d6, hydromorphone-d6, oxymorphone-d3, 6-MAM-d6, codeine-d6, hydrocodone-d6, and oxycodone-d6 working stocks refer to Table 5, the concentration of these seven is 0.200 mg/L.

2) Add to the same flask 0.2 ml of fentanyl-d5, norfentanyl-d5, buprenorphine-d4, and norbuprenorphine-d3 working stocks refer to Table 5, the concentration of these four is 0.020 mg/L, dilute to the mark with methanol.

3. Reagents and Supplies

1) Methanol, ultra-resi grade, J.T. Baker.

2) Acetonitrile, HPLC grade, J.T. Baker

3) Formic Acid, reagent grade, Fluka Chemika

4) Ammonium Formate, reagent grade, Fluka Chemika

5) Concentrated Ammonium Hydroxide, reagent grade, J.T. Baker.

6) 1M Ammonium Formate Solution. Add 6.3 grams of ammonium formate to a 100 ml volumetric flask and dilute to the mark with deionized water.

7) 0.01M Ammonium Carbonate buffer, pH 9.3. Add 3.84 grams of ammonium carbonate to 4.0 L deionized water, slowly add concentrated ammonium hydroxide until pH 9.3 is achieved, using a pH meter.

8) Varian Bond-Elut LRC-C18, 200 mg solid phase extraction columns, or equivalent C18 SPE columns.

9) Double Deionized Water

10) Mobile Phase A, 10 mM ammonium formate, pH 3.0. Add 10.0 ml of 1M ammonium formate solution to 950 ml deionized water and adjust pH to 3.0 by drop wise addition of formic acid, using a pH meter, dilute to 1000 ml. with water.

11) Mobile Phase B, 100% Acetonitrile.

12) Reconstitution Solvent, 97/3, Mobile Phase A/Mobile Phase B.

13) Auto-injector vials, inserts and caps, Shamrock Glass.

14) The Drugs and Deuterated Drugs used in this project were purchased from Cerilliant Corporation, 811 Paloma Dr. #A, Round Rock, TX 78665. Phone: (512) 238-9974

15) The drug-free whole blood used throughout this study is Sheep blood which was obtained from Hemostat Laboratories, P.O. Box 790, Dixon, CA 95620, USA. Phone: (800) 572-6888

IV. Instrumentation

1) MS/MS detector, Applied Biosystems 3200 Qtrap

2) Liquid Chromatograph, Shimadzu Prominence 20 Series binary pumps, degasser, auto sampler, column oven, and controller.

3) Phenomenex Synergi Polar-RP 80A, 150 x 2.0 mm x 4.0 µm HPLC analytical column, or equivalent.

4) Phenomenex Security Guard cartridges, Polar-RP 4.0 x 20 mm, guard column, or equivalent.

5) UCT Positive Pressure Extractor.

6) Zymark, TurboVap LV evaporator.

V. Procedure

A. Sample Preparation

1. Specimen, Blood, Serum, or Plasma

1) Prior to aliquoting whole blood specimen, invert tubes several times to ensure a homogeneous mixture of cells and serum.

2) If the analyte levels are determined to exceed the concentration of the highest calibrator, a dilution of the specimen should be made using drug-free whole blood or plasma.

2. Calibrators

1) Prepare calibrators in porcine whole blood of by adding the appropriate amounts of working stock standards “A”, “B”, or “C”, refer to Table 2. It is preferable to add these solutions to the buffer solution, and then add 1.0 ml of the blood matrix.

a) STD 1 40 mcl of WS “C”

b) STD 2 100 mcl of WS “C”

c) STD 3 40 mcl of WS “B”

d) STD 4 100 mcl of WS “B”

e) STD 5 40 mcl of WS “A”

f) STD 6 70 mcl of WS “A”

g) STD 7 100 mcl of WS “A”

2) Calibrator target values are listed below as mg/L concentrations, except where noted by asterisks, then target values are as mcg/L.

Name	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7
Morphine	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Morphine-3-Glucuronide	0.004	0.010	0.04	0.10	0.40	0.70	1.00
Morphine-6-Glucuronide	0.004	0.010	0.04	0.10	0.40	0.70	1.00
Hydromorphone	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Oxymorphone	0.002	0.005	0.02	0.05	0.20	0.35	0.50
6-MAM	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Codeine	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Hydrocodone	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Oxycodone	0.002	0.005	0.02	0.05	0.20	0.35	0.50
Fentanyl **	0.20	0.50	2.00	5.00	20.00	35	50.00
Norfentanyl **	0.20	0.50	2.00	5.00	20.00	35	50.00
Buprenorphine **	0.10	0.25	1.00	2.50	10.00	17.5	25.00
Norbuprenorphine **	0.10	0.25	1.00	2.50	10.00	17.5	25.00

3. Controls

a) Positive, Low

1) Prepared in-house in large batch lots from drug-free whole blood.

2) Morphine, hydromorphone, oxymorphone, 6-monoacetylmorphine, codeine, hydrocodone, and oxycodone are targeted at 0.01 mg/L. Morphine-3-glucuronide and morphine-6-glucuronide are targeted at 0.02 mg/L. Fentanyl, norfentanyl, buprenorphine, and norbuprenorphine are targeted at 1.0 mcg/L.

b) Positive, High

1) Prepared in-house in large batch lots from drug-free whole blood.

2) Morphine, hydromorphone, oxymorphone, 6-monoacetylmorphine, codeine, hydrocodone, and oxycodone are targeted at 0.20 mg/L. Morphine-3-glucuronide and morphine-6-glucuronide are targeted at

0.40 mg/L. Fentanyl, norfentanyl, buprenorphine, and norbuprenorphine are targeted at 20.0 mcg/L

c) Negative

1) Drug-free whole blood is used for the negative control.

B. Extraction

1) Into respective, pre-labeled 16 X 125mm culture tubes add the following:

a) 3.0 ml of 0.01M ammonium carbonate buffer, pH 9.3.

b) Appropriate amounts of calibrator working stock, as listed above.

c) 100 mcl of combination internal standard.

d) One ml of blank blood for calibrators and controls.

e) One ml of case bloods.

f) Briefly vortex mix, approximately 30 seconds.

2) Ultrasonicate all tubes for 10 minutes.

3) Centrifuge tubes for 10 minutes @ 3600 rpm.

4) Pre-condition the C18 SPE cartridges with 3 ml methanol, 3 ml deionized water, and 3 ml 0.01M ammonium carbonate buffer.

5) Apply the supernatant to the column at a flow rate of 1-2 ml/min.

6) Wash columns twice with 3 ml of 0.01M ammonium carbonate buffer, dry on high air flow for 10 minutes.

7) Gravity drip 3 ml methanol to elute drugs into 13 x 100mm culture tubes.

8) Evaporate solvent in the TurboVap set at 50 deg. C. using air flow.

9) Reconstitute with 100 mcl of the reconstitution solvent.

10) Injection amount varies according to the data acquisition method being used, refer to the appropriate method: "OPS9.dam", "OPS13.dam", or "BupNBFentNF.dam".

C. Instrumental Parameters

1) Chromatographic and mass spectral parameters are attached only for opioid acquisition method entitled "OPS13.dam". All related acquisition methods are clones of this one.

2) Recalibrate method each time new calibrators are run.

3) Position vials in auto-sampler tray and inject following this sequence:

a) Negative Control

b) Calibrators

c) Carryover Check Sample, a re-injection of the Negative Control

d) Low Control

e) Case specimens

f) High Control

D. Calculations

1) Calibration and calculation of the acquired standard, control and unknown data uses the established quantitation application contained in the method, "OPS.qmf". This application contains peak integration parameters, curve type and weighting factor.

2) Once all injections specified in the sequence have been made, create a results table from acquired data files, using the Quantitation Wizard application.

3) Visually check the results table entries, noting analytes' parameters, such as peak shape and integration, the calibration curve's R value and calculated amounts. Also, check individual case chromatograms and calculated amounts.

4) If parameters check out, then proceed to printing out each calibrator, control and case file through the Reports template, "Erie County Medical. dot"

5) Reports will include the following:

a) Header information

b) Results summary table listing internal standard's and analyte's ion transition data.

c) Peak review pages showing a total ion chromatogram and extracted ion chromatograms for internal standards and analytes, calibration summary report, and calibration curves for the analytes.

VI. Acceptance Criteria

A. The Calibration

1) The calibration should consist of a minimum of 5 consecutive, valid calibrators.

2) One failed calibrator may be dropped from the upper and/or lower end of the calibration.

3) The calibration range should bracket the unknown specimens. If unknowns exceed the upper limit of linearity, then appropriate dilutions should be made to bring the sample back within the working range of calibrators.

4) The correlation coefficient (r^2) value should be 0.990 or better.

B. Calibrators

1) Peak shape should be symmetrically shaped and sharp without noticeable leading or trailing edges.

2) Should read within ± 20 % of the weighed-in target value upon back-calculation against the established calibration curve. Note that the lowest calibrator tolerance is ± 30 %.

3) Two transition pairs per analyte and internal standard are monitored and one ratio is determined. Both transitions must be present (analyte and internal standard), the ratio of the quantifying transition to qualifying transition being within ± 20 % of the mean of all calibrators (± 25 % for the lowest calibrator).

4) Should be 70 % resolved with respect to the quantifying (target) peak, or better from the nearest potentially interfering peak.

C. Controls

1) The abundance of the internal standard of the negative and positive controls should be approximately equal.

2) The negative control should read less than 25 % of the value of the lowest valid calibrator. If it fails, then all samples positive for narcotic analgesics will be repeated.

3) The measured value of the positive control must be within ± 20 % of the target value or the established value in the MultiQ control tracking software.

4) The transition ratio criteria must be met, as stated above.

5) If the positive control fails, then all positive samples will be retested.

D. Specimens

1) All criteria for the calibration as a whole, including calibrators and controls, should be met.

2) Ion ratios for the analytes and internal standards in specimens should be within ± 20 % of the mean of all calibrators.

3) The retention time for the unknowns must be within ± 3 % of the corresponding retention time of the calibrators.

4) Values below the limit of detection will be reported as negative.

5) Values above the upper limit of linearity should be appropriately diluted with negative whole blood and retested. The result may also be reported as >ULOL (upper limit of linearity).

6) If retesting of a specimen is necessary, the historic curve is valid provided that negative and 2 positive controls are run concurrently and pass acceptance criteria. Also, sufficient original internal standard solution must be available, and no alterations to the analytical instrument can be made.

7) Signal to noise ratios must equal or exceed 10 for each compound to be reported.

VII. Reporting Criteria

1) Concentrations are reported in mg/L for the nine opiates and mcg/L for the other four (fentanyl, norfentanyl, buprenorphine, and norbuprenorphine).
2) Morphine-3-glucuronide and morphine-6-glucuronide may be reported as "> 1.0 mg/L" if they exceed the upper limit of linearity.

VIII. Assay Parameters

Table 5 (LOD, LOQ, ULOL), (mg/L, mcg/L**)

Name	LOD	LOQ	ULOL
Morphine	0.002	0.002	0.50
Morphine-3-Glucuronide	0.004	0.004	1.00
Morphine-6-Glucuronide	0.004	0.004	1.00
Hydromorphone	0.002	0.002	0.50
Oxymorphone	0.002	0.002	0.50
6-MAM	0.002	0.002	0.50
Codeine	0.002	0.002	0.50
Hydrocodone	0.002	0.002	0.50
Oxycodone	0.002	0.002	0.50
Fentanyl **	0.20	0.20	50.00
Norfentanyl **	0.20	0.20	50.00
Buprenorphine **	0.10	0.10	25.00
Norbuprenorphine **	0.10	0.10	25.00

A. Interference Study

1) The following compounds were ran as neat samples at a concentration of 5.0 mg/L and found not to interfere by eluting at the same retention time or share transitions with the analytes of this assay: dextrorphan, nalbuphine, nalorphine, ibugaine, dextromethorphan, and papaverine.

2) These compounds share transition masses, but elute at different retention times than the analytes of this assay:

   a) Noroxycodone (rt = 7.26 min), shares with oxymorphone (rt = 6.49 min) mrm1 & mrm2.

   b) Norhydrocodone (minor peak rt = 7.60 min, major peak rt = 9.20 min), minor peak shares with hydromorphone (rt = 6.92 min) mrm1, major peak share with hydrocodone (rt = 8.59 min) mrm1 & mrm2.

c) Noroxymorphone (rt = 7.15 min), shares with oxymorphone (rt-6.49 min) mrm1.

d) Noscapine (rt = 11.27 min), shares with norbuprenorphine (rt = 10.6 min) mrm2.

e) Naloxone (rt = 7.73 min), shares with 6MAM (rt = 8.36 min) mrm1 & mrm2.

3) These compounds share transition masses and elute at the same retention time as the analytes of this assay

a) Norcodeine (rt = 7.78 min), shares with codeine (rt = 7.79 min) mrm 1 and mrm 2 at the same retention time, due to manufacturing impurity of reference material (1% codeine).

b) Normorphine (rt = 5.91 min), very slight sharing with morphine (rt = 5.91 min) mrm 1 and mrm 2 at the same retention time, probably due to manufacturing impurity, as in the case of norcodeine.

c) Thebaine (rt = 7.79 min), very slight sharing with codeine (rt = 7.79 min) mrm 1 and mrm 2 at the same retention time.

d) Morphine-N-Oxide (minor peak rt = 5.92 min, major peak rt = 6.36 min), minor peak shares with morphine (rt = 5.91 min) mrm 1 and mrm 2 at the same rt, major peak shares with oxymorphone (rt = 6.49 min) mrm 1 and mrm 2 but rt is slightly prior.

e) Codeine-N-Oxide (minor peak rt = 7.88 min, major peak rt = 7.88 min), minor peak shares with codeine (rt = 7.79 min) mrm 1 and mrm 2 at the same rt, major peak shares with oxycodone (rt = 8.31 min) mrm 1 and mrm 2, but at different rt.

f) 10-Hydroxymorphine (rt = 5.91 min), shares with morphine (rt = 5.91 min) mrm 1 and mrm 2 at the same rt.

g) Diacetylmorphine (major peak rt = 8.36 min, minor peak rt = 5.91 min), major peak shares with 6-MAM (rt = 8.36 min) mrm 1 and mrm 2, at the same rt, minor peak shares with morphine (rt = 5.91 min) mrm 1 and mrm 2, at the same rt. Probably due to methanol instability of reference material.

h) 6-Acetylcodeine (rt = 7.79 min), shares with codeine (rt = 7.79 min) mrm1 and mrm 2, at the same rt. Methanol instability could be a factor, here.

i) Dihydrocodeine (rt = 8.59 min), very slight sharing with hydrocodone (rt = 8.59 min) mrm 1 and mrm 2, at the same rt.

j) Ethylmorphine (minor peak rt = 5.88 min, major peak rt = 7.78 min), minor peak very slightly shares with morphine (rt = 5.91 min) mrm 1 and mrm 2, major peak slightly shares with codeine (rt = 7.79 min) mrm1 and mrm 2, at the same rt.

IX. References

R. Baselt. Disposition of Toxic Drugs and Chemicals in Man. Sixth Edition. Biomedical Publications. Foster City, CA. Copyright 2002.

M. Bogusz, R.D. Maier, M. Erkens, S. Driessen., Determination of morphine and its 3- and 6-glucuronides, codeine, codeine-glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography B, vol 703, 1997: 115-127.

A. Al-Asmari, R. Anderson., Method for quantitation of opioids and their metabolites in autopsy blood by liquid chromatography-tandem mass spectrometry, J. Anal Tox., vol 31, Sept. 2007: 394-408.

M. Gergov, et al., Simultaneous screening and quantitation of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry, Forensic Science International (2009), doi:10.1016/j.forsciint.2009.01.013.

K. Taylor, S. Elliott, A validated hybrid quadruple linear ion-trap LC-MS method for the analysis of morphine and morphine glucuronides applied to opiate deaths, Forensic Science International (2009), doi:10.1016/j.forsciint.2009.02.0

Appendix III

Calibration and Extraction Data

Table 1. Tables of calibrations from 012915 extraction of human liver that has been fortified with known amount of opiates
*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)
Morphine	2	1.595	79.65	5	6.255	125	20	19.6	97.65	50	49.1	98.1
Hydromorphone	2	1.88	94.2	5	5.6	112	20	18.5	92.45	50	50.6	101
Codeine	2	1.82	90.8	5	5.51	110.5	20	18.75	93.95	50	52.9	106
Hydrocodone	2	1.77	88.6	5	5.67	113.5	20	19.35	96.9	50	50.75	101.35
Oxymorphone	2	1.78	89	5	5.65	113	20	19.5	97.4	50	50.7	101.55
Oxycodone	2	1.825	91.3	5	5.57	111.5	20	19.3	96.3	50	50.6	101.45
6-MAM	2	2.01	100.25	5	5.445	109	20	18.2	91	50	50.35	100.5
Morphine-3-Glucuronide	4	3.52	88.1	10	11.5	115	40	38.9	97.2	100	99.8	99.8
Morphine-6-Glucuronide	4	3.75	93.75	10	11.15	111.5	40	39.1	97.75	100	95.7	95.7
Norfentanyl	0.2	0.184	91.95	0.5	0.5765	115	2	1.805	90.45	5	5.11	101.95
Fentanyl	0.2	0.185	92.55	0.5	0.56	112	2	1.87	93.5	5	5.11	102.5
Norbuprenorphine	0.1	0.09385	93.85	0.25	0.2875	115	1	0.898	89.8	2.5	2.53	101.4
Buprenorphine	0.1	0.08765	87.65	0.25	0.2985	119.5	1	0.8985	89.85	2.5	2.595	104

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)
Morphine	200	191.5	95.8	350	369	105.5	500	490	97.95
Hydromorphone	200	200.5	99.95	350	351.5	100.5	500	499	99.7
Codeine	200	201	100.5	350	342	97.65	500	505	101
Hydrocodone	200	198.5	99.35	350	350.5	100.5	500	500	100.2
Oxymorphone	200	196.5	98.2	350	355.5	101.5	500	498	99.5
Oxycodone	200	198.5	99.35	350	352	100.65	500	499	99.65
6-MAM	200	196	98	350	350	99.85	500	505	101
Morphine-3-Glucuronide	400	399	99.45	700	703.5	100.4	1000	998	99.8
Morphine-6-Glucuronide	400	403.5	100.9	700	709.5	101.5	1000	990	99
Norfentanyl	20	19.95	100.05	35	35.2	100.45	50	49.9	99.75
Fentanyl	20	19.9	99.5	35	35.2	100.5	50	49.95	99.8
Norbuprenorphine	10	9.94	99.4	17.5	17.75	101.5	25	24.85	99.2
Buprenorphine	10	9.84	98.4	17.5	17.7	101.5	25	24.9	99.65

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:3 Dilution		
	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)
Morphine	10	12.95	129.5	200	233.5	116.5	0	0	0	0	0.02335	0.2335
Hydromorphone	10	11.85	118.5	200	215	107	0	0	0	0	0	0
Codeine	10	11.25	112.5	200	218	109	0	0	0	0	0	0
Hydrocodone	10	11.7	117	200	223.5	111.5	0	0	0	0	0	0
Oxymorphone	10	11.7	117	200	229	114.5	0	0	0	0	0	0
Oxycodone	10	11.25	112.5	200	205.5	103	0	0	0	0	0	0
6-MAM	10	9.255	92.55	200	193	96.4	0	0.0398	0	0	-	-
Morphine-3-Glucuronide	20	32.9	164.5	400	662.5	165.5	0	0	0	0	3.76	18.8
Morphine-6-Glucuronide	20	16.9	84.45	400	337	84.2	0	0	0	0	0	0
Norfentanyl	1	1.13	113	20	21.75	108.5	0	0	0	0	0	0
Fentanyl	1	1.145	114.5	20	21	105	0	0	0	0	0.0278	0.0278
Norbuprenorphine	1	1.205	120.5	20	22.6	113	0	0	0	0	3.285	3.285
Buprenorphine	1	1.27	127	20	22	110	0	0	0	0	0.2845	0.2845

Table 2. Tables of results from 012915 extraction where various dilutions of a human liver that has been fortified with a known amount of opiates

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Liver 1:6 Dilution (#1)			Liver 1:9 Dilution (#1)			Liver 1:15 Dilution (#1)			Liver 1:30 Dilution (#1)			Liver 1:60 Dilution (#1)		
	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)	Analyte Concentration	*Calculated Concentration	Accuracy (%)
Morphine	83.3	104.95	126	55.6	61.15	110	33.3	56.35	169	16.7	24.05	144.5	8.4	9.925	118.5
Hydromorphone	83.3	66.1	79.35	55.6	38.05	68.4	33.3	29.95	90.05	16.7	12.95	77.6	8.4	4.93	58.7
Codeine	83.3	82.7	99.1	55.6	52.15	93.85	33.3	36.45	109.5	16.7	16.6	99.4	8.4	9.17	109
Hydrocodone	83.3	80.4	96.55	55.6	50.85	91.4	33.3	34.9	104.5	16.7	16.6	99.6	8.4	8.765	104.5
Oxymorphone	83.3	66.9	80.3	55.6	37.05	66.6	33.3	32.15	96.45	16.7	13.5	80.6	8.4	5.39	64.15
Oxycodone	83.3	79.85	95.8	55.6	51.85	93.25	33.3	36.2	109	16.7	17.6	105.5	8.4	8.235	98.05
6-MAM	83.3	-	-	55.6	-	-	33.3	-	-	16.7	-	-	8.4	-	-
Morphine-3-Glucuronide	167	158	94.85	111	100.6	90.7	66.6	64.7	97.2	33.4	33.3	99.45	16.8	17.3	103
Morphine-6-Glucuronide	167	126.5	75.75	111	67.7	60.95	66.6	64.05	96.15	33.4	27.8	83.3	16.8	11.1	66.3
Norfentanyl	8.33	7.94	95.35	5.55	4.605	82.95	3.33	3.4	102.3	1.67	1.575	94.3	0.833	0.834	100.3
Fentanyl	8.33	7.83	93.95	5.55	4.89	88.1	3.33	3.395	102	1.67	1.66	99.5	0.833	0.843	101.5
Norbuprenorphine	4.17	3.28	78.7	2.77	1.64	59.1	1.67	1.55	93	0.833	0.6475	77.75	0.417	0.2665	63.95
Buprenorphine	4.17	2.66	63.75	2.77	1.485	53.6	1.67	1.53	91.7	0.833	0.597	71.7	0.417	0.2125	51

Analytes	Liver 1:6 Dilution (#2)			Liver 1:9 Dilution (#2)			Liver 1:15 Dilution (#2)			Liver 1:30 Dilution (#2)			Liver 1:60 Dilution (#2)		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	83.3	107	128.5	55.6	64.05	115.5	33.3	62.85	189	16.7	23.4	140	8.4	9.43	112.5
Hydromorphone	83.3	61.5	73.8	55.6	38.05	68.45	33.3	32.25	96.8	16.7	11.8	70.75	8.4	4.865	57.95
Codeine	83.3	78.2	93.95	55.6	56.35	101.45	33.3	35.6	107	16.7	17.95	107.5	8.4	7.975	95
Hydrocodone	83.3	77.3	92.8	55.6	51.4	92.4	33.3	34.4	103	16.7	16.1	96.25	8.4	7.55	89.9
Oxymorphone	83.3	58.7	70.5	55.6	37.6	67.6	33.3	33.45	100.3	16.7	13.25	79.25	8.4	4.91	58.45
Oxycodone	83.3	79.85	95.9	55.6	52.9	95.2	33.3	35.2	105.5	16.7	16.4	98.1	8.4	7.525	89.55
6-MAM	83.3	-	-	55.6	-	-	33.3	-	-	16.7	-	-	8.4	-	-
Morphine-3-Glucuronide	167	152.5	91.25	111	100.2	90.35	66.6	66.35	99.45	33.4	32.5	97.3	16.8	15.25	90.95
Morphine-6-Glucuronide	167	118.5	70.95	111	72.45	65.25	66.6	67.95	102.2	33.4	27.35	81.95	16.8	11.4	68
Norfentanyl	8.33	7.65	91.8	5.55	4.86	87.55	3.33	3.35	100.75	1.67	2.08	124.85	0.833	0.744	89.35
Fentanyl	8.33	7.795	93.6	5.55	5.025	90.55	3.33	3.345	100.5	1.67	1.59	95.35	0.833	0.7605	91.3
Norbuprenorphine	4.17	2.835	67.95	2.77	1.655	59.85	1.67	1.645	98.5	0.833	0.65	78.05	0.417	0.275	65.95
Buprenorphine	4.17	2.5	59.9	2.77	1.395	50.35	1.67	1.715	103	0.833	0.5595	67.15	0.417	0.2345	56.1

Table 3. Tables of calibrations from 021215 extraction where whole (sheep) blood calibrators were used as well as a human liver that has been fortified with known amount of opiates

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.49	74.45	5	5.7	114	20	20.05	100.35	50	58.3	117
Hydromorphone	2	1.705	85.4	5	5.445	109	20	19.65	98.1	50	55.9	111.5
Codeine	2	1.825	91.15	5	5.31	106.5	20	19.45	97.25	50	54.35	108.5
Hydrocodone	2	1.625	81.1	5	5.655	113	20	19.75	98.95	50	55.55	111
Oxymorphone	2	1.77	88.45	5	5.41	108.5	20	19.4	96.7	50	55	110
Oxycodone	2	1.58	78.8	5	5.585	111.5	20	20.2	101	50	56.95	114
6-MAM	2	2.045	102	5	5.47	109.5	20	18.6	92.9	50	51.05	102
Morphine-3-Glucuronide	4	3.61	90.3	10	10.55	105.5	40	39.35	98.45	100	109	109
Morphine-6-Glucuronide	4	3.39	84.8	10	10.95	109.5	40	38.95	97.35	100	112.5	112.5

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	185.5	92.9	350	353.5	101	500	502	100.5
Hydromorphone	200	186	92.95	350	361	103	500	497	99.45
Codeine	200	188	94.05	350	362	103.5	500	496	99.2
Hydrocodone	200	188.5	94.2	350	357	101.8	500	499	99.95
Oxymorphone	200	188	94	350	360.5	103.5	500	496.5	99.3
Oxycodone	200	182.5	91.4	350	362.5	103.5	500	497.5	99.5
6-MAM	200	176.5	88.2	350	354.5	101	500	519	103.5
Morphine-3-Glucuronide	400	376	94	700	721.5	103	1000	994	99.4
Morphine-6-Glucuronide	400	378	94.45	700	706	101.05	1000	1006	100.6

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:3 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	14.9	149	200	253	126	0	0	0	0	0	0
Hydromorphone	10	14	140	200	255	127.5	0	0	0	0	0	0
Codeine	10	13.5	135	200	242	121	0	0	0	0	0	0
Hydrocodone	10	13.9	139	200	247.5	123.5	0	0	0	0	0	0
Oxymorphone	10	14.25	142.5	200	258	129	0	0	0	0	0	0
Oxycodone	10	12.8	128	200	229.5	115	0	0	0	0	0	0
6-MAM	10	11.4	114	200	224	112	0	0.1253	0	0	0	0
Morphine-3-Glucuronide	20	23.7	118	400	455.5	114	0	0	0	0	0	0
Morphine-6-Glucuronide	20	24.85	124.5	400	496	124	0	0	0	0	0	0

Analytes	Low Positive Control Liver 1:6			Low Positive Control Liver 1:9			Low Positive Control Liver 1:15		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	18.05	180.5	10	21.65	216.5	10	16.35	163.5
Hydromorphone	10	10.8	108	10	11.9	119	10	9.685	96.85
Codeine	10	10.8	108	10	12.4	124	10	10.55	105.5
Hydrocodone	10	10.22	102.2	10	12.75	127.5	10	10.315	103.15
Oxymorphone	10	9.69	96.9	10	11.5	115	10	9.485	94.85
Oxycodone	10	10.2	102	10	12.35	123.5	10	10.15	101.5
6-MAM	10	-	-	10	-	-	10	-	-
Morphine-3-Glucuronide	20	20	100.25	20	23.35	117	20	19.3	96.35
Morphine-6-Glucuronide	20	19.55	97.6	20	25	125.5	20	21.55	107.4

Analytes	High Positive Control Liver 1:6			High Positive Control Liver 1:9			High Positive Control Liver 1:15		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	319	159.5	200	353.5	177	200	333	166.5
Hydromorphone	200	190.5	95.15	200	206	103	200	205	103
Codeine	200	200	99.7	200	218.5	109	200	206.5	103.5
Hydrocodone	200	191	95.5	200	202	101	200	197.5	98.9
Oxymorphone	200	191	95.65	200	209	104.5	200	197.5	98.8
Oxycodone	200	199	99.55	200	219.5	109.5	200	212	106
6-MAM	200	-	-	200	-	-	200	-	-
Morphine-3-Glucuronide	400	375	93.8	400	398.5	99.8	400	394	98.45
Morphine-6-Glucuronide	400	411	103	400	451	113	400	428	107

Table 4. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 021215 extraction of various dilutions of Case #1

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Case #1				
	Blood	*Liver (1:6 dilution)	*Liver (1:9 dilution)	*Liver (1:15 dilution)	Average Times Higher in Liver than in Blood
Morphine	18	253.8	253.35	284.25	14.656
Hydromorphone	0	0	0	0	0
Codeine	0	0.888	0.869	0	0.879
Hydrocodone	0	0	0	0	0
Oxymorphone	0	0	0	0	0
Oxycodone	0	11.46	10.44	7.905	9.935
6-MAM	0	-	-	-	-
Morphine-3-Glucuronide	190	291.9	349.65	354.75	1.748
Morphine-6-Glucuronide	30	112.2	109.8	109.275	3.681

Analytes	Case #1 (duplicate)				
	Blood	*Liver (1:6 dilution)	*Liver (1:9 dilution)	*Liver (1:15 dilution)	Average Times Higher in Liver than in Blood
Morphine	18	196.5	293.4	299.25	14.614
Hydromorphone	0	0	0	0	0
Codeine	0	0.69	1.224	0	0.638
Hydrocodone	0	0	0	0	0
Oxymorphone	0	0	0	0	0
Oxycodone	0	8.61	12.015	8.955	9.860
6-MAM	0	-	-	-	-
Morphine-3-Glucuronide	190	240.6	357.3	367.5	1.694
Morphine-6-Glucuronide	30	90.3	131.4	112.8	3.717

Table 5. Tables of calibrations from 021315 extraction involving calibrators with whole blood and 1:9 dilution of calf liver as negative control

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.945	97.05	5	5.555	111	20	17.7	88.7	50	51.8	103.5
Hydromorphone	2	1.885	94.3	5	5.31	106	20	19.15	95.7	50	52.5	105
Codeine	2	1.655	82.85	5	5.54	110.5	20	20.35	101.5	50	53.7	107.5
Hydrocodone	2	1.89	94.65	5	5.345	107	20	19.5	97.55	50	51.35	102.5
Oxymorphone	2	1.915	95.75	5	5.525	110.5	20	18.6	92.9	50	51.1	102
Oxycodone	2	1.77	88.4	5	5.41	108.5	20	20	99.85	50	52.75	105.5
6-MAM	2	2.025	101	5	5.28	106	20	18.45	92.3	50	50.6	101
Morphine-3-Glucuronide	4	3.86	96.5	10	10.55	105.5	40	38.4	95.95	100	102.5	102.5
Morphine-6-Glucuronide	4	3.985	99.4	10	10.7	107	40	36.95	92.35	100	101.5	101.5
Norfentanyl	0.2	0.2035	101.5	0.5	0.5275	105.5	2	1.87	93.3	5	4.94	98.55
Fentanyl	0.2	0.18	89.95	0.5	0.545	109	2	1.975	98.75	5	5.205	104
Norbuprenorphine	0.1	0.104	104	0.25	0.263	105	1	0.9055	90.55	2.5	2.505	99.95
Buprenorphine	0.1	0.091	91	0.25	0.3255	130	1	0.6815	68.15	2.5	2.78	111.5

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	195.5	97.7	350	360	103	500	494	98.9
Hydromorphone	200	197	98.5	350	350.5	99.9	500	501	100
Codeine	200	193.5	96.8	350	349.5	99.9	500	503	100.5
Hydrocodone	200	189.5	94.8	350	368.5	105.5	500	491.5	98.2
Oxymorphone	200	189.5	94.8	350	371.5	106.5	500	488.5	97.75
Oxycodone	200	194	96.95	350	353	100.5	500	500.5	100
6-MAM	200	194	97	350	366.5	104.5	500	490.5	98.05
Morphine-3-Glucuronide	400	395.5	98.9	700	704.5	100.5	1000	997	99.7
Morphine-6-Glucuronide	400	392	97.9	700	725	103.5	1000	982.5	98.25
Norfentanyl	20	20.1	100.4	35	35.4	101	50	49.75	99.45
Fentanyl	20	19.3	96.45	35	35.8	102.5	50	49.7	99.35
Norbuprenorphine	10	9.695	96.95	17.5	18.4	105	25	24.5	98
Buprenorphine	10	9.87	98.7	17.5	17.7	101.5	25	24.9	99.6

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	12.55	125.5	200	232.5	116	0	0	0	0	0	0
Hydromorphone	10	12.3	123	200	220.5	110	0	0	0	0	0	0
Codeine	10	11.35	113.5	200	210.5	105.5	0	0	0	0	0	0
Hydrocodone	10	11.55	115.5	200	217	108.5	0	0	0	0	0	0
Oxymorphone	10	13.05	130.5	200	241	120.5	0	0	0	0	0	0
Oxycodone	10	10.9	109	200	208	104	0	0	0	0	0	0
6-MAM	10	10.65	106.5	200	212.5	106	0	0.016	0	0	-	-
Morphine-3-Glucuronide	20	19.3	96.6	400	383	95.65	0	0	0	0	0.1895	0
Morphine-6-Glucuronide	20	21.6	108.5	400	417.5	104.5	0	0	0	0	0	0
Norfentanyl	1	1.06	106	20	20.6	103	0	0.002365	0	0	0.000855	0
Fentanyl	1	1.15	115	20	20.75	103.5	0	0	0	0	0.074	0
Norbuprenorphine	1	1.2	120	20	22.5	112.5	0	0.00785	0	0	0.115	0
Buprenorphine	1	2.005	200.5	20	27.55	138	0	0.01082	0	0	0.0704	0

Table 6. Results from 021315 extraction of calf liver 1:9 dilution fortified with known amount of opiates
*The calculated concentrations are averages from two transitions for each analyte

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	13.45	134.5	100	152.5	152.5	200	364.5	182.5
Hydromorphone	10	7.61	76.1	100	90.45	90.45	200	201.5	100.5
Codeine	10	8.165	81.65	100	91.15	91.15	200	203.5	101.5
Hydrocodone	10	7.64	76.4	100	87.75	87.75	200	194.5	97.15
Oxymorphone	10	7.85	78.5	100	85.7	85.7	200	191.5	95.9
Oxycodone	10	8.06	80.6	100	93.1	93.1	200	207.5	103.5
6-MAM	10	-	-	100	-	-	200	-	-
Morphine-3-Glucuronide	20	15.5	77.5	200	168	84.05	400	382	95.5
Morphine-6-Glucuronide	20	13.8	69.05	200	185.5	92.75	400	400	99.95
Norfentanyl	1	0.81	81	10	8.635	86.35	20	19.65	98.2
Fentanyl	1	0.888	88.8	10	9.02	90.2	20	20.6	102.5
Norbuprenorphine	0.5	0.1975	39.5	5	4.415	88.25	10	9.225	92.25
Buprenorphine	0.5	0.344	68.85	5	5.005	100.25	10	11.65	116.5

Table 7. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 021315 extraction for Case #1 and duplicate Case #1

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Blood	**Case #1** *Liver (1:9 dilution)	**Case #1 (duplicate)** *Liver (1:9 dilution)	Average Times Higher in Liver than in Blood
Morphine	18	253.8	243.9	13.825
Hydromorphone	0	0	0	0
Codeine	0	0	0.056	0.028
Hydrocodone	0	0	0	0
Oxymorphone	0	0	0	0
Oxycodone	0	10.62	10.26	10.440
6-MAM	0	1.186	1.059	1.123
Morphine-3-Glucuronide	190	295.650	286.65	1.532
Morphine-6-Glucuronide	30	103.050	101.25	3.405
Norfentanyl	6.4	14.940	13.905	2.254
Fentanyl	16.6	37.440	36.315	2.222
Norbuprenorphine	0	2.493	1.46	1.977
Buprenorphine	0	0.449	0.316	0.382

Table 8. Tables of calibrations from 030515 extraction for cases #1-10 involving calibrators with whole blood and 1:9 dilution of calf liver

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.645	82.4	5	5.605	112	20	19.7	98.5	50	55.3	110.5
Hydromorphone	2	1.695	84.9	5	5.54	110.5	20	20.05	100.15	50	53.3	106.5
Codeine	2	1.97	98.5	5	5.285	106	20	17.8	89.2	50	53.85	107.5
Hydrocodone	2	1.685	84.3	5	5.46	109.5	20	19.9	99.45	50	55.4	111
Oxymorphone	2	1.83	91.55	5	5.55	111	20	18.85	94.15	50	52.1	104.5
Oxycodone	2	1.64	82	5	5.59	111.5	20	19.95	99.7	50	55.15	110
Morphine-3-Glucuronide	4	3.615	90.4	10	10.6	106	40	39.1	97.75	100	109	109
Morphine-6-Glucuronide	4	3.54	88.45	10	11	110	40	38.55	96.3	100	107	107
Norfentanyl	0.2	0.221	110.5	0.5	0.5295	106	2	1.73	86.5	5	4.86	97.2
Fentanyl	0.2	0.1815	90.8	0.5	0.5425	108.5	2	1.92	95.95	5	5.445	108.5
Norbuprenorphine	0.1	0.0924	92.4	0.25	0.275	110	1	0.981	98.1	2.5	2.555	102.5
Buprenorphine	0.1	0.09355	93.55	0.25	0.284	113.5	1	0.943	94.3	2.5	2.53	101

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	191.5	95.75	350	403.5	115.5	500	503.5	101
Hydromorphone	200	194.5	97.2	350	394.5	113	500	502	100
Codeine	200	197.5	98.7	350	401.5	114.5	500	501	100
Hydrocodone	200	191.5	95.8	350	403	115.5	500	503	101
Oxymorphone	200	198	99.05	350	423.5	121	500	500.5	100
Oxycodone	200	191.5	95.7	350	409.5	117	500	503.5	101
Morphine-3-Glucuronide	400	385.5	96.5	700	783.5	112	1000	1005	100.5
Morphine-6-Glucuronide	400	389.5	97.35	700	746	106.5	1000	1005	100.5
Norfentanyl	20	18.55	92.7	35	39.15	112	50	47.65	95.4
Fentanyl	20	18.05	90.2	35	38.1	108.5	50	48.5	97
Norbuprenorphine	10	9.16	91.6	17.5	19.05	109	25	24.25	97.05
Buprenorphine	10	8.915	89.15	17.5	19.75	112.5	25	23.85	95.4

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	13.85	138.5	200	252	126	0	0	0	0	0	0
Hydromorphone	10	12.45	124.5	200	222.5	111.25	0	0	0	0	0	0
Codeine	10	10.75	107.5	200	162.5	81.25	0	0.1555	0	0	0.168	0
Hydrocodone	10	12.45	124.5	200	228.5	114.25	0	0	0	0	0	0
Oxymorphone	10	12	120	200	261	130.5	0	0.03215	0	0	0.0345	0
Oxycodone	10	12.95	129.5	200	227.5	113.75	0	0	0	0	0	0
Morphine-3-Glucuronide	20	21.25	106.25	400	430.5	107.625	0	0	0	0	1.185	0
Morphine-6-Glucuronide	20	22.2	111	400	427	106.75	0	0.097	0	0	0.089	0
Norfentanyl	1	1.12	112	20	22.85	114.25	0	0.031	0	0	0.081935	0
Fentanyl	1	1.51	151	20	22.25	111.25	0	0	0	0	0.2085	0
Norbuprenorphine	1	1.31	131	20	22.65	113.25	0	0.0132	0	0	0.01705	0
Buprenorphine	1	1.1	110	20	20.55	102.75	0	0.023	0	0	0.04635	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	6.98	69.8	100	77.7	77.7	200	171	85.5
Hydromorphone	10	8	80	100	80.75	80.75	200	176.5	88.25
Codeine	10	8.3	83	100	68.3	68.3	200	137	68.5
Hydrocodone	10	8.68	86.8	100	79.15	79.15	200	166.5	83.25
Oxymorphone	10	7.635	76.35	100	79.5	79.5	200	185.5	92.75
Oxycodone	10	9.345	93.45	100	85.3	85.3	200	178	89
Morphine-3-Glucuronide	20	10.85	54.25	200	90.25	45.125	400	188.5	47.125
Morphine-6-Glucuronide	20	10.31	51.55	200	91.15	45.575	400	195.5	48.875
Norfentanyl	1	0.8945	89.45	10	7.925	79.25	20	15.9	79.5
Fentanyl	1	0.9885	98.85	10	8.095	80.95	20	16.9	84.5
Norbuprenorphine	0.5	0.85	170	5	7.905	158.1	10	16.65	166.5
Buprenorphine	0.5	0.492	98.4	5	5.845	116.9	10	13.55	135.5

Table 9. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 030515 extraction for Cases #1-10. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #1		Times higher in	Case #2		Times higher in	Case #3		Times higher in	Case #4		Times higher in	Case #5		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine	18	237.15	13.175		2.1105	2.1105	100	143.55	1.4355	180	515.25	2.8625		0.2691	0.2691
Hydromorphone		0.54	0.54		51.255	51.255		0	0		0	0		0.306	0.306
Codeine		4.4685	4.4685		168.75	168.75	6	5.9895	0.99825	11	20.97	1.906363636		1.3725	1.3725
Hydrocodone		0	0		0	0		0	0		0	0		0	0
Oxymorphone		0.7209	0.7209	5	83.205	16.641		0.40005	0.40005		0.8577	0.8577		0.59175	0.59175
Oxycodone		7.965	7.965	730	1318.5	1.806164384		0	0		0	0		0	0
Morphine-3-Glucuronide	190	284.4	1.496842105		23.445	23.445	250	557.1	2.2284	330	958.5	2.904545455	12	37.98	3.165
Morphine-6-Glucuronide	30	91.8	3.06		78.2775	78.2775	50	262.8	5.256	30	720.45	24.015		211.356	211.356
Norfentanyl	6.4	16.155	2.52421875		0.15957	0.15957		0.2709	0.2709	0.27	2.0385	7.55		0.27045	0.27045
Fentanyl	16.6	31.905	1.921987952		0.05526	0.05526		0	0	3.1	44.595	14.38548387		0.3177	0.3177
Norbuprenorphine		5.44005	5.44005		5.913	5.913		0.205695	0.205695		2.3787	2.3787		0	0
Buprenorphine		0.5769	0.5769		0.3276	0.3276		0.2304	0.2304		0.3042	0.3042		0.30555	0.30555

Analytes	Case #6		Times higher in	Case #7		Times higher in	Case #8		Times higher in	Case #9		Times higher in	Case #10		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0		0	0	1700	334.8	0.196941176		0	0
Hydromorphone		14.85	14.85		5.382	5.382		28.08	28.08		5.022	5.022	10	46.485	4.6485
Codeine		17.37	17.37		58.905	58.905		50.445	50.445		4.6845	4.6845		418.95	418.95
Hydrocodone		0	0		0	0		0	0		0	0	6	91.8	15.3
Oxymorphone		9.945	9.945		20.07	20.07		3.087	3.087		1.026	1.026	210	281.25	1.339285714
Oxycodone	130	153	1.176923077	420	527.4	1.255714286	220	1003.5	4.561363636	140	8.289	0.059207143	7900	0	0
Morphine-3-Glucuronide		27.72	27.72		31.86	31.86		49.5	49.5	380	164.79	0.433657895		143.1	143.1
Morphine-6-Glucuronide		67.8285	67.8285		78.1425	78.1425		227.997	227.997		72.9	72.9		1.683	1.683
Norfentanyl		0.147555	0.147555		0.15921	0.15921		0.65007	0.65007		1.3905	1.3905		0.90855	0.90855
Fentanyl		0	0		0.1638	0.1638		1.863	1.863		2.56635	2.56635		118.97145	118.97145
Norbuprenorphine		0.2664	0.2664		4.626	4.626		1.39995	1.39995		8.487	8.487		0	0
Buprenorphine		0.2493	0.2493		0.33075	0.33075		0.5607	0.5607		1.7685	1.7685		343.512	343.512

Table 10. Tables of calibrations from 032015 extraction for cases #11-20 involving calibrators with whole blood and 1:9 dilution of calf liver. Standard 6 has been excluded due to errors.
*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.79	89.5	5	5.52	110.5	20	19.05	95.2
Hydromorphone	2	1.875	93.7	5	5.45	109	20	18.8	93.9
Codeine	2	2.08	104	5	5.125	102.5	20	18	90.1
Hydrocodone	2	1.92	95.85	5	5.365	107.5	20	18.65	93.4
Oxymorphone	2	1.79	89.4	5	5.515	110	20	18.95	94.7
Oxycodone	2	1.75	87.45	5	5.485	110	20	19.5	97.35
Morphine-3-Glucuronide	4	3.83	95.8	10	10.7	107	40	37.3	93.35
Morphine-6-Glucuronide	4	3.825	95.7	10	10.7	107	40	37	92.5
Norfentanyl	0.2	0.196	97.9	0.5	0.528	105.5	2	1.88	94
Fentanyl	0.2	0.186	92.95	0.5	0.5395	108	2	1.9	94.8
Norbuprenorphine	0.1	0.08395	83.95	0.25	0.2985	119.5	1	0.9585	95.85
Buprenorphine	0.1	0.0886	88.6	0.25	0.281	112.5	1	0.9445	94.45

Analytes	Standard 4			Standard 5			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	50	53.35	107	200	195.5	97.7	500	502	100
Hydromorphone	50	52.1	104	200	198	99.05	500	501	100
Codeine	50	51.65	103	200	200	100.05	500	499.5	99.9
Hydrocodone	50	52.05	104.5	200	198.5	99.15	500	501	100
Oxymorphone	50	53.75	107.5	200	195.5	97.75	500	501.5	100
Oxycodone	50	54.05	108	200	193.5	96.9	500	503	101
Morphine-3-Glucuronide	100	105	105	400	395.5	98.85	1000	1000	100
Morphine-6-Glucuronide	100	106	106	400	394	98.5	1000	1000	100
Norfentanyl	5	5.135	102.5	20	19.95	99.55	50	50.05	99.95
Fentanyl	5	5.295	106	20	19.65	98.25	50	50.2	100
Norbuprenorphine	2.5	2.53	101.05	10	9.965	99.65	25	25	100
Buprenorphine	2.5	2.655	106	10	9.82	98.2	25	25.1	100

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	11.9	119	200	209.5	104.5	0	0	0	0	0	0
Hydromorphone	10	11	110	200	212.5	106.5	0	0	0	0	0	0
Codeine	10	8.855	88.55	200	133.5	66.9	0	0.4365	0	0	0.429	0
Hydrocodone	10	10.5	105	200	198.5	99.25	0	0.01635	0	0	0.0127	0
Oxymorphone	10	11.7	117	200	232	116	0	0	0	0	0	0
Oxycodone	10	11.6	116	200	202	100.85	0	0	0	0	0	0
Morphine-3-Glucuronide	20	19.55	97.8	400	390.5	97.65	0	0	0	0	0.00665	0
Morphine-6-Glucuronide	20	19.9	99.55	400	382	95.4	0	0.089	0	0	0.0895	0
Norfentanyl	1	1.025	102.5	20	21.5	107.5	0	0.00605	0	0	0.003955	0
Fentanyl	1	1.07	107	20	19	95.05	0	0	0	0	0.119	0
Norbuprenorphine	1	1.21	121	20	25	125	0	0.00695	0	0	0.02478	0
Buprenorphine	1	1.145	114.5	20	21.85	109.5	0	0.003155	0	0	0.03605	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	8.285	82.85	100	72.9	72.9	200	168.5	84.25
Hydromorphone	10	8.455	84.55	100	81.55	81.55	200	183	91.5
Codeine	10	7.69	76.9	100	60.35	60.35	200	122.5	61.25
Hydrocodone	10	8.635	86.35	100	77.95	77.95	200	168.5	84.25
Oxymorphone	10	9.04	90.4	100	82.25	82.25	200	197.5	98.75
Oxycodone	10	9.72	97.2	100	87.9	87.9	200	185.5	92.75
Morphine-3-Glucuronide	20	9.18	45.9	200	84.25	42.125	400	182.5	45.625
Morphine-6-Glucuronide	20	9.275	46.375	200	75.95	37.975	400	174	43.5
Norfentanyl	1	0.872	87.2	10	7.78	77.8	20	16.45	82.25
Fentanyl	1	0.905	90.5	10	7.515	75.15	20	16.25	81.25
Norbuprenorphine	0.5	0.884	176.8	5	8.15	163	10	20.45	204.5
Buprenorphine	0.5	0.9015	180.3	5	7.56	151.2	10	18.4	184

Table 11. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 032015 extraction for cases #11-20. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #11		Times higher in	Case #12		Times higher in	Case #13		Times higher in	Case #14		Times higher in	Case #15		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0	70	242.1	3.458571429		4.239	4.239		0	0
Hydromorphone		0.80775	0.80775	70	12.735	0.181928571		3.078	3.078		6.741	6.741		0	0
Codeine		3.9735	3.9735		4.761	4.761		7.965	7.965		4.581	4.581		3.8655	3.8655
Hydrocodone	30	277.65	9.255		3.456	3.456		0	0		0	0		0.1899	0.1899
Oxymorphone		0	0		0	0		10.314	10.314	20	105.75	5.2875	30	67.635	2.2545
Oxycodone		0	0		0	0		0	0		0.4833	0.4833		0	0
Morphine-3-Glucuronide		28.35	28.35		85.05	85.05	120	241.2	2.01		5.346	5.346		16.8795	16.8795
Morphine-6-Glucuronide		42.0435	42.0435		62.3115	62.3115		62.82	62.82		73.1475	73.1475		0.972	0.972
Norfentanyl		0.05373	0.05373	40.2	27.495	0.683955224	0.42	1.674	3.985714286		0.03114	0.03114	0.45	2.295	5.1
Fentanyl		0.7659	0.7659	112.2	85.14	0.758823529	21.2	210.15	9.912735849		0.3051	0.3051	1.9	7.1865	3.782368421
Norbuprenorphine		28.89	28.89		0.954	0.954		1.2915	1.2915		1.899	1.899		0.4059	0.4059
Buprenorphine		1.34865	1.34865		1.26315	1.26315		0.234	0.234		0.05598	0.05598		0.01791	0.01791

Analytes	Case #16		Times higher in	Case #17		Times higher in	Case #18		Times higher in	Case #19		Times higher in	Case #20		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0		0	0	460	1386	3.013043478	200	1048.5	5.2425
Hydromorphone		0	0		0.63495	0.63495		3.6675	3.6675		3.546	3.546		0.19377	0.19377
Codeine		3.8925	3.8925		3.978	3.978		3.861	3.861	18	40.41	2.245		5.1255	5.1255
Hydrocodone		0.1206	0.1206	30	152.1	5.07		3.6495	3.6495	30	94.5	3.15		0.08784	0.08784
Oxymorphone		0	0		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		11.25	11.25		17.2089	17.2089		26.73	26.73		1660.5	1660.5		1476	1476
Morphine-6-Glucuronide		59.625	59.625		25.605	25.605		35.0505	35.0505		700.2	700.2		551.7	551.7
Norfentanyl	0.53	1.9485	3.676415094		0.0468	0.0468		9.855	9.855		0.04194	0.04194		0.04239	0.04239
Fentanyl	5.7	17.595	3.086842105		0.0441	0.0441	5.8	155.7	26.84482759		0.6768	0.6768		0	0
Norbuprenorphine		0.70245	0.70245		0.29376	0.29376		0.16785	0.16785		0.18711	0.18711		1.70685	1.70685
Buprenorphine		0.0279	0.0279		0.05004	0.05004		0.03429	0.03429		0.07425	0.07425		0.909	0.909

Table 12. Tables of calibrations from 032815 extraction for cases #21-29 involving calibrators with whole blood and 1:9 dilution of calf liver.

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.525	76.45	5	5.53	110.5	20	20.9	104.5	50	57	114
Hydromorphone	2	1.54	76.9	5	5.43	108.5	20	20.9	104.5	50	57.75	115.5
Codeine	2	1.875	93.75	5	5.165	103	20	19.05	95.4	50	55.55	111.5
Hydrocodone	2	1.42	70.95	5	5.525	110.5	20	21.7	108.5	50	58.65	117.5
Oxymorphone	2	1.6	80.05	5	5.475	109.5	20	20.05	99.95	50	58.05	116
Oxycodone	2	1.54	76.95	5	5.455	109	20	21.15	106	50	56.9	114
Morphine-3-Glucuronide	4	3.3	82.55	10	10.75	107.5	40	40.95	102.5	100	112.5	112.5
Morphine-6-Glucuronide	4	3.335	83.35	10	10.7	107	40	40.25	100.45	100	114	114
Norfentanyl	0.2	0.173	86.55	0.5	0.5325	106.5	2	2.01	100.2	5	5.545	111
Fentanyl	0.2	0.1575	78.75	0.5	0.543	109	2	2.08	104	5	5.715	114.5
Norbuprenorphine	0.1	0.082	82	0.25	0.2615	104.7	1	1.0625	106.25	2.5	2.8	112
Buprenorphine	0.1	0.08185	81.85	0.25	0.2635	105.35	1	1.015	101.5	2.5	2.925	117

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	186.5	93.4	350	350.5	100	500	505	101
Hydromorphone	200	187.5	93.85	350	349	99.6	500	505	101
Codeine	200	188.5	94.4	350	358.5	102.5	500	498	99.55
Hydrocodone	200	182	91.1	350	353	101	500	504.5	100.5
Oxymorphone	200	182	91.05	350	361.5	103.5	500	498	99.55
Oxycodone	200	186.5	93.3	350	350.5	100.2	500	505	101
Morphine-3-Glucuronide	400	380.5	95.1	700	694.5	99.15	1000	1015	101.5
Morphine-6-Glucuronide	400	376	94.1	700	700	100.2	1000	1015	101.5
Norfentanyl	20	18.75	93.6	35	35.9	102.5	50	49.85	99.55
Fentanyl	20	18.85	94.1	35	34.65	99.05	50	50.75	101.5
Norbuprenorphine	10	9.415	94.15	17.5	17.55	100.3	25	25.15	100.5
Buprenorphine	10	9.29	92.9	17.5	17.65	100.9	25	25.15	100.5

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	12.95	129.5	200	225.5	113	0	0	0	0	0	0
Hydromorphone	10	12.3	123	200	227.5	114	0	0	0	0	0	0
Codeine	10	9.725	97.25	200	148	74	0	0.00905	0	0	0.0116	0
Hydrocodone	10	13	130	200	232	115.5	0	0	0	0	0	0
Oxymorphone	10	12.65	126.5	200	245	123	0	0	0	0	0	0
Oxycodone	10	13.95	139.5	200	234	117	0	0	0	0	0	0
Morphine-3-Glucuronide	20	21.1	105	400	439	109.5	0	0	0	0	3.735	0
Morphine-6-Glucuronide	20	22.2	111	400	428	107	0	0	0	0	0	0
Norfentanyl	1	1.13	113	20	22.65	113.5	0	0	0	0	0	0
Fentanyl	1	1.195	119.5	20	20.8	104	0	0	0	0	0.0695	0
Norbuprenorphine	1	1.325	132.5	20	25.3	126.5	0	0	0	0	0	0
Buprenorphine	1	1.21	121	20	22.2	111	0	0	0	0	0.00207	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	8.195	81.95	100	80.25	80.25	200	167.5	83.75
Hydromorphone	10	8.915	89.15	100	86.4	86.4	200	179.5	89.75
Codeine	10	7.665	76.65	100	65.1	65.1	200	125.5	62.75
Hydrocodone	10	9.2	92	100	91.85	91.85	200	189	94.5
Oxymorphone	10	9.13	91.3	100	85.2	85.2	200	167.5	83.75
Oxycodone	10	9.71	97.1	100	95.55	95.55	200	193	96.5
Morphine-3-Glucuronide	20	14	70	200	98.75	49.375	400	197.5	49.375
Morphine-6-Glucuronide	20	14.2	71	200	93.85	46.925	400	180	45
Norfentanyl	1	0.9055	90.55	10	7.91	79.1	20	16.85	84.25
Fentanyl	1	0.922	92.2	10	8.03	80.3	20	16.15	80.75
Norbuprenorphine	0.5	0.9155	183.1	5	8.9	178	10	18.65	186.5
Buprenorphine	0.5	0.913	182.6	5	8.265	165.3	10	18.1	181

Table 13. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 032815 extraction for cases #21-29. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #21		Times higher in	Case #22		Times higher in	Case #23		Times higher in	Case #24		Times higher in	Case #25		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine	230	396.45	1.723695652		1.836	1.836	18	113.85	6.325	320	3982.5	12.4453125		0	0
Hydromorphone		0	0		0	0		0	0	0	0.4581	0.4581	5	8.307	1.6614
Codeine	9	17.01	1.89		0.27585	0.27585		0.6786	0.6786	0	7.5645	7.5645		0.6291	0.6291
Hydrocodone		0	0		0	0		0	0	0	0	0	420	845.1	2.012142857
Oxymorphone		0	0		0	0		0	0	0	0	0		0	0
Oxycodone		0	0		0	0		0	0	10	27.135	2.7135		0	0
Morphine-3-Glucuronide		879.3	879.3		29.8935	29.8935		183.15	183.15	unable to analyze	4612.5	-		9.36	9.36
Morphine-6-Glucuronide		542.7	542.7		0	0		129.24	129.24	380	1633.5	4.298684211		0	0
Norfentanyl		0	0		0	0	2.2	15.03	6.831818182		0	0		0	0
Fentanyl		0	0	4.5	59.715	13.27	14.8	142.2	9.608108108		0	0		0	0
Norbuprenorphine		2.511	2.511		0.84015	0.84015		0.01593	0.01593		0.9036	0.9036		2.7783	2.7783
Buprenorphine		0	0		0	0		0	0		0	0		0	0

Analytes	Case #26		Times higher in	Case #27		Times higher in	Case #28		Times higher in	Case #29		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine	260	815.85	3.137884615		0	0	2	8.577	4.2885	3	4.374	1.458
Hydromorphone		0	0		0	0		0	0		0	0
Codeine	6	12.69	2.115		0.162	0.162		0.2088	0.2088		0.2655	0.2655
Hydrocodone		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide	180	583.2	3.24		25.92	25.92		14.85	14.85	20	28.665	1.43325
Morphine-6-Glucuronide	60	303.75	5.0625		0	0		0	0		1.611	1.611
Norfentanyl	1.2	4.3695	3.64125	0.49	3.7395	7.631632653	5.7	35.955	6.307894737		0.13275	0.13275
Fentanyl	10.4	106.2	10.21153846	37.8	182.25	4.821428571	35.8	265.5	7.416201117	2.7	62.55	23.16666667
Norbuprenorphine		0.8901	0.8901		0	0	2.4	20.115	8.38125		11.07	11.07
Buprenorphine		4.059	4.059		0	0	0.44	2.1015	4.776136364		0	0

Table 14. Tables of calibrations from 041815 extraction for cases #30-38 involving calibrators with whole blood and 1:9 dilution of calf liver.

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.335	66.8	5	5.6	112	20	22	110	50	59.15	118.5
Hydromorphone	2	1.505	75.15	5	5.495	109.5	20	21.25	106.5	50	57.2	114
Codeine	2	1.69	84.5	5	5.175	103.5	20	20.3	101.85	50	57.25	114.5
Hydrocodone	2	1.465	73.25	5	5.455	109	20	21.85	109.5	50	57.05	114
Oxymorphone	2	1.395	69.7	5	5.59	112	20	21.6	108	50	58.4	117
Oxycodone	2	1.335	66.7	5	5.58	111.5	20	22.6	113	50	57.9	116
Morphine-3-Glucuronide	4	3.2	80.05	10	10.9	109	40	41.55	104	100	111.5	111.5
Morphine-6-Glucuronide	4	3.25	81.2	10	10.65	106.5	40	41.4	103.5	100	114	114
Norfentanyl	0.2	0.1835	91.75	0.5	0.531	106.5	2	1.945	97.15	5	5.35	107
Fentanyl	0.2	0.137	68.45	0.5	0.561	112	2	2.155	108	5	5.96	119
Norbuprenorphine	0.1	0.06435	64.35	0.25	0.2975	118.5	1	1.03	103	2.5	3.01	120.5
Buprenorphine	0.1	0.0637	63.7	0.25	0.29	116	1	1.055	105.5	2.5	3.06	122.5

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	186	92.9	350	340	97.25	500	513.5	103
Hydromorphone	200	187.5	93.7	350	346.5	99.15	500	508	101.5
Codeine	200	192	96	350	345.5	98.7	500	505	101
Hydrocodone	200	190	94.95	350	337.5	96.45	500	515.5	103
Oxymorphone	200	186.5	93.3	350	347	99.35	500	506.5	101.5
Oxycodone	200	186.5	93.4	350	338	96.5	500	518	103.5
Morphine-3-Glucuronide	400	380	95.1	700	691.5	98.8	1000	1020	102
Morphine-6-Glucuronide	400	382.5	95.65	700	676	96.55	1000	1035	103.5
Norfentanyl	20	19.5	97.5	35	35.1	100	50	50.1	100
Fentanyl	20	18.35	91.8	35	34.1	97.5	50	51.7	103.5
Norbuprenorphine	10	9.33	93.3	17.5	17.2	98.05	25	25.45	102
Buprenorphine	10	9.225	92.25	17.5	17.25	98.4	25	25.45	101.5

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	13.65	136.5	200	239	119.5	0	0	0	0	0.46	0
Hydromorphone	10	11.6	116	200	221	110.5	0	0	0	0	2.645	0
Codeine	10	8.95	89.5	200	131.5	65.65	0	0	0	0	0.03935	0
Hydrocodone	10	12.25	122.5	200	222	111	0	0	0	0	0	0
Oxymorphone	10	12.3	123	200	225.5	112.5	0	0	0	0	1.96	0
Oxycodone	10	12.95	129.5	200	216.5	108	0	0	0	0	0	0
Morphine-3-Glucuronide	20	21	105	400	402	100.5	0	0	0	0	15.7	0
Morphine-6-Glucuronide	20	20.25	101.45	400	383.5	95.85	0	0	0	0	0	0
Norfentanyl	1	1.115	111.5	20	21.35	106.5	0	0	0	0	0.01115	0
Fentanyl	1	1.255	125.5	20	22.1	110.5	0	0	0	0	0.072	0
Norbuprenorphine	1	1.3	130	20	23.75	119	0	0	0	0	0.7645	0
Buprenorphine	1	1.185	118.5	20	23.05	115.5	0	0	0	0	0.024815	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	5.805	58.05	100	63.65	63.65	200	168.5	84.25
Hydromorphone	10	5.77	57.7	100	63.35	63.35	200	172	86
Codeine	10	5.945	59.45	100	54.15	54.15	200	132	66
Hydrocodone	10	7.08	70.8	100	73.7	73.7	200	184.5	92.25
Oxymorphone	10	6.835	68.35	100	76.65	76.65	200	192.5	96.25
Oxycodone	10	6.345	63.45	100	62.15	62.15	200	190	95
Morphine-3-Glucuronide	20	**7.03	35.15	200	89.95	44.975	400	198.5	49.625
Morphine-6-Glucuronide	20	**6.06	30.3	200	67.4	33.7	400	183.5	45.875
Norfentanyl	1	0.6655	66.55	10	6.44	64.4	20	15.4	77
Fentanyl	1	0.8465	84.65	10	7.865	78.65	20	19.75	98.75
Norbuprenorphine	0.5	1.44	288	5	6.92	138.4	10	17.75	177.5
Buprenorphine	0.5	0.7195	143.9	5	6.35	127	10	15.45	154.5

**Used the second transition only for LPC (Beef Liver 1:9) Morphine-3-glucuronide and Morphine-6-glucuronide

Table 15. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 041815 extraction for cases #30-38. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #30		Times higher in	Case #31		Times higher in	Case #32		Times higher in	Case #33		Times higher in	Case #34		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0	30	87.435	2.9145	20	42.66	2.133		0	0	13	42.84	3.295384615
Hydromorphone	230	0	0		0	0		0	0		0	0		0	0
Codeine		1.089	1.089		0	0		1.674	1.674		0	0		0	0
Hydrocodone		426.15	426.15		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		0	0	130	85.59	0.658384615	370	119.25	0.322297297		0	0	18	29.925	1.6625
Morphine-6-Glucuronide		0	0	15	11.25	0.75	60	19.44	0.324		0	0		6.723	6.723
Norfentanyl		0	0		0	0	1.5	4.2705	2.847	0.78	3.042	3.9	2.7	12.015	4.45
Fentanyl		0	0		0	0	14.4	75.51	5.24375	12.1	132.3	10.9338843	44.6	348.3	7.80941704
Norbuprenorphine		0.4887	0.4887		0.1143	0.1143	2.5	14.85	5.94		5.265	5.265		1.557	1.557
Buprenorphine		0	0		0	0	1.7	4.581	2.694705882		0.2547	0.2547		0	0

Analytes	Case #35		Times higher in	Case #36		Times higher in	Case #37		Times higher in	Case #38		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0		0	0	40	150.3	3.7575
Hydromorphone		0	0		0	0		0	0		0	0
Codeine		0	0		0	0		0	0	2	3.033	1.5165
Hydrocodone		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		0	0		1.836	1.836		0	0	290	386.55	1.332931034
Morphine-6-Glucuronide		0	0		0	0		0	0		113.85	113.85
Norfentanyl	1.9	5.562	2.927368421	2.9	30.555	10.5362069	0.51	8.6175	16.89705882	0.97	6.228	6.420618557
Fentanyl	11.2	97.65	8.71875	38.7	410.4	10.60465116	2.4	78.39	32.6625	8.1	205.65	25.38888889
Norbuprenorphine		2.259	2.259	1.1	11.97	10.88181818		2.052	2.052		2.52	2.52
Buprenorphine		0	0	0.78	1.566	2.007692308		0.003213	0.003213		0.3987	0.3987

Table 16. Tables of calibrations from 060915 extraction for cases #39-52 involving calibrators with whole blood and 1:9 dilution of calf liver.

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.13	56.45	5	6.095	121.5	20	20.2	100.8	50	64.55	129
Hydromorphone	2	1.345	67.4	5	5.725	114.5	20	20.3	101.5	50	61.65	123
Codeine	2	1.71	85.5	5	5.145	102.5	20	19.25	96.1	50	60.2	120.5
Hydrocodone	2	1.44	72	5	5.785	115.5	20	19.3	96.4	50	61.3	123
Oxymorphone	2	1.1705	58.6	5	6.055	121	20	20.2	100.7	50	64.05	128
Oxycodone	2	1.29	64.6	5	5.965	119.5	20	19.4	97.35	50	63.75	127.5
Morphine-3-Glucuronide	4	2.895	72.45	10	11.7	117	40	39.05	97.65	100	119	119
Morphine-6-Glucuronide	4	3.04	76	10	10.95	109.5	40	39.4	98.5	100	123	123
Norfentanyl	0.2	0.1605	80.3	0.5	0.5655	113	2	1.875	93.6	5	5.89	118
Fentanyl	0.2	0.1415	70.85	0.5	0.575	115	2	1.975	98.6	5	6.13	123
Norbuprenorphine	0.1	0.0667	66.7	0.25	0.2965	118.5	1	0.975	97.5	2.5	3.095	124
Buprenorphine	0.1	0.06215	62.15	0.25	0.3	120.5	1	0.9725	97.25	2.5	3.195	127.5

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	189	94.35	350	324.5	92.6	500	523	104.5
Hydromorphone	200	189.5	94.7	350	335.5	95.9	500	513	103
Codeine	200	192	95.85	350	342	97.7	500	507	101.5
Hydrocodone	200	188	94.05	350	338.5	96.8	500	512.5	102.5
Oxymorphone	200	181	90.5	350	347.5	99.65	500	507	101.45
Oxycodone	200	183	91.7	350	340.5	97.25	500	513	102.5
Morphine-3-Glucuronide	400	373	93.2	700	696	99.35	1000	1015	101.5
Morphine-6-Glucuronide	400	377.5	94.35	700	670	95.7	1000	1035	103.5
Norfentanyl	20	18.85	94.3	35	35.15	100.3	50	50.25	100.25
Fentanyl	20	18.7	93.55	35	33.95	97.05	50	51.2	102.5
Norbuprenorphine	10	9.47	94.7	17.5	16.9	96.65	25	25.55	102.5
Buprenorphine	10	9.525	95.25	17.5	16.5	94.4	25	25.8	103

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	14.5	145	200	260	130	0	0	0	0	0	0
Hydromorphone	10	12.65	126.5	200	241.5	121	0	0	0	0	0	0
Codeine	10	8.965	89.65	200	139	69.3	0	0	0	0	0	0
Hydrocodone	10	12.15	121.5	200	226.5	113	0	0	0	0	0	0
Oxymorphone	10	12.95	129.5	200	248	123.5	0	0	0	0	0	0
Oxycodone	10	12.9	129	200	241	120.5	0	0	0	0	0	0
Morphine-3-Glucuronide	20	21.9	110	400	438	109.5	0	0	0	0	5.3	0
Morphine-6-Glucuronide	20	21.75	109	400	435.5	109	0	0	0	0	0	0
Norfentanyl	1	1.15	115	20	22.6	113	0	0	0	0	0.012	0
Fentanyl	1	1.315	131.5	20	23.6	118	0	0	0	0	0.575	0
Norbuprenorphine	1	1.47	147	20	25.6	127.5	0	0	0	0	0.459	0
Buprenorphine	1	1.615	161.5	20	27.25	136.5	0	0.003655	0	0	0.5398	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	6.75	67.5	100	88.45	88.45	200	212	106
Hydromorphone	10	7.7	77	100	94.2	94.2	200	206.5	103.25
Codeine	10	7.58	75.8	100	70.65	70.65	200	140.5	70.25
Hydrocodone	10	7.39	73.9	100	82.9	82.9	200	185.5	92.75
Oxymorphone	10	7.7	77	100	89.5	89.5	200	208.5	104.25
Oxycodone	10	9.755	97.55	100	93.15	93.15	200	209	104.5
Morphine-3-Glucuronide	20	16.63	83.15	200	105	52.5	400	217.5	54.375
Morphine-6-Glucuronide	20	5.115	25.575	200	85.2	42.6	400	183.5	45.875
Norfentanyl	1	0.9825	98.25	10	9.055	90.55	20	18.7	93.5
Fentanyl	1	**0.8333	83.3	10	9.58	95.8	20	21.3	106.5
Norbuprenorphine	0.5	1.345	269	5	8.12	162.4	10	20.85	208.5
Buprenorphine	0.5	0.78	156	5	6.81	136.2	10	18.6	186

**Used the second transition only for LPC (Beef Liver 1:9) Fentanyl

Table 17. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 060915 extraction for cases #39-52. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #39		Times higher in	Case #40		Times higher in	Case #41		Times higher in	Case #42		Times higher in	Case #43		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine			0	12	25.83	2.1525			0	5	3.564	0.7128		0	0
Hydromorphone			0		0	0			0		0	0		0	0
Codeine			0		0.1395	0.1395			0		0	0		0	0
Hydrocodone			0	4	3.6135	0.903375			0		0	0	70	177.3	2.532857143
Oxymorphone			0		0	0			0		0	0		0	0
Oxycodone			0		0	0			0		0	0		0	0
Morphine-3-Glucuronide			0	40	43.65	1.09125		0	0	17	14.04	0.825882353		0	0
Morphine-6-Glucuronide			0	5	13.68	2.736		0	0		0	0		0	0
Norfentanyl	6	0.106425	0.0177375	0.62	2.2995	3.708870968	0.51	3.3705	6.608823529	4.6	23.445	5.09673913	0.33	2.313	7.009090909
Fentanyl		38.43	38.43	8.1	66.96	8.266666667	7.3	167.85	22.99315068	47.6	530.1	11.13655462	6.1	67.77	11.10983607
Norbuprenorphine		5.274	5.274		9.144	9.144		8.559	8.559	1.2	10.35	8.625		4.741245	4.741245
Buprenorphine		1.755	1.755		0.73755	0.73755		0.45765	0.45765	0.2	3.6765	18.3825		0.44325	0.44325

Analytes	Case #44		Times higher in	Case #45		Times higher in	Case #46		Times higher in	Case #47		Times higher in	Case #48		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0	13	21.6	1.661538462	10	38.43	3.843	210	475.65	2.265	3	0	0
Hydromorphone		0	0		0	0		0	0		0	0		0	0
Codeine		0	0		0	0		0	0		32.31	32.31		0	0
Hydrocodone	17	66.51	3.912352941		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0	4	2.088	0.522		0	0
Oxycodone		0	0		0	0		0	0	90	101.25	1.125		0	0
Morphine-3-Glucuronide		0	0		62.37	62.37	30	17.1	0.57	330	990	3	16	0.6174	0.0385875
Morphine-6-Glucuronide		0	0		12.15	12.15	6	9.9	1.65	60	577.8	9.63		0	0
Norfentanyl	0.67	7.452	11.12238806		0	0	15.9	50.13	3.152830189	0.92	4.4325	4.817934783	13.2	41.895	3.173863636
Fentanyl	6.2	93.6	15.09677419		3.6675	3.6675	49.4	538.2	10.89473684	72	582.3	8.0875	39.6	226.35	5.715909091
Norbuprenorphine		5.553	5.553		3.447	3.447		0.5868	0.5868		2.236185	2.236185		1.449	1.449
Buprenorphine		0.1575	0.1575		0.130635	0.130635		0.271485	0.271485		0.35415	0.35415		0.06057	0.06057

Analytes	Case #49		Times higher in	Case #50		Times higher in	Case #51		Times higher in	Case #52		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0	190	784.8	4.130526316	19	152.55	8.028947368
Hydromorphone		0	0		0	0		0	0		0	0
Codeine		0	0		0	0	12	16.56	1.38		0	0
Hydrocodone		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		7.605	7.605		0	0	>100	1053	> 10.53	80	103.5	1.29375
Morphine-6-Glucuronide		0	0		0	0	130	238.5	1.834615385		20.88	20.88
Norfentanyl	1.4	10.305	7.360714286	4.2	31.41	7.478571429		0	0	6.4	38.97	6.0890625
Fentanyl	6	121.95	20.325	35.7	579.15	16.22268908		4.284	4.284	18.7	275.4	14.72727273
Norbuprenorphine		2.421	2.421		0.6525	0.6525		1.1925	1.1925		4.923	4.923
Buprenorphine		0.211365	0.211365		0.30402	0.30402		0.28215	0.28215		2.331	2.331

Table 18. Tables of calibrations from 061015 extraction for cases #53-64 involving calibrators with whole blood and 1:9 dilution of calf liver.

*The calculated concentrations are averages from two transitions for each analyte

Analytes	Standard 1			Standard 2			Standard 3			Standard 4		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	2	1.265	63.2	5	6	120	20	20.3	102	50	61.6	123.5
Hydromorphone	2	1.455	72.9	5	5.68	113.5	20	19.7	98.55	50	60.75	121.5
Codeine	2	1.705	85.05	5	5.355	107	20	18.7	93.55	50	59.6	119
Hydrocodone	2	1.325	66.35	5	5.955	119	20	19.8	98.85	50	61.3	123
Oxymorphone	2	1.385	69.4	5	5.955	119.5	20	19.1	95.4	50	60.5	121
Oxycodone	2	1.55	77.5	5	5.495	110	20	19.6	98.05	50	60.8	121.5
Morphine-3-Glucuronide	4	3.165	79.1	10	11.4	114	40	37.4	93.5	100	119	119
Morphine-6-Glucuronide	4	2.955	73.85	10	11.35	113.5	40	39.85	99.6	100	119.5	119.5
Norfentanyl	0.2	0.158	79.05	0.5	0.546	109	2	1.975	98.9	5	5.94	118.5
Fentanyl	0.2	0.135	67.5	0.5	0.5825	116.5	2	2.01	100.4	5	6.185	124
Norbuprenorphine	0.1	0.07555	75.55	0.25	0.2815	112.5	1	0.9635	96.35	2.5	3.04	121.5
Buprenorphine	0.1	0.06735	67.35	0.25	0.2985	119.5	1	0.959	95.9	2.5	3.125	125

Analytes	Standard 5			Standard 6			Standard 7		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	200	184.5	92.15	350	339.5	97.05	500	515	103
Hydromorphone	200	188	94.15	350	338.5	96.85	500	512.5	102.5
Codeine	200	191.5	95.6	350	343	98	500	507.5	101.5
Hydrocodone	200	189.5	94.85	350	331.5	94.7	500	518.5	103.5
Oxymorphone	200	195.5	97.75	350	331.5	94.75	500	513.5	102.5
Oxycodone	200	186	92.85	350	342.5	97.85	500	512	102.5
Morphine-3-Glucuronide	400	377	94.2	700	684.5	97.75	1000	1025	102.5
Morphine-6-Glucuronide	400	373.5	93.35	700	683	97.55	1000	1030	103
Norfentanyl	20	18.8	93.85	35	34.75	99.35	50	50.55	101
Fentanyl	20	18.3	91.5	35	34.25	97.85	50	51.35	103
Norbuprenorphine	10	9.4	94	17.5	17.15	97.95	25	25.45	102
Buprenorphine	10	9.2	92	17.5	17.25	98.65	25	25.45	102

Analytes	Low Positive Control (whole blood)			High Positive Control (whole blood)			Negative Control (whole blood)			Negative Control Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	13.25	132.5	200	230	115	0	0	0	0	0	0
Hydromorphone	10	12.15	121.5	200	242	121	0	0	0	0	0	0
Codeine	10	9.105	91.05	200	130.5	65.25	0	0.023005	0	0	0.013	0
Hydrocodone	10	11.45	114.5	200	224	112	0	0	0	0	0	0
Oxymorphone	10	12.8	128	200	247	123.5	0	0	0	0	0	0
Oxycodone	10	12.85	128.5	200	239	119.5	0	0	0	0	0	0
Morphine-3-Glucuronide	20	20.5	102.5	400	425	106.5	0	0	0	0	4.56	0
Morphine-6-Glucuronide	20	20.9	104	400	442.5	111	0	0	0	0	0	0
Norfentanyl	1	1.195	119.5	20	22.6	113.5	0	0	0	0	0.00575	0
Fentanyl	1	1.26	126	20	23.05	115.5	0	0	0	0	0.63	0
Norbuprenorphine	1	1.35	135	20	25.3	126.5	0	0	0	0	0.06975	0
Buprenorphine	1	1.115	111.5	20	22.95	114.5	0	0	0	0	0.46099	0

Analytes	Low Positive Control Beef Liver 1:9 Dilution			Medium Positive Control Beef Liver 1:9 Dilution			High Positive Control Beef Liver 1:9 Dilution		
	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)	Analyte Concentration (mcg/L)	*Calculated Concentration (mcg/L)	Accuracy (%)
Morphine	10	8.25	82.5	100	79.6	79.6	200	207.5	103.75
Hydromorphone	10	8.24	82.4	100	77.8	77.8	200	204	102
Codeine	10	7.31	73.1	100	56.7	56.7	200	136.5	68.25
Hydrocodone	10	7.345	73.45	100	70.8	70.8	200	190.5	95.25
Oxymorphone	10	9.75	97.5	100	88.05	88.05	200	209	104.5
Oxycodone	10	9.475	94.75	100	85.5	85.5	200	219	109.5
Morphine-3-Glucuronide	20	13.415	67.075	200	92.7	46.35	400	212.5	53.125
Morphine-6-Glucuronide	20	7.945	39.725	200	80.15	40.075	400	193.5	48.375
Norfentanyl	1	0.905	90.5	10	7.095	70.95	20	17.7	88.5
Fentanyl	1	**0.84	84	10	8.215	82.15	20	19.9	99.5
Norbuprenorphine	0.5	**0.757	151.4	5	7.97	159.4	10	19.25	192.5
Buprenorphine	0.5	0.8925	178.5	5	6.78	135.6	10	18.2	182

**took the second transition for LPC (Beef Liver 1:9) Fentanyl & Norbuprenorphine

Table 19. Calculated concentrations of blood analysis and liver analysis (mcg/L) from 061015 extraction for cases #53-64. Cases are prepared and analyzed as 1:9 dilution.

*The Liver concentrations are averages from two transitions for each analyte

Analytes	Case #53		Times higher in	Case #54		Times higher in	Case #55		Times higher in	Case #56		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine	7	37.125	5.303571429		0	0		0	0		0	0
Hydromorphone		0	0		0	0		0	0		0	0
Codeine		1.0854	1.0854		0.4077	0.4077		0.14319	0.14319		0.162	0.162
Hydrocodone		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide	6	25.92	4.32		0	0		0	0		0	0
Morphine-6-Glucuronide		10.17	10.17		0	0		0	0		0	0
Norfentanyl	0.77	5.1255	6.656493506	0.35	2.322	6.634285714	35.4	97.65	2.758474576	0.92	4.5675	4.964673913
Fentanyl	20.3	276.75	13.63300493	10	143.1	14.31	53	326.7	6.164150943	6.9	67.68	9.808695652
Norbuprenorphine	0.69	18.09	26.2173913		2.9835	2.9835	6.6	50.895	7.711363636		1.89765	1.89765
Buprenorphine	1.2	17.595	14.6625			0	1.5	34.65	23.1		0	0

Analytes	Case #57		Times higher in	Case #58		Times higher in	Case #59		Times higher in	Case #60		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0		0	0		0	0	4	2.421	0.60525
Hydromorphone		0	0		0	0		0	0		0	0
Codeine		0.34425	0.34425		0.2061	0.2061		0.8262	0.8262		0.2376	0.2376
Hydrocodone		0	0		0	0	60	218.25	3.6375		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		0	0		0	0		0	0	7	4.473	0.639
Morphine-6-Glucuronide		0	0		0	0		0	0		0	0
Norfentanyl	1.2	4.635	3.8625	2.6	12.96	4.984615385		0.07983	0.07983	5.4	22.05	4.083333333
Fentanyl	18.6	143.55	7.717741935	8.6	41.76	4.855813953	2.6	20.565	7.909615385	35.9	391.5	10.90529248
Norbuprenorphine		1.8405	1.8405		2.092635	2.092635		0.963	0.963		1.91565	1.91565
Buprenorphine		0.07389	0.07389		0	0		0	0		0.3897	0.3897

Analytes	Case #61		Times higher in	Case #62		Times higher in	Case #63		Times higher in	Case #64		Times higher in
	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood	Blood	*Liver	Liver than Blood
Morphine		0	0	90	186.3	2.07	150	450.9	3.006	280	378.9	1.353214286
Hydromorphone		0	0		0	0		0	0		0	0
Codeine		0.3069	0.3069	3	4.815	1.605	7	11.385	1.626428571	8	15.615	1.951875
Hydrocodone		0	0		0	0		0	0		0	0
Oxymorphone		0	0		0	0		0	0		0	0
Oxycodone		0	0		0	0		0	0		0	0
Morphine-3-Glucuronide		0	0	850	568.8	0.669176471	210	540	2.571428571	90	918	10.2
Morphine-6-Glucuronide		0	0	160	166.5	1.040625	30	499.5	16.65	13	377.1	29.00769231
Norfentanyl	1.4	4.311	3.079285714		0.01899	0.01899		0.3438	0.3438		0.4554	0.4554
Fentanyl	23.9	74.7	3.125523013		1.4265	1.4265	2.7	25.38	9.4	1	2.2545	2.2545
Norbuprenorphine		3.33	3.33		1.4985	1.4985		3.1086	3.1086		4.41	4.41
Buprenorphine		0.4374	0.4374		0	0		0.2322	0.2322		0.3276	0.3276

Table 20. Table of the concentration (mcg/L) of each component in the low and high positive calibrator controls which are made in-house at the Erie County Toxicology Lab using whole [sheep] blood.

Analyte	Low Positive Lot #	Low Positive Control	High Positive Lot #	High Positive Control
Morphine	1501211030BK	11.75	1501211045BK	210.1
Hydromorphone	1501211030BK	11.15	1501211045BK	207.8
Codeine	1501211030BK	11.49	1501211045BK	199.2
Hydrocodone	1501211030BK	11.5	1501211045BK	211.3
Oxymorphone	1501211030BK	11.36	1501211045BK	214
Oxycodone	1501211030BK	11.08	1501211045BK	201.5
6-MAM	1501211030BK	9.761	1501211045BK	201.5
Morphine-3-Glucuronide	1501211030BK	19.489	1501211045BK	385.33
Morphine-6-Glucuronide	1501211030BK	20.989	1501211045BK	408.33
Norfentanyl	1501211030BK	1.062	1501211045BK	20.62
Fentanyl	1501211030BK	1.134	1501211045BK	20.28
Norbuprenrphine	1501211030BK	1.149	1501211045BK	21.43
Buprenorphine	1501211030BK	1.159	1501211045BK	21.88

Table 21. Concentrations for calibration standards (STD) # 1-7 (mcg/L).

Analytes	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7
Morphine	2	5	20	50	200	350	500
Hydromorphone	2	5	20	50	200	350	500
Codeine	2	5	20	50	200	350	500
Hydrocodone	2	5	20	50	200	350	500
Oxymorphone	2	5	20	50	200	350	500
Oxycodone	2	5	20	50	200	350	500
Morphine-3-Glucuronide	4	10	40	100	400	700	1000
Morphine-6-Glucuronide	4	10	40	100	400	700	1000
Norfentanyl	0.2	0.5	2	5	20	35	50
Fentanyl	0.2	0.5	2	5	20	35	50
Norbuprenorphine	0.1	0.25	1	2.5	10	17.5	25
Buprenorphine	0.1	0.25	1	2.5	10	17.5	25

Table 22. Concentrations for Beef liver Calibration standards (mcg/L).

Analytes	Low Positive Control Beef Liver 1:9 Dilution	Medium Positive Control Beef Liver 1:9 Dilution	High Positive Control Beef Liver 1:9 Dilution
Morphine	10	100	200
Hydromorphone	10	100	200
Codeine	10	100	200
Hydrocodone	10	100	200
Oxymorphone	10	100	200
Oxycodone	10	100	200
Morphine-3-Glucuronide	20	200	400
Morphine-6-Glucuronide	20	200	400
Norfentanyl	1	10	20
Fentanyl	1	10	20
Norbuprenorphine	0.5	5	10
Buprenorphine	0.5	5	10

Appendix IV

Case History

Case Dates range from September 2013 – April 2015
1 case from 2013, 22 cases from 2014, and 41 cases from 2015

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
1	Morphine	26.35	18	237.15	13.175	*Decedent:* **White/Male/23 Years 67" 139 lbs.**
1	Hydromorphone	0.06		0.54		
1	Codeine	0.4965		4.4685		*Manner of Death:* **Accident**
1	Hydrocodone	0		0		*Cause of Death:* **Acute combined fentanyl, heroin, cocaine, amphetamine, methylphenidate, and ethanol intoxication**
1	Oxymorphone	0.0801		0.7209		
1	Oxycodone	0.885		7.965		
1	6-MAM	0		0		The setting of this suspected drug overdose death is a rental car parked in a parking lot of Walgreens Pharmacy. Two (2) packets of white substance were found in the decedent's jacket upper left pocket and a small case containing needles, Q-tips and other drug paraphernalia was found in the rear of the vehicle and were taken by police. He is in a "moderate" state of decomposition.
1	Morphine-3-Glucuronide	31.6	190	284.4	1.497	
1	Morphine-6-Glucuronide	10.2	30	91.8	3.060	
1	Norfentanyl	1.795	6.4	16.155	2.524	
1	Fentanyl	3.545	16.6	31.905	1.922	
1	Norbuprenorphine	0.60445		5.44005		
1	Buprenorphine	0.0641		0.5769		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
2	Morphine	0.2345		2.1105		*Decedent:* **White/Female/36 Years 64" 140 lbs.**
2	Hydromorphone	5.695		51.255		
2	Codeine	18.75		168.75		*Manner of Death:* **Accident**
2	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with oxycodone and methadone**
2	Oxymorphone	9.245	5	83.205	16.641	
2	Oxycodone	146.5	730	1318.5	1.806	Probable overdose death (prescriptions) is a bathroom of a residence. On the bathroom sink is a syringe with a pink liquid and blood. Also on the sink are three syringes of Sodium Chloride and two bottle of Oxycodone; She has bruises and small scabs consistent with needle injection sites on her hips and legs. There is evidence of medical intervention about her in the form of cardiac pads, an endotracheal tube with a strap and an intraosseous line in her right lower leg. She is warm to the touch. Rigor mortis is not appreciated. Liver mortis is posterior and blanches. Livor mortis is consistent with her found position. She's had the flu the last few days. Medications taken from the scene: 2 bottles of Oxycodone (they do not belong to her) and 3 syringes of Sodium Chloride.
2	6-MAM	0		0		
2	Morphine-3-Glucuronide	2.605		23.445		
2	Morphine-6-Glucuronide	8.6975		78.2775		
2	Norfentanyl	0.01773		0.15957		
2	Fentanyl	0.00614		0.05526		
2	Norbuprenorphine	0.657		5.913		
2	Buprenorphine	0.0364		0.3276		
3	Morphine	15.95	100	143.55	1.436	*Decedent:* **White/Male/24 Years 66" 146 lbs.**
3	Hydromorphone	0		0		
3	Codeine	0.6655	6	5.9895	0.998	*Manner of Death:* **Accident**
3	Hydrocodone	0		0		*Cause of Death:* **Acute combined cocaine and heroin intoxication**
3	Oxymorphone	0.04445		0.40005		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
3	Oxycodone	0		0		Possible overdose. The residence has no door locks or door handles and is a vacant building. There is no evidence of forced entry or foul play. The residence is tidy for a squatter house. The scene has drug paraphernalia including used needles, clean needles, crack pipes, a tan substance, a white substance and alcohol pads. The ambient temperature is 72 degrees. There is evidence of medical therapy in the form of EKG leads on the back of his arms and legs. Decedent had a medical history of diabetes.
3	6-MAM	0		0		
3	Morphine-3-Glucuronide	61.9	250	557.1	2.228	
3	Morphine-6-Glucuronide	29.2	50	262.8	5.256	
3	Norfentanyl	0.0301		0.2709		
3	Fentanyl	0		0		
3	Norbuprenorphine	0.022855		0.205695		
3	Buprenorphine	0.0256		0.2304		
4	Morphine	57.25	180	515.25	2.863	*Decedent:* **White/Female/21 Years 62" 147 lbs.** *Manner of Death:* **Accident** *Cause of Death:* **Acute heroin and fentanyl intoxication** Found face down on her bed. She has a small amount of blood found in her right antecubital fossa. She has bruises and small scabs consistent with needle injection sites on her arms and legs. She is warm to the touch. Rigor mortis not appreciable. Livor mortis is posterior and blanches. Livor mortis is consistent with her found position. The decedent has a past medical history prescription drug and heroin abuse and previous suicide attempts. Her last overdose was in 2013, when Police and EMS used Narcan to revive her but she refused any further medical treatment and refused to go to the hospital. Day before- the decedent came home from a four day binge of using heroin. Hydrocodone pills were missing. Decedent and she said she took them so she could sell them to buy more heroin. Decedent found next day face down on bed.
4	Hydromorphone	0		0		
4	Codeine	2.33	11	20.97	1.906	
4	Hydrocodone	0		0		
4	Oxymorphone	0.0953		0.8577		
4	Oxycodone	0		0		
4	6-MAM	0	3	0		
4	Morphine-3-Glucuronide	106.5	330	958.5	2.905	
4	Morphine-6-Glucuronide	80.05	30	720.45	24.015	
4	Norfentanyl	0.2265	0.27	2.0385	7.550	
4	Fentanyl	4.955	3.1	44.595	14.385	
4	Norbuprenorphine	0.2643		2.3787		
4	Buprenorphine	0.0338		0.3042		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
5	Morphine	0.0299		0.2691		*Decedent:* **White/Female/0 Days 10" 453 grams**
5	Hydromorphone	0.034		0.306		
5	Codeine	0.1525		1.3725		*Manner of Death:* **Undetermined circumstances**
5	Hydrocodone	0		0		*Cause of Death:* **Complications of prematurity**
5	Oxymorphone	0.06575		0.59175		
5	Oxycodone	0		0		
5	6-MAM	0		0		Gestational age is 22 weeks.
5	Morphine-3-Glucuronide	4.22	12	37.98	3.165	The decedent's mother has a history of marijuana and heroin use; Mom tested positive for opiates and marijuana. She used heroin 6 days ago. The mother was not taking prenatal vitamins.
5	Morphine-6-Glucuronide	23.484		211.356		
5	Norfentanyl	0.03005		0.27045		
5	Fentanyl	0.0353		0.3177		
5	Norbuprenorphine	0		0		
5	Buprenorphine	0.03395		0.30555		
6	Morphine	0		0		*Decedent:* **White/Female/46 Years 67" 122 lbs.**
6	Hydromorphone	1.65		14.85		
6	Codeine	1.93		17.37		*Manner of Death*: **Natural**
6	Hydrocodone	0		0		*Cause of Death:* **Hypertensive and atherosclerotic cardiovascular disease**
6	Oxymorphone	1.105		9.945		
6	Oxycodone	17	130	153	1.177	Alive when incident occurred-ems defibrillated on scene. Scooter accident recently with only abrasions. Multiple drugs for cervical/lumbar issues. No history of drug abuse.
6	6-MAM	0		0		
6	Morphine-3-Glucuronide	3.08		27.72		
6	Morphine-6-Glucuronide	7.5365		67.8285		
6	Norfentanyl	0.016395		0.147555		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
6	Fentanyl	0		0		
6	Norbuprenorphine	0.0296		0.2664		
6	Buprenorphine	0.0277		0.2493		
7	Morphine	0		0		*Decedent:* **White/Female/33 Years 59.5" 183 lbs.**
7	Hydromorphone	0.598		5.382		
7	Codeine	6.545		58.905		*Manner of Death:* **Accident**
7	Hydrocodone	0		0		*Cause of Death:* **Oxycodone and methadone intoxication**
7	Oxymorphone	2.23		20.07		
7	Oxycodone	58.6	420	527.4	1.256	Decedent had a medical history of vulvar cancer, obesity and smoking. Discharged home with several pain medications. She was prescribed Oxycodone. She had been taking her medications and drinking alcohol. She went to bed and 15 minutes later her family heard her vomiting and when they went to check on her she was unresponsive. Medications: Methadone, Oxycodone, Lyrica, desipramine, and Senna. It was stated that decedent's residence is a known drug house.
7	6-MAM	0		0		
7	Morphine-3-Glucuronide	3.54		31.86		
7	Morphine-6-Glucuronide	8.6825		78.1425		
7	Norfentanyl	0.01769		0.15921		
7	Fentanyl	0.0182		0.1638		
7	Norbuprenorphine	0.514		4.626		
7	Buprenorphine	0.03675		0.33075		
8	Morphine	0		0		*Decedent:* **White/Female/53 Years 64" 123 lbs.**
8	Hydromorphone	3.12		28.08		
8	Codeine	5.605		50.445		*Manner of Death:* **Suicide**
8	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with metformin, oxycodone, diphenhydramine, clonazepam, risperidone and trazodone**
8	Oxymorphone	0.343		3.087		
8	Oxycodone	111.5	220	1003.5	4.561	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
8	6-MAM	0		0		Smells heavily of cigarette smoke. On a table in the kitchen there are several empty cans of beer and bottles of iced tea. There is a bag of prescription medications belonging to the decedent. Decedent had a medical history of diabetes mellitus, gastro esophageal reflux disease, chronic obstructive pulmonary disease, depression, and tobacco abuse. Medications found at the scene include Advair, Metformin, Rhinocort, Oxycocet, Trazadone, Lansoprazole, Rosuvastatin, Clonazepam, Senokot, Soflax, Amoxapine, Uniphyl, and Risperidone. All medications except Metformin appear to have been taken less frequently than prescribed. Metformin appears to have been taken more frequently than prescribed. Also found were over the counter medications Melatonin and Dimenhydrinate. The decedent had threatened self-harm in the past, but seemed fine after he had induced vomiting.
8	Morphine-3-Glucuronide	5.5		49.5		
8	Morphine-6-Glucuronide	25.333		227.997		
8	Norfentanyl	0.07223		0.65007		
8	Fentanyl	0.207		1.863		
8	Norbuprenorphine	0.15555		1.39995		
8	Buprenorphine	0.0623		0.5607		
9	Morphine	37.2	1700	334.8	0.197	*Decedent:* **White/Female/52 Years 65.5" 240 lbs.**
9	Hydromorphone	0.558		5.022		
9	Codeine	0.5205		4.6845		*Manner of Death:* **Accident**
9	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with morphine and oxycodone**
9	Oxymorphone	0.114		1.026		
9	Oxycodone	0.921	140	8.289	0.059	*Date of Death:* **1/22/14**
9	6-MAM	0		0		
9	Morphine-3-Glucuronide	18.31	380	164.79	0.434	Decedent is a known drug user
9	Morphine-6-Glucuronide	8.1		72.9		Condition of body: decomposed.
9	Norfentanyl	0.1545		1.3905		Welfare check on the decedent as she has not been heard from in three days.
9	Fentanyl	0.28515		2.56635		
9	Norbuprenorphine	0.943		8.487		
9	Buprenorphine	0.1965		1.7685		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
10	Morphine	0		0		*Decedent:* **White/Female/64 Years 65" 99 lbs.**
10	Hydromorphone	5.165	10	46.485	4.649	
10	Codeine	46.55		418.95		*Manner of Death:* **Suicide**
10	Hydrocodone	10.2	6	91.8	15.300	*Cause of Death:* **Acute oxycodone and hydrocodone intoxication**
10	Oxymorphone	31.25	210	281.25	1.339	
10	Oxycodone	0	7900	0	0.000	*Date of Death:* **7/30/14**
10	6-MAM	0		0		
10	Morphine-3-Glucuronide	15.9		143.1		Floor next to the bed are three cans of beer and an ashtray with cigarette butts. On a chair next to the bed are prayer cards, cigarettes, a manila folder, a television remote control, a doll and an empty prescription bottle of Oxycodone, (the pills belonged to family member). Medications taken from the scene: Oxycodone. Advanced decomposition and maggots on her face.
10	Morphine-6-Glucuronide	0.187		1.683		
10	Norfentanyl	0.10095		0.90855		
10	Fentanyl	13.21905		118.97145		
10	Norbuprenorphine	0		0		
10	Buprenorphine	38.168		343.512		
11	Morphine	0		0		*Decedent:* **White/Female/75 Years 64" 113 lbs.**
11	Hydromorphone	0.08975		0.80775		
11	Codeine	0.4415		3.9735		*Manner of Death:* **Undetermined circumstances**
11	Hydrocodone	30.85	30	277.65	9.255	*Cause of Death:* **Undetermined**
11	Oxymorphone	0		0		
11	Oxycodone	0		0		Decedent has a medical history of a seizure disorder, diabetes, alcohol, marijuana and cocaine abuse. The decedent has no physician of record and is taking no medications. CPR was initiated and the decedent was intubated and given Epinephrine x3, Calcium and Sodium Bicarbonate. An intraosseous device was established in the left tibia and the decedent was given 1mg of Epinephrine, 2mg of Narcan, 1mg of Calcium and Sodium Bicarbonate.
11	6-MAM	0		0		
11	Morphine-3-Glucuronide	3.15		28.35		
11	Morphine-6-Glucuronide	4.6715		42.0435		
11	Norfentanyl	0.00597		0.05373		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
11	Fentanyl	0.0851		0.7659		
11	Norbuprenorphine	3.21		28.89		
11	Buprenorphine	0.14985		1.34865		
12	Morphine	0				*Decedent:* **White/Male/56 Years 61" 83 lbs.**
12	Hydromorphone	1.415	70	12.735	0.18192	
12	Codeine	0.529		4.761		*Manner of Death:* **Accident**
12	Hydrocodone	0.384		3.456		*Cause of Death:* **Fentanyl and hydromorphone intoxication**
12	Oxymorphone	0		0		
12	Oxycodone	0		0		Found the decedent dead on the floor under the kitchen table. Crack pipe by feet. Syringe with 5 cc clear fluid on the table. Possibly Old Fentanyl patches in cabinet. No evidence of crimes or break-in. Syringe and suspected drugs. No prescription drugs. No suicide note. Past medical history: known to use drugs; no illnesses - never saw doctor. No recent hospitalizations. Smoker (chain).
12	6-MAM	0				
12	Morphine-3-Glucuronide	9.45		85.05		
12	Morphine-6-Glucuronide	6.9235		62.3115		
12	Norfentanyl	3.055	40.2	27.495	0.68395	
12	Fentanyl	9.46	112.2	85.14	0.75882	
12	Norbuprenorphine	0.106		0.954		
12	Buprenorphine	0.14035		1.26315		
13	Morphine	26.9	70	242.1	3.45857	*Decedent:* **White/Female/28 Years 65" 124 lbs.**
13	Hydromorphone	0.342		3.078		
13	Codeine	0.885		7.965		*Manner of Death*: **Accident**
13	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with fentanyl and heroin**
13	Oxymorphone	1.146		10.314		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
13	Oxycodone	0		0		Probable double overdose death is a motel room. (2 decedents found on bed). A syringe is found in the other decedent's right hand and another is found on an end table; One of the end tables are two cans and one bottle of unopened beer, mild state of decomposition.
13	6-MAM	0		0		
13	Morphine-3-Glucuronide	26.8	120	241.2	2.01	
13	Morphine-6-Glucuronide	6.98		62.82		
13	Norfentanyl	0.186	0.42	1.674	3.98571	
13	Fentanyl	23.35	21.2	210.15	9.91273	
13	Norbuprenorphine	0.1435		1.2915		
13	Buprenorphine	0.026		0.234		
14	Morphine	0.471		4.239		*Decedent:* **White/Male/45 Years 71" 237 lbs.**
14	Hydromorphone	0.749		6.741		
14	Codeine	0.509		4.581		*Manner of Death:* **Accident**
14	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with alprazolam, oxymorphone, ethanol and 7-aminoclonazepam**
14	Oxymorphone	11.75	20	105.75	5.2875	
14	Oxycodone	0.0537		0.4833		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
14	6-MAM	0		0		Obese white male. Three bottles of prescription medications are found on the kitchen counter. There is an insulin needle in one of the kitchen drawers and an empty beer can in the sink. Six empty beer cans are found in a dresser drawer in the decedent's bedroom. Clear discharge coming from his mouth and what appears to be dried white foam on his lips. Decedent had a medical history of depression, anxiety, gastro esophageal reflux disease, kidney injury (not specified), benzodiazepine dependence, cocaine, ethanol and prescription drug abuse. The decedent also had made previous comments of suicidal ideations and had been admitted several times for substance abuse Medications found at the scene include Lisinopril and Clonazepam. These medications appear to have been taken as prescribed. Day before death-decedent appeared heavily intoxicated, but denied any alcohol or drug use. Had been treated for alcohol abuse several times, but never remained sober for more than a few months.
14	Morphine-3-Glucuronide	0.594		5.346		
14	Morphine-6-Glucuronide	8.1275		73.1475		
14	Norfentanyl	0.00346		0.03114		
14	Fentanyl	0.0339		0.3051		
14	Norbuprenorphine	0.211		1.899		
14	Buprenorphine	0.00622		0.05598		
15	Morphine	0		0		*Decedent:* **White/Male/24 Years 72.5" 191 lbs.**
15	Hydromorphone	0		0		
15	Codeine	0.4295		3.8655		*Manner of Death:* **Accident**
15	Hydrocodone	0.0211		0.1899		*Cause of Death:* **Acute combined oxymorphone, fentanyl, 7-aminoclonazepam, and quetiapine intoxication**
15	Oxymorphone	7.515	30	67.635	2.2545	
15	Oxycodone	0		0		
15	6-MAM	0		0		Decedent went out with friend, came home-found in bedroom later with emesis. EMS intubated and given 5 rounds of Epinephrine and 4 mg of narcan with no effect.
15	Morphine-3-Glucuronide	1.8755		16.8795		
15	Morphine-6-Glucuronide	0.108		0.972		
15	Norfentanyl	0.255	0.45	2.295	5.1	
15	Fentanyl	0.7985	1.9	7.1865	3.78236	
15	Norbuprenorphine	0.0451		0.4059		
15	Buprenorphine	0.00199		0.01791		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
16	Morphine	0		0		*Decedent:* **White/Male/36 Years 71" 236 lbs.**
16	Hydromorphone	0		0		
16	Codeine	0.4325		3.8925		*Manner of Death:* **Accident**
16	Hydrocodone	0.0134		0.1206		*Cause of Death:* **Acute combined fentanyl, methadone, 7-aminoclonazepam, amphetamine, methylphenidate, gapabenpin and citalopram intoxication**
16	Oxymorphone	0		0		
16	Oxycodone	0		0		
16	6-MAM	0		0		Apparent overdose at residence. A wrapper for a Fentanyl patch and a piece of tin foil with burn marks on it were collected from next to the bed where the decedent was found. There are numerous bottles of prescription medications. Medical history of opiate abuse (on Methadone treatment), depression, gastro esophageal reflux disease, and a groin abscess. He had been smoking Fentanyl, slept then decedent took Gabapentin when they awoke (unknown how many).
16	Morphine-3-Glucuronide	1.25		11.25		
16	Morphine-6-Glucuronide	6.625		59.625		
16	Norfentanyl	0.2165	0.53	1.9485	3.67641	
16	Fentanyl	1.955	5.7	17.595	3.08684	
16	Norbuprenorphine	0.07805		0.70245		
16	Buprenorphine	0.0031		0.0279		
17	Morphine	0		0		*Decedent:* **White/Male/24 Years 72" 158 lbs.**
17	Hydromorphone	0.07055		0.63495		
17	Codeine	0.442		3.978		*Manner of Death:* **Suicide**
17	Hydrocodone	16.9	30	152.1	5.07	*Cause of Death:* **Acute paroxetine intoxication**
17	Oxymorphone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
17	Oxycodone	0		0		Possible suicide. Pink tinged emesis stains are noted on a pillow, a sheet and on a comforter on the bed. A glass containing pink liquid residue (suspected wine) is on top of a dresser in the bedroom. Several text's written on papers (about death) are seen hanging on the wall and in a notebook. Foam and bloody discharge is coming from his nostrils and mouth. Medical history of : Drug abuse (marijuana and cocaine), Alcohol abuse, Depression/Anxiety (years)
17	6-MAM	0		0		
17	Morphine-3-Glucuronide	1.9121		17.2089		
17	Morphine-6-Glucuronide	2.845		25.605		
17	Norfentanyl	0.0052		0.0468		
17	Fentanyl	0.0049		0.0441		
17	Norbuprenorphine	0.03264		0.29376		
17	Buprenorphine	0.00556		0.05004		
18	Morphine	0		0		*Decedent:* **White/Male/27 Years 70" 173 lbs.**
18	Hydromorphone	0.4075		3.6675		
18	Codeine	0.429		3.861		*Manner of Death:* **Accident**
18	Hydrocodone	0.4055		3.6495		*Cause of Death:* **Acute fentanyl intoxication**
18	Oxymorphone	0		0		
18	Oxycodone	0		0		Possible overdose death. Decedent found in vehicle was locked and the keys were in the on position. Passenger seat is a straw cut in half, a piece of foil with burn marks and a cigarette lighter is in the decedent's right hand. In the middle console is a fentanyl patch. No medical history and no cardiac problems that they know of. There was a statement that the decedent had heart condition and was taking Vioxx.
18	6-MAM	0		0		
18	Morphine-3-Glucuronide	2.97		26.73		
18	Morphine-6-Glucuronide	3.8945		35.0505		
18	Norfentanyl	1.095		9.855		
18	Fentanyl	17.3	5.8	155.7	26.8448	
18	Norbuprenorphine	0.01865		0.16785		
18	Buprenorphine	0.00381		0.03429		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
19	Morphine	154	460	1386	3.013043478	*Decedent:* **White/Male/26 Years 65.5" 226 lbs.**
19	Hydromorphone	0.394		3.546		
19	Codeine	4.49	18	40.41	2.245	*Manner of Death:* **Accident**
19	Hydrocodone	10.5	30	94.5	3.15	*Cause of Death:* **Acute combined heroin and clonazepam intoxication**
19	Oxymorphone	0		0		
19	Oxycodone	0		0		Suspected overdose death. Excessive drug use. Several bags of suspected heroin, a cooking spoon and several syringes were found at the scene. Several packets that are commonly used to package heroin were noted on the floor. On the desk, next to the decedent's face, was a bottle cap (which is commonly utilized for drug usage) as well as a capped syringe that was on the desk. A bottle of Clonazepam (prescribed to the decedent) was also there as well. In a bathroom cabinet, two bottles of Clonazepam and a large metal container with traces of what appeared to be heroin. On the living room table, there was an empty bottle of hydrocodone prescribed to the decedent as well as a bottle labeled Clonazepam (but found to contain Gabapentin). Other drug paraphernalia was found throughout the apartment (glass marijuana pipes, etc.) There did not appear to be any suspicious circumstances surrounding the decedents death and it appeared to be an unintentional drug overdose.
19	6-MAM	0	11	0		
19	Morphine-3-Glucuronide	184.5		1660.5		
19	Morphine-6-Glucuronide	77.8		700.2		
19	Norfentanyl	0.00466		0.04194		
19	Fentanyl	0.0752		0.6768		
19	Norbuprenorphine	0.02079		0.18711		
19	Buprenorphine	0.00825		0.07425		
20	Morphine	116.5	200	1048.5	5.2425	*Decedent: White/Male/17 Years 66.5" 283 lbs.*
20	Hydromorphone	0.02153		0.19377		
20	Codeine	0.5695		5.1255		*Manner of Death:* **Suicide**
20	Hydrocodone	0.00976		0.08784		*Cause of Death:* **acute combined morphine, clonazepam, diazepam, temazepam, and venlafaxine intoxication**
20	Oxymorphone	0		0		
20	Oxycodone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
20	6-MAM	0		0		Apparent overdose death at residence. Soda bottle filled with cigarette butts, a bowl filled with a green leafy substance, and some pill bottles. Bottle of Temazepam issued on 11/21/2014 with a quantity of 30, dose of one per day, with one pill remaining. There are additional pill bottles on the table. Decedent had a history of major depressive disorder, anxiety and insomnia. He smoked one pack of cigarettes a day and smoked marijuana daily for anxiety. He was a binge drinker. Decedent attempted to hang himself in the past. Medications taken from the scene included the Temazepam mentioned above, Venlafaxine, Buspirone, Sumatriptan, and Abilify.
20	Morphine-3-Glucuronide	164		1476		
20	Morphine-6-Glucuronide	61.3		551.7		
20	Norfentanyl	0.00471		0.04239		
20	Fentanyl	0		0		
20	Norbuprenorphine	0.18965		1.70685		
20	Buprenorphine	0.101		0.909		
21	Morphine	44.05	230	396.45	1.72369	*Decedent:* **White/Male/34 Years 77.5" 228 lbs.**
21	Hydromorphone	0		0		
21	Codeine	1.89	9	17.01	1.89	*Manner of Death:* **Accident**
21	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with heroin and diazepam**
21	Oxymorphone	0		0		
21	Oxycodone	0		0		
21	6-MAM	0	8			
21	Morphine-3-Glucuronide	97.7		879.3		
21	Morphine-6-Glucuronide	60.3		542.7		
21	Norfentanyl	0		0		
21	Fentanyl	0		0		
21	Norbuprenorphine	0.279		2.511		
21	Buprenorphine	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
22	Morphine	0.204		1.836		*Decedent:* **Hispanic/Male/47 Years 65" 172 lbs.**
22	Hydromorphone	0		0		
22	Codeine	0.03065		0.27585		*Manner of Death:* **Accident**
22	Hydrocodone	0		0		*Cause of Death:* **Acute combined fentanyl, cocaine, and ethanol intoxication**
22	Oxymorphone	0		0		
22	Oxycodone	0		0		The decedent smoked a pack of cigarettes per day, drank alcohol daily, abused cocaine, and had a low blood oxygen level.
22	6-MAM	0				
22	Morphine-3-Glucuronide	3.3215		29.8935		
22	Morphine-6-Glucuronide	0		0		
22	Norfentanyl	0		0		
22	Fentanyl	6.635	4.5	59.715	13.27	
22	Norbuprenorphine	0.09335		0.84015		
22	Buprenorphine	0		0		
23	Morphine	12.65	18	113.85	6.325	*Decedent:* **White/Male/28 Years 71" 191 lbs.**
23	Hydromorphone	0		0		
23	Codeine	0.0754		0.6786		*Manner of Death:* **Accident**
23	Hydrocodone	0		0		*Cause of Death:* **Acute heroin and fentanyl intoxication**
23	Oxymorphone	0		0		
23	Oxycodone	0		0		Decedent had a medical history of alcohol use, Heroin use and subdural hematoma. He had a surgical history of an adenoidectomy.
23	6-MAM	0		0		
23	Morphine-3-Glucuronide	20.35		183.15		
23	Morphine-6-Glucuronide	14.36		129.24		
23	Norfentanyl	1.67	2.2	15.03	6.83181	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
23	Fentanyl	15.8	14.8	142.2	9.608108108	
23	Norbuprenorphine	0.00177		0.01593		
23	Buprenorphine	0		0		
24	Morphine	442.5	320	3982.5	12.445	*Decedent:* **White/Female/45 Years 64" 159 lbs.**
24	Hydromorphone	0.0509		0.4581		
24	Codeine	0.8405		7.5645		*Manner of Death:* **Accident**
24	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with morphine, oxycodone and clonazepam**
24	Oxymorphone	0		0		
24	Oxycodone	3.015	10	27.135	2.7135	
24	6-MAM	0		0		Decedent had a past psychiatric history of adjustment disorder, anxiety, and panic disorder. after polysubstance ingestion with intent to kill herself. The decedent had a total of 3 overdoses in the past. She also had a history of alcohol and cocaine abuse.
24	Morphine-3-Glucuronide	512.5	unable to analyze	4612.5		
24	Morphine-6-Glucuronide	181.5	380	1633.5	4.29868	
24	Norfentanyl	0		0		
24	Fentanyl	0		0		
24	Norbuprenorphine	0.1004		0.9036		
24	Buprenorphine	0		0		
25	Morphine	0		0		*Decedent:* **White/Male/52 Years 72" 148 lbs.**
25	Hydromorphone	0.923	5	8.307	1.6614	
25	Codeine	0.0699		0.6291		*Manner of Death:* **Accident**
25	Hydrocodone	93.9	420	845.1	2.01214	*Cause of Death:* **Acute intoxication with hydrocodone, ethanol and gabapentin**
25	Oxymorphone	0		0		
25	Oxycodone	0		0		Gastro esophageal reflux disease, smoking, alcohol abuse and a stomach ulcer.
25	6-MAM	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
25	Morphine-3-Glucuronide	1.04		9.36		
25	Morphine-6-Glucuronide	0		0		
25	Norfentanyl	0		0		
25	Fentanyl	0		0		
25	Norbuprenorphine	0.3087		2.7783		
25	Buprenorphine	0		0		
26	Morphine	90.65	260	815.85	3.13788	*Decedent:* **White/Male/45 Years 73" 231 lbs.**
26	Hydromorphone	0		0		
26	Codeine	1.41	6	12.69	2.115	*Manner of Death:* **Accident**
26	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with heroin and fentanyl**
26	Oxymorphone	0		0		
26	Oxycodone	0		0		Syringes were found in his cigarette pack. He has a homemade sign that says "Homeless".
26	6-MAM	0	3	0		
26	Morphine-3-Glucuronide	64.8	180	583.2	3.24	
26	Morphine-6-Glucuronide	33.75	60	303.75	5.0625	
26	Norfentanyl	0.4855	1.2	4.3695	3.64125	
26	Fentanyl	11.8	10.4	106.2	10.2115	
26	Norbuprenorphine	0.0989		0.8901		
26	Buprenorphine	0.451		4.059		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
27	Morphine	0		0		*Decedent* **White/Female/28 Years 65.5" 188 lbs.**
27	Hydromorphone	0		0		
27	Codeine	0.018		0.162		*Manner of Death:* **Accident**
27	Hydrocodone	0		0		*Cause of Death:* **Acute combined fentanyl, clonazepam, alprazolam, and citalopram intoxication**
27	Oxymorphone	0		0		
27	Oxycodone	0		0		
27	6-MAM	0		0		the decedent smoked, but not often, and that she was not a habitual drug abuser. Medications found at the scene include Ferocon, Omeprazole, Anucort, Lomedia, Clotrimazole, Clonazepam, Ibuprofen, Escitalopram, Hydroxyzine, Lidocaine viscous fluid, and pre-natal vitamins.
27	Morphine-3-Glucuronide	2.88		25.92		
27	Morphine-6-Glucuronide	0		0		
27	Norfentanyl	0.4155	0.49	3.7395	7.63163	
27	Fentanyl	20.25	37.8	182.25	4.82142	
27	Norbuprenorphine	0		0		
27	Buprenorphine	0		0		
28	Morphine	0.953	2	8.577	4.2885	*Decedent:* **White/Female/42 Years 62" 138 lbs.**
28	Hydromorphone	0		0		
28	Codeine	0.0232		0.2088		*Manner of Death:* **Accident**
28	Hydrocodone	0		0		*Cause of Death:* **Acute fentanyl intoxication**
28	Oxymorphone	0		0		
28	Oxycodone	0		0		In the bathroom, there is a small amount of blood on the toilet seat, in the toilet and on a piece of toilet paper on the bathroom sink. Also in the bathroom is a couch cushion, a syringe next to the toilet, two empty packets of heroin in the garbage can, a syringe cap and a shoelace on the bathroom sink. medical history of asthma and heroin abuse.
28	6-MAM	0		0		
28	Morphine-3-Glucuronide	1.65		14.85		
28	Morphine-6-Glucuronide	0		0		
28	Norfentanyl	3.995	5.7	35.955	6.30789	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
28	Fentanyl	29.5	35.8	265.5	7.41620	
28	Norbuprenorphine	2.235	2.4	20.115	8.38125	
28	Buprenorphine	0.2335	0.44	2.1015	4.77613	
29	Morphine	0.486	3	4.374	1.458	*Decedent:* **White/Male/22 Years 75.5" 284 lbs.**
29	Hydromorphone	0		0		
29	Codeine	0.0295		0.2655		*Manner of Death:* **Accident**
29	Hydrocodone	0		0		*Cause of Death:* **Acute fentanyl and morphine intoxication**
29	Oxymorphone	0		0		
29	Oxycodone	0		0		The decedent had a history of narcotic/prescription drug abuse. the drug Fentanyl was mentioned in the conversation when asked about drug history.
29	6-MAM	0		0		
29	Morphine-3-Glucuronide	3.185	20	28.665	1.43325	
29	Morphine-6-Glucuronide	0.179		1.611		
29	Norfentanyl	0.01475		0.13275		
29	Fentanyl	6.95	2.7	62.55	23.1666	
29	Norbuprenorphine	1.23		11.07		
29	Buprenorphine	0		0		
30	Morphine	0		0		*Decedent:* **White/Female/44 Years 62.5" 148 lbs.**
30	Hydromorphone	0		0		
30	Codeine	0.121		1.089		*Manner of Death:* **Accident**
30	Hydrocodone	47.35	230	426.15	1.853	Cause of Death: **Acute combined methadone, hydrocodone, diazepam, alprazolam, pregabalin and methocarbamol intoxication**
30	Oxymorphone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
30	Oxycodone	0		0		Medical history of prescription drug and alcohol abuse and chronic neck, back and knee pain from previous motor vehicle accidents. Medications: Methadone, Lyrica, Norco, Xanax, Robaxin, Ambien, Chantix, Amitiza, Lactulose, Zolpidem and Ibuprofen.
30	6-MAM	0		0		
30	Morphine-3-Glucuronide	0		0		
30	Morphine-6-Glucuronide	0		0		
30	Norfentanyl			0		
30	Fentanyl			0		
30	Norbuprenorphine	0.0543		0.4887		
30	Buprenorphine			0		
31	Morphine	9.715	30	87.435	2.915	*Decedent:* **White/Male/50 Years 70" 221 lbs.**
31	Hydromorphone	0		0		
31	Codeine	0		0		*Manner of Death:* **Natural**
31	Hydrocodone	0		0		*Cause of Death:* **Chronic substance abuse**
31	Oxymorphone	0		0		
31	Oxycodone	0		0		The decedent had a medical history of lumbar osteoarthritis, bilateral knee osteoarthritis, chronic obstructive pulmonary disease, alcohol use, smoker, bronchitis, lumbosacral sprain status post laminectomy, left total knee replacement, anxiety, hypertension, asthma and dermatitis.
31	6-MAM	0		0		
31	Morphine-3-Glucuronide	9.51	130	85.59	0.658	
31	Morphine-6-Glucuronide	1.25	15	11.25	0.750	
31	Norfentanyl			0		
31	Fentanyl			0		
31	Norbuprenorphine	0.0127		0.1143		
31	Buprenorphine			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
32	Morphine	4.74	20	42.66	2.133	*Decedent:* **White/Female/47 Years 62.5" 176 lbs.**
32	Hydromorphone	0		0		
32	Codeine	0.186		1.674		*Manner of Death:* **Accident**
32	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with fentanyl, heroin, cocaine, amitriptyline, methadone, buprenorphine, pregabalin and mordiazepam**
32	Oxymorphone	0		0		
32	Oxycodone	0		0		
32	6-MAM	0		0		Past medical history of :
32	Morphine-3-Glucuronide	13.25	370	119.25	0.322	Drug use (on Suboxone)
32	Morphine-6-Glucuronide	2.16	60	19.44	0.324	Smoking
32	Norfentanyl	0.4745	1.5	4.2705	2.847	Insomnia (on Lyrica and unknown sleep aids)
32	Fentanyl	8.39	14.4	75.51	5.244	
32	Norbuprenorphine	1.65	2.5	14.85	5.940	
32	Buprenorphine	0.509	1.7	4.581	2.695	
33	Morphine	0		0		*Decedent:* **White/Male/29 Years 75" 277 lbs.**
33	Hydromorphone	0		0		
33	Codeine	0		0		*Manner of Death:* **Accident**
33	Hydrocodone	0		0		*Cause of Death:* **Acute fentanyl intoxication**
33	Oxymorphone	0		0		
33	Oxycodone	0		0		Medical history of testing positive for Cannabinoids, high triglycerides, lymphocytes, monocytes and white blood cells, Heroin use and low back pain that extended down his left leg. He had been seen at ECMC for detox.
33	6-MAM	0		0		
33	Morphine-3-Glucuronide	0		0		
33	Morphine-6-Glucuronide	0		0		
33	Norfentanyl	0.338	0.78	3.042	3.900	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
33	Fentanyl	14.7	12.1	132.3	10.934	
33	Norbuprenorphine	0.585		5.265		
33	Buprenorphine	0.0283		0.2547		
34	Morphine	4.76	13	42.84	3.295	*Decedent:* **White/Male/32 Years 71.5" 162 lbs.**
34	Hydromorphone	0		0		
34	Codeine	0		0		*Manner of Death:* **Accident**
34	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with fentanyl, morphine and alprazolam**
34	Oxymorphone	0		0		
34	Oxycodone	0		0		Decedent has used prescription drugs since he was a young teenager and has a medical history of marijuana, alcohol and heroin use.
34	6-MAM	341.5		3073.5		
34	Morphine-3-Glucuronide	3.325	18	29.925	1.663	
34	Morphine-6-Glucuronide	0.747		6.723		
34	Norfentanyl	1.335	2.7	12.015	4.450	
34	Fentanyl	38.7	44.6	348.3	7.809	
34	Norbuprenorphine	0.173		1.557		
34	Buprenorphine			0		
35	Morphine	0		0		*Decedent:* **White/Male/29 Years 74.5" 200 lbs.**
35	Hydromorphone	0		0		
35	Codeine	0		0		*Manner of Death:* **Accident**
35	Hydrocodone	0		0		*Cause of Death:* **Acute combined fentanyl, carisoprodol, ketamine, and alprazolam intoxication**
35	Oxymorphone	0		0		
35	Oxycodone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
35	6-MAM	0		0		Medications found at the scene include Gabapentin, Duexis, and Carisoprodol. Carisoprodol appears to have been taken more frequently than prescribed. Gabapentin and Duexis appear to have been taken less frequently than prescribed. Decedent had a medical history of prescription drug abuse and C4-T1 disc injuries from a motor vehicle accident.
35	Morphine-3-Glucuronide	0		0		
35	Morphine-6-Glucuronide	0		0		
35	Norfentanyl	0.618	1.9	5.562	2.927	
35	Fentanyl	10.85	11.2	97.65	8.719	
35	Norbuprenorphine	0.251		2.259		
35	Buprenorphine			0		
36	Morphine			0		*Decedent:* **White/Male/36 Years 67" 164 lbs.**
36	Hydromorphone			0		
36	Codeine			0		*Manner of Death:* **Accident**
36	Hydrocodone			0		*Cause of Death:* **Acute fentanyl and buprenorphine intoxication**
36	Oxymorphone			0		
36	Oxycodone			0		The decedent had a history of heroin abuse, smoked and used alcohol. He was prescribed Zubsolv (Buprenorphine and Naloxone sublingual tablets).
36	6-MAM			0		
36	Morphine-3-Glucuronide	0.204	-	1.836		
36	Morphine-6-Glucuronide			0		
36	Norfentanyl	3.395	2.9	30.555	10.536	
36	Fentanyl	45.6	38.7	410.4	10.605	
36	Norbuprenorphine	1.33	1.1	11.97	10.882	
36	Buprenorphine	0.174	0.78	1.566	2.008	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
37	Morphine			0		*Decedent:* **White/Male/33 Years 69.5" 222 lbs.**
37	Hydromorphone			0		
37	Codeine			0		*Manner of Death:* **Accident**
37	Hydrocodone			0		*Cause of Death:* **Acute intoxication with fentanyl, cocaine and alprazolam**
37	Oxymorphone			0		
37	Oxycodone			0		There was a top of a baggie on the floor to the left of the decedent; there were two needles and a baggie in the garbage can to the left of the decedent and razor blades on the table next to the bed.
37	6-MAM			0		
37	Morphine-3-Glucuronide			0		
37	Morphine-6-Glucuronide			0		
37	Norfentanyl	0.9575	0.51	8.6175	16.897	
37	Fentanyl	8.71	2.4	78.39	32.663	
37	Norbuprenorphine	0.228		2.052		
37	Buprenorphine	0.000357		0.003213		
38	Morphine	16.7	40	150.3	3.758	*Decedent:* **White/Male/32 Years 69.5" 330 lbs.**
38	Hydromorphone	0		0		
38	Codeine	0.337	2	3.033	1.517	*Manner of Death:* **Accident**
38	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with heroin and fentanyl**
38	Oxymorphone	0		0		
38	Oxycodone	0		0		Past medical history of :
38	6-MAM	0		0		Drug use (started using lortabs for aching joints when he was 500-600 lbs., later graduating to Heroin)
38	Morphine-3-Glucuronide	42.95	290	386.55	1.333	
38	Morphine-6-Glucuronide	12.65		113.85		Smoking
38	Norfentanyl	0.692	0.97	6.228	6.421	Obesity (330 lbs. after losing weight)

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
38	Fentanyl	22.85	8.1	205.65	25.389	
38	Norbuprenorphine	0.28		2.52		
38	Buprenorphine	0.0443		0.3987		
39	Morphine					*Decedent:* **White/Female/29 Years 64" 153 lbs.**
39	Hydromorphone					
39	Codeine					*Manner of Death:* **Accident**
39	Hydrocodone					*Cause of Death:* **Acute intoxication with fentanyl, gabapentin, alprazolam, and topiramate**
39	Oxymorphone					
39	Oxycodone					
39	6-MAM					Medical history of poly-substance abuse (heroin, prescription medications, and crack cocaine). There is a bottle of water, a bottle of hydrogen peroxide, several packs of cigarettes, a lighter, an ashtray, a small plastic Zip Lock baggie, and a bottle of prescription medications belonging to one of the home owners. The prescription is for Alprazolam, but the bottle contains Sucralfate (prescribed to the same resident). There is a bloody tissue under the bed and a cap for a needle in the decedent's purse which is on the floor. A prescription bottle of Xanax belonging to one of the residents was found by Police in the kitchen. Police report it appeared that the medication was being taken as prescribed.
39	Morphine-3-Glucuronide					
39	Morphine-6-Glucuronide					
39	Norfentanyl	0.011825	6	0.106425	0.018	
39	Fentanyl	4.27		38.43		
39	Norbuprenorphine	0.586		5.274		
39	Buprenorphine	0.195		1.755		
40	Morphine	2.87	12	25.83	2.153	*Decedent:* **White/Female/35 Years 63" 181 lbs.**
40	Hydromorphone	0		0		
40	Codeine	0.0155		0.1395		*Manner of Death:* **Accident**
40	Hydrocodone	0.4015	4	3.6135	0.903	*Cause of Death:* **Acute intoxication with fentanyl, cocaine, alprazolam, morphine and hydrocodone**
40	Oxymorphone	0		0		
40	Oxycodone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
40	6-MAM	0		0		Past medical history of :
40	Morphine-3-Glucuronide	4.85	40	43.65	1.091	Drug abuse (crack cocaine/pills)
40	Morphine-6-Glucuronide	1.52	5	13.68	2.736	Medications found at the scene include :
40	Norfentanyl	0.2555	0.62	2.2995	3.709	Ibuprofen
40	Fentanyl	7.44	8.1	66.96	8.267	Amoxicillin
40	Norbuprenorphine	1.016		9.144		Nitrofurantoin
40	Buprenorphine	0.08195		0.73755		
41	Morphine			0		*Decedent:* **Black/Male/21 Years 70" 319 lbs.**
41	Hydromorphone			0		
41	Codeine			0		*Manner of Death:* **Accident**
41	Hydrocodone			0		*Cause of Death:* **Acute fentanyl intoxication**
41	Oxymorphone			0		
41	Oxycodone			0		There is a small blue bag - "Overdose Prevention Rescue Kit"- on the night stand. decedent has a medical history of morbid obesity, ethanol abuse, heroin abuse and hallucinogenic drug use.
41	6-MAM			0		
41	Morphine-3-Glucuronide			0		
41	Morphine-6-Glucuronide			0		
41	Norfentanyl	0.3745	0.51	3.3705	6.609	
41	Fentanyl	18.65	7.3	167.85	22.993	
41	Norbuprenorphine	0.951		8.559		
41	Buprenorphine	0.05085		0.45765		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
42	Morphine	0.396	5	3.564	0.713	*Decedent:* **White/Male/44 Years 72" 217 lbs.**
42	Hydromorphone	0		0		
42	Codeine	0		0		*Manner of Death:* **Accident**
42	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with fentanyl, alprazolam, clonazepam, morphine and buprenorphine**
42	Oxymorphone	0		0		
42	Oxycodone	0		0		
42	6-MAM	0		0		Decedent had a history of drug abuse (Heroin) and an abscess to the right neck.
42	Morphine-3-Glucuronide	1.56	17	14.04	0.826	
42	Morphine-6-Glucuronide	0		0		
42	Norfentanyl	2.605	4.6	23.445	5.097	
42	Fentanyl	58.9	47.6	530.1	11.137	
42	Norbuprenorphine	1.15	1.2	10.35	8.625	
42	Buprenorphine	0.4085	0.2	3.6765	18.383	
43	Morphine	0		0		*Decedent:* **White/Male/33 Years 69" 205 lbs.**
43	Hydromorphone	0		0		
43	Codeine	0		0		*Manner of Death:* **Accident**
43	Hydrocodone	19.7	70	177.3	2.533	*Cause of Death:* **Acute mixed drug intoxication including fentanyl, hydrocodone and meprobamate**
43	Oxymorphone	0		0		
43	Oxycodone	0		0		
43	6-MAM	0		0		Past medical history of: Drug abuse (ongoing for approximately 10 years). the decedent began smoking marijuana, then progressed to taking pills and eventually started using heroin.
43	Morphine-3-Glucuronide	0		0		
43	Morphine-6-Glucuronide	0		0		
43	Norfentanyl	0.257	0.33	2.313	7.009	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
43	Fentanyl	7.53	6.1	67.77	11.110	
43	Norbuprenorphine	0.526805		4.741245		
43	Buprenorphine	0.04925		0.44325		
44	Morphine	0		0		*Decedent:* **White/Male/49 Years 72.5" 212 lbs.**
44	Hydromorphone	0		0		
44	Codeine	0		0		*Manner of Death:* **Accident**
44	Hydrocodone	7.39	17	66.51	3.912	*Cause of Death:* **Acute combined fentanyl, cocaine, hydrocodone, lorazepam, citalopram, and ethanol intoxication**
44	Oxymorphone	0		0		
44	Oxycodone	0		0		
44	6-MAM	0		0		The decedent's ex-wife reported he had a medical history of alcohol, tobacco and crack cocaine abuse.
44	Morphine-3-Glucuronide	0		0		
44	Morphine-6-Glucuronide	0		0		
44	Norfentanyl	0.828	0.67	7.452	11.122	
44	Fentanyl	10.4	6.2	93.6	15.097	
44	Norbuprenorphine	0.617		5.553		
44	Buprenorphine	0.0175		0.1575		
45	Morphine	2.4	13	21.6	1.662	*Decedent:* **White/Female/48 Years 63" 175 lbs.**
45	Hydromorphone	0		0		
45	Codeine	0		0		*Manner of Death:* **Accident**
45	Hydrocodone	0		0		*Cause of Death:* **Acute mixed drug intoxication including heroin, cocaine, ethanol, clonazepam and gabapentin**
45	Oxymorphone	0		0		
45	Oxycodone	0		0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
45	6-MAM	0		0		Decedent had a medical history of alcohol use, Heroin use and prescription drug abuse (unknown what type of pills).
45	Morphine-3-Glucuronide	6.93		62.37		
45	Morphine-6-Glucuronide	1.35		12.15		
45	Norfentanyl			0		
45	Fentanyl	0.4075		3.6675		
45	Norbuprenorphine	0.383		3.447		
45	Buprenorphine	0.014515		0.130635		
46	Morphine	4.27	10	38.43	3.843	*Decedent* **White/Female/26 Years 64" 116 lbs.**
46	Hydromorphone	0		0		
46	Codeine	0		0		*Manner of Death:* **Accident**
46	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication by the combined effects of fentanyl, heroin, alporozolam, clonazepam and cyclobenzaprine**
46	Oxymorphone	0		0		
46	Oxycodone	0		0		
46	6-MAM	0		0		Decedent has a past medical history of :
46	Morphine-3-Glucuronide	1.9	30	17.1	0.570	Drug abuse, Smoker, Bronchial asthma (intermittent), Anxiety and depression, Chronic nausea, vomiting and weight loss. There was a statement that the decedent did not have any primary care physician at the time of death.
46	Morphine-6-Glucuronide	1.1	6	9.9	1.650	
46	Norfentanyl	5.57	15.9	50.13	3.153	
46	Fentanyl	59.8	49.4	538.2	10.895	Medications found at the scene include :
46	Norbuprenorphine	0.0652		0.5868		D-Amphetamine, Bupropion, Paroxetine, Metronidazole
46	Buprenorphine	0.030165		0.271485		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
47	Morphine	52.85	210	475.65	2.265	*Decedent:* **Hispanic/Female/17 Years 62" 146 lbs.**
47	Hydromorphone	0		0		
47	Codeine	3.59		32.31		*Manner of Death:* **Accident**
47	Hydrocodone	0		0		*Cause of Death:* **Acute mixed drug intoxication including fentanyl, heroin, cocaine, oxycodone, oxymorphone, hydroxyzine and lorazepam**
47	Oxymorphone	0.232	4	2.088	0.522	
47	Oxycodone	11.25	90	101.25	1.125	*Date of Death:* **3/7/2015**
47	6-MAM	0		0		
47	Morphine-3-Glucuronide	110	330	990	3.000	The decedent had a history of asthma, left bicipital tendonitis and was a marijuana smoker. She was seen January 2015 at the hospital for possible Methicillin-resistant Staphylococcus aureus (MRSA). The housemate is a known an IV heroin user. Decedent had her 4 wisdom teeth pulled on 3-5-15. She received medication for pain.
47	Morphine-6-Glucuronide	64.2	60	577.8	9.630	
47	Norfentanyl	0.4925	0.92	4.4325	4.818	
47	Fentanyl	64.7	72	582.3	8.088	
47	Norbuprenorphine	0.248465		2.23618		Medications: Oxycodone/Acetaminophen 5-325 mg (40 filled 3-5-15, 20 remaining), Etodolac 400mg (15 filled 3-5-15, 13 remain), Lorazepam 1mg (30 filled 2-20-15, 14 remain)
47	Buprenorphine	0.03935		0.35415		
48	Morphine		3	0		*Decedent:* **White/Male/34 Years 72" 168 lbs.**
48	Hydromorphone			0		
48	Codeine			0		*Manner of Death:* **Accident**
48	Hydrocodone			0		*Cause of Death:* **Fentanyl, cocaine and morphine intoxication**
48	Oxymorphone			0		
48	Oxycodone			0		On the kitchen counter is a spoon with a white substance on it. In a bedroom on the dresser and in the garbage can are syringes. Also found in the bedroom is a white substance in a small plastic bag on a jacket. decedent has a medical history of Hepatitis C and heroin abuse.
48	6-MAM			0		
48	Morphine-3-Glucuronide	0.0686	16	0.6174	0.039	
48	Morphine-6-Glucuronide			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
48	Norfentanyl	4.655	13.2	41.895	3.174	
48	Fentanyl	25.15	39.6	226.35	5.716	
48	Norbuprenorphine	0.161		1.449		
48	Buprenorphine	0.00673		0.06057		
49	Morphine			0		*Decedent:* **Hispanic/Male/35 Years 63.5" 207 lbs.**
49	Hydromorphone			0		
49	Codeine			0		*Manner of Death:* **Accident**
49	Hydrocodone			0		*Cause of Death:* **Acute combined fentanyl and bupropion intoxication**
49	Oxymorphone			0		
49	Oxycodone			0		He has defects on both arms and antecubital fossae consistent with needle injection sites. The decedent has a medical history of sleep apnea, high cholesterol and heroin abuse. Medication taken from the scene: two bottles of Omeprazole DR, two bottles of Bupropion HCL XL and two vials of Naloxone.
49	6-MAM			0		
49	Morphine-3-Glucuronide	0.845		7.605		
49	Morphine-6-Glucuronide			0		
49	Norfentanyl	1.145	1.4	10.305	7.361	
49	Fentanyl	13.55	6	121.95	20.325	
49	Norbuprenorphine	0.269		2.421		
49	Buprenorphine	0.023485		0.211365		
50	Morphine			0		*Decedent:* **White/Male/24 Years 72" 164 lbs.**
50	Hydromorphone			0		
50	Codeine			0		*Manner of Death:* **Accident**
50	Hydrocodone			0		*Cause of Death:* **Acute mixed drug intoxication including fentanyl, clonazepam and amphetamine**
50	Oxymorphone			0		
50	Oxycodone			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
50	6-MAM			0		The decedent has a history of heroin abuse.
50	Morphine-3-Glucuronide			0		A syringe and an elastic cord found in bathroom.
50	Morphine-6-Glucuronide			0		
50	Norfentanyl	3.49	4.2	31.41	7.479	
50	Fentanyl	64.35	35.7	579.15	16.223	
50	Norbuprenorphine	0.0725		0.6525		
50	Buprenorphine	0.03378		0.30402		
51	Morphine	87.2	190	784.8	4.131	*Decedent:* **Other/Male/31 Years 70.5" 259 lbs.**
51	Hydromorphone	0		0		
51	Codeine	1.84	12	16.56	1.380	*Manner of Death*: **Accident**
51	Hydrocodone	0		0		*Cause of Death:* **Acute heroin intoxication**
51	Oxymorphone	0		0		
51	Oxycodone	0		0		
51	6-MAM	0		0		
51	Morphine-3-Glucuronide	117	>1.0	1053		
51	Morphine-6-Glucuronide	26.5	130	238.5	1.835	
51	Norfentanyl			0		
51	Fentanyl	0.476		4.284		
51	Norbuprenorphine	0.1325		1.1925		
51	Buprenorphine	0.03135		0.28215		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
52	Morphine	16.95	19	152.55	8.029	*Decedent:* **White/Male/32 Years 74" 163 lbs.**
52	Hydromorphone	0		0		
52	Codeine	0		0		*Manner of Death:* **Accident**
52	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication with heroin and fentanyl**
52	Oxymorphone	0		0		
52	Oxycodone	0		0		There are several prescription medications found on the kitchen counter and in a dresser drawer. There is a cell phone, scale, pipe, and small plastic baggie on a table in the kitchen area. There is a small container of marijuana in a dresser drawer. On the coffee table there is a straw with white residue on it. There is a belt and a spoon with residue and a small piece of cotton in it on the floor near the decedent. the decedent had a medical history of IV drug abuse and recent left inguinal hernia repair. No other medical history is known. Medications found at the scene include Escitalopram, Brintellix, Zolpidem, Trazodone, Tramadol, Bupropion, and Lortab.
52	6-MAM	0		0		
52	Morphine-3-Glucuronide	11.5	80	103.5	1.294	
52	Morphine-6-Glucuronide	2.32		20.88		
52	Norfentanyl	4.33	6.4	38.97	6.089	
52	Fentanyl	30.6	18.7	275.4	14.727	
52	Norbuprenorphine	0.547		4.923		
52	Buprenorphine	0.259		2.331		
53	Morphine	4.125	7	37.125	5.304	*Decedent:* **White/Male/36 Years 73" 284 lbs.**
53	Hydromorphone	0		0		
53	Codeine	0.1206		1.0854		*Manner of Death*: **Accident**
53	Hydrocodone	0		0		*Cause of Death:* **Acute intoxication by the combined effects of fentanyl, morphine, buprenorphine and citalopram**
53	Oxymorphone	0		0		
53	Oxycodone	0		0		There is an empty baggie, a pen cap and a cell phone. All have a white powdery substance on them. A tissue with 3 white pills (TCL 370 are OTC pain relievers) and 17 blue pills (Mylan A1 are Alprazolam) was found in a bowl on the stand/bookcase. The decedent had an unknown history.
53	6-MAM	0		0		
53	Morphine-3-Glucuronide	2.88	6	25.92	4.320	
53	Morphine-6-Glucuronide	1.13		10.17		
53	Norfentanyl	0.5695	0.77	5.1255	6.656	

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
53	Fentanyl	30.75	20.3	276.75	13.633	Medications: Citalopram and Pantoprazole.
53	Norbuprenorphine	2.01	0.69	18.09	26.217	
53	Buprenorphine	1.955	1.2	17.595	14.663	
54	Morphine			0		*Decedent:* **White/Male/46 Years 67" 175 lbs.**
54	Hydromorphone			0		
54	Codeine	0.0453		0.4077		*Manner of Death:* **Accident**
54	Hydrocodone			0		*Cause of Death:* **Acute intoxication with fentanyl and ethanol**
54	Oxymorphone			0		
54	Oxycodone			0		Drug paraphernalia (needles, spoon w/ reside) found in bathroom. Known IV drug user.
54	6-MAM			0		
54	Morphine-3-Glucuronide			0		
54	Morphine-6-Glucuronide			0		
54	Norfentanyl	0.258	0.35	2.322	6.634	
54	Fentanyl	15.9	10	143.1	14.310	
54	Norbuprenorphine	0.3315		2.9835		
54	Buprenorphine			0		
55	Morphine			0		*Decedent:* **White/Male/35 Years 71" 192 lbs.**
55	Hydromorphone			0		
55	Codeine	0.01591		0.14319		*Manner of Death:* **Accident**
55	Hydrocodone			0		*Cause of Death:* **Acute mixed drug intoxication including fentanyl, buprenorphine, alprazolam, clonazepam and ethanol**
55	Oxymorphone			0		
55	Oxycodone			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
55	6-MAM			0		The decedent had a history of substance abuse (specifically opiates), alcohol abuse, hepatitis C infection (diagnosed 2008), anxiety, depression, and panic disorder. His medications in 2012 included Cymbalta 30 mg twice a day, Xanax 2 mg twice a day, and Methadone 70 mg daily.
55	Morphine-3-Glucuronide			0		
55	Morphine-6-Glucuronide			0		
55	Norfentanyl	10.85	35.4	97.65	2.758	Decedent had a documented intentional overdose of the combination of heroin, methadone, and benzodiazepines.
55	Fentanyl	36.3	53	326.7	6.164	
55	Norbuprenorphine	5.655	6.6	50.895	7.711	
55	Buprenorphine	3.85	1.5	34.65	23.100	
56	Morphine			0		*Decedent:* **Black/Male/31 Years 73" 273 lbs.**
56	Hydromorphone			0		
56	Codeine	0.018		0.162		*Manner of Death:* **Accident**
56	Hydrocodone			0		*Cause of Death:* **Acute Fentanyl intoxication**
56	Oxymorphone			0		
56	Oxycodone			0		There is a yellow packet/paper (presumptive heroin) on the bathroom floor; several packs of cigarettes and empty bottles of alcohol (beer, wine and liquor) are found on the fireplace mantle and a hookah smoking device is found in a box in the dining room. decedent had a history of bipolar and major depressive disorders, chronic back pain, multiple psychiatric admissions to the Hospital, suicidal ideations including thoughts of overdosing on pills, polysubstance abuse/dependence (benzodiazepines, crack cocaine, marijuana and prescription narcotics), cocaine overdose within the past three years, attention deficit hyperactivity disorder, gonorrhea, genital herpes, used alcohol, and smoked. He was not currently prescribed narcotic medications, but used them when he left the house and often returned home intoxicated. He has been prescribed Lortab, Thorazine, Seroquel, Lamictal, Naprosyn, and Flexeril.
56	6-MAM			0		
56	Morphine-3-Glucuronide			0		
56	Morphine-6-Glucuronide			0		
56	Norfentanyl	0.5075	0.92	4.5675	4.965	
56	Fentanyl	7.52	6.9	67.68	9.809	
56	Norbuprenorphine	0.21085		1.89765		
56	Buprenorphine			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
57	Morphine			0		*Decedent:* **White/Male/32 Years 69" 208 lbs.**
57	Hydromorphone			0		
57	Codeine	0.03825		0.34425		*Manner of Death:* **Accident**
57	Hydrocodone			0		*Cause of Death:* **Acute Fentanyl and cocaine intoxication**
57	Oxymorphone			0		
57	Oxycodone			0		Decedent has a past medical history of :
57	6-MAM			0		Drug abuse (crack, heroin and pills) for years
57	Morphine-3-Glucuronide			0		Smoking (also chewed tobacco)
57	Morphine-6-Glucuronide			0		Anxiety/depression
57	Norfentanyl	0.515	1.2	4.635	3.863	
57	Fentanyl	15.95	18.6	143.55	7.718	
57	Norbuprenorphine	0.2045		1.8405		
57	Buprenorphine	0.00821		0.07389		
58	Morphine			0		*Decedent:* **White/Male/42 Years 68" 205 lbs.**
58	Hydromorphone			0		
58	Codeine	0.0229		0.2061		*Manner of Death*: **Accident**
58	Hydrocodone			0		*Cause of Death:* **Acute intoxication with fentanyl and cocaine**
58	Oxymorphone			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
58	Oxycodone			0		There are two glassine pipes, numerous plastic baggy "knots", "roaches", Q-tips, three round orange loose pills (Ranitidine Hydrochloride), a lighter, playing cards, multiple cigarettes packs and an ashtray with butts found on the coffee table. There is a plastic baggy with a white rock-like substance found in one of the cigarette packs, another baggy with a white powder-like substance is found in the front right pocket of a pair of denim jeans and a bottle of vodka with only a small amount left in it is found on the couch. decedent had a history of heartburn, heavy alcohol use (2 bottles of vodka/week for many years), marijuana use, and smoked.
58	6-MAM			0		
58	Morphine-3-Glucuronide			0		
58	Morphine-6-Glucuronide			0		
58	Norfentanyl	1.44	2.6	12.96	4.985	
58	Fentanyl	4.64	8.6	41.76	4.856	
58	Norbuprenorphine	0.232515		2.092635		
58	Buprenorphine			0		
59	Morphine	0		0		*Decedent:* **White/Male/37 Years 72" 236 lbs.**
59	Hydromorphone	0		0		
59	Codeine	0.0918		0.8262		*Manner of Death:* **Accident**
59	Hydrocodone	24.25	60	218.25	3.638	*Cause of Death:* **Acute mixed drug intoxication including fentanyl, cocaine, hydrocodone, alprazolam and pregabalin**
59	Oxymorphone	0		0		
59	Oxycodone	0		0		*Date of Death:* **4/1/2015**
59	6-MAM	0		0		
59	Morphine-3-Glucuronide	0		0		The decedent smokes 3/4 pack per day of cigarettes and he drinks occasional alcohol. The following pill bottles were found in the decedent's jacket. Alprazolam, filled on 3/31/2015 quantity of 75 with 5 remaining; Zolpidem, filled on 3/31/2015 quantity of 30 with 30 remaining; Hydrocodone 10-325, filled on 3/31/2015, quantity of 90 with 0 remaining; and Lyrica, filled on 3/31/2015, quantity of 90 with 89 remaining.
59	Morphine-6-Glucuronide	0		0		
59	Norfentanyl	0.00887		0.07983		
59	Fentanyl	2.285	2.6	20.565	7.910	
59	Norbuprenorphine	0.107		0.963		
59	Buprenorphine			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
60	Morphine	0.269	4	2.421	0.605	*Decedent:* **White/Male/22 Years 65" 136 lbs.**
60	Hydromorphone	0		0		
60	Codeine	0.0264		0.2376		*Manner of Death:* **Accident**
60	Hydrocodone	0		0		*Cause of Death:* **Fentanyl intoxication**
60	Oxymorphone	0		0		
60	Oxycodone	0		0		The decedent has a medical history of prescription drug and heroin abuse. There are multiple glassine baggies and 2 loose white pills on top of a small safe on the living room floor. There are multiple capped needles in an open safe. There is a needle near the couch on the living room floor.
60	6-MAM	0		0		
60	Morphine-3-Glucuronide	0.497	7	4.473	0.639	
60	Morphine-6-Glucuronide	0		0		
60	Norfentanyl	2.45	5.4	22.05	4.083	
60	Fentanyl	43.5	35.9	391.5	10.905	
60	Norbuprenorphine	0.21285		1.91565		
60	Buprenorphine	0.0433		0.3897		
61	Morphine			0		*Decedent:* **Hispanic/Male/40 Years 72" 208 lbs.**
61	Hydromorphone			0		
61	Codeine	0.0341		0.3069		*Manner of Death:* **Accident**
61	Hydrocodone			0		*Cause of Death:* **Fentanyl intoxication**
61	Oxymorphone			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
61	Oxycodone			0		There is a bag of needles on a shelf. There is a capped needle, a cap, lighter, and 10 empty wax paper bindles on the bathroom sink. There is a spoon with charring on it and a needle on the floor near the decedent. Dried blood is noted on the bathroom sink and toilet seat. Prescription medications are found on a dresser in the kitchen. Decedent had a medical history of endocarditis, anxiety, tobacco abuse and poly-substance abuse. Medications found at the scene include Zolpidem and Alprazolam. These medications appear to have been taken more frequently than prescribed.
61	6-MAM			0		
61	Morphine-3-Glucuronide			0		
61	Morphine-6-Glucuronide			0		
61	Norfentanyl	0.479	1.4	4.311	3.079	
61	Fentanyl	8.3	23.9	74.7	3.126	
61	Norbuprenorphine	0.37		3.33		
61	Buprenorphine	0.0486		0.4374		
62	Morphine	20.7	90	186.3	2.070	*Decedent:* **White/Female/28 Years 62" 177 lbs.** *Manner of Death:* **Accident** *Cause of Death:* **Acute heroin intoxication** The decedent has a past medical history of : Substance abuse Smoking The decedent used 5-7 bags of heroin per day in the past. Decedent used marijuana since age 16, used cocaine since age 18, and used opiates daily since age 19. She had been in and out of rehab at E.C.M.C. and has had no family history of suicide attempts.
62	Hydromorphone	0		0		
62	Codeine	0.535	3	4.815	1.605	
62	Hydrocodone	0		0		
62	Oxymorphone	0		0		
62	Oxycodone	0		0		
62	6-MAM	0		0		
62	Morphine-3-Glucuronide	63.2	850	568.8	0.669	
62	Morphine-6-Glucuronide	18.5	160	166.5	1.041	
62	Norfentanyl	0.00211		0.01899		
62	Fentanyl	0.1585		1.4265		
62	Norbuprenorphine	0.1665		1.4985		
62	Buprenorphine			0		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
63	Morphine	50.1	150	450.9	3.006	*Decedent:* **White/Male/32 Years 68.5" 170 lbs.**
63	Hydromorphone	0		0		
63	Codeine	1.265	7	11.385	1.626	*Manner of Death:* **Accident**
63	Hydrocodone	0		0		*Cause of Death:* **Acute combined heroin and fentanyl intoxication**
63	Oxymorphone	0		0		
63	Oxycodone	0		0		The decedent has a medical history of heroin abuse, depression and Hepatitis C. In the bedroom on the sink is white powder on a piece of paper, a spoon and a syringe cap. On the floor is a purple shoe lace and a pair of slippers. A syringe and a pair of glasses are in the decedent's right hand; the needle tip is missing.
63	6-MAM	0		0		
63	Morphine-3-Glucuronide	60	210	540	2.571	
63	Morphine-6-Glucuronide	55.5	30	499.5	16.650	
63	Norfentanyl	0.0382		0.3438		
63	Fentanyl	2.82	2.7	25.38	9.400	
63	Norbuprenorphine	0.3454		3.1086		
63	Buprenorphine	0.0258		0.2322		

Case #	Analyte Name	Concentration in liver (1:9) dilution(mcg/L)*	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio	Case History
64	Morphine	42.1	280	378.9	1.353	*Decedent:* **White/Female/40 Years 64" 159 lbs.**
64	Hydromorphone	0		0		
64	Codeine	1.735	8	15.615	1.952	*Manner of Death:* **Accident**
64	Hydrocodone	0		0		*Cause of Death:* **Acute combined heroin, fentanyl, diazepam, venlafaxine, gabapentin, diphenhydramine, and hydroxyzine intoxication**
64	Oxymorphone	0		0		
64	Oxycodone	0		0		
64	6-MAM	0		0		Decedent had a history of chronic obstructive pulmonary disease (COPD), pulmonary embolism in June 2014 and March 2015, mild emphysema, non-traumatic seizure disorder due to a brain tumor s/p surgery, chronic neck pain (C4, C5, C6 disc herniation), gastro esophageal reflux disease, polysubstance abuse (benzodiazepines and heroin), prescription drug misuse, kidney stones, cholecystectomy, hysterectomy, right breast lumpectomy, lithotripsy, craniectomy, anxiety, depression, post-traumatic stress disorder, and she smoked. She was seen in the emergency room numerous times 2015; once for a pulmonary embolism, another for COPD exacerbation, a third following a motor vehicle accident on 4/4/2015 and others for drug use/overdose with at least one suggested as being intentional. She was prescribed Pristiq, Warfarin, Gabapentin, Amoxicillin/Clavulanate and Diazepam.
64	Morphine-3-Glucuronide	102	90	918	10.200	
64	Morphine-6-Glucuronide	41.9	13	377.1	29.008	
64	Norfentanyl	0.0506		0.4554		
64	Fentanyl	0.2505	1	2.2545	2.255	
64	Norbuprenorphine	0.49		4.41		
64	Buprenorphine	0.0364		0.3276		

* Average of two transitions

Appendix V

Backtracking Oxycodone & Hydrocodone in Case # 47 & 59, Respectively

Table 1. Pharmacokinetic data for Oxycodone and Hydrocodone (Baselt, 2014).

	Oxycodone	**Hydrocodone**
Half-life (t ½)	3.4 – 8.8 hours	3.4 – 8.8 hours
Volume of Distribution (V_d)	3.3 – 4.7 L/Kg	3.3 – 4.7 L/Kg
Bioavailability (F_b)	0.25 = 25%	0.25 = 25%
Therapeutic Adult Oral Dose	2.5 – 5 mg every 4-6 hours	5 – 10 mg every 4-6 hours (45 mg suggested daily maximum)
Therapeutic Extended Release Oral Dose	10 – 80 mg every 12 hours	10 – 50 mg every 12 hours
Subcutaneous, Intramuscular, or Intravenous Injection	1 – 10 mg every 4-6 hours	
Minimum Lethal Adult Dose		100 mg
Toxicity Level	0.1 – 8.0 mg/L	0.12 – 3.6 mg/L

Case #47

Decedent: Hispanic/Female/17 Years 62" 146 lbs. (66.2 Kg)

Manner of Death: Accident
Cause of Death: Acute mixed drug intoxication including fentanyl, heroin, cocaine, oxycodone, oxymorphone, hydroxyzine and lorazepam

Date of Death 3/7/15

Filled **Oxycodone** 3/5/15
Original quantity 40 pills
20 pills left 3/7/15
5 mg / 325 mg (oxycodone/acetaminophen)

Filled (1mg) **Lorazopam** 2/20/15
Original quantity 30 pills
14 pills left 3/7/15

Filled **Etodolac** (NSAID-nonsteroidal anti-inflammatory) 3/5/15
Original quantity 15 pills
13 pills left 3/7/15

The decedent had a history of asthma, left bicipital tendonitis and was a marijuana smoker. She was seen January 2015 at the hospital for possible Methicillin-resistant Staphylococcus aureus (MRSA). The housemate is a known an IV heroin user. Decedent had her 4 wisdom teeth pulled on 3-5-15. She received medication for pain. Also decedent tested positive for cocaine and fentanyl.

Table 2. Opiate concentrations in the blood and liver for Case #47.

Case #	Analyte Name	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio
47	Morphine	210	475.65	2.265
47	Hydromorphone		0	
47	Codeine		32.31	
47	Hydrocodone		0	
47	Oxymorphone	4	2.088	0.522
47	Oxycodone	90	101.25	1.125
47	6-MAM		0	
47	Morphine-3-Glucuronide	330	990	3.000
47	Morphine-6-Glucuronide	60	577.8	9.630
47	Norfentanyl	0.92	4.4325	4.818
47	Fentanyl	72	582.3	8.088
47	Norbuprenorphine		2.236185	
47	Buprenorphine		0.35415	

Blood Level Oxycodone = 90 mcg/L = 0.09 mg/L

Liver Level Oxycodone = 101.25 mcg/L = 0.10125 mg/L

Blood Level Hydrocodone = 0

Liver Level Hydrocodone = 0

Oxycodone Dosage Calculations

5 mg each x 20 pills = 100 mg

Probable dosage administered by decedent = V_d x Weight x Blood level

= (1.8 L/kg) (66.2 kg) (0.09 mg/L)

= 10.72 mg oxycodone in decedent

Using blood concentration

Intravenous delivery = 100% F_b = 10.72 mg

Other route of administration = 25% F_b (multiply by 4 to backtrack) = 42.88 mg

Probable dosage administered by decedent = V_d x weight x Blood level

= (1.8 L/kg) (66.2 kg) (.10125 mg/L)

= 12.06 mg oxycodone in decedent

Using liver concentration

Intravenous delivery = 100% F_b = 12.06 mg

Other route of administration = 25% F_b (multiply by 4 to backtrack) = 48.24 mg

Case #59

Decedent: White/Male/37 Years 72" 236 lbs. (107.048 kg)
Manner of Death: Accident
Cause of Death: Acute mixed drug intoxication including fentanyl, cocaine, hydrocodone, alprazolam and pregabalin

Date of Death 4/1/15

Filled **Hydrocodone** 3/31/15
Original quantity 90 pills
0 pills left 4/1/15
10 mg / 325 mg (hydrocodone/acetaminophen)

Filled Alprazolam (Xanax) 3/31/15
Original quantity 75 pills
5 pills left 4/1/15

Filled **Zolpidem** 3/31/15
Original quantity 30 pills
30 pills left 4/1/15

Filled **Lyrica** 3/31/15
Original quantity 90 pills
89 pills left 4/1/15

The decedent smokes 3/4 pack per day of cigarettes and he drinks occasional alcohol.

Also decedent tested positive for cocaine and fentanyl.

Table 3. Opiate concentrations in the blood and liver for Case #59.

Case #	Analyte Name	Blood Concentration (mcg/L)	Actual Concentration in Liver (mcg/L)	Liver: Blood Ratio
59	Morphine		0	
59	Hydromorphone		0	
59	Codeine		0.8262	
59	Hydrocodone	60	218.25	3.638
59	Oxymorphone		0	
59	Oxycodone		0	
59	6-MAM		0	
59	Morphine-3-Glucuronide		0	
59	Morphine-6-Glucuronide		0	
59	Norfentanyl		0.07983	
59	Fentanyl	2.6	20.565	7.910
59	Norbuprenorphine		0.963	
59	Buprenorphine		0	

Blood Level Oxycodone = 0

Liver Level Oxycodone = 0

Blood Level Hydrocodone = 60 mcg/L = 0.06 mg/L

Liver Level Hydrocodone = 218.25 mgc/L = 0.21825 mg/L

Oxycodone Dosage Calculations

10 mg each x 90 pills = 900 mg

Probable dosage administered by decedent = V_d x weight x Blood level

= (3.30 L/kg) (107.048 kg) (0.06 mg/L)

= 21.20 mg oxycodone in decedent

Using blood concentration

Intravenous delivery = 100% F_b = 21.20 mg

Other route of administration = 25% F_b (multiply by 4 to backtrack) = 84.80 mg

Probable dosage administered by decedent = V_d x weight x Blood level

= (3.30 L/kg) (107.048 kg) (0.21825 mg/L)

= 77.10 mg oxycodone in decedent

Using liver concentration

Intravenous delivery = 100% F_b = 77.10 mg

Other route of administration = 25% F_b (multiply by 4 to backtrack) = 308.4 mg