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A Mechanism Based Forensic Investigation into the Postmortem Redistribution of Morphine

A dissertation presented

By

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ABSTRACT

Postmortem redistribution (PMR) of drugs and their metabolites refers to the changes that occur in drug concentrations after death. Postmortem drug concentrations are also known to show variations depending on sampling site as well as characteristics of the drugs themselves. Similar to antemortem pharmacokinetics, PMR is affected by lipophilicity, degree of ionization (e.g. pk_a) and volume of distribution (V_d) [1, 2]. While it was originally thought that the primary source of drug redistribution was diffusion from the cardiac tissues, recent research shows that the redistribution from solid organs such as the lungs, liver, and myocardium is a major contributor [3]. One complication in the interpretation of postmortem blood drug concentration is whether the measured drug concentration accurately reflects the concentration at death. It is important for the field of forensics to continue to research commonly used and/or abused drugs to provide further data into postmortem redistribution and establish significant reference literature. This literature is particularly valuable in medicolegal investigations since medical examiners and coroners use this information to determine a cause and manner of death.

Heroin is a widely abused substance, involved in a large number of deaths annually. According to the CDC (Center for Disease Control and Prevention), in 2011 there was a 47% increase in heroin-related deaths and a 39% increase was observed between 2012 and 2013. Often morphine (metabolite of heroin), is treated as an indicator for heroin use and concentrations are often used to determine the cause of death [4]. Due to the increased prevalence of opioid use in the United States, establishing a relationship or understanding of postmortem drug concentrations among various samples collected at autopsy is essential. Postmortem distribution patterns of morphine have been established in blood, vitreous humor, brain and other samples from human autopsy material, and previously conducted animal studies. However, there are inconsistencies (e.g. gender, dose, sample site collection) among these

studies.

My dissertation provides an extensive insight into the postmortem redistribution of morphine, as well as the effect of fentanyl administration on morphine redistribution, that can be used throughout the forensic toxicology and pathology field to aide in the interpretation of toxicological results.

My dissertation research not only advances the understanding into the postmortem redistribution of morphine but provides an LC-MS/MS method for the analysis of morphine (MOR), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), normorphine (NM), fentanyl (Fent) and norfentanyl (NF). This LC-MS/MS method offers a rapid and sensitive analysis with minimal sample preparation, simple mobile phase composition and small sample volume. This method was validated using a fit-for use method validation using SWGTOX guidelines that is suitable for use in forensic, clinical and research laboratories.

ACKNOWLEDGMENT

This work was the culmination of many hours (years), students, colleagues and family providing so much patience, love, support and time to help one person pursue their lifelong dream. First and foremost, I would like to express my sincere appreciation and gratitude to my advisor Dr. Kim for his tremendous guidance and continuous support through what seemed like an endless journey. I still remember the day I met with him to discuss the crazy possibility of him accepting me into his lab as a full time PhD student while working full time. It was a discussion that stayed with me my entire journey; if he was willing to give me a chance, I should be confident in myself and my dream. It has been an honor to work under his mentorship and I will be forever grateful for this opportunity and everything I have learned through the years.

Secondly, I would like to thank my committee members, Dr. Samuel Gatley, Dr. David Janero, Dr. Adam Hall and Dr. Elizabeth Laposata for their time, support and feedback from my proposal, colloquium, progress report and this dissertation. I am grateful for their comments and suggestions, which not only challenged me to step outside my comfort zone but broadened my horizons as a scientist.

I am grateful to all my colleagues in Dr. Kim's lab, especially Mansi Tolia and Ji Hyun Lim. Without their willingness to assist me on weekends, their acceptance of me needing their assistance at crazy hours, whether it was 4am on Saturday or 6pm on Sunday, I would not have been able to accomplish everything presented in this dissertation. They were a constant support system during those long weekends and I am truly grateful for their dedication and support to my dissertation.

Last but not least, I would like to thank my family who were always there to support me with their endless love and trust. They were there to pick me up and help me push through the tough times; when I wanted to quit, when I did not think I was smart enough to do this or when I just needed to vent about a failed experiment or something that did not go as planned. While there may have been more times they needed to pick me up and help me refocus, the fact I get to share this great accomplish with them gives me great joy.

While this journey may have seemed like it was never going to come to an end, I will never regret for a minute doing it. While I can say with confidence, if I had to do it over again, I would not work full time and get a PhD, this journey has made me grow as a person and get stronger by the day. I will never forget my journey at Northeastern University.

BIOGRAPHICAL SKETCH

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Juhascik M, **Gleba J**, Jenkins A *(2012)*. Formation of Benzoylecgonine Isopropyl Ester Following Solid-Phase Extraction. J Anal Toxicol 36(2): 141-143

Forensic Characterization of Mitragynine in Kratom Extracts.-Master's Thesis- Advisor: Dr. Adam Hall

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Gleba J, Kim J. A Mechanism Based Approach into the Postmortem Redistribution of Morphine.

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LIST OF ABBREVIATIONS

%	Percent
% CV	Coefficient of Variation
µCi	Microcurie
µg	Microgram
µL	Microliter
ºC	Degree Celsius
6-AM	6-Acetylmorphine
ACN	Acetonitrile
ANOVA	Analysis of Variance
APCI	Atmospheric Pressure Chemical Ionization
BBB	Blood Brain Barrier
CDC	Center for Disease Control and Prevention
CID	Collision Induced Dissociation
CNS	Central Nervous System
CO_2	Carbon Dioxide
CPM	Counts per Minute
DLU	Digital Light Units
d subscript #	Deuterated (e.g D_5)
ESI	Electrospray Ionization
FB	Femoral Blood
FENT	Fentanyl
g	Gram
GC/MS	Gas Chromatography Mass Spectrometry
h	Hour
HB	Heart Blood
HPLC	High Performance Liquid Chromatography
IM	Intramuscular
IN	Intranasal
IS	Internal Standard
IV	Intravenous
kg	Kilogram
L	Liter
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography- Mass Spectrometry/Mass Spectrometry
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
LSC	Liquid Scintillation Counting
M3G	Morphine-3-Glucuronide
M6G	Morphine-6-Glucuronide

mCi	Millicurie
ME	Medical Examiner
mg	Milligram
MIN	Minute
mL	Milliliter
mm	Millimeter
MOR	Morphine
MPA	Mobile Phase A
MPB	Mobile Phase B
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
NF	Norfentanyl
ng	Nanogram
NM	Normorphine
NS	Nothing Significant
PK	Pharmacokinetics
pKa	Acid Dissociation Constant
PMPK	Postmortem Pharmacokinetics
PMR	Postmortem Redistribution
SEM	Standard Error of Mean
SRM	Selected Reaction Monitoring
SWGTOX	Scientific Working Group for Forensic Toxicology
$t_{1/2}$	Half Life
UHPLC	Ultra High Performance Liquid Chromatography
V_d	Volume of Distribution

Chapter 1. Introduction

1.1. Background and Significance

A major challenge faced by postmortem forensic toxicologists is to establish whether a drug caused or contributed to the death. This issue is resolved by a combination of the following: an analysis of the medical history, consideration of the effects of the presumed dose or measured concentration of drug, the role of other drugs and their pharmacokinetics (i.e. the time course of drug concentrations), and the exclusion of other potential causes. It is thus critical that medical examiners or coroners have an accurate interpretation of drug levels found in various locations in the body. Although a number of studies have reported on the ratios of the levels of common drugs of abuse in postmortem tissue versus blood levels in an attempt to evaluate postmortem drug distribution, we still do not fully understand the significance of postmortem drug levels as antemortem indicators. Because clinical information is derived from antemortem blood or serum/plasma levels, accurate interpretation of intoxication requires that appropriate samples be collected at autopsy that best represent the antemortem blood levels for potential drugs of abuse. However, relationships between antemortem and postmortem blood levels for many drugs have not been established.

The postmortem redistribution (PMR) of drugs and their metabolites refers to changes in drug concentrations in the blood and other matrices that occur after death. In postmortem toxicology, the severity or lethality of intoxication for a given drug is derived from blood concentrations determined during testing and comparison to reference values - therapeutic, toxic or lethal – which exist for these levels. These values are calculated using antemortem specimens, for which the total amount administered (i.e. following a single administration), can be evaluated and taken into account. In contrast, the postmortem pharmacokinetic characteristics of a given

substance, and the total amount used by a decedent, may not be available, thus reference values cannot be directly correlated.

In my dissertation, I have addressed gaps in our understanding of the PMR phenomenon in relation to morphine and its metabolites, and mechanisms at the interface between forensic chemistry and toxicology when decedents are under the influence of multiple drugs. In particular, I proposed to focus on the PMR of morphine relevant to postmortem medical examination. The following have been addressed:

1.2. Specific Aims

Aim 1: To evaluate the tissue distribution pattern of morphine in healthy rats using autoradiography. Radioactivity was measured in various organs (lung, kidney, liver and brain) using imaging software and beta counting with tissue digestion to characterize the postmortem distribution pattern of morphine. **(Chapter 3)**

Aim 2: To develop and validate an LC-MS/MS method for the detection and confirmation of morphine, morphine-3-glucuronide, morphine-6-glucuronide, normorphine, fentanyl and norfentanyl. I developed a rapid and sensitive method using LC-MS/MS for the detection and quantitation of morphine and metabolites as well as fentanyl and its metabolites following SWGTOX guidelines, which aided in the quantitative determination of concentrations used for interpretation and comparison purposes. **(Chapter 4)**

Aim 3: To establish an antemortem pharmacokinetic profile of morphine and its metabolites. The pharmacokinetic evaluation demonstrates how MOR metabolized over an

hour time period. Pharmacokinetic profile was used to determine optimal sacrifice time to be used in subsequent chapters. **(Chapter 5)**

Aim 4: To evaluate the *in vivo* postmortem pharmacokinetics and tissue distribution of morphine after intravenous administration. The pharmacokinetic evaluation demonstrates how morphine and its primary metabolites (M3G, M6G and NM) are redistributed in rats after death. A comparison between the various tissues and blood were made to determine the extent of PMR. **(Chapter 6)**

Aim 5: To evaluate the various factors that can affect the PMR of morphine. A pharmacokinetic study demonstrated how morphine is redistributed in rats after death when fentanyl is co-administered. A comparison between the various tissues and blood collected in aim 3 and those collected in aim 4 was made to determine the extent to which fentanyl affects the PMR of morphine. Further evaluation of data obtained in aim 4 was performed to look into the effect of gender and drug injection time on morphine PMR. **(Chapter 7)**

Chapter 2. Literature Review

2.1. Drug induced death information in the United States

Postmortem examination reveals important information about the cause of and contribution to drug-related death. The primary question asked of postmortem forensic toxicologists is usually 'Did a drug cause or contribute to the death?' The answer comes from a combination of the following: analyzing medical history, considering the likely effects of the presumed dose or the measured concentration of drug, assessing the role of other drugs and their pharmacokinetic (i.e. the time course of drug concentrations) factors, and excluding other potential causes. Often, pathologists or toxicologists are requested to estimate the amount of drug present at the time of death or the number of tablets consumed. This assumes that the drug concentration found at postmortem examination is a reliable estimate of the amount present at the time of death. However, there is almost no evidence to support such an extrapolation. Literature is limited in the correlation of antemortem and postmortem concentrations among several sampling sites (e.g. femoral blood, heart blood and tissues).

2.2. Accurate interpretation of drug concentrations is critical for postmortem examination

As a forensic toxicologist, I know about the importance of providing the medical examiner or coroner with an accurate interpretation of drug levels found in various locations in the body. In an attempt to evaluate PMR, ratios of the levels of common drugs of abuse in postmortem tissue to those in blood have been reported, but whether postmortem drug levels are accurate indicators of antemortem levels remains uncertain. Since clinical information is derived from blood levels, it is necessary to select appropriate samples that best represent antemortem blood

levels in order to accurately interpret intoxication. However, the relationships between antemortem and postmortem blood levels for drugs of abuse have not been established. Thus, referencing kinetic studies in which postmortem redistribution is examined for a given drug or drug class will provide a scientific basis for interpretation, and will greatly impact the field of forensic toxicology.

In postmortem toxicology, the severity or lethality of intoxication by a given drug is interpreted from blood concentrations for which therapeutic, toxic or lethal reference values exist [2]. These values are often calculated on the basis of antemortem specimens, which enable evaluation of the total amount administered (following a single administration). This calculation also takes into account the pharmacokinetic characteristics of a given molecule, however, the total amount used by the decedent is usually not known, and thus reference values cannot be directly correlated. A complication in the interpretation of postmortem blood drug concentration is the issue of whether the measured drug concentration accurately reflects the concentration at death, and to what extent it contributed to the cause and/or manner of death.

2.3. The need to account for postmortem redistribution in the interpretation of toxicological analyses

Postmortem redistribution (PMR) of drugs and their metabolites refers to changes in drug concentrations that occur between the time of death and specimen collection [5] – such changes can be attributed to the pharmacokinetic and chemical properties of the drug, orientation of the body, putrefaction, drug dosage and interval between drug ingestion and death. Moreover, as seen with antemortem pharmacokinetics, PMR is affected by lipophilicity (lipid solubility), degree of ionization/charge (e.g. pKa) and the volume of distribution [2, 3]. It was originally thought that the primary source of drug redistribution was diffusion from the cardiac tissues;

however, recent research shows that redistribution from solid organs such as the lungs and liver is also possible [6]. Therefore, it is important to address postmortem redistribution, and establish more reliable reference values for drugs that are commonly used and/or abused. These values will significantly improve the assessments made by medical examiners and coroners in determining the cause and manner of death.

Heroin is a widely abused substance, which is involved in a large number of deaths annually. According to the CDC (Center for Disease Control and Prevention), heroin-related deaths increased by 47% in 2011, and by 39% between 2012 and 2013. The estimated rate of unintentional opioid-related overdoses death in 2015 was 25.8 deaths per 100,000 residents. This represents a 26% increase from a rate of 20.4 deaths per 100,000 residents in 2014 [4]. Figure 2.1 shows the trend in the annual number of confirmed and estimated cases of opioid-related overdose deaths for all intents from 2000-2018. The data brief published by the Massachusetts Department of Public Health, also estimated as of January 15, 2019, there would be an additional 104-117 deaths in 2017 and 320 to 394 in 2018 once these cases are finalized [7].

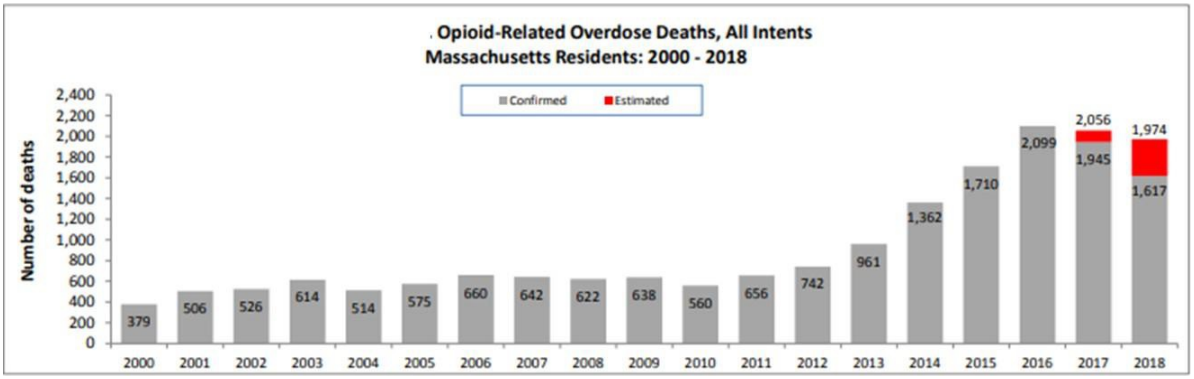

Figure 2.1: Massachusetts Department of Public Health published data brief on opioid-related overdose deaths from 2000-2018. [7]

Heroin is a pro-drug, with a low affinity for opioid receptors. Shortly after administration, heroin is de-acetylated to 6-acetylmorphine (6-AM), which is further hydrolyzed to morphine. Heroin itself has a half-life of approximately 2-6 min in blood, and is rarely detected in human samples. 6-AM has a half-life of approximately 6-25 minutes, and detection of 6-AM in blood samples indicates very recent heroin intake [8]. Morphine has a half-life of approximately 2-3 hours, and is glucuronidated to M3G and M6G (which is an active metabolite). Due to the short half-life of heroin and 6-AM, morphine concentrations are routinely used in postmortem toxicology to indicate heroin abuse.

Deaths due to the abuse of heroin and morphine still present a formidable problem in forensic toxicology. Interpreting the results of such analyses is often difficult. PMR patterns of morphine have been established for blood, vitreous humor, brain and other samples from human autopsy material, but the relationship of these levels to antemortem values has not been extensively studied [2,9,10].

2.4. Morphine Pharmacokinetics

Morphine is the most abundant analgesic opiate found in opium and is a potent pain reliever. Morphine is used in clinical pain relief but is also used illicitly for recreational purposes among drug users due to its euphoric effect. It is highly addictive and can cause intense physical dependence that leads to abuse of the substance. Morphine is obtained from the seedpod extract or opium found in the poppy plant, Papaver somniferum, and is available in the following forms: injectable, pill, oral solution as well as a suppository. Once injected or ingested, morphine enters the bloodstream which carries it to the brain and other parts of the body where it activates opiate receptors to exert the effects of the drug. Three types of opiate receptor μ (mu), κ (kappa) and □ (delta) are recognized; they are approximately 70% homologous. There are two mu, three kappa, and two delta subtypes. Differences between receptor occur mainly at the N and C terminal

ends. The mu receptor is thought to be the most important, because it is where morphine (and like drugs) exerts its effect. When a mu receptor binds with an agonist, such as morphine, a G protein attached to the third intracellular loop of the receptor is activated (opiate receptors have seven loops, also called transmembrane domains) [11]. A fourth opiates receptor, referred to as the sigma receptor, is now recognized as a completely unrelated entity [12].

One of the clinical effects of morphine is papillary constriction due to its excitatory action on the parasympathetic nerves that supply the pupil. Respiratory depression also occurs as mu agonists exert effects on brain stem respiratory centers. Small doses of morphine depress the respiratory rate, while large doses cause respiratory arrest, which is the accepted mechanism of death in cases of narcotic overdose. Nausea and vomiting are also associated with mu receptor activation [13,14].

Activation of kappa receptors also produces analgesia but it simultaneously induces nausea and dysphoria. They bind to an endogenously occurring ligand called dynorphin. Some believe dynorphin may play an important role in determining an individual's risk for addiction [15,16].

Delta receptor activation also produces analgesia but it can also cause seizures. Delta receptors normally bind to enkephalins, which are responsible for relieving pain [12].

2.4.1. Absorption

Morphine can be given orally, or subcutaneous, intramuscular injection or intravenous injection. Morphine is also administered via epidural, either as an individual dose or continuous infusion. Morphine concentrations generally peak at 2 – 7.8 minutes with a reported half-life of 109 – 287 minutes and are detectable in plasma for much longer than heroin and 6-AM. [17, 18]. The oral bioavailability of morphine is quite low due to extensive first pass hepatic metabolism.

2.4.2. Distribution

The volume of distribution (V_d) of morphine ranges from 2 to 5 L/kg in humans although values as high as 7 L/kg have been observed [19]. Owing to the relatively large V_d of morphine (3 – 5 L/kg) [20], less than 2% of a given dose is to be found circulating in blood. The volume of distribution is defined as the amount of drug in the body divided by the plasma drug concentration. V_d increases when the plasma concentration is reduced, with the total drug amount unchanged; this indicates that the drug distributes preferentially to tissues relative to plasma. After IV administration morphine is rapidly distributed to the tissues receiving the highest blood flow (i.e. the lung, kidney, spleen and muscle) and tissue concentrations generally reflect the relative blood flow [21]. Morphine crosses the blood brain barrier (BBB) at a much slower rate than heroin and 6-AM [22]. During life (antemortem levels), it is estimated that approximately half of the morphine circulating in plasma is protein bound although other estimates are more conservative (20 – 35%) [23]. Since the amount of morphine able to enter the brain is dependent on the amount of free morphine circulating in the plasma, concentrations in this matrix are typically low 249]. The accumulation of morphine in fat has been reported [25]. M3G and M6G are highly polarized and minimally lipophilic therefore their ability to cross the BBB is significantly less than that of morphine. Due to the V_d's of M3G (0.14 L/kg) and M6G (0.15 L/kg) being low very little is often observed in tissues however M6G is sometimes observed in the brain. At physiological pH, morphine, a weak base with the pKa of about 8.0, is primarily ionized [5]. About 10-20% of morphine's molecules are unionized and the ionized form does not favor passage through the lipid membrane.

2.4.3. Metabolism

Morphine is a metabolite of heroin which has a half-life of approximately 2-3 hours and is

metabolized via glucuronidation to M3G and M6G. Morphine is metabolized by CYP450 enzymes resulting in the formation of NM however this metabolism accounts for 6% of metabolite formation; thus inhibition/induction or genetic polymorphisms of CYP450 enzymes should have little-to-no effect on the metabolism or clearance of morphine. The major pathway of this agent is the UGT2B7 metabolic pathway, which chiefly forms glucuronides. While the glucuronidation process typically inactivates drugs, extensive research with morphine suggests that the chief metabolites of morphine (M3G and M6G; representing 50- 60% and 10% of the total metabolites, respectively) have the potential for significant clinical effects. M3G has no analgesic properties but may have central nervous system (CNS) toxicity that includes irritability, hallucinations, and allodynia [26,27], whereas M6G is an active metabolite, which exhibits up to a 50-fold greater potency than the parent drug morphine in terms of its analgesic properties [26,27]. A small amount of morphine, on the order of 5%, is N- demethylated by hepatic CYP3A4 and to a lesser extent CYP2C8 to form normorphine. This metabolite is pharmacologically active but less potent than morphine and present in lower concentrations [11, 28].

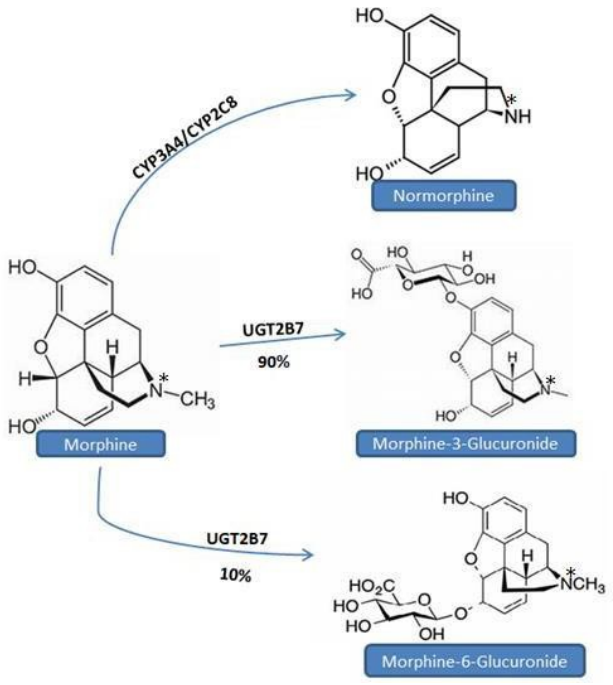

Figure 2.2: Metabolism profile of morphine. * indicates location of C14 labeling (Chapter 3)

2.5. "Poly-drug exposure" or drug-drug interaction is common in real life.

Fentanyl has invaded the opioid crisis across the United States within the last couple of years. The United States has seen an increase in the number of opioid-related deaths, thought to be due to heroin; however, research determined that the opioid epidemic was not solely related to heroin, but was caused by a combination of heroin and fentanyl. Fentanyl can be synthesized cheaply, but still be sold for the same price as heroin. Thus street level drug manufacturers, to make as much money as possible and generate a large repeat customer following, began to "cut" heroin with fentanyl, which in turn led users to experience a better high. Over time, heroin has been replaced with fentanyl, but still being sold as heroin. Users are unaware of what they are consuming, and the increased potency of fentanyl has led to an increase in overdoses across the country. An added problem for medical examiners and toxicologists is that very few studies have addressed how heroin and fentanyl, taken in combination, affect postmortem toxicology

results.

2.6. Fentanyl Pharmacokinetics

Fentanyl is a fast-acting potent synthetic opioid introduced in the U.S. in the early 1960s for use as an anesthetic supplement. It interacts with the opioid mu 1 and 2 receptors located in the brain, spinal cord, and smooth muscle. Respiratory depression is similar to that observed with other receptor agonists but the onset is more rapid. As also observed with morphine, delayed onset respiratory depression can be observed and may be attributed to enterohepatic circulation. Fentanyl is highly lipophilic and therefore crosses the BBB rapidly. Therapeutic use involves the CNS, producing pharmacological actions as pain relief and sedation. In addition to its uses as an anesthetic agent, fentanyl is prescribed clinically to treat chronic pain.

2.6.1. Absorption

Fentanyl is highly lipid soluble and rapidly crosses the blood brain barrier. Fentanyl has a pKa of 8.4 and is 84.4% plasma protein bound [29].

2.6.2. Distribution

Fentanyl is highly lipophilic and is rapidly distributed to tissues such as the brain, heart, kidneys, and lungs followed by slower movement into muscle and fat. Fentanyl is approximately 80% bound to plasma proteins, principally α-l-acid glycoprotein. The V_d ranges from 3 to 9 L/kg. The plasma half-life is 3 to 12 hours [30]. The half-life ($t_{1/2}$) for equilibration between the plasma and cerebrospinal fluid is approximately 5 min. The levels in the plasma decrease rapidly owing to redistribution of fentanyl from highly perfused tissue groups to other tissues such as muscle and fat. As saturation of less perfused tissue occurs, the duration of effect of fentanyl

approaches the length of their elimination $t_{1/2}$ of 3-4 hours. Fentanyl undergoes hepatic metabolism and renal excretion. With the use of higher doses, the drugs accumulate and the clearance mechanisms become saturated and fentanyl becomes longer acting. The clearance rate for fentanyl is 13 mL/min/kg however this decreases with age [31].

2.6.3. Metabolism

Fentanyl is rapidly metabolized by the liver to the inactive metabolites, norfentanyl, hydoxyfentanyl and hydroxynorfentanyl. Results presented by Labroo et al. [32] indicate the primary route of hepatic metabolism for fentanyl is CYP3A4. Approximately 85% of an intravenous dose is excreted in the urine over a 3-4 day period, with 0.4-0.6% excreted unchanged, and 26-55% excreted as norfentanyl.

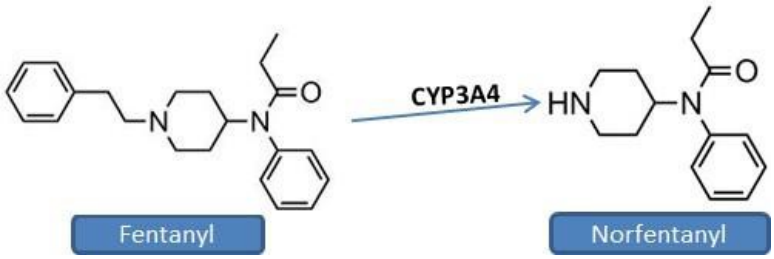

Figure 2.3: Metabolism profile of fentanyl

2.7. Postmortem Changes and Redistribution

After death, as cell and tissue autolysis advances, cell membranes are broken down and drugs released from their binding sites can redistribute via passive diffusion from areas of high concentration in the tissues to areas of lower concentration in adjacent tissues and blood [33]. In general the concentrations recorded in the central and heart vessels (pulmonary artery and vein and vena cava) are more subject to postmortem increases than peripheral sites which are not in

proximity to the central organs and gastrointestinal tract in which drugs accumulate antemortem [3,33,34]. Drugs sequestered in the pulmonary circulation can diffuse through the thin-walled pulmonary veins and significantly elevate concentrations in the left ventricle [35]. As the postmortem interval increases so does the likelihood that site- and time dependent changes in drug concentrations will have occurred [36]. Eventually the peripheral blood achieves equilibrium with central blood [37] and in cases of advanced decomposition the concentrations of drugs measured in the heart and peripheral blood are similar [36]. Authors also reported comparable central and peripheral concentrations following extensive resuscitation attempts. It is generally agreed that in order to avoid misinterpretation of cardiac blood drug concentrations which may have been falsely elevated post-mortem, blood samples should be collected from peripheral sites, which are less subject to change in the early post-mortem period [38]. The recommended site for sampling is the femoral vein and this should be cross-clamped proximally prior to sampling to avoid drawing blood with elevated drug concentrations from the proximal iliac vein and vena cava [38].

The advancement of putrefaction can contribute significantly to changes in drug concentrations. Invasion of the corpse by aerobic and anaerobic bacteria of the gastrointestinal tract, oral cavity and lungs is known to occur during putrefaction [39, 40]. These bacteria are capable of producing and/or metabolizing many compounds in postmortem blood and tissues. It has been established that many drug metabolites are actively deconjugated (e.g. converted from M3G back to morphine) by intestinal, bacterial enzymes resulting in enterohepatic recirculation and artefactual elevation of the free form of the drug [41]. Further, residual metabolic enzyme activity, variable with the nature of the enzyme involved, occurs in the early postmortem period [2]. Continuing drug metabolism and metabolite deconjugation during the postmortem interval is an important consideration when interpreting parent drug to metabolite ratios.

2.8. Postmortem Change and Redistribution of Morphine

It is not surprising that morphine has been extensively studied in postmortem pharmacokinetics (PMPK). In 1988, William Sawyer and Robert Forney conducted a study to look into the postmortem disposition of morphine in rats [41]. The authors explored the antemortem and postmortem distribution of morphine in rats for the purpose of establishing whether drug distribution is altered after death. Samples were examined for free and total morphine concentration at 0-96 hours after death with morphine being administered at regular intervals antemortem. All groups of rats studied showed a significant ($P<0.05$) increase in postmortem cardiac blood morphine concentrations. These changes were detectable within 5 minutes after death in blood specimens. Increased morphine levels were also observed at 24 and 96 hours after death in liver, heart tissue and forebrain, while morphine levels in urine decreased. However, Sawyer and Forney only looked into one morphine concentration (5 mg/kg). In 1992, Koren and Klein [42] performed a similar study to Sawyer and Forney using 4 mg/kg of morphine with euthanasia occurring 2 hours after administration of morphine. Yet again, the scope of their study was limited and only the cavity blood was measured. They also concluded that there is substantial redistribution of morphine after death with elevated postmortem levels being an inaccurate representation of antemortem concentrations. However, conflicting information exists about the extent of PMR in humans. Gerostamouls et al. [9] found that in 40 heroin related deaths there was no significant difference between antemortem admission and autopsy blood concentrations, and while there was a trend for higher concentrations in heart blood compared to femoral blood or subclavian blood, the difference was not significant.

On the contrary, others [35, 36, 43, 44] have found that morphine does exhibit PMR, and reported differences between central and peripheral concentrations of morphine, M3G and M6G. These studies used data from human studies in which an opioid (mainly heroin) overdose was suspected.

One possible explanation for the discrepancies among studies is the difference in sampling site, sampling time and pH among samples. Various studies [1, 2, 3, 9, 10] only focused on the central site concentrations rather than peripheral and other tissues. Since both morphine and heroin undergo hepatic metabolism one would expect higher concentration in the inferior vena cava as compared with femoral vein. While representative of what may occur in the field of forensic science, the interval between the time of death and time of postmortem sample collection varies among studies, leading to inaccurate comparisons and conclusions. The concentrations of free morphine can increase significantly in the postmortem period due to hydrolysis of the morphine glucuronides [35, 45]. Escherichia coli, one of the most predominant bacteria present in intestinal flora, is an important source of β-glucuronidase [46], and is known to deconjugate morphine glucuronides, particularly M3G [47], in putrefying blood and tissues[35]. The hydrolysis of morphine glucuronides back to free morphine during the post-mortem interval can alter the ratios (e.g. M3G: MOR) significantly as a function of time.

2.9. Relating Postmortem Blood Morphine Concentrations to Toxicity

One of the major problems in the interpretation of morphine concentrations is that the fatal concentrations reported in the literature often overlap the stated therapeutic (0.08 - 0.12 mg/L) and toxic concentrations (0.15 - 0.5 mg/L). Tolerance to both the pharmacological and respiratory depressant effects of morphine occurs rapidly and morphine concentrations

obtained at autopsy may be misinterpreted if concentrations presumed to be fatal in non-tolerant individuals are applied to active heroin users or to individuals undergoing chronic pain treatment with opioids who have built up tolerance to the drug [48]. Heroin overdose as cause of death may be evident in cases involving heroin body packing, where blood morphine concentrations as high as 120 mg/L have been reported [49] however in a great majority of heroin overdose cases the morphine concentrations recorded at autopsy are, in fact, lower than or similar to those recorded in living intoxicated addicts or heroin users who have died of causes other than overdose [24, 50]. Darke et al. [45] observed substantial overlap in the blood morphine concentrations measured in heroin overdose fatalities (median: 0.35 mg/L; 0.08 – 3.2 mg/L; n = 39) with those measured in living addicts receiving maintenance heroin (median: 0.09 mg/L; 0.05 – 1.45 mg/L; n = 100). Only four of the 39 heroin fatalities had morphine concentrations exceeding the highest concentration measured in the current users. Low blood morphine in cases of heroin overdose has been largely attributed to periods of abstinence resulting in loss of tolerance and/or the concomitant use of other drugs. Research has shown that following a period of incarceration, and thus a period of drug abstinence or reduced use, there is a particularly acute risk of drug-related death, in the first few weeks following release [51, 52]. These studies report the involvement of opioids in the vast majority of deaths.

2.10. Study Rationale

It is important for the field of forensics to continue to research commonly used and/or abused drugs to provide further data into postmortem redistribution and establish significant reference literature. This literature will become important as medical examiners and coroners use this information to determine a cause of death. Given the instability of morphine in blood

during the postmortem period due to bacterial action and other factors, there is a need for more accurate and reliable characterization of substance concentrations at the time of death as it is critical for medical examiners or coroners to determine the cause and manner of death or at least provide evidence of drug misuse or abuse. The information gained from the postmortem pharmacokinetics of morphine can be correlated (with caution) with other drugs similar in nature.

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Chapter 3. Investigation into Whole-Body Distribution of Morphine using Autoradiography

3.1 Introduction

Knowledge of local drug concentrations, in other words, pharmacokinetics (PK) and distribution to tissues and cells is of critical importance for researchers and toxicological studies. The bio distribution of drugs in the body after oral or systemic administrations is of value for both guiding toxicological studies and for identifying sites of retention that can be used to account for pharmacokinetic behavior. These drug levels can be quantified using autoradiography and/or scintillation counting for radiolabeled drugs, provided that the presence of radiolabeled metabolites can be accounted for. The use of radiolabeled drug compounds, the combination of organ digestion with liquid scintillation counting (LSC) of organ homogenates and autoradiography provides a detailed visual picture as well as quantitative results that can be evaluated by a researcher.

Sven Ullberg pioneered the autoradiography technique in 1954, which relied on the placement of whole-body tissue sections on x-ray film [1, 2]. Newer imaging studies have employed phosphor imaging plates [3]. These have several advantages over film, including an order-of-magnitude greater sensitivity, linearity of response, and greater dynamic range, although the maximal spatial resolution is lower. Phosphor imaging thus produces digital images of radioactivity distributed within the tissues sections, with a resolution of about 50 microns [4, 5]. This is a good match for rodent whole-body autoradiography. The sequence of events in a whole-body autoradiographic study may be briefly summarized as follows. Mice or rats are injected intravenously with a labeled compound; each animal receives a single dose. After various time-intervals, the animals are euthanized and rapidly deep-frozen. Sagittal sections are prepared at different levels though the whole frozen body. The frozen sections are placed against

imaging plates. The plates are stored in the freezer for sufficient time to generate a useful latent image, which is developed using a phosphor imaging scanner. Local concentrations of radioactivity in the images are estimated using appropriate software (e.g. OPTIquant).

The attractive feature of autoradiography is that, as an imaging modality, it indicates the distribution of radioactivity throughout the whole section. That is, it provides estimates of radioactivity concentration at every x, y coordinate. However, autoradiography is more uncertain in the z dimension, perpendicular to the surface of the section, because radiation emanating from the surface is reduced than that from deeper parts of the section, and contributes less to the signal recorded by the phosphor imaging plate. It is thus convenient to complement autoradiographic imaging with liquid scintillation counting (LSC) that allows quantification of radioactivity in tissue punches from regions of interest indicated in the images. LSC involves dissolution of tissue samples in a "solubilizer" that is then intimately mixed with solution containing compounds (scintillators) that emit light when stimulated by beta radiation. In this way, the energy of each beta particle is converted with high efficiency into a shower of light photons, which can be detected using a pair of photomultiplier tubes. Counts are recorded when both photomultipliers simultaneously detect light photons in the appropriate energy range [6-8], allowing the instrument to ignore light photons arising from chemical processes in the samples.

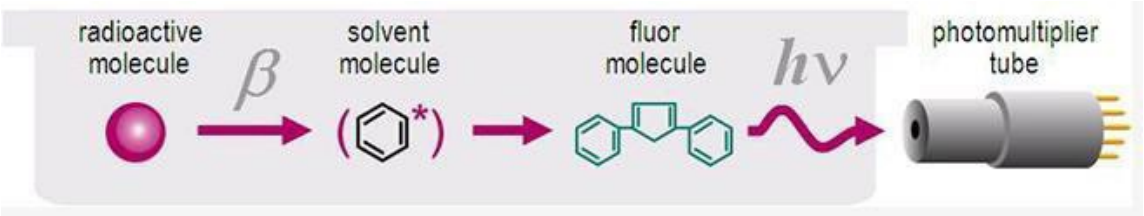


Figure 3.1: Schematic overview of the scintillation process (from Perkin Elmer Website) [9]

A previous study of ^{14}C-morphine distribution through the central nervous system (CNS) was performed in 1985 in which rats were studied after intrathecal injection through catheters

ending at the lumbar level [10]. Whole body autoradiography was performed and spinal cord levels and brain region were studied. To my knowledge this is the only study performed that looked at the distribution of ^{14}C-morphine. In contrast to the present research it was focused solely on the spinal cord region.

In my dissertation, whole body autoradiography with phosphor imaging autoradiography was employed followed by organ digestion and analysis using LSC after intravenous administration of ^{14}C-morphine to look at the distribution pattern of morphine throughout the body over various time periods and determine if morphine will be detectable at various postmortem intervals.

An autoradiography approach was taken to get prior knowledge of what tissues would be most suitable for use in subsequent PMR studies. By performing whole body autoradiography and not focusing on specific organ/s, it allows for the full tissue distribution pattern to be evaluated. It is common for researchers to focus on the organs such as the liver, brain and kidney, which are commonly used for toxicological purposes but I wanted to see if there was an organ outside of these that would provide valuable morphine concentration values that could be used for interpretation.

3.2 Materials and Methods

3.2.1 Ethics Statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Northeastern University Institutional Animal Care and Use Committee.

3.2.2 Animals and Drugs

Tissue dissection and counting studies were performed using Fischer F344 rats (Harlan) weighing approximately 90 g. Male and female rats were fed facility chow and water *ad libitum*. Morphine [N-methyl-^{14}C] (0.1 mCi/mL in ethanol) was obtained from American Radiolabeled Chemicals (St. Louis, MO).

3.2.3 Preparation of dosing solution

Morphine [N-methyl-^{14}C] stock solution was evaporated down under air to remove ethanol and dissolved with injectable saline. Target injection concentration was 5 µCi/20 g body weight (or 29 µg as morphine/20 g) of morphine [N-methyl-^{14}C]. Dosing solution was analyzed using LSC to ensure reconstitution in saline was successful.

3.2.4 Treatment of rats with Morphine [N-methyl-^{14}C]

Rats were lightly anesthetized with isoflurane, and administered 4 µCi of [N-methyl-^{14}C] morphine via the lateral tail vein. The rats were then euthanized by isoflurane overdose 60 minutes post-injection (with the exception of the rat expected to be collected at 0 hour postmortem interval as rat died immediately following intravenous injection) of [N-methyl-^{14}C] morphine. Rats were placed in a supine position for various postmortem intervals (0, 4, 8, 24 hours) prior to being flash frozen in a dry ice slurry.

3.2.5 Whole body autoradiography

The frozen carcass was sectioned using a band saw and the frozen sections (shown in Figure 3.2) were exposed to an imaging plate and placed in the freezer for 24 hours. After 24

hours, the imaging plates were removed and scanned in a phosphor imager (Perkin Elmer Cyclone Plus) to image radioactivity.

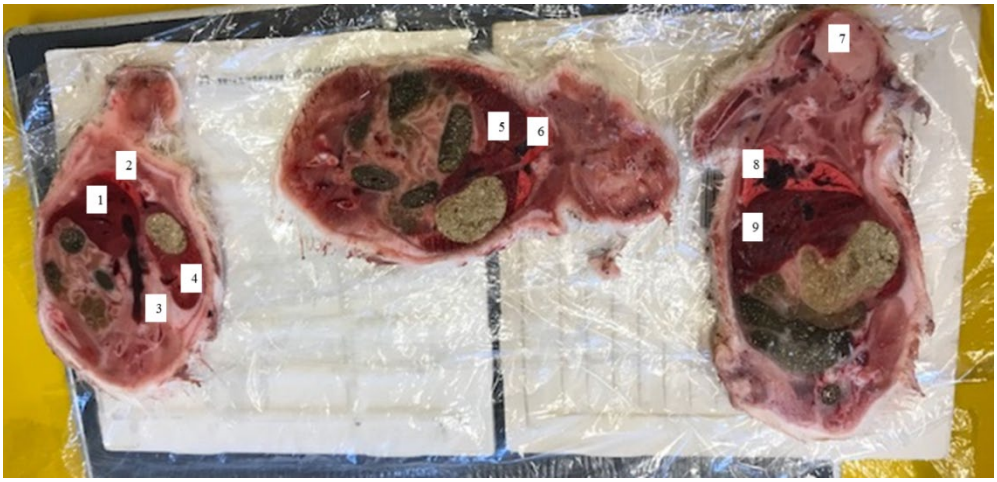


Figure 3.2: Slices were placed on imaging plate and placed in the freezer for 24 hours. Numbers with corresponding location: 1) Liver, 2) Lung, 3) Blood clot, 4) Kidney, 5) Liver, 6) Lungs, 7) Brain, 8) Lungs, 9) Liver

3.2.6 [N-methyl-^{14}C] Morphine postmortem redistribution in rats

To enable quantification of radioactivity levels in sections, tissue punches were taken of various organs (brain, lung, heart and liver) followed by weighing, digestion of the tissues using Solvable and heated for 48 hours on a slide warmer. After 48 hours, hydrogen peroxide (30%) was added (in 100 µL increments) to decolorize the digestions and samples were allowed to sit for 24 hours at room temperature to ensure complete decay of hydrogen peroxide. Scintillation cocktail (Ultima Gold, Fisher Scientific) was added and counting was performed using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation counter). Counting was performed in triplicate over three days and CPM measurements were averaged.

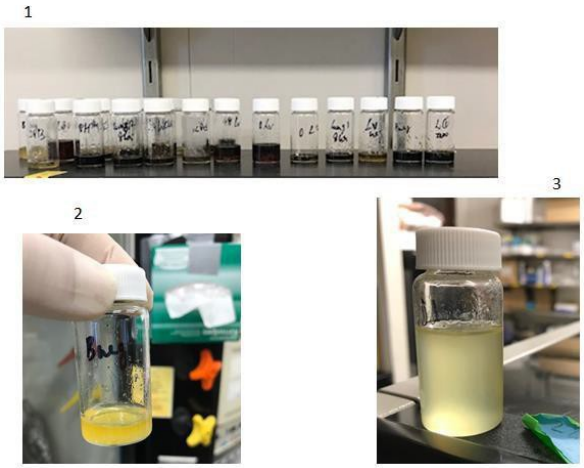


Figure 3.3: Digestion of tissue samples post imaging. 1) Immediately after addition of solvable 2) After addition of hydrogen peroxide and remaining at room temperature for 24 hours 3) After addition of liquid scintillation fluid prior to reading

3.3 Results and Conclusions

Whole-body autoradiography images (Figure 3.4) collected were evaluated for levels of [N-methyl-^{14}C] morphine present in the various organs.

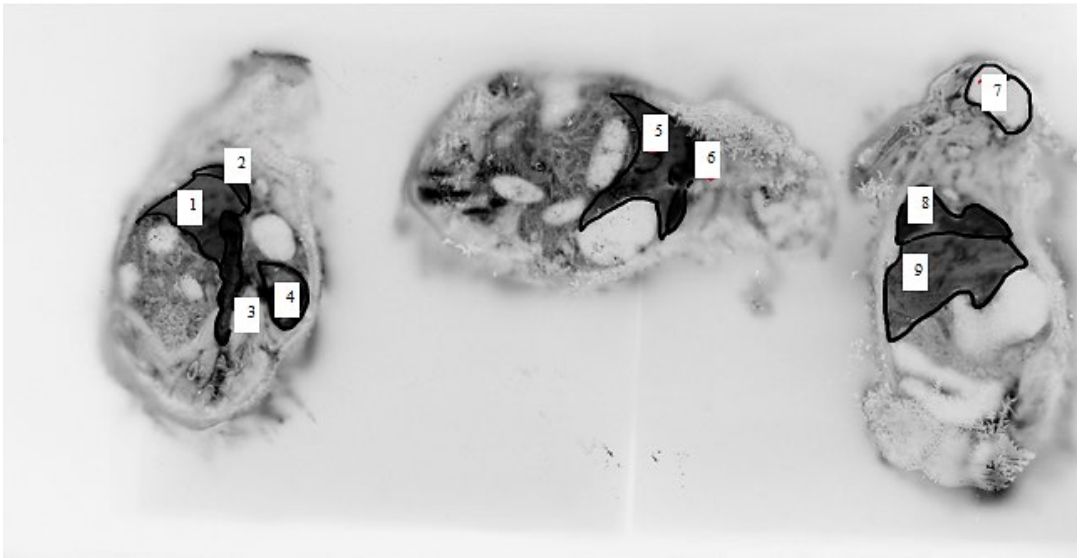
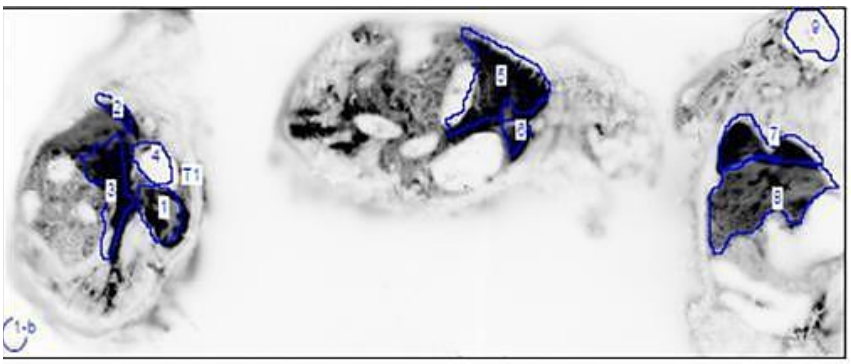


Figure 3.4: After imaging time period, images were collected. Numbers with corresponding location: 1, 9) Liver 2, 6, 8) Lung(s) 3) Blood clot 4) Kidney 5) Liver 7) Brain

Pixel values (Figure 3.5) for regions of interest drawn over the images using OptiQuant software were compiled and evaluated based on intensity level.



ID	DLU
1	547,226,888
2	157,932,642
3	1,066,283,659
4	30,643,716
5	1,795,566,332
6	227,517,581
7	945,541,962
8	14,370,444,452
9	42,962,957

Figure 3.5: Representative autoradiographic images of rat sagittal sections generated by the Cyclone instrument's OPTIquant software, with regions-of-interest drawn by hand. The Table displays total image intensities for the regions, in terms of "digital light units" (DLU). Values of DLU are proportional to surface carbon-14 concentrations in the regions. The images were used to guide collection of tissue punches for radioactivity quantitation using liquid scintillation counting. Region ID: 1) Kidney 2, 6, 7) Lung 3, 5, 8) Liver and Inferior Vena Cava 4) Food Matter 5, 8) Liver 9) Brain.

Tissue punches (additional data provided in appendix A) were collected from whole-body sections above, digested and counted. Results were compiled, converted to pmol/mg values using CPM/mg (from tissue digestion data) and specific activity of [N-methyl-^{14}C] morphine (55 mCi/mmol) and evaluated over the various postmortem intervals.

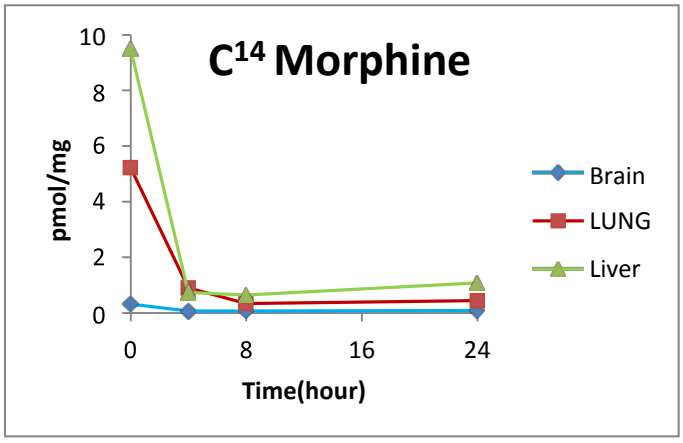


Figure 3.6: Tissue punches were collected after imaging was complete, digested, decolorized and counted

Due to the detection of [N-methyl-^{14}C] morphine in the liver, lung and brain in the whole body autoradiography images and in digested tissue samples, it was determined that these tissues are suitable for use in future postmortem redistribution studies (Chapters 6 and 7). The detection of [N-methyl-^{14}C] morphine in these organs during this study coincides with the published distribution and metabolism profile of morphine [11-13].

The tissue digestion and corresponding count, as shown in Figure 3.6, demonstrate a rapid decrease from time zero to 4 hour in all tissues, however, the concentrations leveled out from 4 hours to 24 hours. It is possible the rapid decrease seen from the zero hour to 4 hour interval is over-estimated due to reduction in post-injection time interval for zero hour. This possible overestimation does not affect the results of this chapter as the focus was on morphine tissue distribution and detection over the course of various postmortem intervals.

Heart tissue was also collected, digested and evaluated for [N- methyl-^{14}C] morphine. [N-methyl-^{14}C] Morphine was present in these samples however it was determined that the heart tissue would not provide additional information in future PMR studies (Chapters 6 and 7) as heart blood would be collected.

Limitations of this study include the use of only one rat per time point as well as lack of calibration standards. Future studies to include the use of multiple rats per time point would establish if the data presented here is reproducible.

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Chapter 4. Development of an LC-MS/MS Method for the Quantitation of Morphine and its Metabolites and Fentanyl and its Metabolite

4.1 Introduction

As opioids play a significant role in the field of forensic toxicology, a number of methods for their determination in various biological matrices of forensic importance have been published [1-4]. Most of these methods employ a sample preparation technique such as liquid-liquid or solid-phase extraction. Protein precipitation techniques or "dilute-and-shoot" methods provide a rapid and simple sample pretreatment, but suffer from the potentially adverse effects of ion suppression [5]. Fentanyl and/or its major metabolite, norfentanyl, have been determined using gas chromatography mass spectrometry (GC/MS) [6, 7], LC-MS/MS [8, 9] and immunoassays [10, 11]. However, high performance liquid chromatography (HPLC) and immunoassays do not offer the high sensitivity required for low dose studies of fentanyl, while GC/MS yields good sensitivity but requires long run times [12-14].

Liquid chromatography (LC) has become the leading separation technique in chromatography due to its flexibility, accuracy, and efficiency when compared to other techniques such as gas chromatography. Although LC achieves the physical separation of multiple components in a mixture, mass spectrometry (MS) offers information about their structural identity. The addition of tandem MS technology further improves the specificity and accuracy of the detection method. LC-MS/MS methods provide significant advantages such as reduced analytical run time, reduced sample size, elimination of extensive sample preparation thus resulting in cost savings for laboratories [15]. Analysis by LC- MS/MS has become widespread among clinical toxicology, forensic toxicology and the pharmaceutical industry for these reasons [16]. With respect to morphine and fentanyl, current literature provides separate methods for the quantitation of morphine and fentanyl which leads to the consumption of additional sample, the

need for additional analysis time as well as presents a redundancy among instrumental and extraction methods [12, 13]. Also, it is not common that their active and inactive metabolites, including M3G, M6G and NM, are evaluated as part of routine postmortem toxicology casework. The addition of these analytes to current methods can provide additional information to medical examiners and coroners about survival time of an individual.

The aim of this study was to develop a sensitive LC-MS/MS method for the simultaneous determination of MOR, M3G, M6G, NM, FENT and NF in limited samples of blood and tissue from rats combined with minimal sample treatment to allow for the detection of all analytes which is currently not available to my knowledge.

The validated method described in this chapter was used in the determination of drug and metabolite concentrations in all samples analyzed in this study (results presented in Chapters 5, 6, 7).

4.2 LC-MS/MS

An Agilent 1200 series LC system (Capillary HPLC pump, high-performance autosampler and vacuum degasser) equipped with an Agilent 6460 triple quadrupole was employed using Agilent Masshunter acquisition software (Version B.04.00). The analytical column was a Raptor Biphenyl LC column (50x2.1mm 5.0 µm) that was purchased from Restek (Bellefonte, PA).

Generally, the instrument consists of a liquid chromatograph (LC) attached to a tandem mass spectrometer (MS/MS). Compounds are initially separated by LC according to their interaction with the chemical coating of the stationary phase and the solvent eluting through the column (mobile phase).

The LC column chosen for this analysis employs a biphenyl stationary phase combined

with a superficially porous silica particle for separation. The Biphenyl is particularly adept to separating compounds that are hard to resolve or that elute early on C18 or other phenyl chemistries [17]. This is important for our analysis due to M3G and M6G being early eluters as well as difficult to separate. The superficially porous particles allow for decreased analysis time as well as improved separation in comparison to using standard HPLC instruments resulting in methods similar in nature to ultra-high performance liquid chromatography (UHPLC) [17]. After LC separation, the sample enters the mass spectrometer.

The triple quadrupole mass spectrometer consists of an ion source, enhanced desolvation technology, followed by ion optics that transfers the ions to the first quadrupole (see figure 4.1). In the Agilent 6460, an electrospray ion source is used where the analyte is simultaneously ionized and desolvated from the liquid matrix. The desolvated ions enter the mass spectrometer via an inert capillary tube (position 2 on Figure 4.1) [18].

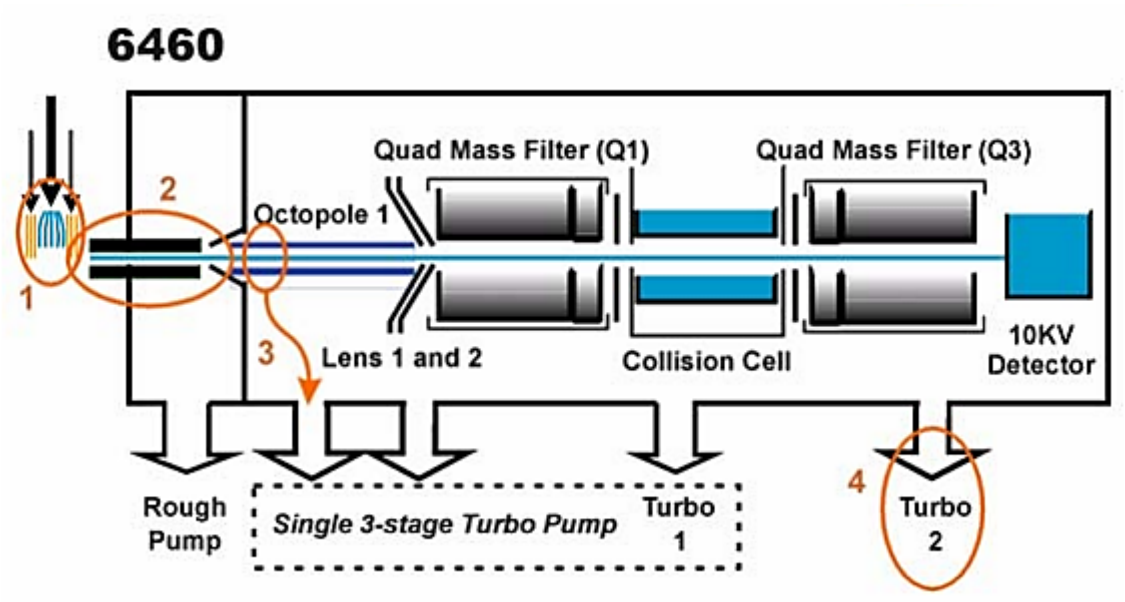

Figure 4.1: Agilent 6460 triple quadrupole mass spectrometer diagram [18]

The ions next pass though optics and into the first quadrupole analyzer. The quadrupole consists of four parallel hyperbolic rods through which selected ions are filtered based on mass

to charge ratio before reaching a collision cell where they are fragmented. The collision cell is typically called the second quadrupole, but in the Agilent 6460 instrument, it is actually a hexapole filled with nitrogen, the same gas used in the ion source. The fragment ions are formed in the collision cell then sent to the third quadrupole for a second filtering stage to enable isolation and examination of multiple precursor to product ion transitions. Finally, the ions that pass through the third quadrupole are detected using a high energy detector [18].

Quadrupoles allow users to perform MS/MS in serval ways: product ion scan, precursor ion scan, full scan and selected reaction monitoring (SRM)/multiple reacting monitoring (MRM). Due to its usefulness in quantitative measurements for analytes present in complex mixtures MRM was used. MRM is conceptually the same as a product ion scan in which fragments of the collision induced dissociation (CID) process are known for a target analyte [19]. However, instead of scanning the second mass spectrometer in a broad mass range (as done with product ion scan), the two mass analyzers are adjusted to monitor one or more chosen precursor–product pairs of the analyte. Further a triggered dynamic MRM was used which sets criteria for primary MRMs which trigger confirmatory (secondary) MRMs to be acquired for a compound [18, 19]. If the abundance of the primary MRMs are higher than the set thresholds and other criteria (e.g. retention time) are met, then the confirmatory (or secondary) MRMs are acquired.

Advantages of tandem mass spectrometry include the ability to analyze a wider range of compounds; it is more selective and specific for drugs compared to GC/MS. MS/MS mode is not used for general drug screening but for specific analyte quantitation. The drug of interest must be ionized and the fragmentation of the parent ion must produce a stable fragmentation pattern of qualifier ions, which are used to identify and confirm the presence of an individual drug.

LC-MS/MS was chosen as the analytical method of choice because of its common use in

numerous forensic and toxicology laboratories across the nation [16]. Although LC achieves the physical separation of multiple components in a mixture, MS offers information about their structural identity. The addition of tandem MS technology further improves the specificity and accuracy of the detection method.

4.3 Analyte Quantitation

Analyte quantitation was achieved by means of an internal standard calibration graph plotting analyte response divided by IS response versus concentration in calibration standards. Because of the degree of ionization in MS and the potential for drug loss during sample preparation, the use of an internal standard (IS) is vital when performing quantitative measurements. Even with analyte losses during sample preparation the ratio of the sample to the IS will remain constant and the measured concentration should reflect more accurately that of the original biological sample. The concentration of an analyte in an unknown sample may be calculated against the corresponding calibration curve providing the detector response is proportional to analyte concentration in the calibration standards.

Deuterated internal standards were chosen for use in this assay for all analytes except normorphine as a deuterated internal standard is not available. The use of deuterated internal standards is recommended as they are have similar chemical and physical properties as the target analyte and should perform identically during extraction and instrumentation analysis [20]. The IS concentration (100 ng/mL M3G-d_3, MOR-d_6, M6G-d_3 and 10 ng/mL NF-d_5, FENT-d_5)was based on a concentration giving a peak response that was approximately equal to the response obtained from the non-deuterated analogue at the mid-point of the calibration range.

4.4 Instrument Optimization

4.4.1 Liquid Chromatography Optimization

First, two columns were tested: Ultra Biphenyl (50x2.1mm, 3µm) and Raptor Biphenyl (50x2.1mm, 5µm). The ultra-biphenyl column was not acceptable for analysis due to high back pressure created due to use of capillary HPLC. The Raptor Biphenyl was selected for method validation. A flow rate of 300µL/min was selected to accommodate the maximum column pressure of the instrument.

4.4.2 Mass Spectrometry Optimization

Precursor ion and product ion transitions for each analyte and internal standard (Table 4.1) were determined using Agilent Optimizer software (version B.04.00). MRM transitions (detailed chromatography in appendix B) were identified based on highest sensitivity and specific discrimination between co-eluting compounds.

Table 4.1: Precursor ions along with their qualitative and quantitative transitions for all analytes and internal standards

Peak	Analyte	Quant Transition (m/z)	Qualifier Transitions (m/z)	Fragmentor (V)	Collison Energy(V)
1	M3G-d_3	465.2-289.2	465.2-201.1	130	29,49
2	M3G	462.2-286.1	462.2-201.1	185	29,49
3	Normorphine	272.3-152.1	272.1-165.1	160	60,41
4	Morphine-d_6	292.2-153.1	292.2-128.2	135	30
5	Morphine	286.1-152.0	286.1-128.1	135	30
6	M6G-d_3	465.2-289.2	465.2-165.1	190	29,60
7	M6G	462.2-286.2	462.2-165.0	200	29,60
8	Norfentanyl-d_5	238.1-84.1	N/A	135	30
9	Norfentanyl	233.1-84.1	233.1-150.1	135	30
10	Fentanyl-d_5	342.4-188.3	342.4-105.1	135	30
11	Fentanyl	337.2-105.2	337.2-188.1	135	30

Figure 4.2 shows the LC-MS/MS chromatogram of the level 4 calibrator.

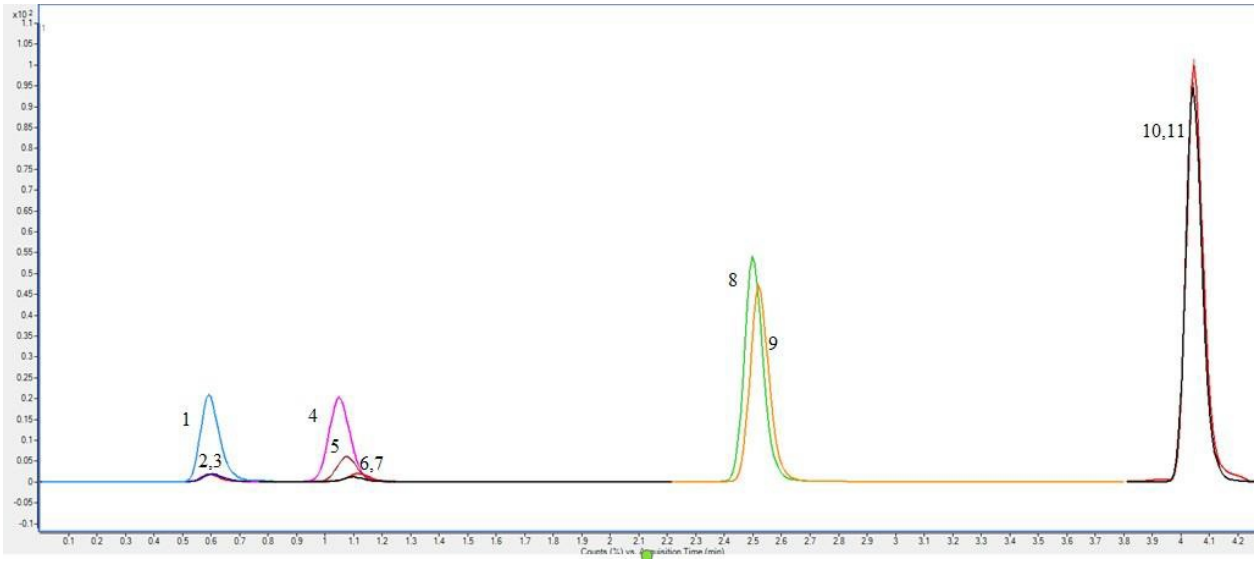

Figure 4.2: LC-MS/MS ion chromatogram of level 4 calibrator (target concentration of 100 ng/mL MOR, M3G, M6G, NM and 10 ng/mL NF, Fent). Each peak represents the quantitative transition ion (qualitative transition ion not shown). Analyte and internal standard identities: (1) M3G-d3, (2) M3G, (3) NM, (4) MOR-d6, (5) MOR, (6) M6G-d3, (7) M6G, (8) NF-d_5, (9) NF, (10) FENT-d_5, (11) FENT

4.5 Sample Preparation

4.5.1 Biological Matrices

Biological matrices used during method validation were collected from drug free rats. Blood was preserved with heparin (1000U/mL) and stored frozen. Tissue specimens were stored frozen without added preservative.

4.5.2 Modified crash and shoot extraction

Calibrators, controls and samples were all treated the same during the method validation. Internal standard (deuterated target analytes except for normorphine) was added to 100 µL of sample (unless run diluted) followed by the addition of 400 µL of acetonitrile while vortexing. After centrifugation, supernatant was collected, evaporated at 50℃ under a stream of air and

reconstituted with 50 µL of mobile phase A and B mixture (95:5) and injected onto LC-MS/MS. Modified crash and shoot method resulted in poor extraction of urine samples and poor chromatography on LC-MS/MS (see appendix F) therefore urine was not included in further studies.

4.6 Method Validation

4.6.1 Summary

A fit-for-purpose method validation was performed by assessing the following criteria: bias and precision, linear dynamic range, dilution integrity and carryover. Criteria for acceptable control performance were as follows: average bias <20%, within- and between-run precision coefficient of variation <15%, to meet accuracy requirement, the calculated concentration of the control had to have a coefficient of variation of <15% from the target concentration. Carryover was evaluated by analyzing extracted blank matrix after the highest calibrator ((target concentration of 1000 ng/mL MOR, M3G, M6G, NM and 100 ng/mL NF, Fent).).

4.6.2 Methods

4.6.2.1 Materials

High performance liquid chromatography grade acetonitrile (ACN) and formic acid (99%) were purchased from Fisher Scientific. Certified reference standards of MOR, M3G, M6G, NM, FENT, and NF were purchased from Cerilliant (Round Rock, TX). Internal standards were MOR-d6, M3G-d3, M6G-d3, FENT-d5 and NF-d5 from Cerilliant. Double deionized water was made in house.

4.6.2.2 Standard Solutions

Stock and dilute calibrator solutions (1.0 µg/mL M3G, NM, MOR, M6G/0.10 NF, Fent

µg/mL and 0.05 µg/mL M3G, NM, MOR, M6G/0.005 NF, Fent µg/mL) and stock control solution (1.0 µg/mL M3G, NM, MOR, M6G/0.10 NF, Fent µg/mL) were prepared by dilution of purchased certified reference material in acetonitrile and were stored in freezer for up to 6 months. All calibration standards (10-1000 ng/mL M3G, NM, MOR, M6G, 1.0-100 ng/mL NF, Fent) were prepared by serial dilution in pooled rat blood.

Matrix matched quality controls (75 ng/mL and 350 ng/mL M3G, NM, MOR, M6G, 7.5 ng/mL and35 ng/mL NF, Fent) were run along with the calibration curve. All standards and quality controls were spiked with a stock internal standard solution (1.0 µg/mL M3G-d_3, MOR-d_6, M6G-d_3/0.10 µg/mL NF-d_5, and Fent-d_5) to a final concentration of 100 ng/mL M3G-d_3, MOR-d_6, M6G-d_3 and 10 ng/mL/NF-d_5, and Fent-d_5. MOR- d_6 was used as the internal standard for normorphine as a deuterated internal standard is not available.

4.6.2.3 LC-MS/MS Parameters

Separation of morphine and its metabolites, fentanyl and its metabolite was achieved with a Raptor Biphenyl (50x2.1 mm, 5µm) analytical column. Mobile phase A (MPA) consisted of 0.1% formic acid in water. Mobile Phase B (MPB) consisted of 0.1% formic acid in acetonitrile. MPA and MPB were held for 2.0 min at 95/5%. MPA was ramped down from 95% to 80% over 0.80 minutes following by a gradual ramp down to 10% over 1.2 minutes, then held for 0.5 min and finally returned to 95% and held for the remainder of the time for a total run time of 8.0 min. Electrospray ionization in positive ion mode was used. Source parameters were maintained for nitrogen gas temperature (300ºC), gas flow (5.0 L/min) and a capillary voltage (3500 V). Detection was accomplished using a triggered dynamic MRM scan function.

4.6.3 Validation Parameters

4.6.3.1 Linear dynamic range

All calibration curves (see appendix B) except fentanyl and norfentanyl were calculated using a quadratic fit with 1/x weighting regression analysis with the resulting range of calibration of 10-1000 ng/mL. Fentanyl and norfentanyl calibration curves were calculated using a linear fit with 1/x weighting regression analysis with a resulting calibration range of 1.0-100 ng/mL. LOD was only tested at 50% of the lowest calibrator (5.0 ng/mL MOR, M3G, M6G, NF/0.5 ng/mL NF and Fent) and meet acceptable reporting criteria (Table 4.2). Concentrations below this level were not relevant for the research being performed (see appendix C for negative matrix data).

Table 4.2: Retention times, limit of detection, lower limit of quantitation, and linear/quadratic range for all analytes along with corresponding internal standards

Analyte	Internal Standard	Retention Time(min)	LOD (ng/mL)	LLOQ (ng/mL)	Linear/Quadratic Range (ng/mL)
M3G	M3G-d_3	0.463	5.0	10	10-1000
Normorphine	Morphine-d_6	0.599	5.0	10	10-1000
Morphine	Morphine-d_6	1.013	5.0	10	10-1000
M6G	M6G-d_3	1.066	5.0	10	10-1000
Norfentanyl	Norfentanyl-d_5	2.507	0.5	1.0	1.0-100
Fentanyl	Fentanyl-d_5	4.046	0.5	1.0	1.0-100

4.6.3.2 Carryover

Carryover was evaluated by analyzing extracted blank matrix after the highest calibrator. Analyte response less than 10% of the response of the level 1 calibrator was deemed acceptable. Carryover was not observed after the highest calibrator.

4.6.3.3 Bias and Precision

All analytes in this method passed the criteria for acceptable control performance listed above for bias and precision, shown in Table 4.3.

Table 4.3: Intra- and interday bias and precision

			Bias (%)		Precision (%CV)	
Analyte	Expected Concentrations (ng/mL)	Mean (ng/mL)	Intraday	Interday	Intraday	Interday
M3G	75	78	5.33	3.42	4.56	11.87
	350	360	3.64	2.80	3.74	9.39
Normorphine	75	75	-1.60	-0.38	6.38	6.83
	350	353	0	0.73	2.63	4.74
Morphine	75	75	-3.47	0.51	6.22	8.12
	350	364	-1.00	4.12	1.86	7.67
M6G	75	75	10.67	0.07	3.51	10.89
	350	355	-1.07	1.56	4.36	6.91
Norfentanyl	7.5	7.2	-6.67	-3.67	3.35	6.82
	35	34	-1.43	-2.27	5.55	4.55
Fentanyl	7.5	7.6	-2.40	1.13	2.80	6.60
	35	36	-1.43	2.32	5.02	5.77

4.6.3.4 Dilution Integrity

Dilution integrity was evaluated by determining the coefficient of variation for each target concentration as each dilution ratio performed (x2, x4 and x10). All dilutions meet acceptable criteria.

4.7 Results and Discussion

The LC-MS/MS method developed for the analysis of morphine, morphine-3-glucuronide, morphine-6-glucuronide, normorphine, fentanyl and norfentanyl offers a rapid and sensitive analysis with minimal sample preparation, simple mobile phase composition and small sample volume that is suitable for using in forensic, clinical and research laboratories.

The limit of detection was established to be 5.0 ng/mL for MOR, M3G and M6G and 0.5 ng/mL for Fent and NF which meets guidelines set forth for LOD or meets common practice standards for postmortem toxicology, workplace drug testing, clinical toxicology and sexual assault testing [21,22].

The modified crash and shoot extraction method employed in this chapter reduces the sample preparation time from approximately 2-4 hours for a standard solid phase extraction to less than 1 hour. The reduction in sample preparation time increases the efficiency and reduces cost for laboratories when compared to current extraction techniques. In addition, the sample volume required for this method is 100 µL which is $1/10^{th}$ the standard sample volume used in most laboratories (1 mL). The reduction in sample volume allows for the samples that could not previously be tested due to increased limit of detection when running reduced sample volume to be tested. Finally, the modified method reduces the consumption of solvents and other consumables used during solid phase extraction or liquid liquid extraction procedures. Therefore this method not only saves a laboratory analyst time there will be a cost savings.

The reduced sample volume as well as minimal sample preparation demonstrates advances to LC-MS/MS methods which are currently available. The LOQ, linear range, precision, and accuracy validated for this method would permit its use for a variety of forensic and clinical applications.

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Chapter 5. Antemortem Pharmacokinetics of Morphine

5.1 Introduction

Although PK parameters have previously been established for morphine, it was critical to perform an antemortem pharmacokinetic tissue distribution analysis using parameters specific to my study (e.g. specific dose and rat model). The tissue distribution of morphine and its metabolites provides a guide for determination of optimal time for detection of all analytes that can be used as sacrifice time in future PMR studies. It is important to assess the concentration detected using the specific dose to be used for PMR studies, as they must fall within the calibration range set forth in the instrumental method (Chapter 3). The information obtained from the antemortem pharmacokinetic study provided critical relationships and decision points which were the basis for method plans used in PMR studies.

Morphine is a drug commonly used in the management of moderate to severe nociceptive pain, such as pain due to cancer, surgery or trauma. In order to establish the relationship between the pharmacokinetic parameters detailed below and dosage, two doses were studied, one estimated in the clinical dose range and a dose near high clinical dose range. The recommended starting parenteral dose for adults >50kg body weight is 15 mg every 3-4 hours whereas for adults <50 kg body weight, the starting parenteral dose is 0.1 mg/kg every 3-4 hours with standard doses ranging from 2 to 10 mg IV [1, 2]. It is known that morphine can produce wide spectrum of unwanted effects, including respiratory depression, nausea, vomiting, dizziness and hypotension [1]. It is important to consider the PK effect of morphine and how they are affected by varying dose in order to reduce the possibility of the effects listed above. Morphine is well absorbed through the gastrointestinal mucosa where it undergoes substantial hepatic first-pass effect and therefore, results in relatively low bioavailability (~25%). Peak plasma drug

concentrations are reached 30 to 90 min after oral administration and 15 to 20 minutes after subcutaneous or intramuscular administration [3]. After absorption, morphine rapidly distributes within the body, including the brain. Morphine has a large volume of distribution, and only one third of the circulating amount binds to plasma proteins. Morphine has a high systemic clearance and an accordingly short half-life of approximately two hours. The primary site of morphine metabolism is the liver, where it undergoes rapid glucuronidation [3, 4]. However, extrahepatic metabolism of the drug can account for up to 30% of its total clearance. This extrahepatic metabolism may play a relatively important role in morphine metabolism in patients with severe liver failure. In the liver, the principal metabolite is M3G [3]. This metabolite has no pharmacological effect and is primarily excreted in the urine. Another important metabolite is M6G, which appears to be at least as potent as morphine [2]. M6G has a half-life of approximately 1-2 hours. This active metabolite is also excreted in the urine and may accumulate significantly in case of renal insufficiency. Less than 10% of the dose is excreted unchanged in the urine.

Table 5.1: Previously established pharmacokinetic parameters of morphine [3, 4]

Morphine pharmacokinetic parameters	
Clearance (CL)	20-30 mL/min/kg
Volume of distribution (Vd)	2-5 L/kg
Half-life (t1/2)	1.3-6.7 h

The goal of this study was to evaluate the distribution of morphine over an 8 hour time period as well at establish a PK profile for metabolite formation. The PK profiles for morphine and its metabolites were then used to establish the appropriate dose as well as time period best suited for sacrifice to be used in subsequent chapters. It was critical to determine a time period and dose that would provide quantifiable concentrations of morphine and its metabolites that can be detected by the instrumental parameters set forth in Chapter 4.

5.2 Materials and Methods

5.2.1 Ethics Statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Northeastern University Animal Care and Use Committee.

5.2.2 Animals and Drugs

The experiments were conducted on male and female Fisher F344 (Harlan) rats weighing approximately 210 g. Male and female rats were fed facility chow and water *ad libitum*. Morphine sulfate (25 mg/mL) was obtained from Henry Schein (Indianapolis, IN) and diluted to appropriate concentrations for the two different dosing groups (1.0 and 10 mg/kg) with sterile saline. Rats were lightly anesthetized with isoflurane, and administered morphine intravenously via the right tail vein with dosing volumes of 1.0 mL/kg.

5.2.3 Blood Sampling

Blood samples of approximately 100 µL were collected via the right tail vein at predefined time points as follows: 1, 5, 15, 30, 60, 120, 240, 360 and 480 min. The samples were collected in microcentrifuge tubes (2.0 mL) and stored at -80℃ until instrumental analysis was performed.

5.2.4 Instrumental Analysis

Blood samples were analyzed for MOR, M3G, M6G, NM, FENT and NF concentrations using the method described in Chapter 3.

5.3 Results and Discussion

5.3.1 Selection of morphine dose

Morphine was detectable up to 4 hours post injection in the 1.0 and 10 mg/kg dose studies. M3G was detectable up to 4 hours post injection in the 1 mg/kg study but for 8 hours in the 10 mg/kg study. M6G in 1 mg/kg and NM in the 1 mg/kg and 10 mg/kg studies were not detectable. M6G was detectable for up to 15 min post-injection in the 10 mg/kg study. Based on this information, the 10 mg/kg dose was chosen to be used for future studies to ensure morphine and metabolite detection.

5.3.2 Selection of time point for euthanasia

A sacrifice (Figure 5.1) time of 60 minutes was chosen for use during future studies based on concentrations detected over the antemortem time course study. Morphine was detectable at concentrations that fell within or close to the linear range of the instrumental

method without the need for further dilution. Choosing a concentration that falls within the linear curve becomes important as it reduces the need for repeat instrumental analysis and the possibility of needing to eliminate an animal time point (during PMR evaluation) due to concentrations above the calibration curve. It was deemed acceptable to use 60 minutes as sacrifice time even though M6G and NM were not detectable as morphine PMR is the main focus of medical examiner and coroner evaluation. Evaluation of M3G concentrations at 60 minutes was similar to morphine and feel within the linear range for the instrumental assay.

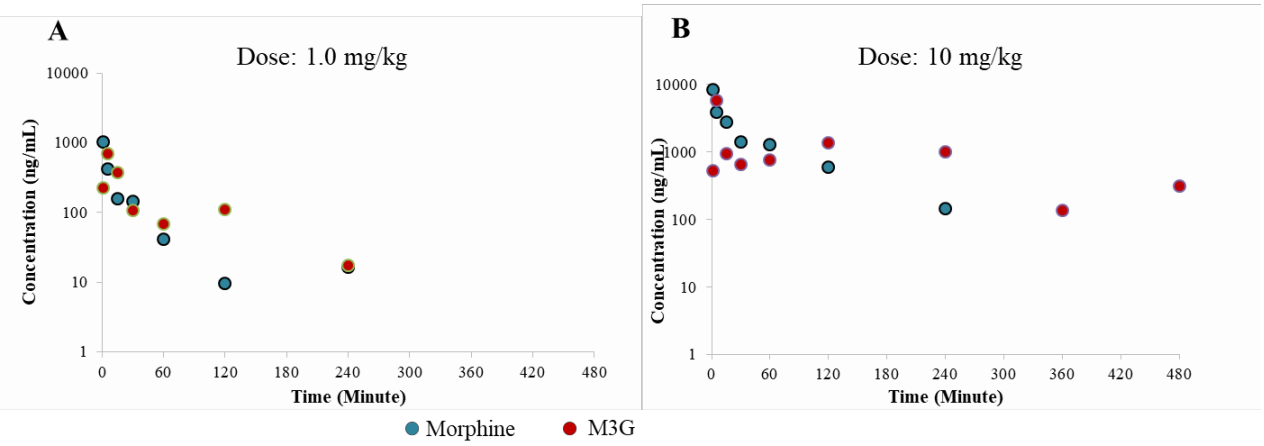

Figure 5.1: Morphine and metabolite pharmacokinetic profile after intravenous injection. A: 10 mg/kg dose B: 1.0 mg/kg dose

5.4 Conclusions

It was determined that 60 min post-injection time range will provide the most probative values for use with postmortem redistribution studies while allowing for concentrations to fall within the calibration curve parameters. While morphine was detectable at earlier time points, the concentrations were outside the linear range and would require a dilution be performed during postmortem redistribution studies. The dose selected for future studies was 10 mg/kg. This dose was chosen over 1.0 mg/kg due to the higher concentration detected at the 60 minute sacrifice interval. Also, the purpose of my dissertation was to develop reference literature that

was useful for toxicologist, medical examiners and coroners when overdose is suspected, therefore it was imperative to choose a dose that was high enough to replicate overdose like situations without overdosing the rats themselves.

Chapter 5 References

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Chapter 6. Postmortem Redistribution of Morphine and its Metabolites

6.1 Introduction

In most medical examiner (ME) offices, bodies are transported to the office and refrigerated upon arrival before examination, which may occur 24 hours or even longer after receipt of the body. Postmortem redistribution is a well described phenomenon in postmortem toxicology, and it has been shown to occur within the first 24 hours after death. Although the issue of postmortem redistribution is important for most drugs, it is of particular importance for opioids/opiates, in which the concentrations can vary greatly between patients with chronic pain and opioid-naïve patients because of tolerance. Morphine is a commonly prescribed and commonly encountered opioid medication found in postmortem examinations. It has been long accepted that drugs with a volume of distribution greater than 3 to 4 L/kg are expected to redistribute postmortem [1-3]. Morphine's volume of distribution is 2 to 5 L/kg, which means it falls into this category.

Animal studies with rat models have shown that there is indeed an increase in postmortem heart blood concentrations over time [4,5] although a more recent study with swine showed that total morphine concentrations do not change over time or between sampling sites [6]. Human studies regarding morphine have also presented conflicting information as to whether or not PMR exists for morphine [7-9]. One possible explanation for these discrepancies is the difference in sampling sites, sampling time, specimen pH and unknown dose (or low dose studied), especially in human studies. In response to these issues, the present study was designed to assess both spatial and temporal changes in postmortem redistribution of morphine by quantitative instrumental analysis of commonly collected postmortem samples (heart blood, femoral blood, liver, brain and lung tissue) from rats over a 24 hour postmortem interval. The collection of multiple postmortem samples, those which are routinely evaluated in postmortem

casework, provides a more comprehensive view and understanding of morphine PMR, thus providing medical examiners, coroners and toxicologists with better supporting data to use during the interpretation and evaluation of autopsy toxicology results. Further, in comparison to other studies, this study was performed in a controlled environment with reproducible conditions and methods with only one variable that was changing, postmortem interval. This controlled study allows for a more precise evaluation that cannot be obtained when determining PMR using autopsy samples where there are several unknown variables (e.g. dose, postmortem interval and body conditions).

This study provided a basis for morphine PMR using an approach novel to the PMR investigation. The use of a mechanism based approach to PMR, not previously reported in literature, and provides information that is not available from current studies which focus on central to peripheral ratios as well as peripheral to tissue ratio. This approach which involved the collection of various tissues, femoral and heart blood samples was representative of what is collected during autopsy.

6.2 Materials and Methods

6.2.1 Ethics Statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Northeastern University Institutional Animal Care and Use Committee.

6.2.2 Animals and Drugs

Male and female Fischer F344 (Harlan) rats were fed facility chow and water *ad libitum*. Morphine sulfate (25 mg/mL) was obtained from Henry Schein (Indianapolis, IN) and diluted to

appropriate concentration (10 mg/kg) with sterile saline.

6.2.3 Administration and sample collection

Rats were injected intravenously through the right tail vein with 10 mg/kg of morphine sulfate while under light anesthesia using isoflurane. Blood was collected via the left tail vein 5 min post-injection as well as immediately prior to euthanasia. Prior to being euthanized, heparin (1000 U/mL) was injected vial the tail vein to prevent extensive clotting. One hour post injection, rats were euthanized via CO_2 and placed in supine position at room temperature for desired postmortem interval (0, 8, 16, and 24 hours). Heart blood, femoral blood, liver, lung and brain were collected and immediately analyzed. Femoral blood was collected via needle stick and pooled from both the left and right femoral veins (due to limited volume). Heart blood was collected by direct cardiac stick. Liver sample was collected from the upper right quadrant and lung was collected from the right side. The whole brain was collected. Left kidney and urine samples were collected but testing was not performed as part of this study.

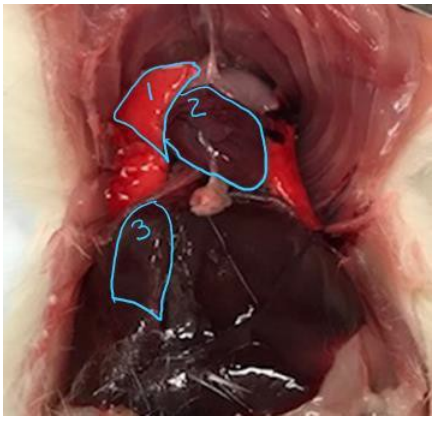

Figure 6.1: Visual depiction of collection sites. 1) Lung 2) Site for Cardiac Puncture 3) Liver

6.2.4 Instrumental Analysis

Blood samples were analyzed for MOR, M3G, M6G, NM, FENT and NF concentrations using the method described in Chapter 3.

6.2.5 Statistical Analysis

Graphical representation of data was achieved using Microsoft Excel 2010. Group comparisons performed using one-way ANOVA followed by Tukey's post-hoc analysis using SigmaPlot 12.3, when applicable ($p<0.05$).

6.3 Results and Discussion

6.3.1 Postmortem redistribution of morphine and metabolites

Graphical representation of the results for the PMR study of morphine are shown in Figure 6.2 (additional data provided in appendix D and E) One-way ANOVA with Tukey's post-hoc analysis was employed to evaluate the difference observed across the postmortem interval in each matrix as well as difference at each postmortem interval across the matrices. Morphine concentrations in femoral blood decreased over time, but this pattern was less obvious in heart

blood (Figure 6.2.A). In contrast, the concentrations of morphine did not change with time in the liver or brain. Interestingly, there was a rapid decrease in morphine concentrations in the lung for the first 8 h postmortem with a leveling out between 8 and 24 hours. This pattern was similar to the time course of morphine concentrations in femoral blood (distal to the lung), but not in heart blood (proximal). This excludes a possibility of postmortem diffusion of morphine between lung and heart. These results indicate that morphine undergoes tissue-specific PMR, especially in femoral blood and lung, but not directly influenced by heart blood, liver or brain.

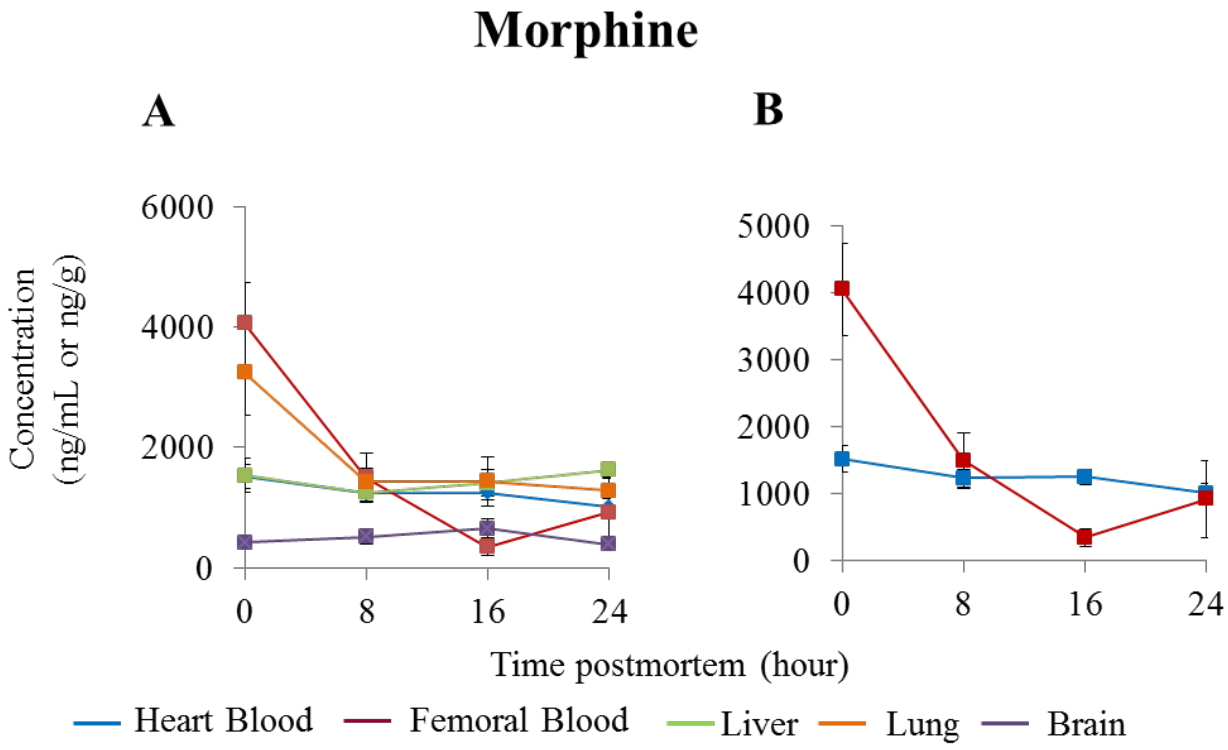

Figure 6.2: (A) Concentration vs time profile of morphine in various postmortem matrices at different time points. (B) Heart blood and femoral blood concentration vs time profile extracted from A. Data are expressed in mean (SEM).

While it is important to understand the relationship or distribution of morphine across the various postmortem intervals in one matrix, which aides in the ability to establish what the concentration may have been at time 0 if samples were collected 24 hours later, the relationship

of the matrices to each other at a given postmortem interval is equally as important as autopsy samples are all collected at the same time. During extended postmortem intervals, the ability to collect blood specimens is reduced or volume becomes limited due to extent of postmortem decomposition therefore tissue samples (e.g., liver, lung or brain) are collected and used for toxicological analysis. Toxicologists, ME or coroners will then use those results to assist in determining cause of death. The ability to say that a liver concentration is equivalent to heart blood or femoral blood is important. A one-way ANOVA using Tukey's post-hoc analysis was performed using the data presented in Figure 6.2 to assess the relationship between the sample matrices at each postmortem interval. Results are presented in Table 6.1

Table 6.1: One-way ANOVA with Tukey post-hoc analysis. Results are presented by postmortem interval at each matrix. NS, Not Significant; HB, heart blood; FB, femoral blood.

	Statistical Test: One-Way ANOVA				P<0.05
Morphine					
Postmortem Interval	**HB**	**FB**	**LIVER**	**BRAIN**	**LUNG**
0	FB and Lung	Brain, HB, Liver	FB, Lung	FB, Lung	Brain, HB, Liver
8	NS	Brain	Brain	FB, Lung, Liver	Brain
16	NS	NS	NS	NS	NS
24	NS	NS	Brain	Liver, Lung	Brain

Based on the results presented in the table above, the most significant difference between matrixes was observed at 0 hour. It is important to note that femoral blood (which is most commonly used for quantitation) and lung were statistically different from brain, heart blood and liver levels at 0 hour postmortem. These were also the two matrices that demonstrated PMR across the entire postmortem interval. At 8 hour postmortem, brain was determined to be

statistically significant from FB, lung and liver. Interestingly, there was nothing significant at 16 hours across all matrices but brain was determined to be significantly different from liver and lung again at 24 hours. It is important to note that at the later postmortem time intervals, concentrations among matrix were determined to be insignificant indicating the longer the postmortem interval the reduced PMR observed therefore the ability to use any matrix for analysis is possible. Additional considerations would need to be employed when using alternative matrices to heart blood and femoral blood at shorter postmortem intervals.

Published literature [10] presents the idea that postmortem diffusion of drugs from the bladder into the femoral blood occurs over prolonged postmortem intervals. This does not appear to be the reason for PMR in my study because, based on this theory, one would expect to see an increase in morphine concentrations over the postmortem intervals; however a decrease was observed (Figure 6.2). It is possible that there is a significant drop of morphine concentrations during the first 8 hours (0-8 hour period) due to diffusion from the bladder which occurs early in the postmortem interval (due to concentration gradient) however further research into the concentrations present in the urine at all time points should be performed. The PMR of morphine in femoral blood could be affected by the amount of urine contained in the bladder during the postmortem interval which could lead to increased diffusion (large urine volume) or the opposite, limited diffusion (small urine volume), across the concentration gradient. Another possible explanation for the PMR seen in femoral blood is blood from the femoral vein, is hypothesized to distribute morphine to the surrounding muscle due to the decomposition or breakdown of the femoral vein structure. Current literature [11-13] suggests that drug concentrations in skeletal muscle are relatively similar to those in the blood at the equilibrium stage (between the late absorption and late excretion phases) of drugs between the two matrices. However, further studies demonstrated large variations between the blood concentration and the skeletal sample

concentration and a need for further evaluation as to why the variation was occurring [14]. My research did not evaluate the morphine concentration present in the leg muscle and should be evaluated as future research to establish if diffusion is occurring.

A study by Moriya and Hashimoto [15] demonstrated that when bodies are in a supine position, basic drugs in the lungs diffuse rapidly postmortem into the left cardiac chambers via the pulmonary venous blood rather than simply diffusing across the concentration gradient. This explains the rapid decrease observed from the 0 hour to 8 hour postmortem interval. However, there was no corresponding increase in heart blood concentration at the 8 hour postmortem interval in lung tissue. It appears that equilibrium was established between the two organs since heart blood and lung concentrations were comparable from 8 to 24 hour postmortem. I hypothesize that the rapid decrease occurred between the 0 hour and 8 hour postmortem interval and further investigation and studies to include postmortem intervals such as 2 hours and 4 hours post sacrifice should be performed.

It is common practice in postmortem toxicology to perform screening analysis in the heart blood and quantitative analysis in the femoral blood. If only heart blood or femoral blood is available, then all testing is performed in that specimen and it becomes critical to understand the differences in concentration among sampling site.

Figure 6.2B details the time course of morphine in heart blood and femoral blood. While there was a graphical difference observed between the heart blood and femoral blood at 0 and 16 hour postmortem intervals, it was determined using one-way ANOVA that the difference observed graphically at 16 hour interval was not statistically significant.

Results presented in Figure 6.2 demonstrate consistencies and inconsistencies among previously published literature. Sawyer and Forney [4] concluded that there was a significant increase in postmortem cardiac blood morphine concentrations as well as increased morphine levels in liver

tissue at 24 hours. My results do not corroborate with these findings. Cardiac blood concentrations in the current study remained constant across the 24 hour time interval and there was no change in the concentration at 24 hour postmortem in liver. There are several differences between their study and my current study, including dose and gender; Sawyer and Forney used 5 mg/kg and male rats only however similarities include CO_2 euthanasia and intravenous injection. A study conducted by Gerostamouls et al. [16] was performed in human autopsy samples and concluded concentrations of morphine in heart blood were higher than femoral blood but the difference was not significant. In my study, femoral blood concentrations showed higher concentrations than heart blood for morphine at 0 and 8 hours postmortem intervals but lower concentrations at 16 and 24 hour postmortem intervals. While it may seem my study does not support the conclusion of Gerostamouls, their study was conducted using autopsy samples where heroin overdose was suspected and the average postmortem interval studied was 59 hours therefore based on this information, my data supports their conclusion.

The time courses of M3G are shown in Figure 6.3. M3G levels in the liver were much higher than those in other tissues, which is likely because morphine is extensively metabolized and converted to M3G in the liver. Further, levels of M3G in the brain were lower in comparison to other samples studied, which is likely due to the increased polarity and reduced ability to cross the BBB. It is notable that M3G levels in the heart were generally higher than those in the femoral blood, which is different from the kinetics of morphine. There were no significant changes in M3G levels over time in tissues studied however as assessed by one-way ANOVA, M3G levels in the liver were statistically significant from other matrices at all postmortem intervals. This plays an important role if toxicologist, ME or coroners are using the metabolite profile in liver to make conclusions about how long an individual may have been using previous to death or if blood and other tissues are not available for interpretation or comparison purposes.

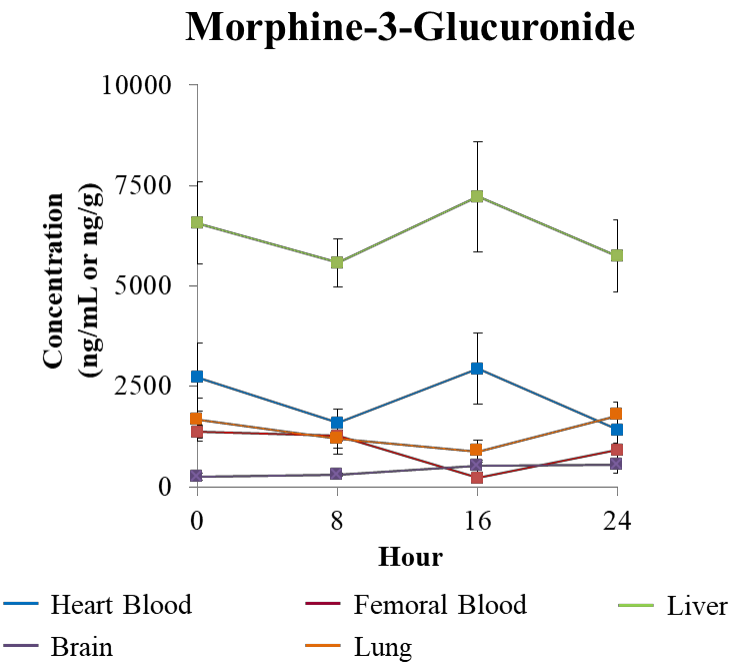

Figure 6.3: Concentration vs time profile of M3G in various postmortem matrices at different postmortem intervals (hour). Data are expressed in mean (SEM).

The time courses of normorphine are shown in Figure 6.4. Again, normorphine levels were highest in the liver, while other tissues exhibited low levels of normorphine (Figure 6.4.A-1; concentrations shown up to 300 ng/mL or ng/g). There was no obvious pattern of PMR in normorphine in any of these tissues except femoral blood graphically. There was a rapid increase in normorphine levels from 8 to 16 h followed by a decrease at 24 h. However this change was determined to be insignificant by statistical analysis. Although a pattern was not observed graphically across the postmortem interval, a one-way ANOVA performed to assess the relationship between matrices at each time points proved that the HB and liver at 8 hour as well as liver in comparison to HB, brain and lung at 24 hours were statistically different.

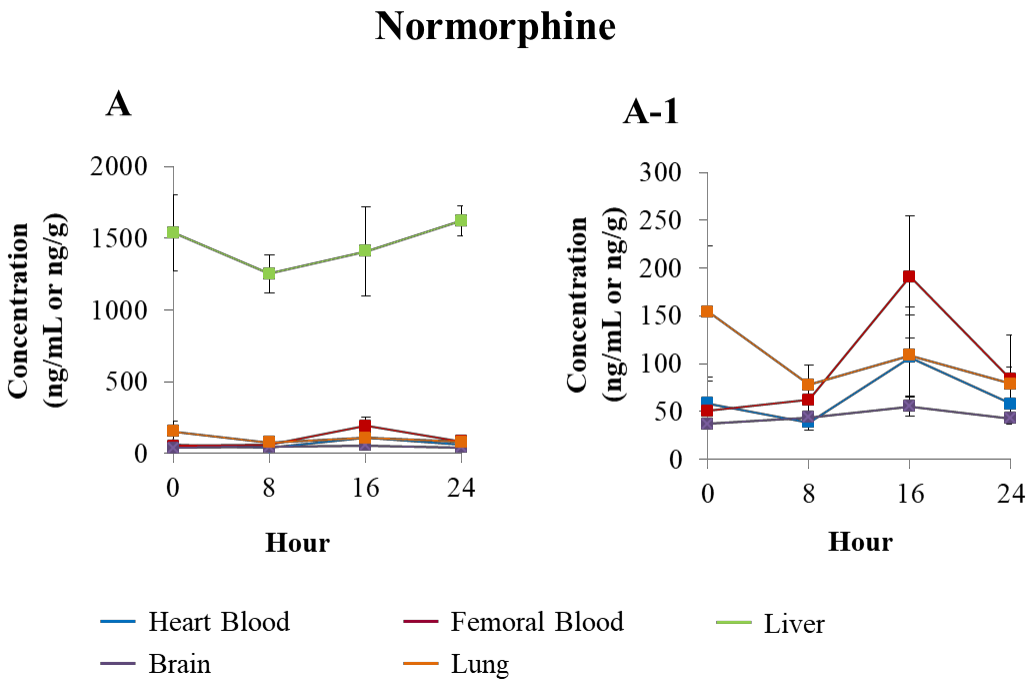

Figure 6.4: Concentration vs time profile of NM in various postmortem matrices at different postmortem intervals (hour). Data are expressed in mean (SEM).

6.4 Mass Balance

6.4.1 Methods

Mass balance is described as the calculated difference between administered dose and the dose recovered. A mass balance study is a source of information about the disposition of drug. The analyses include identifying metabolites that could contribute to the drug's pharmacological effect and/or toxicity effect. This study was performed using the data collected throughout this chapter, including morphine and its metabolites in liver, brain, lung, heart blood and femoral blood. Concentration results reported were converted to percent of administered dose in terms of nmol amount, using tissue concentrations for each analyte in wet tissues (e.g. nmol/g) and blood concentrations (nmol/mL or nmol/g) with an assumption that mL is the same as gram (density is 1.0). Residual blood content in each tissue was calculated (see section 7.5.1). Blood concentration

(nmol/mL) was multiplied by blood content (g blood/g wet tissue) to determine nmol of analyte in blood inside respective tissue. Analyte amount in blood inside respective tissue (nmol) was then subtracted from that in wet tissue (nmol) to obtain nmol of analyte in blood-free tissue. Heart blood and femoral blood concentration (ng/mL) were converted to nmol using molecular weight of analyte and expected blood volume (1% for heart blood and 8% for femoral blood of body weight) as reported in literature [17, 18]. Intravenous dose of morphine (10 mg/kg) was converted to nmol using rat body weight and molecular weight of morphine. The amount of analyte in tissue (nmol) was divided by nmol of dose and converted to percent; results are shown in Table 6.2.

6.4.2 Results/Discussion

It was concluded that total amount of dose recovered across the postmortem intervals studied was less than 5%. However, all analytes, except morphine, in the individual matrices demonstrated % dose that was consistent across the postmortem interval. Morphine demonstrated a steady decrease in percentage over the postmortem interval in femoral blood however there was no corresponding increase in metabolite concentration. Given this information, it is expected that morphine redistributes to organs (i.e. fat, muscle) that were not collected in this experiment. This drop in percentage in femoral blood morphine levels accounts for the drop from approximately 5% to 2% seen in total percentage recovered.

In a paper published by Geza Bodor [19], patients were administered 0.125 mg/kg of morphine and 2 hours later 20 ng/mL of morphine was seen in the blood. Given this information and a volume of distribution of 3 L/kg of morphine, the calculated amount of dose excreted is approximately 52%. Therefore, the low mass balance obtained in this study cannot be explained solely by excretion. It is likely that the low mass balance results from minimal sample collection as this study did not account for morphine or metabolite concentration in other organs (e.g. kidney, intestine, fat or muscle). Future

studies involving the collection of all organs as well as urine and feces would provide a better basis for mass balance of morphine and its metabolites.

Table 6.2: Mass balance results in rats following IV administration of 10 mg/kg dose of morphine. N/A: not enough data points collected to be included in analysis. * Femoral blood is assumed to represent circulating blood (8% of body weight). Levels of analytes in other tissues are shown at % dose in blood-free tissue.

	Femoral Blood			Heart Blood			Liver			Lung			Brain			Total
Postmortem Interval	**MOR (%)**	**M3G (%)**	**NM (%)**	**MOR (%)**	**M3G (%)**	**NM (%)**	**MOR (%)**	**M3G (%)**	**NM (%)**	**MOR (%)**	**M3G (%)**	**NM (%)**	**MOR (%)**	**M3G (%)**	**NM (%)**	%
0	3.25	0.51	0.04	0.15	0.17	0.01	0.47	0.05	0.12	0.05	0.02	0.002	N/A	0.005	0.001	4.848
8	1.2	0.15	0.05	0.12	0.10	0.004	0.45	0.02	0.03	0.04	0.02	0.001	0.02	0.004	0.001	2.210
16	0.28	0.12	0.11	0.12	0.18	0.01	0.46	0.03	0.06	0.04	0.02	0.001	0.02	0.009	0.001	1.461
24	0.74	0.45	0.07	0.10	0.09	0.01	0.67	0.05	0.07	0.02	0.04	0.002	0.01	0.01	0.001	2.303

6.5 Conclusions

Based on the results of this study, it can be concluded that PMR does exist for morphine in the femoral blood and lung tissue across the postmortem interval. However, further studies into the characterization of the redistribution are necessary to understand the mechanism behind this redistribution. Further, heart blood and femoral blood morphine concentrations are comparable to liver and lung concentration (after 8 hours postmortem). Therefore, if liver or lung samples (assuming postmortem interval can be estimated) are the only sample available for testing, toxicologists, medical examiners and coroners can compare reported concentrations to previously published heart blood values to interpret and establish cause of death. Brain tissues could provide an underestimation of concentration in comparison to heart blood or femoral blood however concentrations are only significantly different from femoral blood levels at 0 and 8 hour interval. At longer postmortem intervals, it can be concluded all matrices can be used for toxicology testing and will provide concentrations consistent to each other which will allow for a liver result to be compared to a blood reference value. All interpretation should be done with caution and take into account other circumstances of the death such as temperature, naive vs chronic users and position of the body. Further evaluation into the possibility that factors outside of the drug properties (e.g. pKa, lipophilicty and V_d), such as drug-drug interaction, body position, injection time and gender can change the PMR of morphine and its metabolites will increase our understanding about the complexity in morphine PMR.

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Chapter 7. Factors that Effect Postmortem Redistribution of Morphine

7.1 Introduction

It is well known and detailed in literature that properties of a drug (volume of distribution, lipophilicity, and pKa) contribute greatly to drug PMR. Literature states that drugs that are basic in nature, highly lipophilic with a volume of distribution greater than 3 L/kg will undergo PMR [1-5]. However, factors such as gender, drug injection time and drug-drug interaction have not been studied in regards to postmortem redistribution. Researchers have proposed that gender may play a role however rat studies performed using one gender [6, 7] and while autopsy studies looked at both genders, gender was not considered as a factor [8]. Human autopsy samples have been studied in cases where multiple drug were identified [2, 9-11] for the presence of PMR however individual drugs were evaluated based on their drug properties not the possibility that they effect each other. The conditions of the body, drug characteristics (e.g. PMR), specimen site analysis, drug-drug interaction and decedent demographic information are factors which need to be considered in the proper interpretation of a toxicology result performed on an autopsy specimen. It is important to evaluate these characteristics in a controlled environment as to observe the relationships without external or unknown factors.

As part of my dissertation, an evaluation of injection time, gender, drug–drug interaction and their resultant effect on morphine PMR was performed. A look into how these factors affect the postmortem redistribution of morphine and its metabolites provided supplemental information to the postmortem redistribution pattern of these analytes established in Chapter 6 that can aide medical examiners, coroners and toxicologists in their interpretation of autopsy toxicology results.

7.2 Effect of fentanyl administration on the postmortem redistribution of morphine

7.2.1 Materials and Methods

7.2.1.1 Ethics Statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Northeastern University Animal Care and Use Committee.

7.2.1.2 Animals and drugs

Male and female Fischer F344 (Harlan) rats were fed facility chow and water *ad libitum*. Morphine sulfate (25 mg/mL) and fentanyl citrate (50 µg/mL) was obtained from Henry Schein (Indianapolis, IN) and diluted to appropriate concentrations (10 mg/kg and 10 µg/kg respectively) with sterile saline.

7.2.1.3 Administration and sample collection

Rats were injected intravenously through the right tail vein with 10 mg/kg of morphine sulfate and 10 µg/kg fentanyl citrate while under light anesthesia using isoflurane. Blood was collected via the left tail vein 5 minute post-injection as well as immediately prior to euthanasia. Prior to being euthanized, heparin (1000 U/mL) was injected vial the tail vein to prevent extensive clotting. One hour post injection, rats were euthanized via CO_2 and placed in supine position at room temperature for desired postmortem interval (0, 8, 16, and 24 hours). Heart blood, femoral blood, liver, lung and brain were collected and immediately analyzed. Femoral blood was collected via needle stick and pooled from both the left and right femoral veins (due to limited volume). Heart blood was collected by direct cardiac stick. Liver sample was collected

from the upper right quadrant and lung was collected from the right side. The whole brain was collected. Left kidney and urine samples were collected but testing was not performed as part of this study (see Chapter 6 Figure 6.1).

7.2.1.4 Instrumental Analysis

Blood samples were analyzed for MOR, M3G, M6G, NM, FENT and NF concentrations using the method described in Chapter 3.

7.2.1.5 Statistical Analysis

Graphical representation of data was achieved out using Microsoft Excel 2010. Group-wise comparisons were performed using a two-way ANOVA followed by Tukey's post hoc analysis, if applicable ($p<0.05$).

7.2.2 Results and Discussion

Graphical differences were observed across all postmortem intervals for liver and femoral blood samples however co-administration of fentanyl had an opposite effect on morphine PMR in femoral blood than liver samples. The addition of fentanyl reduced the PMR or change in concentration observed across the 24 hour postmortem interval in femoral blood as well as caused an overall reduction in amount of morphine detected in the samples. The addition of fentanyl caused a reduction in morphine concentration across the 0, 8 and 16 hour postmortem intervals in the liver while still consistent with the pattern observed with morphine only. However an increase in concentration was observed at 24 hour interval.

Heart blood, brain and lung samples demonstrated a consistent response for both studies therefore PMR was not observed.

Statistical analysis using two-way ANOVA of all data resulted in significance ($p<0.05$) being observed at the 0 hour interval in the femoral blood, lung and liver samples only, all other matrices demonstrated insignificant variation.

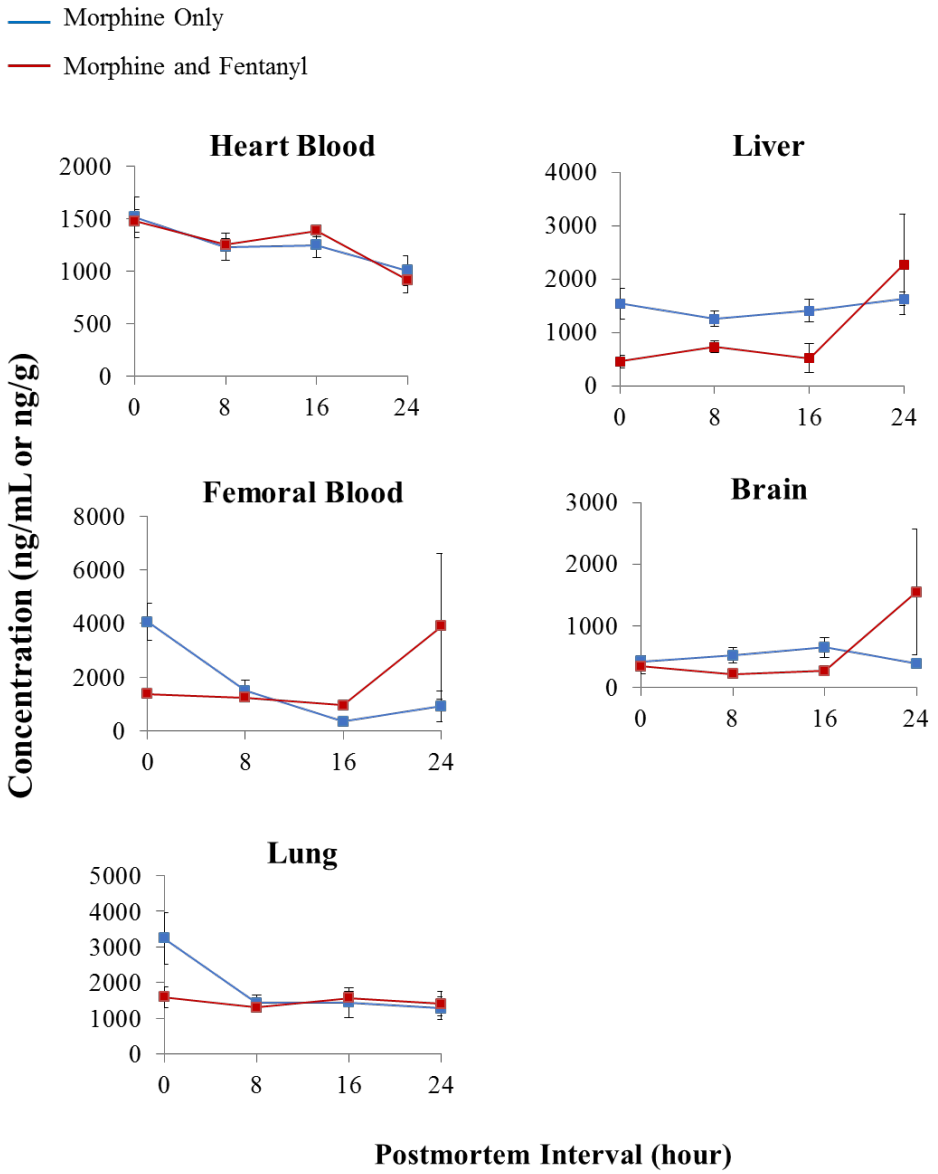

Figure 7.1: Co-administration of morphine and fentanyl and effects on morphine PMR. Data are expressed as mean (SEM)

Overall, fentanyl affected the 0 hour concentration of morphine in femoral blood, liver and lung but did not affect the heart blood concentration, therefore analysis of heart blood concentrations for morphine when fentanyl is suspected would provide concentrations equivalent to those with only morphine identified For other tissues, the fentanyl effect is more pronounced at the 0 h interval in comparison to other time points, it is possible this effect occurs due to the effect of fentanyl on morphine metabolism during the first hour after death (when enzyme activity remains). Additionally, it is important to address that fentanyl appears to cause an increase in morphine concentration at the 24 hour interval in the brain, femoral blood and liver however the SEM attributed to these data points indicates this data needs further investigation and the results are inconclusive for interpretation. Further evaluation of data (see appendix G and H for additional data) to include morphine metabolite concentrations when fentanyl is administered should be performed to determine if the lower concentration detected for morphine in the liver, femoral blood and lung is due to increased rate of metabolism or if the lower concentration is due to a slower tissue distribution in general due to lower heart rate, blood pressure and respiratory depression.

The limitation of the current study is that we used only one dose of morphine and one dose of fentanyl. Therefore, we were unable to characterize the dose effect of fentanyl (dose-response relationship). Future studies with varying doses should be performed to determine if these results are consistent among various co-administration variables.

7.3 Effects of Gender on PMR of Morphine

The effects of gender on morphine and its metabolites PMR are shown in Figure 7.2.

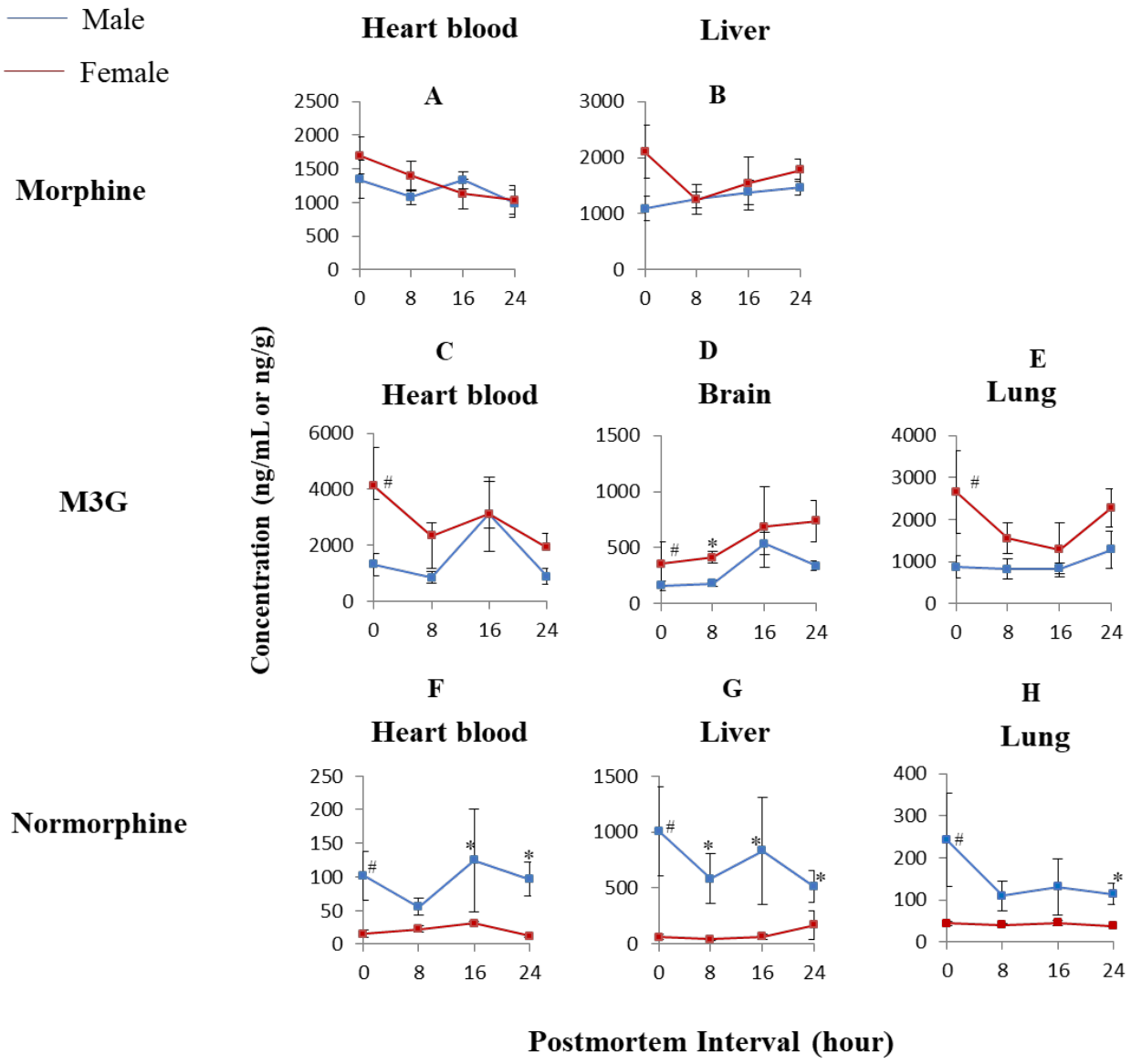

Figure 7.2: Gender effect on PMR of morphine and metabolites. Data are expressed as mean (SEM). * indicates significance at specific postmortem interval, # indicates significance in relation to drug across entire postmortem interval study.

Morphine levels were higher females in heart blood and liver at 0 hour as well as in heart blood at 8 hour however remaining time points demonstrated a consistent concentration (Figure 7.2 A-B).

M3G demonstrated higher levels in females then in males as shown in Figure 7.2 C-E while distribution patterns were similar. Figure 7.2 F-H demonstrates that NM concentrations were higher in males then females across all time points and matrices. Interestingly, NM concentrations in females were low and consistent across the interval whereas males fluctuated. PMR was observed across the full postmortem interval studied for M3G and NM in all matrices as assessed by one-way ANOVA with Tukey post- hoc analysis. PMR was not observed across the postmortem interval studied in any matrix. Significant differences between males and females were also observed at 8 hour M3G in brain, 16 and 24 hour NM in heart blood, 8, 16, 24 hour NM in liver and 24 hour NM in lung. Femoral blood was not presented in Figure 7.2 due to the limited number of sampling points available between male and female study. Reduced number of sampling points was a result increased difficulty in collecting samples at later time points (16 and 24 hours) as well as rat death resulting in low N numbers.

It has been reported that morphine has a delayed onset of action in females and lower concentrations are necessary to achieve the same effects as observed in males however the mechanism as to why is unknown [12-14]. While the concentrations of morphine are consistent across the genders over the course of the postmortem interval, pharmacodynamic characteristics such as delayed sedation, agitation and dilated pupils, were observed in female rats across this study. The delayed onset can also account for slower metabolism or delayed metabolism in females. Higher NM concentrations in males as compared to females support this theory. However, Soldin [15] reported that CYP3A4 metabolism occurs at a faster rate in females the males. It is possible formation of NM happened readily and was excreted therefore accounting for the lower concentrations observed [16]. Further research into the mechanism behind the decreased NM concentration in females compared to males should be performed.

However, M3G did not demonstrate higher concentrations in males as expected based on metabolism occurring more quickly. Studies performed [12-14] have reported that serum levels for M3G in females are consistently 3 times higher than males however there are no reports as to the mechanism behind this. It is possible this is related to the amount of fat present in the body. Females tend to have a higher body fat percentage than males and M3G is more fat soluble then NM and MOR. Rats studied during my dissertation were 3-4 months old but it was noted that female rats had more fat surrounding organs then males.

It can be concluded that gender effects are insignificant when evaluating postmortem samples for morphine; however, gender plays a key role in interpretation when interpreting and drawing conclusions for morphine metabolites.

7.4 Effect of Time of Administration

7.4.1 Methods

Over the course of the study (Chapter 6), initially injections were performed over the course of a 24 hour period with some injections occurring in the morning hours and others occurring in the evening hours. Upon evaluation of this data, it was observed that concentrations difference occurred at similar postmortem interval time points based on the injection time point and further studies were performed. Although time of injection may not be known during autopsy examination, a look into whether circadian rhythm may play a role in metabolism and effect PMR of morphine and its metabolites was of interest. The administration and sample collection procedure described in Chapter 6 Section 6.2.3 was modified to allow for 1 set of rats to be injected between 10-11 pm and a second set between 10-11 am. In addition to the graphical representation shown in Figure 7.3, a t-test was performed to determine if a difference was observed between the two studies.

7.4.2 Results and Discussion

It should be noted that results of study were affected by number of rats per time point. Due to death of rats upon intravenous injection, 0 hour rats in evening injection study (all matrices) have an N=1. In addition, due to insufficient sample volume available, femoral blood plots for morning injection rats at 16 h for MOR and NM as well as 24 h for MOR, M3G and NM have an N=1 as well. Data with N=1 were not presented in the figures below.

Graphical representation of the results for the effect of injection time on morphine and its metabolites PMR are shown in Figure 7.3. Due to incomplete results presented, a t-test was performed on data points where AM and PM injections were available. There was a significant difference at 16 and 24 hour intervals in liver for M3G. Based on information provided, while there is not a statistical significant difference observed, there is a concentration difference among the postmortem interval between AM and PM injections.

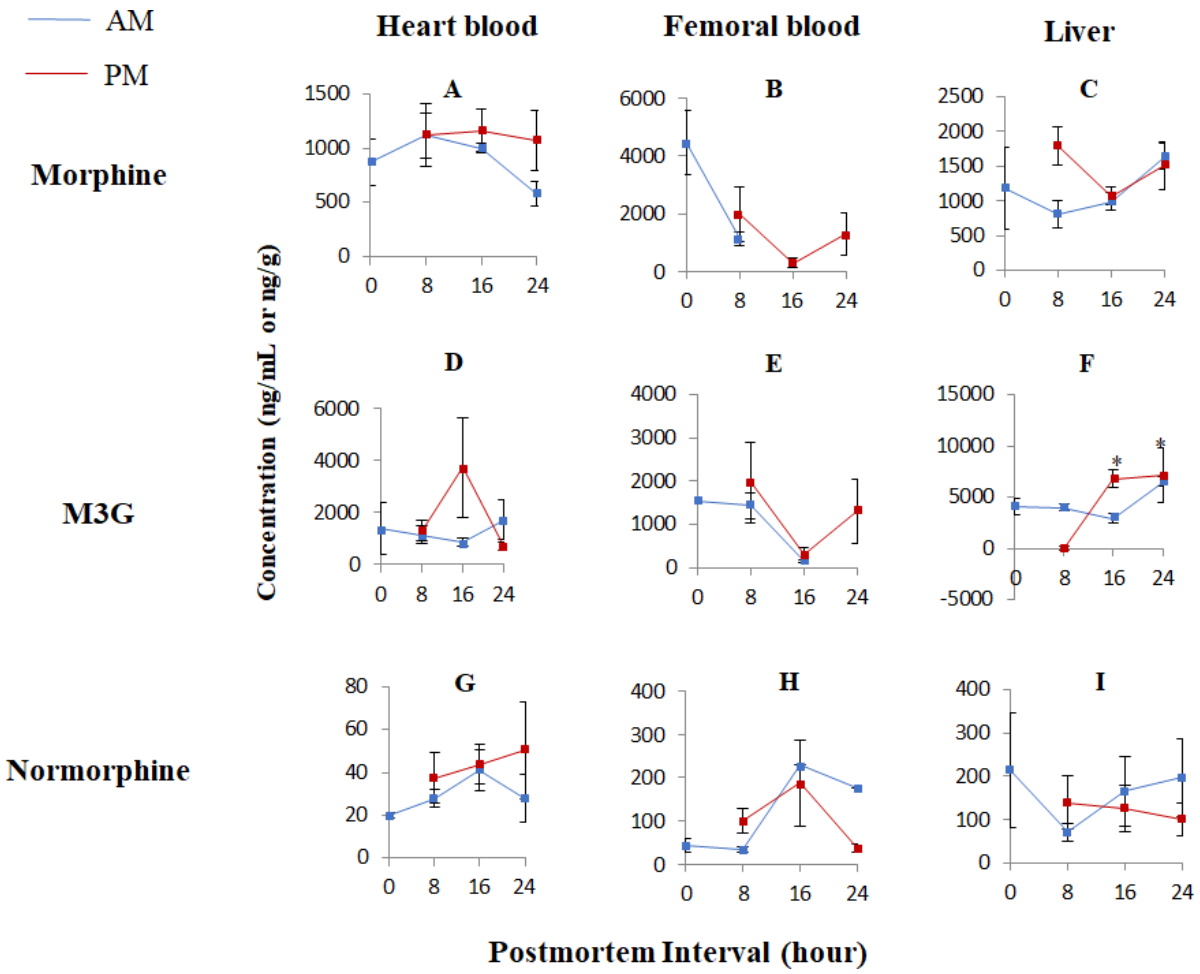

Figure 7.3: AM and PM injection comparison for morphine and metabolites. Data are expressed as mean. * indicated significant difference.

As a result of this study, it can be concluded that concentration variation does exist but was deemed to be insignificant with the exception of M3G at 16 and 24 hours. This adds a level of difficulty when it comes to interpreting autopsy toxicology results as the time of death is not always available let alone the time the decedent used the drug. Given this information, it can be concluded that there is no difference in postmortem kinetics of morphine therefore the elimination of morphine (by metabolism and/or excretion) during the first hour after injection is key to determining the extent of variation or change in morphine and metabolites concentrations. The extent of metabolism or excretion of morphine and its metabolites could be attributed to the

metabolic activity difference during morning and evening hours (e.g circadian rhythm). Rats are nocturnal animals, thus they sleep more during the day and are awake at night. Given this information, the tissue distribution and subsequent metabolism/excretion in rats during the morning hours would be slowed due to decreased heart rate and blood flow. This is supported by the AM injections having a higher level of morphine in femoral blood (close to injection site) in comparison to PM injections but lower levels of morphine in heart blood and liver. It should be taken into consideration that all rats were brought into the laboratory prior to injection in which lights were on but an acclimation period was not performed. Additional studies need to be performed to determine why the M3G concentrations in heart blood were so varied. A large SEM was observed for 16 hour M3G time point, it would be important to include additional studies surrounding this time point (e.g. 12 and 18 hours) to more precisely characterize this peak. The results of this analysis, lead to modifications in morphine PMR studies performed in Chapter 6 and 7 to perform the intravenous injection of all rats in the morning and within 3 hours of each other.

7.5 Effect of Residual Blood Volume

7.5.1 Methods

In my studies, exsanguination was not performed prior to collection of organs and samples were not dried prior to analysis. Since morphine concentrations in blood at time zero are higher than those in other organs, it is plausible that residual blood retained in each organ could influence PMR. Tissue samples collected during the PMR study in Chapter 6 which had a corresponding femoral blood concentration were evaluated. Tissue weights (ng/g) and femoral blood concentrations (ng/mL) reported in Chapter 6 were converted to ng amounts using the expected tissue weight and residual blood volume in the tissue (as reported in literature) [17, 18, 19], respectively. Once ng amounts were calculated, blood amount was subtracted from liver amount and divided by dry tissue amount (g), resulting in an ng/g dry tissue drug concentration.

7.5.2 Results and Discussion

A look into the effect of residual blood on the tissue concentration was performed and presented in Figure 7.4. Residual blood volume did not have an effect on the PMR of morphine, M3G or normorphine with the exception of 0 hour morphine concentrations in lung and 16 hour normorphine concentrations in brain. The change in concentration from 0 hour to 8 hour postmortem seen in the lung was decreased for tissue with blood volume removed. Lung and femoral blood concentrations demonstrated a parallel relationship (between zero and 8 hours postmortem), shown in Figure 6.2. This relationship can be accounted as a result of the blood volume present in the lung contributing to over lung concentration in the early postmortem interval (see Figure 7.4). Normorphine in brain is more susceptible to PMR with a decrease occurring between 8 and 16 hours postmortem however the SEM at 16 hours is large and may account for the difference observed.

Morphine concentrations in the brain at zero hour demonstrated negative results and are not shown in Figure 7.4. The negative results indicated that the concentration of morphine in the brain at 0 hours is largely affected by the residual blood in the tissue in early postmortem intervals. It is proposed that the concentration in the blood remaining in the brain during this time period may not be the same as femoral blood due to lack of circulation or distribution throughout the body.

Overall, the majority of concentrations in the blood containing tissues and without blood volume were equivalent. When the concentrations were not approximately equal, the blood containing tissues were higher in concentration. This would be expected as femoral blood or circulating blood is expected to contain morphine and its metabolites. Despite the fact that tissue samples typically collected for toxicological analysis are not dried or exsanguinated prior to testing, understanding the effect of the residual blood volume on results is important. Based on the results presented in Figure 7.4, there would not be a large effect on concentration and interpretation shall not be affected.

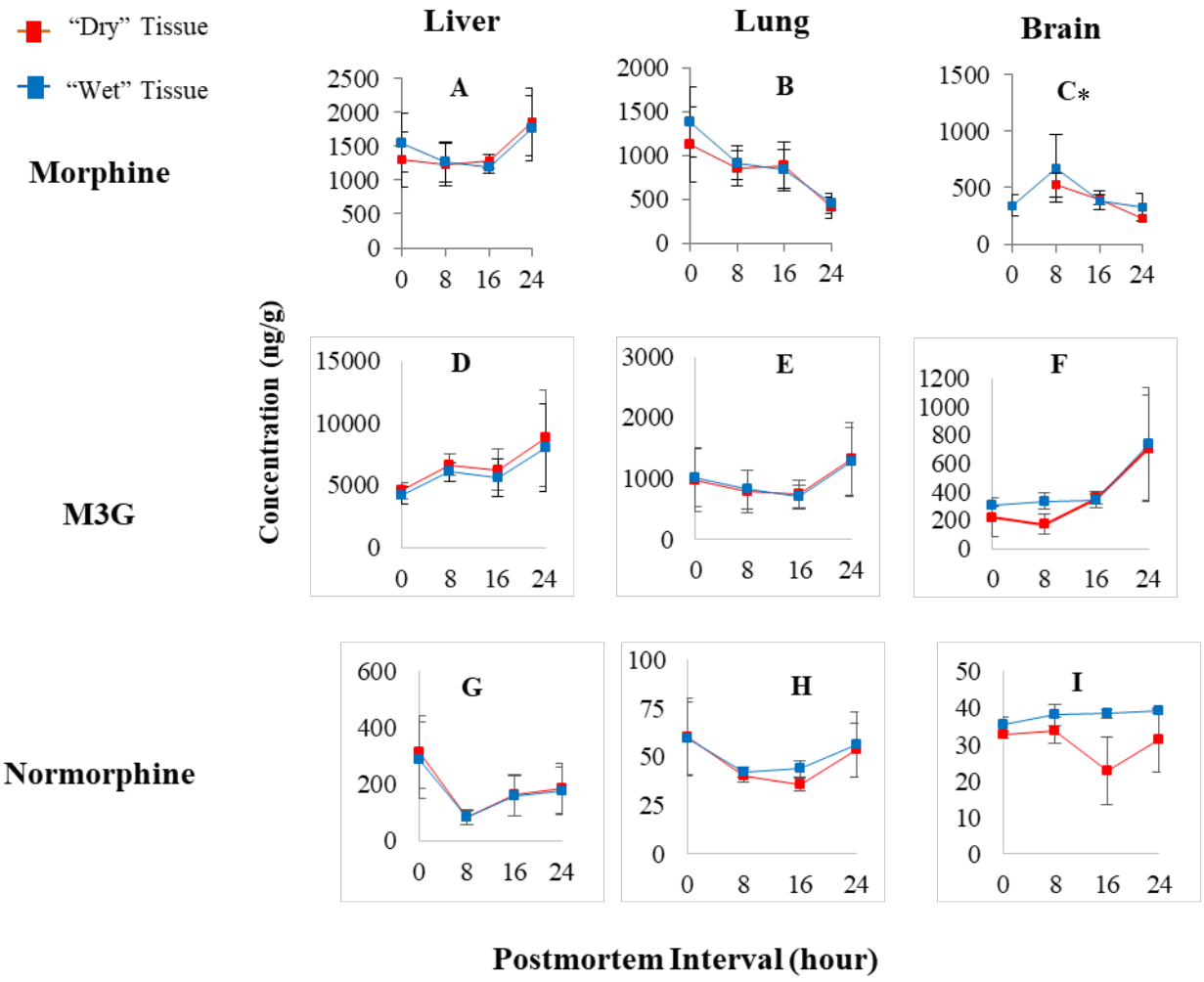

Figure 7.4: Concentration time profiles comparing "wet" tissue and "dry" tissue. "Wet" tissue is defined as blood containing where as "dry" tissue is defined as without residual blood volume. *data is incomplete due to residual effect at time zero.

7.6 Conclusion

This study demonstrated that there are factors that can affect PMR of morphine and its metabolites that are not related to the drug properties itself. It is easy to rely on drug properties as the explanation for drug PMR however this study also indicates that there are factors unrelated to the drug properties that can modify the PMR of a drug. Further investigations into the mechanism behind these factors are warranted to improve our understanding about PMR of morphine.

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Chapter 8. Overall Conclusion

8.1 Conclusion

My dissertation provided a basis for morphine PMR using an approach novel to the PMR investigation. The use of a mechanism based approach to PMR using statistical and graphical analysis is novel and provides information not previously presented in PMR literature. Previous studies focused on central to peripheral ratios as well as peripheral to tissue ratio. My dissertation focused on the changes in morphine and metabolite concentrations which vary amongst tissues and fluids over the course of the postmortem interval. An approach which involved the collection of various tissues, femoral and heart blood samples was representative of what is performed during autopsy in comparison to previous research which focused on a single sample collection site. Finally, factors which routinely affect PMR were investigated using a controlled study instead of interpreting results from autopsy samples.

The co-administration study of morphine and fentanyl is important to the current public health crisis in which the number of opioid deaths is on the rise and medical examiners/coroners are seeing more heroin/morphine/fentanyl combination deaths, something which is not presented or investigated in literature specific to postmortem samples.

8.2 Future Studies

Future studies to look into additional conditions which may affect PMR such as temperature, additional body conditions, use of additional matrices (e.g. muscle or fat), extended PMR intervals, various storage conditions of the carcass (frozen or refrigerator), as well as additional concentrations of morphine, will provide valuable information to supplement this work and provide medical examiners, coroners and toxicologists useful data and publications that can be references and cited when making conclusions in regards to

autopsy toxicology results involving morphine. Additionally a more thorough co-administration study should be performed encompassing different drugs and/or alcohol as well varying concentrations. It is common to see multiple drug as well as alcohol identified at time of death. Co-administration studies with 2 or more drugs would be beneficial to the field of postmortem toxicology for this reason. Further, due to recent increase in use of NARCAN, by first responders and medical personnel, a look into the effects of NARCAN on the postmortem redistribution and concentration levels of morphine and its metabolites would be of interest to toxicologists, medical examiners and coroners.

8.3 Funding

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Appendix A

Individual Data from [N-methyl-^{14}C] Morphine Tissue Digestion Study

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Abbreviations

CPM: Counts per Minute

MG: Milligram

Table A-1 : Individual Data from [N-methyl-^{14}C] Morphine Tissue Digestion Study

Time Point	Tissue Sample	Weight (mg)	Date	H#	CPM Count	% Lumex	Date	H#	CPM Count	% Lumex	Date	H#	CPM Count	% Lumex	Average CPM	CPM/mg
Negative	Brain	601	2/11/2018	157	979	15.07	2/12/2018	158	929	6.73	2/14/2018	164	2178	60.25	1362	2
	Lung(1)	511.5	2/11/2018	135	252	12.88	2/12/2018	139	324	3.03	2/14/2018	143	349	2.48	308	1
	Lung(2)	257.9	2/11/2018	126	111	26.36	2/12/2018	134	139	11.19	2/14/2018	141	136	11.89	128	0
	Heart	238.8	2/11/2018	218	203	43.80	2/12/2018	218	137	10.63	2/14/2018	221	146	5.38	162	1
	Liver a	210.2	2/11/2018	173	221	52.20	2/12/2018	177	161	26.35	2/14/2018	175	152	17.68	178	1
Died Before 1 Hour	Brain	389.5	2/11/2018	118	13802	0.77	2/12/2018	124	13561	0.19	2/14/2018	124	13583	0.45	13648	35
	Lung(1)	312.5	2/11/2018	203	163371	0.06	2/12/2018	201	159968	0.02	2/14/2018	200	155845	0.01	159728	511
	Lung(2)	64.5	2/11/2018	138	41089	0.13	2/12/2018	136	41266	0.03	2/14/2018	136	40947	0.02	41100	637
	Heart(clot)	48.3	2/11/2018	146	34812	0.18	2/12/2018	147	35210	0.05	2/14/2018	146	35353	0.03	35125	727
	Liver a		2/11/2018	280	174574	0.17	2/12/2018	268	170758	0.03	2/14/2018	251	156360	0.02	167231	
	Liver b		2/11/2018	268	184728	0.14	2/12/2018	262	173922	0.02	2/14/2018	239	157204	0.01	171951	
	Total Liver	210.2	2/11/2018	548			2/12/2018		344680		2/14/2018		313563		219414	1044
	Kidney	279.3	2/11/2018	168	189854	0.07	2/12/2018	172	187460	0.02	2/14/2018	167	182370	0.01	186561	668
4 Hour	Brain	258.3	2/11/2018	107	775	1.40	2/12/2018	113	1901	0.36	2/14/2018	112	2396	0.17	1691	7
	Lung(1)		2/11/2018	221	9050	0.53	2/12/2018	222	8787	0.11	2/14/2018	217	8412	0.05	8750	
	Lung 1b		2/11/2018	202	8043	0.58	2/12/2018	202	7678	0.11	2/14/2018	201	7520	0.05	7747	
	Total Lung	166.1	2/11/2018		17093				16464				15932		16496	99
	Heart	412.7	2/11/2018	184	11548	0.59	2/12/2018	186	11101	0.19	2/14/2018	183	11102	0.09	11250	27
	Liver a		2/11/2018	268	49046	0.34	2/12/2018	243	51493	0.04	2/14/2018	225	49067	0.02	49868	
	Liver b		2/11/2018	250	50980	0.25	2/12/2018	232	52066	0.03	2/14/2018	22	49523	0.01	50856	
	Liver c		2/11/2018	268	54773	0.30	2/12/2018	245	56496	0.03	2/14/2018	221	53942	0.02	55070	

	Total Liver	1306.2	2/11/2018		105753		2/12/2018		108562		2/14/2018		103465		105926	81

Time Point	Tissue Sample	Weight (mg)	Date	H#	CPM Count	% Lumex	Date	H#	CPM Count	% Lumex	Date	H#	CPM Count	% Lumex	Average CPM	CPM/mg
8 Hour	Brain	312.9	2/11/2018	111	2410	3.89	2/12/2018	118	2385	0.95	2/14/2018	121	2323	0.96	2373	8
	Lung(1)	94.3	2/11/2018	159	3723	0.86	2/12/2018	161	3699	0.23	2/14/2018	163	3667	0.12	3696	39
	Lung(2)	213.7	2/11/2018	194	7830	0.87	2/12/2018	198	7453	0.22	2/14/2018	197	7253	0.10	7512	35
	Blood Clot	490.9	2/11/2018	246	11200	0.65	2/12/2018	245	10721	0.18	2/14/2018	237	9943	0.08	10621	22
	Liver a		2/11/2018	214	30960	0.19	2/12/2018	212	29716	0.05	2/14/2018	206	27342	0.02	29339	
	Liver b		2/11/2018	232	31708	0.17	2/12/2018	226	30374	0.04	2/14/2018	216	27550	0.02	29877	
	Total Liver	837.8	2/11/2018	446	62668		2/12/2018		60090		2/14/2018		54892		59216	71
24 hour	Brain	380	2/11/2018	118	3672	6.11	2/12/2018	129	3311	0.48	2/14/2018	131	3300	0.32	3427	9
	Lung(1)	156	2/11/2018	153	5785	5.59	2/12/2018	169	8218	0.46	2/14/2018	174	8599	0.17	7534	48
	Heart	386.1	2/11/2018	221	19284	0.54	2/12/2018	223	19064	0.12	2/14/2018	225	18876	0.05	19074	49
	Liver a		2/11/2018	205	40961	0.18	2/12/2018	203	39665	0.05	2/14/2018	202	37551	0.03	39392	
	Liver b		2/11/2018	200	39758	0.14	2/12/2018	200	38758	0.04	2/14/2018	199	36770	0.02	38429	
	Total Liver	657.1	2/11/2018	405	80719		2/12/2018		78423		2/14/2018		74320		77820	118

Appendix B

Supporting Documentation for LC-MS/MS Quantitation Method Validation

Table of Contents

Abbreviations

FENT: Fentanyl

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry

MOR: Morphine

M3G: Morphine-3-Glucuronide

M6G: Morpine-6-Glucuronide

NF: Norfentanyl

NM: Normorphine

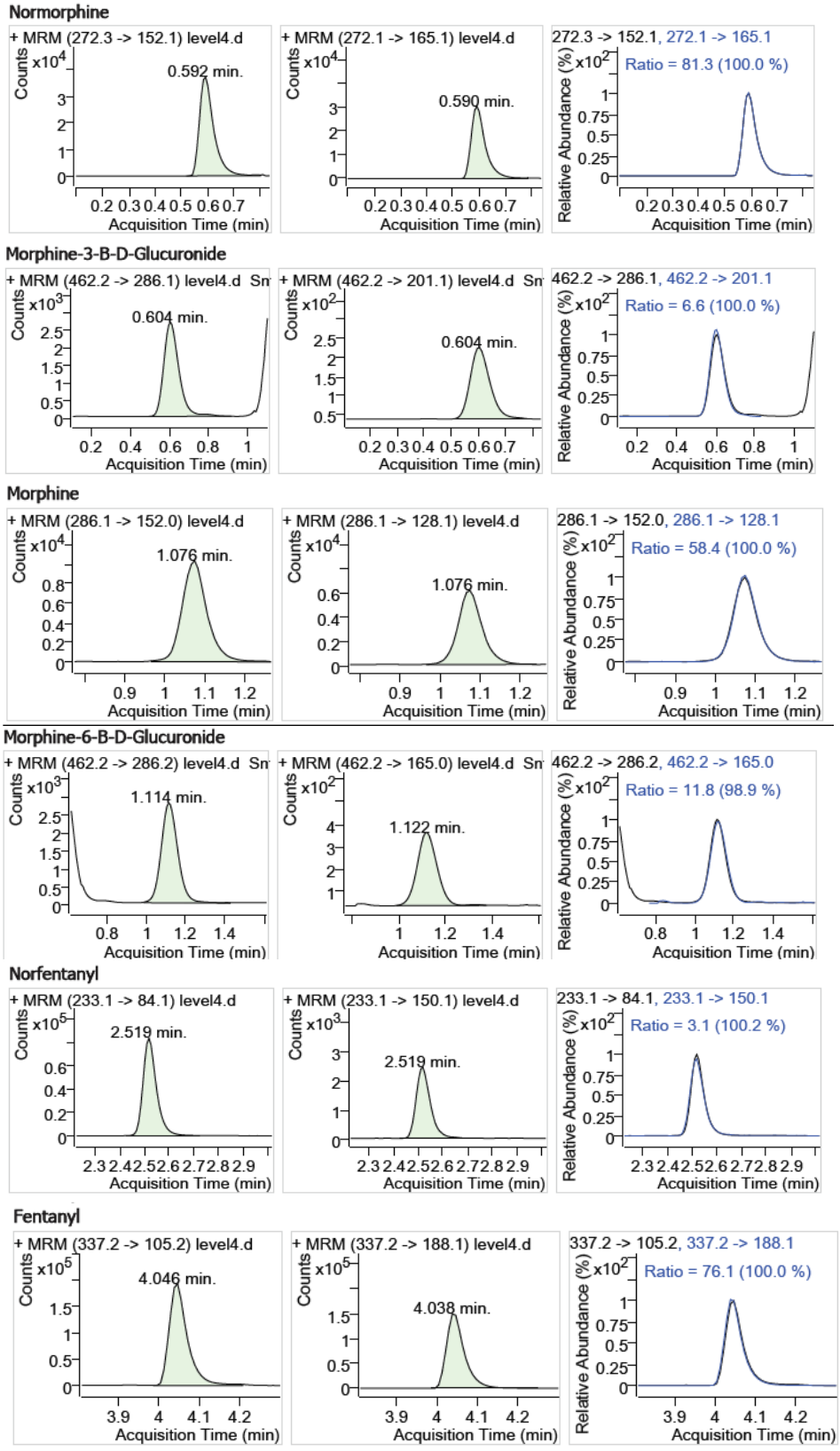


Figure B-1: LC-MS/MS quantifier/qualifier transitions of MOR, M3G, M6G, NM at 100 ng/mL and Fent, NF at 10 ng/mL

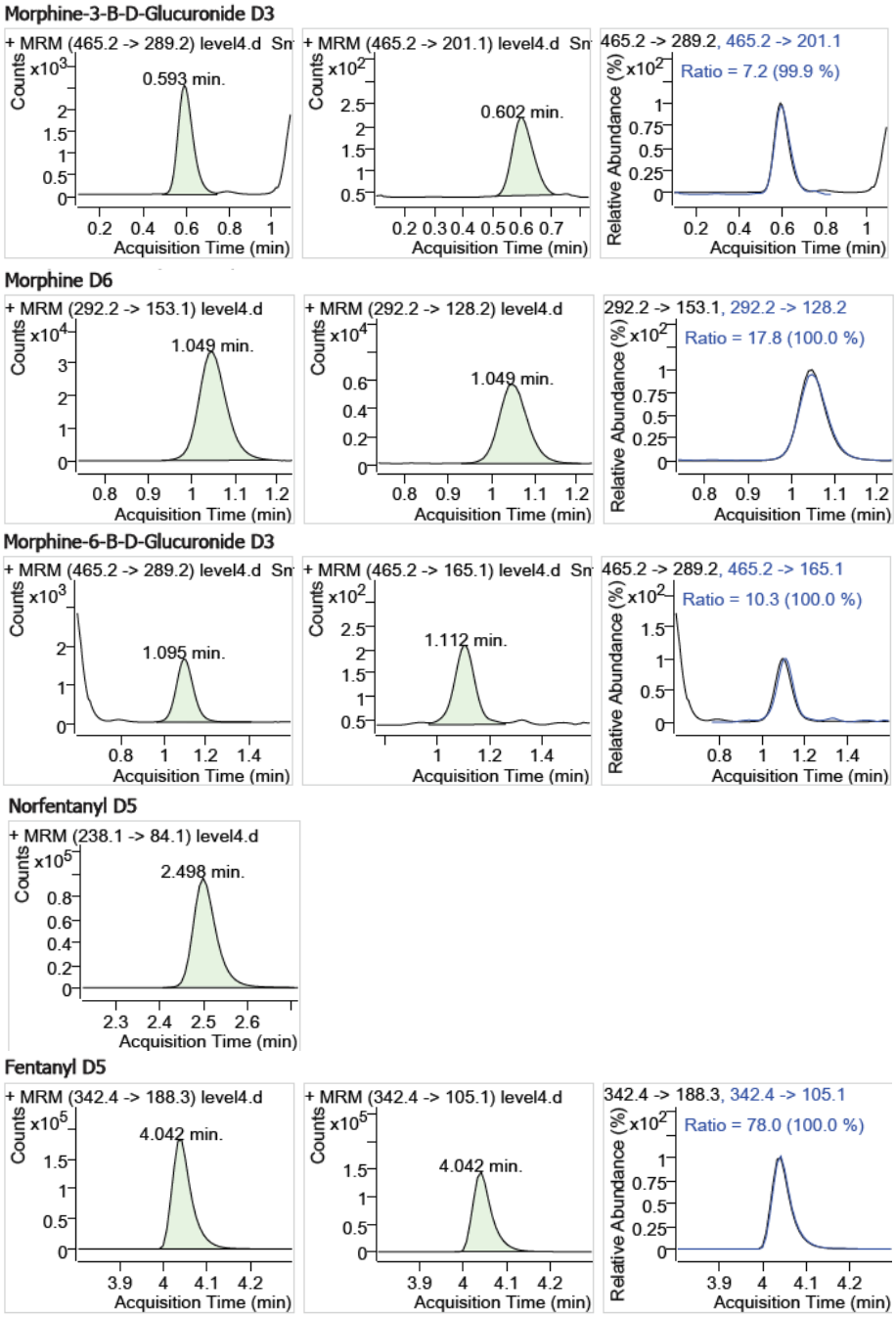


Figure B-2: LC-MS/MS transitions of MOR-d6, M3G-d3, M6G-d3 and Fent-d5, NF-d5

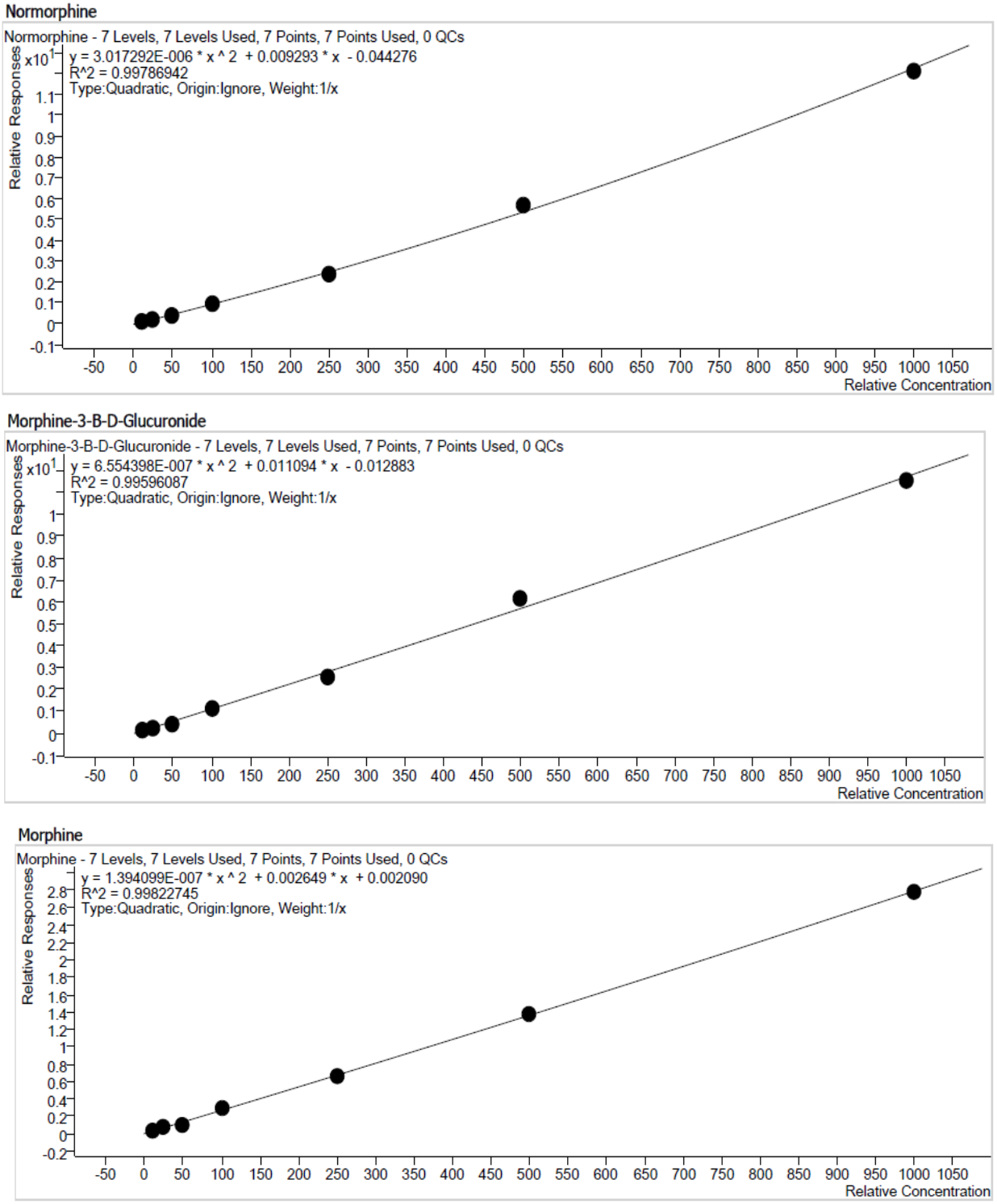


Figure B-3 Calibration Curves for MOR, M3G and NM

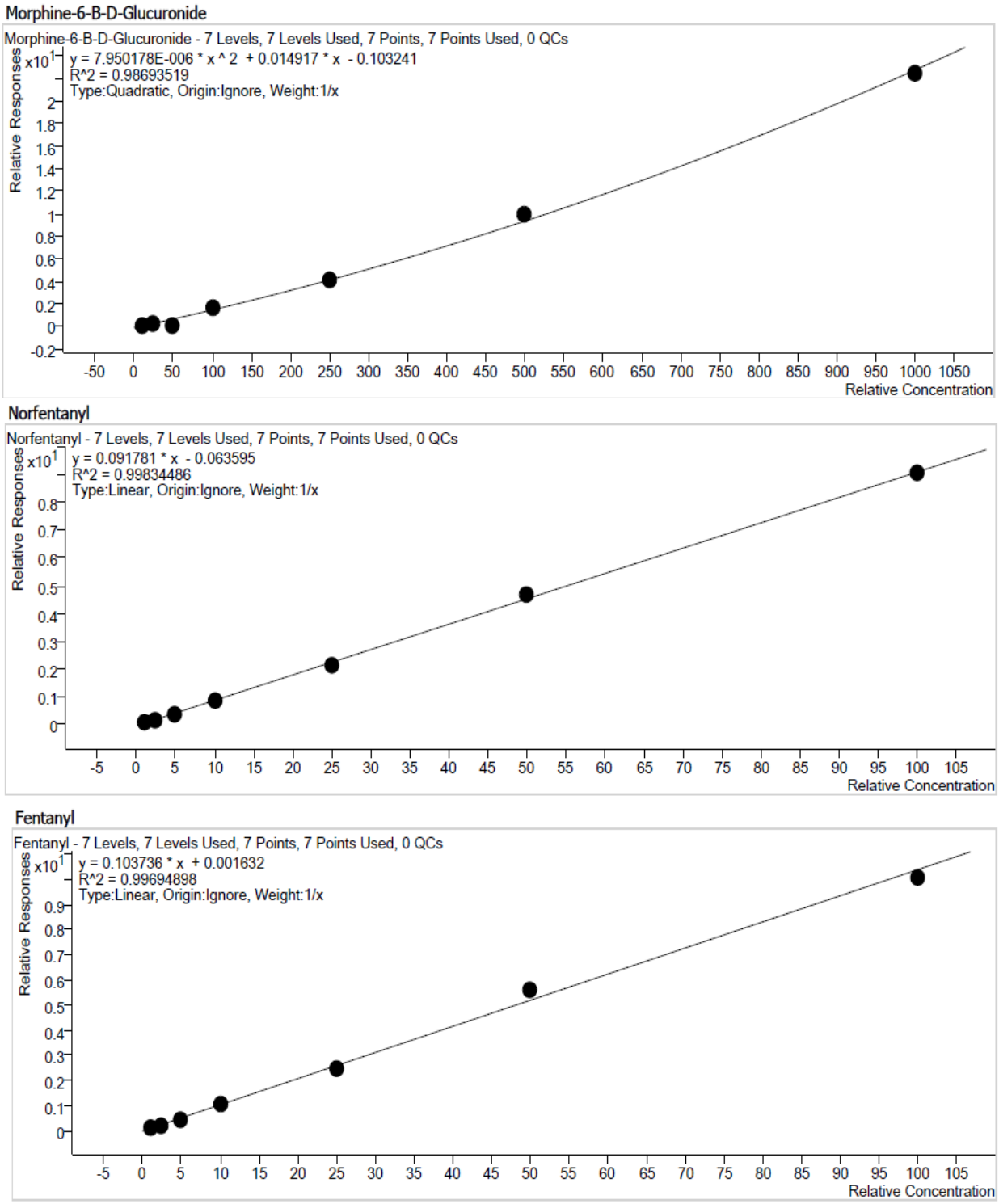


Figure B-4: Calibration Curves for M6G, Fent and NF

Appendix C

Quantitative Analysis Reports for LC-MS/MS Quantitation of Negative Matrices

Table of Contents

Abbreviations

Conc: Concentration

RT: Retention Time

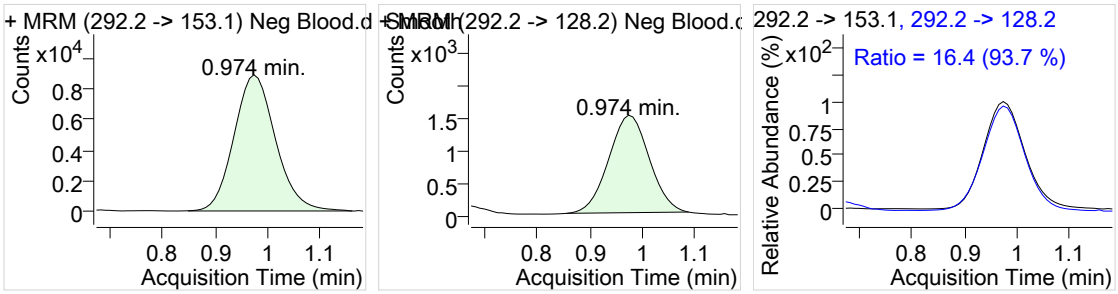
Quantitative Analysis Report

Agilent Technologies

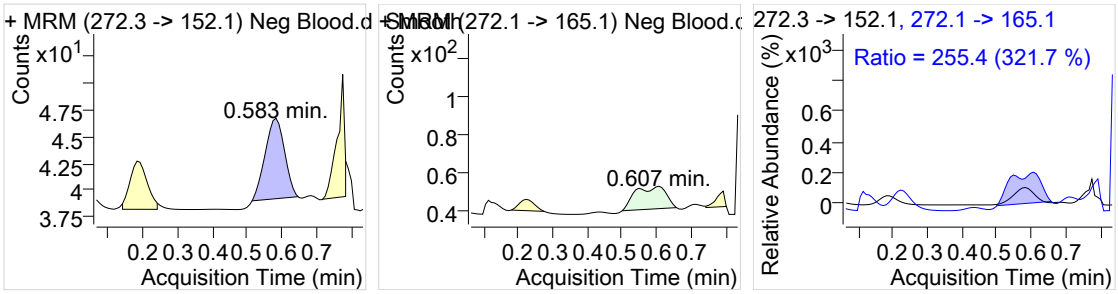
Sample Name: : Neg Blood
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\01052019\Neg Blood.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 1/6/2019 4:34:06 AM
Dilution : 1.0
Operator :
Sample Position : P2-B5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.97	49055			
	292.2 -> 128.2		8042	16.4	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.58	29			3.9 ng/ml
	272.1 -> 165.1		74	*255.4	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.58	8821			
	465.2 -> 201.1		513	5.8	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.74	543			0.0 ng/ml
	462.2 -> 201.1				5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.97	49055			
	292.2 -> 128.2		8042	16.4	13.1 - 21.9	
Morphine	286.1 -> 152.0	*1.07	458			0.0 ng/ml
	286.1 -> 128.1		287	62.5	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.02	17895			
	465.2 -> 165.1		1914	10.7	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.74	554			23.7 ng/ml
	462.2 -> 165.0		68	*12.3	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.47	283121			
Norfentanyl	233.1 -> 84.1	2.49	909			0.3 ng/ml
	233.1 -> 150.1		25	2.7	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.97	472048			
	342.4 -> 105.1		359968	76.3	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.10	1904			0.2 ng/ml
	337.2 -> 188.1		73	*3.8	72.8 - 121.3	

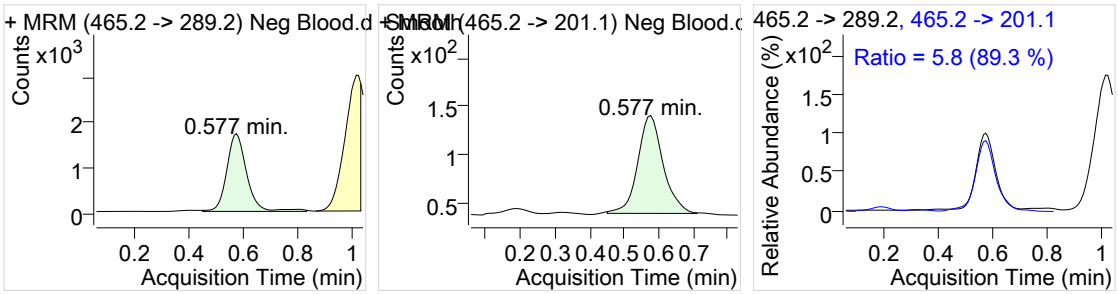
Morphine D6



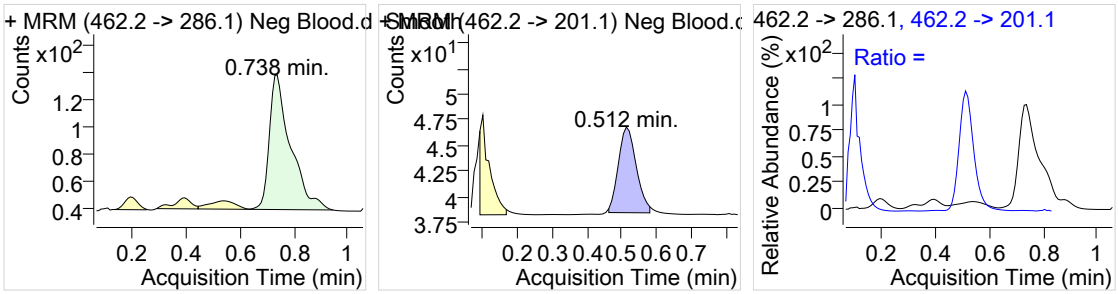
Normorphine



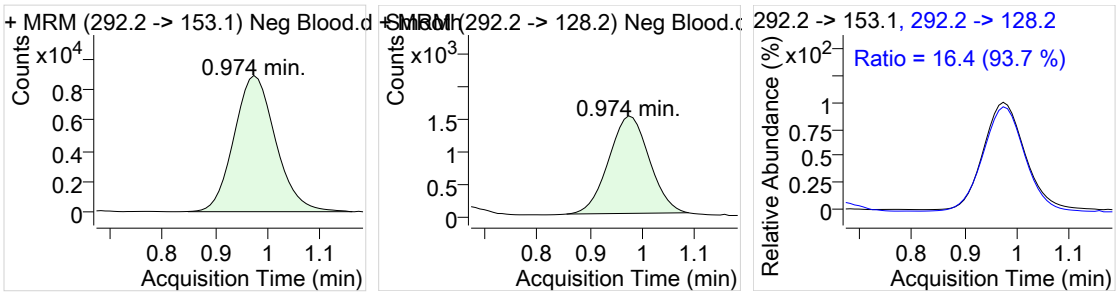
Morphine-3-B-D-Glucuronide D3



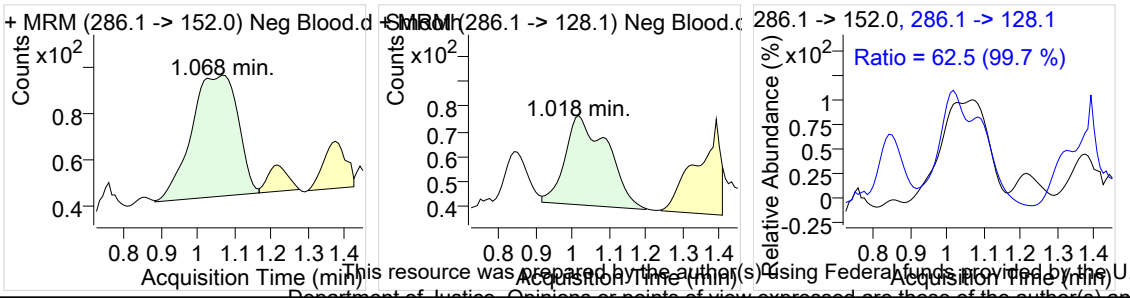
Morphine-3-B-D-Glucuronide



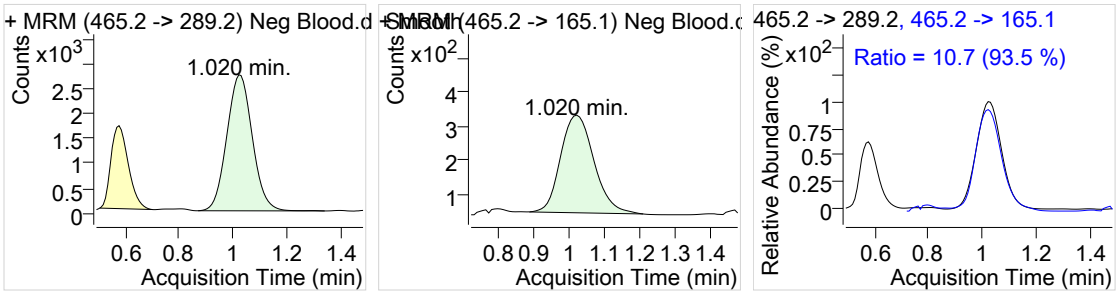
Morphine D6



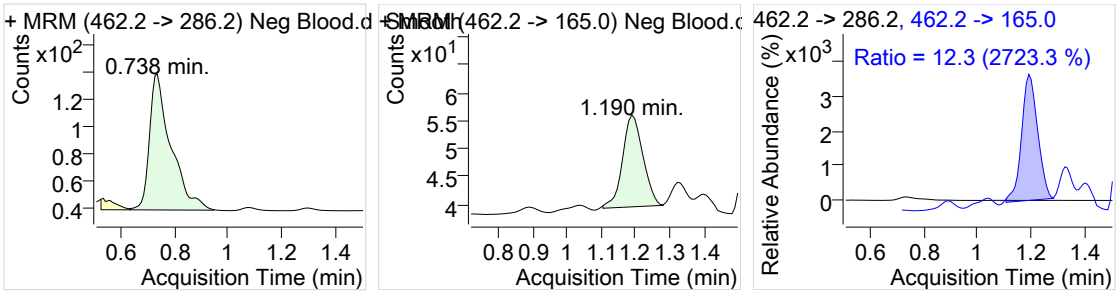
Morphine



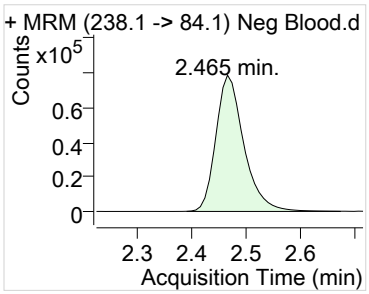
Morphine-6-B-D-Glucuronide D3



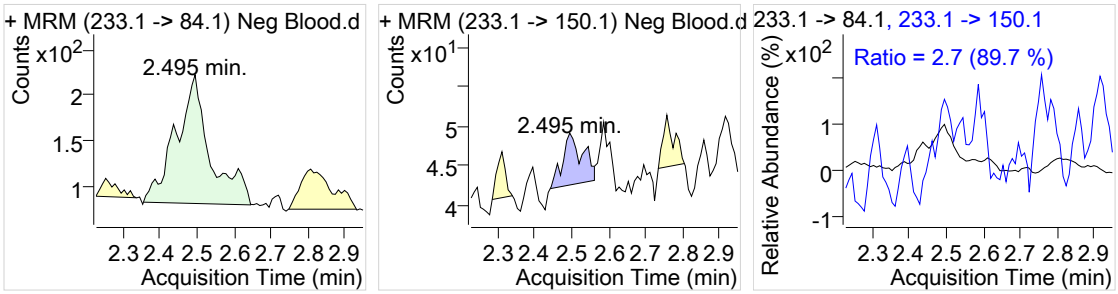
Morphine-6-B-D-Glucuronide



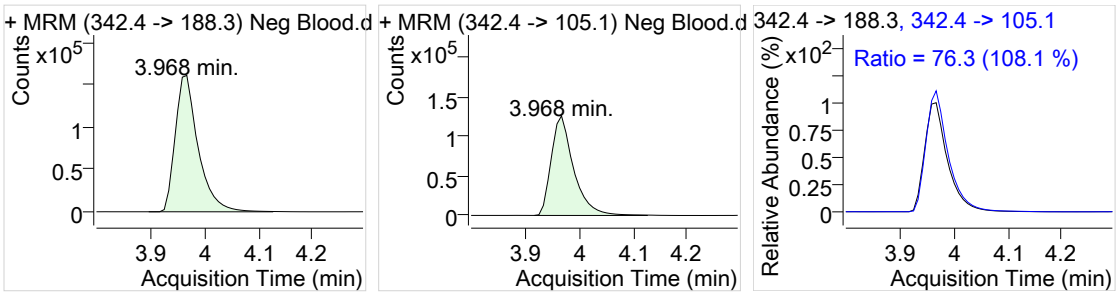
Norfentanyl D5



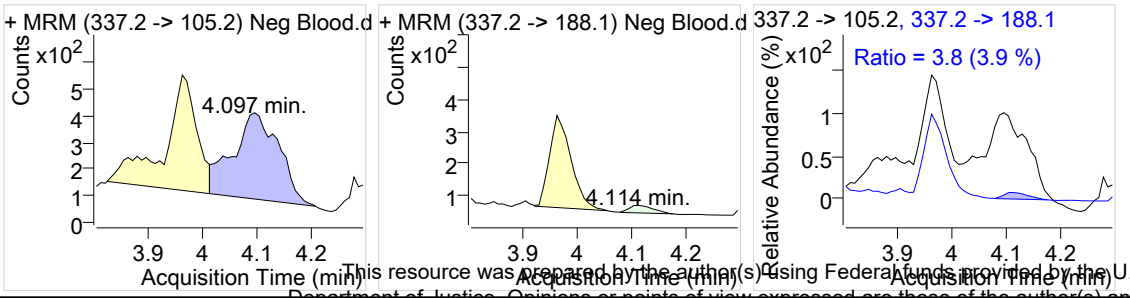
Norfentanyl



Fentanyl D5



Fentanyl



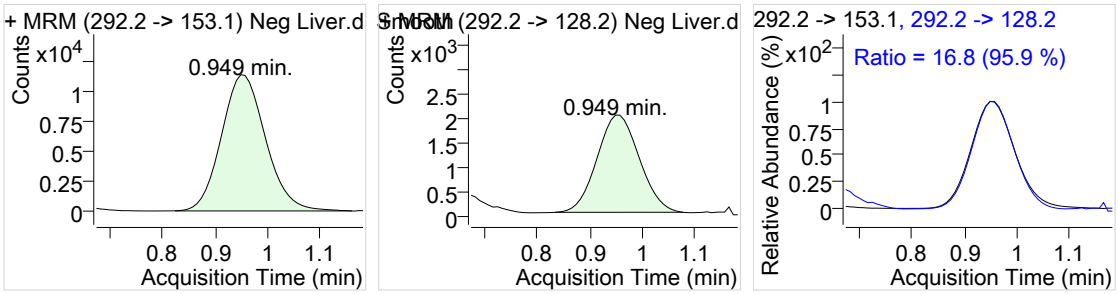
Quantitative Analysis Report

Agilent Technologies

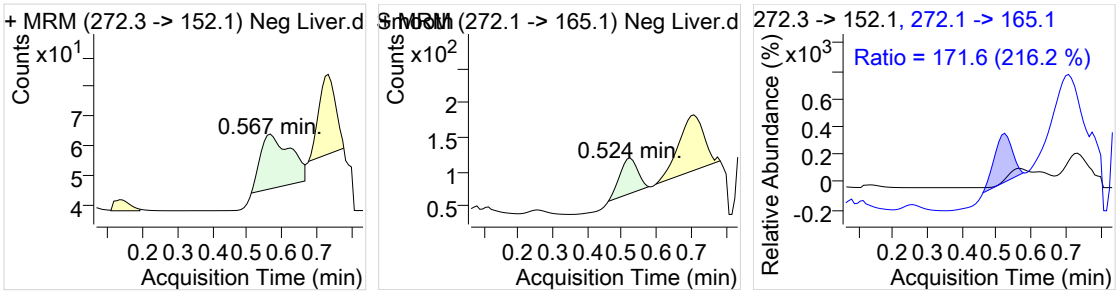
Sample Name:	:	Neg Liver
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\Neg Liver.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 4:43:33 AM
Dilution	:	1.0
Operator	:	
Sample Position	:	P2-B6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.95	66096			
	292.2 -> 128.2		11096	16.8	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.57	109			3.9 ng/ml
	272.1 -> 165.1		187	*171.6	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	21118			
	465.2 -> 201.1		1133	5.4	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	4544			0.0 ng/ml
	462.2 -> 201.1		429	*9.4	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.95	66096			
	292.2 -> 128.2		11096	16.8	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.98	9817			49.4 ng/ml
	286.1 -> 128.1		5924	60.3	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	59999			
	465.2 -> 165.1		5882	9.8	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	2764			24.3 ng/ml
	462.2 -> 165.0				0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.46	342507			
Norfentanyl	233.1 -> 84.1	2.50	932			0.3 ng/ml
	233.1 -> 150.1		33	3.6	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.98	457808			
	342.4 -> 105.1		336931	73.6	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	3.98	11795			0.4 ng/ml
	337.2 -> 188.1		1943	*16.5	72.8 - 121.3	

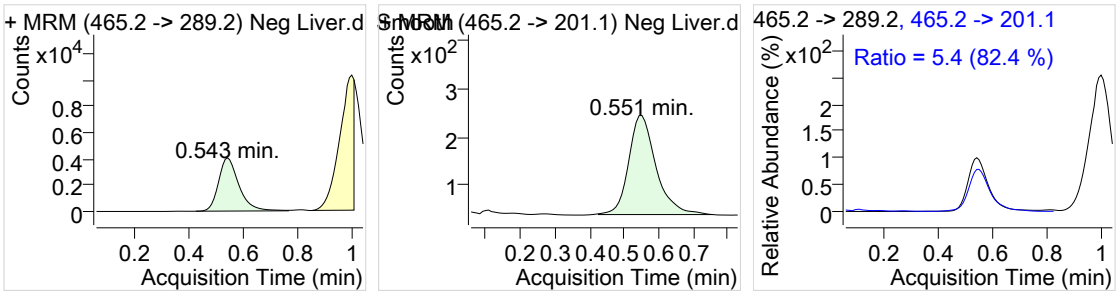
Morphine D6



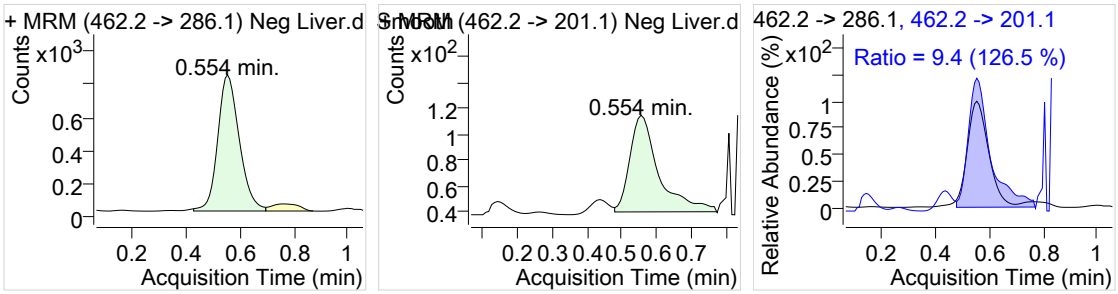
Normorphine



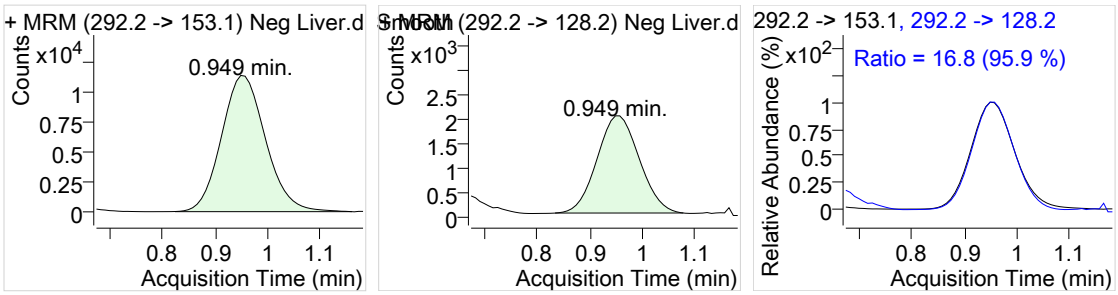
Morphine-3-B-D-Glucuronide D3



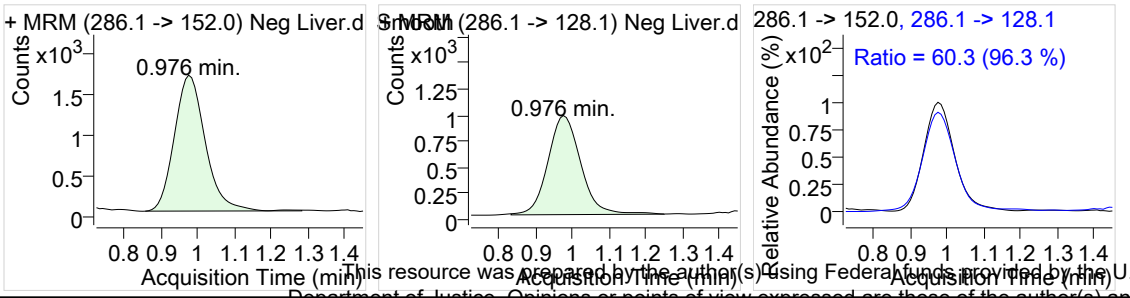
Morphine-3-B-D-Glucuronide



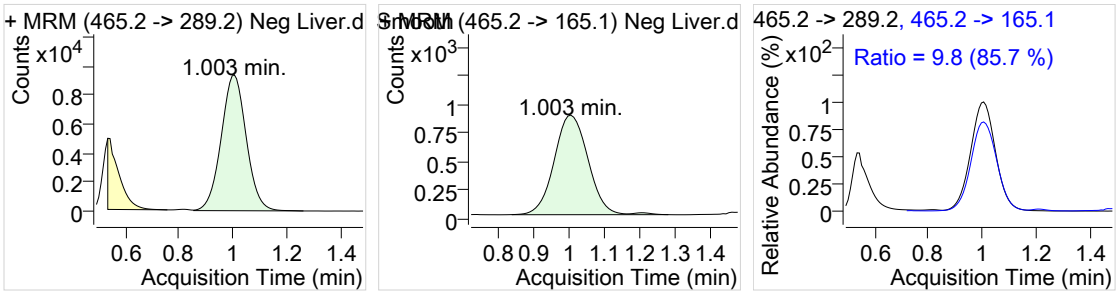
Morphine D6



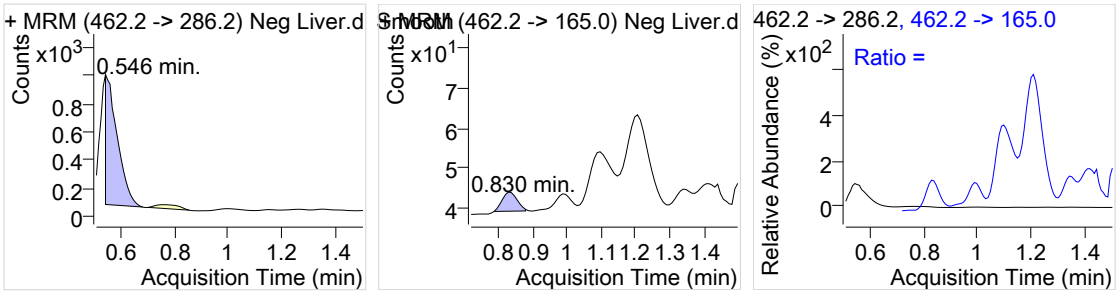
Morphine



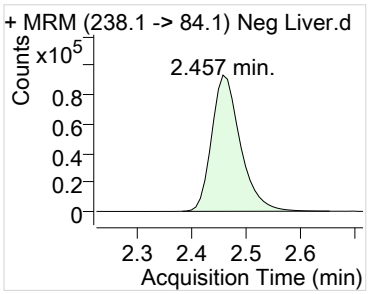
Morphine-6-B-D-Glucuronide D3



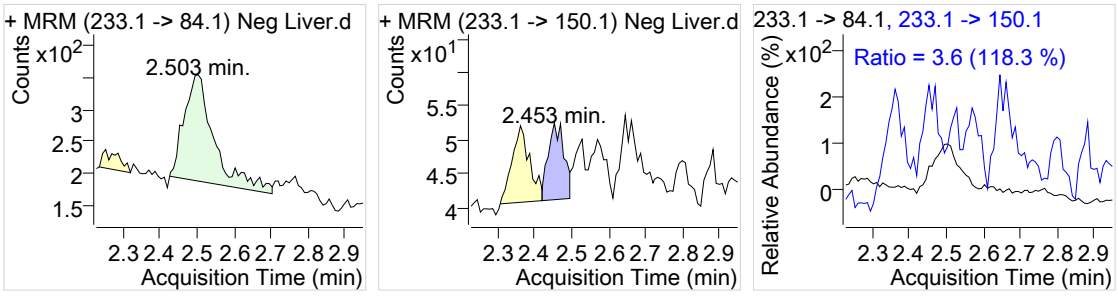
Morphine-6-B-D-Glucuronide



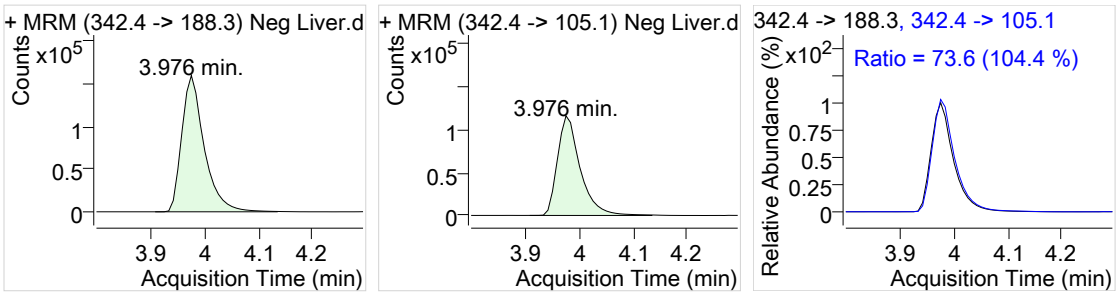
Norfentanyl D5



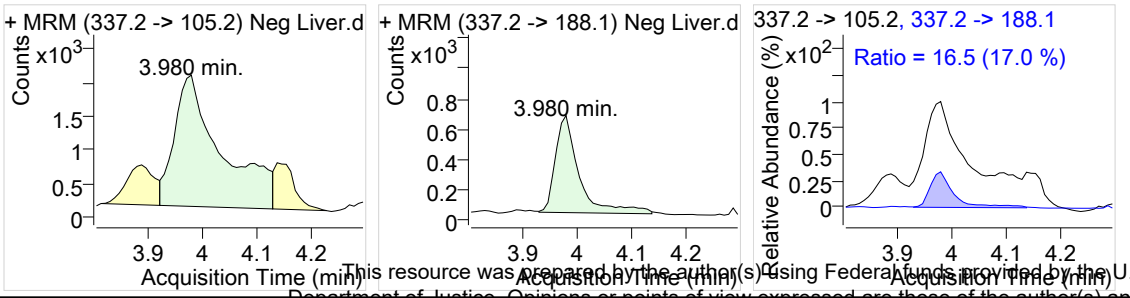
Norfentanyl



Fentanyl D5



Fentanyl



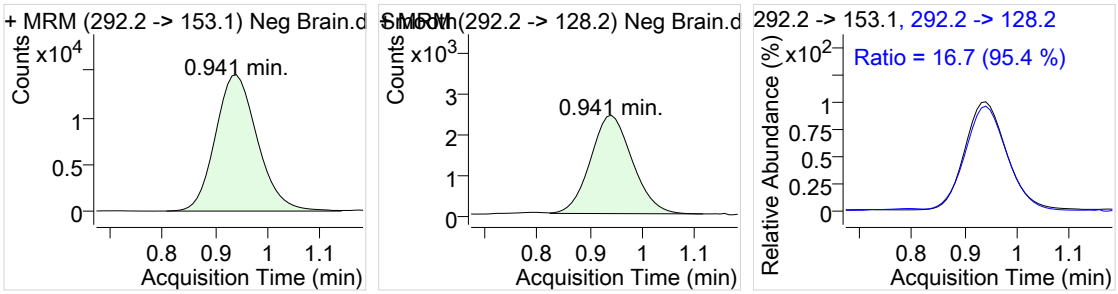
Quantitative Analysis Report

Agilent Technologies

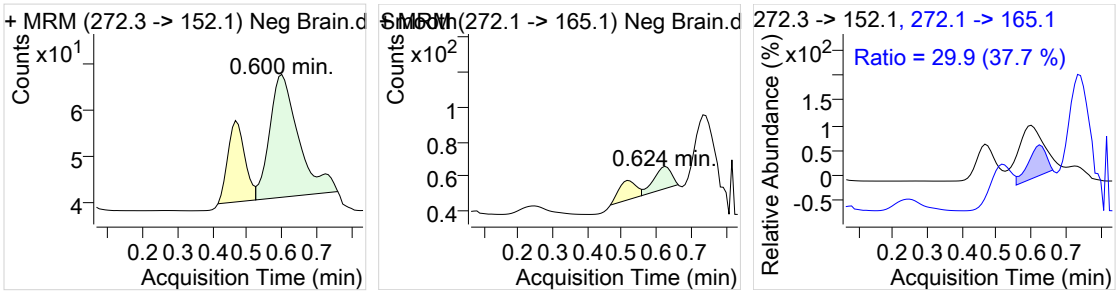
Sample Name:	:	Neg Brain
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\Neg Brain.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 5:01:00 AM
Dilution	:	1.0
Operator	:	
Sample Position	:	P2-B7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.94	81151			
	292.2 -> 128.2		13558	16.7	13.1 - 21.9	
Normorphine	272.3 -> 152.1	*0.60	155			3.9 ng/ml
	272.1 -> 165.1		46	*29.9	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	61502			
	465.2 -> 201.1		4254	6.9	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.76	339			0.0 ng/ml
	462.2 -> 201.1		32	*9.5	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.94	81151			
	292.2 -> 128.2		13558	16.7	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.96	1380			2.7 ng/ml
	286.1 -> 128.1		908	65.8	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	95073			
	465.2 -> 165.1		8481	8.9	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.76	260			22.7 ng/ml
	462.2 -> 165.0		37	*14.4	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.47	393634			
Norfentanyl	233.1 -> 84.1	2.51	639			0.3 ng/ml
	233.1 -> 150.1		15	2.3	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.97	1078608			
	342.4 -> 105.1		788447	73.1	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	3.97	7994			0.2 ng/ml
	337.2 -> 188.1		1955	*24.5	72.8 - 121.3	

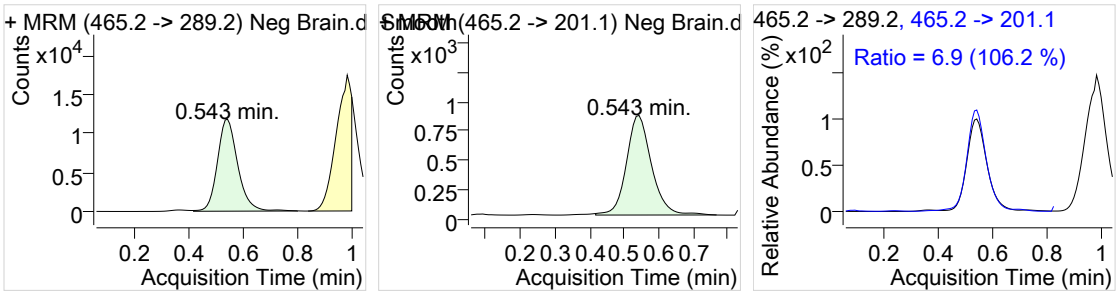
Morphine D6



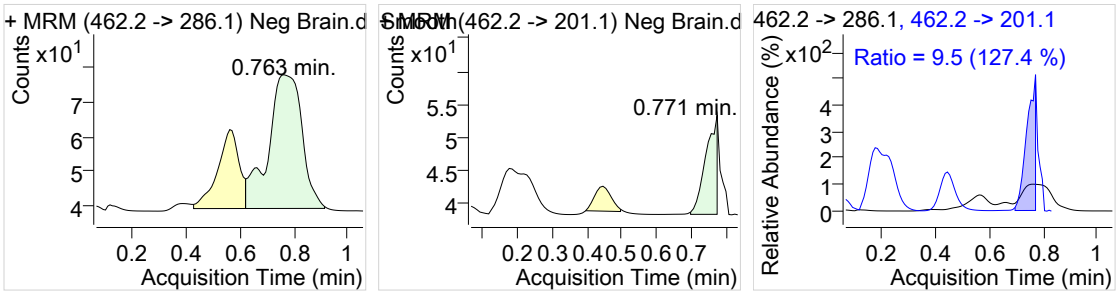
Normorphine



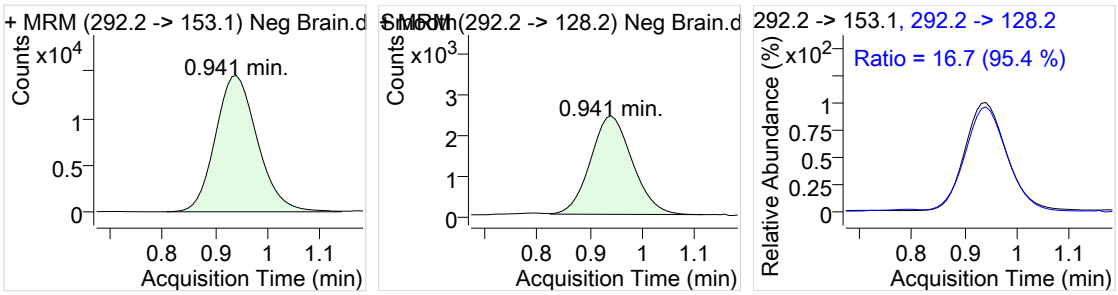
Morphine-3-B-D-Glucuronide D3



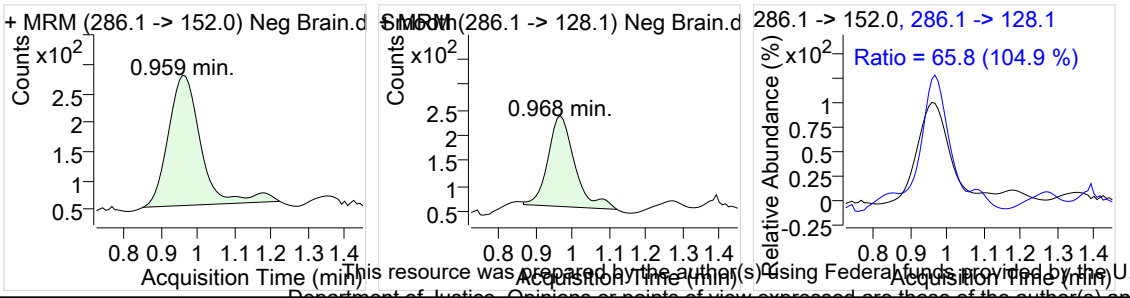
Morphine-3-B-D-Glucuronide



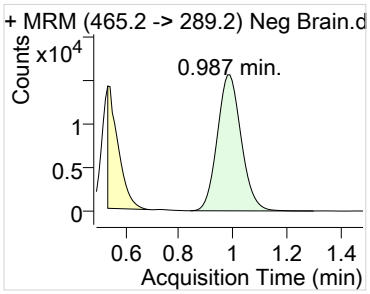
Morphine D6

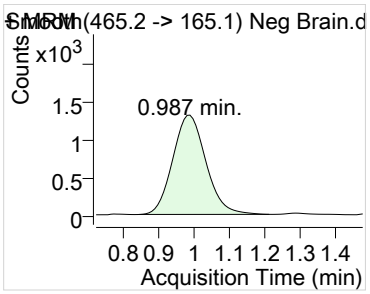


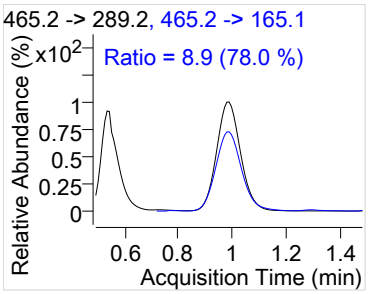
Morphine



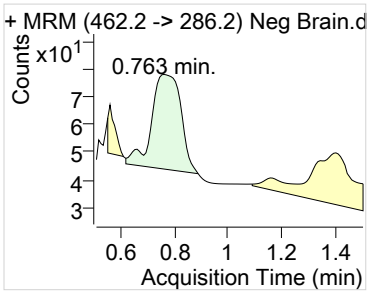
Morphine-6-B-D-Glucuronide D3

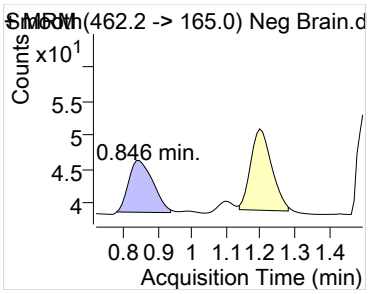


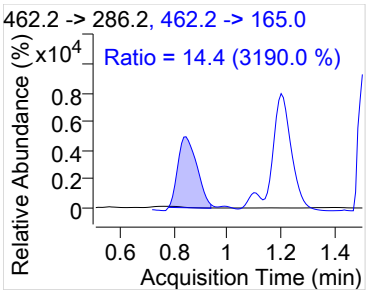




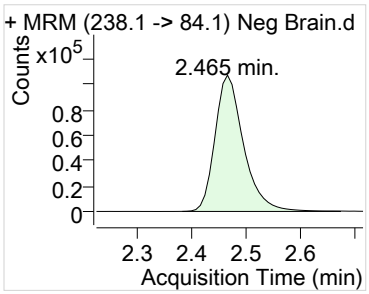
Morphine-6-B-D-Glucuronide



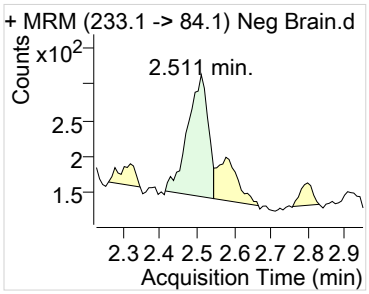


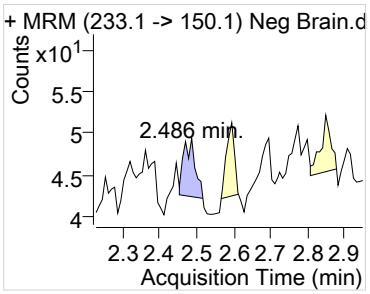


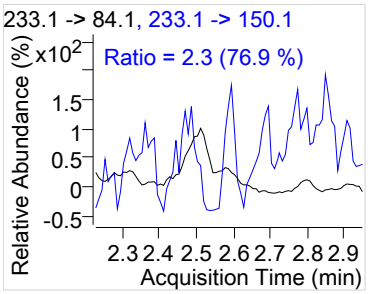
Norfentanyl D5



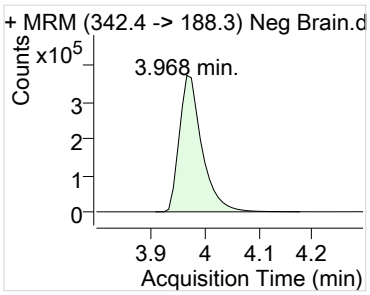
Norfentanyl

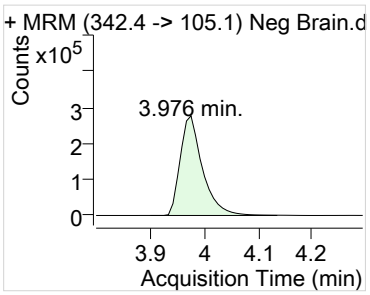


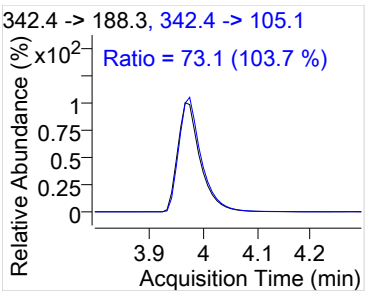




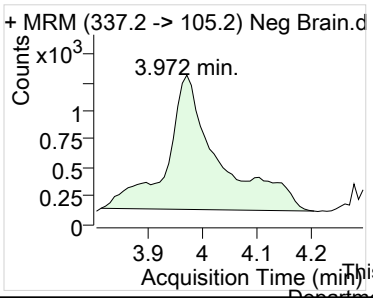
Fentanyl D5

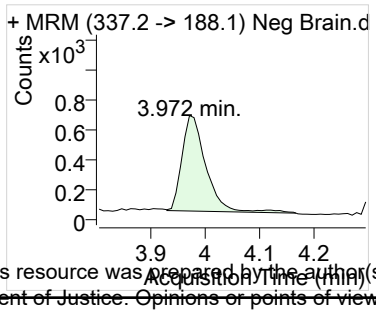


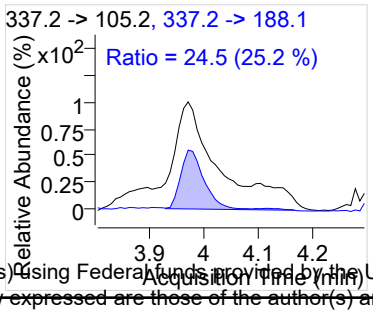




Fentanyl







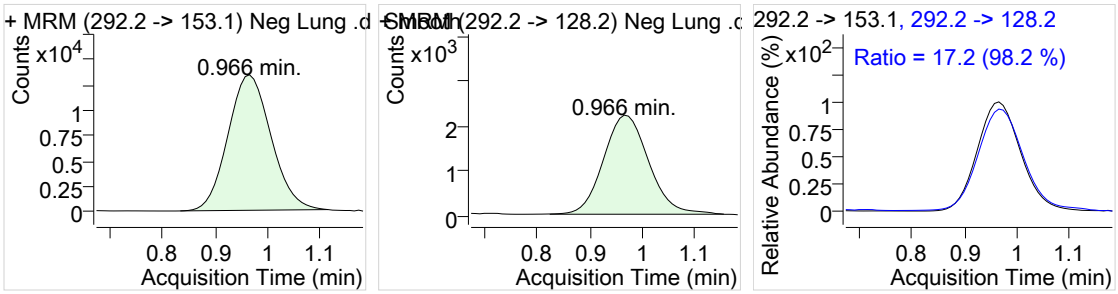
Quantitative Analysis Report

Agilent Technologies

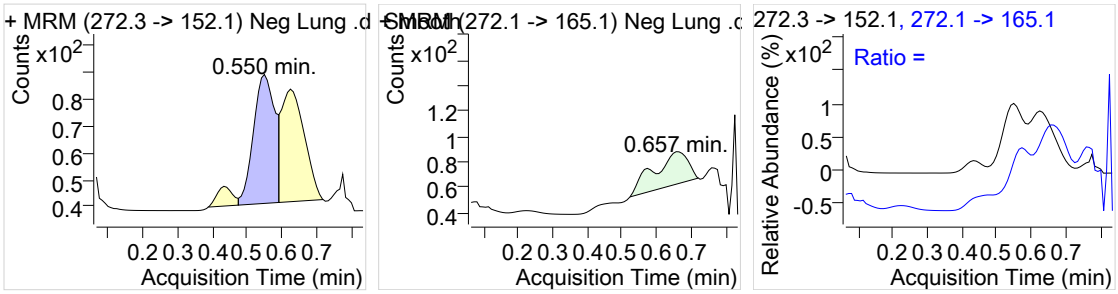
Sample Name:	:	Neg Lung
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\Neg Lung .d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 5:02:28 AM
Dilution	:	1.0
Operator	:	
Sample Position	:	P2-B8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.97	76906			
	292.2 -> 128.2		13216	17.2	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.55	203			3.9 ng/ml
	272.1 -> 165.1				59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	64432			
	465.2 -> 201.1		4827	7.5	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	2769			0.0 ng/ml
	462.2 -> 201.1		259	*9.4	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.97	76906			
	292.2 -> 128.2		13216	17.2	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.99	1717			4.6 ng/ml
	286.1 -> 128.1		868	50.6	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.01	91991			
	465.2 -> 165.1		9106	9.9	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	2141			23.4 ng/ml
	462.2 -> 165.0				0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.47	393438			
Norfentanyl	233.1 -> 84.1	2.49	649			0.3 ng/ml
	233.1 -> 150.1		42	*6.4	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.97	1043031			
	342.4 -> 105.1		751716	72.1	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	3.97	4345			0.2 ng/ml
	337.2 -> 188.1		1681	*38.7	72.8 - 121.3	

Morphine D6



Normorphine



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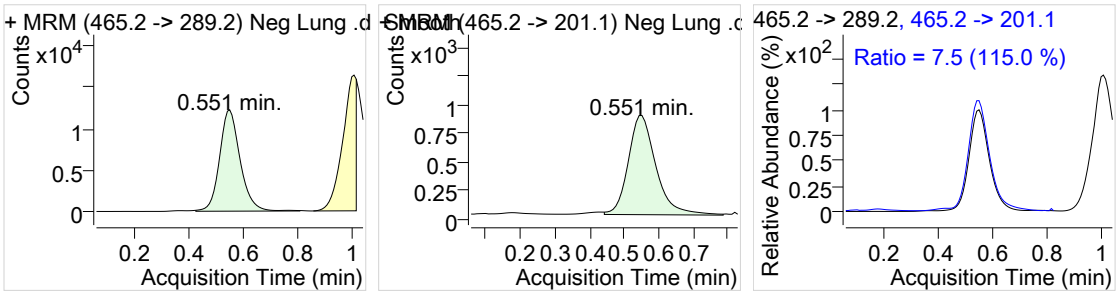
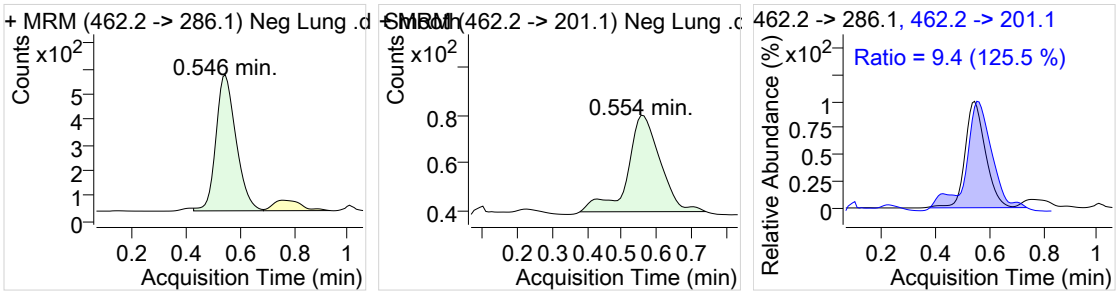


Morphine-3-B-D-Glucuronide



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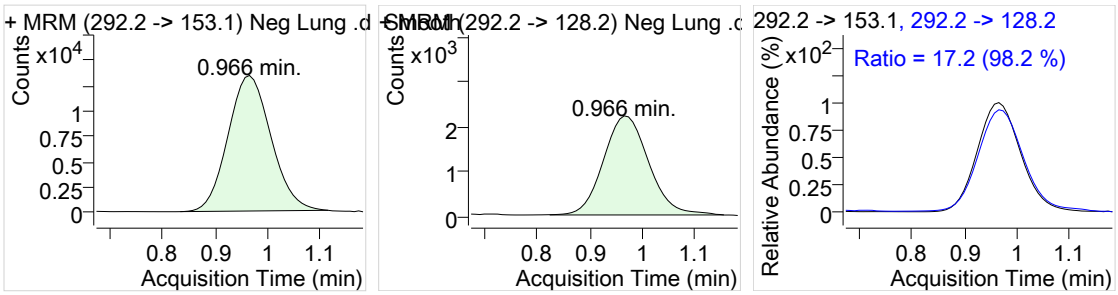
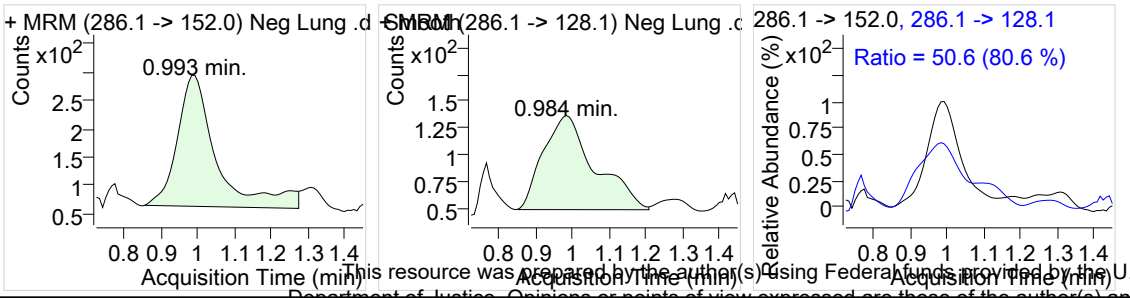


Morphine



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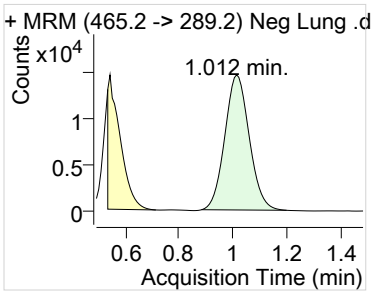
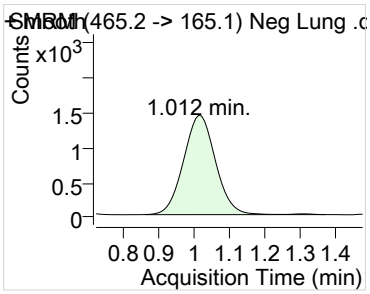
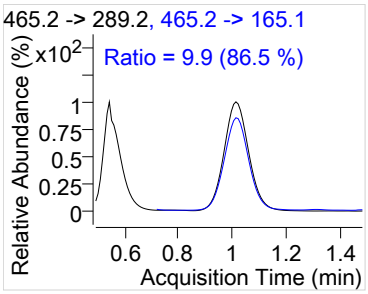
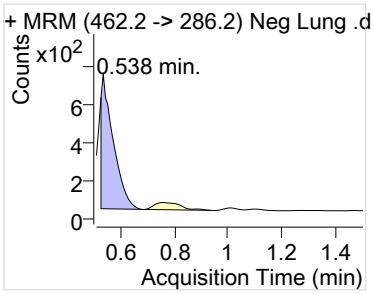
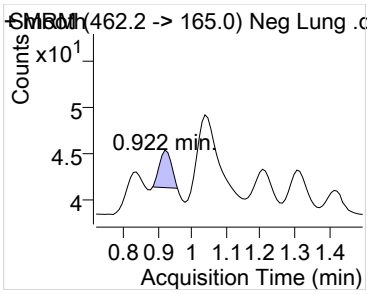


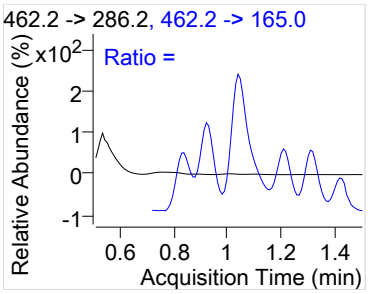




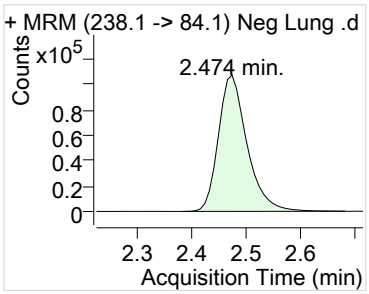
Morphine-6-B-D-Glucuronide



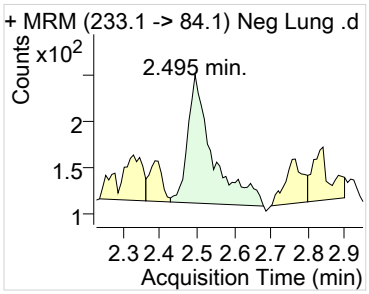


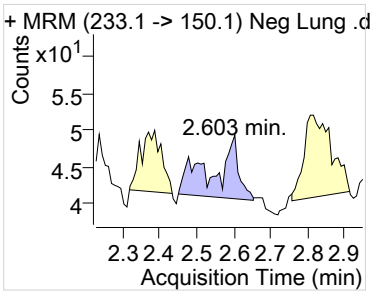


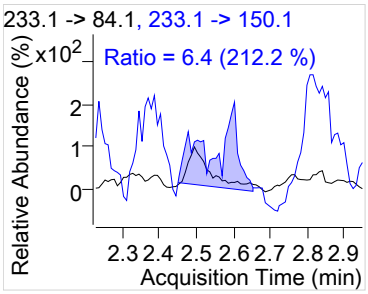
Norfentanyl D5



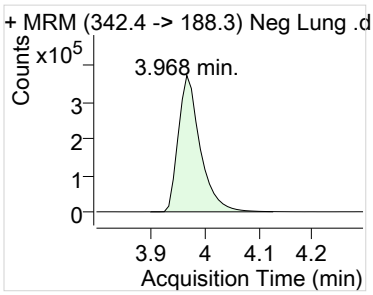
Norfentanyl

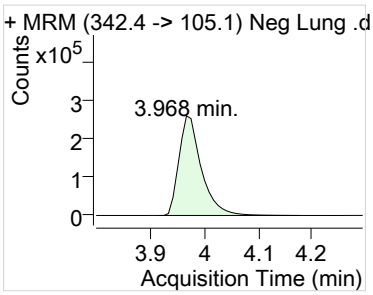


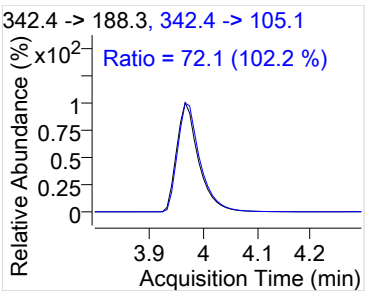




Fentanyl D5







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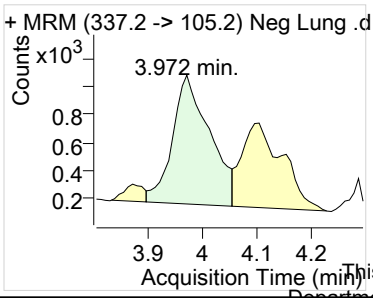

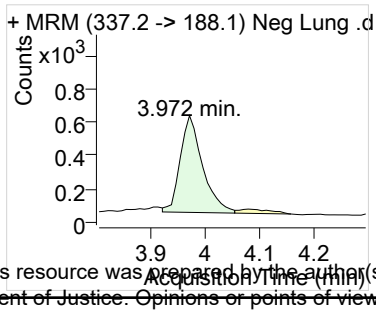

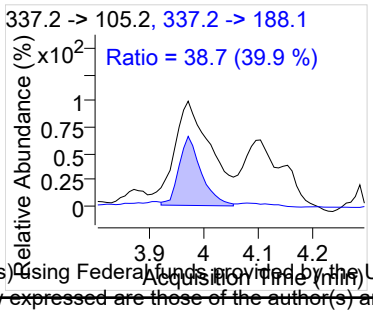

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Appendix D

LC-MS/MS Quantitative Analysis Reports for Matrices at Different Postmortem Intervals

Table of Contents

Abbreviations

Conc: Concentration

FB: Femoral Blood

HB: Heart Blood

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry

RT: Retention Time

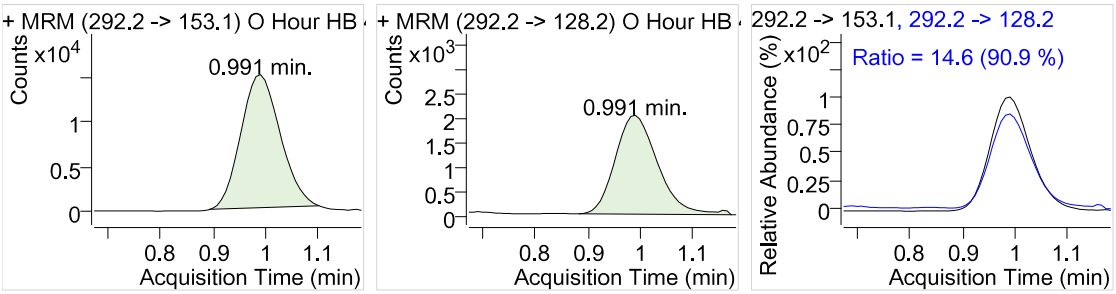
Quantitative Analysis Report

Agilent Technologies

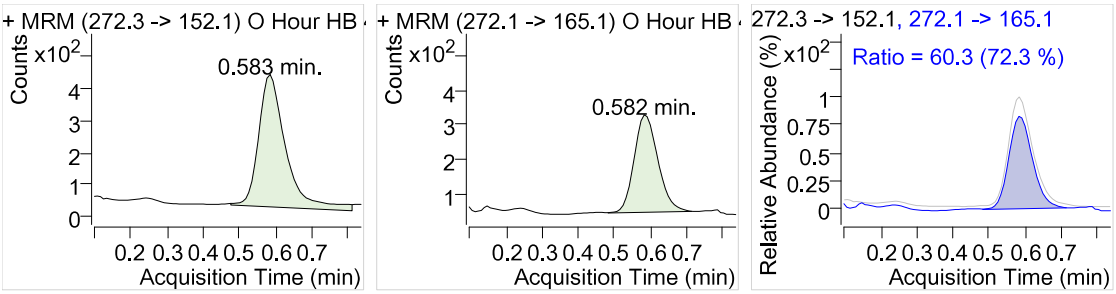
Sample Name:	:	O Hour HB 4
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07072018\O Hour HB 4.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 12:40:57 PM
Dilution	:	2.6
Operator	:	
Sample Position	:	P1-C1

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.99	77515			
	292.2 -> 128.2		11327	14.6	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.58	2166			21.4 ng/ml
	272.1 -> 165.1		1305	*60.3	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.58	2118			
	465.2 -> 201.1		243	*11.5	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.59	28336			2355.5 ng/ml
	462.2 -> 201.1		2253	*8.0	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	0.99	77515			
	292.2 -> 128.2		11327	14.6	12.9 - 19.3	
Morphine	286.1 -> 152.0	1.02	66209			1078.8 ng/ml
	286.1 -> 128.1		45391	68.6	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.05	2882			
	465.2 -> 165.1		305	*10.6	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.97	144			0.0 ng/ml
	462.2 -> 165.0		60	*41.8	0.5 - 0.8	

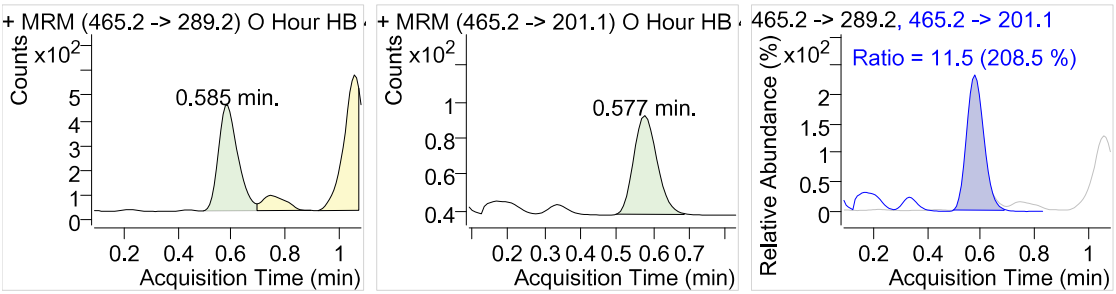
Morphine D6



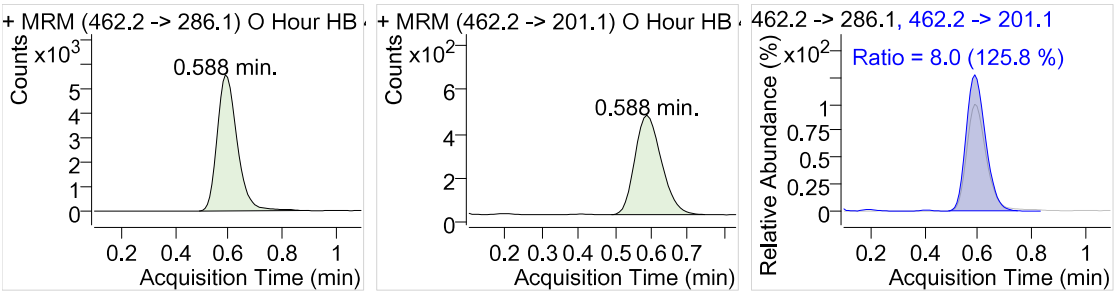
Normorphine



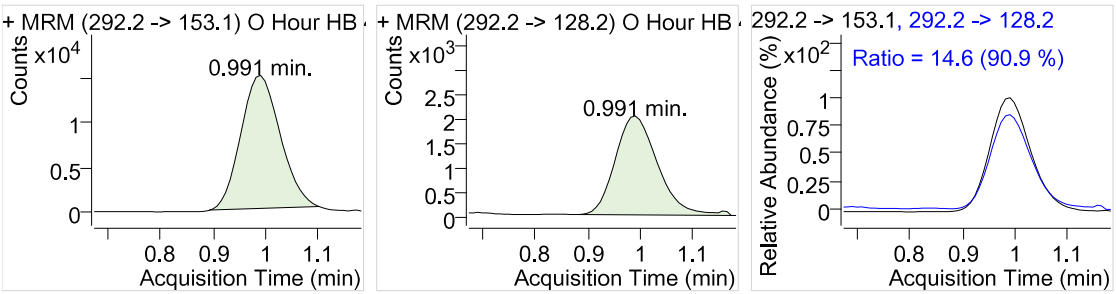
Morphine-3-B-D-Glucuronide D3



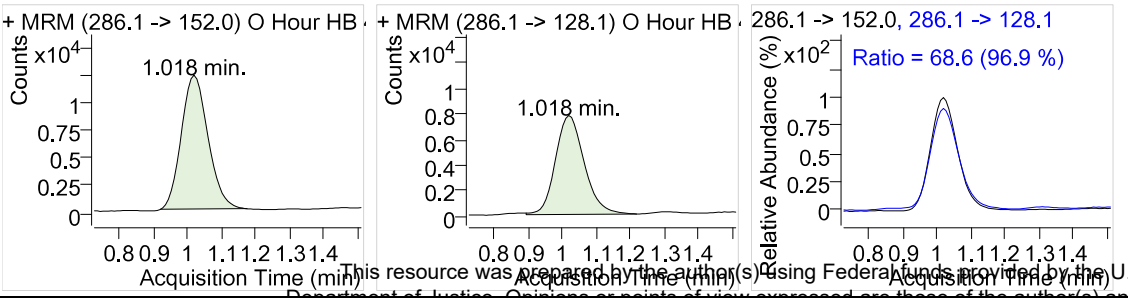
Morphine-3-B-D-Glucuronide



Morphine D6



Morphine



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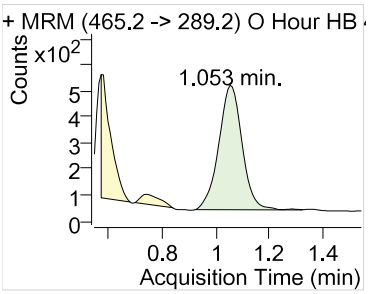
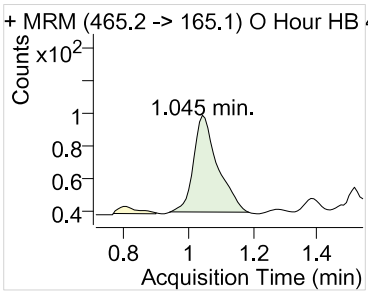
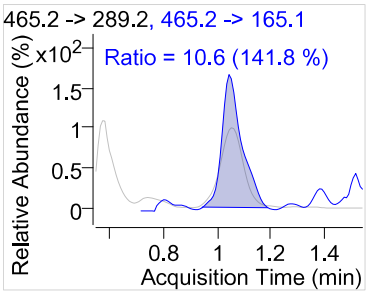
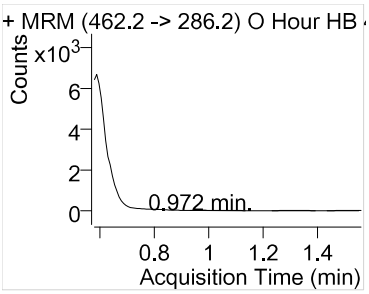
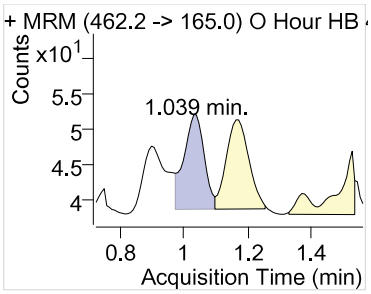


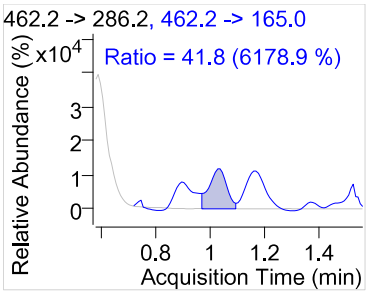




Morphine-6-B-D-Glucuronide







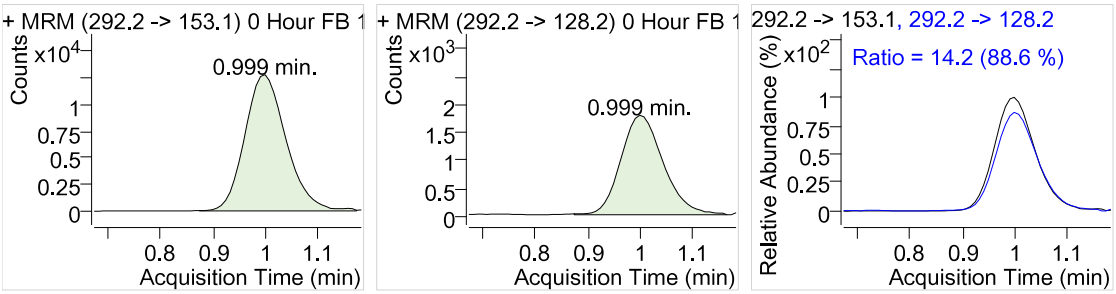
Quantitative Analysis Report

Agilent Technologies

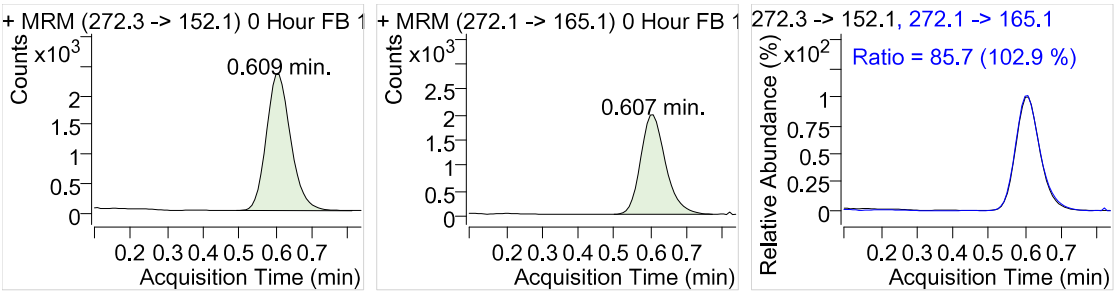
Sample Name:	:	0 Hour FB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07072018\0 Hour FB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 12:42:24 PM
Dilution	:	3.9
Operator	:	
Sample Position	:	P1-C2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	1.00	70012			
	292.2 -> 128.2		9975	14.2	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.61	10942			37.9 ng/ml
	272.1 -> 165.1		9377	85.7	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.60	5042			
	465.2 -> 201.1		532	*10.6	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.60	3239			524.1 ng/ml
	462.2 -> 201.1		196	6.0	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	1.00	70012			
	292.2 -> 128.2		9975	14.2	12.9 - 19.3	
Morphine	286.1 -> 152.0	1.03	191386			4789.2 ng/ml
	286.1 -> 128.1		123018	64.3	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.07	2611			
	465.2 -> 165.1		202	7.7	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.60	1865			495.7 ng/ml
	462.2 -> 165.0		44	*2.4	0.5 - 0.8	

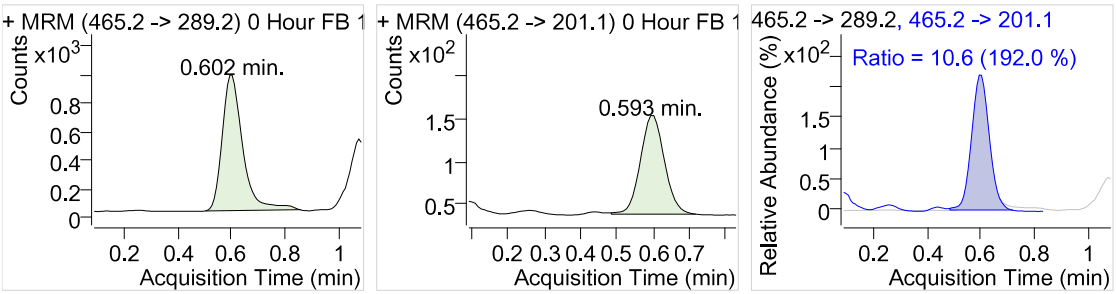
Morphine D6



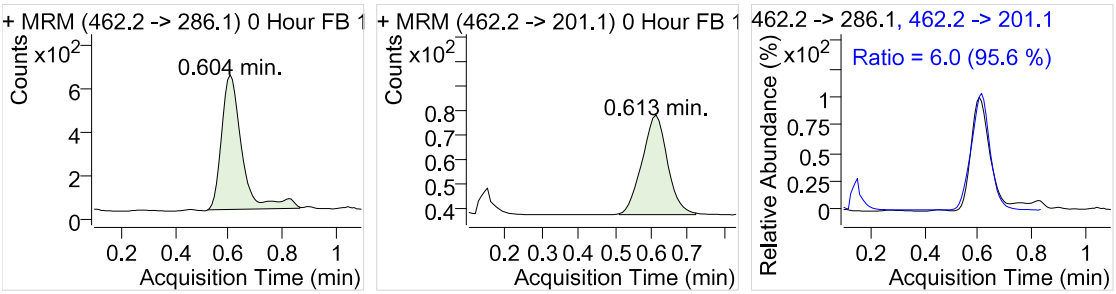
Normorphine



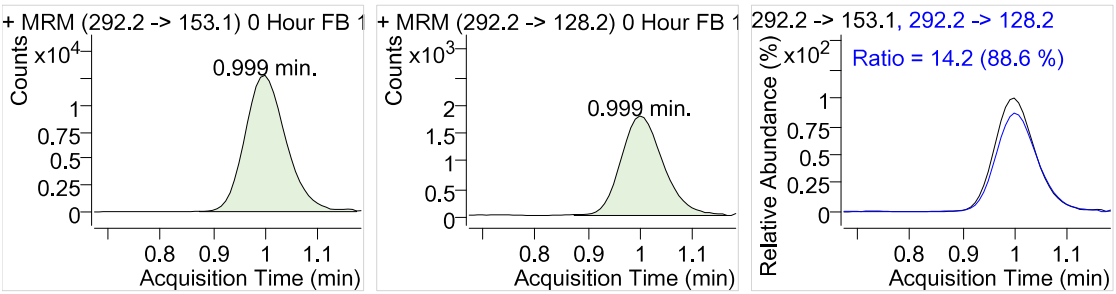
Morphine-3-B-D-Glucuronide D3



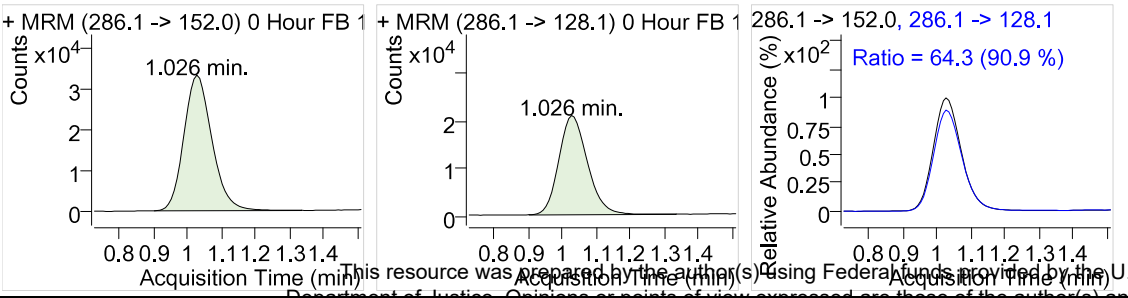
Morphine-3-B-D-Glucuronide



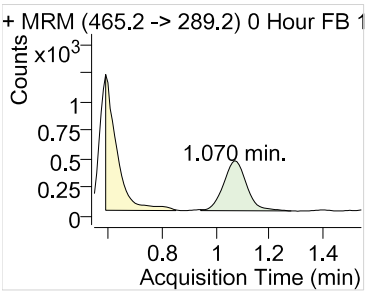
Morphine D6

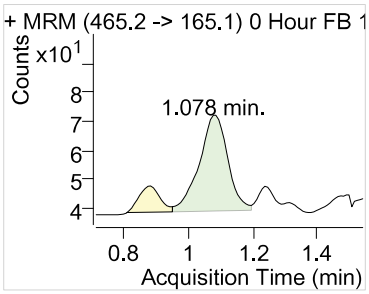


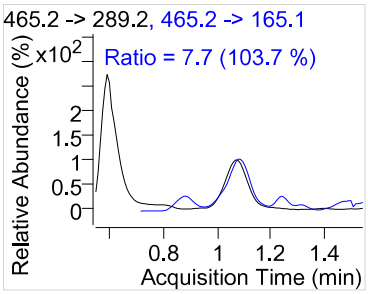
Morphine



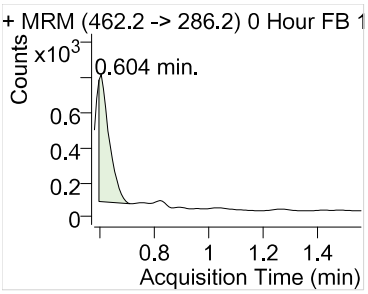
Morphine-6-B-D-Glucuronide D3

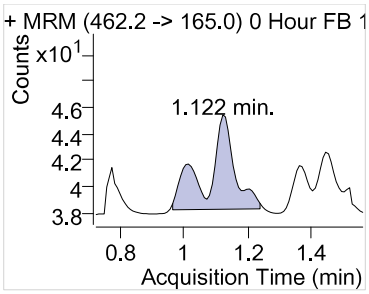


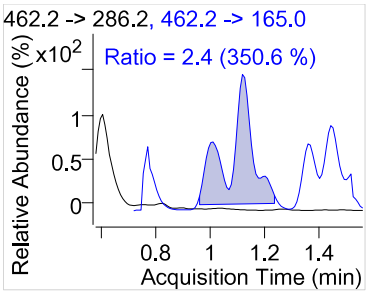




Morphine-6-B-D-Glucuronide







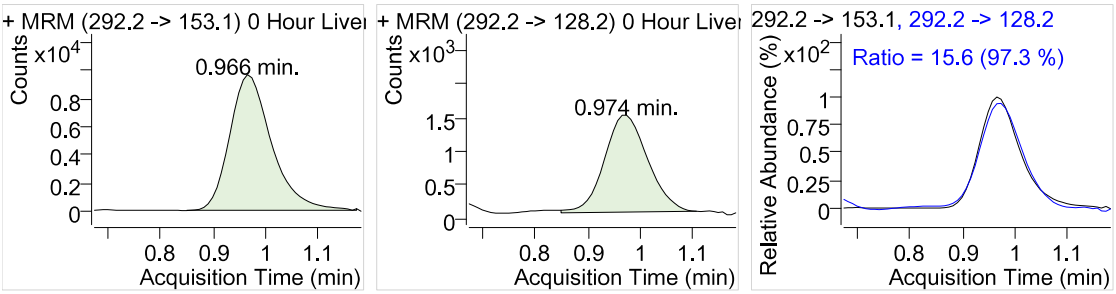
Quantitative Analysis Report

Agilent Technologies

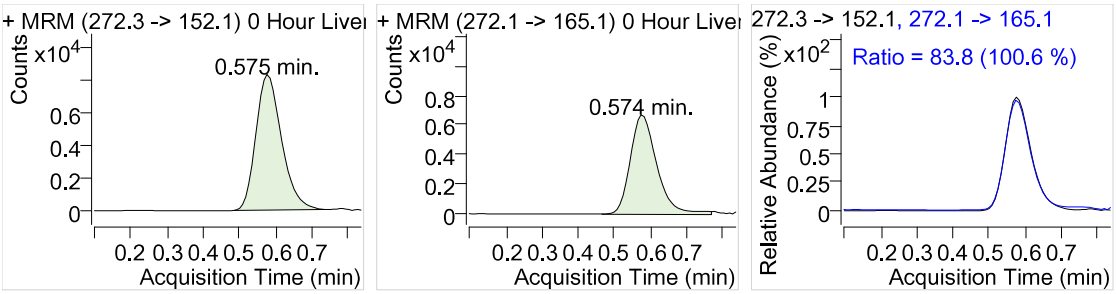
Sample Name: : 0 Hour Liver 3
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\07072018\0 Hour Liver 3.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/8/2018 1:47:00 PM
Dilution : 4.0
Operator :
Sample Position : P1-C8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.97	53406			
	292.2 -> 128.2		8351	15.6	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.58	40666			66.4 ng/ml
	272.1 -> 165.1		34059	83.8	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.57	78491			
	465.2 -> 201.1		5173	6.6	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.58	4492716			8035.3 ng/ml
	462.2 -> 201.1		290331	6.5	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	0.97	53406			
	292.2 -> 128.2		8351	15.6	12.9 - 19.3	
Morphine	286.1 -> 152.0	0.99	478238			13259.0 ng/ml
	286.1 -> 128.1		287140	60.0	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.05	84539			
	465.2 -> 165.1		6883	8.1	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	1.06	67077			554.8 ng/ml
	462.2 -> 165.0		9369	*14.0	0.5 - 0.8	

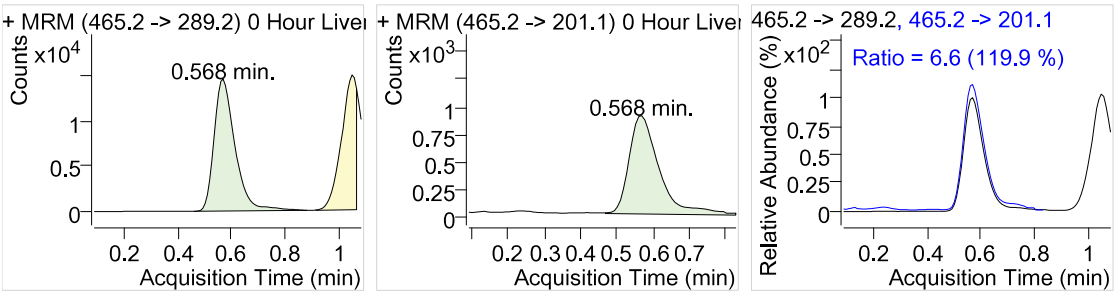
Morphine D6



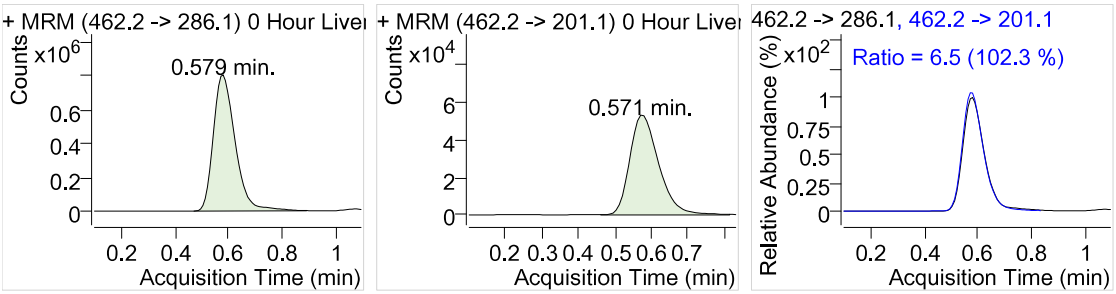
Normorphine



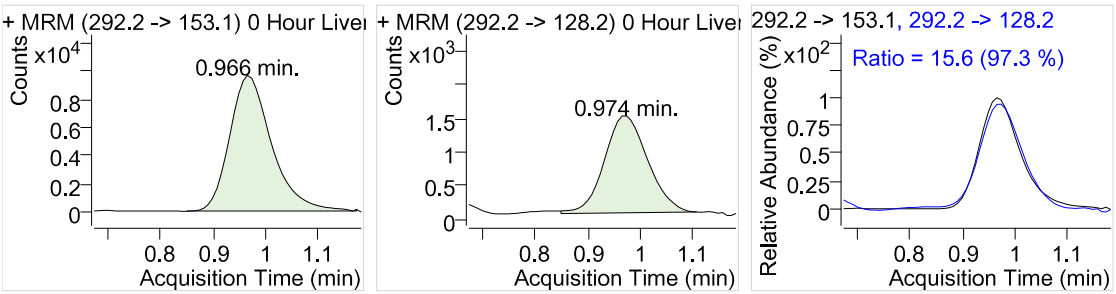
Morphine-3-B-D-Glucuronide D3



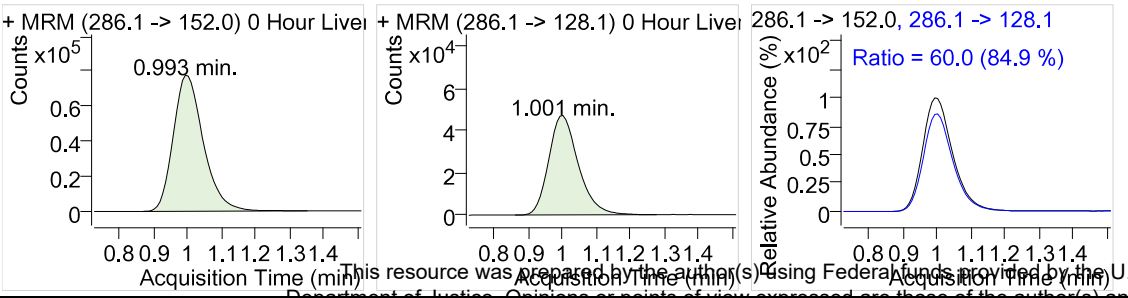
Morphine-3-B-D-Glucuronide



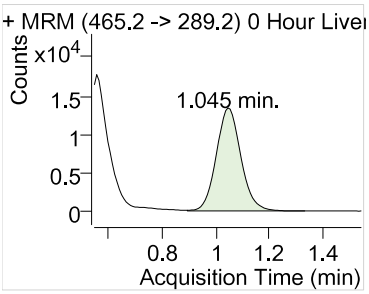
Morphine D6

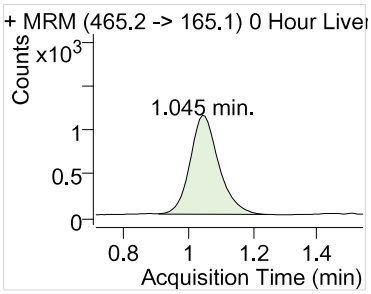


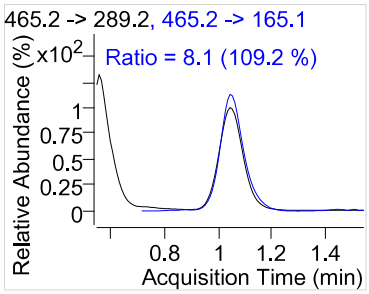
Morphine



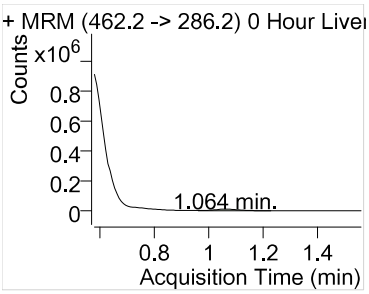
Morphine-6-B-D-Glucuronide D3

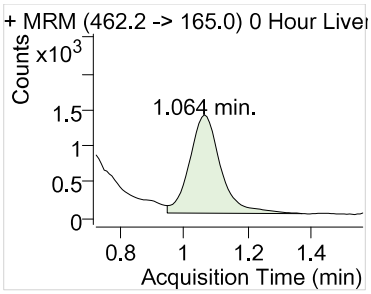


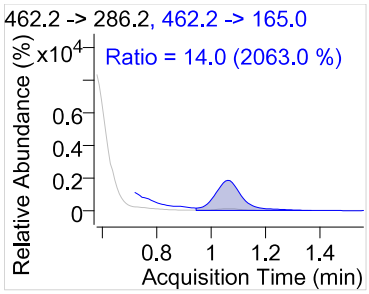




Morphine-6-B-D-Glucuronide





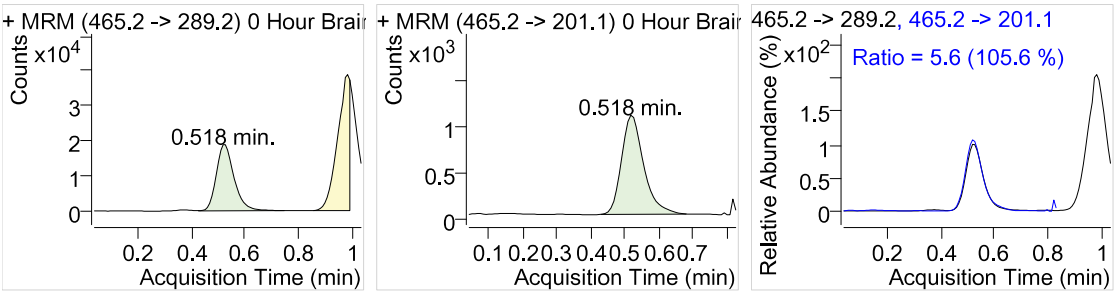


Quantitative Analysis Report

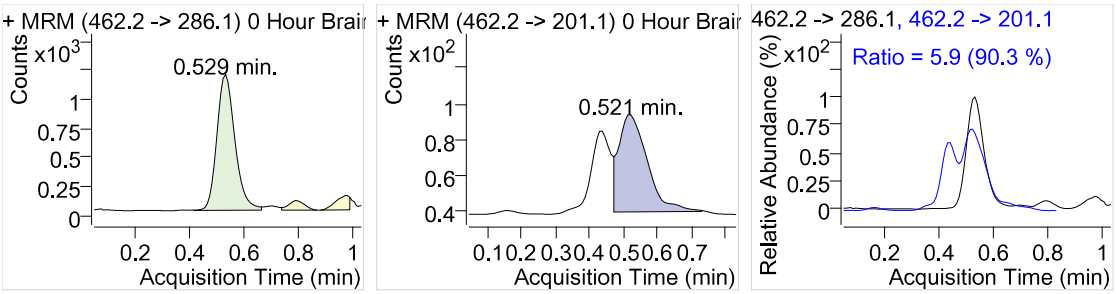
Sample Name: : 0 Hour Brain 1
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\0 Hour Brain 1.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/7/2018 8:44:26 PM
Dilution : 4.0
Operator :
Sample Position : P1-B6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.52	86640			
	465.2 -> 201.1		4885	5.6	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.53	5413			62.6 ng/ml
	462.2 -> 201.1		319	5.9	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.92	88372			
	292.2 -> 128.2		14724	16.7	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.54	1871			39.4 ng/ml
	272.1 -> 165.1		1815	97.0	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.92	88372			
	292.2 -> 128.2		14724	16.7	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.95	44904			1034.5 ng/ml
	286.1 -> 128.1		26852	59.8	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	197940			
	465.2 -> 165.1		14938	7.5	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	0.97	671			33.8 ng/ml
	462.2 -> 165.0		123	*18.3	7.1 - 10.6	

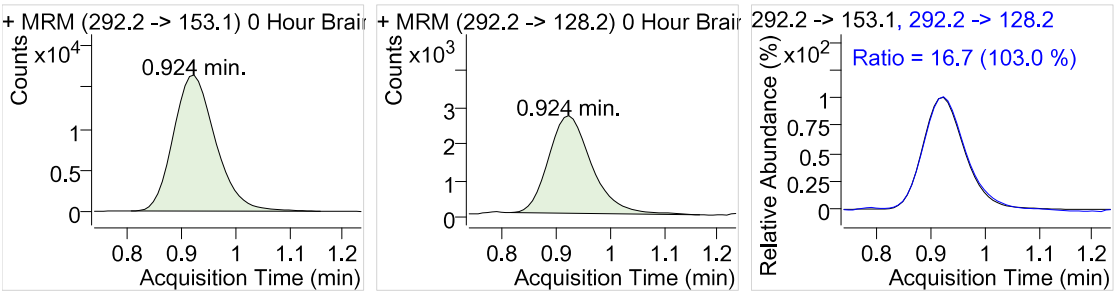
Morphine-3-B-D-Glucuronide D3



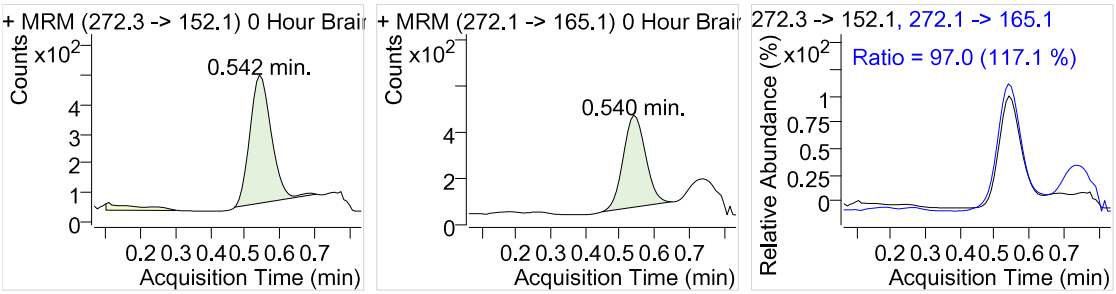
Morphine-3-B-D-Glucuronide



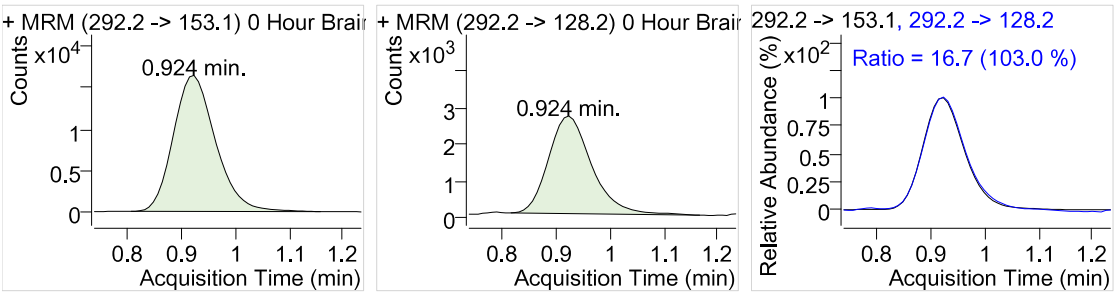
Morphine D6



Normorphine



Morphine D6



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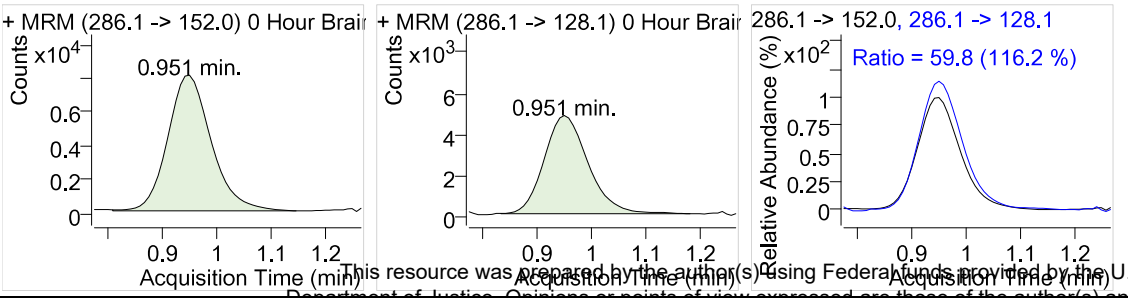

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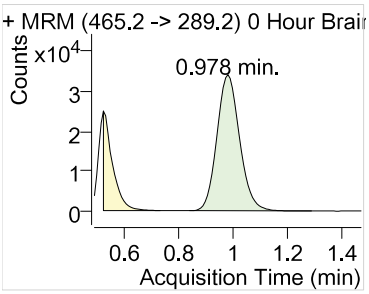
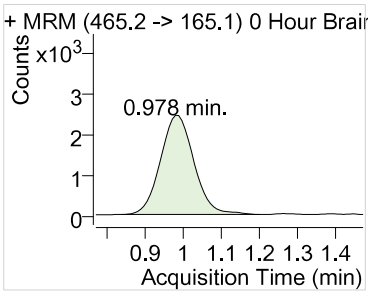
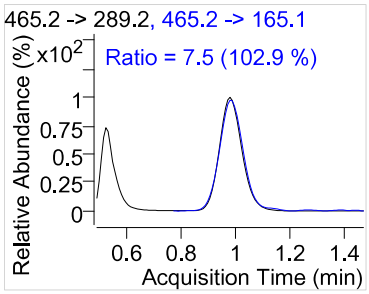
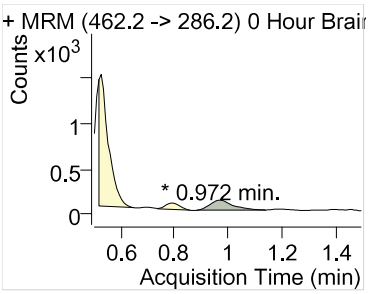
Morphine-6-B-D-Glucuronide D3

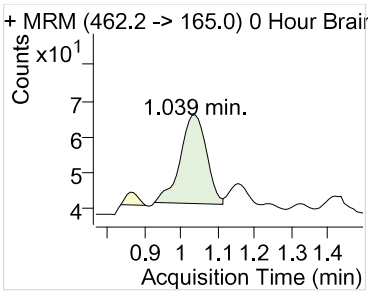


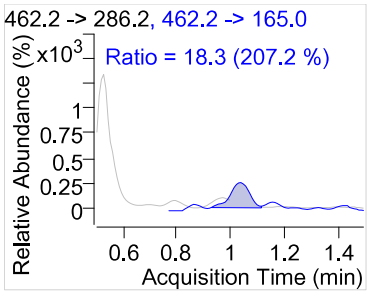




Morphine-6-B-D-Glucuronide





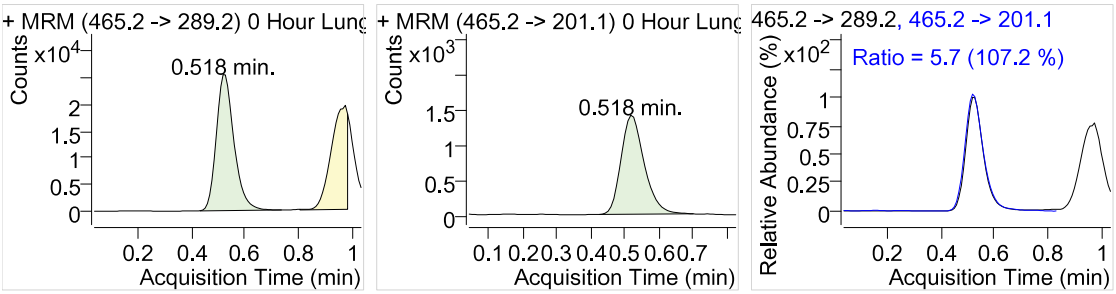


Quantitative Analysis Report

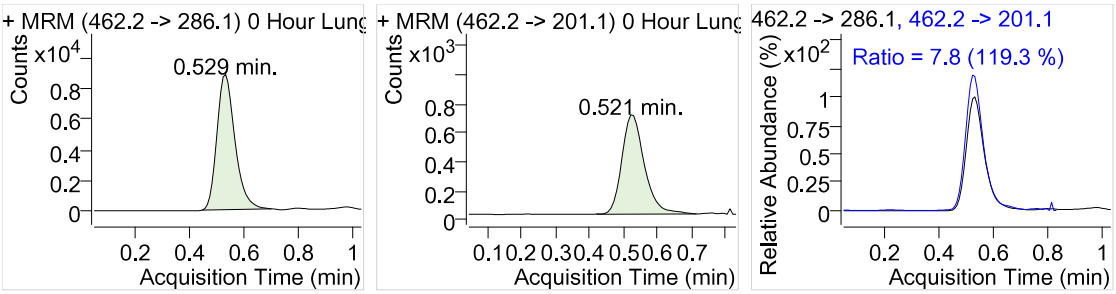
Sample Name: : 0 Hour Lung 1
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\0 Hour Lung 1.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/7/2018 8:53:59 PM
Dilution : 4.0
Operator :
Sample Position : P1-B7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.52	115798			
	465.2 -> 201.1		6623	5.7	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.53	40471			212.2 ng/ml
	462.2 -> 201.1		3148	7.8	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.89	37736			
	292.2 -> 128.2		6294	16.7	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.54	29398			71.1 ng/ml
	272.1 -> 165.1		24154	82.2	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.89	37736			
	292.2 -> 128.2		6294	16.7	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.92	402705			18639.6 ng/ml
	286.1 -> 128.1		235609	58.5	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.96	117823			
	465.2 -> 165.1		9454	8.0	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.52	28492			102.4 ng/ml
	462.2 -> 165.0		67	*0.2	7.1 - 10.6	

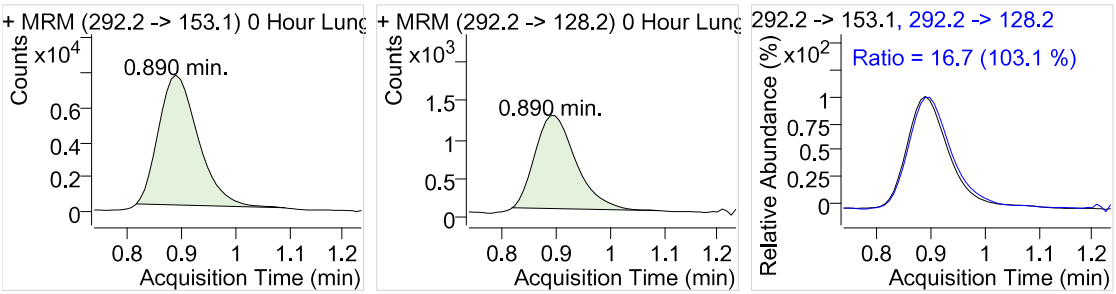
Morphine-3-B-D-Glucuronide D3



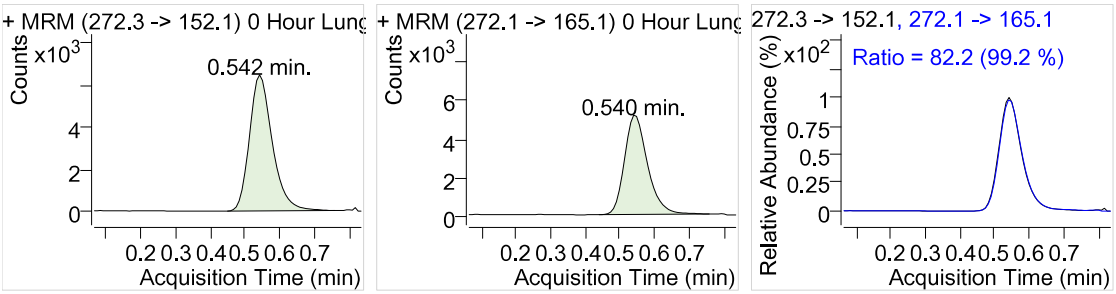
Morphine-3-B-D-Glucuronide



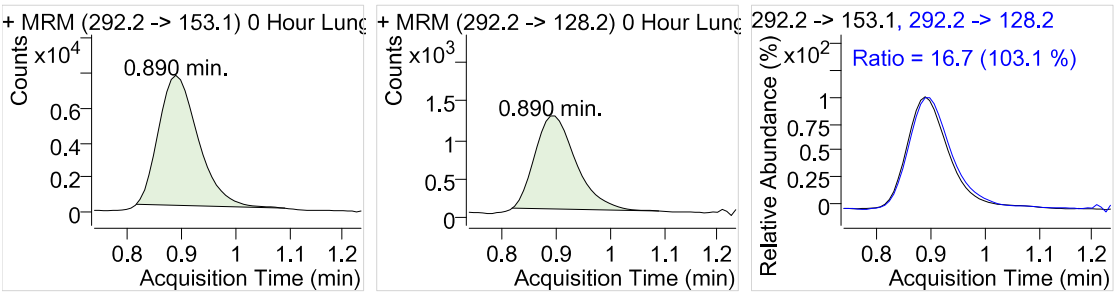
Morphine D6



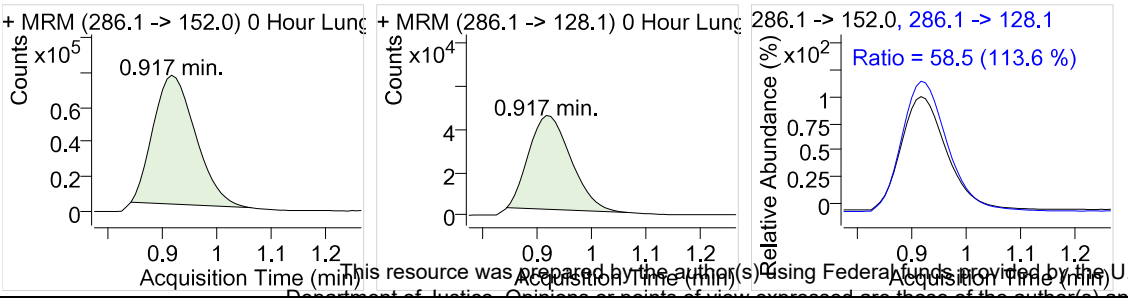
Normorphine



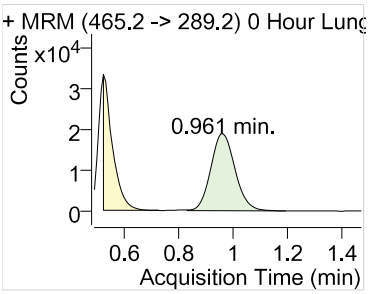
Morphine D6

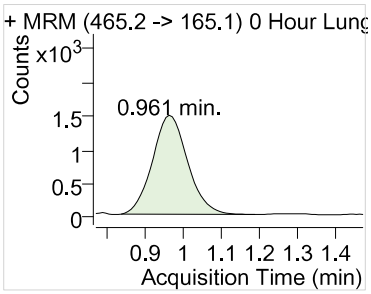


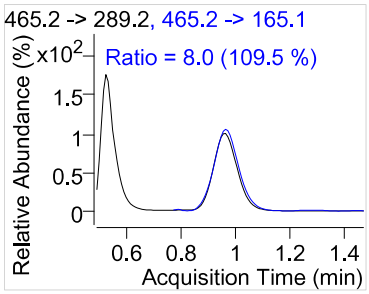
Morphine



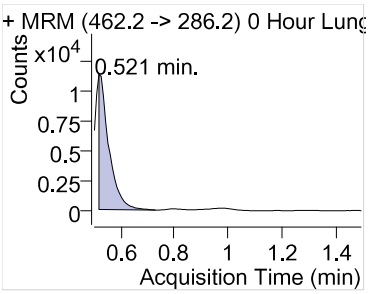
Morphine-6-B-D-Glucuronide D3

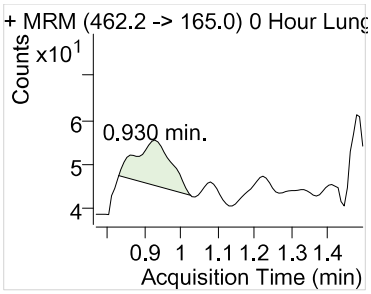


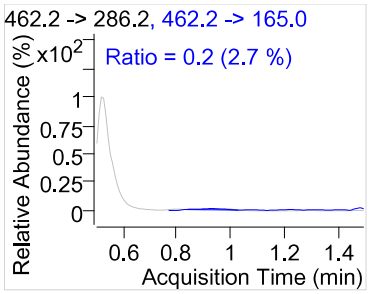




Morphine-6-B-D-Glucuronide





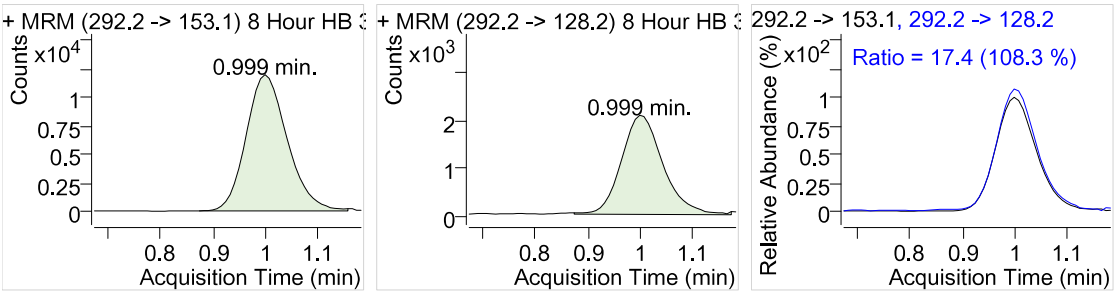


Quantitative Analysis Report

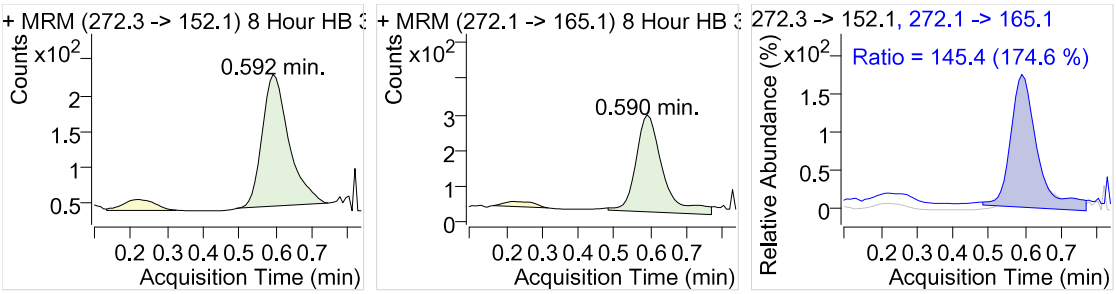
Sample Name: : 8 Hour HB 3
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\07072018\8 Hour HB 3.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/8/2018 4:38:08 PM
Dilution : 2.1
Operator :
Sample Position : P1-E9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	1.00	65803			
	292.2 -> 128.2		11454	17.4	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.59	951			17.5 ng/ml
	272.1 -> 165.1		1383	*145.4	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.59	3490			
	465.2 -> 201.1		205	5.9	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.60	18456			1165.9 ng/ml
	462.2 -> 201.1		1218	6.6	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	1.00	65803			
	292.2 -> 128.2		11454	17.4	12.9 - 19.3	
Morphine	286.1 -> 152.0	1.03	44500			718.9 ng/ml
	286.1 -> 128.1		26516	59.6	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.06	2622			
	465.2 -> 165.1		624	*23.8	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.60	11997			900.8 ng/ml
	462.2 -> 165.0		113	*0.9	0.5 - 0.8	

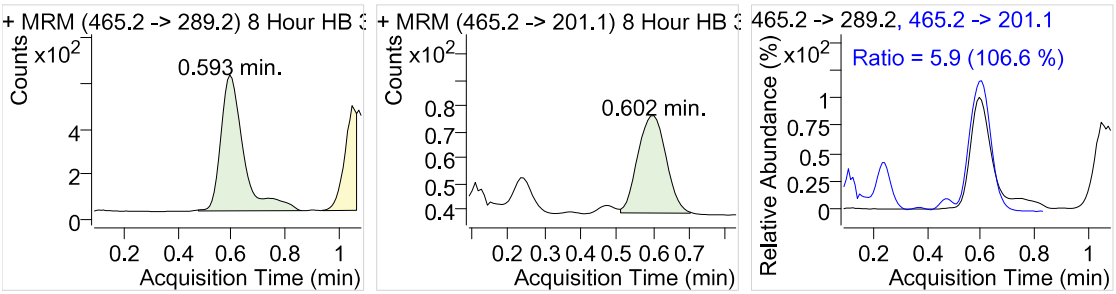
Morphine D6



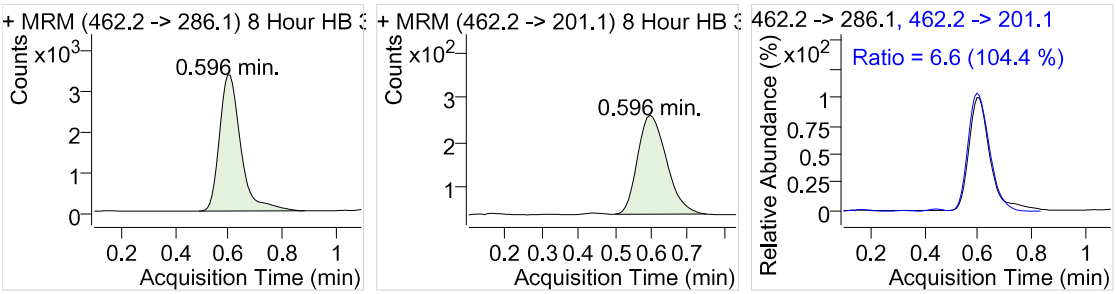
Normorphine



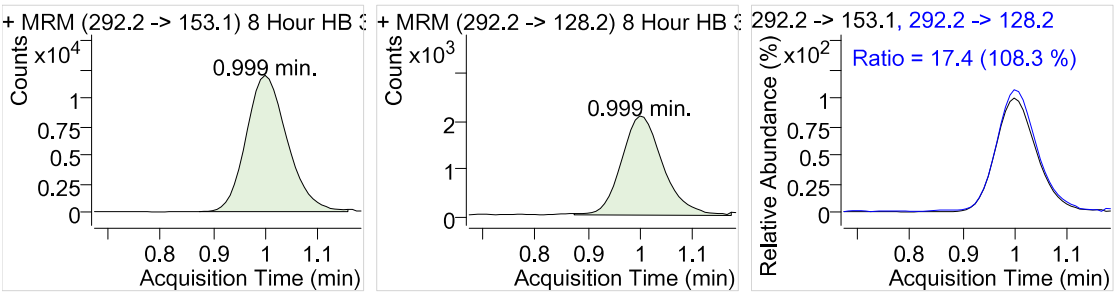
Morphine-3-B-D-Glucuronide D3



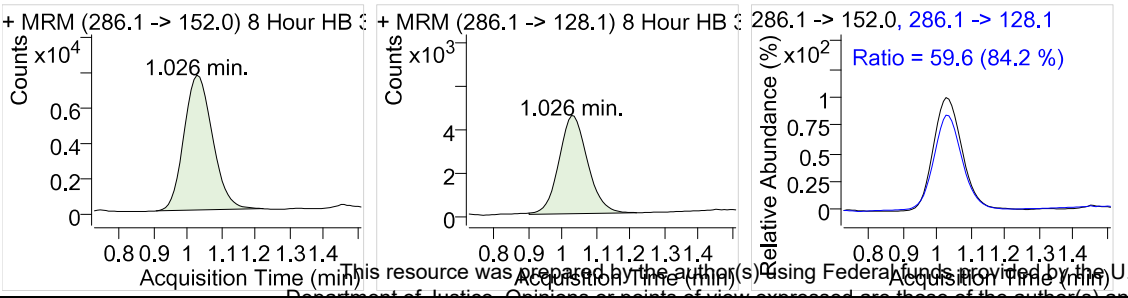
Morphine-3-B-D-Glucuronide



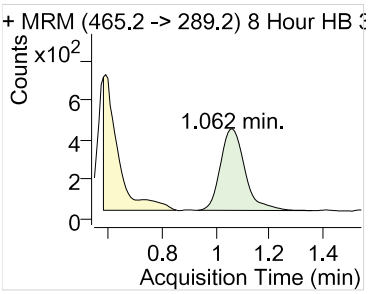
Morphine D6

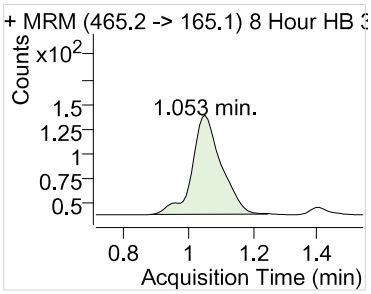


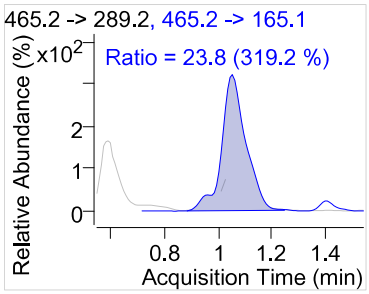
Morphine



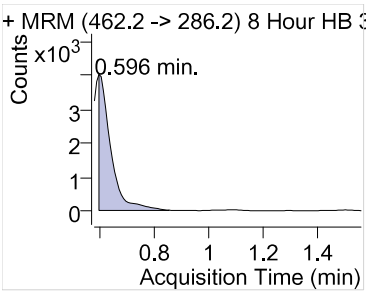
Morphine-6-B-D-Glucuronide D3

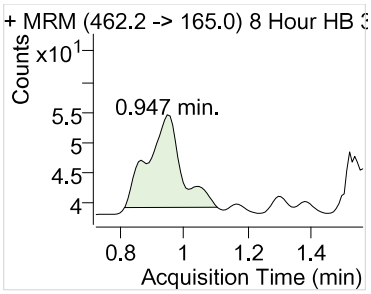


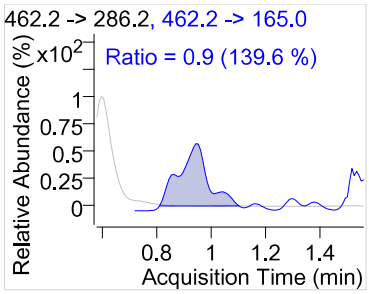




Morphine-6-B-D-Glucuronide







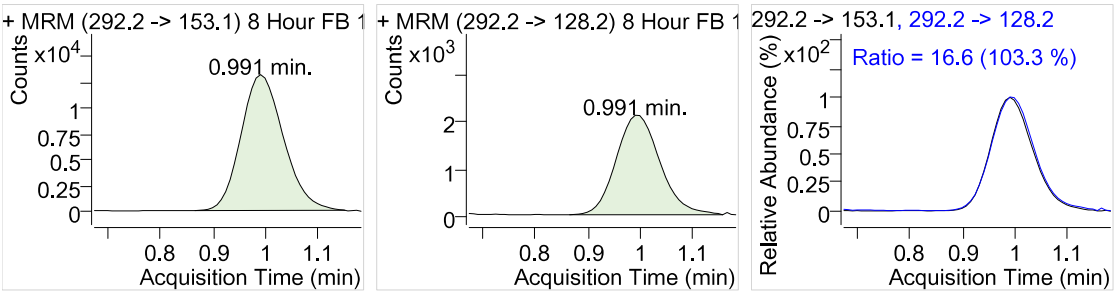
Quantitative Analysis Report

Agilent Technologies

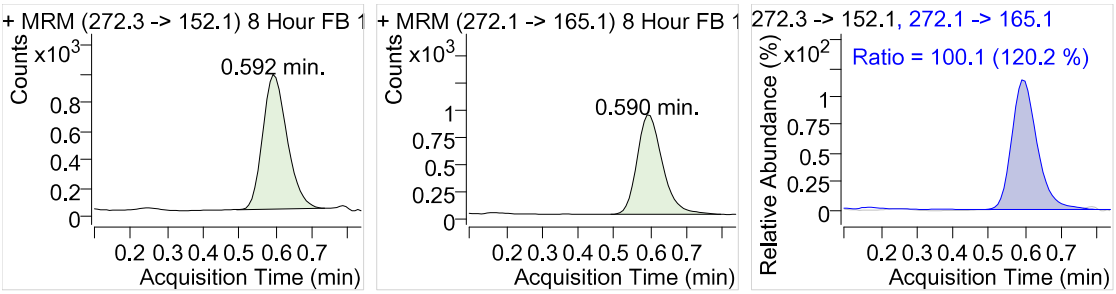
Sample Name:	:	8 Hour FB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07072018\8 Hour FB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 4:56:56 PM
Dilution	:	4.8
Operator	:	
Sample Position	:	P1-F2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.99	73807			
	292.2 -> 128.2		12260	16.6	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.59	4459			42.0 ng/ml
	272.1 -> 165.1		4462	*100.1	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.59	57169			
	465.2 -> 201.1		3374	5.9	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.60	2726			0.0 ng/ml
	462.2 -> 201.1		227	*8.3	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	0.99	73807			
	292.2 -> 128.2		12260	16.6	12.9 - 19.3	
Morphine	286.1 -> 152.0	1.02	24726			809.9 ng/ml
	286.1 -> 128.1		15206	61.5	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.05	25546			
	465.2 -> 165.1		2134	8.4	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.60	1139			0.0 ng/ml
	462.2 -> 165.0				0.5 - 0.8	

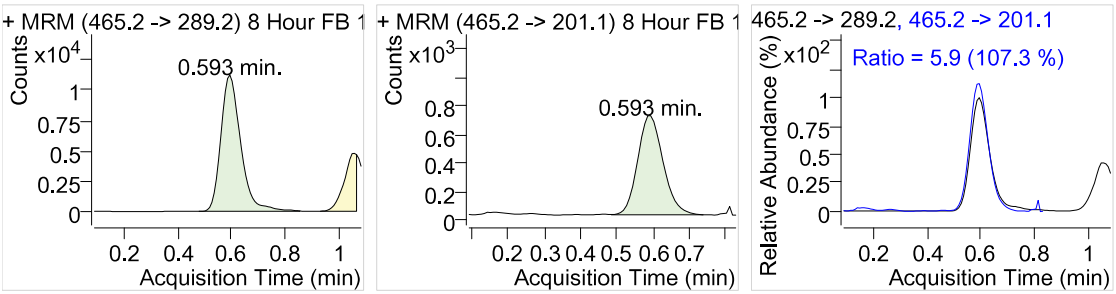
Morphine D6



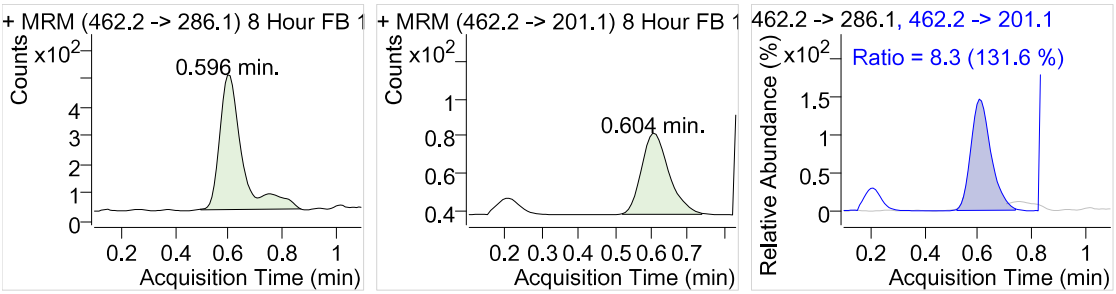
Normorphine



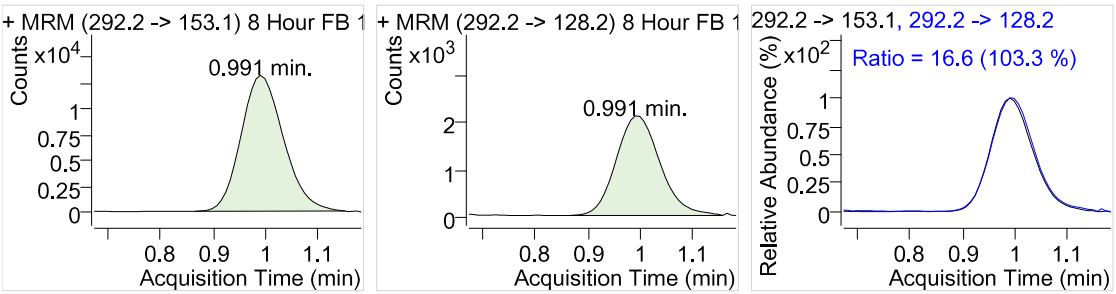
Morphine-3-B-D-Glucuronide D3



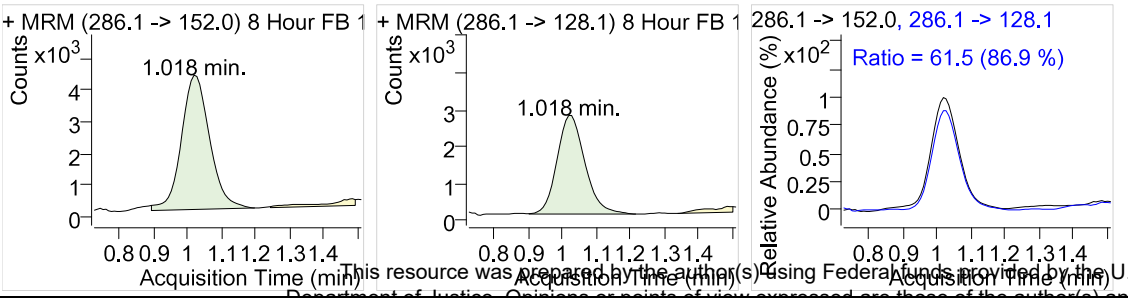
Morphine-3-B-D-Glucuronide



Morphine D6



Morphine



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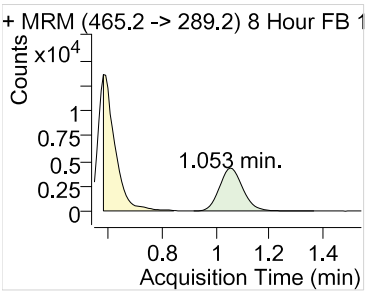
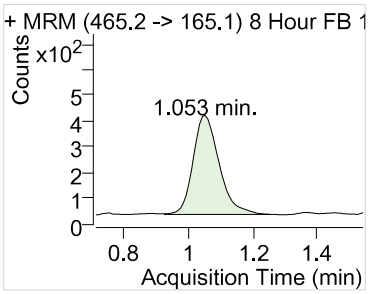
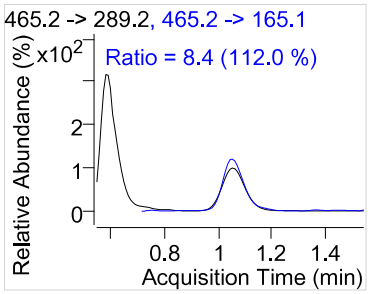
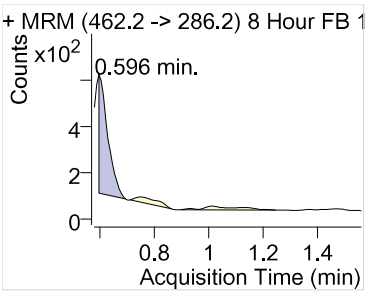
Morphine-6-B-D-Glucuronide D3

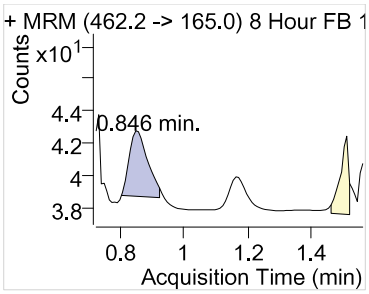


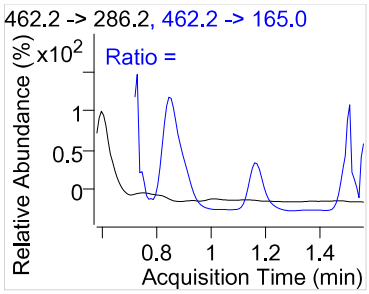




Morphine-6-B-D-Glucuronide







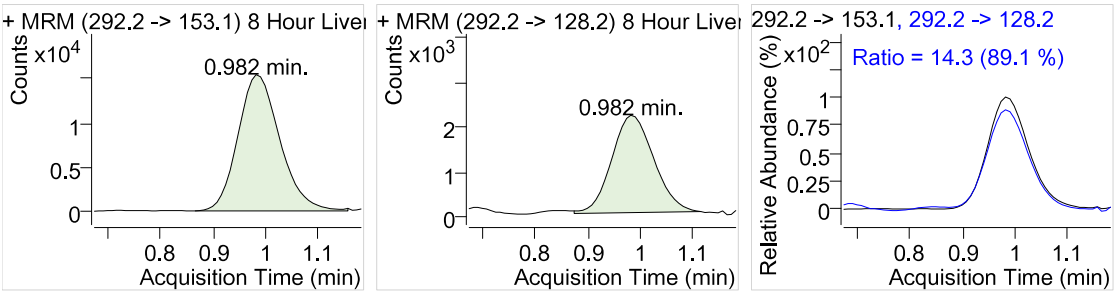
Quantitative Analysis Report

Agilent Technologies

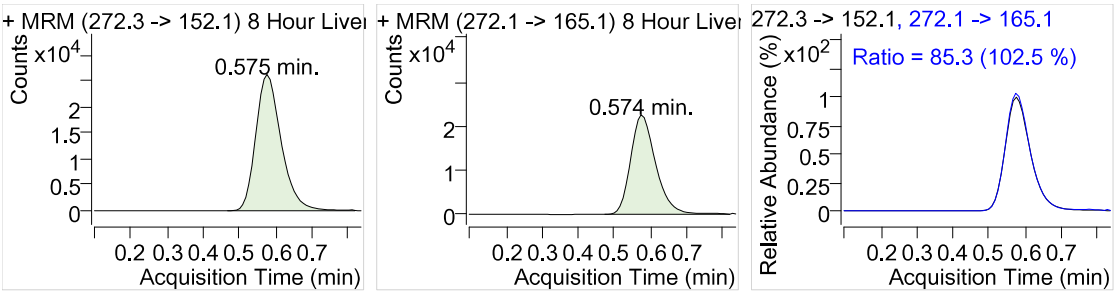
Sample Name:	:	8 Hour Liver 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07072018\8 Hour Liver 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 5:42:42 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-F6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.98	85237			
	292.2 -> 128.2		12214	14.3	12.9 - 19.3	
Normorphine	272.3 -> 152.1	0.58	127897			99.5 ng/ml
	272.1 -> 165.1		109152	85.3	66.6 - 99.9	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.57	56422			
	465.2 -> 201.1		3281	5.8	4.4 - 6.6	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.58	529078			3034.4 ng/ml
	462.2 -> 201.1		31218	5.9	5.1 - 7.6	
Morphine D6	292.2 -> 153.1	0.98	85237			
	292.2 -> 128.2		12214	14.3	12.9 - 19.3	
Morphine	286.1 -> 152.0	1.01	23364			549.8 ng/ml
	286.1 -> 128.1		14910	63.8	56.6 - 84.9	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.05	107268			
	465.2 -> 165.1		8555	8.0	6.0 - 9.0	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	1.07	19696			146.8 ng/ml
	462.2 -> 165.0		1694	*8.6	0.5 - 0.8	

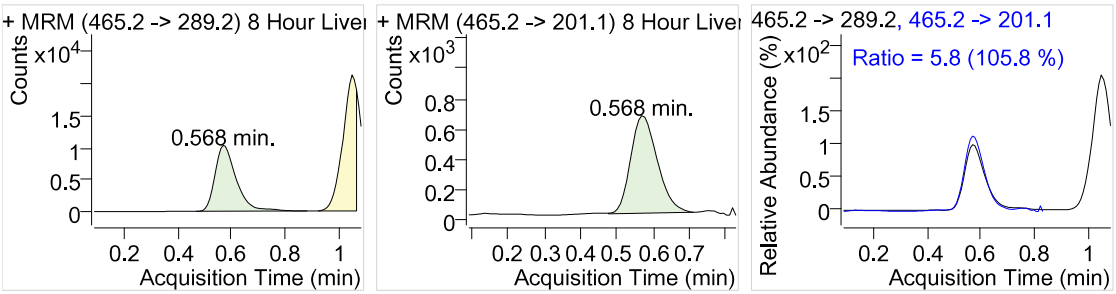
Morphine D6



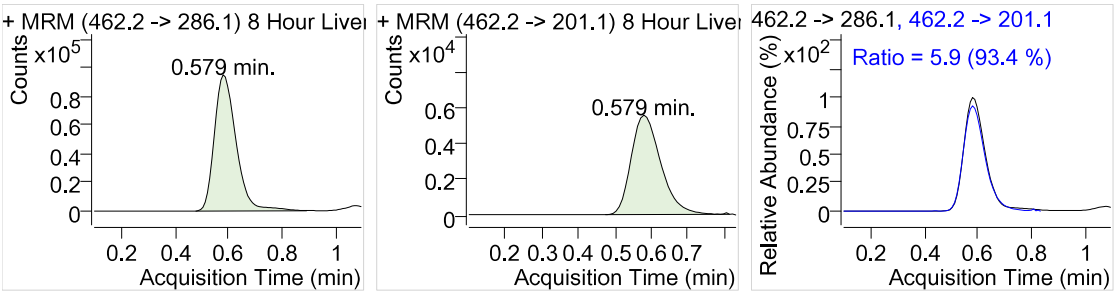
Normorphine



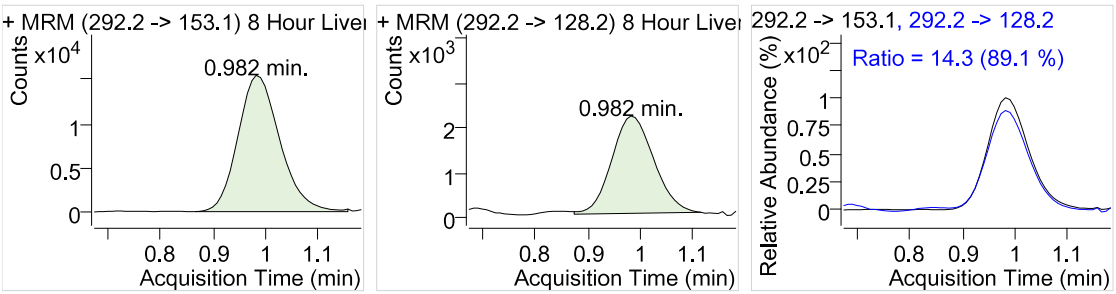
Morphine-3-B-D-Glucuronide D3



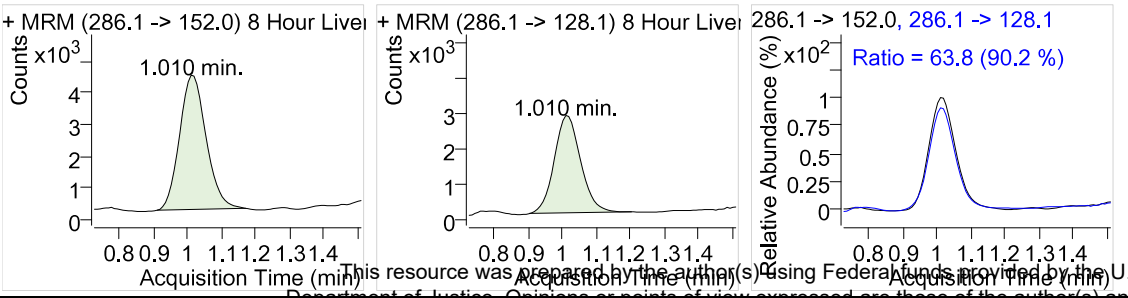
Morphine-3-B-D-Glucuronide



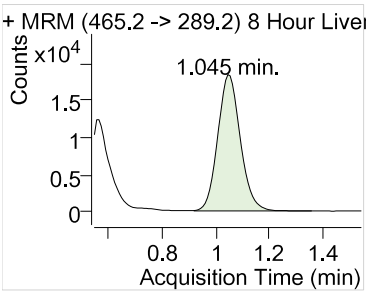
Morphine D6

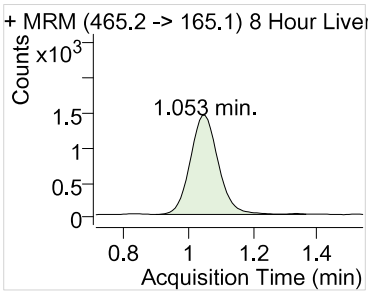


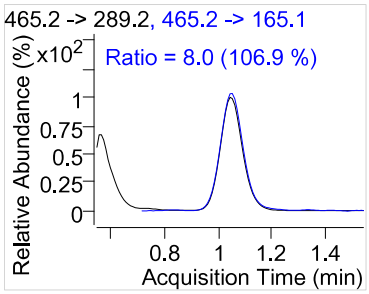
Morphine



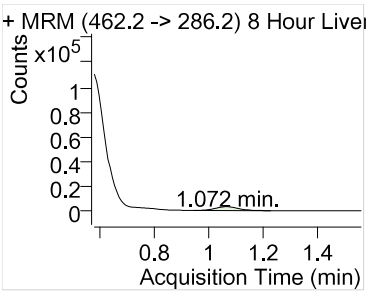
Morphine-6-B-D-Glucuronide D3

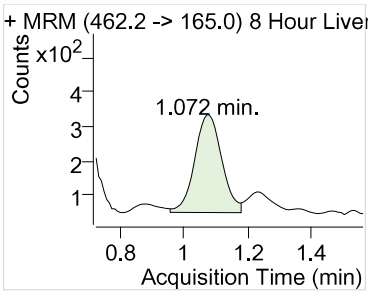


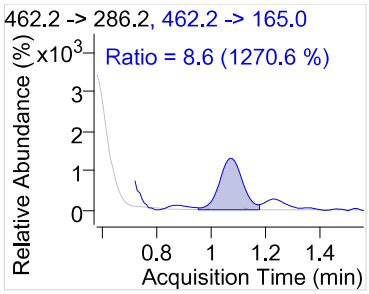




Morphine-6-B-D-Glucuronide







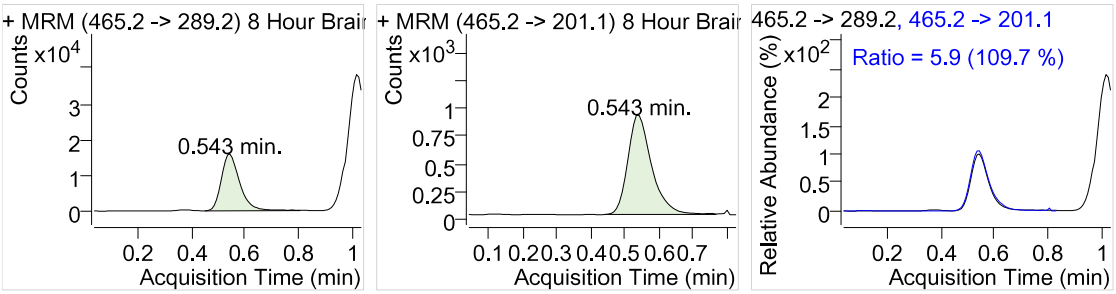
Quantitative Analysis Report

Agilent Technologies

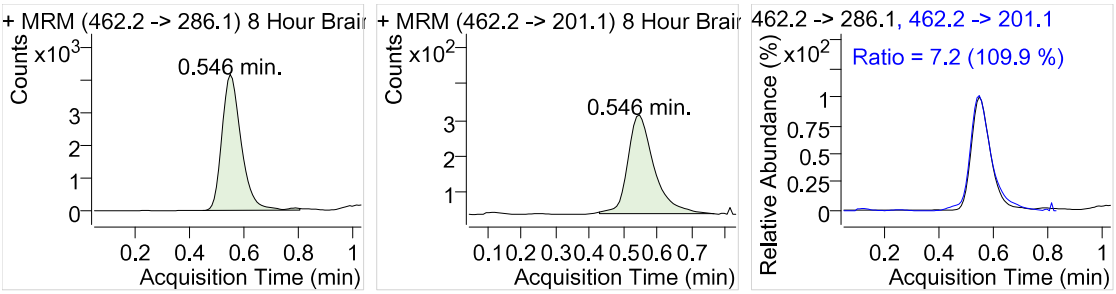
Sample Name:	:	8 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\8 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 10:47:01 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	75197			
	465.2 -> 201.1		4403	5.9	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	20588			172.8 ng/ml
	462.2 -> 201.1		1476	7.2	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.96	97610			
	292.2 -> 128.2		13951	14.3	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.56	7766			41.9 ng/ml
	272.1 -> 165.1		4710	*60.6	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.96	97610			
	292.2 -> 128.2		13951	14.3	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.98	28743			597.9 ng/ml
	286.1 -> 128.1		19224	*66.9	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.01	187406			
	465.2 -> 165.1		14330	7.6	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	1.02	943			34.3 ng/ml
	462.2 -> 165.0		104	*11.0	7.1 - 10.6	

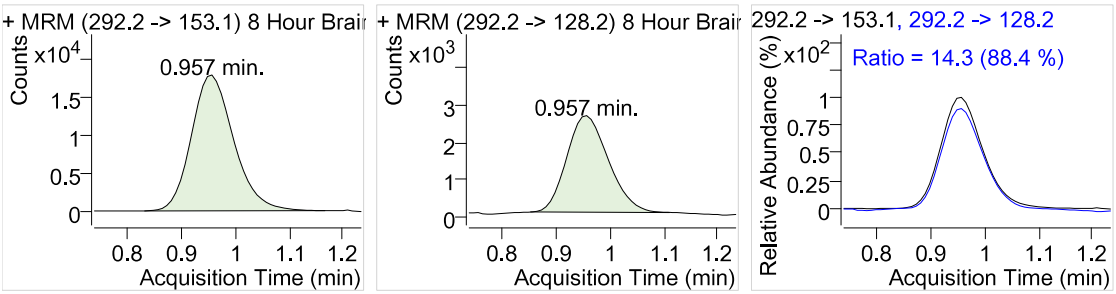
Morphine-3-B-D-Glucuronide D3



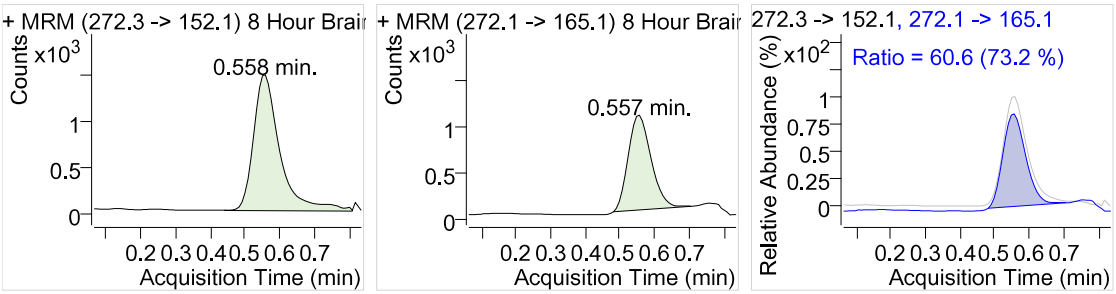
Morphine-3-B-D-Glucuronide



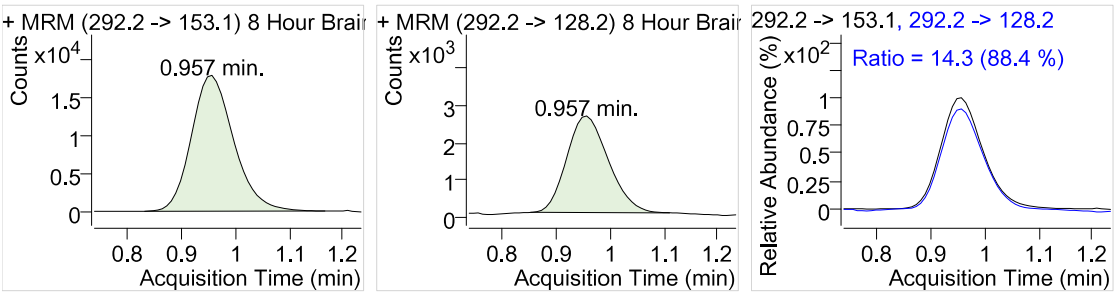
Morphine D6



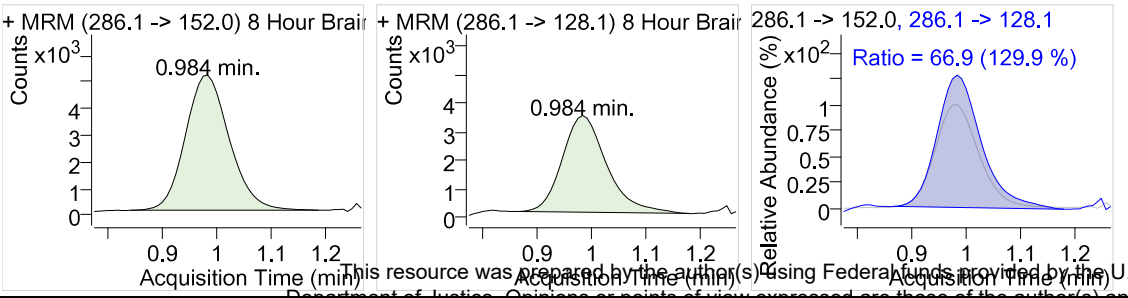
Normorphine



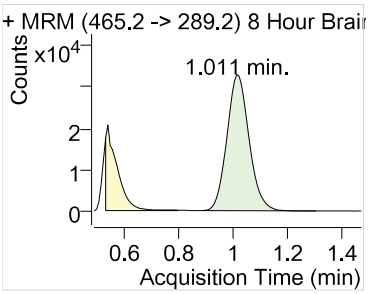
Morphine D6

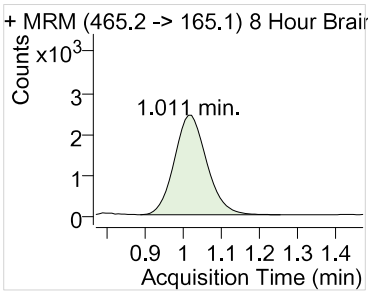


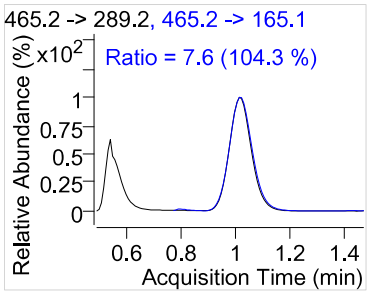
Morphine



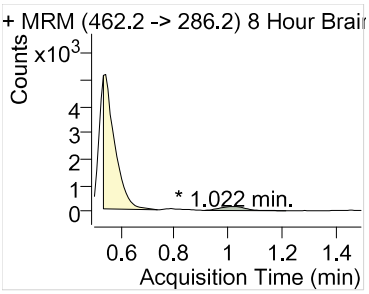
Morphine-6-B-D-Glucuronide D3

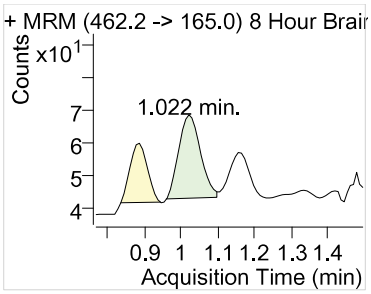


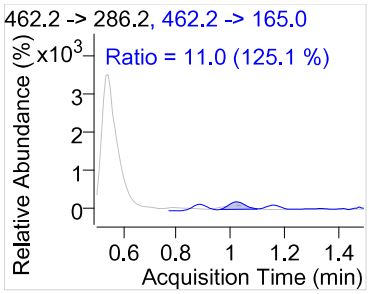




Morphine-6-B-D-Glucuronide







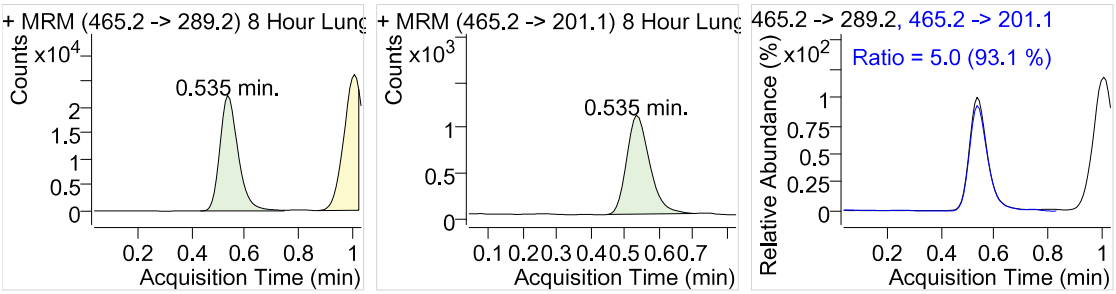
Quantitative Analysis Report

Agilent Technologies

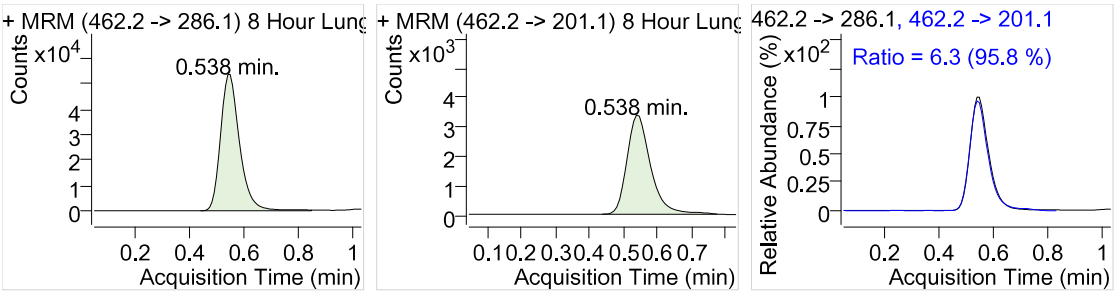
Sample Name:	:	8 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\8 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 10:56:33 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	103823			
	465.2 -> 201.1		5161	5.0	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	260225			1315.9 ng/ml
	462.2 -> 201.1		16265	6.3	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.94	63165			
	292.2 -> 128.2		9320	14.8	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.56	100082			104.8 ng/ml
	272.1 -> 165.1		79734	79.7	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.94	63165			
	292.2 -> 128.2		9320	14.8	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.98	52393			1684.1 ng/ml
	286.1 -> 128.1		33654	*64.2	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	134644			
	465.2 -> 165.1		11562	8.6	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	166942			380.7 ng/ml
	462.2 -> 165.0		29	*0.0	7.1 - 10.6	

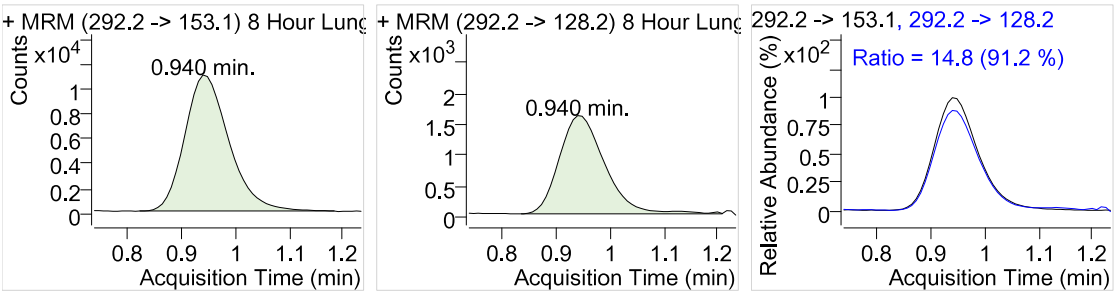
Morphine-3-B-D-Glucuronide D3



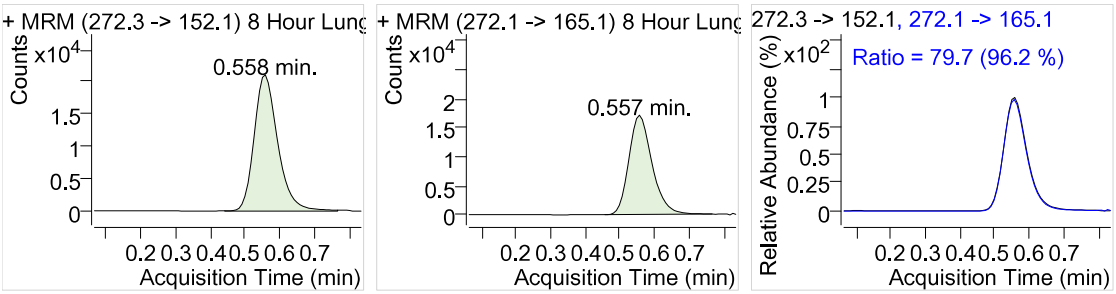
Morphine-3-B-D-Glucuronide



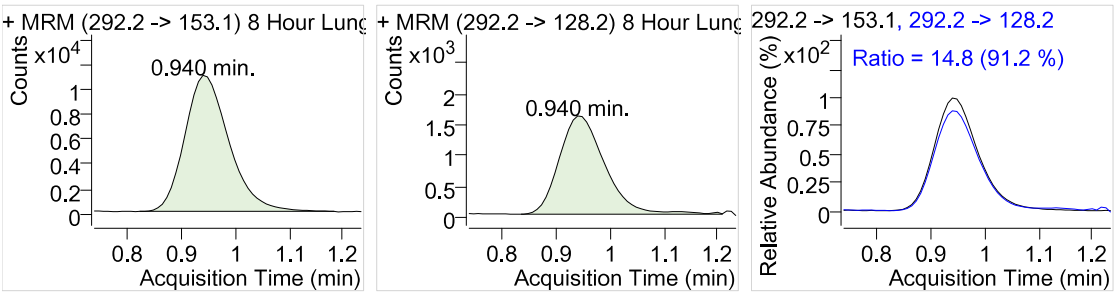
Morphine D6



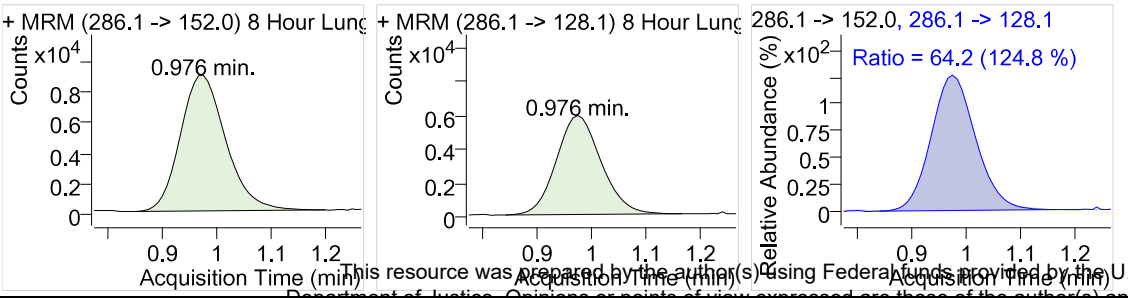
Normorphine



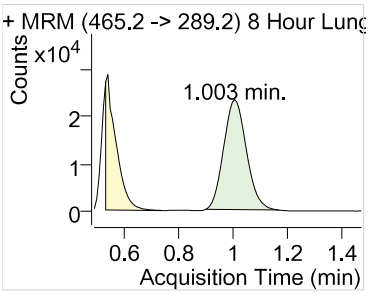
Morphine D6

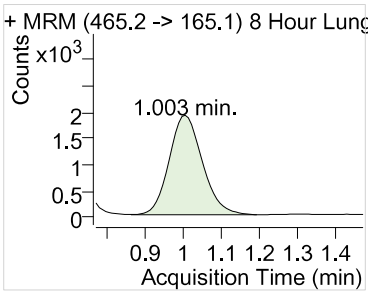


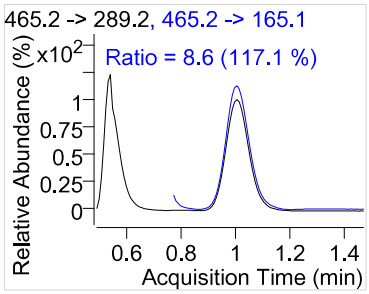
Morphine



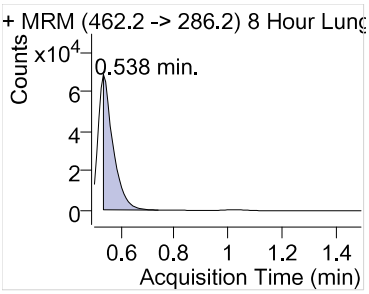
Morphine-6-B-D-Glucuronide D3

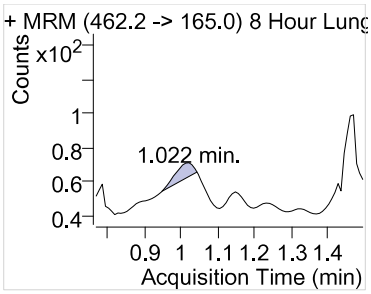


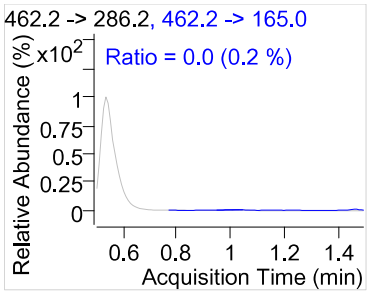




Morphine-6-B-D-Glucuronide







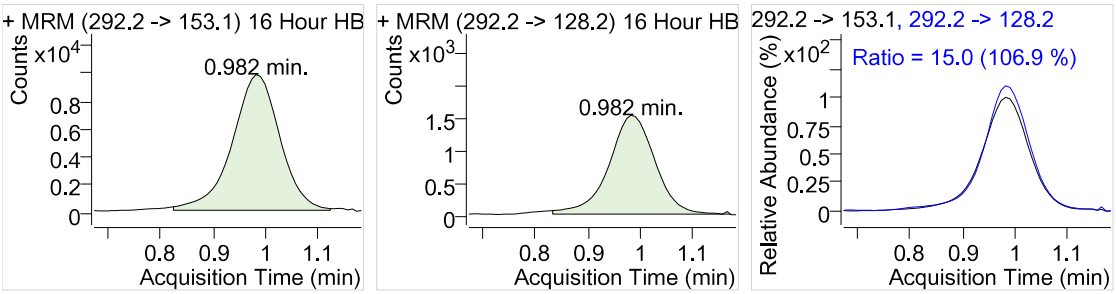
Quantitative Analysis Report

Agilent Technologies

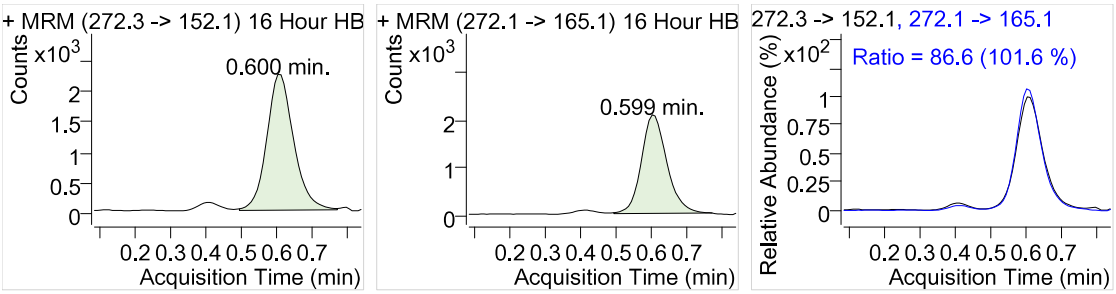
Sample Name:	:	16 Hour HB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\16 Hour HB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/14/2018 5:22:41 PM
Dilution	:	5.1
Operator	:	
Sample Position	:	P1-B9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.98	61961			
	292.2 -> 128.2		9307	15.0	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.60	11856			59.5 ng/ml
	272.1 -> 165.1		10266	86.6	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	*0.61	6361			
	465.2 -> 201.1		575	9.0	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.61	22065			841.4 ng/ml
	462.2 -> 201.1		1352	6.1	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.98	61961			
	292.2 -> 128.2		9307	15.0	11.2 - 16.9	
Morphine	286.1 -> 152.0	1.01	20530			1012.4 ng/ml
	286.1 -> 128.1		13017	63.4	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.05	4724			
	465.2 -> 165.1		727	15.4	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.61	14311			1106.1 ng/ml
	462.2 -> 165.0		13	*0.1	11.0 - 16.5	

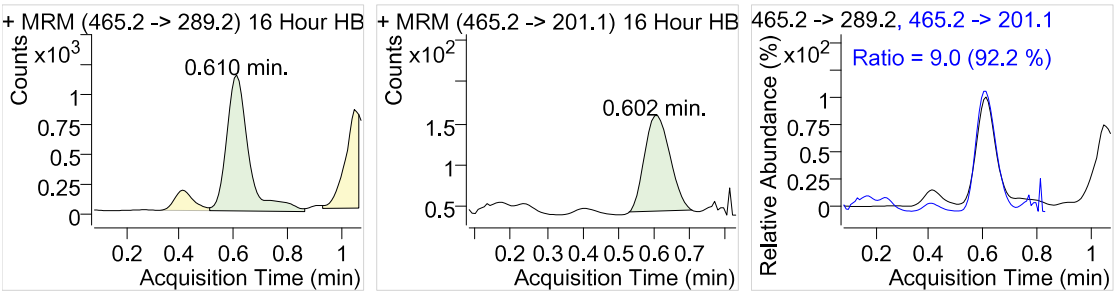
Morphine D6



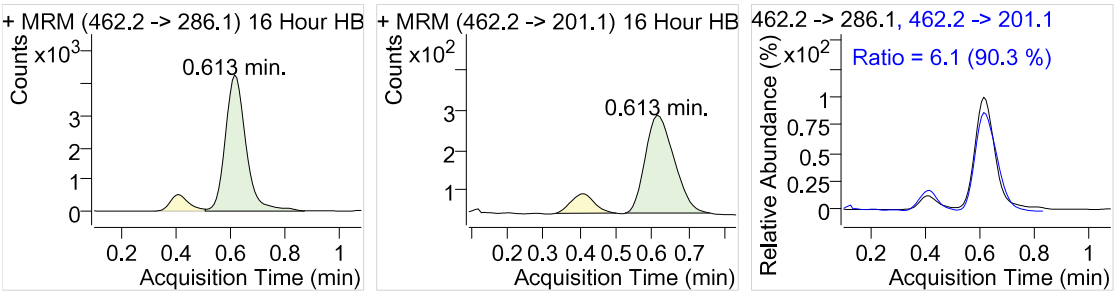
Normorphine



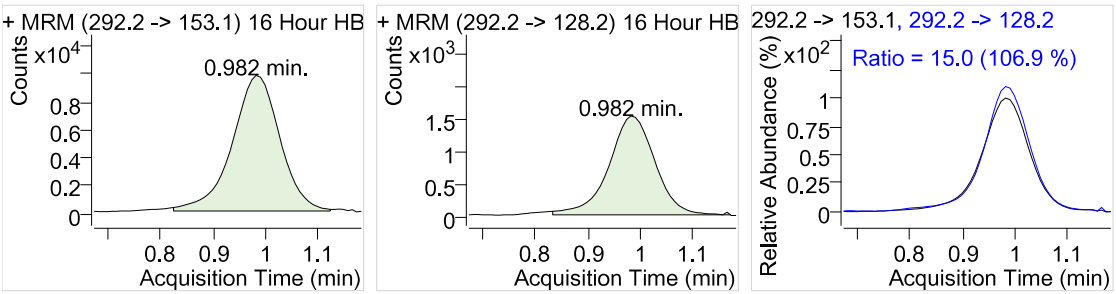
Morphine-3-B-D-Glucuronide D3



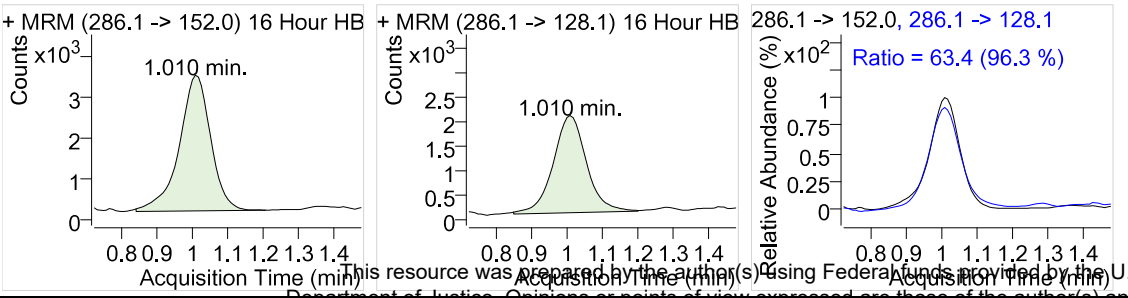
Morphine-3-B-D-Glucuronide



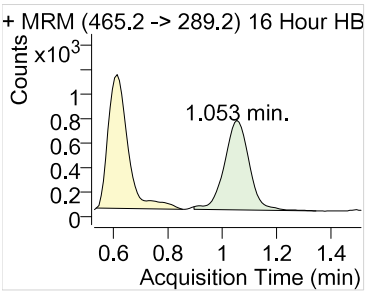
Morphine D6

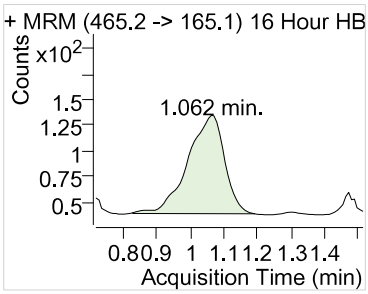


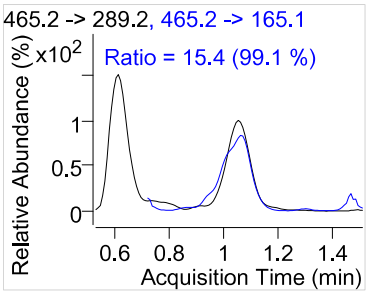
Morphine



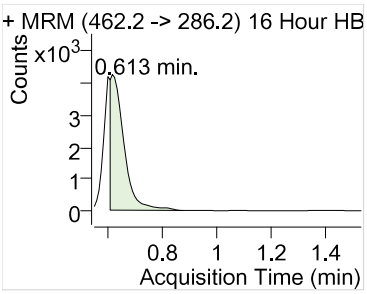
Morphine-6-B-D-Glucuronide D3

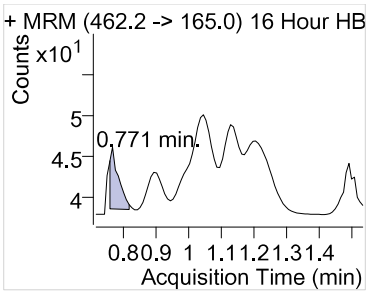


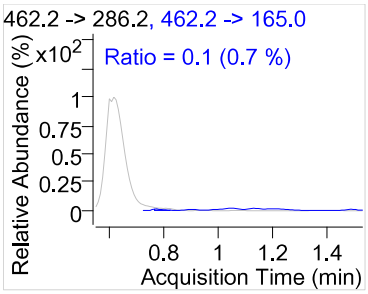




Morphine-6-B-D-Glucuronide







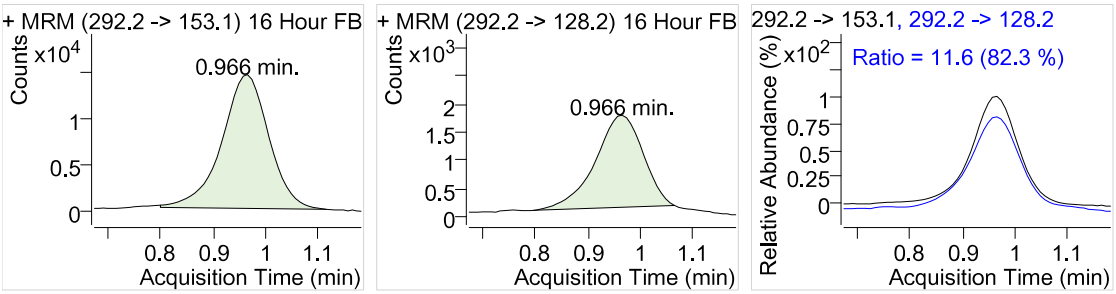
Quantitative Analysis Report

Agilent Technologies

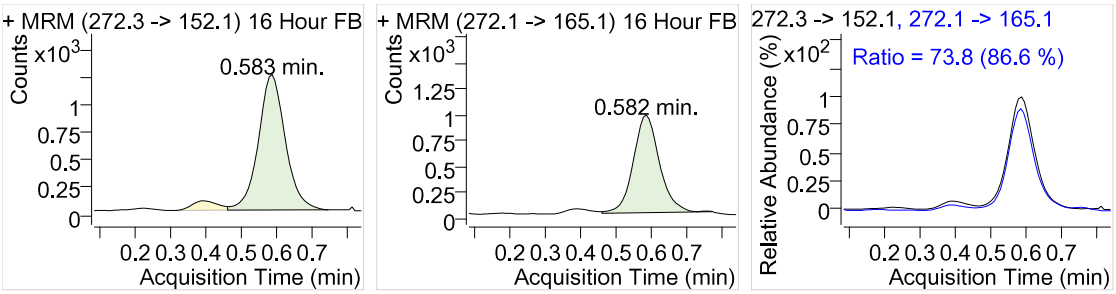
Sample Name:	:	16 Hour FB 2
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\16 Hour FB 2.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/14/2018 6:01:53 PM
Dilution	:	6.3
Operator	:	
Sample Position	:	P1-C5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.97	89850			
	292.2 -> 128.2		10389	11.6	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.58	6538			62.4 ng/ml
	272.1 -> 165.1		4827	73.8	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.58	21646			
	465.2 -> 201.1		2344	10.8	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.59	12314			211.8 ng/ml
	462.2 -> 201.1		741	6.0	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.97	89850			
	292.2 -> 128.2		10389	11.6	11.2 - 16.9	
Morphine	286.1 -> 152.0	0.99	26349			1114.0 ng/ml
	286.1 -> 128.1		14875	56.5	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.03	11157			
	465.2 -> 165.1		1755	15.7	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.58	8219			469.5 ng/ml
	462.2 -> 165.0		26	*0.3	11.0 - 16.5	

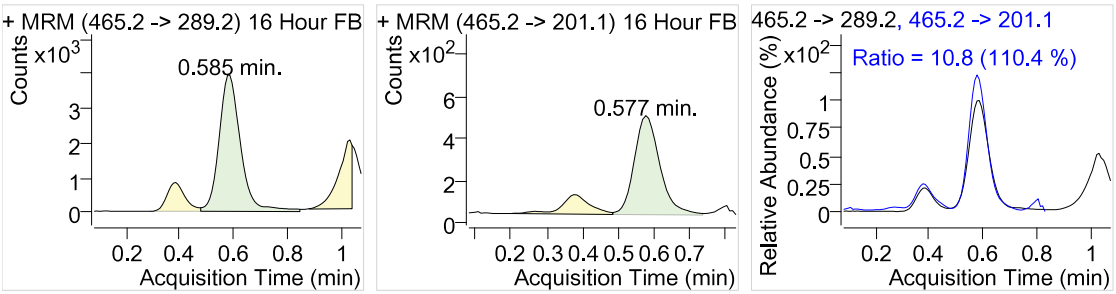
Morphine D6



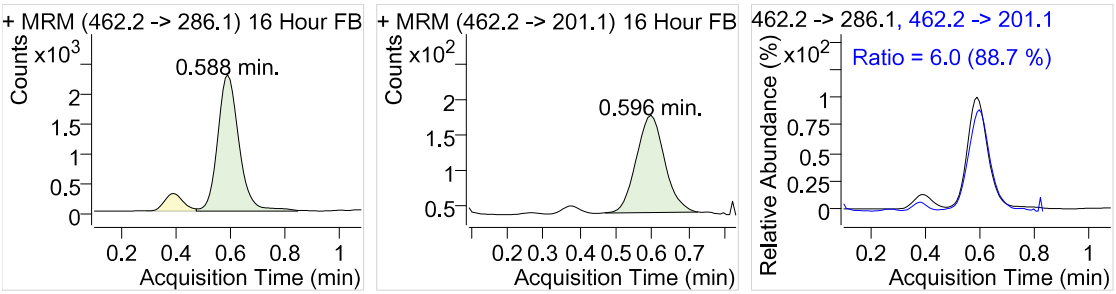
Normorphine



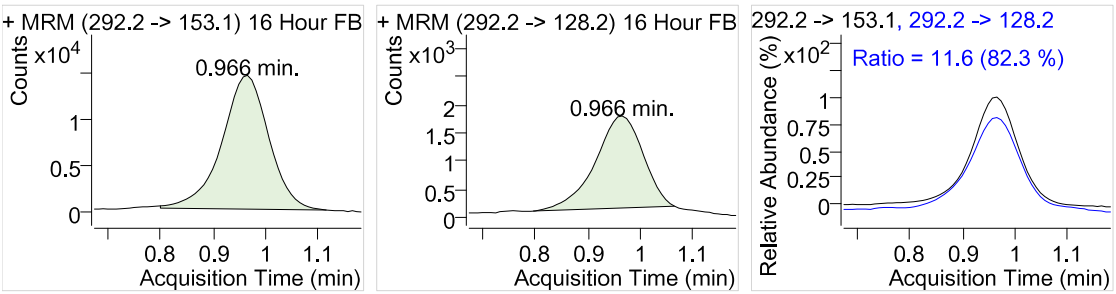
Morphine-3-B-D-Glucuronide D3



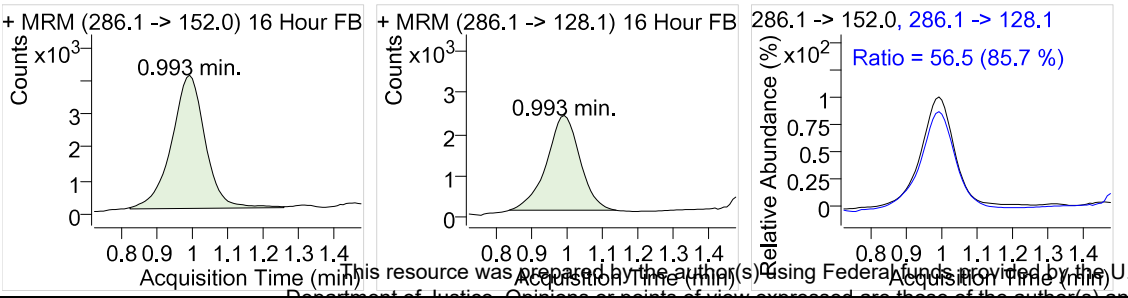
Morphine-3-B-D-Glucuronide



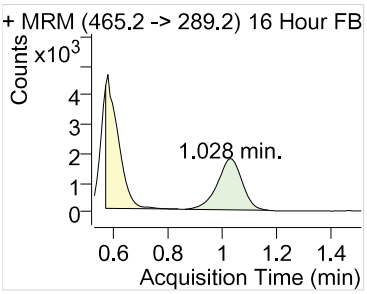
Morphine D6

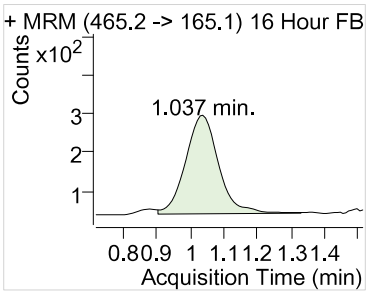


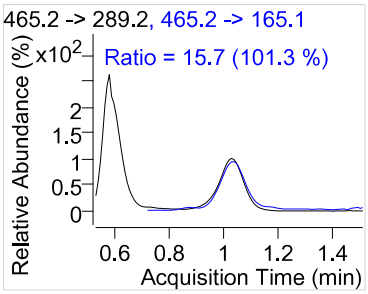
Morphine



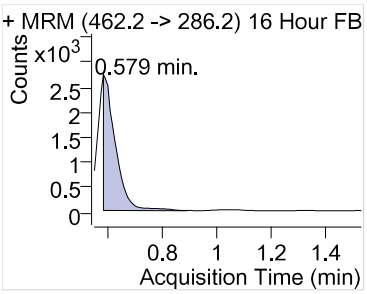
Morphine-6-B-D-Glucuronide D3

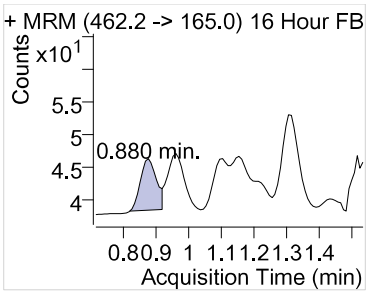


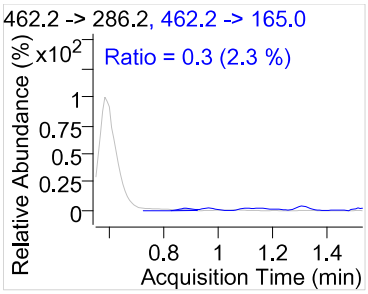




Morphine-6-B-D-Glucuronide







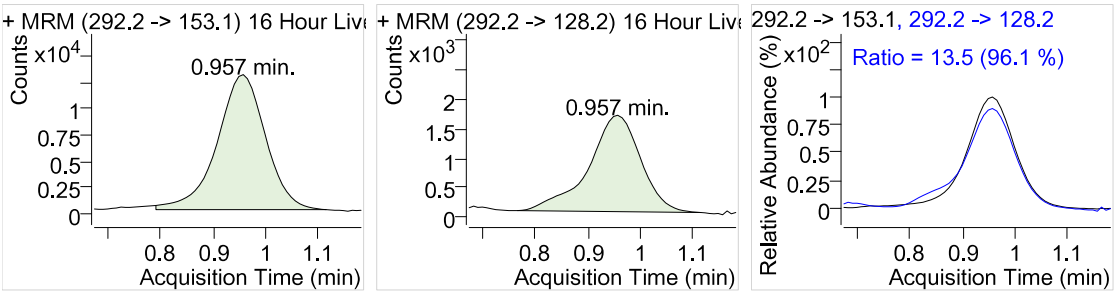
Quantitative Analysis Report

Agilent Technologies

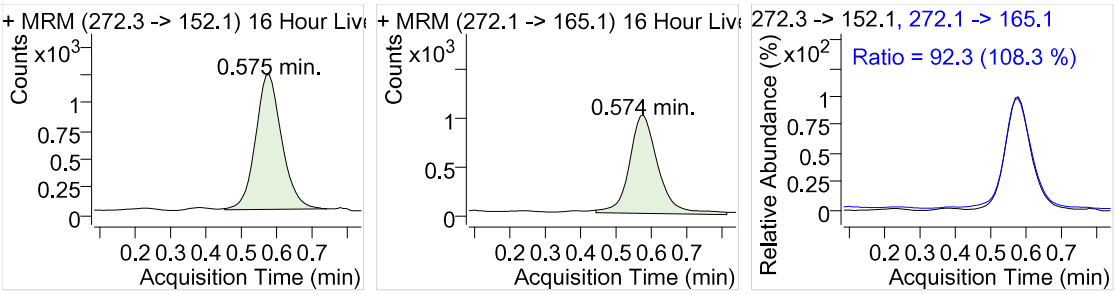
Sample Name:	:	16 Hour Liver 3
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\16 Hour Liver 3.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/14/2018 6:39:42 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.96	84183			
	292.2 -> 128.2		11366	13.5	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.58	6228			40.0 ng/ml
	272.1 -> 165.1		5750	92.3	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.57	19186			
	465.2 -> 201.1		1822	9.5	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.58	365685			2526.5 ng/ml
	462.2 -> 201.1		20782	5.7	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.96	84183			
	292.2 -> 128.2		11366	13.5	11.2 - 16.9	
Morphine	286.1 -> 152.0	0.98	36049			1023.6 ng/ml
	286.1 -> 128.1		20875	57.9	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.02	46368			
	465.2 -> 165.1		5484	*11.8	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.58	244041			1232.1 ng/ml
	462.2 -> 165.0		255	*0.1	11.0 - 16.5	

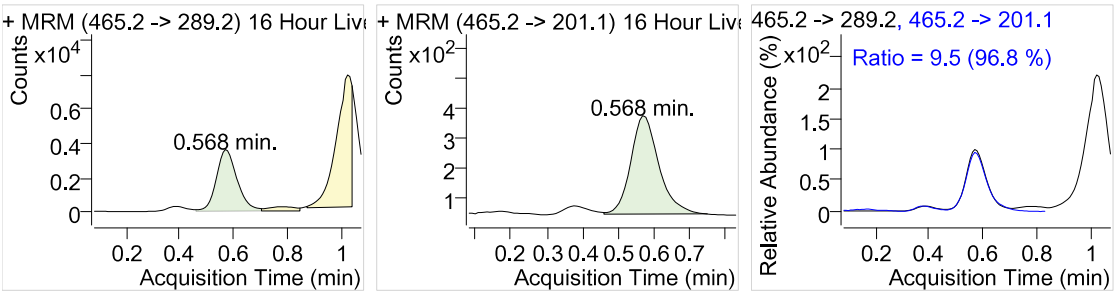
Morphine D6



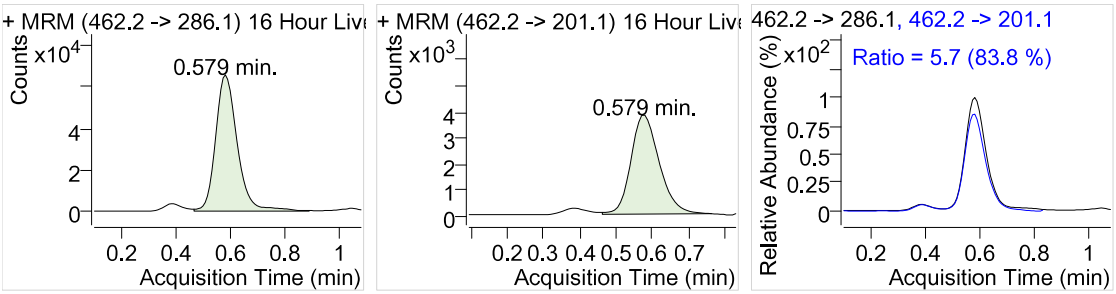
Normorphine



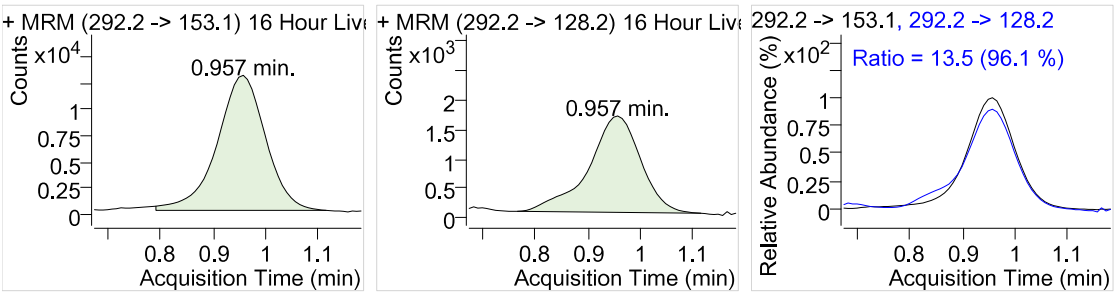
Morphine-3-B-D-Glucuronide D3



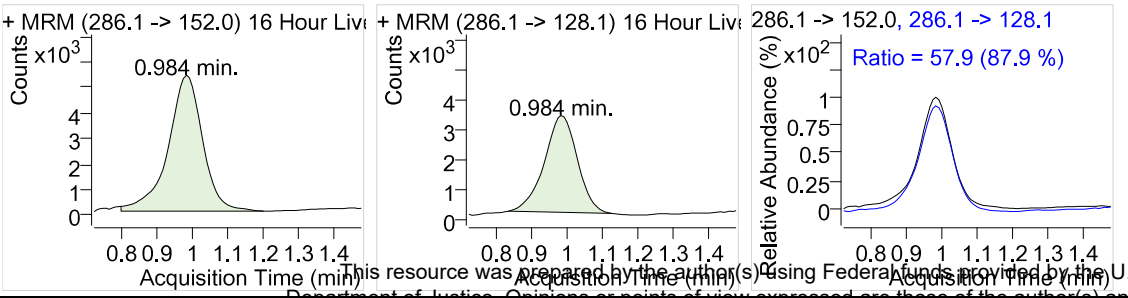
Morphine-3-B-D-Glucuronide



Morphine D6



Morphine



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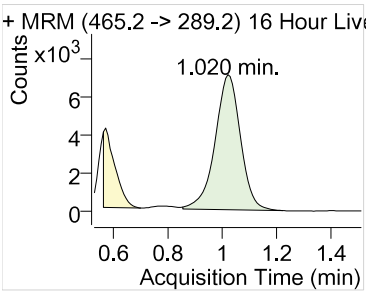
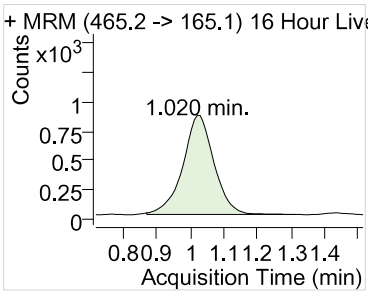
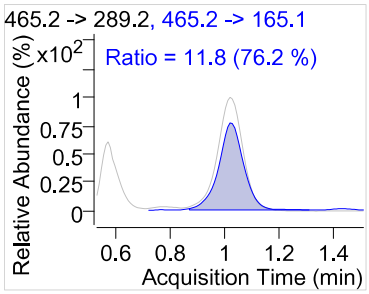
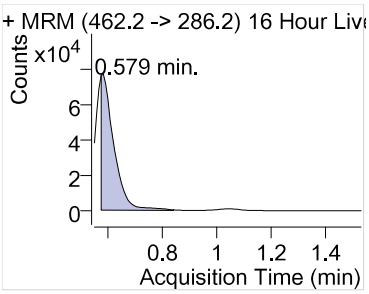
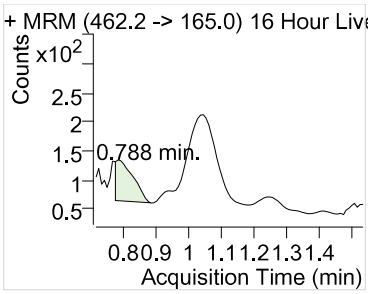


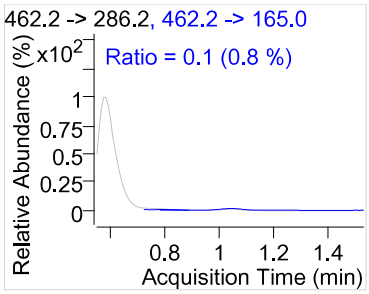




Morphine-6-B-D-Glucuronide







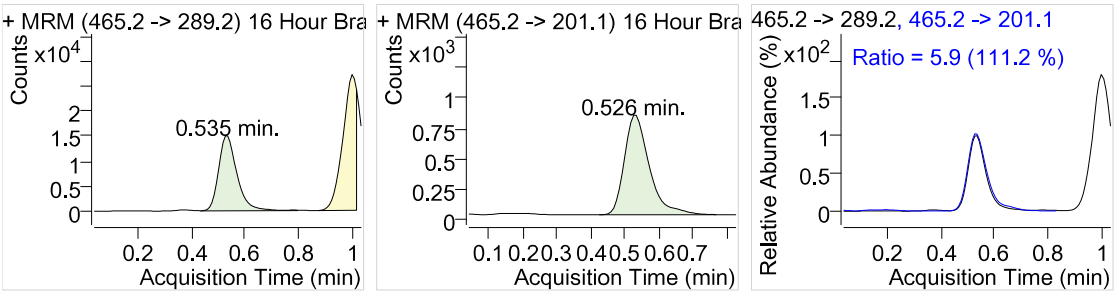
Quantitative Analysis Report

Agilent Technologies

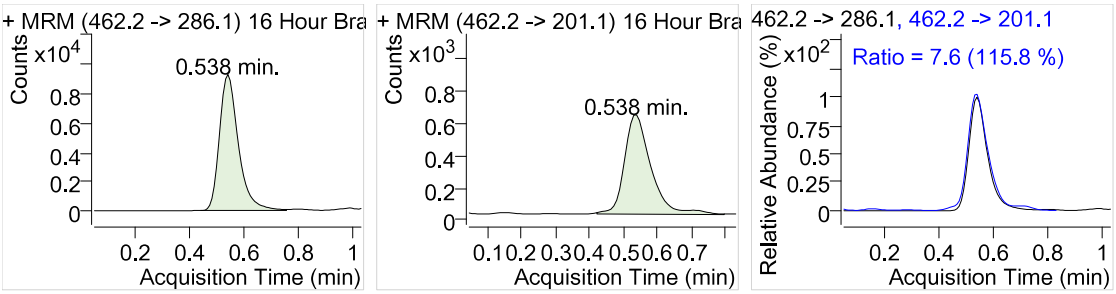
Sample Name:	:	16 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\16 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 1:19:36 AM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-E2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	70663			
	465.2 -> 201.1		4194	5.9	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	44744			359.4 ng/ml
	462.2 -> 201.1		3379	7.6	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.94	80488			
	292.2 -> 128.2		12962	16.1	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.55	4872			41.1 ng/ml
	272.1 -> 165.1		3334	68.4	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.94	80488			
	292.2 -> 128.2		12962	16.1	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.97	22240			560.6 ng/ml
	286.1 -> 128.1		13582	61.1	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	130655			
	465.2 -> 165.1		10113	7.7	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.53	31264			101.7 ng/ml
	462.2 -> 165.0		40	*0.1	7.1 - 10.6	

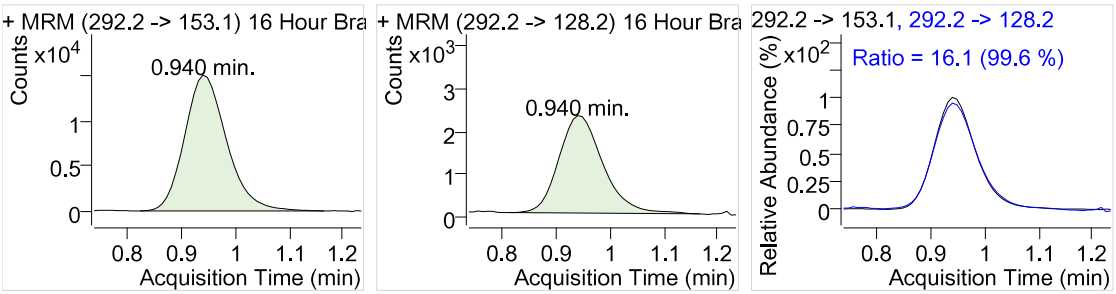
Morphine-3-B-D-Glucuronide D3



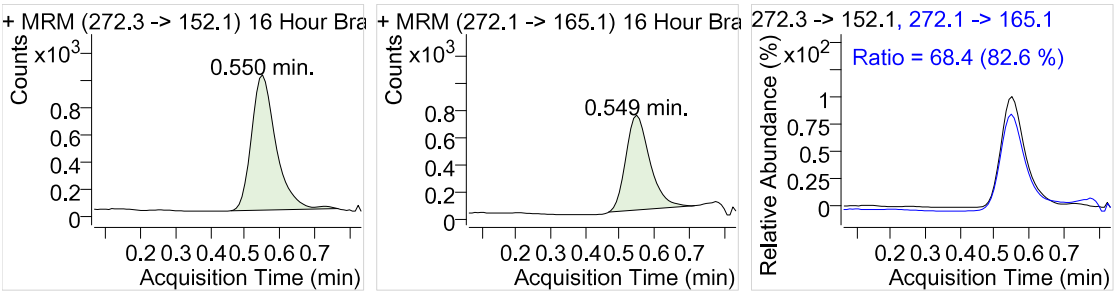
Morphine-3-B-D-Glucuronide



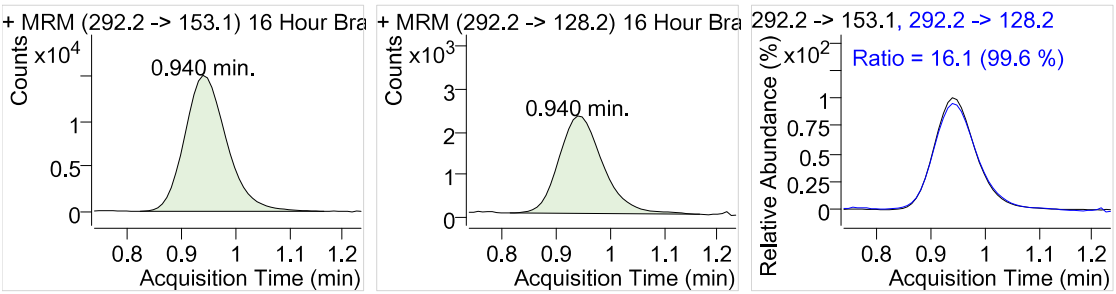
Morphine D6



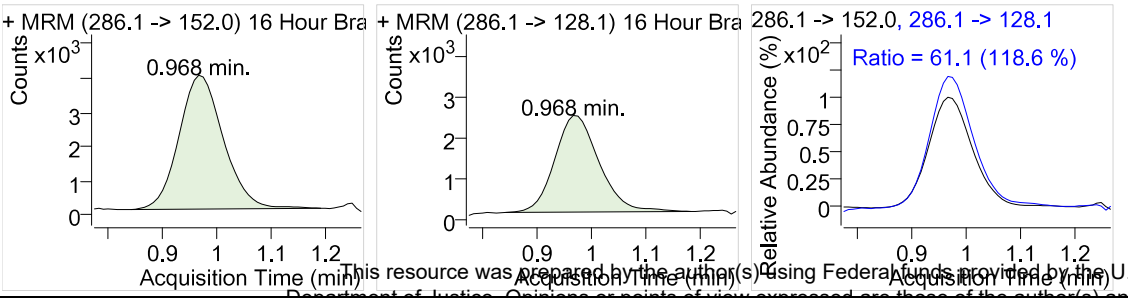
Normorphine



Morphine D6



Morphine



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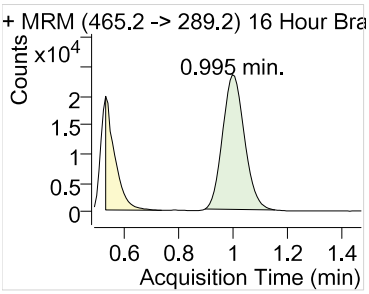
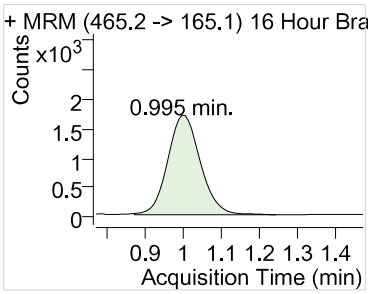
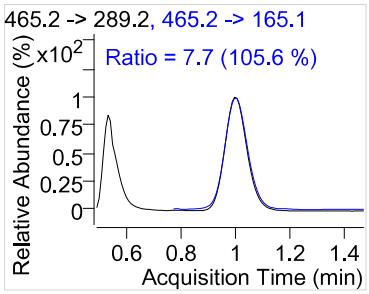
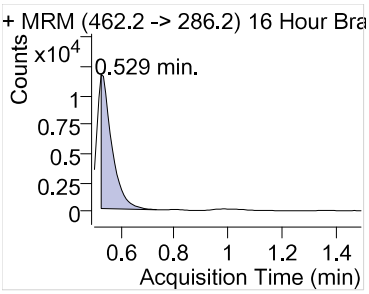
Morphine-6-B-D-Glucuronide D3

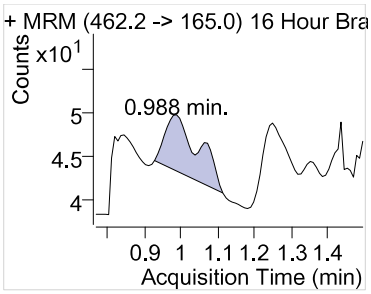


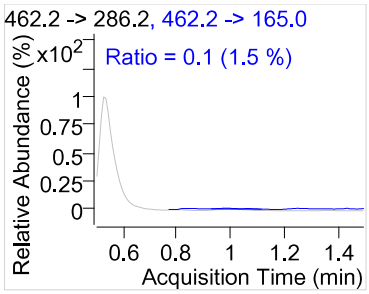




Morphine-6-B-D-Glucuronide







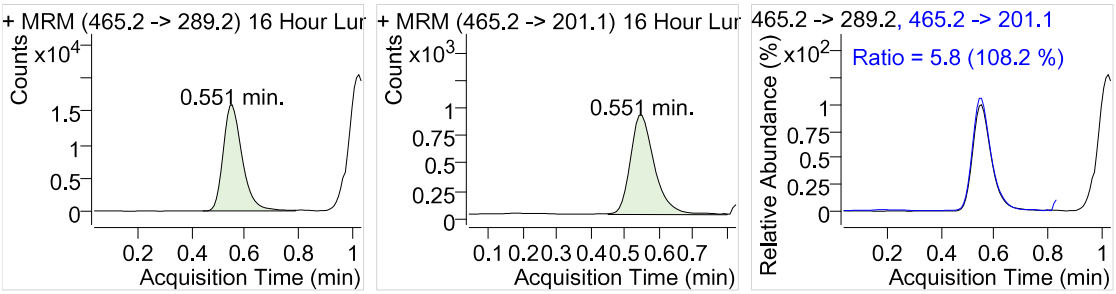
Quantitative Analysis Report

Agilent Technologies

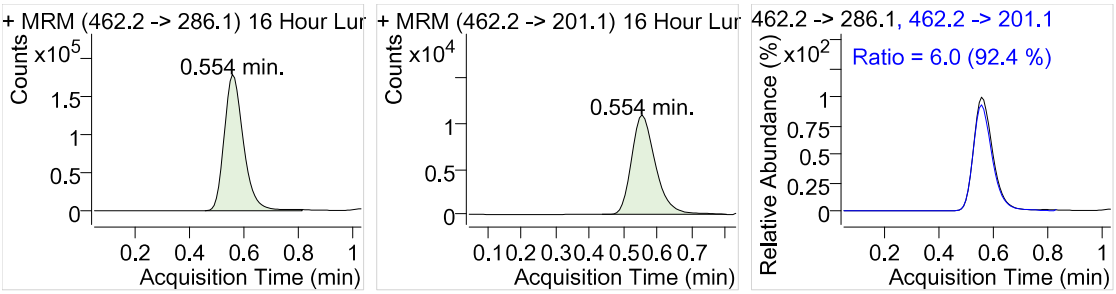
Sample Name:	:	16 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\16 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 1:29:07 AM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-E3

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	77861			
	465.2 -> 201.1		4499	5.8	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	884768			5523.3 ng/ml
	462.2 -> 201.1		53339	6.0	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.96	61645			
	292.2 -> 128.2		10359	16.8	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.57	73014			88.0 ng/ml
	272.1 -> 165.1		58617	80.3	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.96	61645			
	292.2 -> 128.2		10359	16.8	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.99	38994			1287.0 ng/ml
	286.1 -> 128.1		22397	57.4	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.02	106837			
	465.2 -> 165.1		8631	8.1	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	608098			1486.1 ng/ml
	462.2 -> 165.0		105	*0.0	7.1 - 10.6	

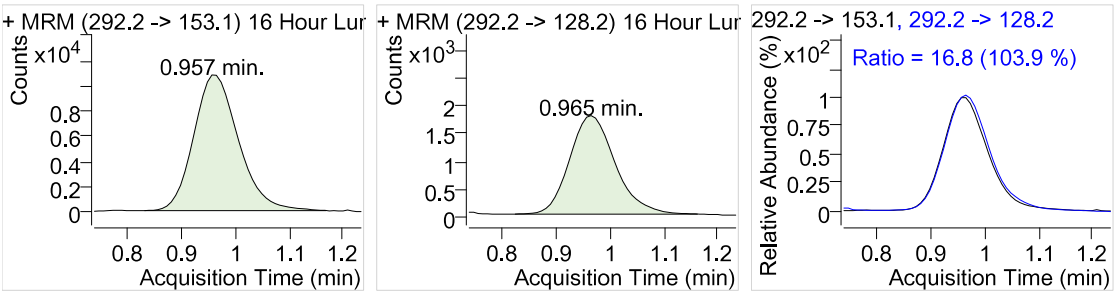
Morphine-3-B-D-Glucuronide D3



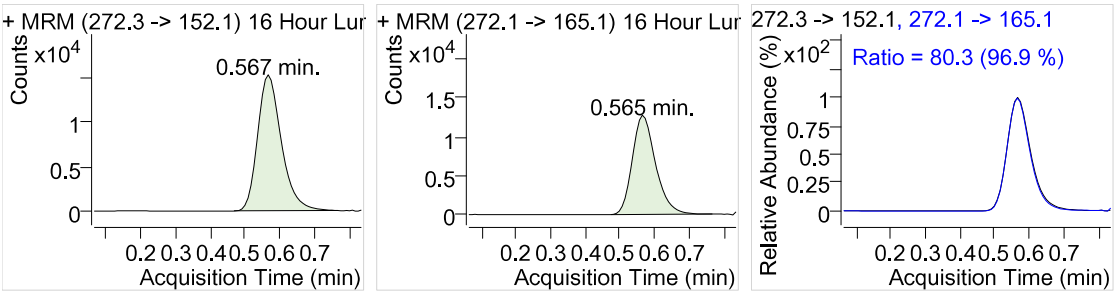
Morphine-3-B-D-Glucuronide



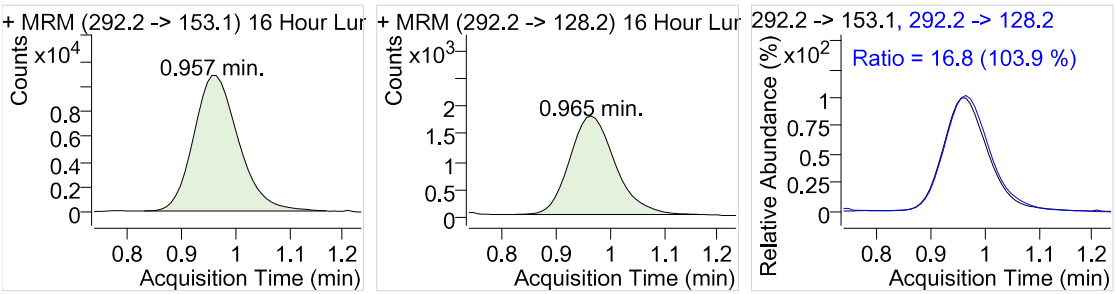
Morphine D6



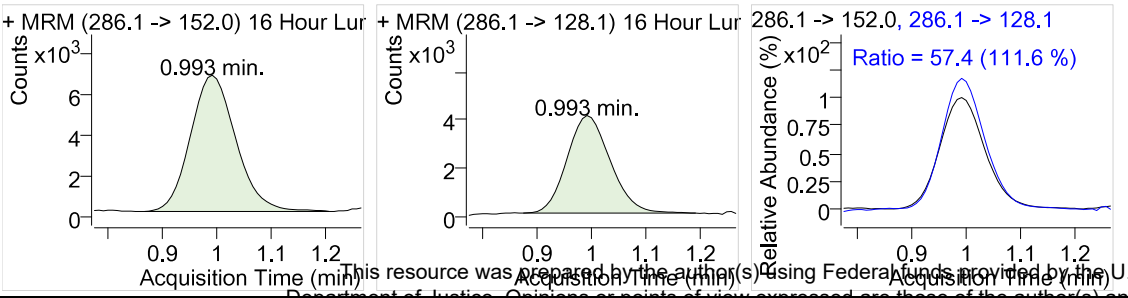
Normorphine



Morphine D6



Morphine



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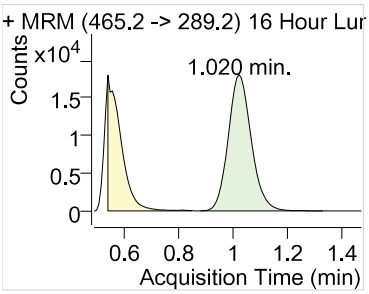
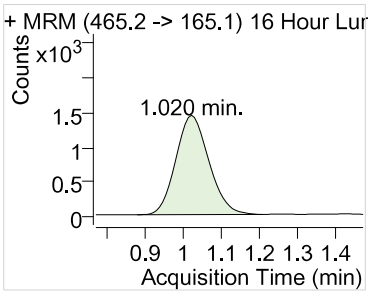
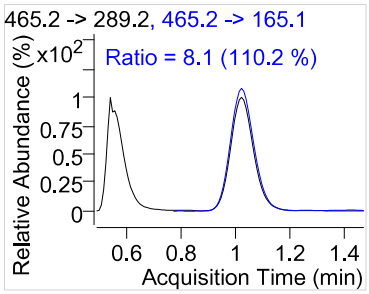
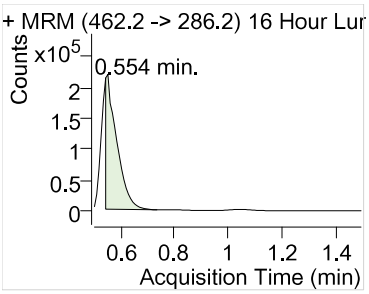
Morphine-6-B-D-Glucuronide D3

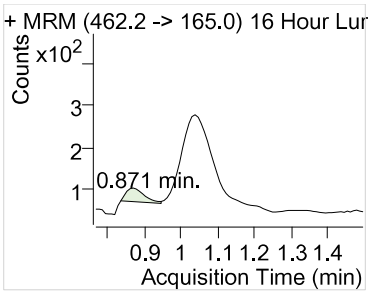


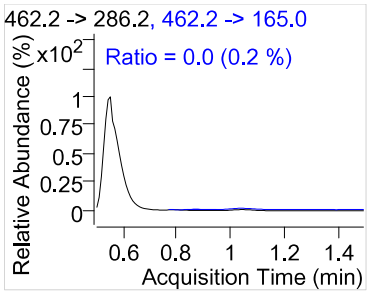




Morphine-6-B-D-Glucuronide







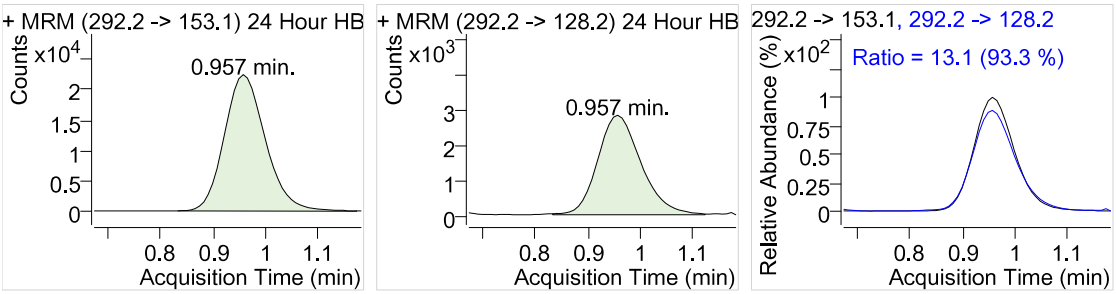
Quantitative Analysis Report

Agilent Technologies

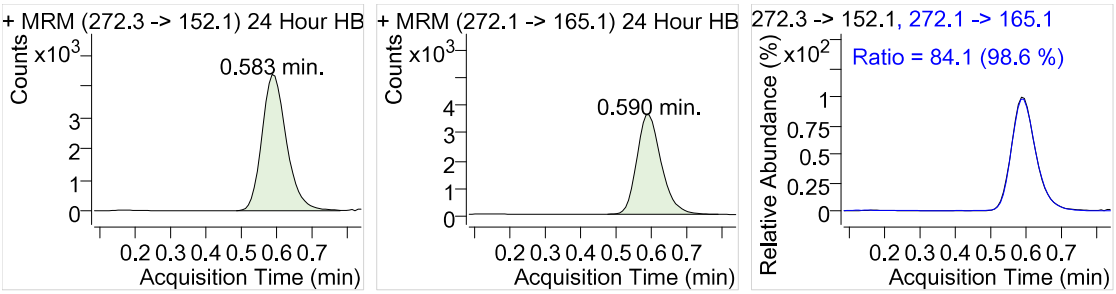
Sample Name:	:	24 Hour HB 2
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\24 Hour HB 2.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/14/2018 9:29:49 PM
Dilution	:	3.9
Operator	:	
Sample Position	:	P1-E9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.96	122745			
	292.2 -> 128.2		16089	13.1	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.58	20884			44.5 ng/ml
	272.1 -> 165.1		17556	84.1	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.58	7221			
	465.2 -> 201.1		738	10.2	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.58	11843			336.4 ng/ml
	462.2 -> 201.1		759	6.4	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.96	122745			
	292.2 -> 128.2		16089	13.1	11.2 - 16.9	
Morphine	286.1 -> 152.0	0.98	26926			520.9 ng/ml
	286.1 -> 128.1		18189	67.6	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.01	5183			
	465.2 -> 165.1		563	*10.9	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.57	7002			486.1 ng/ml
	462.2 -> 165.0		46	*0.7	11.0 - 16.5	

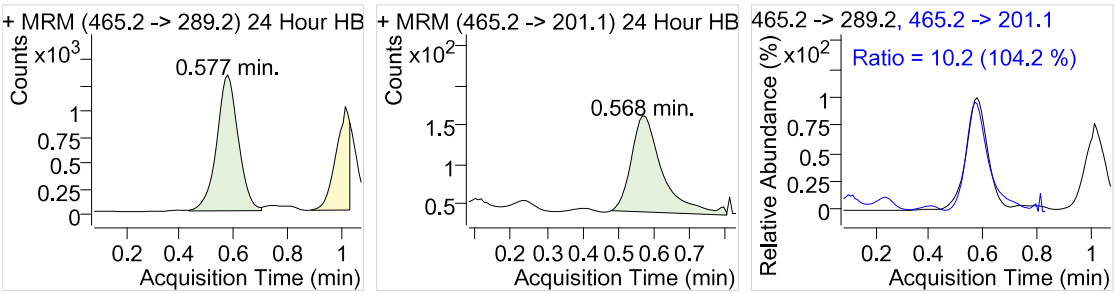
Morphine D6



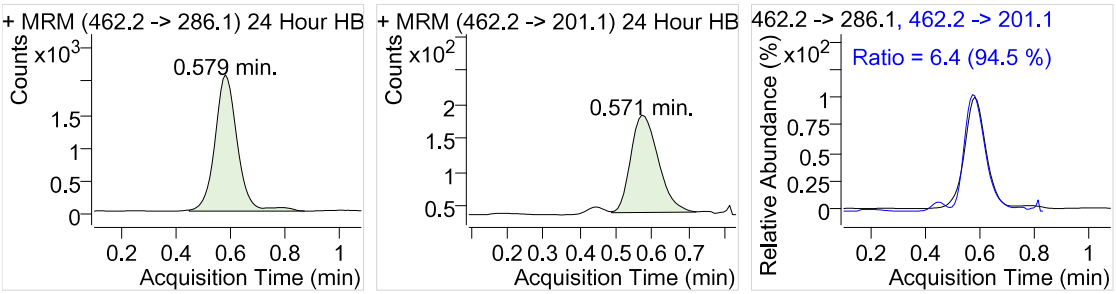
Normorphine



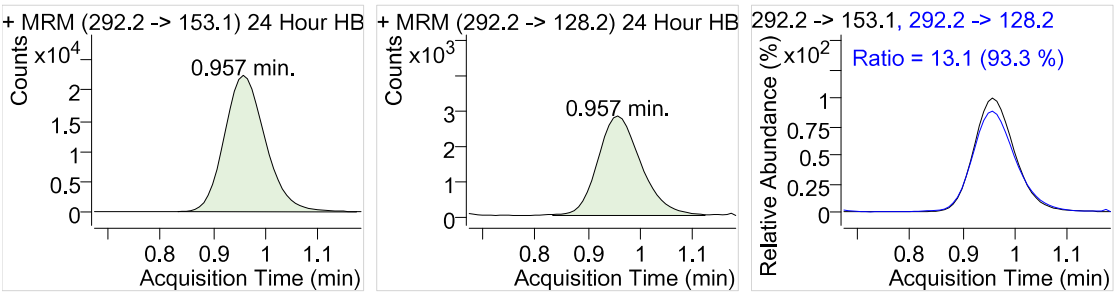
Morphine-3-B-D-Glucuronide D3



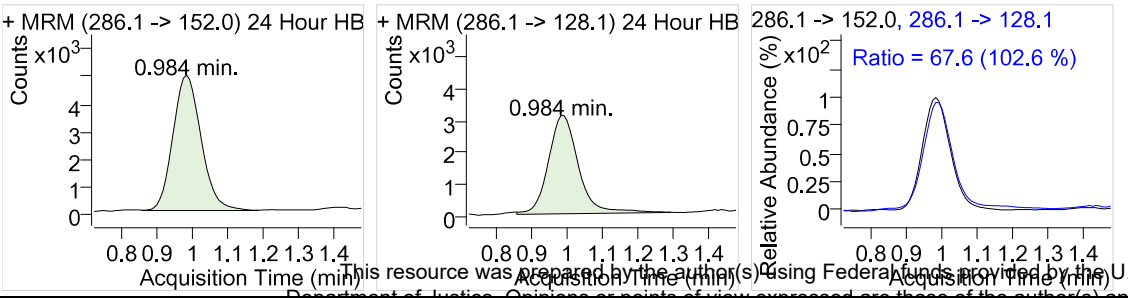
Morphine-3-B-D-Glucuronide



Morphine D6



Morphine



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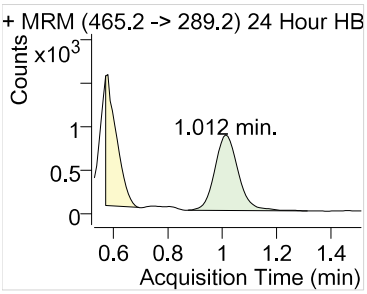
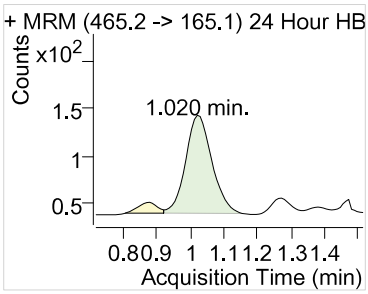
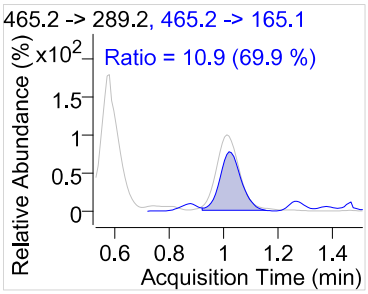
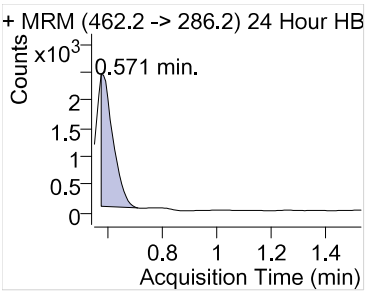
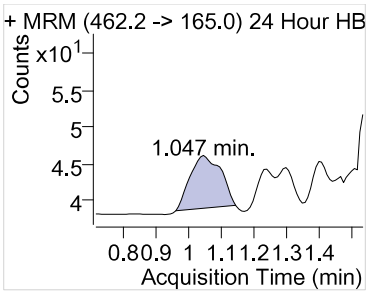


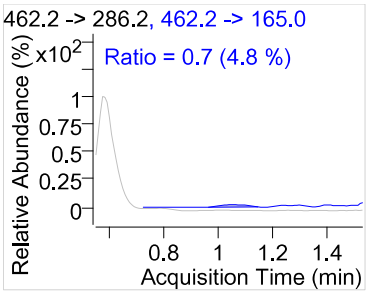




Morphine-6-B-D-Glucuronide







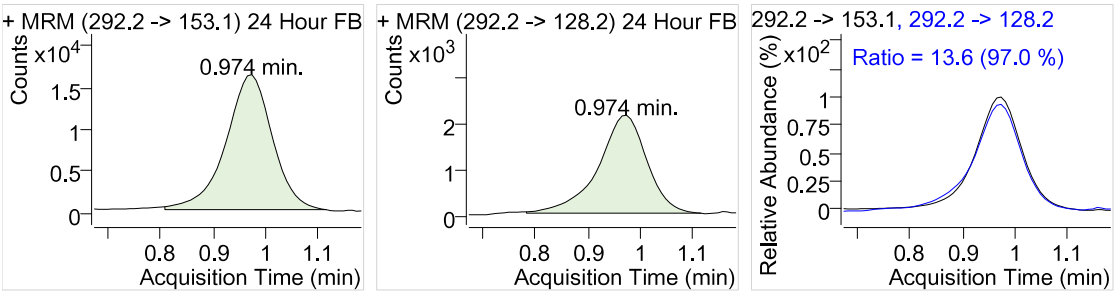
Quantitative Analysis Report

Agilent Technologies

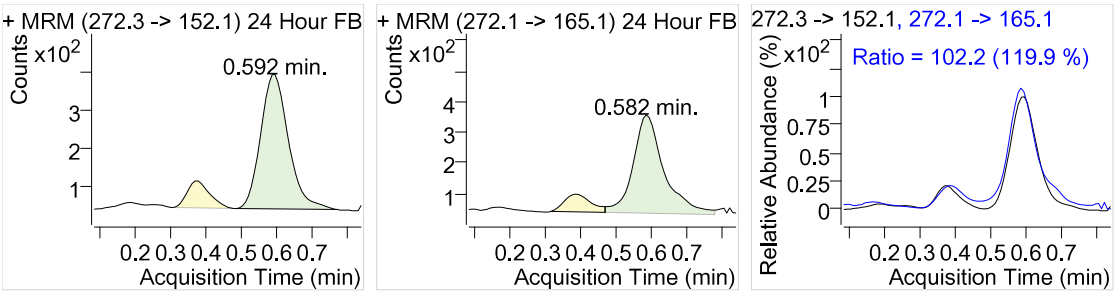
Sample Name:	:	24 Hour FB 2
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\24 Hour FB 2.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/14/2018 9:58:11 PM
Dilution	:	19.2
Operator	:	
Sample Position	:	P1-F3

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.97	100956			
	292.2 -> 128.2		13759	13.6	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.59	1913			176.3 ng/ml
	272.1 -> 165.1		1954	102.2	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.58	49482			
	465.2 -> 201.1		4020	8.1	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.60	7465			224.8 ng/ml
	462.2 -> 201.1		406	5.4	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.97	100956			
	292.2 -> 128.2		13759	13.6	11.2 - 16.9	
Morphine	286.1 -> 152.0	1.00	1720			148.2 ng/ml
	286.1 -> 128.1		1618	*94.1	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.03	14908			
	465.2 -> 165.1		2034	13.6	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.59	4114			338.5 ng/ml
	462.2 -> 165.0		24	*0.6	11.0 - 16.5	

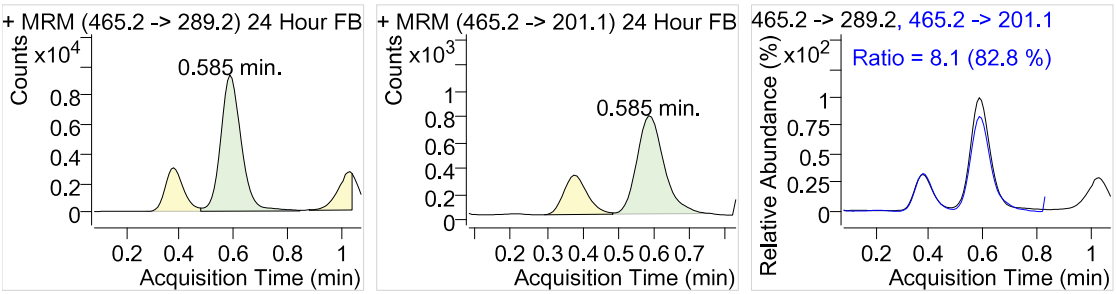
Morphine D6



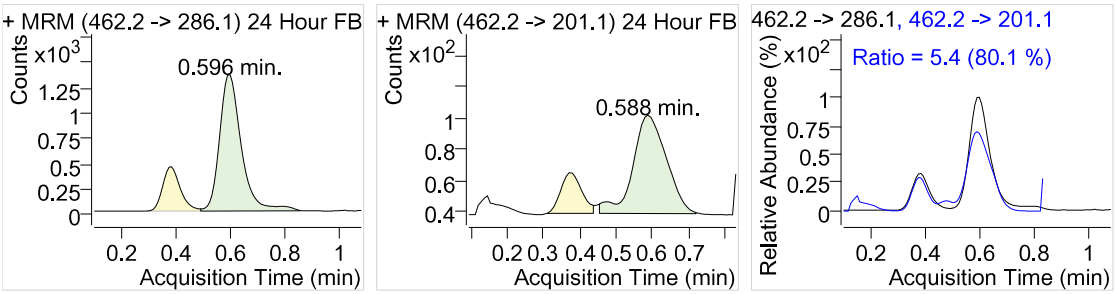
Normorphine



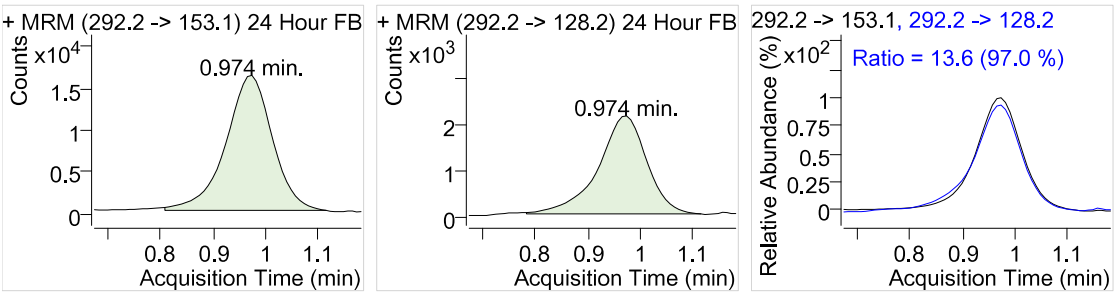
Morphine-3-B-D-Glucuronide D3



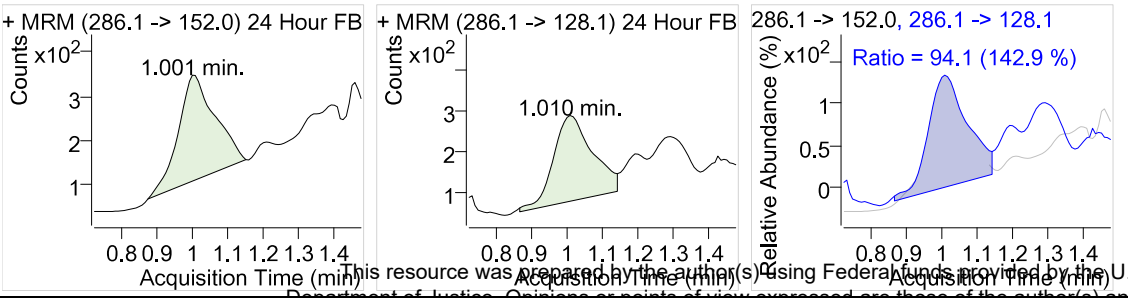
Morphine-3-B-D-Glucuronide



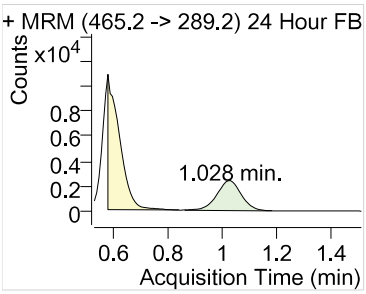
Morphine D6

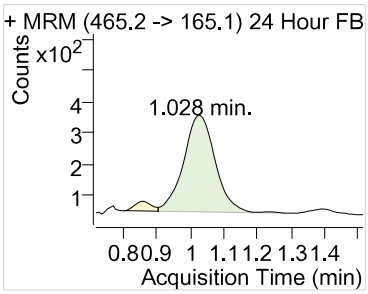


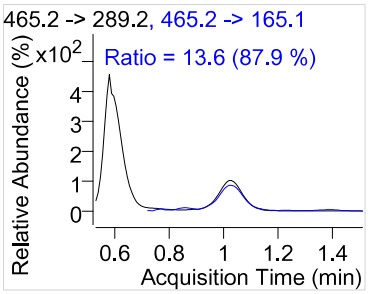
Morphine



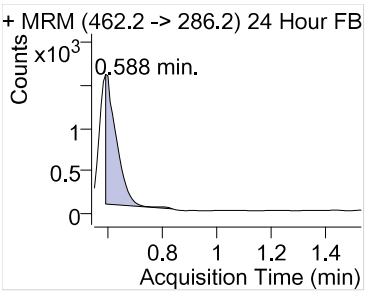
Morphine-6-B-D-Glucuronide D3

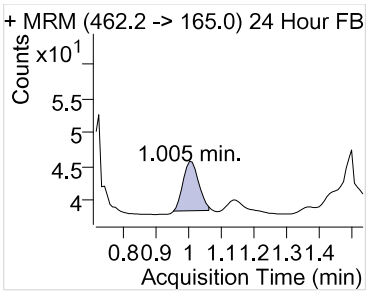


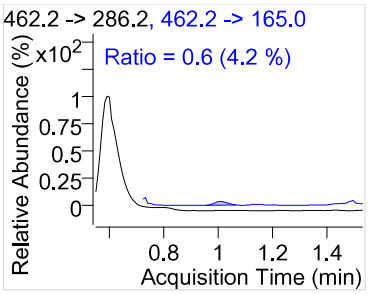




Morphine-6-B-D-Glucuronide





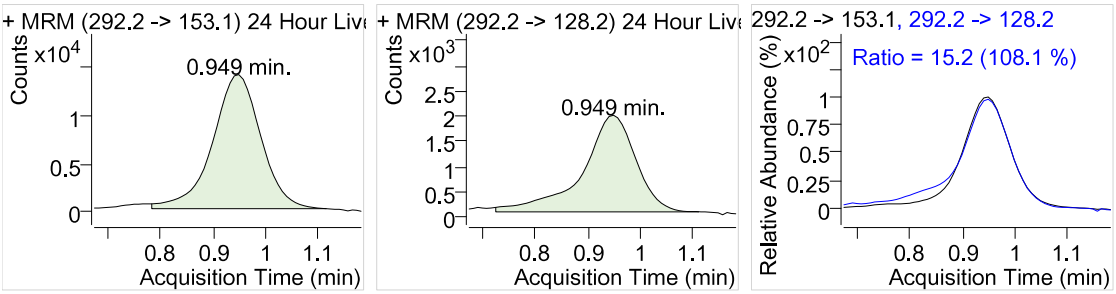


Quantitative Analysis Report

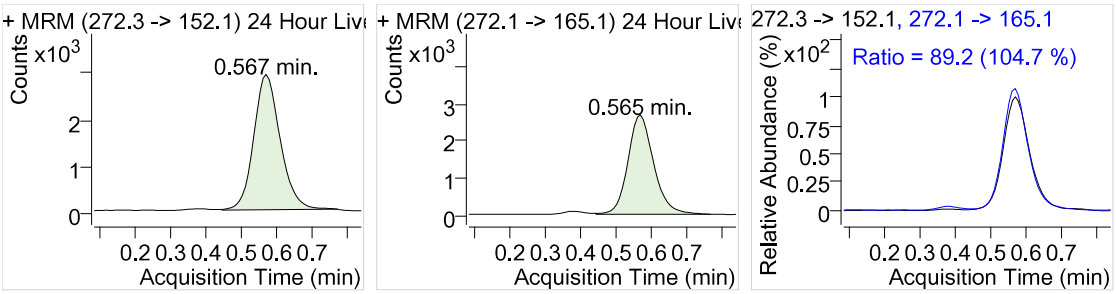
Sample Name: : 24 Hour Liver 3
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\07142018c\24 Hour Liver 3.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/14/2018 10:26:29 PM
Dilution : 4.0
Operator :
Sample Position : P1-F6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.95	90082			
	292.2 -> 128.2		13684	15.2	11.2 - 16.9	
Normorphine	272.3 -> 152.1	0.57	15148			45.7 ng/ml
	272.1 -> 165.1		13516	89.2	68.2 - 102.3	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	18347			
	465.2 -> 201.1		1968	10.7	7.8 - 11.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.57	1315727			6047.6 ng/ml
	462.2 -> 201.1		73003	5.5	5.4 - 8.1	
Morphine D6	292.2 -> 153.1	0.95	90082			
	292.2 -> 128.2		13684	15.2	11.2 - 16.9	
Morphine	286.1 -> 152.0	0.98	41355			1092.8 ng/ml
	286.1 -> 128.1		24472	59.2	52.7 - 79.0	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.01	36673			
	465.2 -> 165.1		4290	*11.7	12.4 - 18.6	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.57	890055			2915.5 ng/ml
	462.2 -> 165.0		2478	*0.3	11.0 - 16.5	

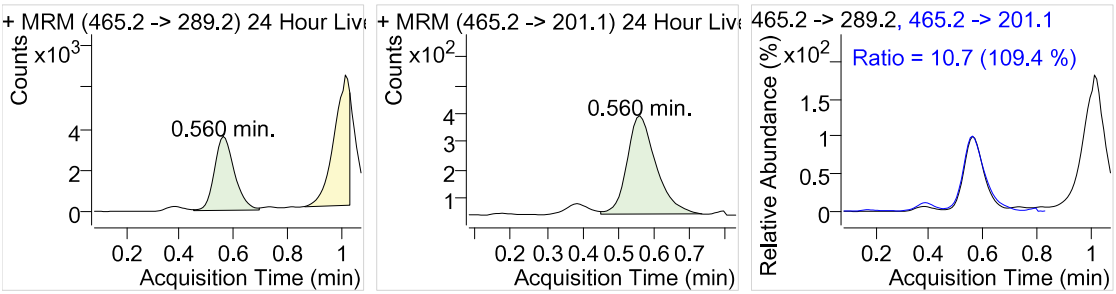
Morphine D6



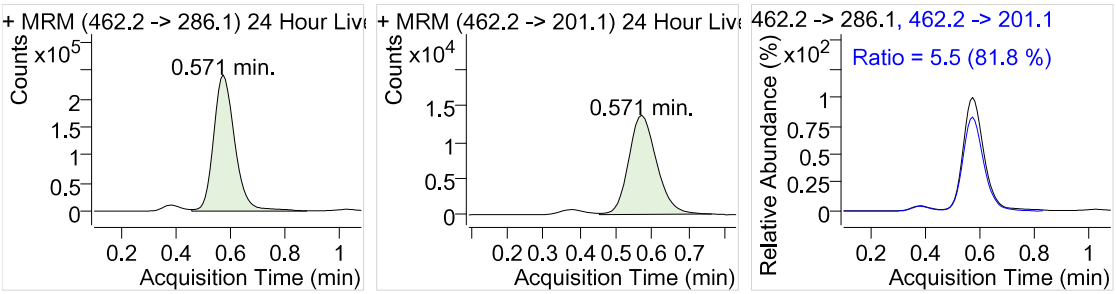
Normorphine



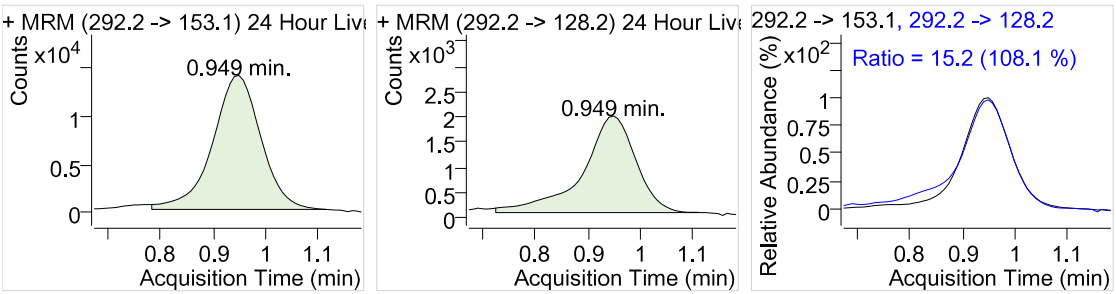
Morphine-3-B-D-Glucuronide D3



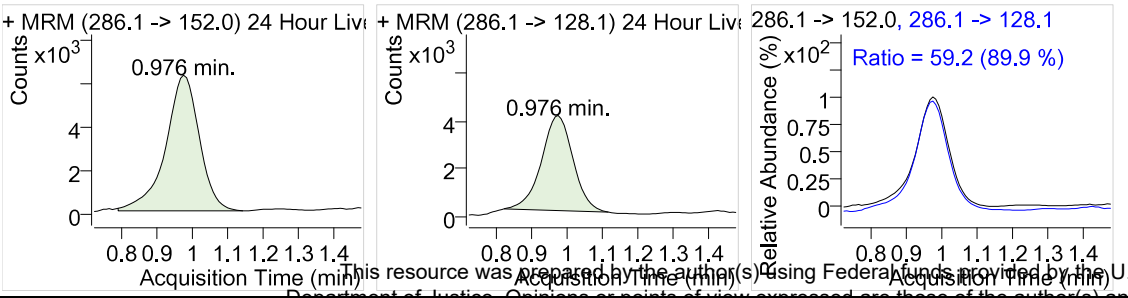
Morphine-3-B-D-Glucuronide



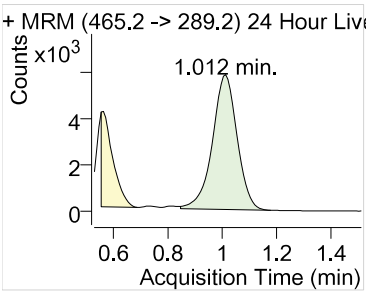
Morphine D6

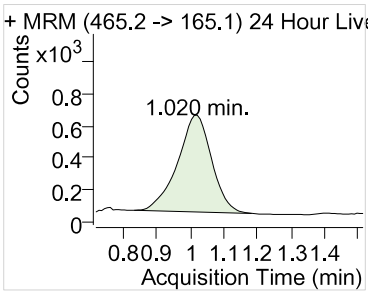


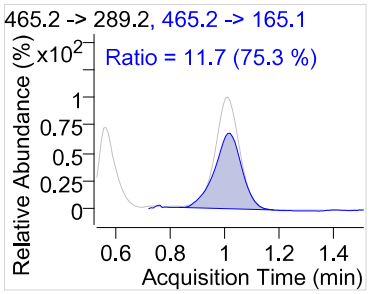
Morphine



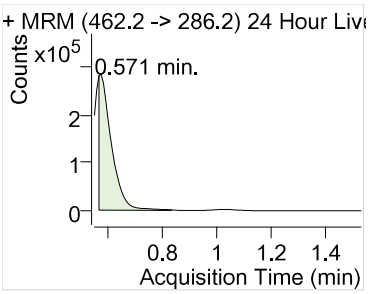
Morphine-6-B-D-Glucuronide D3

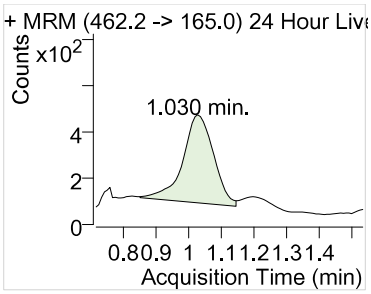


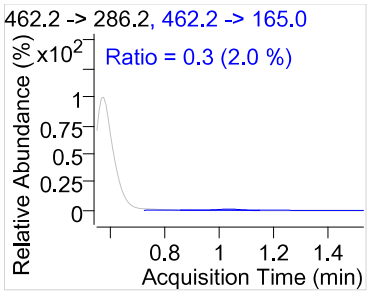




Morphine-6-B-D-Glucuronide







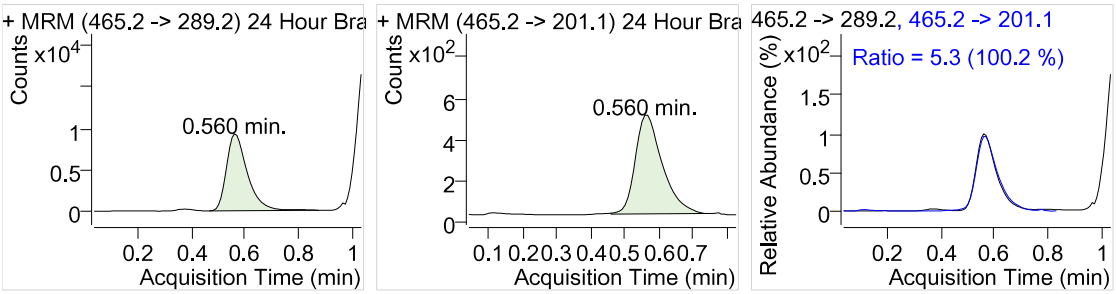
Quantitative Analysis Report

Agilent Technologies

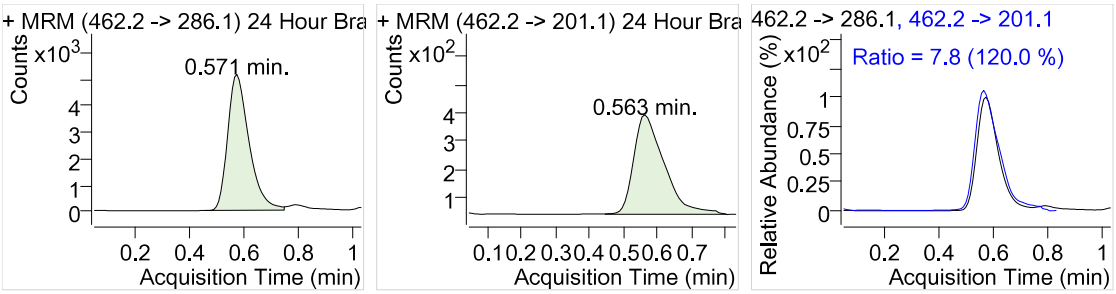
Sample Name:	:	24 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\24 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 3:51:56 AM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-F6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	49620			
	465.2 -> 201.1		2654	5.3	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.57	28329			327.1 ng/ml
	462.2 -> 201.1		2217	7.8	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	*0.99	87698			
	292.2 -> 128.2		12800	14.6	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.56	1881			39.4 ng/ml
	272.1 -> 165.1		1089	*57.9	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	*0.99	87698			
	292.2 -> 128.2		12800	14.6	12.9 - 19.4	
Morphine	286.1 -> 152.0	*1.02	21028			485.6 ng/ml
	286.1 -> 128.1		12966	61.7	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	*1.04	100553			
	465.2 -> 165.1		7550	7.5	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	19612			89.0 ng/ml
	462.2 -> 165.0		5	*0.0	7.1 - 10.6	

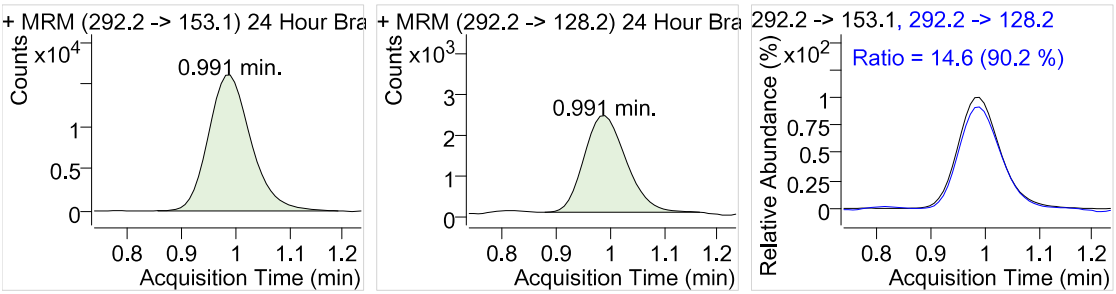
Morphine-3-B-D-Glucuronide D3



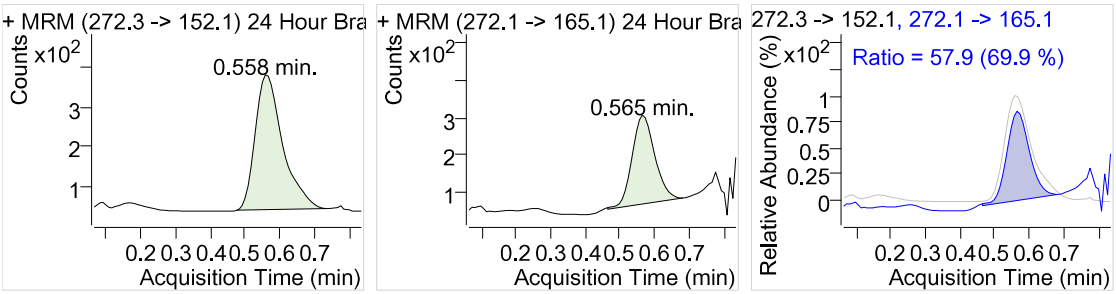
Morphine-3-B-D-Glucuronide



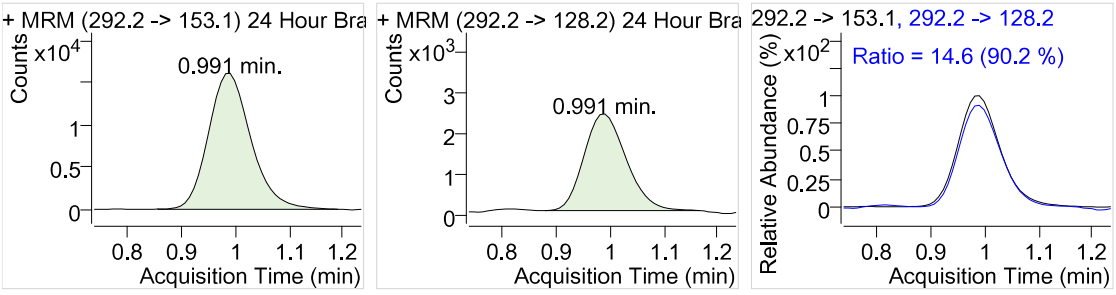
Morphine D6



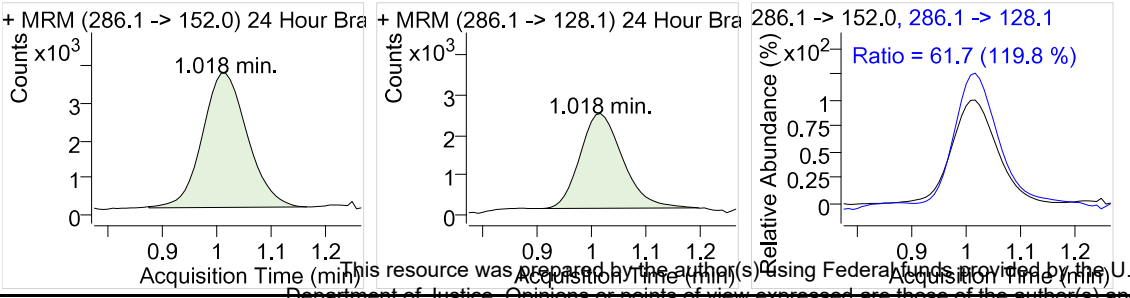
Normorphine



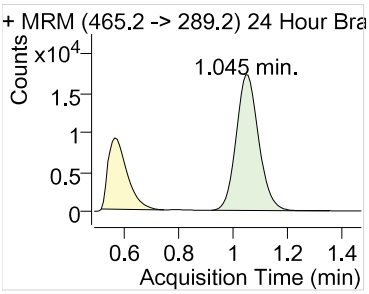
Morphine D6

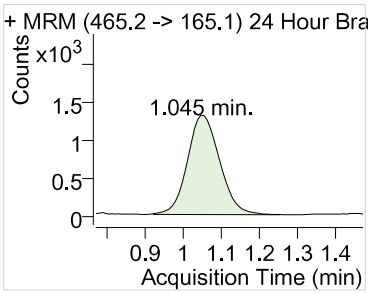


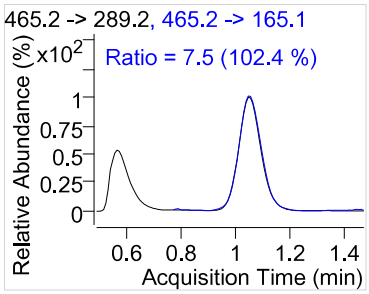
Morphine



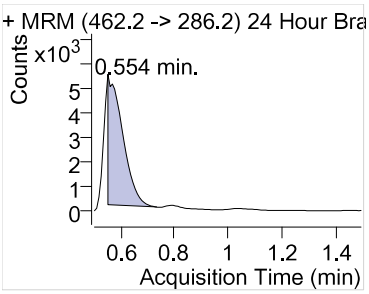
Morphine-6-B-D-Glucuronide D3

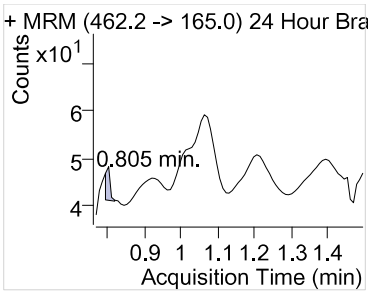


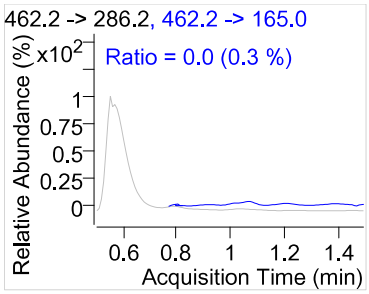




Morphine-6-B-D-Glucuronide





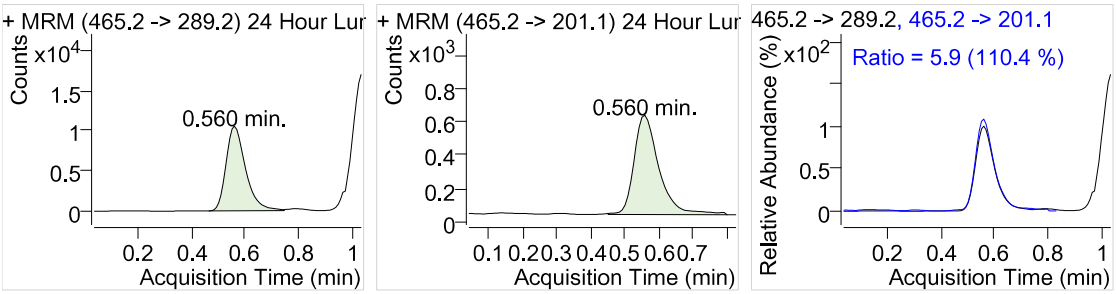


Quantitative Analysis Report

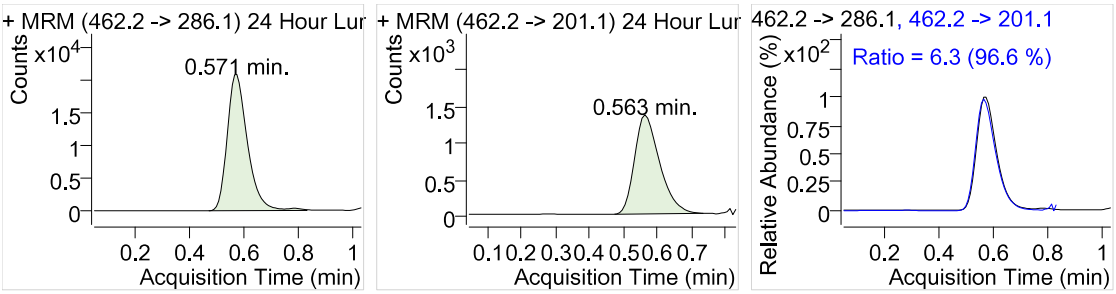
Sample Name: : 24 Hour Lung 1
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\24 Hour Lung 1.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/8/2018 3:53:29 AM
Dilution : 4.0
Operator :
Sample Position : P1-F7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	52329			
	465.2 -> 201.1		3085	5.9	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.57	109345			1105.3 ng/ml
	462.2 -> 201.1		6888	6.3	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	*0.97	46485			
	292.2 -> 128.2		8395	18.1	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.57	42749			77.0 ng/ml
	272.1 -> 165.1		33849	79.2	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	*0.97	46485			
	292.2 -> 128.2		8395	18.1	12.9 - 19.4	
Morphine	286.1 -> 152.0	*1.00	62132			2692.7 ng/ml
	286.1 -> 128.1		37013	59.6	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	*1.04	87206			
	465.2 -> 165.1		6529	7.5	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	76263			280.4 ng/ml
	462.2 -> 165.0		23	*0.0	7.1 - 10.6	

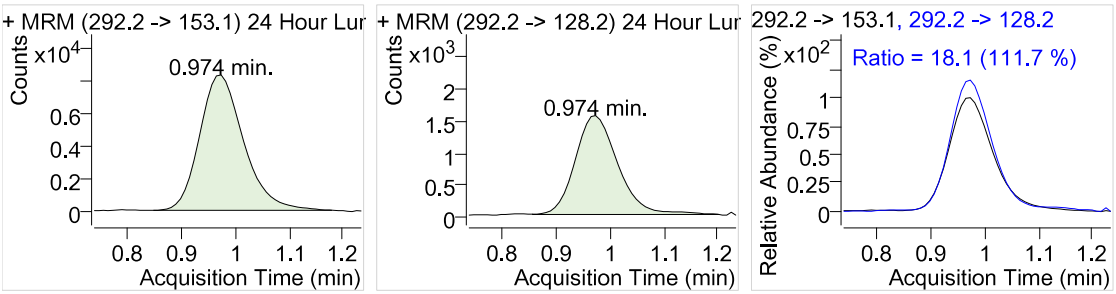
Morphine-3-B-D-Glucuronide D3



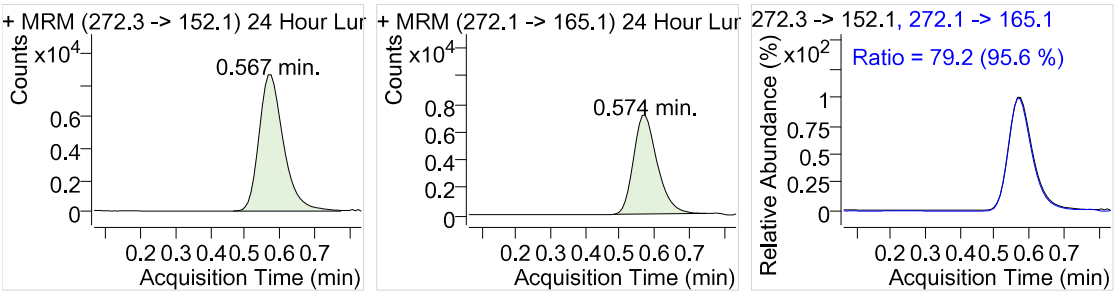
Morphine-3-B-D-Glucuronide



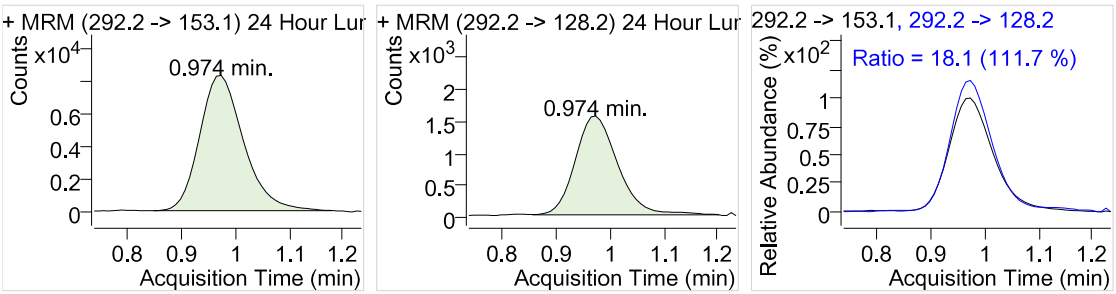
Morphine D6



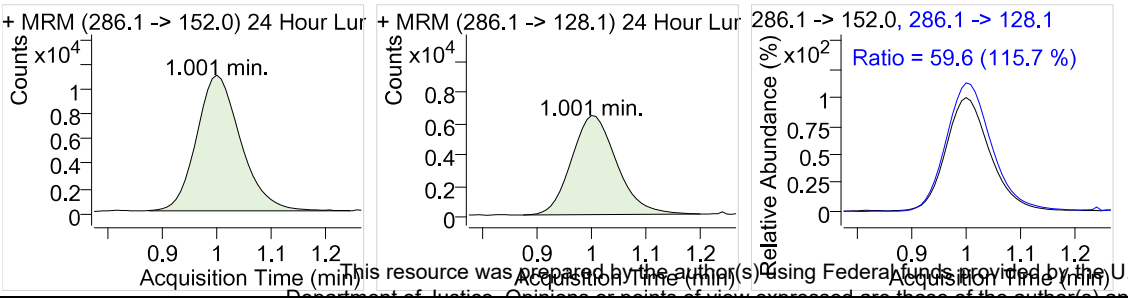
Normorphine



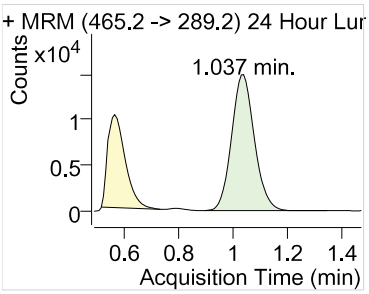
Morphine D6

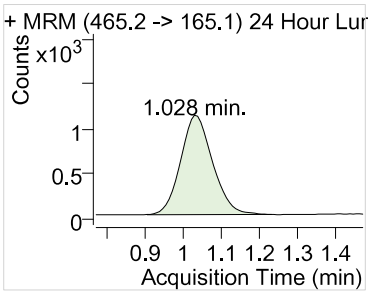


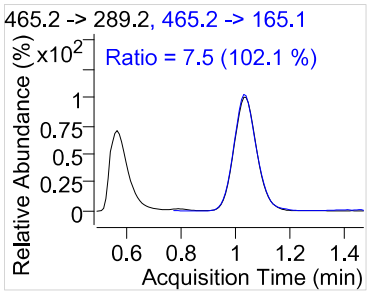
Morphine



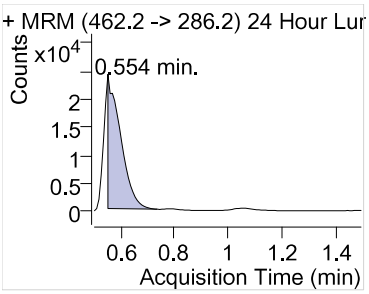
Morphine-6-B-D-Glucuronide D3

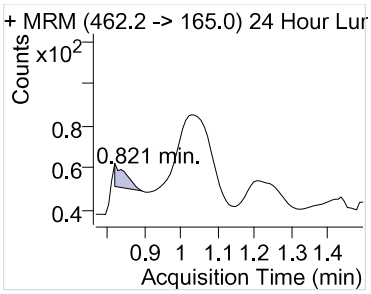


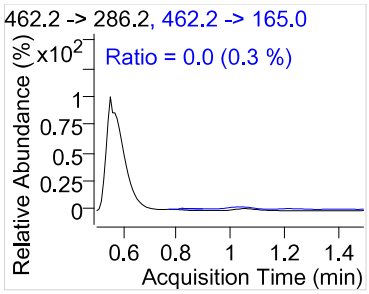




Morphine-6-B-D-Glucuronide







Appendix E

Concentration- Time Data of Morphine and It Metabolites Collected During Morphine Only Study

Table of Contents

Abbreviations

BW: Body Weight

HB: Heart Blood

F: Female

FB: Femoral Blood

M: Male

MOR: Morphine

M3G: Morphine-3-Glucuronide

M6G: Morphine-6-Glucuronide

NM: Normorphine

PM: Postmortem

Table E-1: Individual Data from the Morphine Only Study for Heart Blood and Femoral Blood Samples

Date	Injection Time	PM Interval	BW	Sex	M(HB)	NM(HB)	M3G(HB)	M(FB)	NM(FB)	M3G(FB)
2/10/2018	1:54pm	0	116	F	2066	8.3	3779			
2/10/2018	2:15pm	0	210	F	1406	4.7	2248			
4/15/2018	2:25pm	0	236	F	2228	27	8107			
7/5/2018	1212am	0	189	F	1079	21	2355	5561	29	1579
2/10/2018	9:50am	8	205	F						
2/10/2018	10:10am	8	127	F	1943	16	2934			
4/15/2018	10:16am	8	236	F	1433	17	4383			
6/2/2018	1046pm	8	277	F	1565	13	2252	3056	56	393
6/2/2018	1049pm	8	181	F	743	36	1525	2753	88	2724
7/5/2018	1015am	8	196	F	719	18	1166	1800	30	1746
7/5/2018	1019am	8	199	F	1950	36	1781	1201	18	1139
4/15/2018	4:57pm	16	217	F	1697		5868			
6/2/2018	1128pm	16	179	F	1203	28	4257	129	11	565
6/2/2018	1132pm	16	170	F	694	36	1228		473	256
7/5/2018	1132am	16	238	F	915	28	1157	643	166	119
2/10/2018	12:00pm	24	132	F	1852	12	1451			
2/10/2018	12:25pm	24	198	F						
4/15/2018	12:18pm	24	231	F	1208		3001			
6/2/2018	935pm	24	169	F	1210	9.2	707	2050	27	2044
6/2/2018	946pm	24	177	F	1048	19	518			
7/5/2018	941am	24	224	F	483	9.0	2380			
7/5/2018	945am	24	192	F	417	8.3	3528			
4/15/2018	1:55pm	0	440	M	1160	156	1662			
4/15/2018	2:10pm	0	405	M	1567	169	2165			
6/2/2018	1154pm	0	303	M	1983	64	1074	2523	77	1034
7/5/2018	1147am	0	388	M	659	18	367	4789	38	
7/5/2018	1154am	0	420	M				3352	59	1500
2/10/2018	10:38am	8	180	M	1164	69	779			
4/15/2018	9:39am	8	412	M						
4/15/2018	9:55am	8	390	M	1324	104	1251			
6/2/2018	1003pm	8	314	M	1383	70	1118	120	156	297
6/2/2018	1007pm	8	315	M	776	31	299			
7/5/2018	959am	8	379	M	1036	24	187	810	42	
7/5/2018	1004am	8	360	M	778	34	1429	736	43	
4/15/2018	4:29pm	16	417	M	1708	145	3179			
4/15/2018	4:44pm	16	510	M	1462	518	2888			
6/2/2018	1120pm	16	450	M	1644	71	8969	471	101	196
6/2/2018	1123pm	16	289	M	1105	40	452		166	85
7/5/2018	1122am	16	415	M	1012	59	841	134	230	98
7/5/2018	1126am	16	391	M	1062	35	555			212
2/10/2018	12:45pm	24	150	M						
4/15/2018	11:47am	24	460	M	1240	206	600			
4/15/2018	12:05pm	24	408	M	1216	105	2194			
6/2/2018	913pm	24	430	M	1691	109	1121			
6/2/2018	925pm	24	304	M	336	65	478	581	48	472

7/5/2018	926am	24	337	M	906	50	629	148	176	225
7/5/2018	930am	24	393	M	521	44	336			

Table E-2: Individual Data from the Morphine Only Study for Liver, Brain and Lung Samples

Date	Injection Time	PM Interval	BW	Sex	M(Liver)	NM(Liver)	M3G(Liver)	M(Brain)	NM(Brain)	M3G(Brain)	M(Lung)	NM(Lung)	M3G(Lung)
2/10/2018	1:54pm	0	116	F	947	29	4440	239		71	2978	36	1002
2/10/2018	2:15pm	0	210	F	1945	40	6970	380		127	5491	50	2415
4/15/2018	2:25pm	0	236	F	3190	134	11203	1035	64	918	7073	56	5482
7/5/2018	1212am	0	189	F	2332	40	4434	288	33	308	1756	34	1740
2/10/2018	9:50am	8	205	F	1250	32	5231	395		671	2363	47	2950
2/10/2018	10:10am	8	127	F	931	29	2929	281		271	2855		2537
4/15/2018	10:16am	8	236	F	1585	101	6080	379	66	438	1592	39	1402
6/2/2018	1046pm	8	277	F	1660	42	7231	1832	54	436	1832	54	436
6/2/2018	1049pm	8	181	F	1354	36	7174	471	40	432	471	40	432
7/5/2018	1015am	8	196	F	576	35	3936	194	33	423	459	33	1184
7/5/2018	1019am	8	199	F	1384	35	4672	176	32	222	1130	34	1924
4/15/2018	4:57pm	16	217	F	2912	145	16588	1440	60	1753	4258	64	3124
6/2/2018	1128pm	16	179	F	1411	38	9060	371	39	447	371	39	447
6/2/2018	1132pm	16	170	F	827	35	5424	307	39	330	307	39	330
7/5/2018	1132am	16	238	F	1024	40	2526	181	36	212	962	39	1240
2/10/2018	12:00pm	24	132	F	2371	38	3796	519		429	4296	46	1924
2/10/2018	12:25pm	24	198	F	2014	31	1970	328		236	3080		3179
4/15/2018	12:18pm	24	231	F	1423	956	7408	670	56	775	1593	26	2384
6/2/2018	935pm	24	169	F	2494	36	15068	579	39	1527	579	39	1527
6/2/2018	946pm	24	177	F	1370	35	5401	206	40	186	206	40	186
7/5/2018	941am	24	224	F	1093	46	6048	195	36	1055	409	39	3231
7/5/2018	945am	24	192	F	1675	40	7263	120	36	953	406	38	3536
4/15/2018	1:55pm	0	440	M	1421	1932	9204	359	27	85	3940	598	1090
4/15/2018	2:10pm	0	405	M	1339	1993	10366	458	28	102	4109	412	1552
6/2/2018	1154pm	0	303	M	1475	498	4704	607	41	100	607	41	100
7/5/2018	1147am	0	388	M	352	130	2477	158	33	303	851	48	414
7/5/2018	1154am	0	420	M	842	474	5284	318	35	218	2353	114	1220
2/10/2018	10:38am	8	180	M	1185	656	2841	322	32	162	2551	196	1443
4/15/2018	9:39am	8	412	M	785	1070	4604	530	57	231	1798	122	695
4/15/2018	9:55am	8	390	M	1420	1648	10464	1272	69	299	1936	277	1717
6/2/2018	1003pm	8	314	M	2623	204	7563	598	42	173	598	42	173

Date	Injection Time	PM Interval	BW	Sex	M(Liver)	NM(Liver)	M3G(Liver)	M(Brain)	NM(Brain)	M3G(Brain)	M(Lung)	NM(Lung)	M3G(Lung)
6/2/2018	1007pm	8	315	M	1528	277	7958	397	40	98	397	40	98
7/5/2018	959am	8	379	M	550	100	3034	202	33	227	725	43	603
7/5/2018	1004am	8	360	M	692	115	4292	243	33	72	1230	49	1042
4/15/2018	4:29pm	16	417	M	2126	2301	10770	1435	131	493	2622	320	860
4/15/2018	4:44pm	16	510	M	1814	2647	9003	1228	93	857	2555	403	997
6/2/2018	1120pm	16	450	M	1120	235	7194	561	41	359	561	41	359
6/2/2018	1123pm	16	289	M	908	196	5403	345	40	285	345	40	285
7/5/2018	1122am	16	415	M	1196	315	3796	437	37	349	1457	56	764
7/5/2018	1126am	16	391	M	745	145	2460	239	37	154	915	49	443
2/10/2018	12:45pm	24	150	M	1625	495	1514	390	31	333	2502	219	2755
4/15/2018	11:47am	24	460	M	1181	1109	3422	559	60	164	1279	171	435
4/15/2018	12:05pm	24	408	M	1498	938	7706	873	62	379	1538	144	456
6/2/2018	913pm	24	430	M	1333	174	4388	486	39	327	486	39	327
6/2/2018	925pm	24	304	M	844	158	3604	225	40	233	225	40	233
7/5/2018	926am	24	337	M	1964	327	5412	180	38	458	568	89	2104
7/5/2018	930am	24	393	M	1829	375	7420	133	38	461	777	95	2674

Appendix F

Quantitative Analysis Reports for Urine Matrix LC-MS/MS Data Generated Using Negative Urine Matrix as well as Collected at Various Postmortem Intervals. Extraction and LC-MS/MS Method performed as Described in Dissertation.

Table of Contents

Abbreviations

Conc: Concentration

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry LC: Low Control HC: High Control

RT: Retention Time

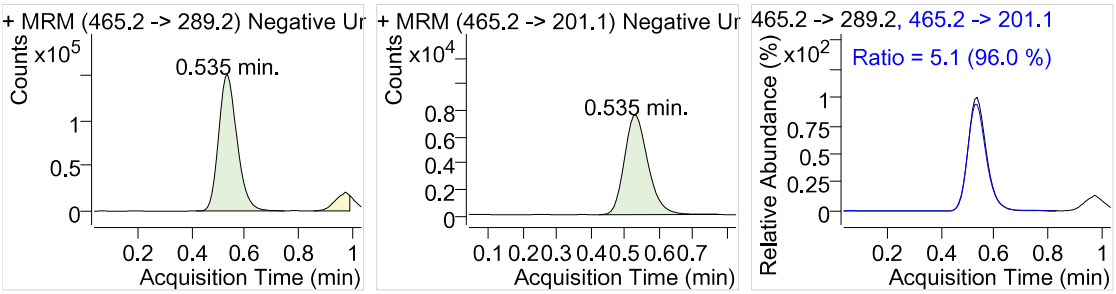
Quantitative Analysis Report

Agilent Technologies

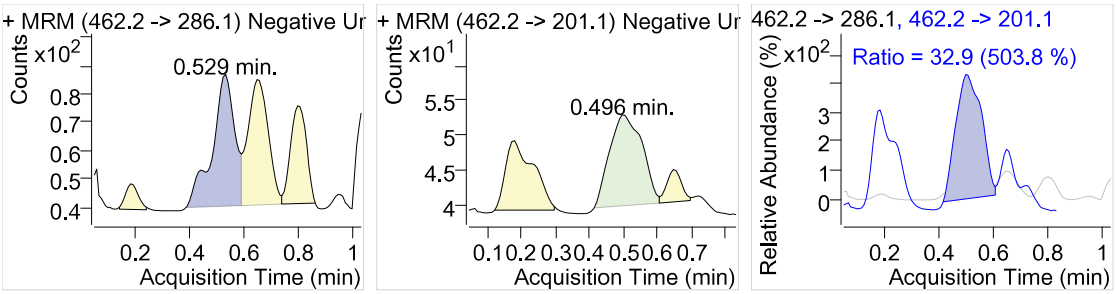
Sample Name:	:	Negative Urine
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\Negative Urine.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 8:23:52 PM
Dilution	:	1.0
Operator	:	
Sample Position	:	P1-B3

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	718075			
	465.2 -> 201.1		36799	5.1	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.53	265			7.5 ng/ml
	462.2 -> 201.1		87	*32.9	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.92	82815			
	292.2 -> 128.2		12802	15.5	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.55	762			9.7 ng/ml
	272.1 -> 165.1		530	69.6	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.92	82815			
	292.2 -> 128.2		12802	15.5	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.94	404			0.0 ng/ml
	286.1 -> 128.1		138	*34.2	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.97	103632			
	465.2 -> 165.1		7876	7.6	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.65	132			8.3 ng/ml
	462.2 -> 165.0				7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.43	374319			
Norfentanyl	233.1 -> 84.1	2.45	1692			0.9 ng/ml
	233.1 -> 150.1		64	3.8	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.02	1599164			
	342.4 -> 105.1		869717	54.4	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.02	7240			0.4 ng/ml
	337.2 -> 188.1		8422	116.3	100.6 - 150.9	

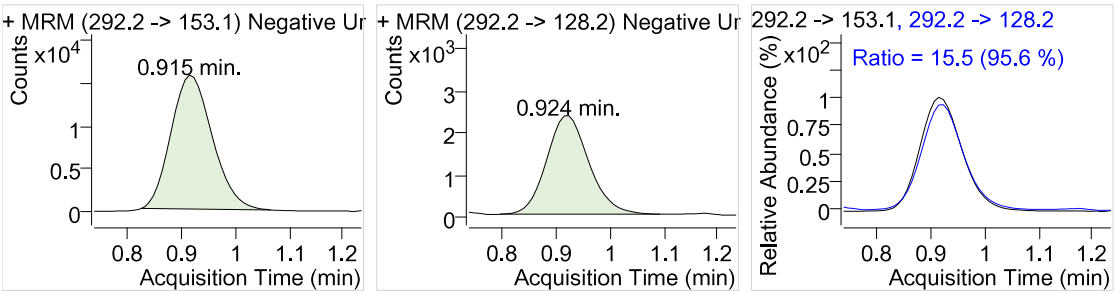
Morphine-3-B-D-Glucuronide D3



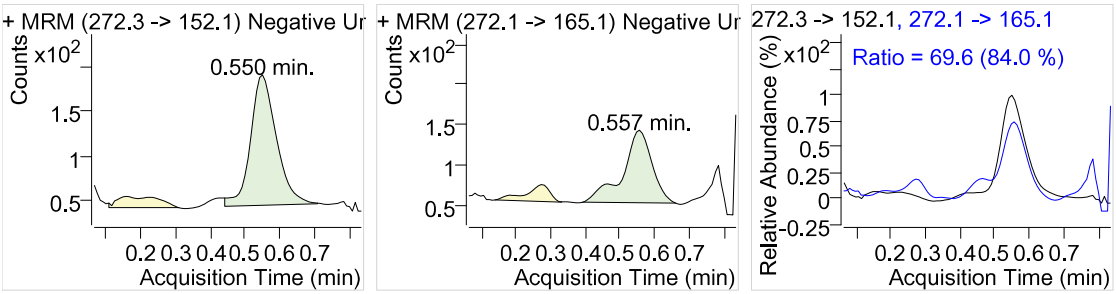
Morphine-3-B-D-Glucuronide



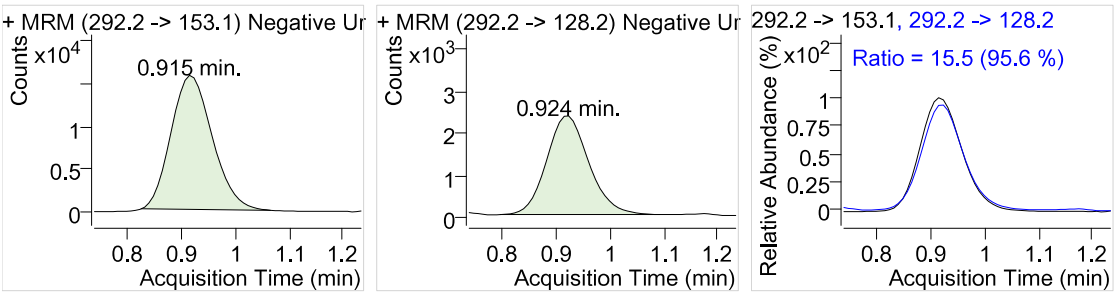
Morphine D6



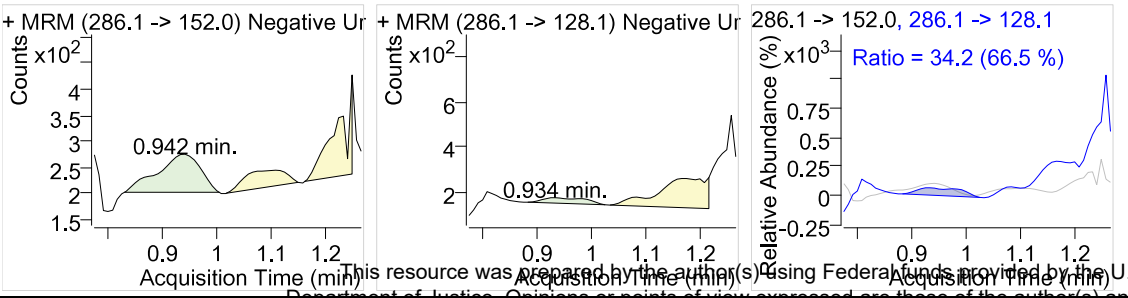
Normorphine



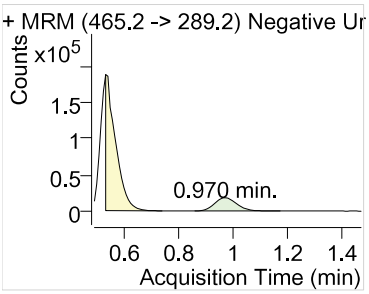
Morphine D6

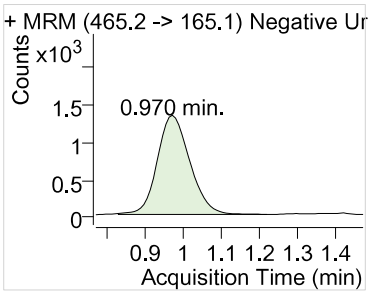


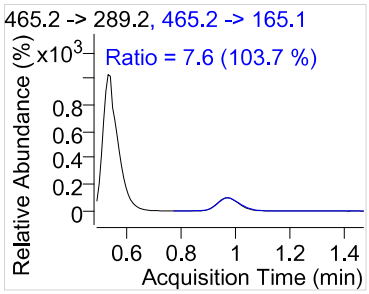
Morphine



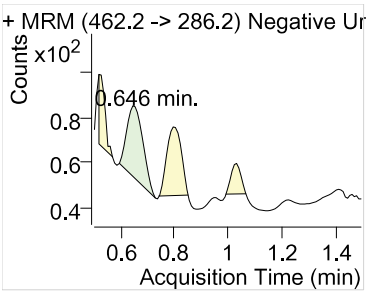
Morphine-6-B-D-Glucuronide D3

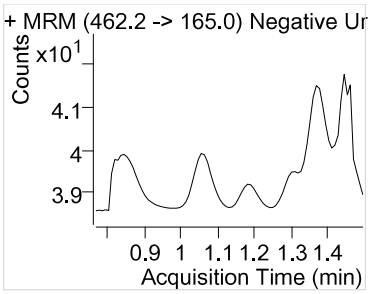


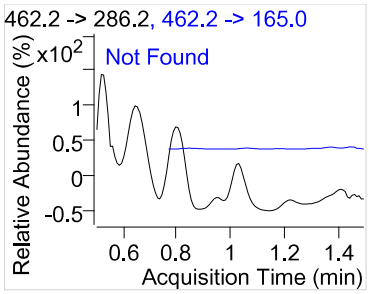




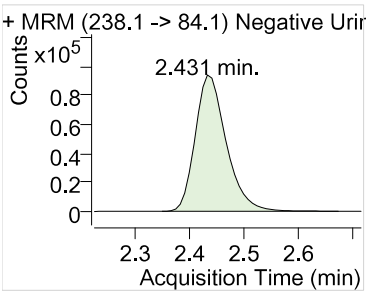
Morphine-6-B-D-Glucuronide



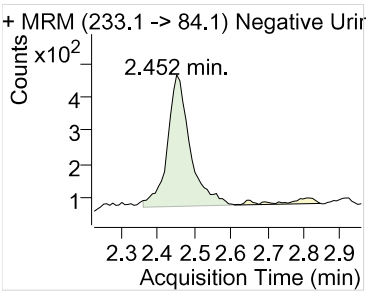


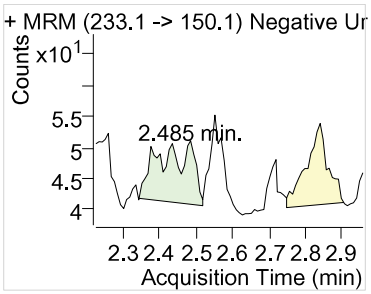


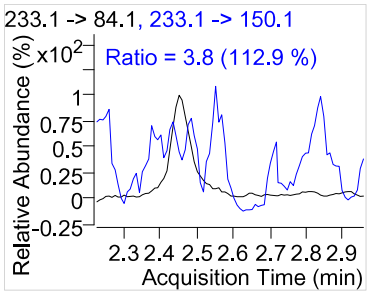
Norfentanyl D5



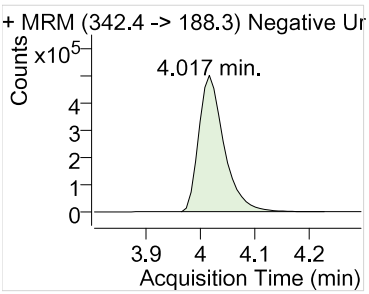
Norfentanyl

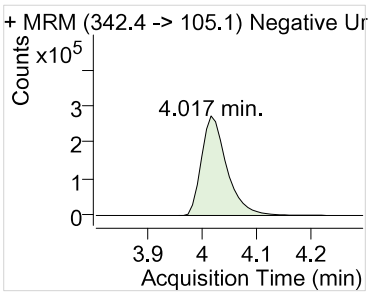


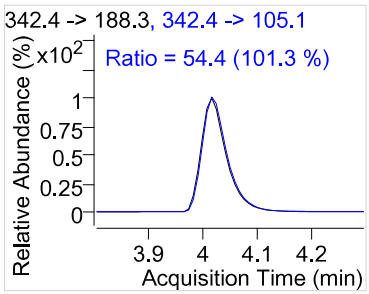




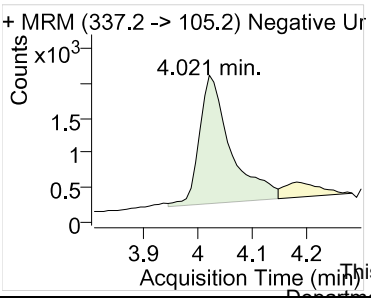
Fentanyl D5

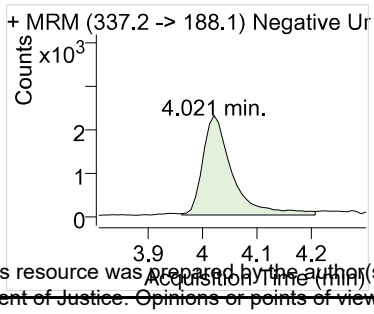


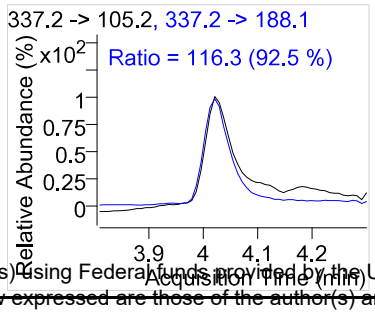




Fentanyl







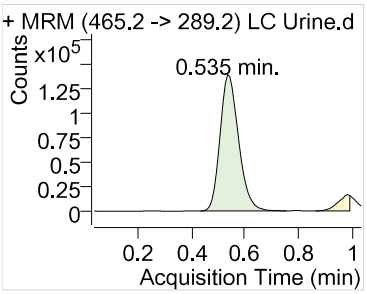
Quantitative Analysis Report

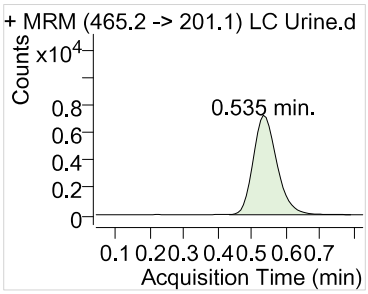
Agilent Technologies

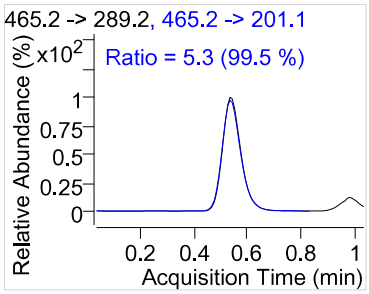
Sample Name:	:	LC Urine
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\LC Urine.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 8:33:22 PM
Dilution	:	1.0
Operator	:	
Sample Position	:	P1-B4

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	665697			
	465.2 -> 201.1		35368	5.3	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	267678			59.9 ng/ml
	462.2 -> 201.1		16307	6.1	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.92	83734			
	292.2 -> 128.2		14295	17.1	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.56	531643			76.3 ng/ml
	272.1 -> 165.1		439359	82.6	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.92	83734			
	292.2 -> 128.2		14295	17.1	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.95	12981			77.7 ng/ml
	286.1 -> 128.1		7281	56.1	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	80043			
	465.2 -> 165.1		6698	8.4	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	1.00	28939			34.1 ng/ml
	462.2 -> 165.0		2992	10.3	7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.43	411695			
Norfentanyl	233.1 -> 84.1	2.45	247205			7.5 ng/ml
	233.1 -> 150.1		7308	3.0	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.02	1602289			
	342.4 -> 105.1		868920	54.2	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.02	813170			7.3 ng/ml
	337.2 -> 188.1		1016038	124.9	100.6 - 150.9	

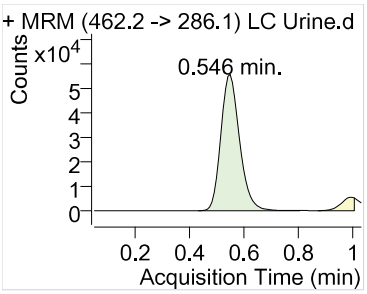
Morphine-3-B-D-Glucuronide D3

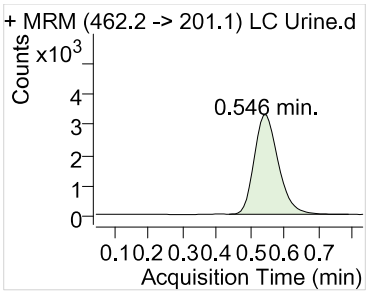


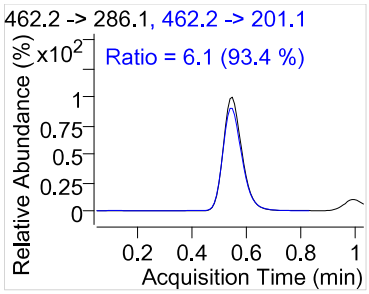




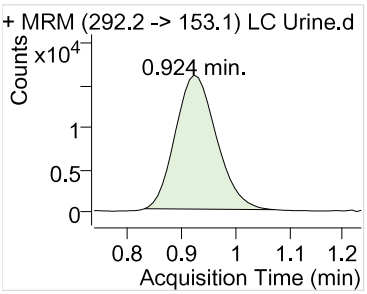
Morphine-3-B-D-Glucuronide

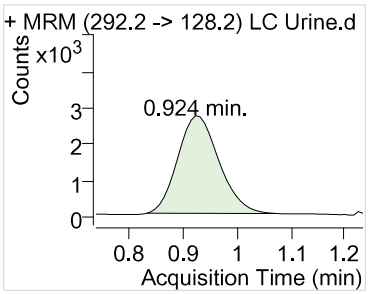


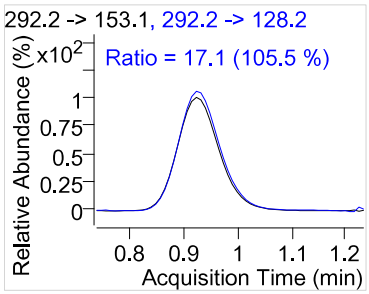




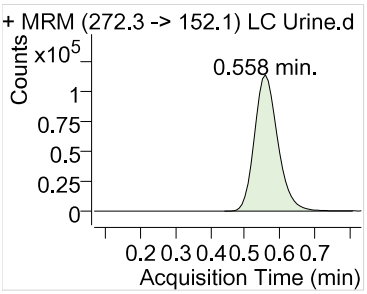
Morphine D6

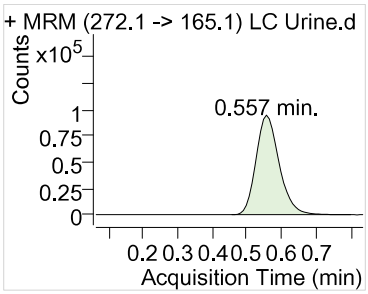


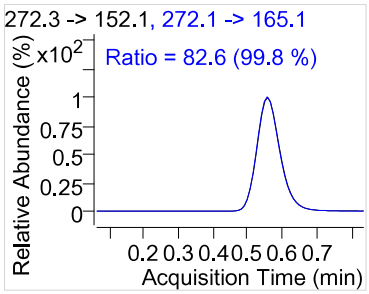




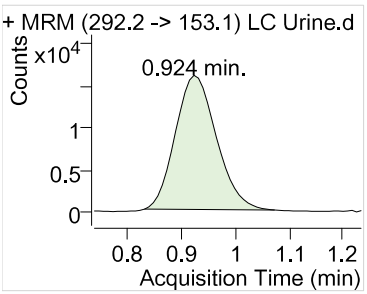
Normorphine

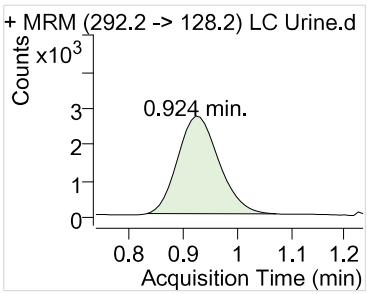


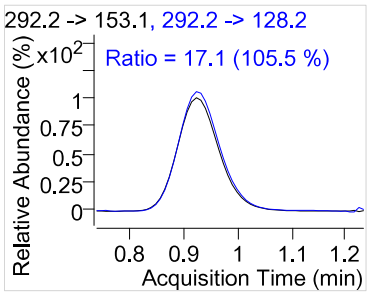




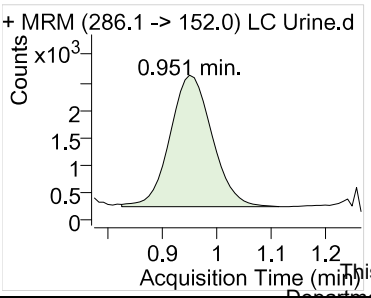
Morphine D6

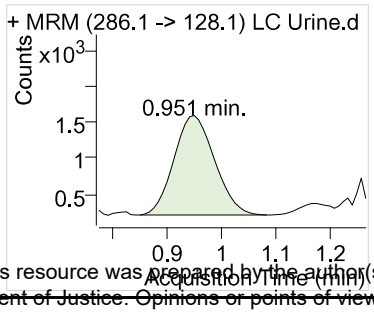


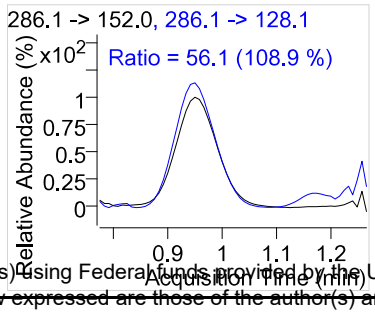




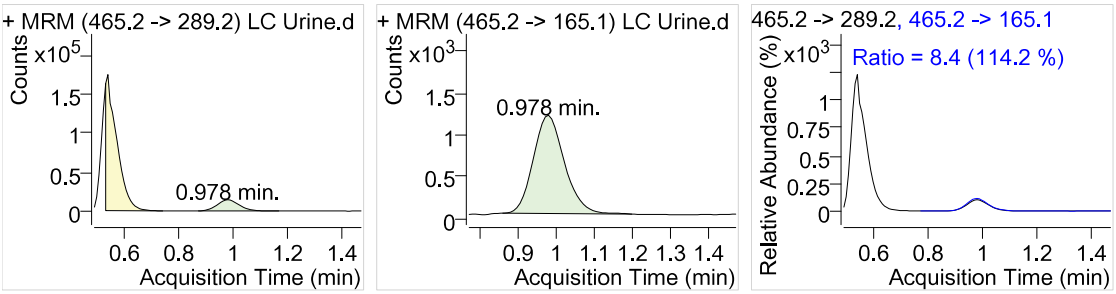
Morphine



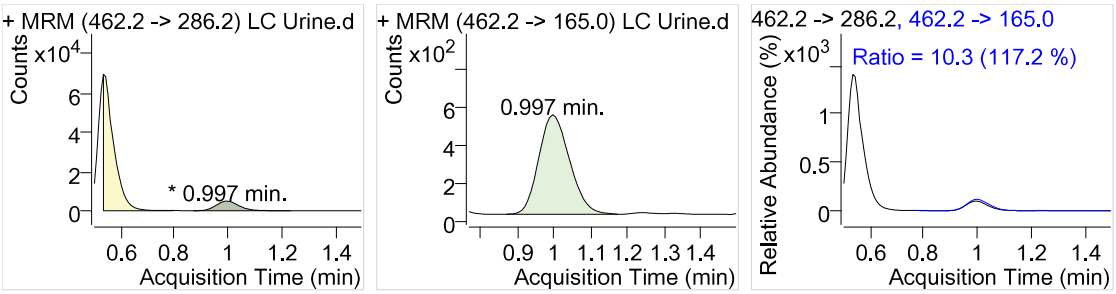




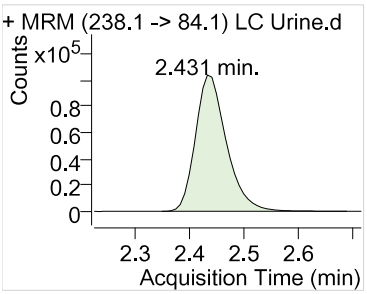
Morphine-6-B-D-Glucuronide D3



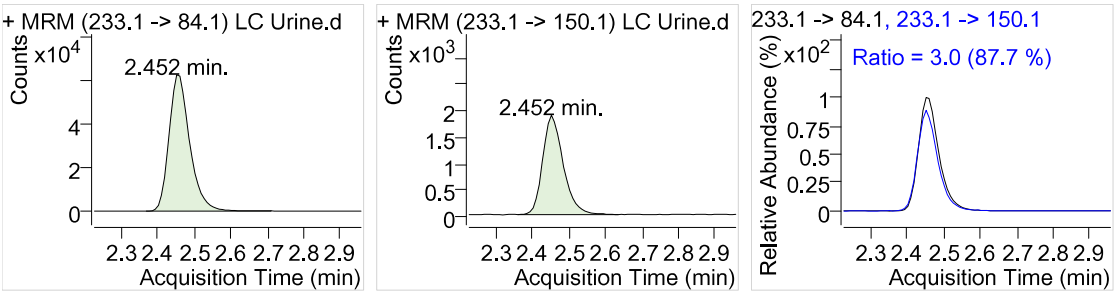
Morphine-6-B-D-Glucuronide



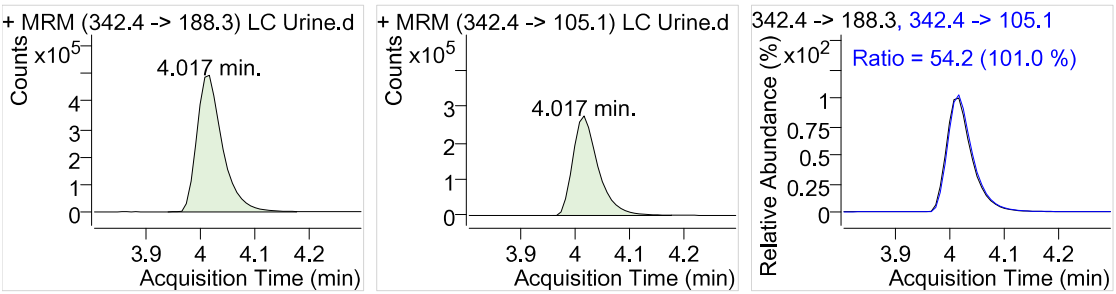
Norfentanyl D5



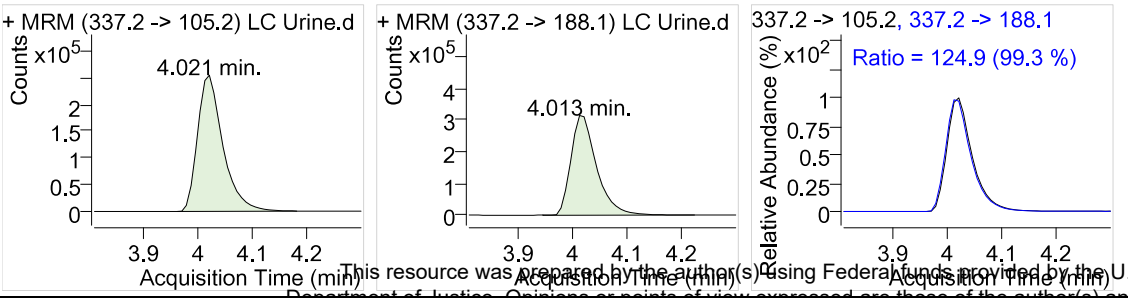
Norfentanyl



Fentanyl D5



Fentanyl



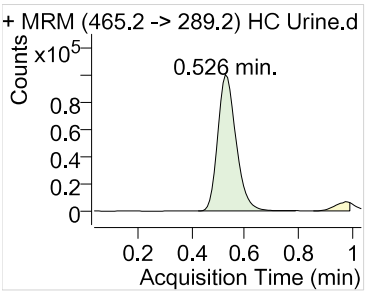
Quantitative Analysis Report

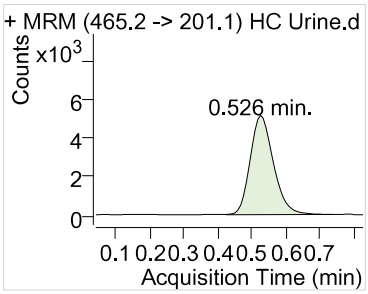
Agilent Technologies

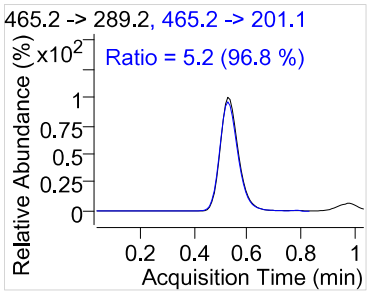
Sample Name:	:	HC Urine
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\HC Urine.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 8:34:56 PM
Dilution	:	1.0
Operator	:	
Sample Position	:	P1-B5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	472852			
	465.2 -> 201.1		24422	5.2	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	1169961			324.9 ng/ml
	462.2 -> 201.1		73177	6.3	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.92	78378			
	292.2 -> 128.2		11946	15.2	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.55	2275115			320.5 ng/ml
	272.1 -> 165.1		1883232	82.8	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.92	78378			
	292.2 -> 128.2		11946	15.2	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.94	51568			334.6 ng/ml
	286.1 -> 128.1		29029	56.3	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.97	35765			
	465.2 -> 165.1		2782	7.8	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	0.99	131816			252.9 ng/ml
	462.2 -> 165.0		10937	8.3	7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.43	398965			
Norfentanyl	233.1 -> 84.1	2.45	1206412			34.3 ng/ml
	233.1 -> 150.1		36678	3.0	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.02	1295170			
	342.4 -> 105.1		683500	52.8	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.02	3121030			33.3 ng/ml
	337.2 -> 188.1		3849852	123.4	100.6 - 150.9	

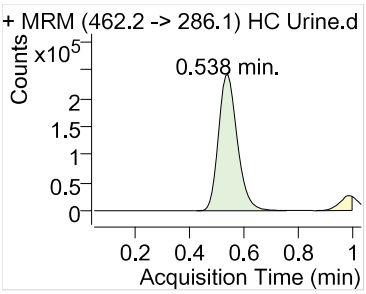
Morphine-3-B-D-Glucuronide D3

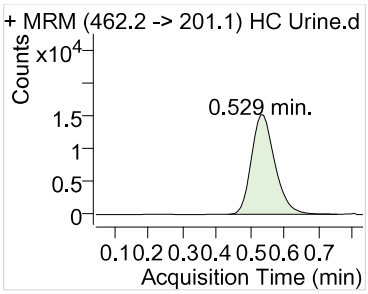


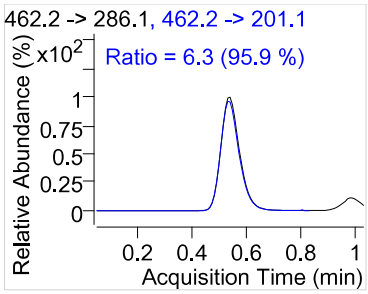




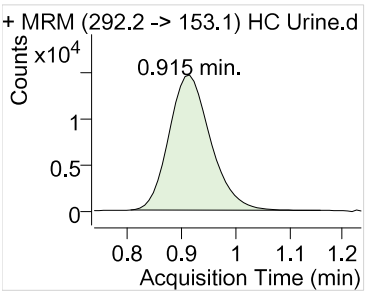
Morphine-3-B-D-Glucuronide

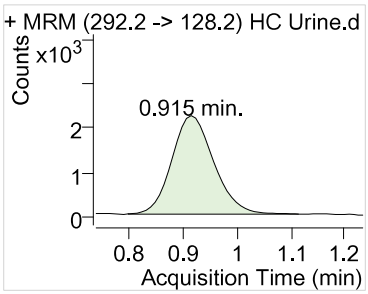


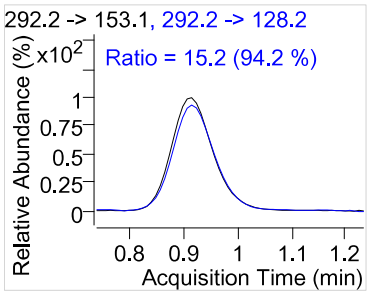




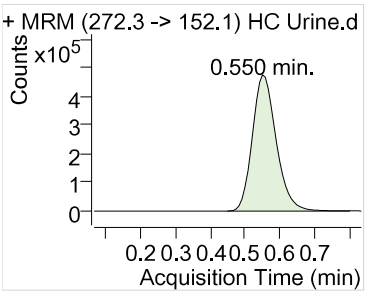
Morphine D6

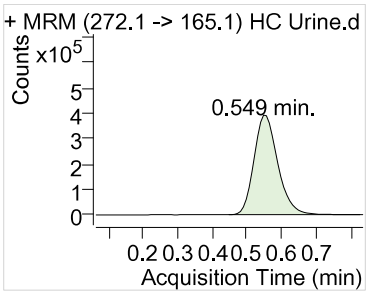


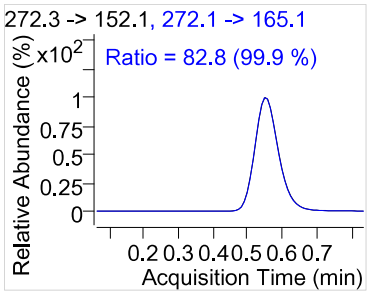




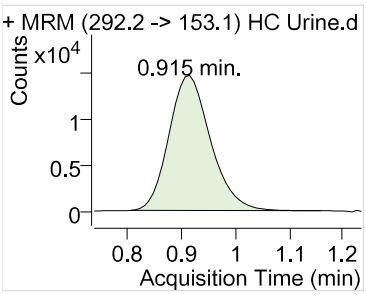
Normorphine

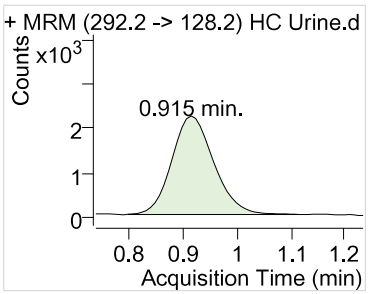


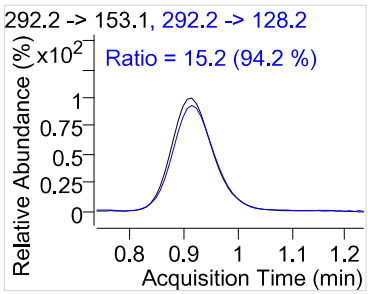




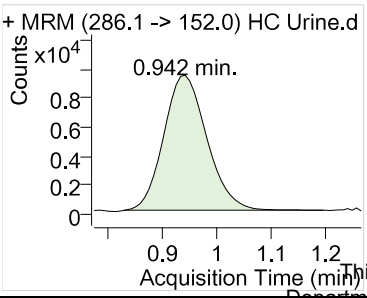
Morphine D6

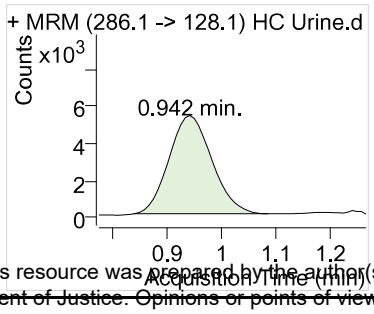


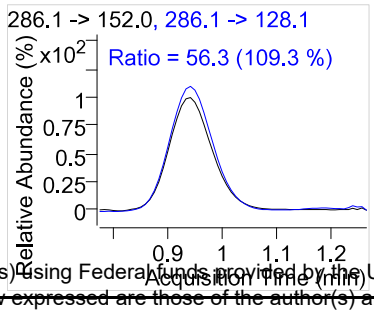




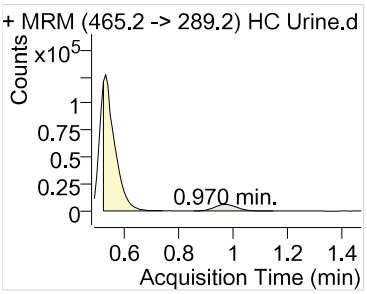
Morphine

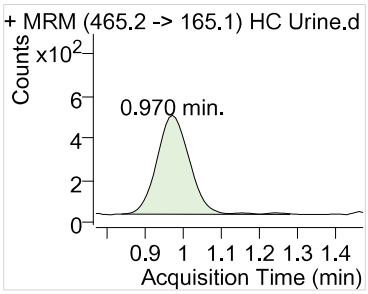


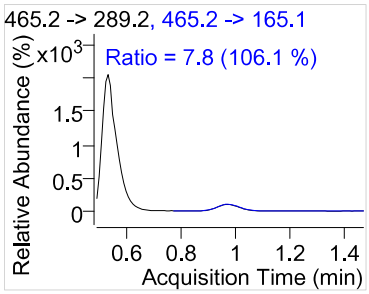




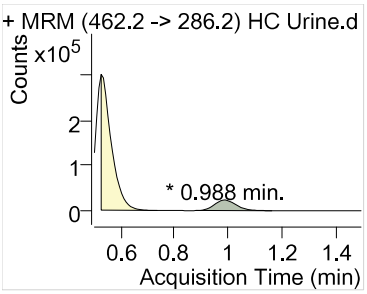
Morphine-6-B-D-Glucuronide D3

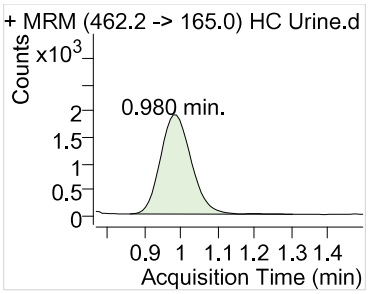


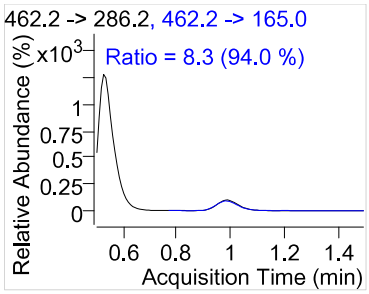




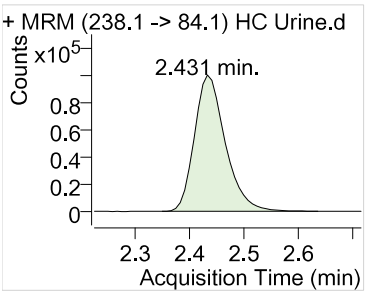
Morphine-6-B-D-Glucuronide



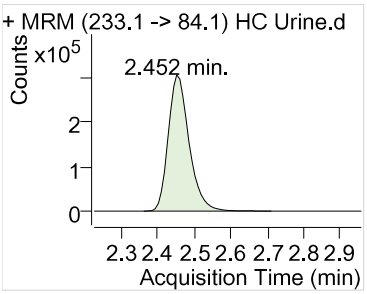


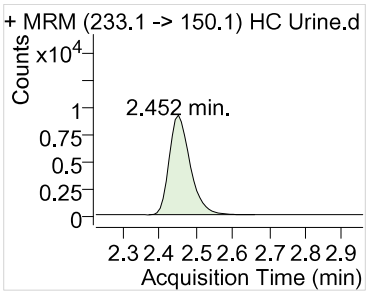


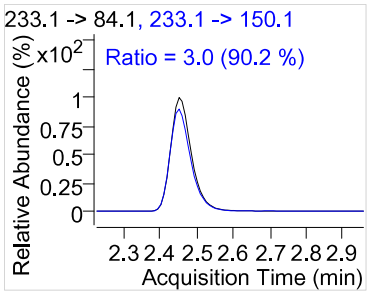
Norfentanyl D5



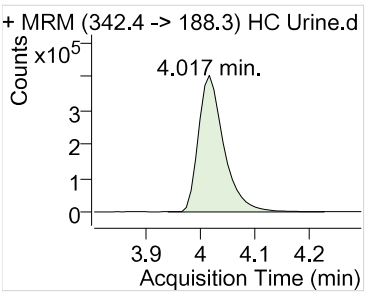
Norfentanyl

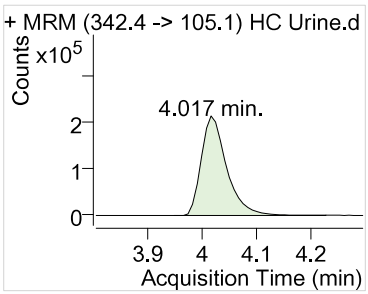


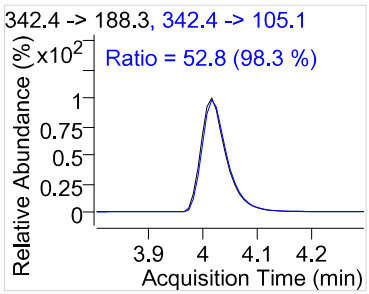




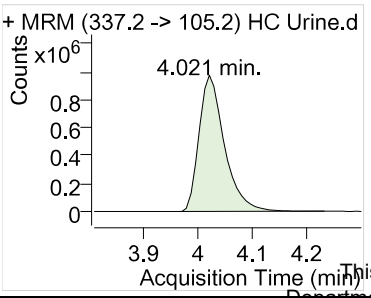
Fentanyl D5

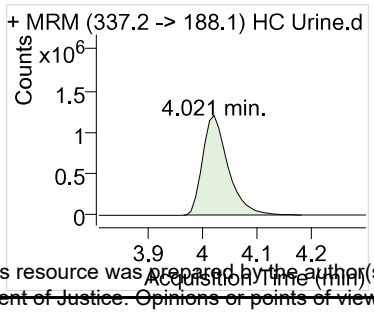


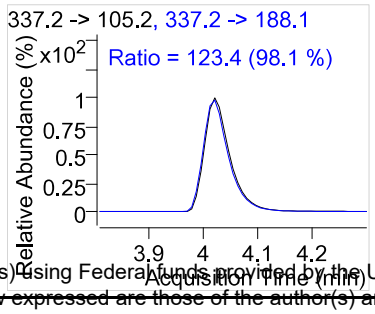




Fentanyl







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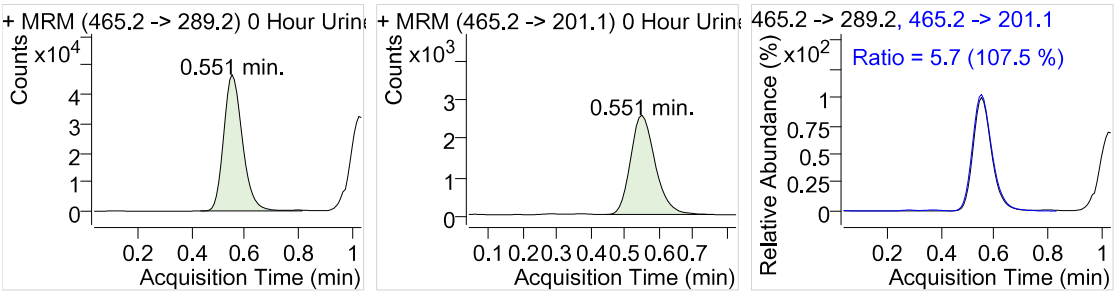
Quantitative Analysis Report

Agilent Technologies

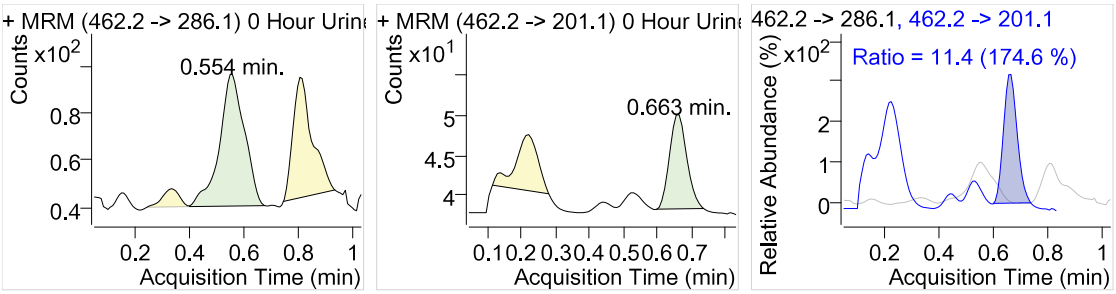
Sample Name:	:	0 Hour Urine 4
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\0 Hour Urine 4.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/7/2018 10:19:56 PM
Dilution	:	10.0
Operator	:	
Sample Position	:	P1-C7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	225013			
	465.2 -> 201.1		12915	5.7	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	359			77.0 ng/ml
	462.2 -> 201.1		41	*11.4	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.97	88051			
	292.2 -> 128.2		13056	14.8	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.57	501			97.0 ng/ml
	272.1 -> 165.1				66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.97	88051			
	292.2 -> 128.2		13056	14.8	12.9 - 19.4	
Morphine	286.1 -> 152.0	*1.04	790			21.3 ng/ml
	286.1 -> 128.1		646	*81.8	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	*1.03	168250			
	465.2 -> 165.1		12291	7.3	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*1.38	816			85.6 ng/ml
	462.2 -> 165.0				7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.45	596294			
Norfentanyl	233.1 -> 84.1	2.48	2238			9.0 ng/ml
	233.1 -> 150.1		67	3.0	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.02	1154362			
	342.4 -> 105.1		673905	58.4	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.02	7491			4.0 ng/ml
	337.2 -> 188.1		4693	*62.6	100.6 - 150.9	

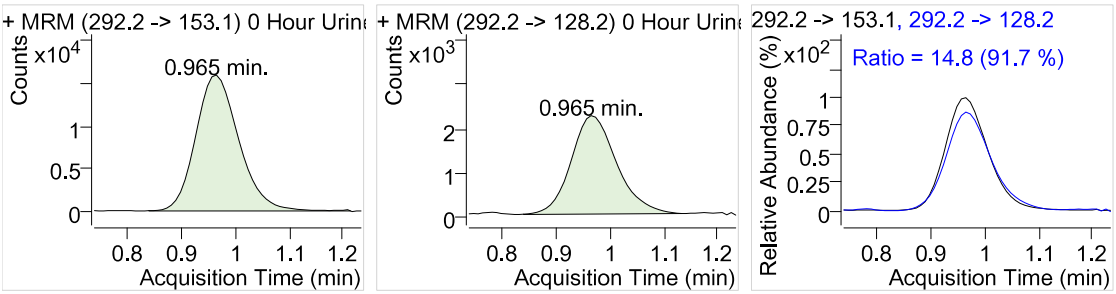
Morphine-3-B-D-Glucuronide D3



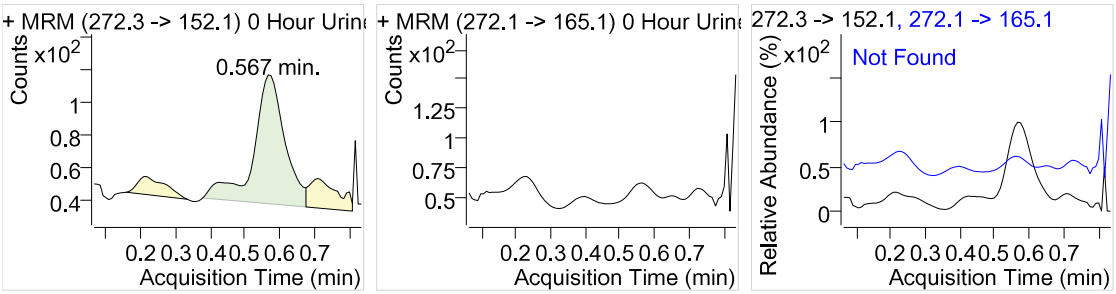
Morphine-3-B-D-Glucuronide



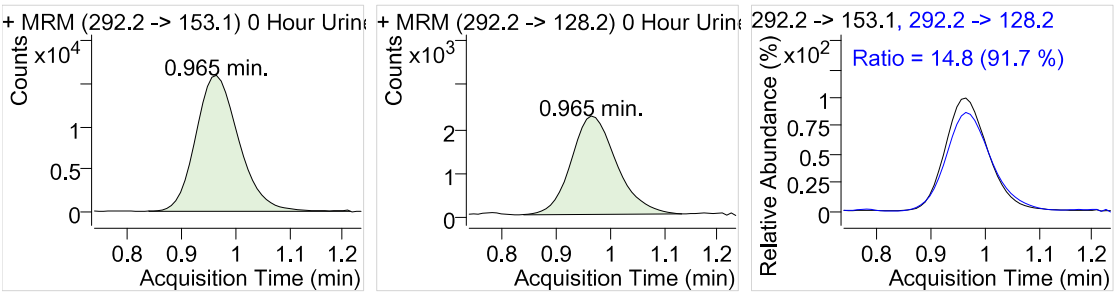
Morphine D6



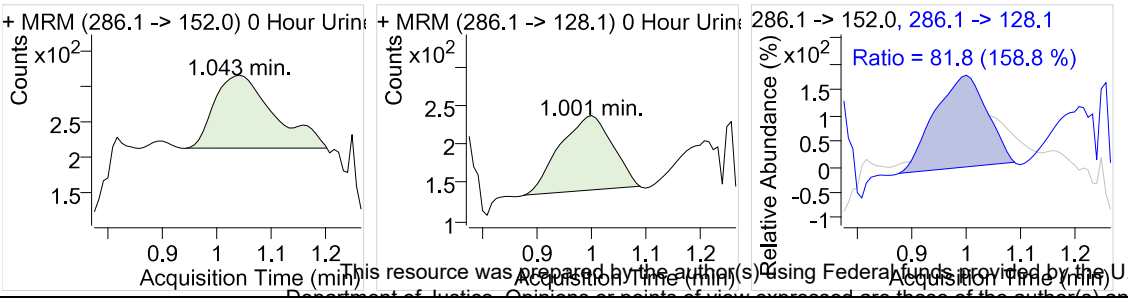
Normorphine



Morphine D6



Morphine



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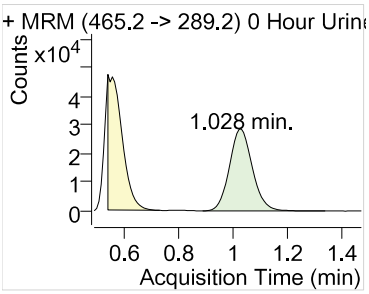
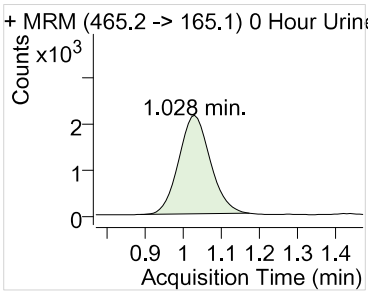
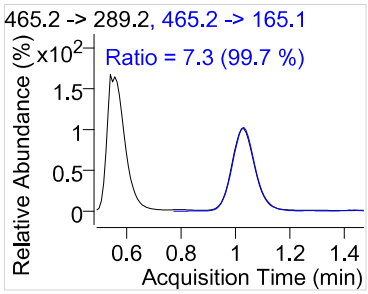
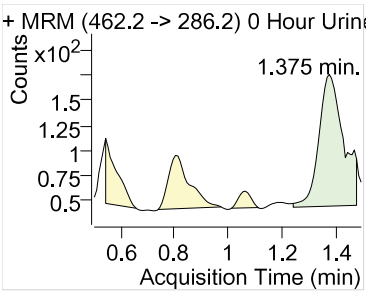
Morphine-6-B-D-Glucuronide D3

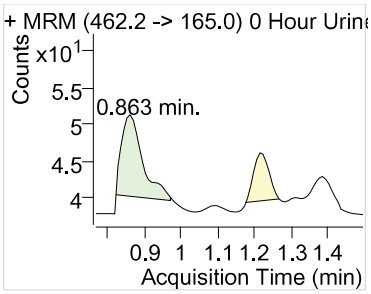


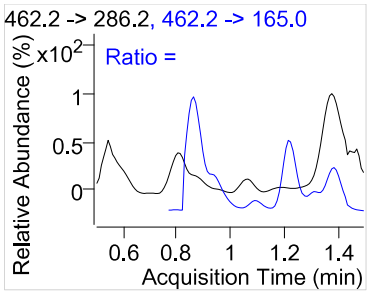




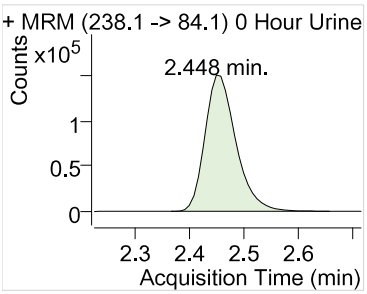
Morphine-6-B-D-Glucuronide



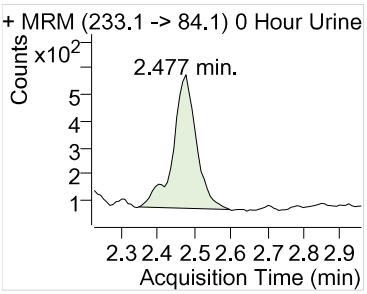


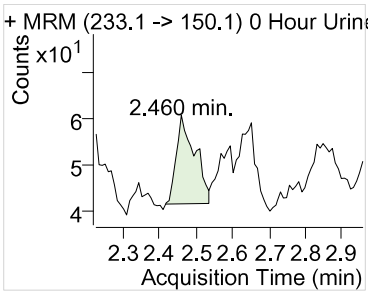


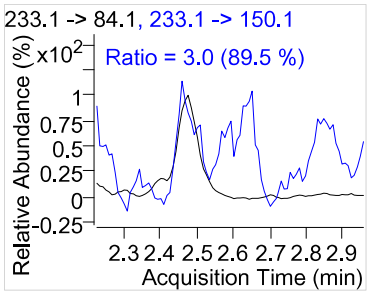
Norfentanyl D5



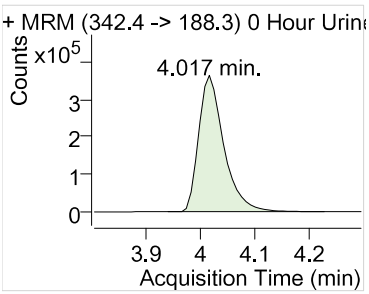
Norfentanyl

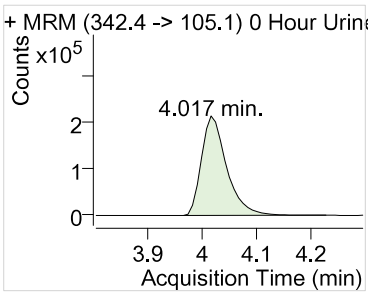


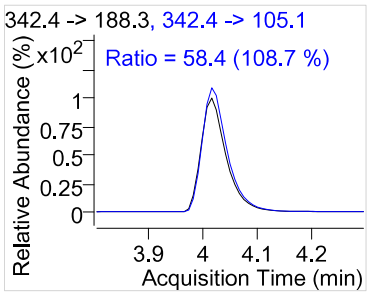




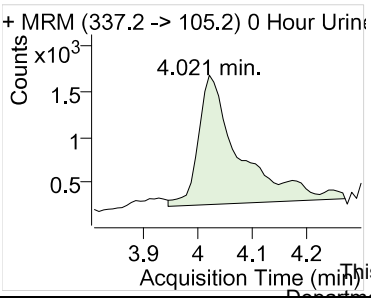
Fentanyl D5

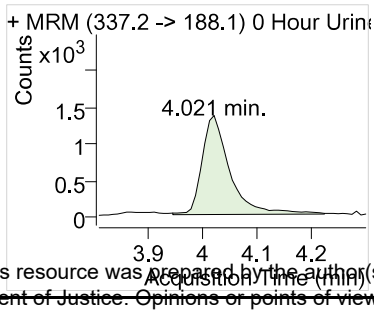


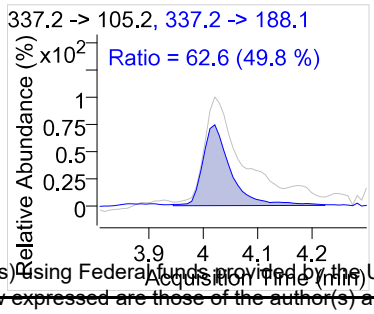




Fentanyl







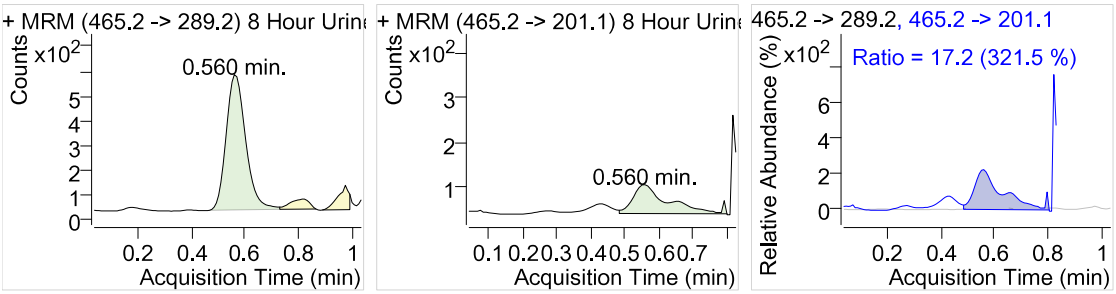
Quantitative Analysis Report

Agilent Technologies

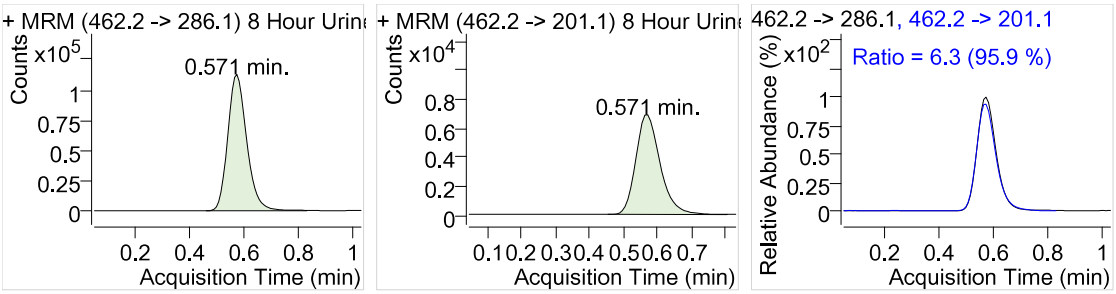
Sample Name:	:	8 Hour Urine 3
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\8 Hour Urine 3.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 12:14:21 AM
Dilution	:	10.0
Operator	:	
Sample Position	:	P1-D7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	2716			
	465.2 -> 201.1		466	*17.2	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.57	549977			143200.3 ng/ml
	462.2 -> 201.1		34382	6.3	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	*0.84	1052			
	292.2 -> 128.2		582	*55.3	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.58	169528			20236.0 ng/ml
	272.1 -> 165.1		141108	83.2	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	*0.84	1052			
	292.2 -> 128.2		582	*55.3	12.9 - 19.4	
Morphine	286.1 -> 152.0	*0.88	4569094			2239863.5 ng/ml
	286.1 -> 128.1		2842598	*62.2	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.97	309			
	465.2 -> 165.1		2342	*756.8	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	406602			147725.9 ng/ml
	462.2 -> 165.0		28	*0.0	7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.50	617354			
Norfentanyl	233.1 -> 84.1	2.51	2670			9.1 ng/ml
	233.1 -> 150.1		234	*8.8	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.02	910862			
	342.4 -> 105.1		568842	62.5	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.02	8466			4.4 ng/ml
	337.2 -> 188.1		3920	*46.3	100.6 - 150.9	

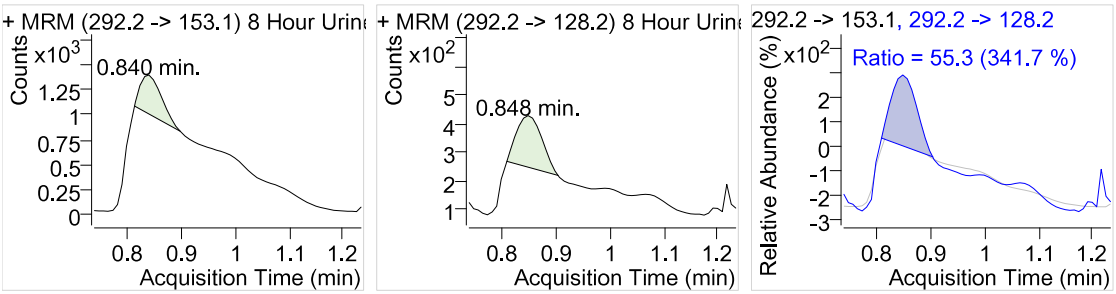
Morphine-3-B-D-Glucuronide D3



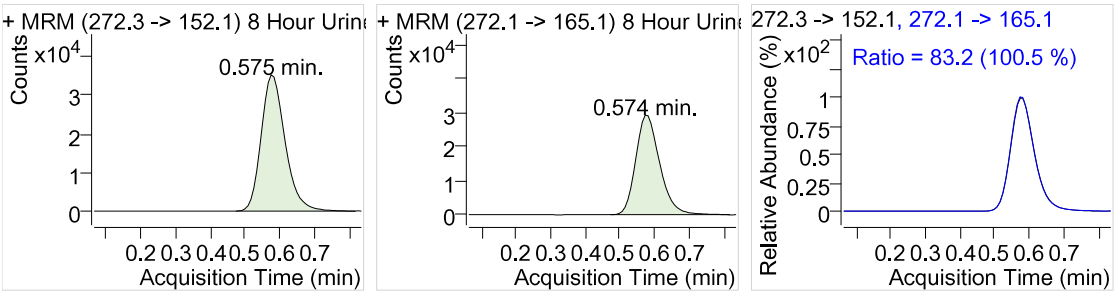
Morphine-3-B-D-Glucuronide



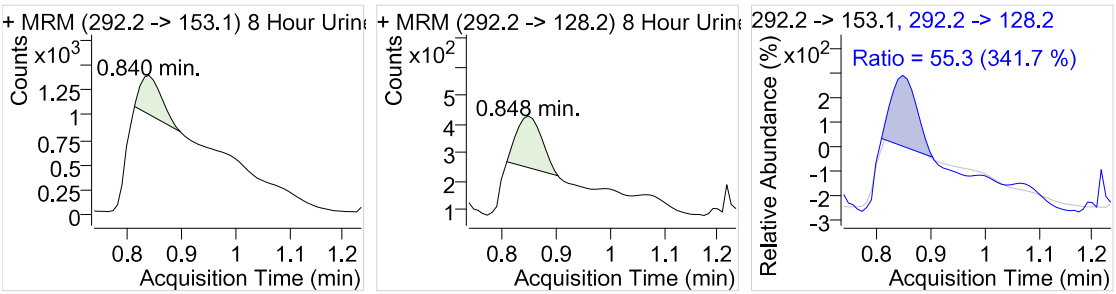
Morphine D6



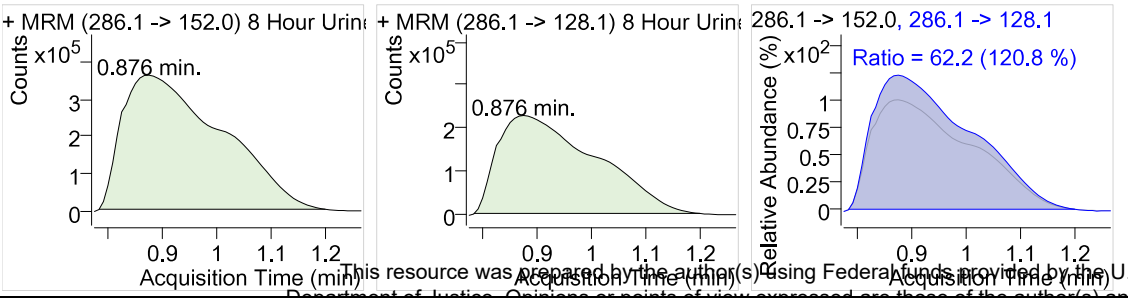
Normorphine



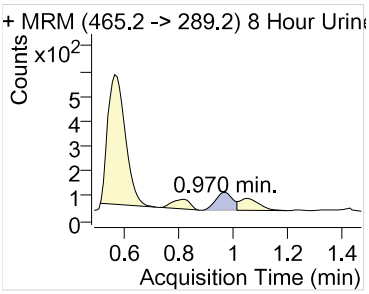
Morphine D6

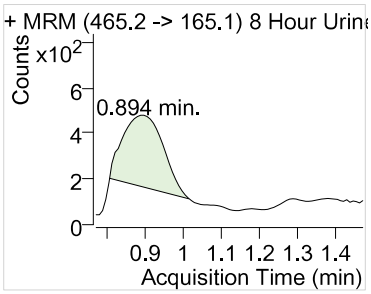


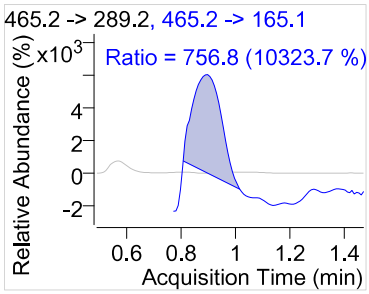
Morphine



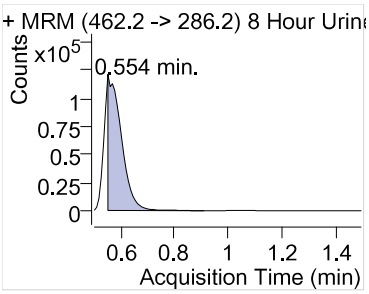
Morphine-6-B-D-Glucuronide D3

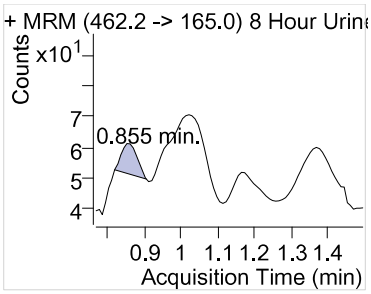


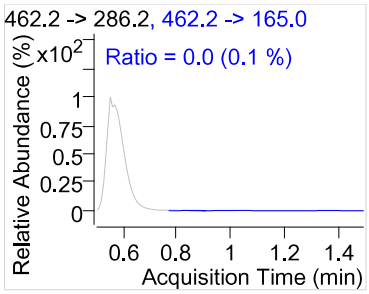




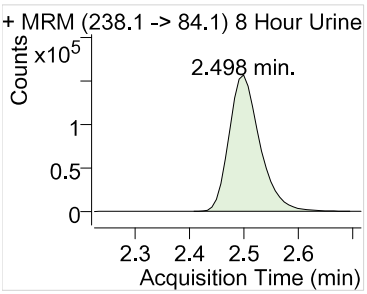
Morphine-6-B-D-Glucuronide



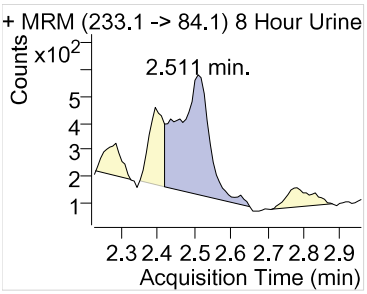


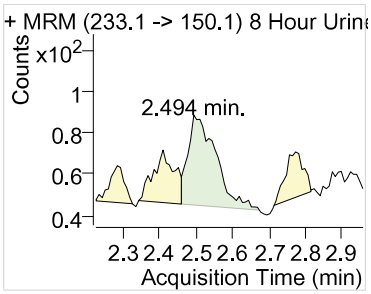


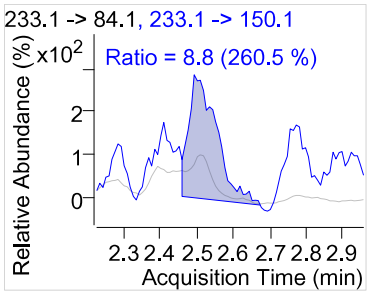
Norfentanyl D5



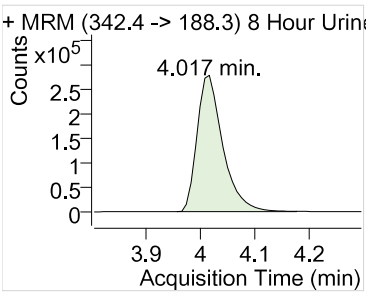
Norfentanyl

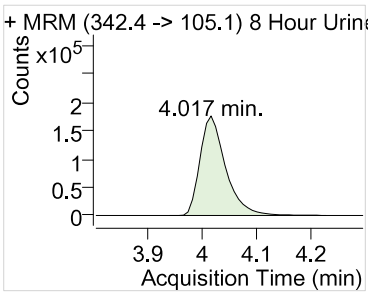


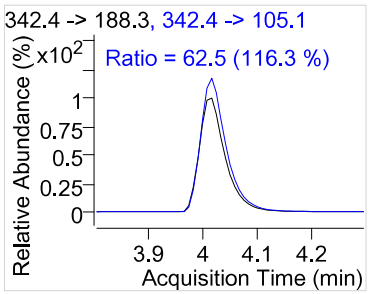




Fentanyl D5







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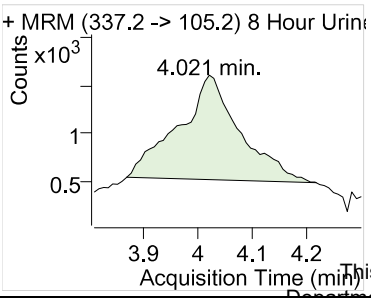

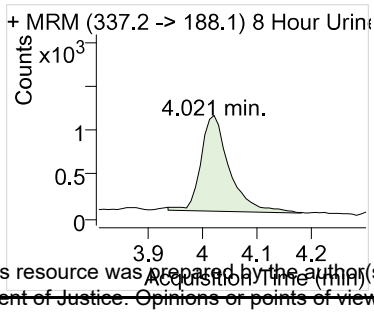

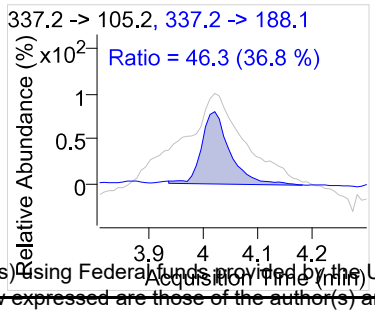

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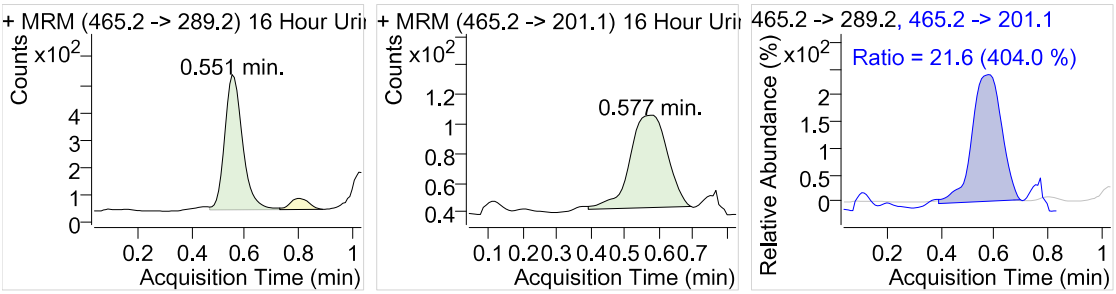
Quantitative Analysis Report

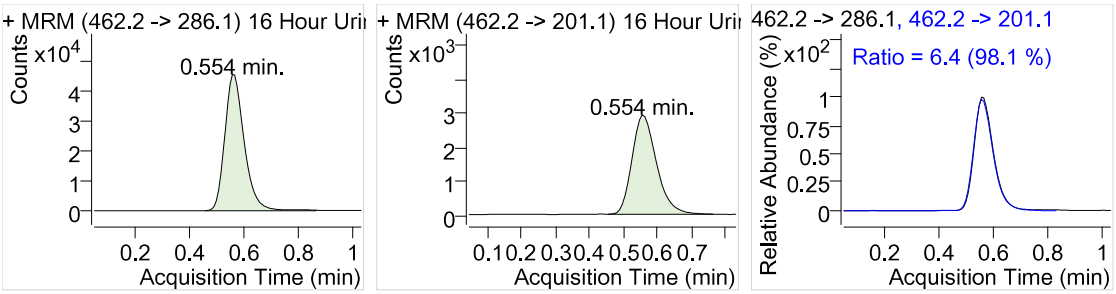
Sample Name: : 16 Hour Urine 1
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\16 Hour Urine 1.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 7/8/2018 1:30:38 AM
Dilution : 10.0
Operator :
Sample Position : P1-E4

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	2257			
	465.2 -> 201.1		487	*21.6	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	228386			87235.0 ng/ml
	462.2 -> 201.1		14615	6.4	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.90	26494			
	292.2 -> 128.2		4086	15.4	12.9 - 19.4	
Normorphine	272.3 -> 152.1	0.57	4870068			23940.9 ng/ml
	272.1 -> 165.1		4106752	84.3	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.90	26494			
	292.2 -> 128.2		4086	15.4	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.93	1871572			201852.5 ng/ml
	286.1 -> 128.1		1157347	*61.8	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	*1.03	644			
	465.2 -> 165.1		2132	*331.3	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	163301			58399.0 ng/ml
	462.2 -> 165.0		24	*0.0	7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.46	629323			
Norfentanyl	233.1 -> 84.1	2.49	2777			9.1 ng/ml
	233.1 -> 150.1		162	*5.9	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.01	1329939			
	342.4 -> 105.1		805458	60.6	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.01	5104			3.6 ng/ml
	337.2 -> 188.1		5477	107.3	100.6 - 150.9	

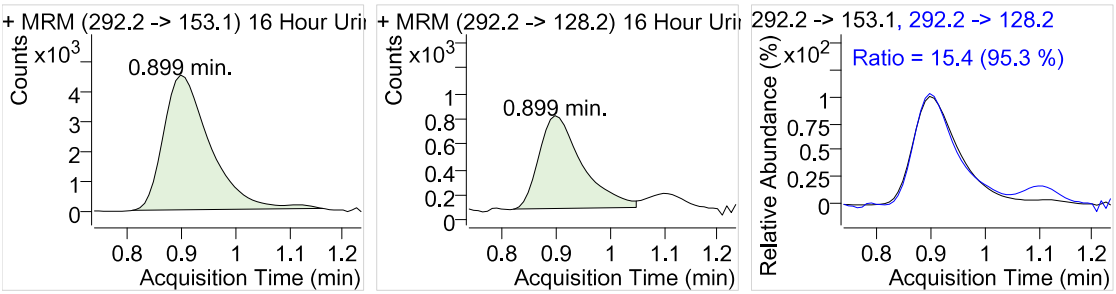
Morphine-3-B-D-Glucuronide D3



Morphine-3-B-D-Glucuronide



Morphine D6



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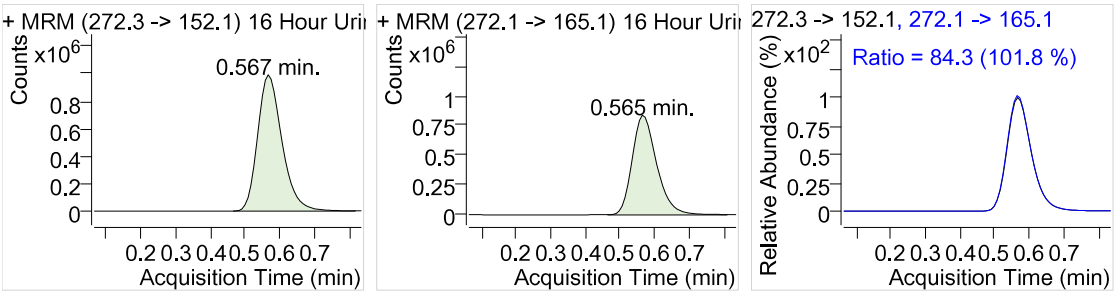

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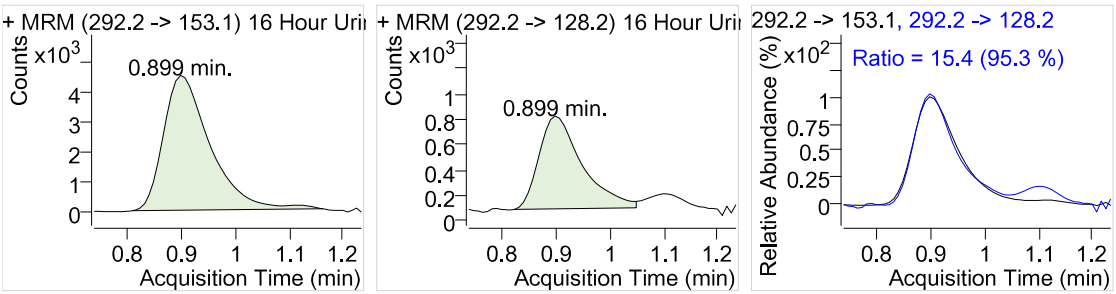

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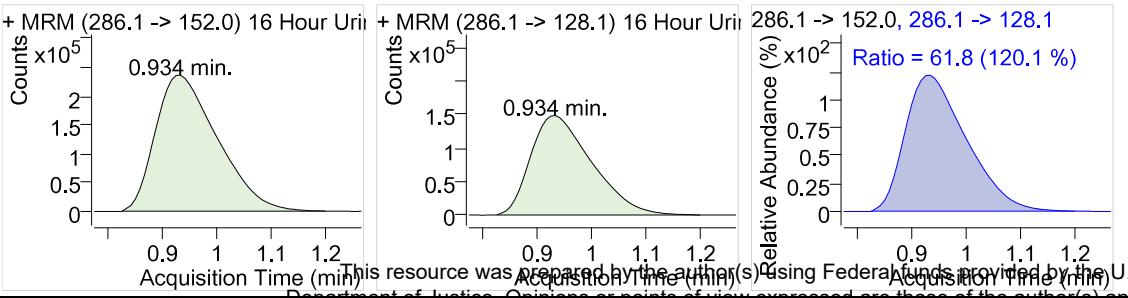
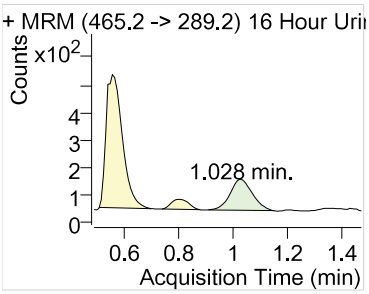
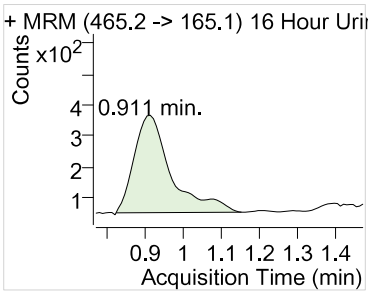
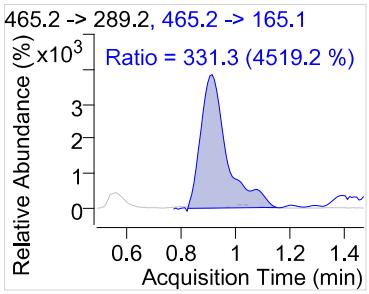


Morphine-6-B-D-Glucuronide D3







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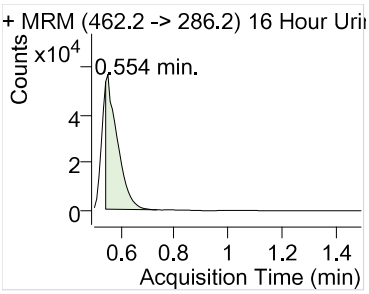

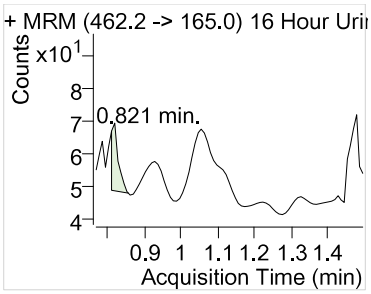

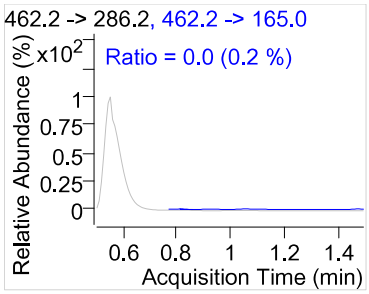

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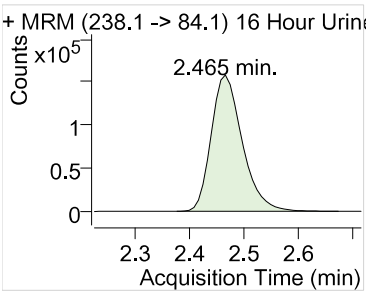

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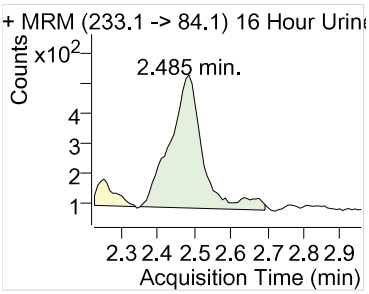

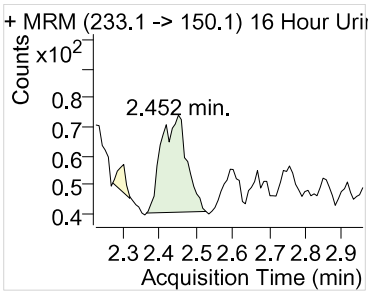

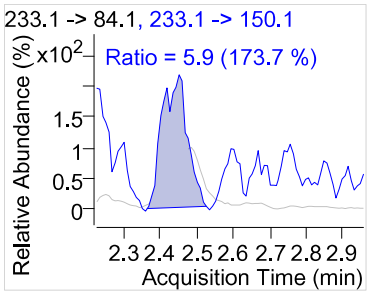

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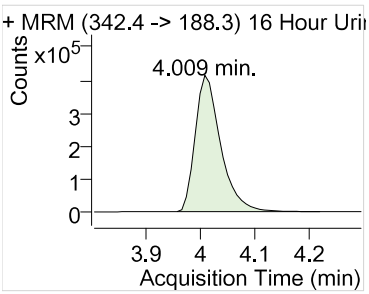

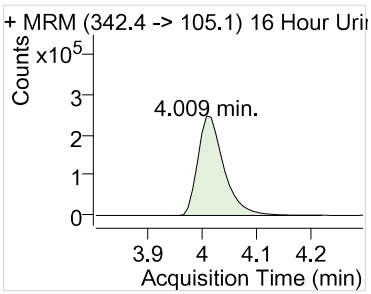

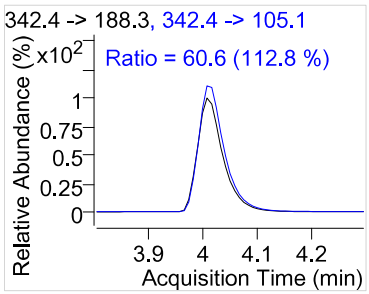

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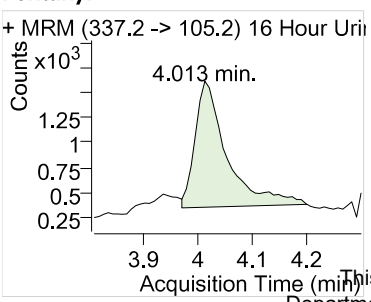
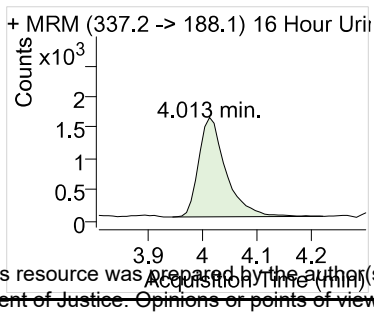
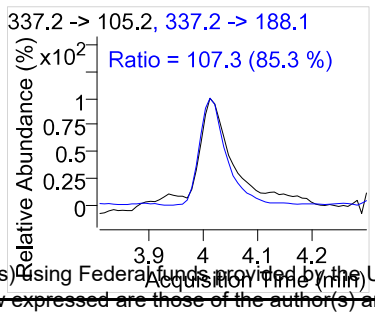






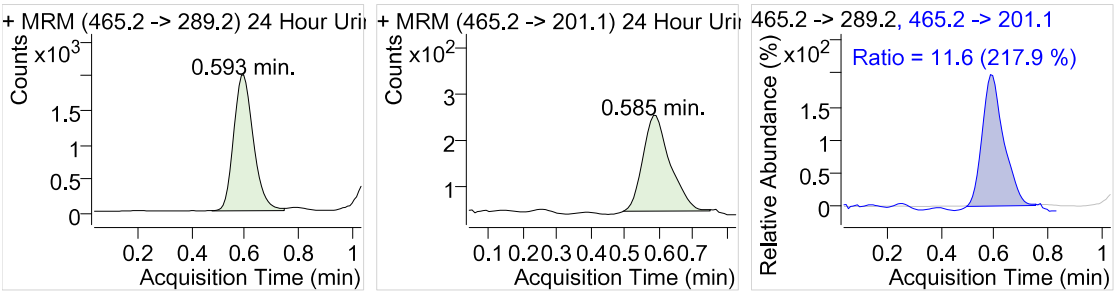
Quantitative Analysis Report

Agilent Technologies

Sample Name:	:	24 Hour Urine 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\Juneday2\24 Hour Urine 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	7/8/2018 4:02:58 AM
Dilution	:	10.0
Operator	:	
Sample Position	:	P1-F8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	*0.59	9996			
	465.2 -> 201.1		1163	*11.6	4.3 - 6.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.60	334817			36136.5 ng/ml
	462.2 -> 201.1		22568	6.7	5.2 - 7.8	
Morphine D6	292.2 -> 153.1	0.96	39515			
	292.2 -> 128.2		7078	17.9	12.9 - 19.4	
Normorphine	272.3 -> 152.1	*0.59	1486633			4157.5 ng/ml
	272.1 -> 165.1		1246751	83.9	66.3 - 99.4	
Morphine D6	292.2 -> 153.1	0.96	39515			
	292.2 -> 128.2		7078	17.9	12.9 - 19.4	
Morphine	286.1 -> 152.0	0.99	1300522			116209.9 ng/ml
	286.1 -> 128.1		793317	61.0	41.2 - 61.8	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	*1.05	2865			
	465.2 -> 165.1		114	*4.0	5.9 - 8.8	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.60	303866			34127.2 ng/ml
	462.2 -> 165.0		161	*0.1	7.1 - 10.6	
Norfentanyl D5	238.1 -> 84.1	2.46	747928			
Norfentanyl	233.1 -> 84.1	2.48	2250			8.9 ng/ml
	233.1 -> 150.1		140	*6.2	2.7 - 4.0	
Fentanyl D5	342.4 -> 188.3	4.01	1894156			
	342.4 -> 105.1		1093731	57.7	42.9 - 64.4	
Fentanyl	337.2 -> 105.2	4.01	8993			3.8 ng/ml
	337.2 -> 188.1		7106	*79.0	100.6 - 150.9	

Morphine-3-B-D-Glucuronide D3



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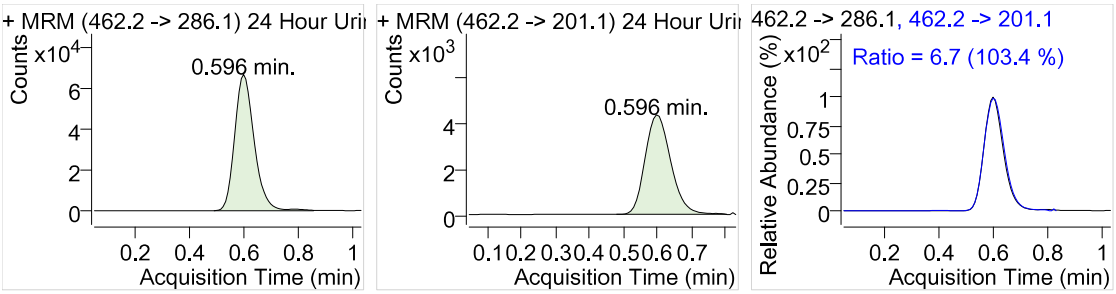

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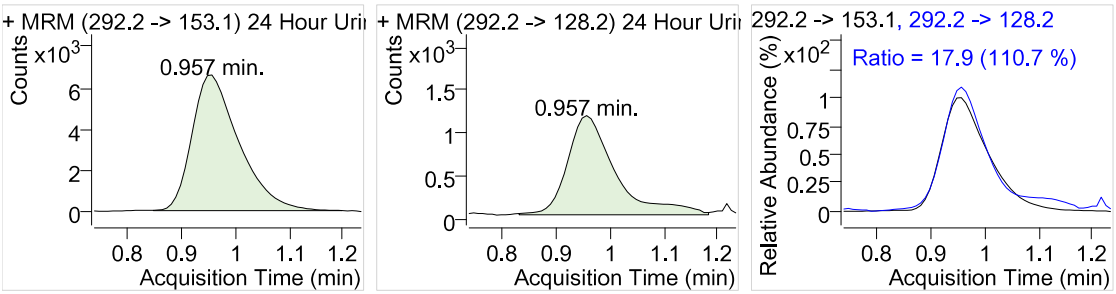

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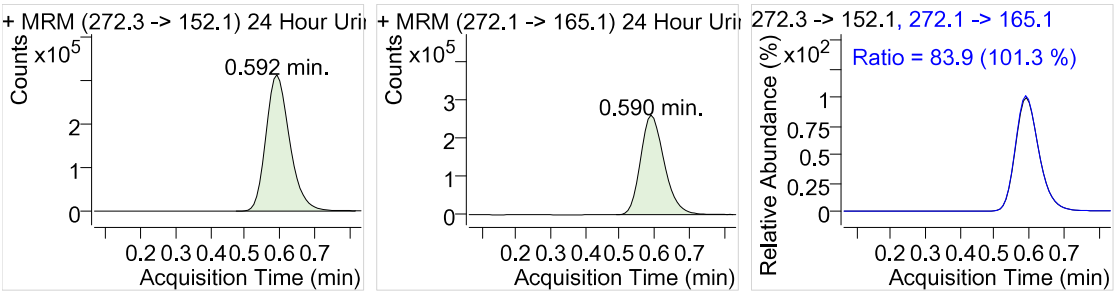

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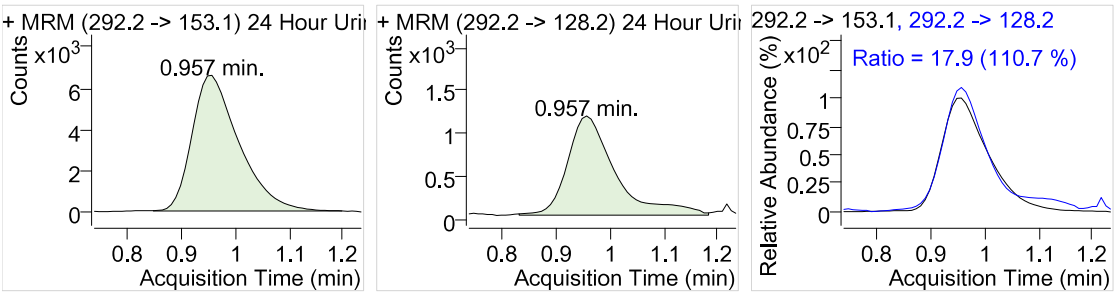

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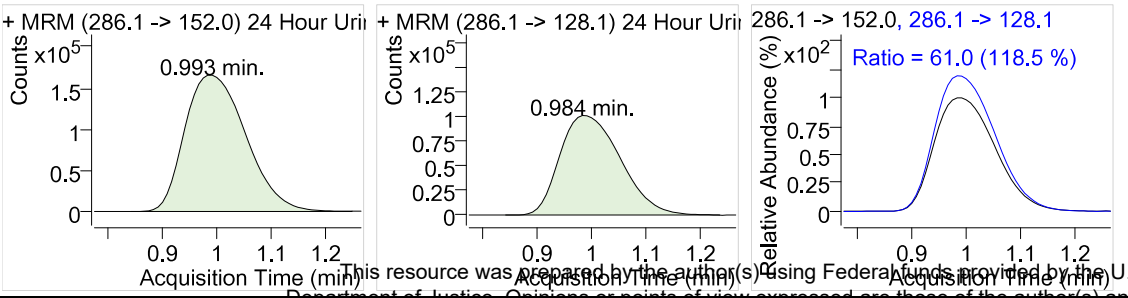

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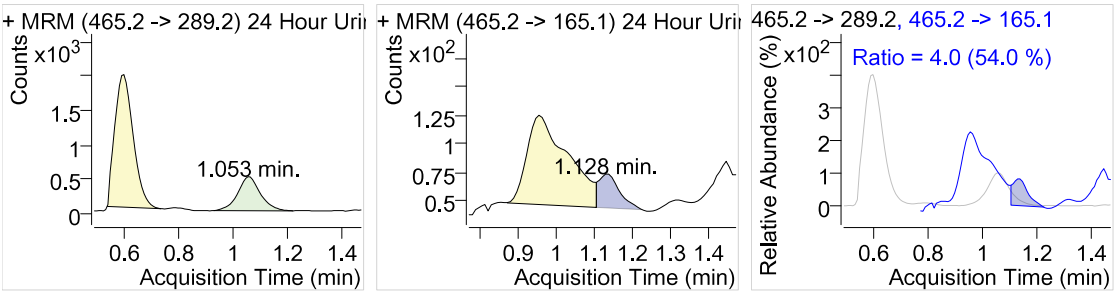
Morphine-6-B-D-Glucuronide D3



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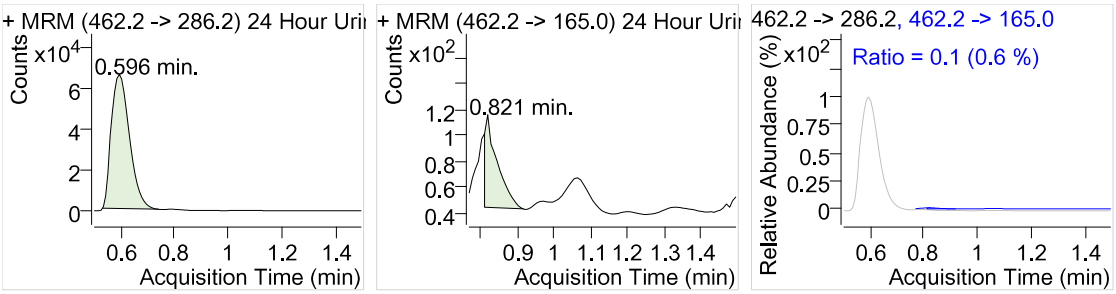
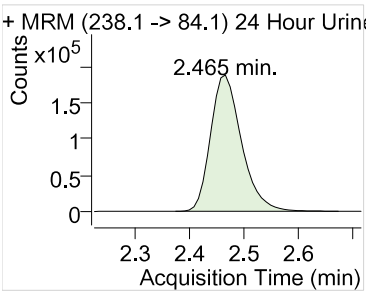
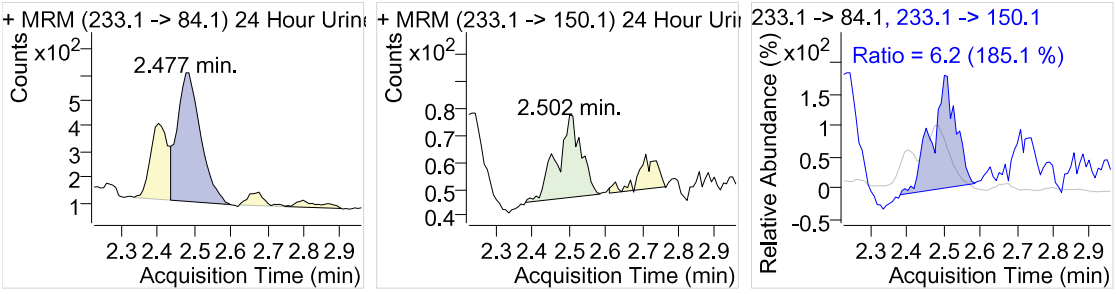


Norfentanyl D5



Norfentanyl



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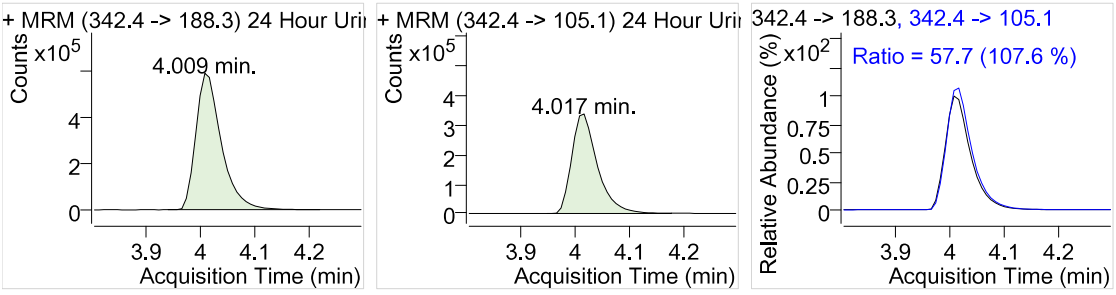

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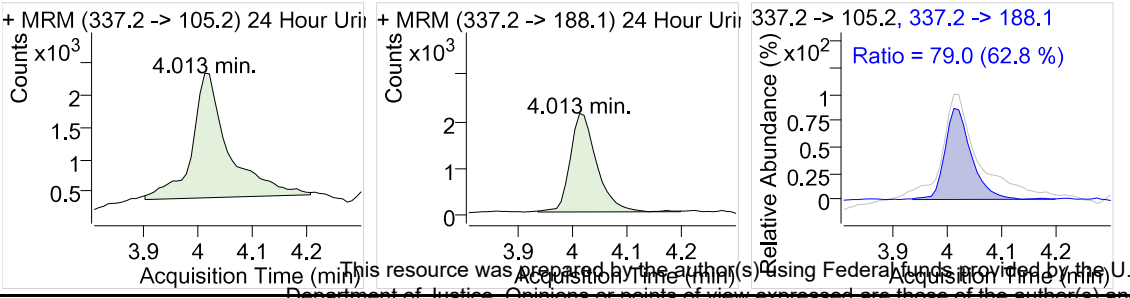


Appendix G

Quantitative Analysis Reports for Co-Administration of Morphine and Fentanyl Study

Table of Contents

Abbreviations

Conc: Concentration

FB: Femoral Blood

HB: Heart Blood

RT: Retention Time

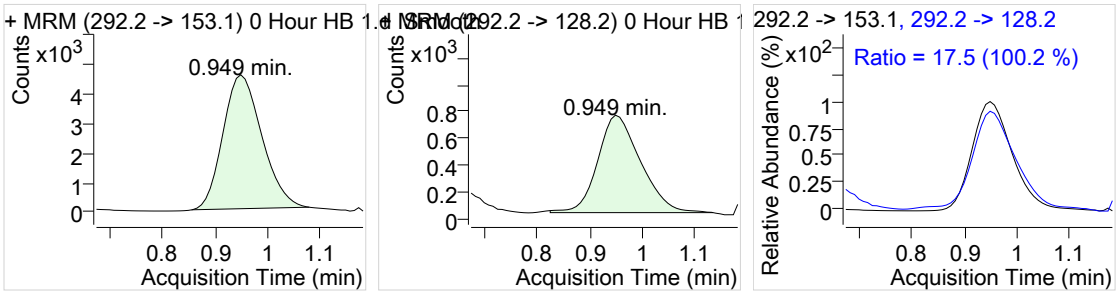
Quantitative Analysis Report

Agilent Technologies

Sample Name:	:	0 Hour HB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\0 Hour HB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/5/2019 8:11:56 PM
Dilution	:	0.5
Operator	:	
Sample Position	:	P1-B7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.95	23392			
	292.2 -> 128.2		4104	17.5	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.57	100416			42.8 ng/ml
	272.1 -> 165.1		78051	77.7	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	9120			
	465.2 -> 201.1		733	*8.0	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.56	169514			1242.1 ng/ml
	462.2 -> 201.1		12545	7.4	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.95	23392			
	292.2 -> 128.2		4104	17.5	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.98	110341			963.9 ng/ml
	286.1 -> 128.1		65154	59.0	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	31200			
	465.2 -> 165.1		3167	10.2	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.56	115534			90.2 ng/ml
	462.2 -> 165.0		295	*0.3	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.43	253450			
Norfentanyl	233.1 -> 84.1	2.45	14847			0.5 ng/ml
	233.1 -> 150.1		596	*4.0	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	4.00	424878			
	342.4 -> 105.1		323252	76.1	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.01	49040			0.9 ng/ml
	337.2 -> 188.1		43162	88.0	72.8 - 121.3	

Morphine D6



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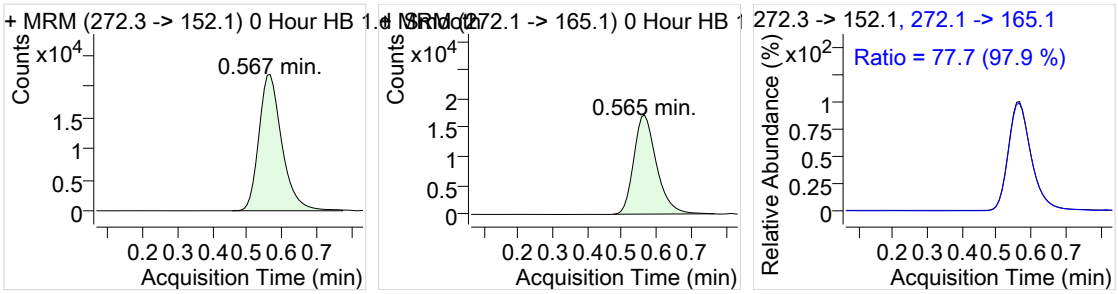

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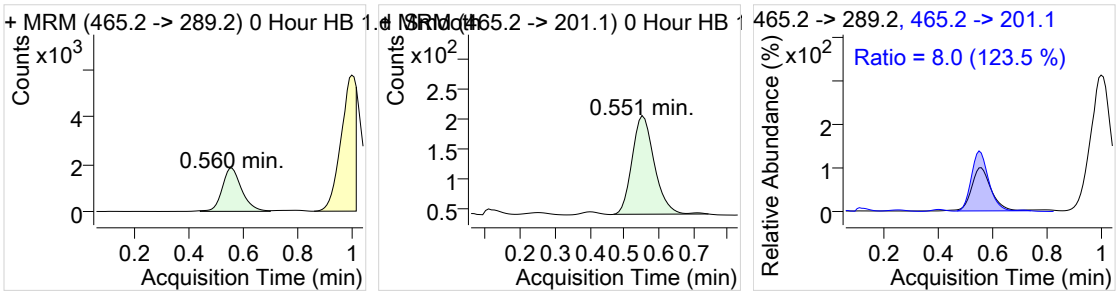

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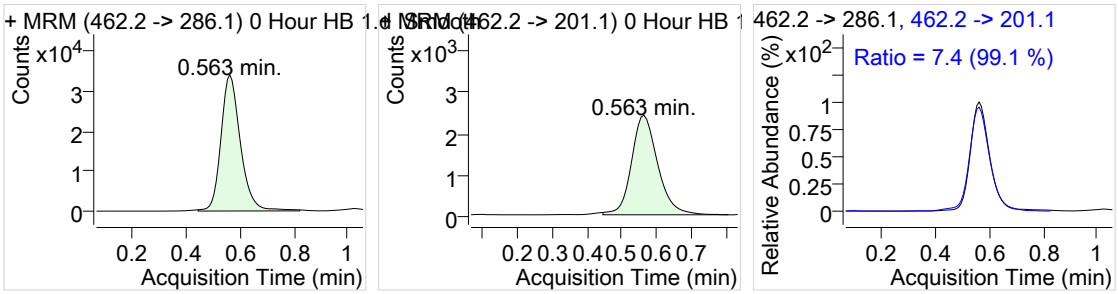

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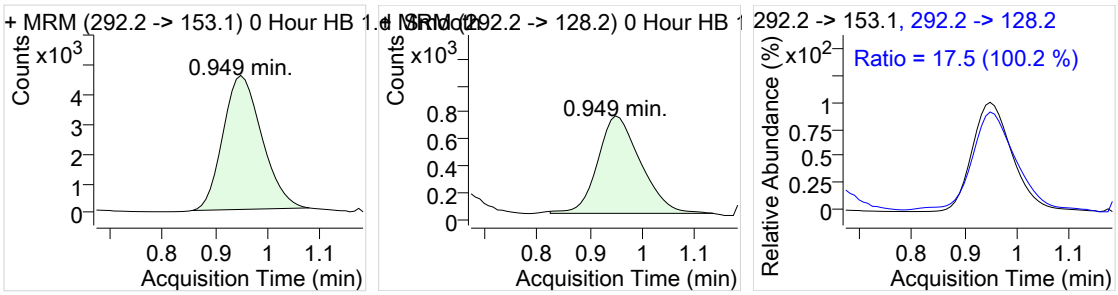

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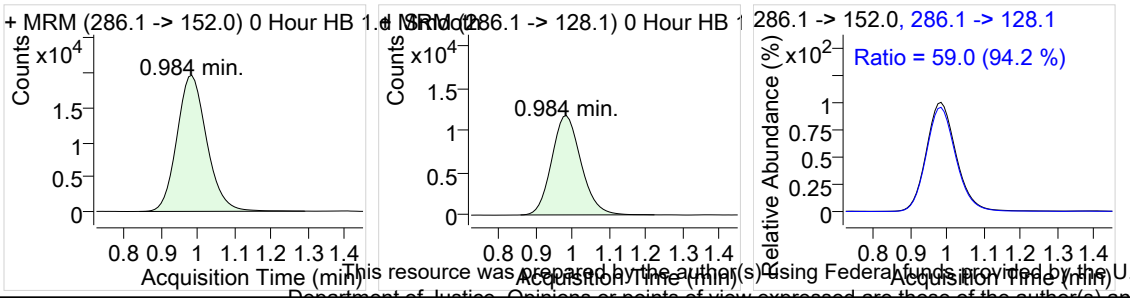
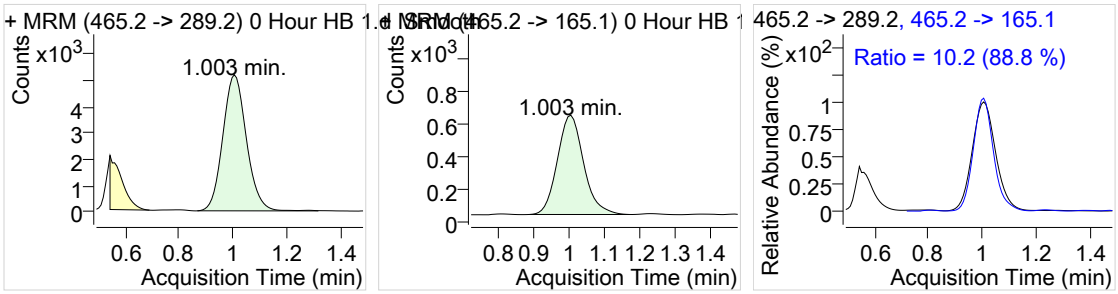
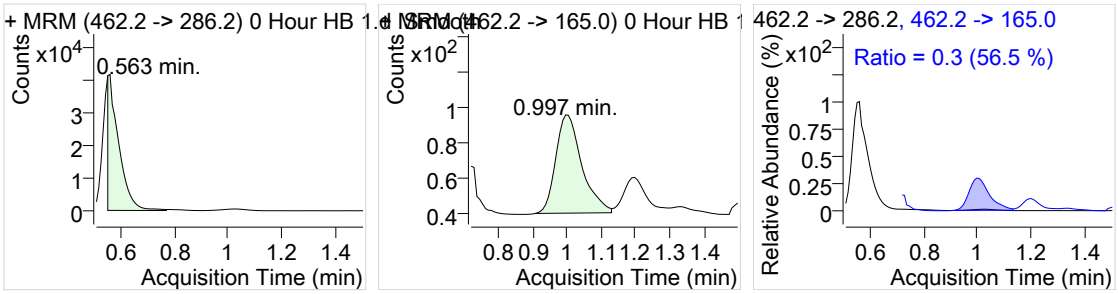


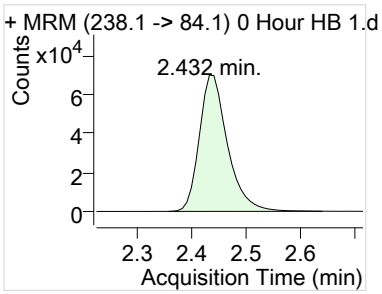
Morphine-6-B-D-Glucuronide D3



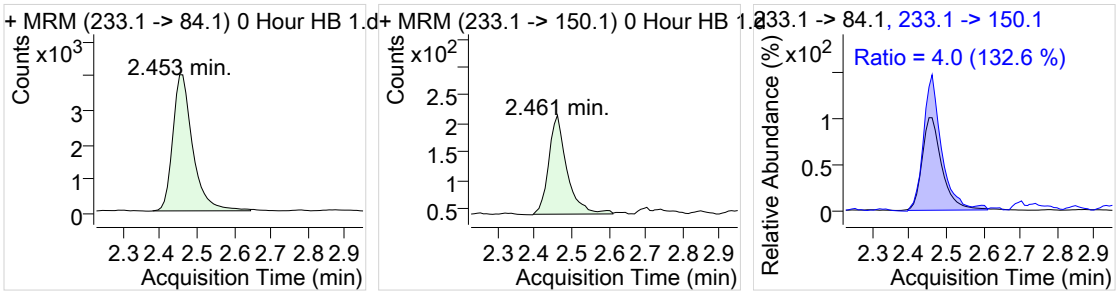
Morphine-6-B-D-Glucuronide



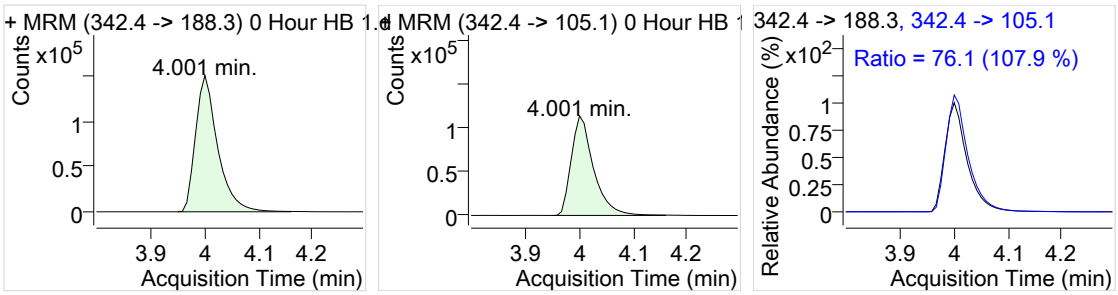
Norfentanyl D5



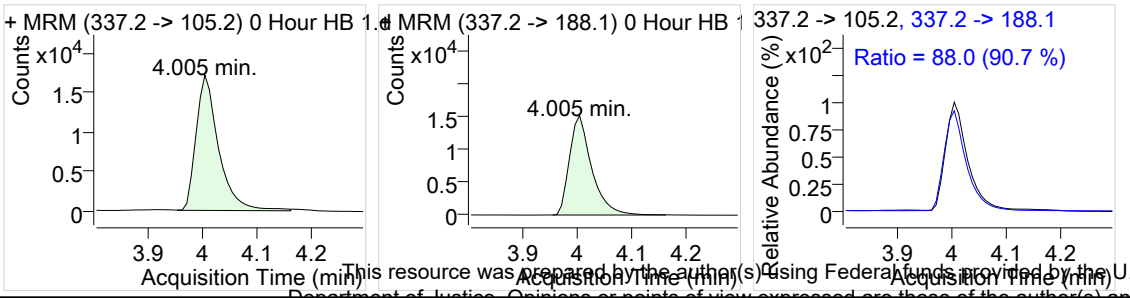
Norfentanyl



Fentanyl D5



Fentanyl



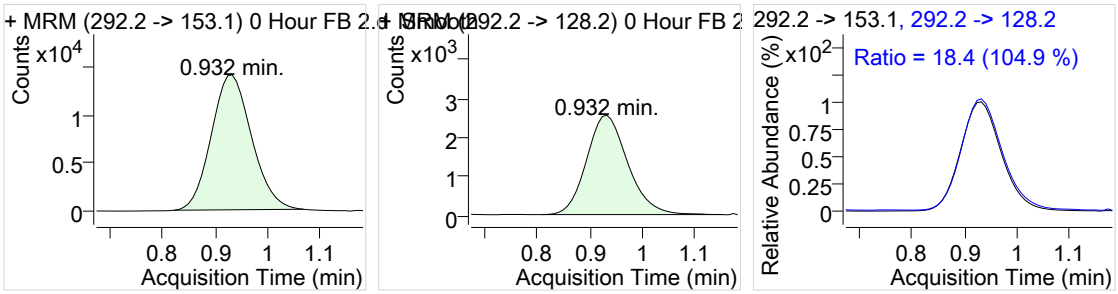
Quantitative Analysis Report

Agilent Technologies

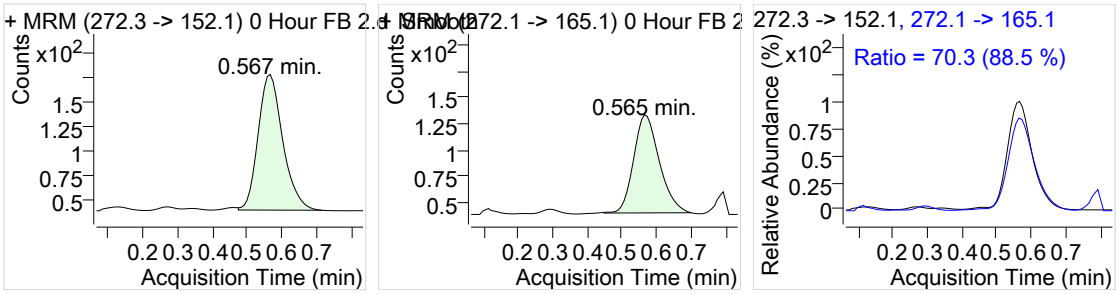
Sample Name:	:	0 Hour FB 2
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\0 Hour FB 2.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/5/2019 8:51:09 PM
Dilution	:	8.8
Operator	:	
Sample Position	:	P1-C2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.93	75251			
	292.2 -> 128.2		13824	18.4	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.57	682			35.5 ng/ml
	272.1 -> 165.1		479	70.3	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	50896			
	465.2 -> 201.1		3131	6.2	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.56	51945			787.8 ng/ml
	462.2 -> 201.1		4262	8.2	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.93	75251			
	292.2 -> 128.2		13824	18.4	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.95	15272			602.9 ng/ml
	286.1 -> 128.1		9625	63.0	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.97	27951			
	465.2 -> 165.1		3118	11.2	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	30810			554.0 ng/ml
	462.2 -> 165.0		41	*0.1	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.43	292200			
Norfentanyl	233.1 -> 84.1	2.45	514			2.5 ng/ml
	233.1 -> 150.1		53	*10.3	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.99	127240			
	342.4 -> 105.1		99738	78.4	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.00	446			1.3 ng/ml
	337.2 -> 188.1		545	*122.4	72.8 - 121.3	

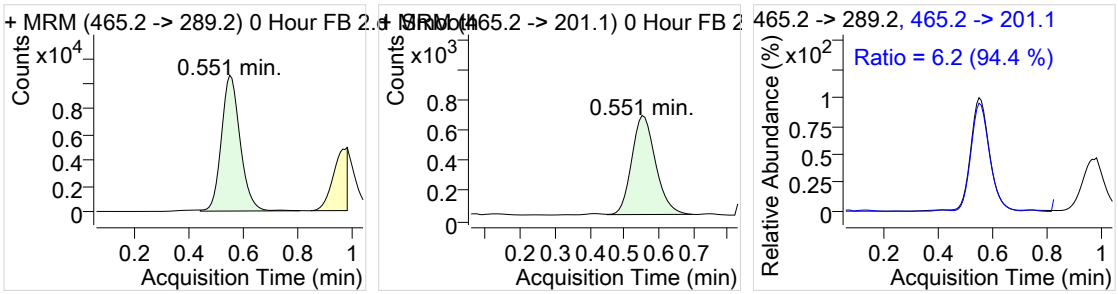
Morphine D6



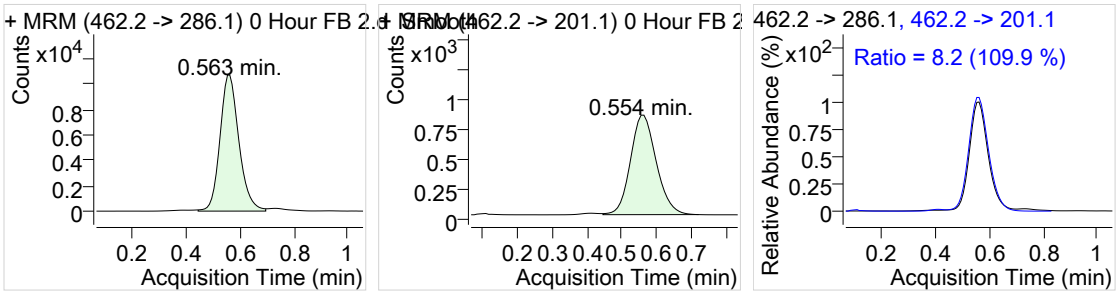
Normorphine



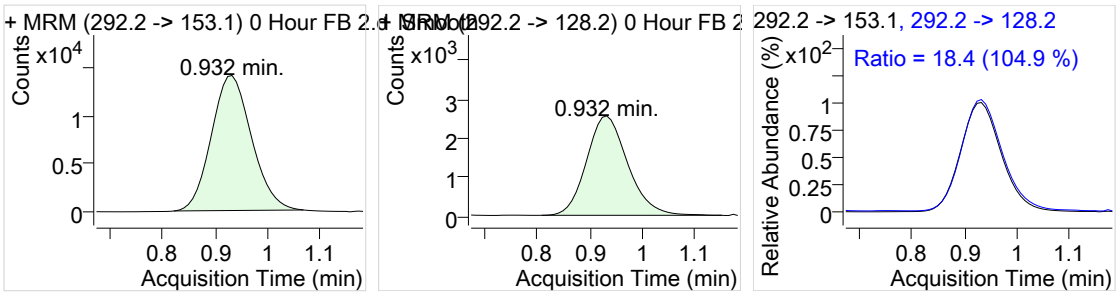
Morphine-3-B-D-Glucuronide D3



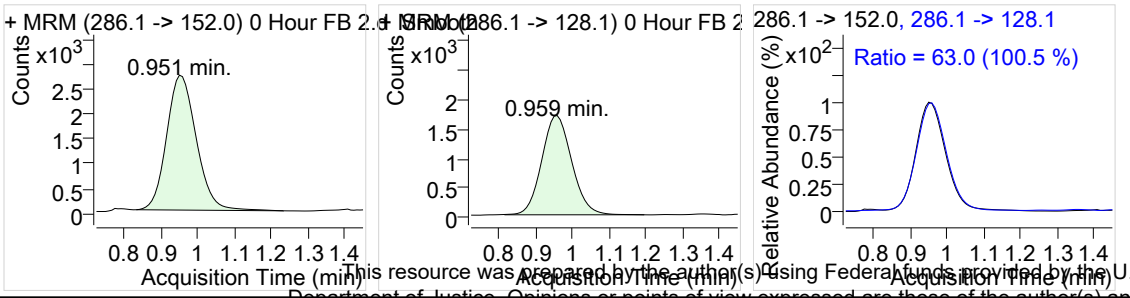
Morphine-3-B-D-Glucuronide



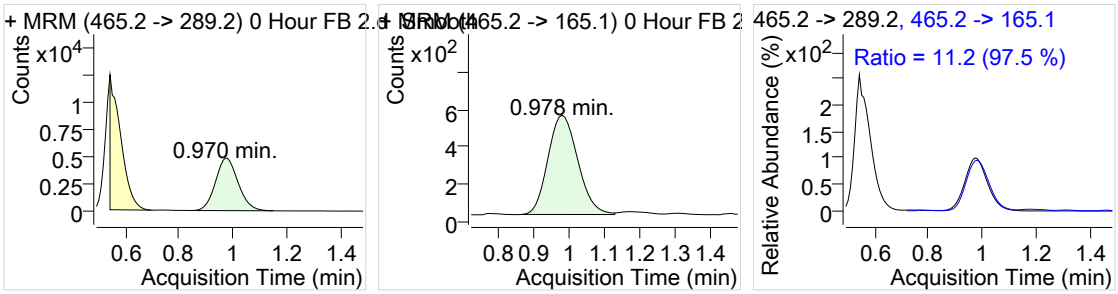
Morphine D6



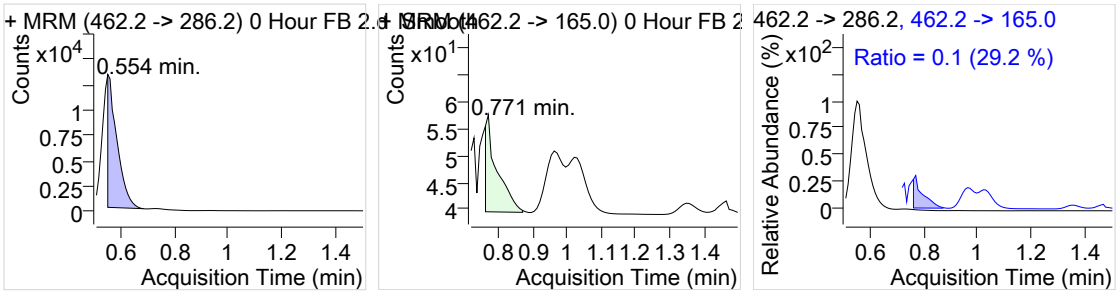
Morphine



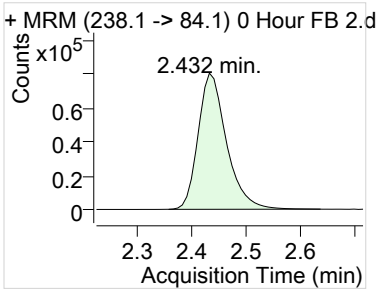
Morphine-6-B-D-Glucuronide D3



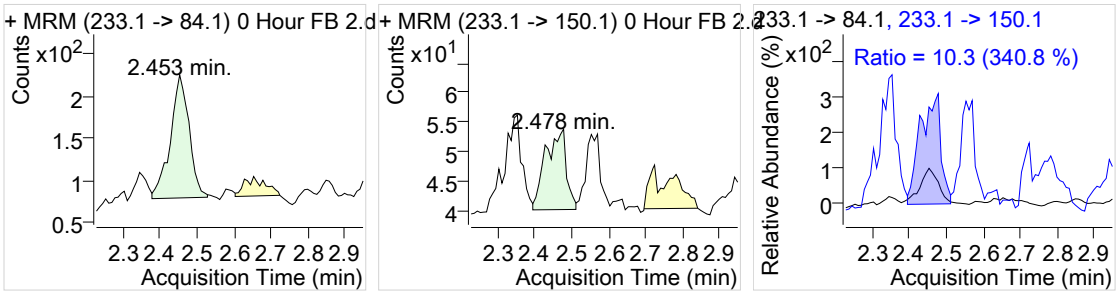
Morphine-6-B-D-Glucuronide



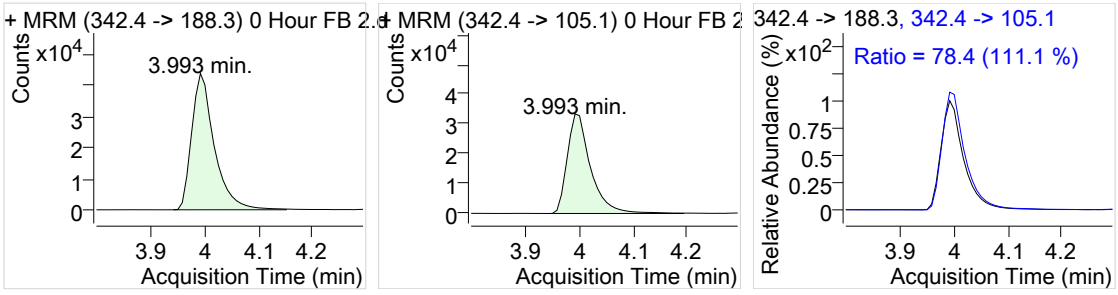
Norfentanyl D5



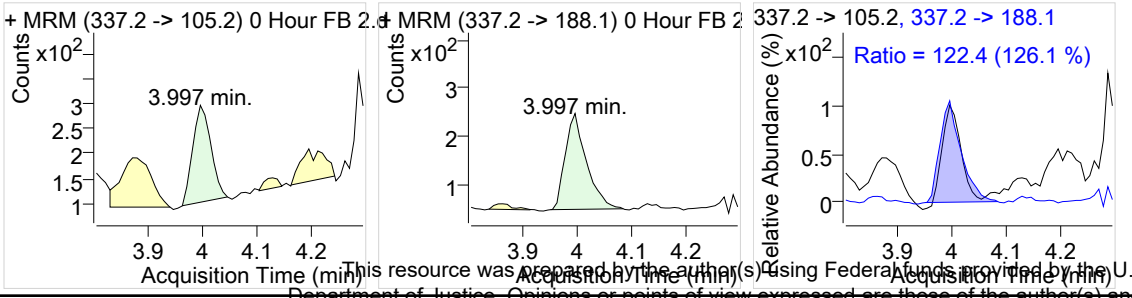
Norfentanyl



Fentanyl D5



Fentanyl



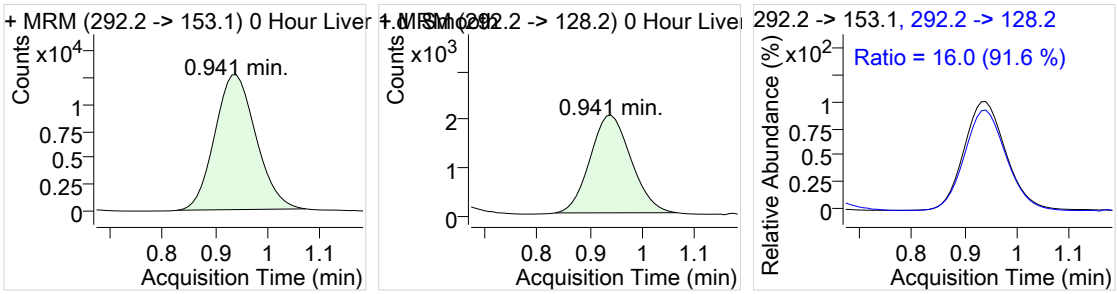
Quantitative Analysis Report

Agilent Technologies

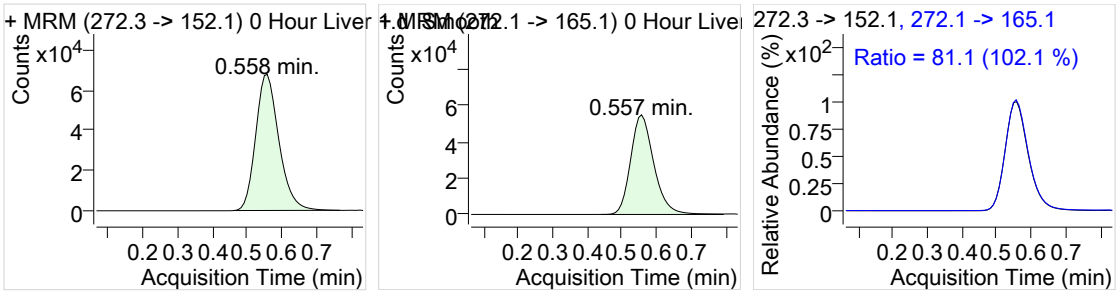
Sample Name:	:	0 Hour Liver 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\0 Hour Liver 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/5/2019 9:19:32 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C4

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.94	68642			
	292.2 -> 128.2		11004	16.0	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.56	314151			337.2 ng/ml
	272.1 -> 165.1		254643	81.1	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	61239			
	465.2 -> 201.1		3958	6.5	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	504865			3995.1 ng/ml
	462.2 -> 201.1		34829	6.9	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.94	68642			
	292.2 -> 128.2		11004	16.0	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.97	24000			484.4 ng/ml
	286.1 -> 128.1		14021	58.4	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	118257			
	465.2 -> 165.1		12597	10.7	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	338803			528.7 ng/ml
	462.2 -> 165.0		1461	0.4	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.44	342211			
Norfentanyl	233.1 -> 84.1	2.46	17736			3.3 ng/ml
	233.1 -> 150.1		512	2.9	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.99	449739			
	342.4 -> 105.1		346975	77.2	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.00	16139			2.3 ng/ml
	337.2 -> 188.1		9278	*57.5	72.8 - 121.3	

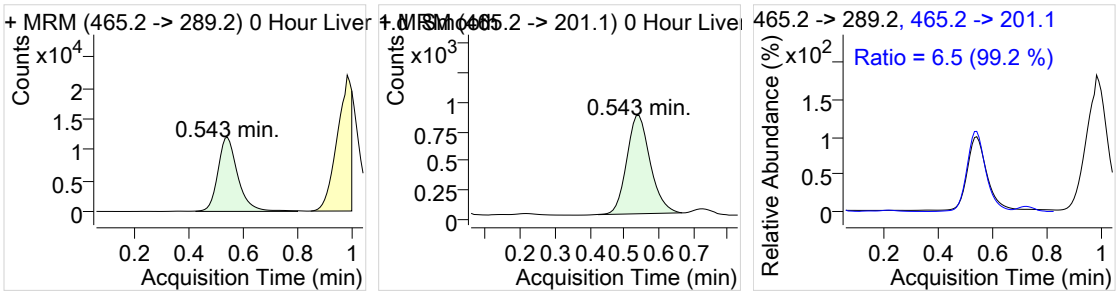
Morphine D6



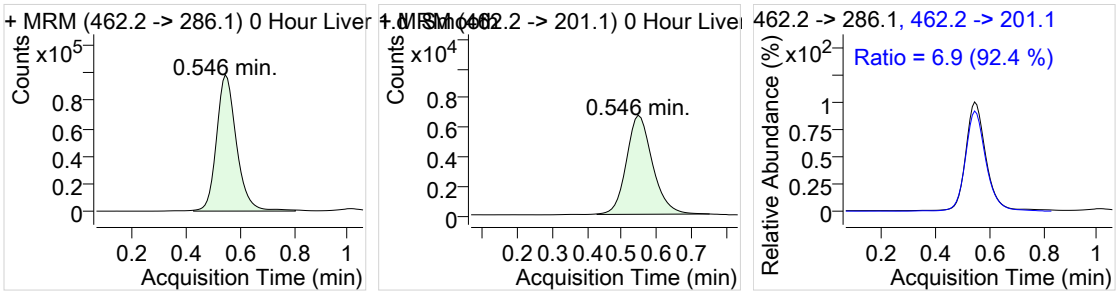
Normorphine



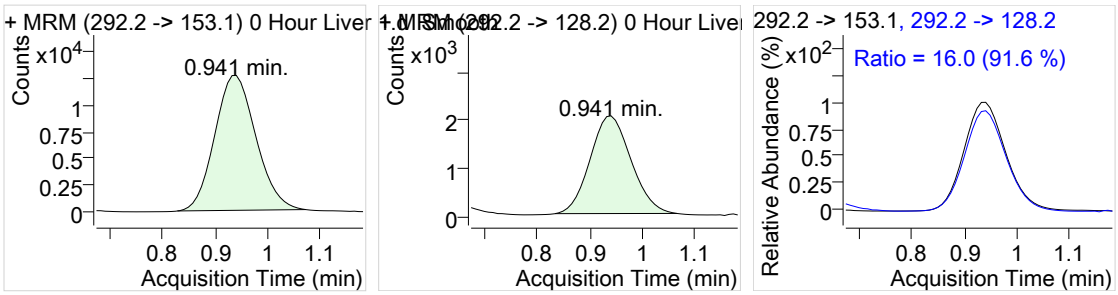
Morphine-3-B-D-Glucuronide D3



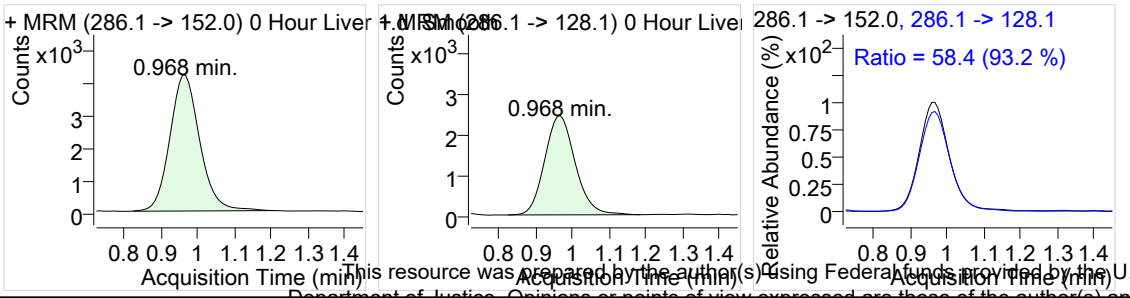
Morphine-3-B-D-Glucuronide



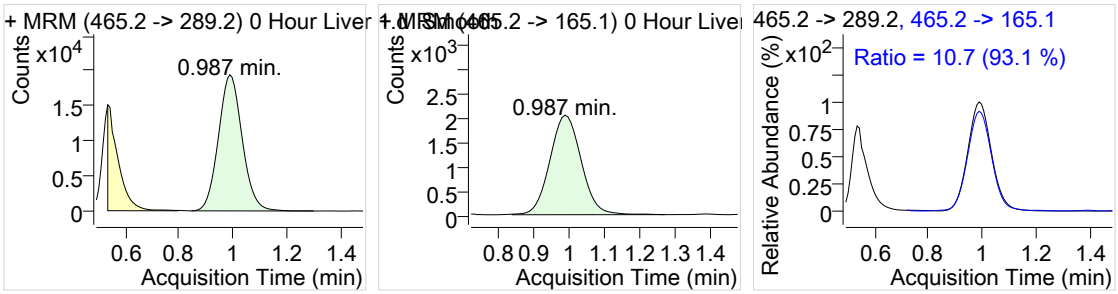
Morphine D6



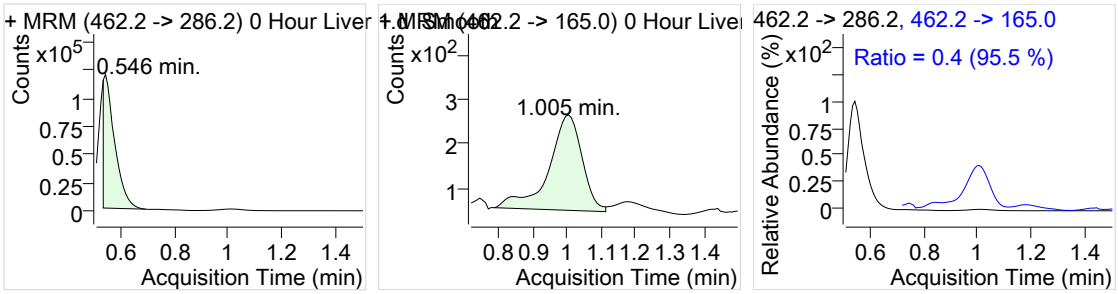
Morphine



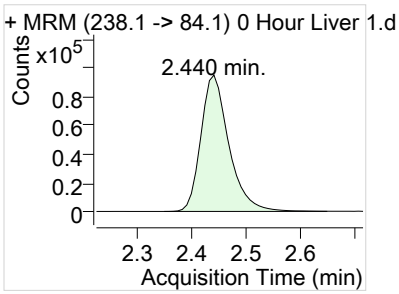
Morphine-6-B-D-Glucuronide D3



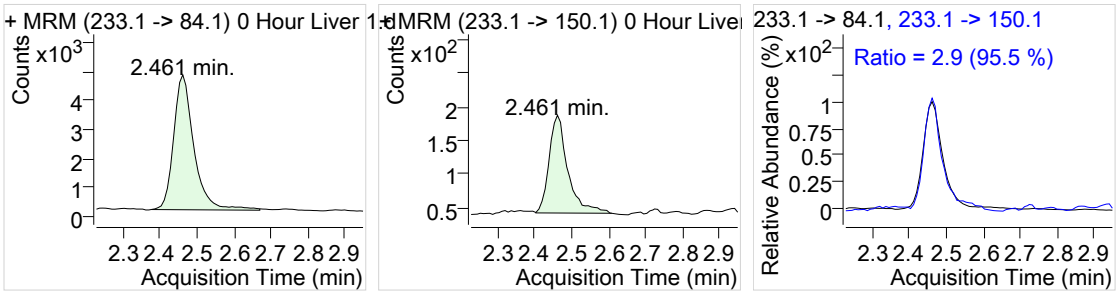
Morphine-6-B-D-Glucuronide



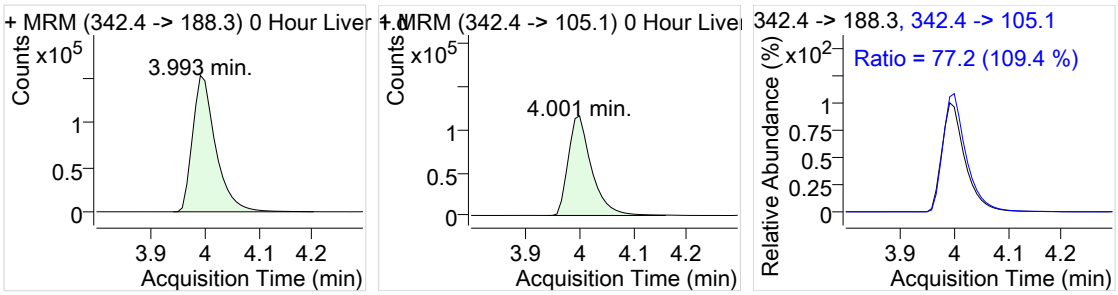
Norfentanyl D5



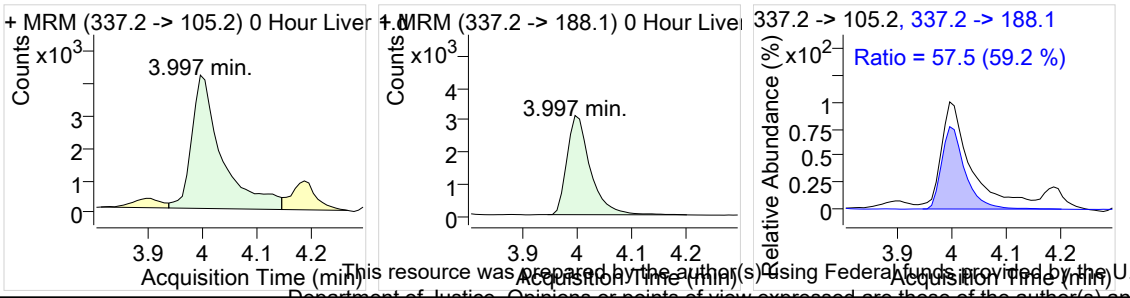
Norfentanyl



Fentanyl D5



Fentanyl



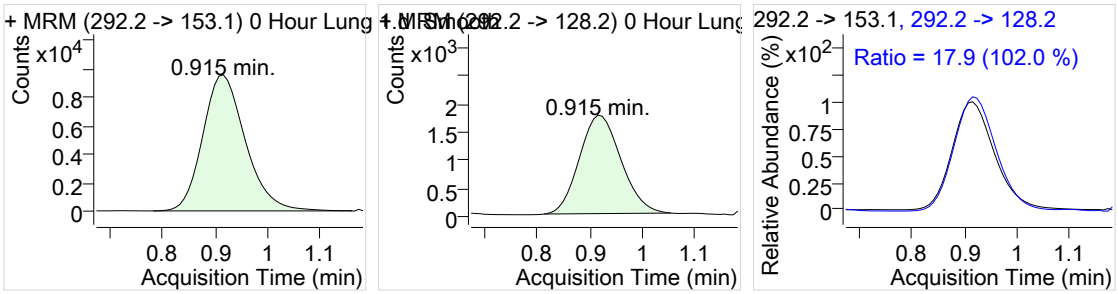
Quantitative Analysis Report

Agilent Technologies

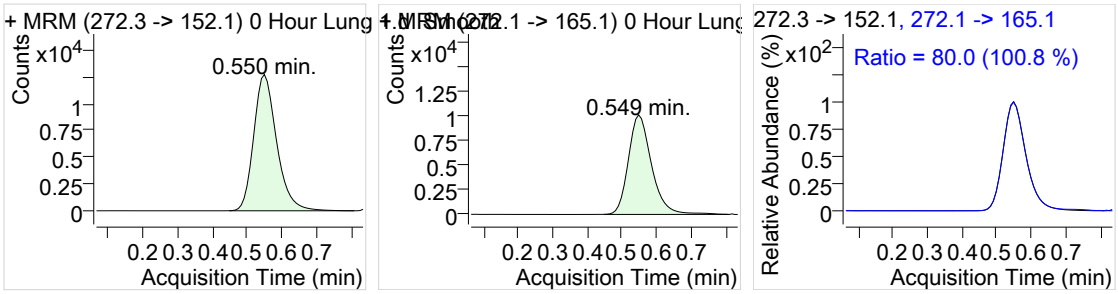
Sample Name:	:	0 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\0 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/5/2019 9:55:50 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.92	54197			
	292.2 -> 128.2		9679	17.9	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.55	58231			91.0 ng/ml
	272.1 -> 165.1		46592	80.0	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	81370			
	465.2 -> 201.1		4997	6.1	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	93229			422.3 ng/ml
	462.2 -> 201.1		6678	7.2	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.92	54197			
	292.2 -> 128.2		9679	17.9	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.94	68798			1816.4 ng/ml
	286.1 -> 128.1		40989	59.6	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.96	112647			
	465.2 -> 165.1		12343	11.0	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	64539			173.9 ng/ml
	462.2 -> 165.0		26	*0.0	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.44	372980			
Norfentanyl	233.1 -> 84.1	2.46	9093			2.1 ng/ml
	233.1 -> 150.1		273	3.0	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.99	893477			
	342.4 -> 105.1		660039	73.9	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.00	61789			4.0 ng/ml
	337.2 -> 188.1		56484	91.4	72.8 - 121.3	

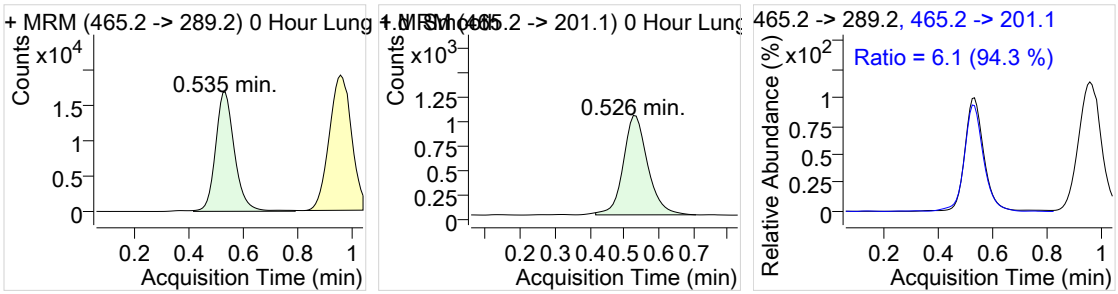
Morphine D6



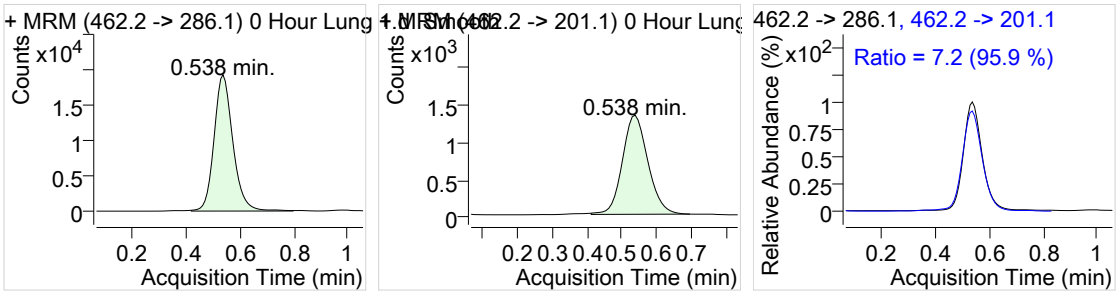
Normorphine



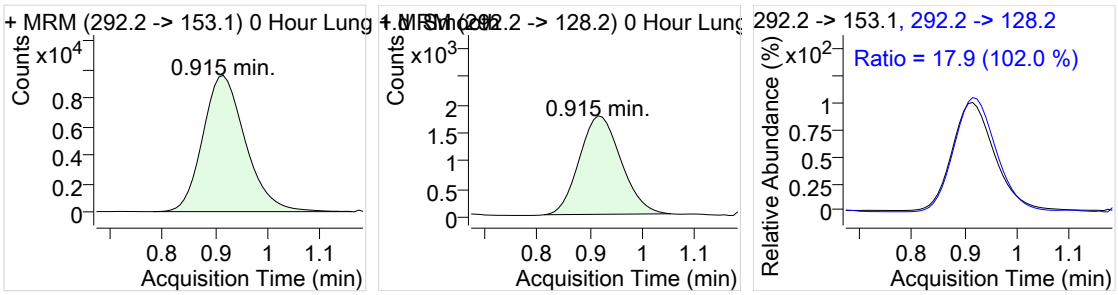
Morphine-3-B-D-Glucuronide D3



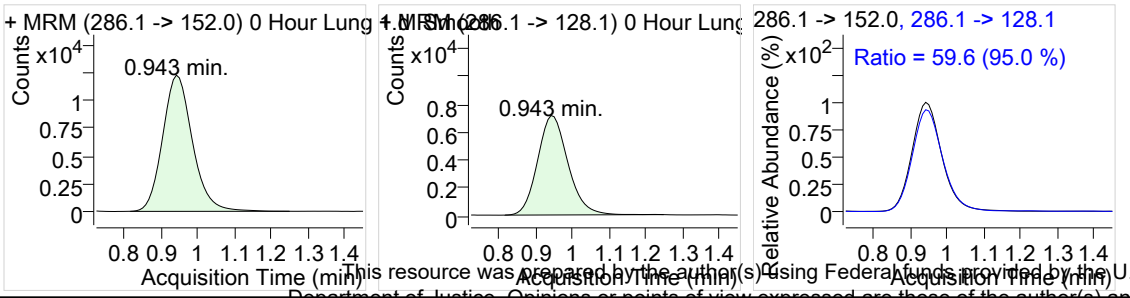
Morphine-3-B-D-Glucuronide



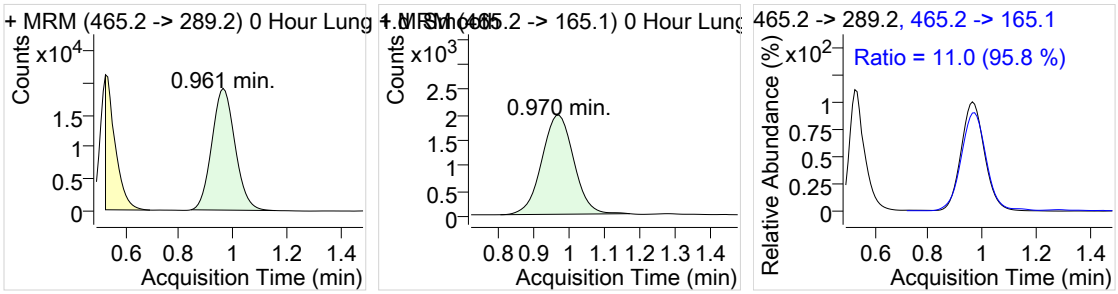
Morphine D6



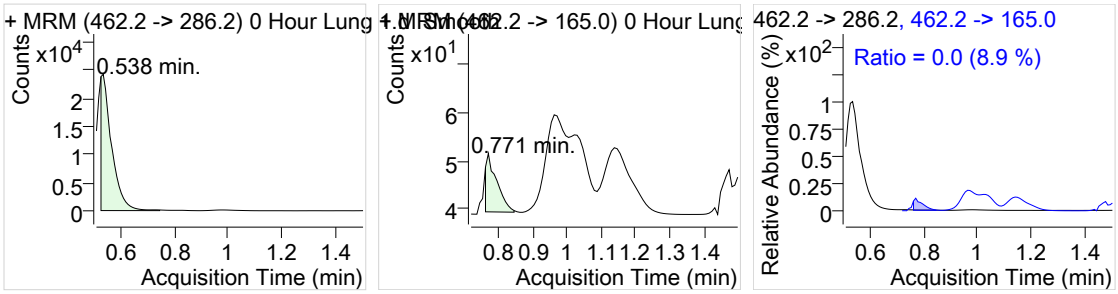
Morphine



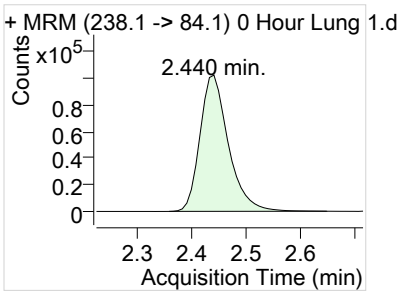
Morphine-6-B-D-Glucuronide D3



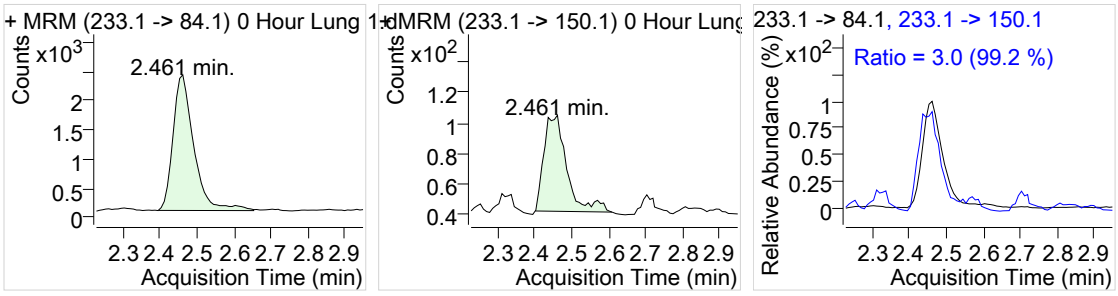
Morphine-6-B-D-Glucuronide



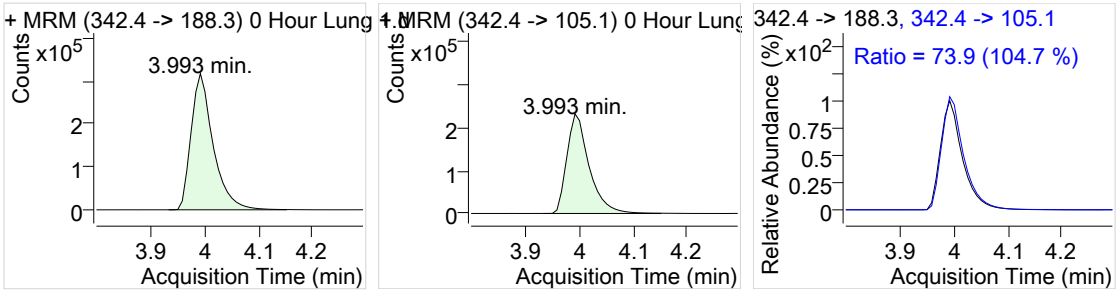
Norfentanyl D5



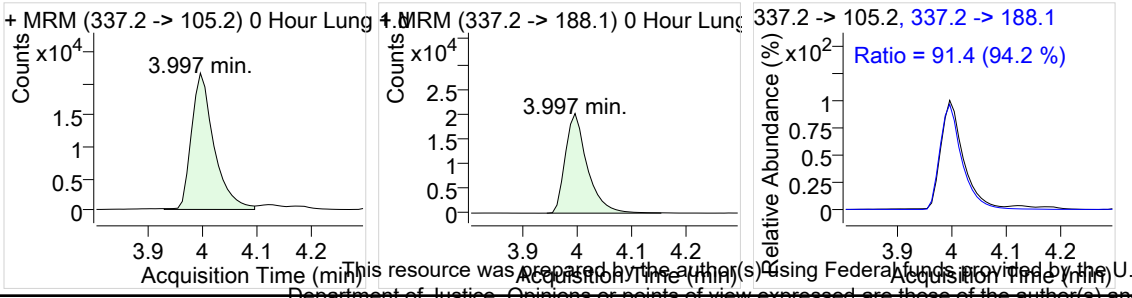
Norfentanyl



Fentanyl D5



Fentanyl



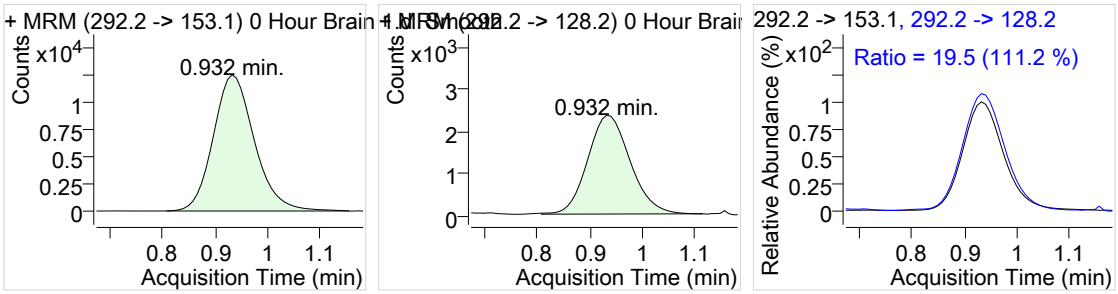
Quantitative Analysis Report

Agilent Technologies

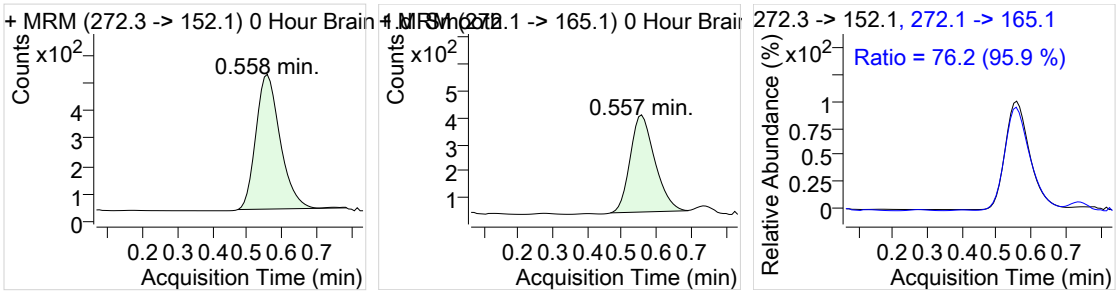
Sample Name:	:	0 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01052019\0 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/5/2019 10:05:16 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine D6	292.2 -> 153.1	0.93	68062			
	292.2 -> 128.2		13248	19.5	13.1 - 21.9	
Normorphine	272.3 -> 152.1	0.56	2359			18.0 ng/ml
	272.1 -> 165.1		1797	76.2	59.5 - 99.2	
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	61972			
	465.2 -> 201.1		4165	6.7	5.2 - 7.8	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	8700			0.0 ng/ml
	462.2 -> 201.1		631	7.2	5.6 - 9.3	
Morphine D6	292.2 -> 153.1	0.93	68062			
	292.2 -> 128.2		13248	19.5	13.1 - 21.9	
Morphine	286.1 -> 152.0	0.96	10796			211.9 ng/ml
	286.1 -> 128.1		6457	59.8	47.0 - 78.4	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	110501			
	465.2 -> 165.1		10810	9.8	8.6 - 14.3	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	5747			97.9 ng/ml
	462.2 -> 165.0		20	0.4	0.3 - 0.6	
Norfentanyl D5	238.1 -> 84.1	2.44	353189			
Norfentanyl	233.1 -> 84.1	2.46	1371			1.2 ng/ml
	233.1 -> 150.1		56	*4.1	2.3 - 3.8	
Fentanyl D5	342.4 -> 188.3	3.99	933863			
	342.4 -> 105.1		688747	73.8	52.9 - 88.2	
Fentanyl	337.2 -> 105.2	4.00	29249			2.1 ng/ml
	337.2 -> 188.1		27809	95.1	72.8 - 121.3	

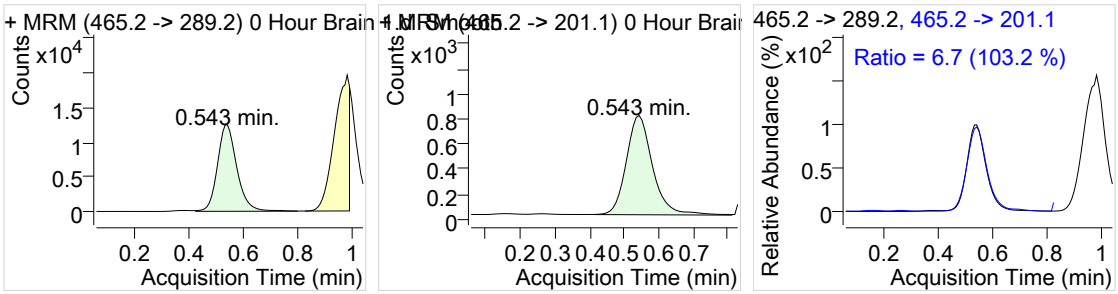
Morphine D6



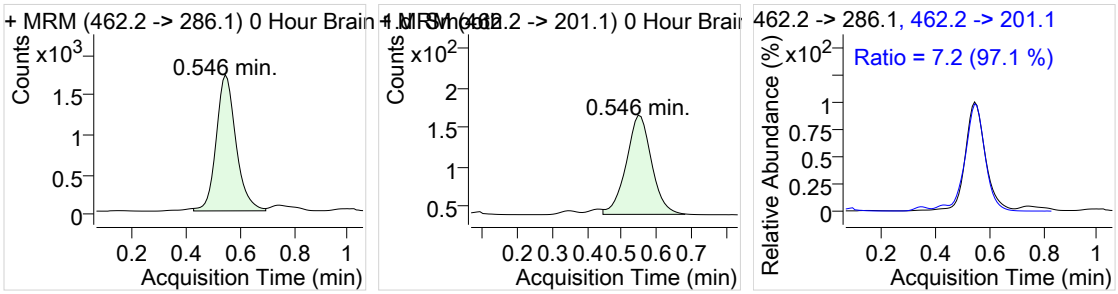
Normorphine



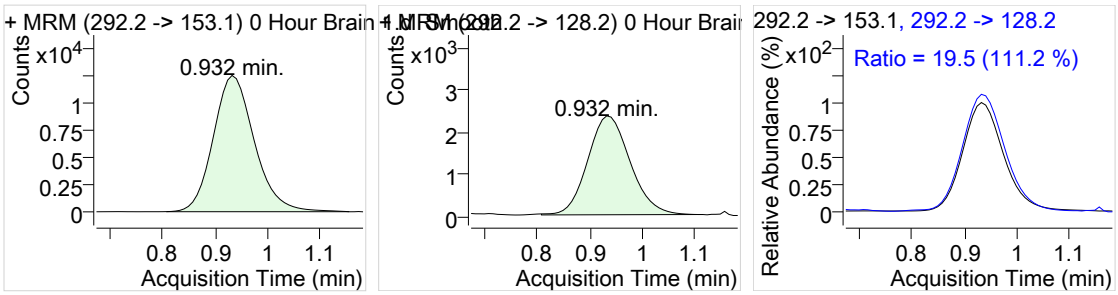
Morphine-3-B-D-Glucuronide D3



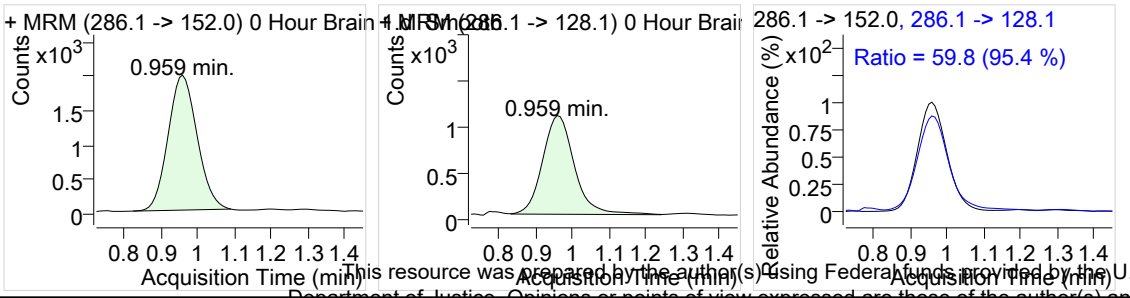
Morphine-3-B-D-Glucuronide



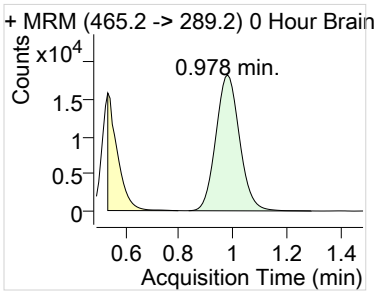
Morphine D6

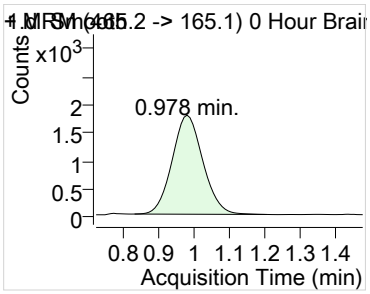


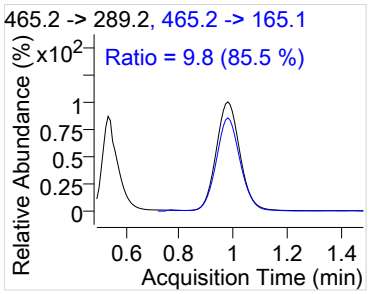
Morphine



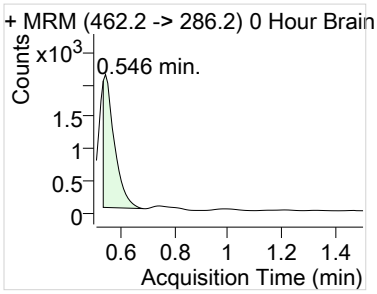
Morphine-6-B-D-Glucuronide D3

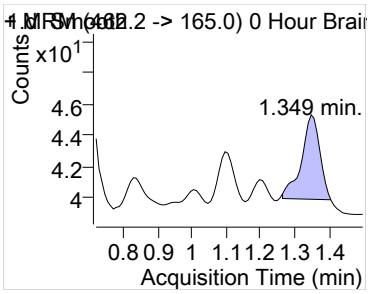


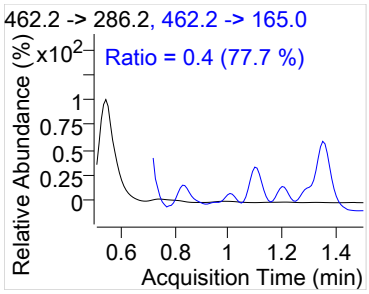




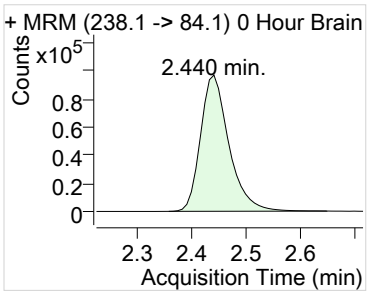
Morphine-6-B-D-Glucuronide



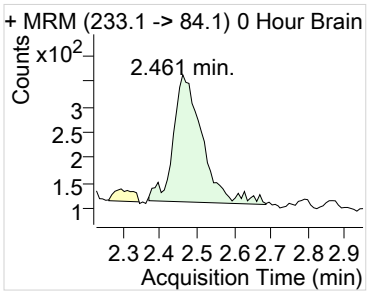


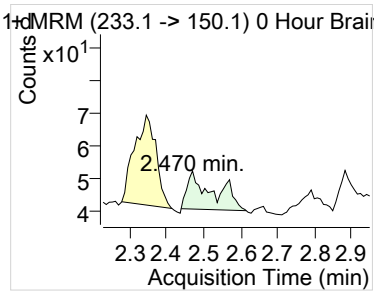


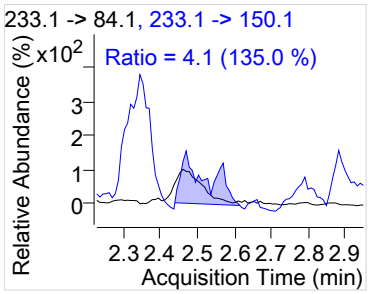
Norfentanyl D5



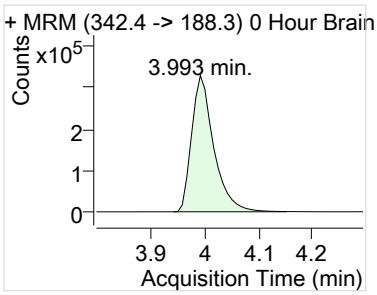
Norfentanyl

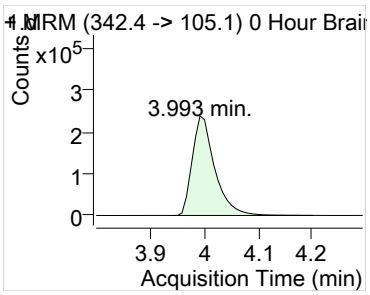


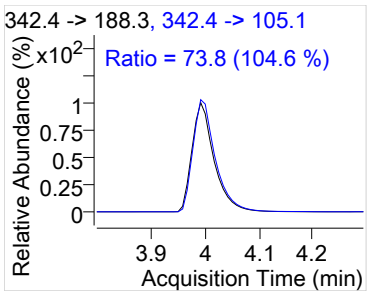




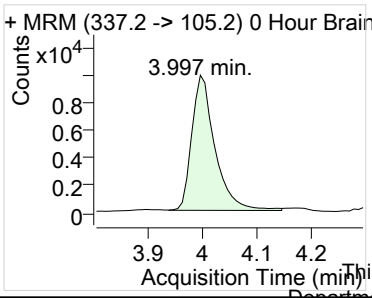
Fentanyl D5

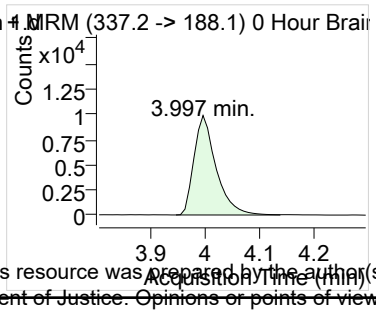


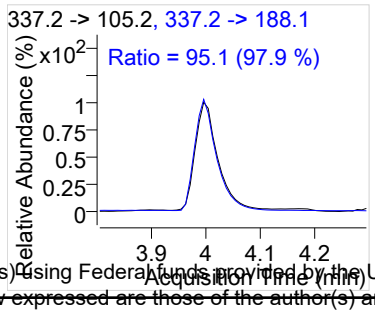




Fentanyl







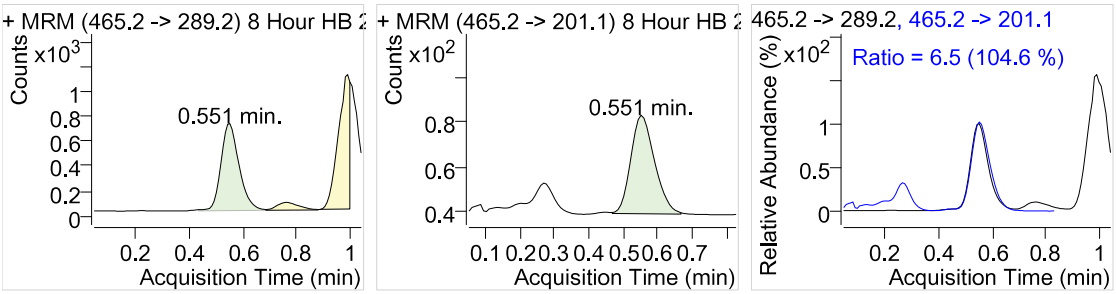
Quantitative Analysis Report

Agilent Technologies

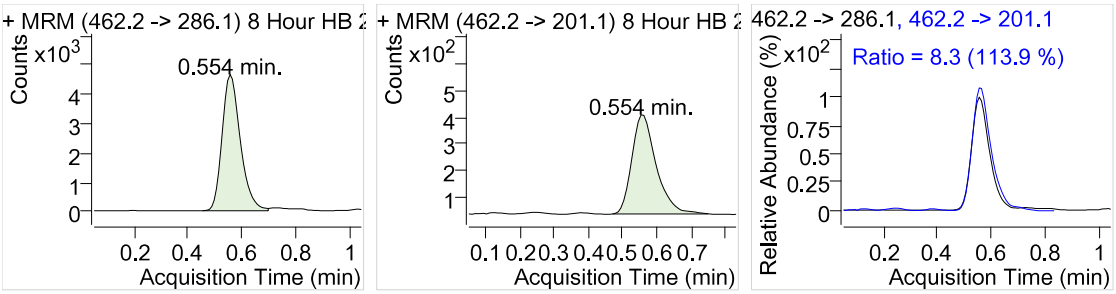
Sample Name: : 8 Hour HB 2
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\01062019\8 Hour HB 2.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 1/6/2019 12:37:57 PM
Dilution : 1.4
Operator :
Sample Position : P1-B4

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	3264			
	465.2 -> 201.1		211	6.5	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	21590			1273.8 ng/ml
	462.2 -> 201.1		1802	8.3	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.94	39359			
	292.2 -> 128.2		7252	18.4	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.56	763			10.1 ng/ml
	272.1 -> 165.1		704	92.2	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.94	39359			
	292.2 -> 128.2		7252	18.4	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.97	120571			1522.4 ng/ml
	286.1 -> 128.1		74725	62.0	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	5665			
	465.2 -> 165.1		722	*12.7	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	14729			362.9 ng/ml
	462.2 -> 165.0		39	*0.3	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	350095			
Norfentanyl	233.1 -> 84.1	2.49	1964			0.6 ng/ml
	233.1 -> 150.1		120	*6.1	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	533498			
	342.4 -> 105.1		403321	75.6	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.97	30287			1.3 ng/ml
	337.2 -> 188.1		24429	80.7	77.3 - 116.0	

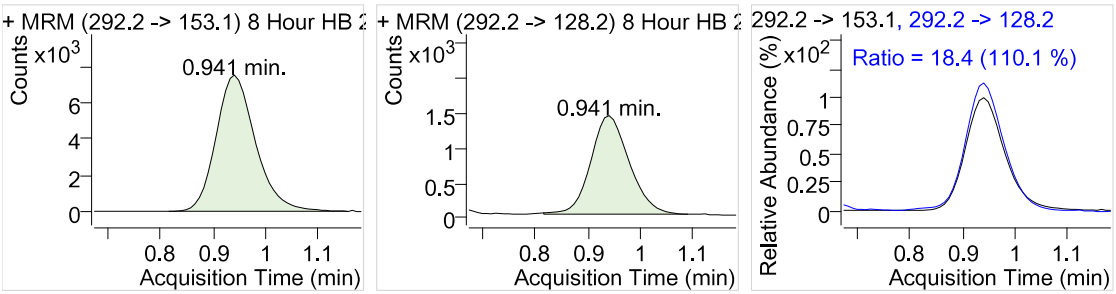
Morphine-3-B-D-Glucuronide D3



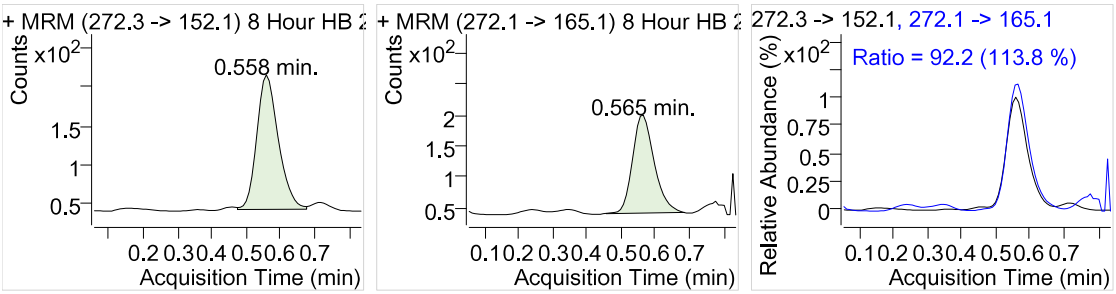
Morphine-3-B-D-Glucuronide



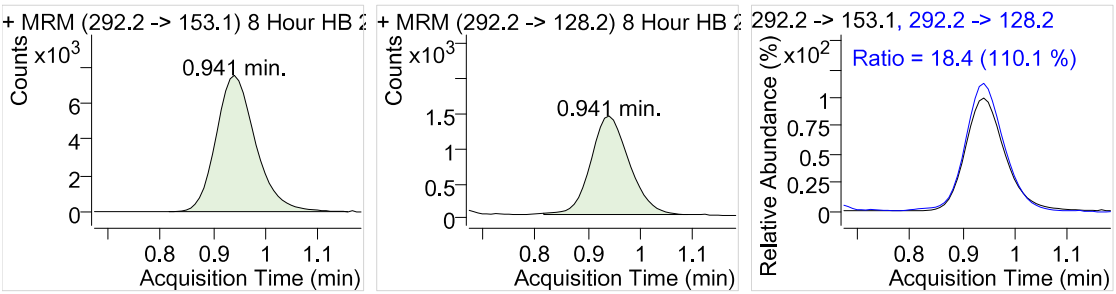
Morphine D6



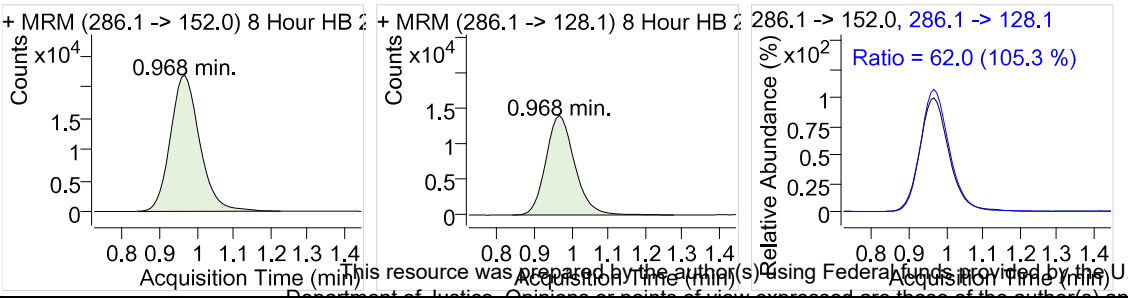
Normorphine



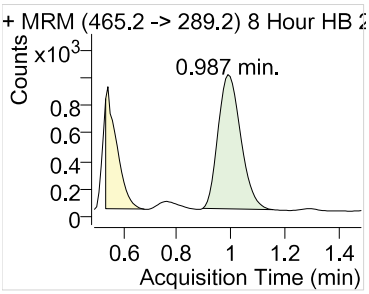
Morphine D6

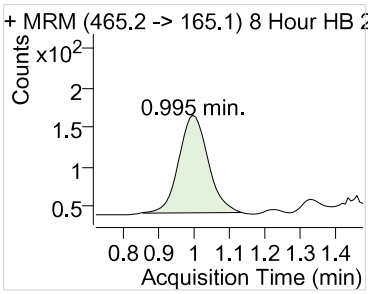


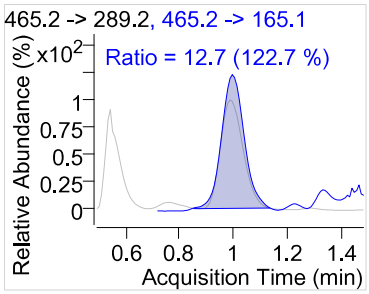
Morphine



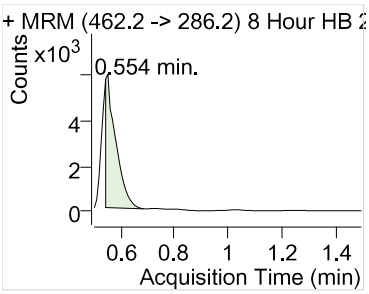
Morphine-6-B-D-Glucuronide D3

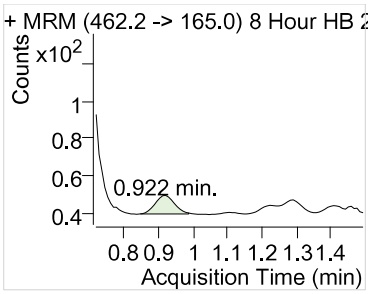


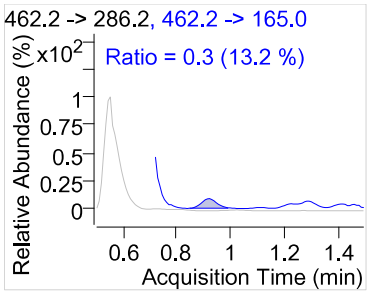




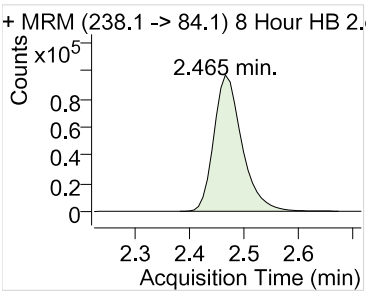
Morphine-6-B-D-Glucuronide



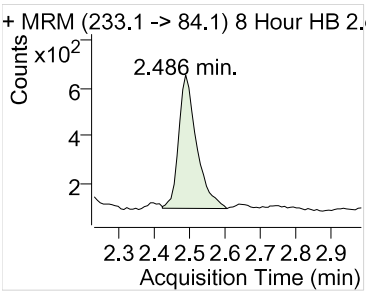


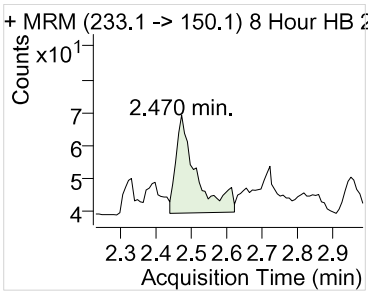


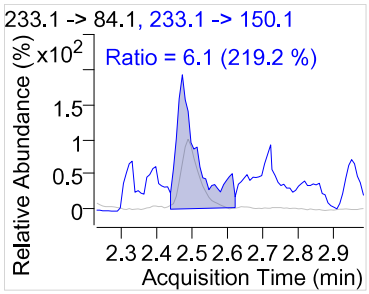
Norfentanyl D5



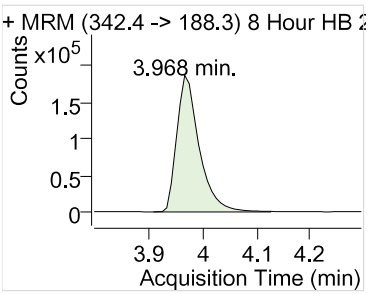
Norfentanyl

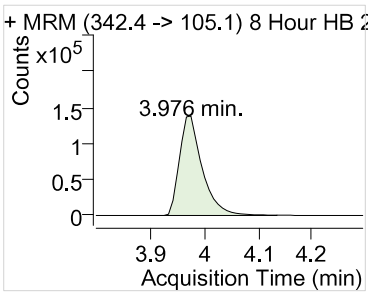


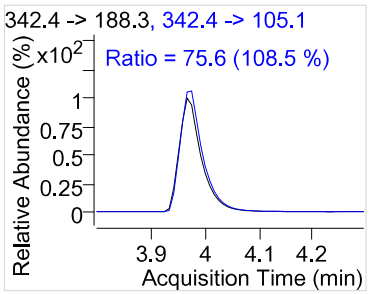




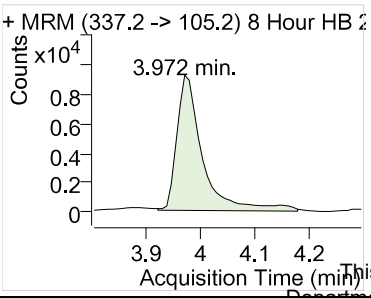
Fentanyl D5

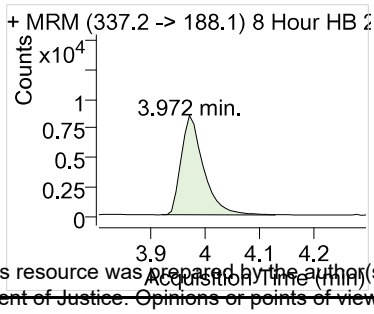


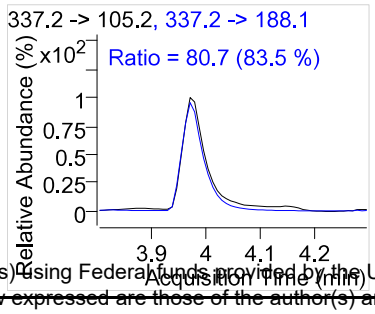




Fentanyl







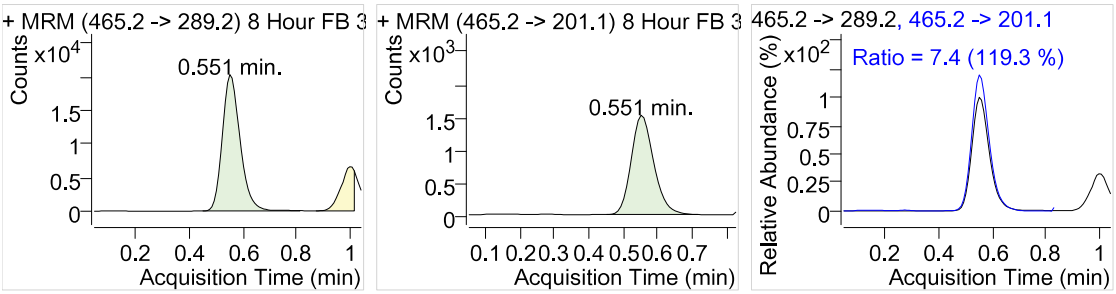
Quantitative Analysis Report

Agilent Technologies

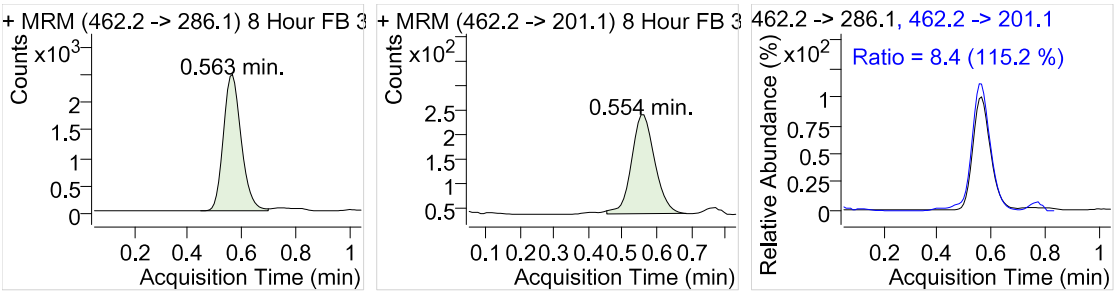
Sample Name:	:	8 Hour FB 3
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\8 Hour FB 3.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 12:58:13 PM
Dilution	:	13.5
Operator	:	
Sample Position	:	P1-B7

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	92659			
	465.2 -> 201.1		6831	7.4	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.56	11260			341.7 ng/ml
	462.2 -> 201.1		951	8.4	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.95	80100			
	292.2 -> 128.2		12554	15.7	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.57	622			95.1 ng/ml
	272.1 -> 165.1		416	66.8	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.95	80100			
	292.2 -> 128.2		12554	15.7	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.97	12808			765.9 ng/ml
	286.1 -> 128.1		7155	55.9	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	32049			
	465.2 -> 165.1		3581	11.2	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	8276			454.2 ng/ml
	462.2 -> 165.0		25	*0.3	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.46	334686			
Norfentanyl	233.1 -> 84.1	2.48	340			5.6 ng/ml
	233.1 -> 150.1		24	*7.1	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	186972			
	342.4 -> 105.1		144001	77.0	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.97	942			3.9 ng/ml
	337.2 -> 188.1		1262	*134.0	77.3 - 116.0	

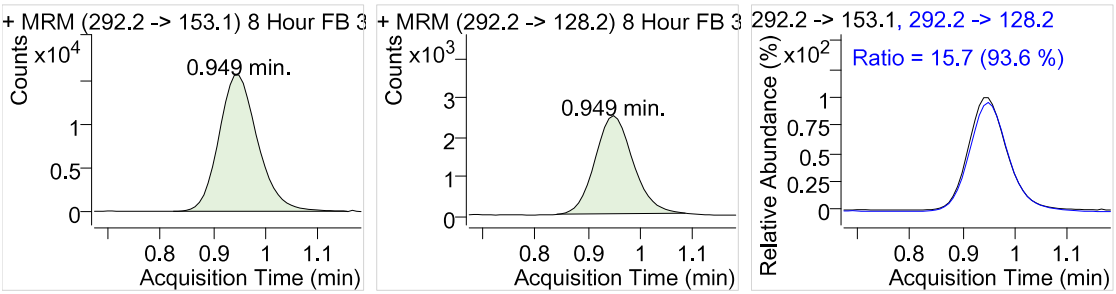
Morphine-3-B-D-Glucuronide D3



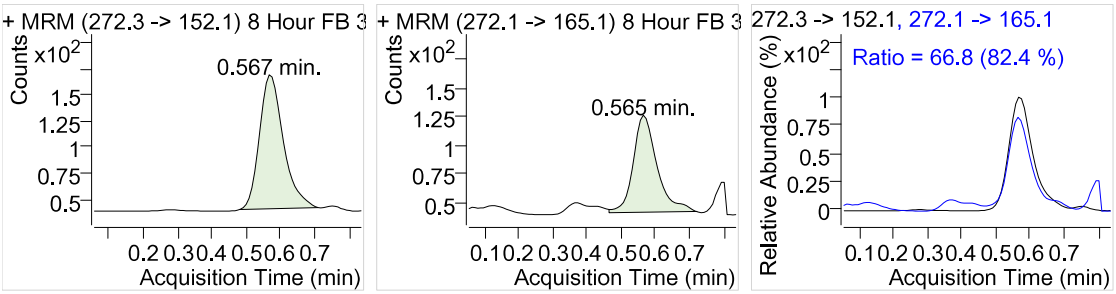
Morphine-3-B-D-Glucuronide



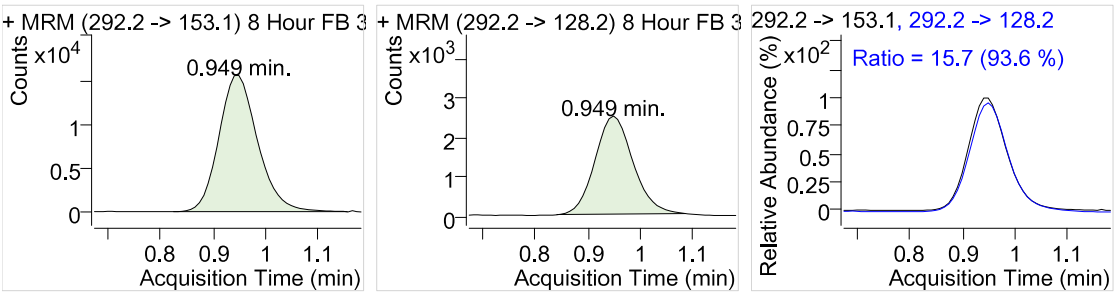
Morphine D6



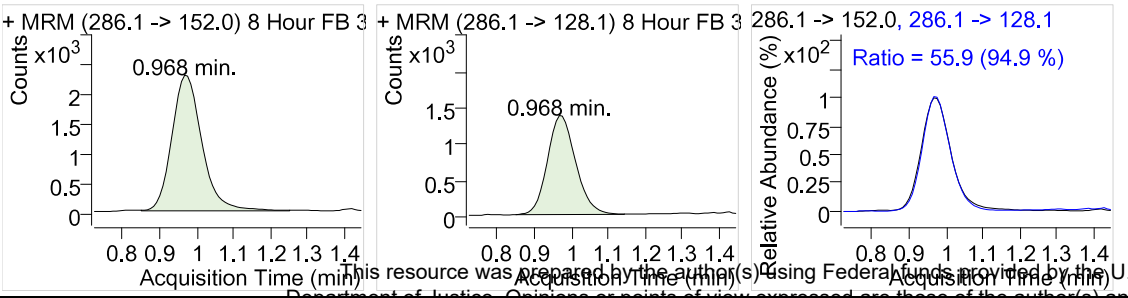
Normorphine



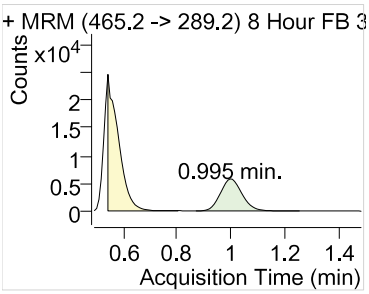
Morphine D6

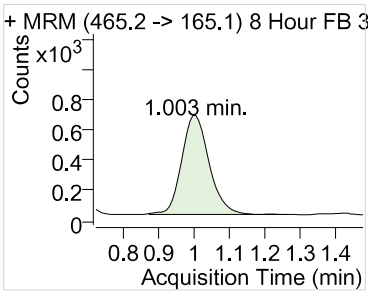


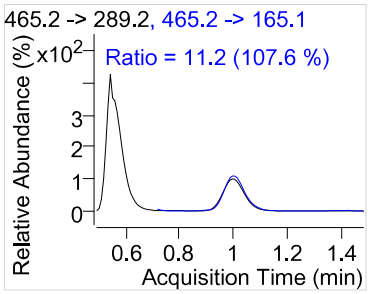
Morphine



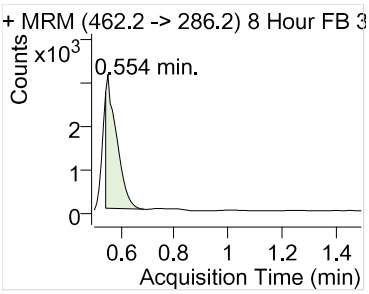
Morphine-6-B-D-Glucuronide D3

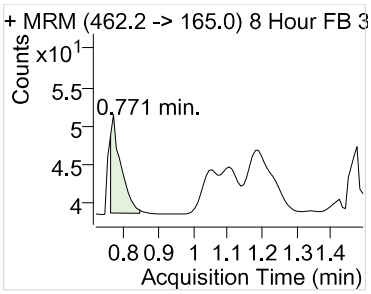


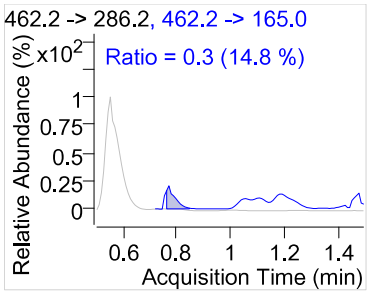




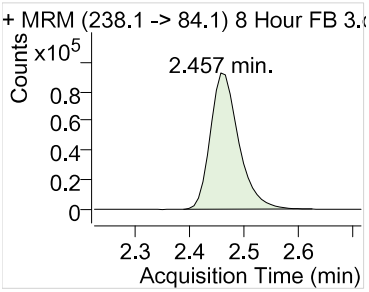
Morphine-6-B-D-Glucuronide



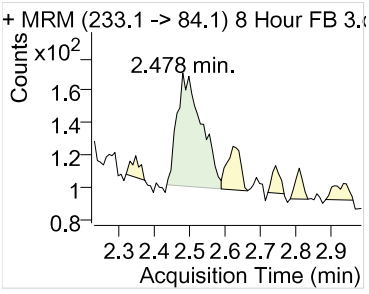


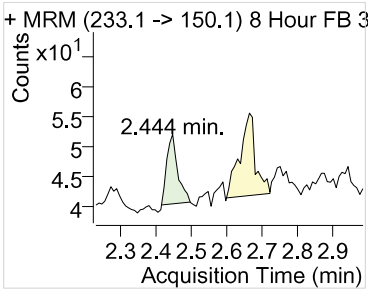


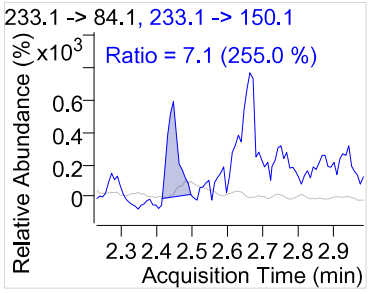
Norfentanyl D5



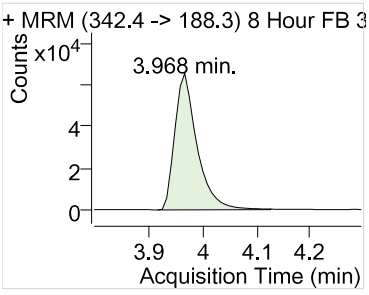
Norfentanyl

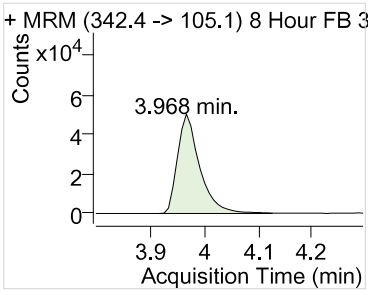


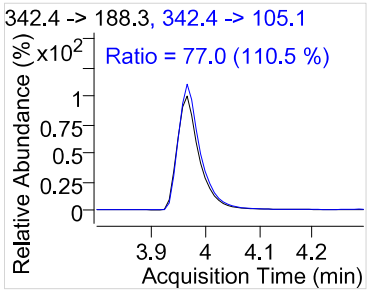




Fentanyl D5







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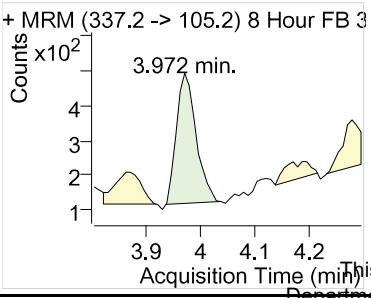
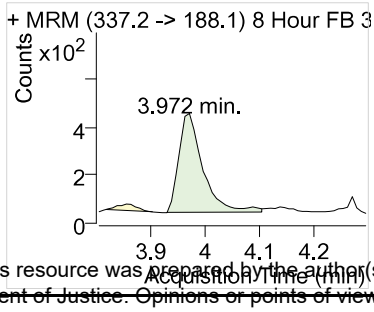
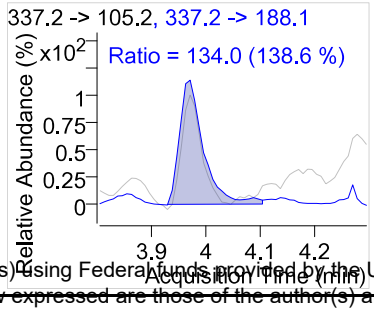






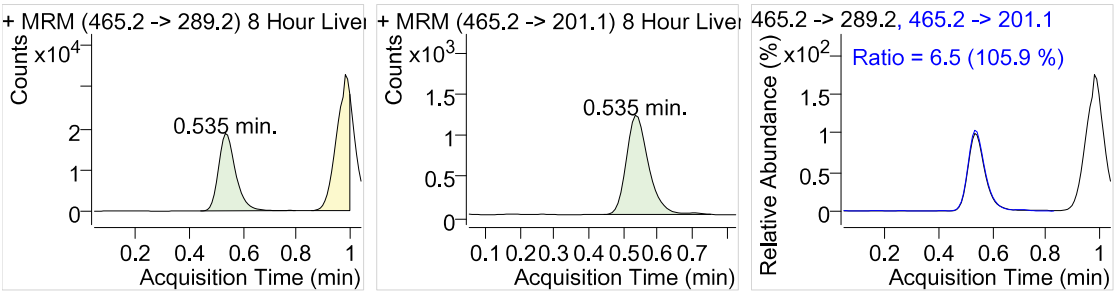
Quantitative Analysis Report

Agilent Technologies

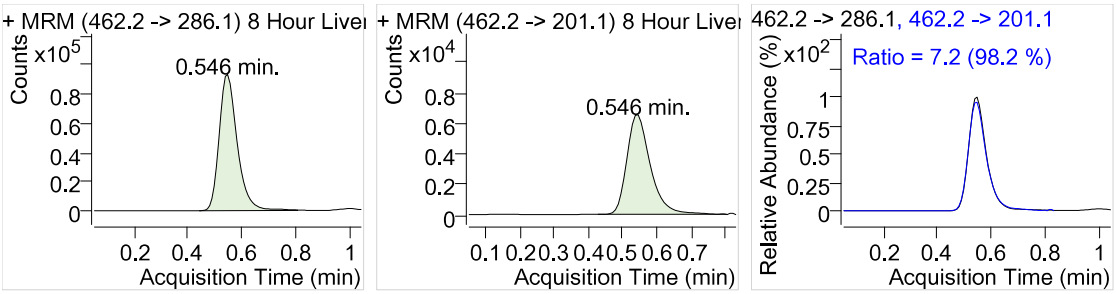
Sample Name:	:	8 Hour Liver 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\8 Hour Liver 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 1:07:41 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-B8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	82830			
	465.2 -> 201.1		5420	6.5	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	431974			2734.9 ng/ml
	462.2 -> 201.1		31092	7.2	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.92	79075			
	292.2 -> 128.2		12642	16.0	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	118235			119.8 ng/ml
	272.1 -> 165.1		96967	82.0	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.92	79075			
	292.2 -> 128.2		12642	16.0	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.95	16778			306.5 ng/ml
	286.1 -> 128.1		11695	69.7	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	150110			
	465.2 -> 165.1		13891	9.3	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	302468			811.5 ng/ml
	462.2 -> 165.0		1277	*0.4	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	373494			
Norfentanyl	233.1 -> 84.1	2.49	6450			2.3 ng/ml
	233.1 -> 150.1		266	*4.1	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	492088			
	342.4 -> 105.1		366320	74.4	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	11221			2.1 ng/ml
	337.2 -> 188.1		7575	*67.5	77.3 - 116.0	

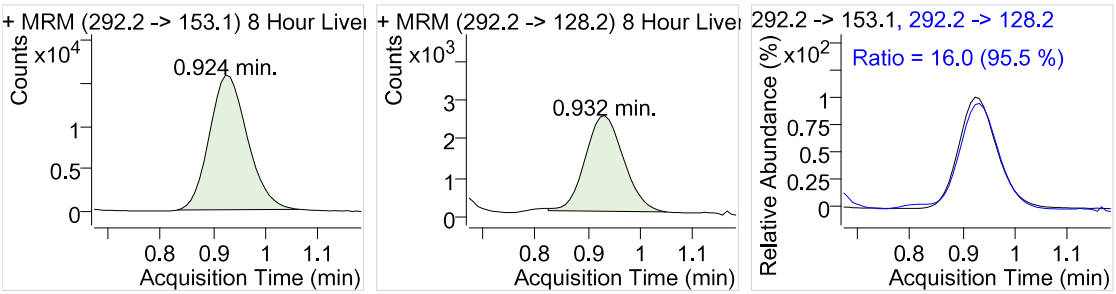
Morphine-3-B-D-Glucuronide D3



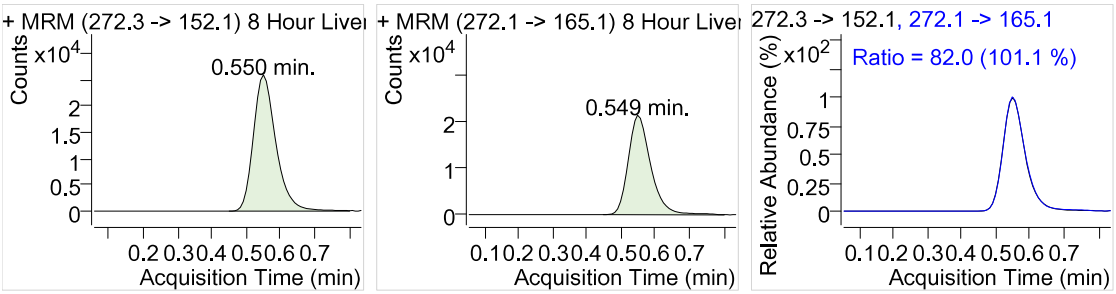
Morphine-3-B-D-Glucuronide



Morphine D6



Normorphine



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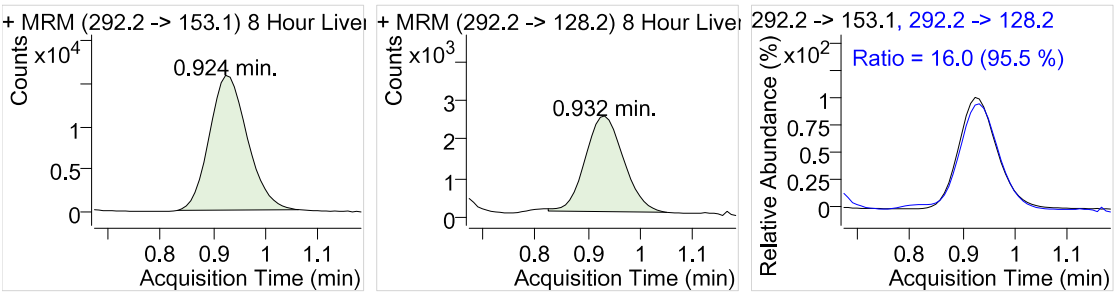
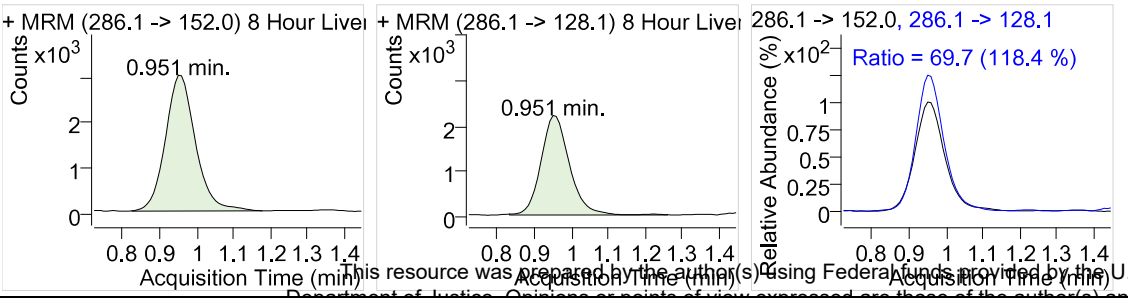
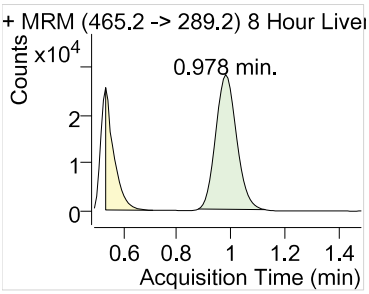
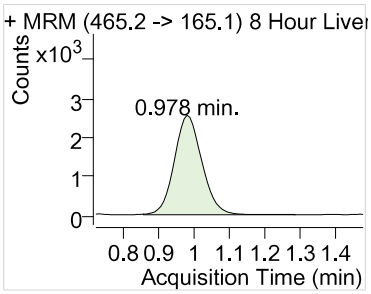


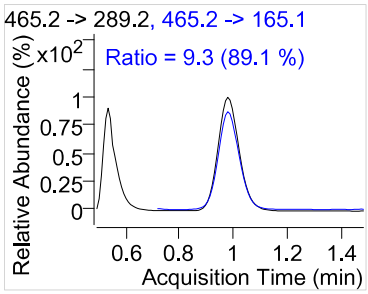
Morphine



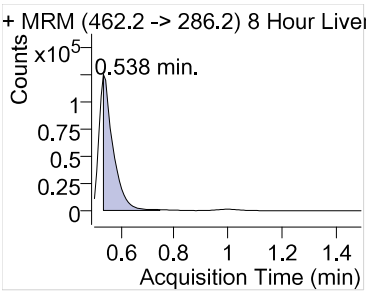
Morphine-6-B-D-Glucuronide D3

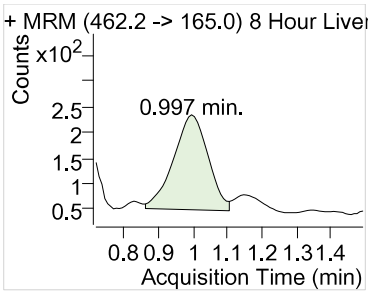


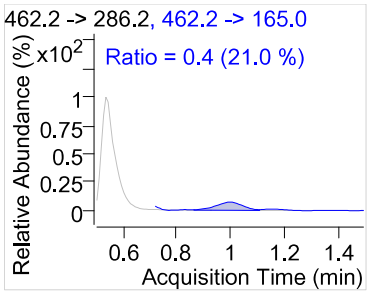




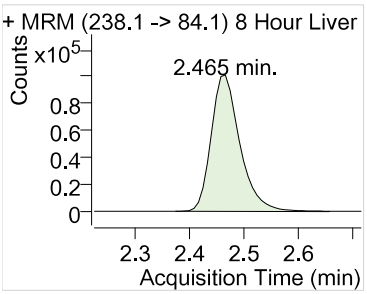
Morphine-6-B-D-Glucuronide



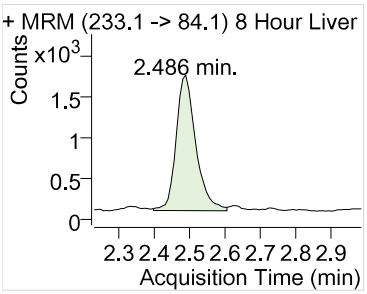


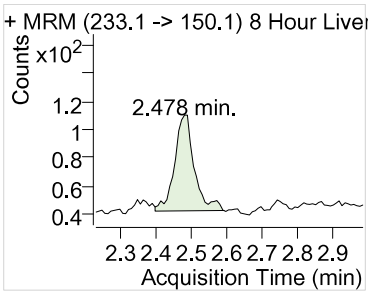


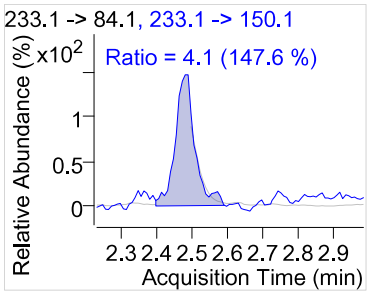
Norfentanyl D5



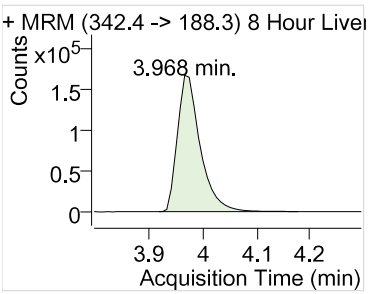
Norfentanyl

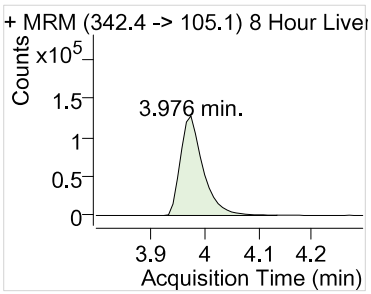


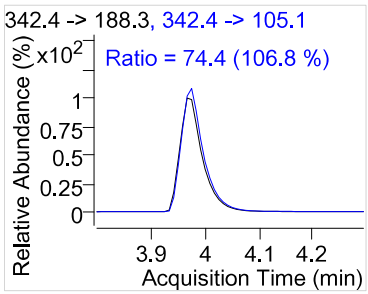




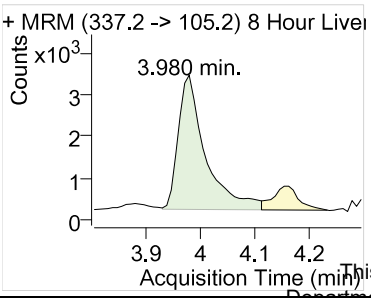
Fentanyl D5

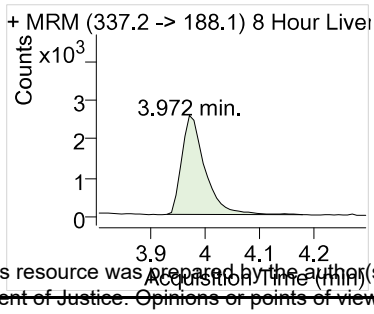


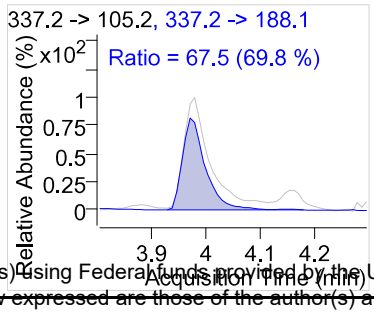




Fentanyl







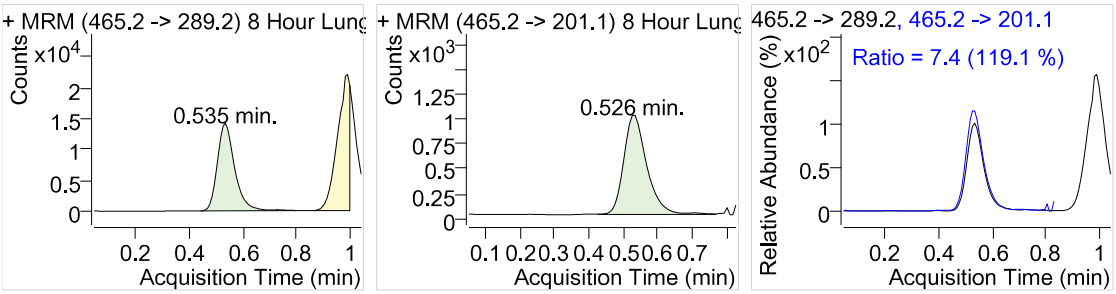
Quantitative Analysis Report

Agilent Technologies

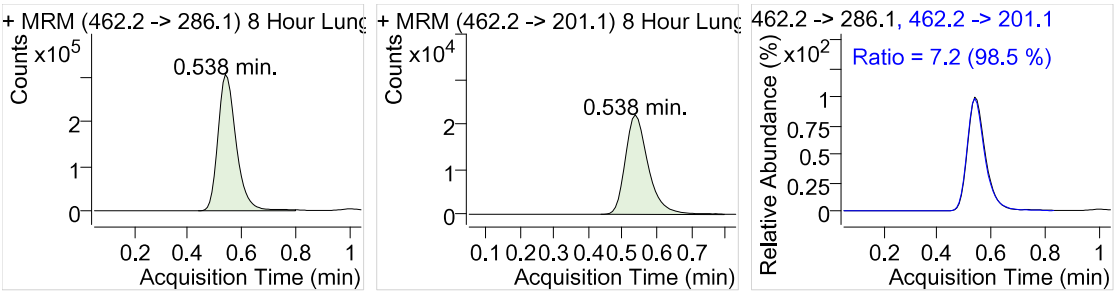
Sample Name:	:	8 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\8 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 1:43:58 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	63194			
	465.2 -> 201.1		4651	7.4	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	1425075			11815.5 ng/ml
	462.2 -> 201.1		102849	7.2	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.92	34248			
	292.2 -> 128.2		6385	18.6	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	52781			122.6 ng/ml
	272.1 -> 165.1		42500	80.5	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.92	34248			
	292.2 -> 128.2		6385	18.6	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.96	45453			1954.5 ng/ml
	286.1 -> 128.1		26324	57.9	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	105107			
	465.2 -> 165.1		9825	9.3	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	1042845			3848.9 ng/ml
	462.2 -> 165.0		2550	*0.2	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	281796			
Norfentanyl	233.1 -> 84.1	2.49	2527			2.0 ng/ml
	233.1 -> 150.1		114	*4.5	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	644435			
	342.4 -> 105.1		469036	72.8	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.99	25623			2.9 ng/ml
	337.2 -> 188.1		20469	79.9	77.3 - 116.0	

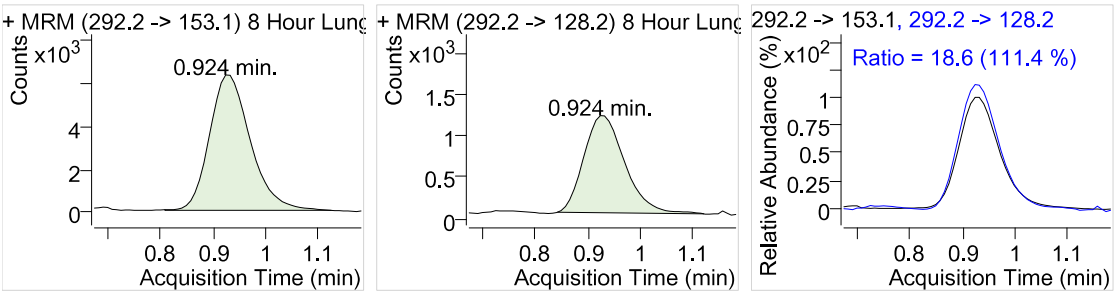
Morphine-3-B-D-Glucuronide D3



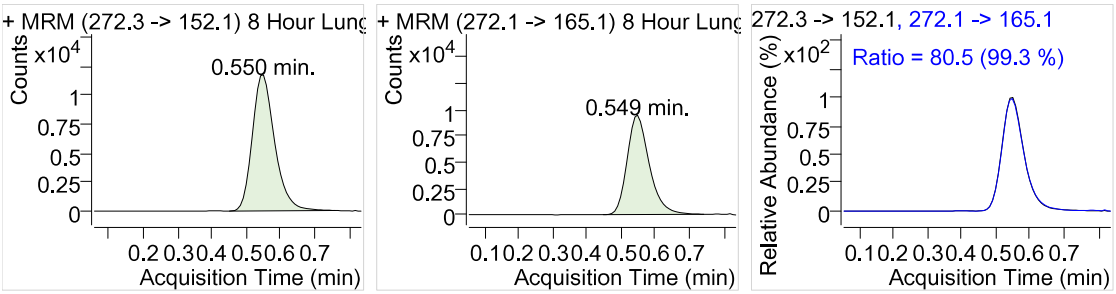
Morphine-3-B-D-Glucuronide



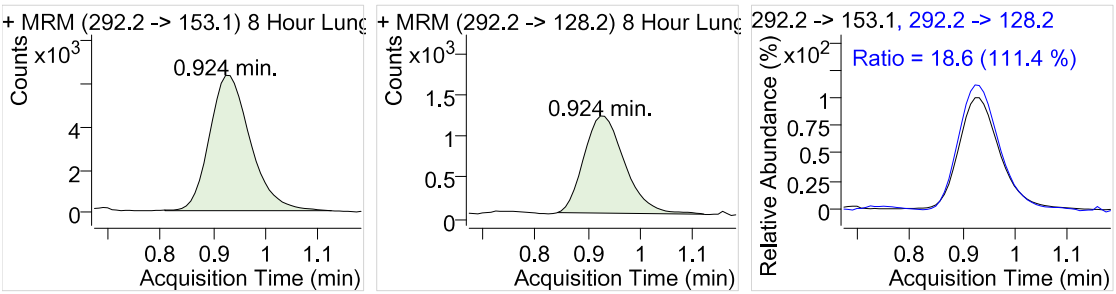
Morphine D6



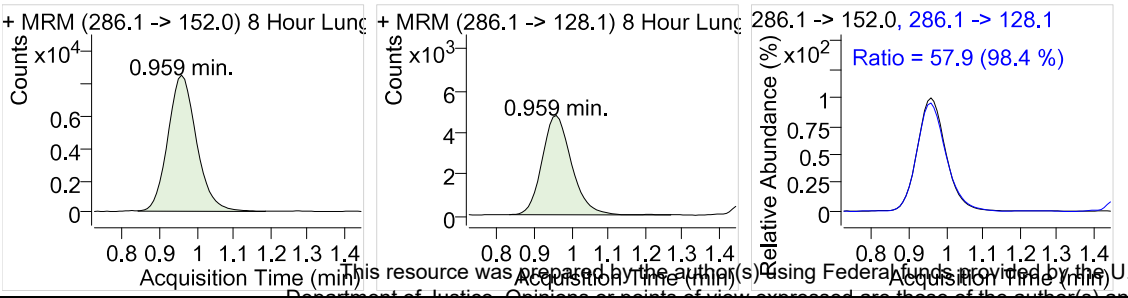
Normorphine



Morphine D6



Morphine



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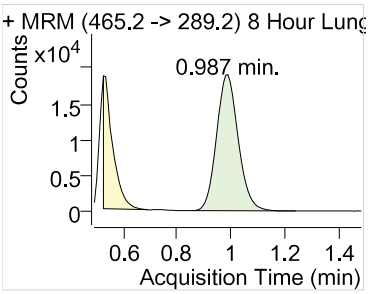
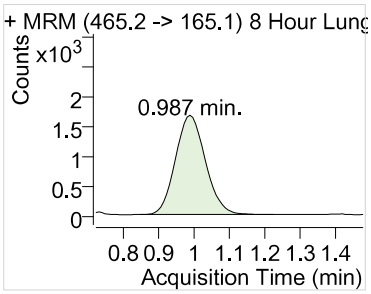
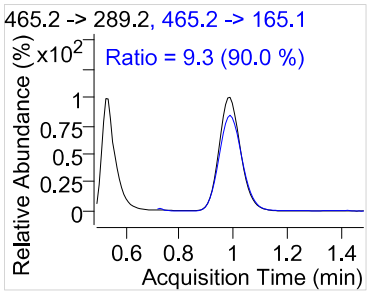
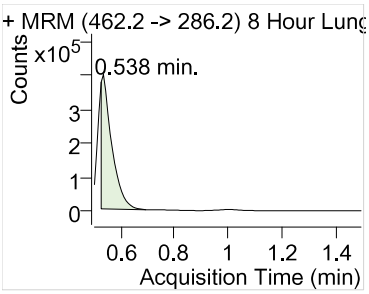
Morphine-6-B-D-Glucuronide D3

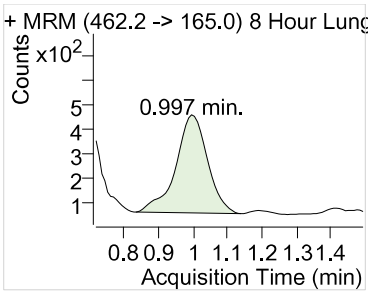


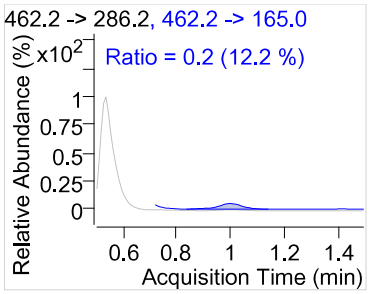




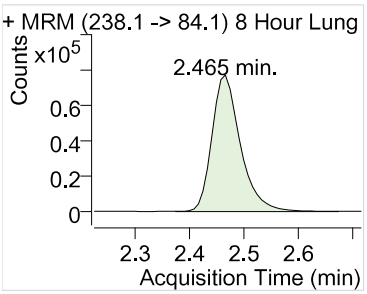
Morphine-6-B-D-Glucuronide



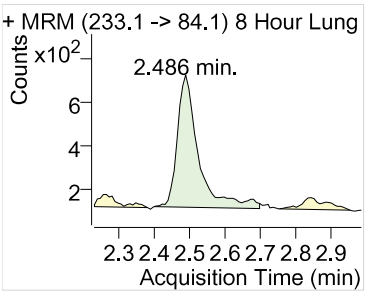


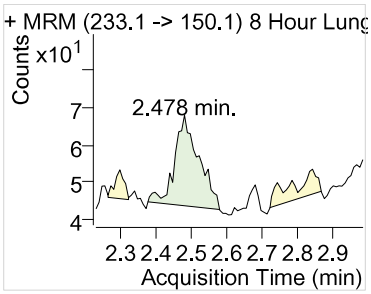


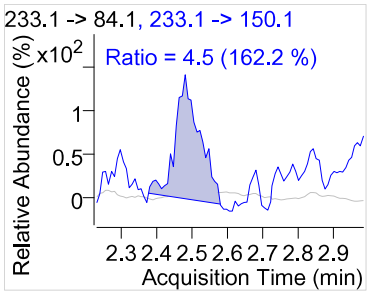
Norfentanyl D5



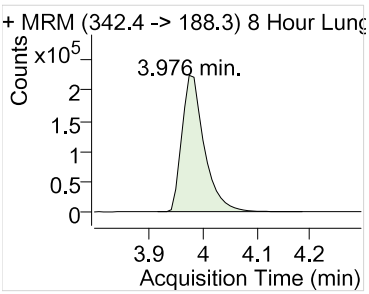
Norfentanyl

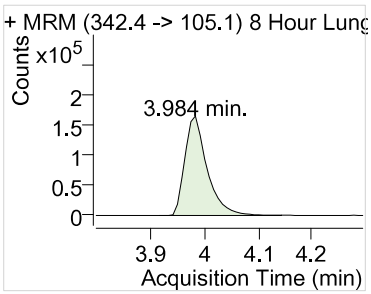


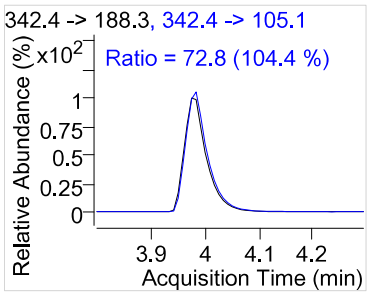




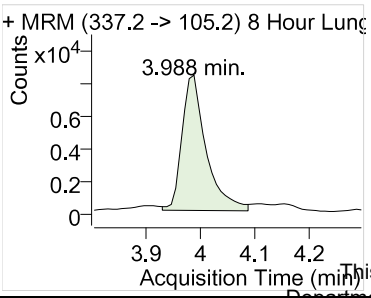
Fentanyl D5

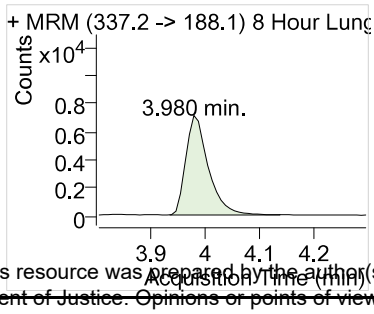


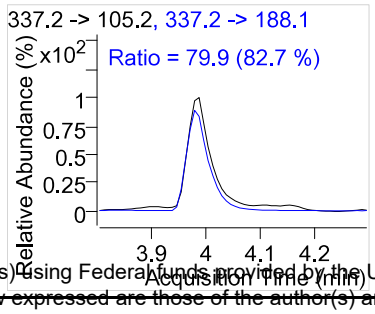




Fentanyl







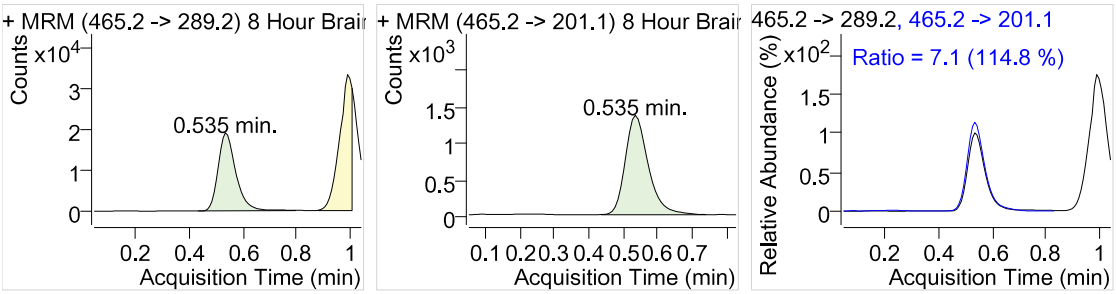
Quantitative Analysis Report

Agilent Technologies

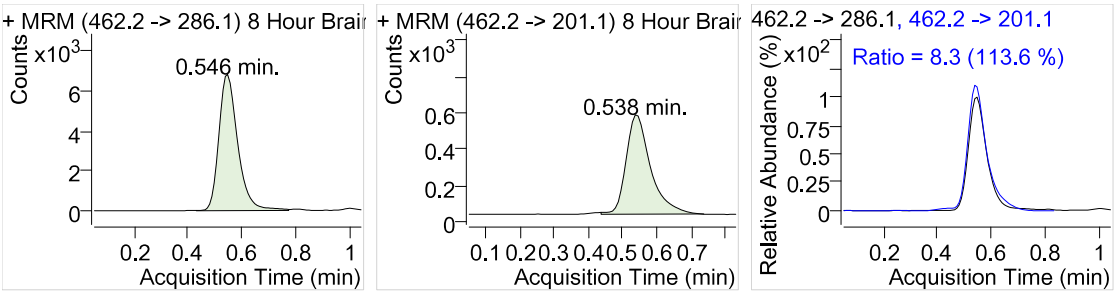
Sample Name:	:	8 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\8 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 1:53:26 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-C3

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	88112			
	465.2 -> 201.1		6254	7.1	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	32563			213.4 ng/ml
	462.2 -> 201.1		2712	8.3	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.94	78440			
	292.2 -> 128.2		13443	17.1	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	2698			29.8 ng/ml
	272.1 -> 165.1		2303	85.4	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.94	78440			
	292.2 -> 128.2		13443	17.1	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.97	12090			217.9 ng/ml
	286.1 -> 128.1		7856	65.0	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	157190			
	465.2 -> 165.1		14875	9.5	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	20859			86.1 ng/ml
	462.2 -> 165.0		29	*0.1	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	394328			
Norfentanyl	233.1 -> 84.1	2.49	1409			1.8 ng/ml
	233.1 -> 150.1		38	2.7	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	1010132			
	342.4 -> 105.1		728861	72.2	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	32655			2.6 ng/ml
	337.2 -> 188.1		28118	86.1	77.3 - 116.0	

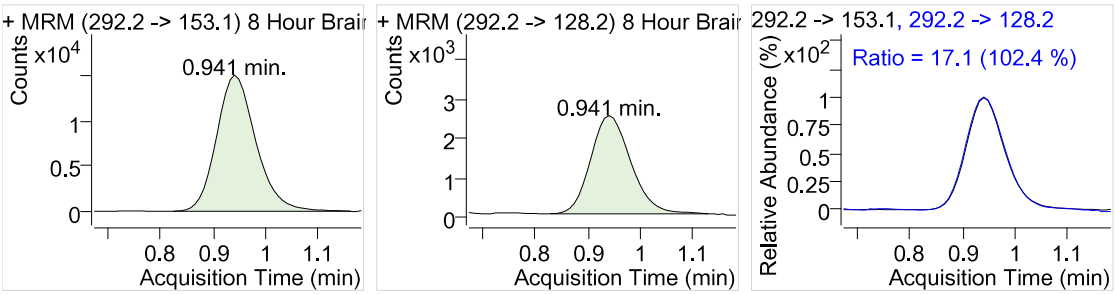
Morphine-3-B-D-Glucuronide D3



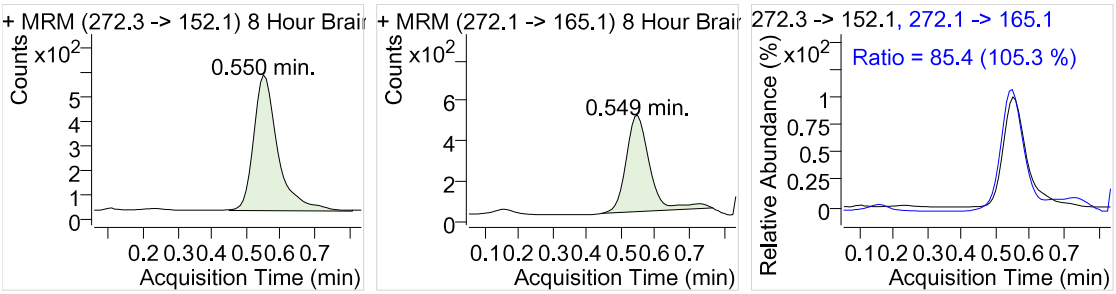
Morphine-3-B-D-Glucuronide



Morphine D6



Normorphine



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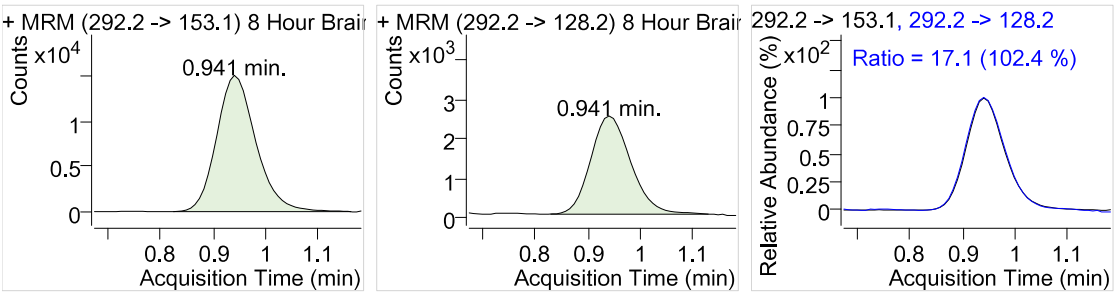
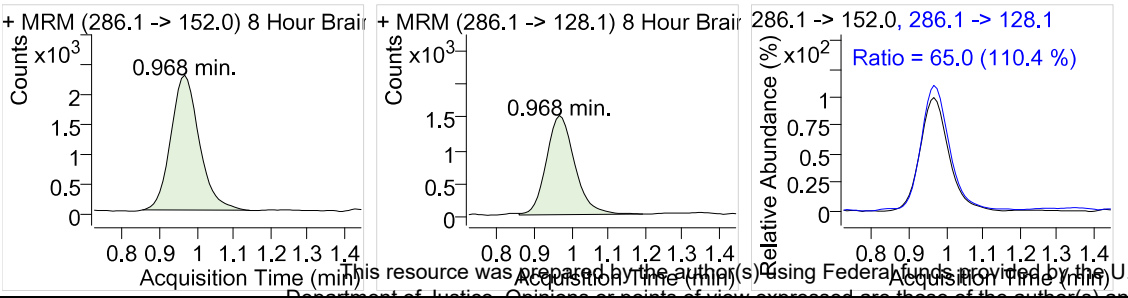


Morphine



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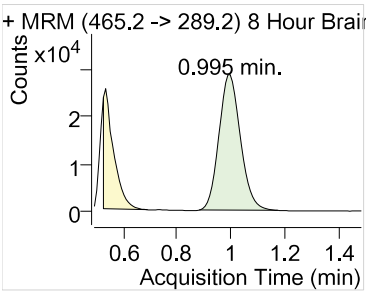
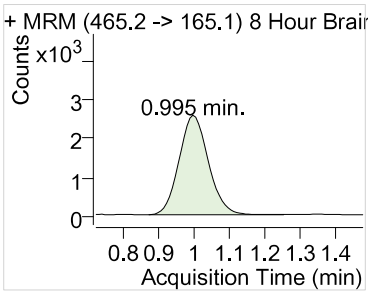
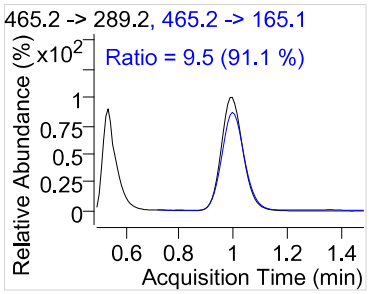
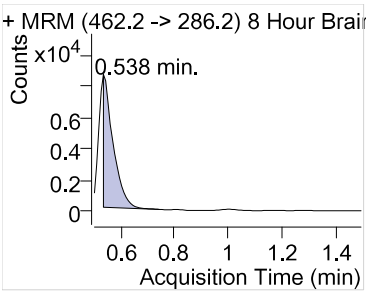
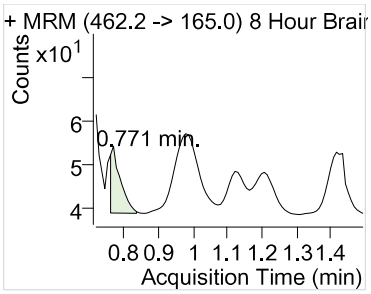


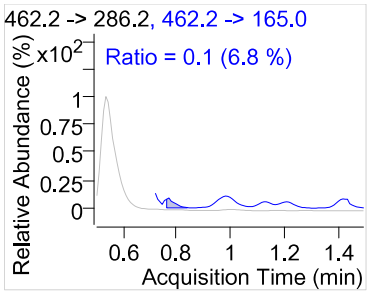




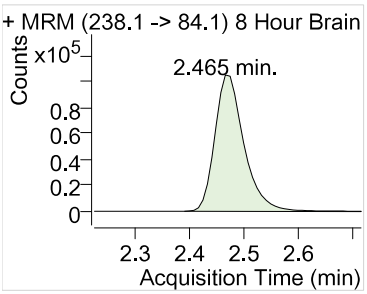
Morphine-6-B-D-Glucuronide



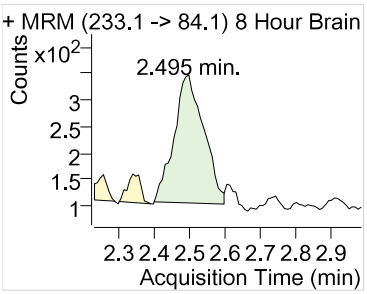


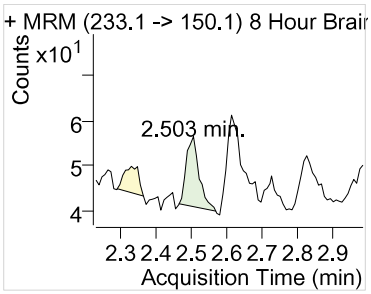


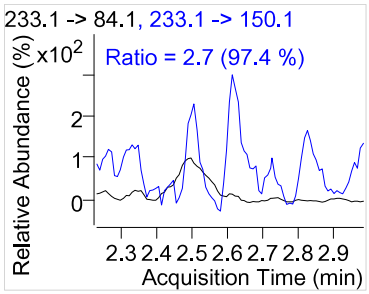
Norfentanyl D5



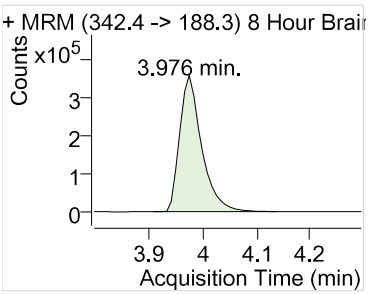
Norfentanyl

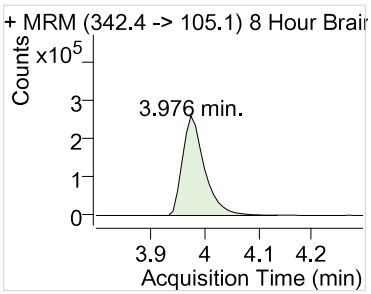


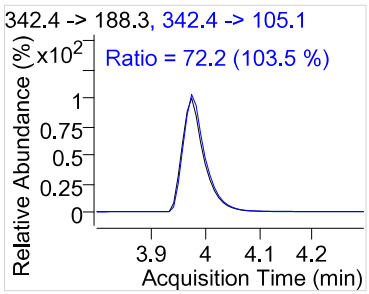




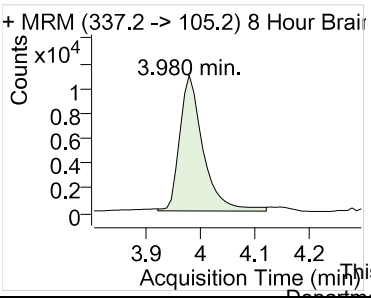
Fentanyl D5

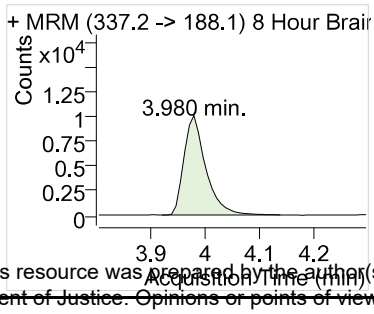


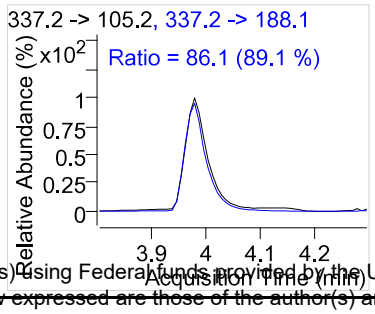




Fentanyl







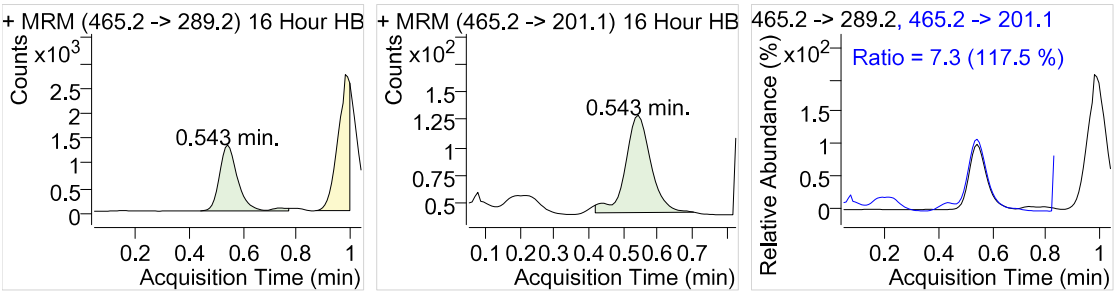
Quantitative Analysis Report

Agilent Technologies

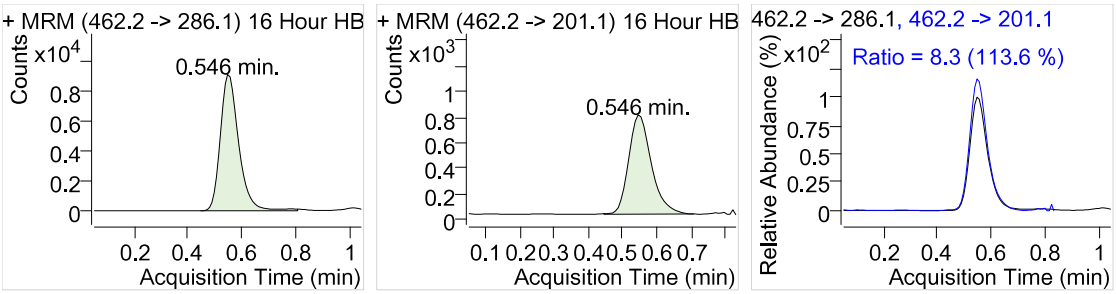
Sample Name:	:	16 Hour HB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\16 Hour HB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 2:32:37 PM
Dilution	:	1.3
Operator	:	
Sample Position	:	P1-C8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	6234			
	465.2 -> 201.1		453	7.3	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	42760			1266.9 ng/ml
	462.2 -> 201.1		3560	8.3	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.93	40163			
	292.2 -> 128.2		7152	17.8	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	108469			64.6 ng/ml
	272.1 -> 165.1		84095	77.5	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.93	40163			
	292.2 -> 128.2		7152	17.8	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.96	177516			2028.6 ng/ml
	286.1 -> 128.1		105359	59.4	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	13064			
	465.2 -> 165.1		1366	10.5	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	31099			316.6 ng/ml
	462.2 -> 165.0		157	*0.5	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	390656			
Norfentanyl	233.1 -> 84.1	2.49	6097			0.8 ng/ml
	233.1 -> 150.1		203	3.3	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	927685			
	342.4 -> 105.1		668547	72.1	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	103495			2.2 ng/ml
	337.2 -> 188.1		95952	92.7	77.3 - 116.0	

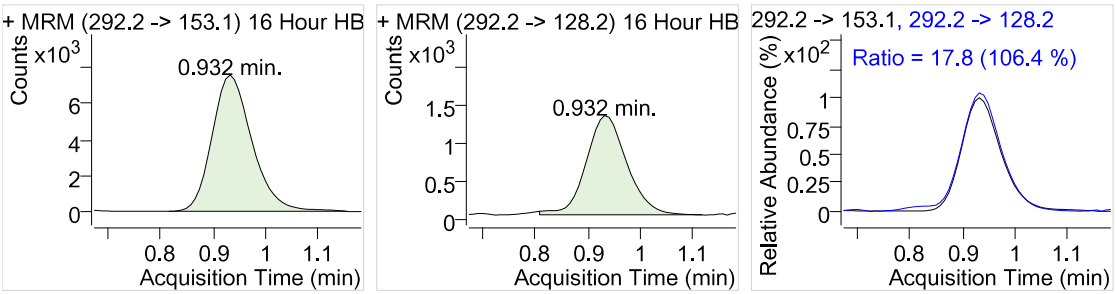
Morphine-3-B-D-Glucuronide D3



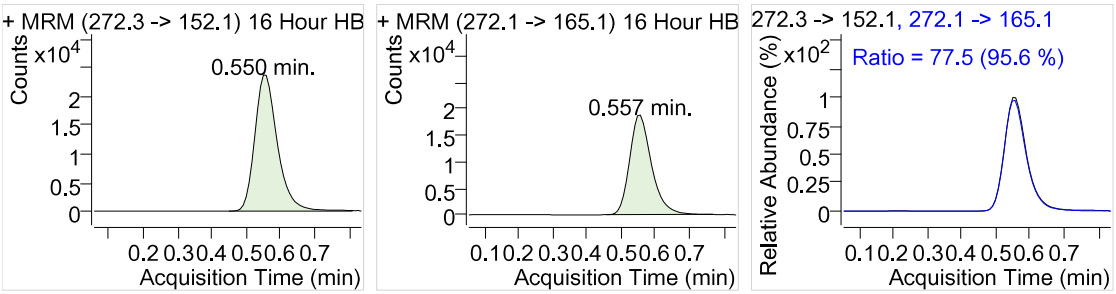
Morphine-3-B-D-Glucuronide



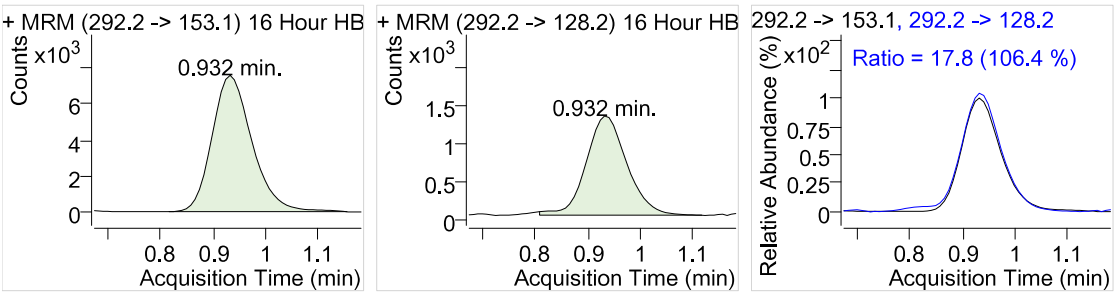
Morphine D6



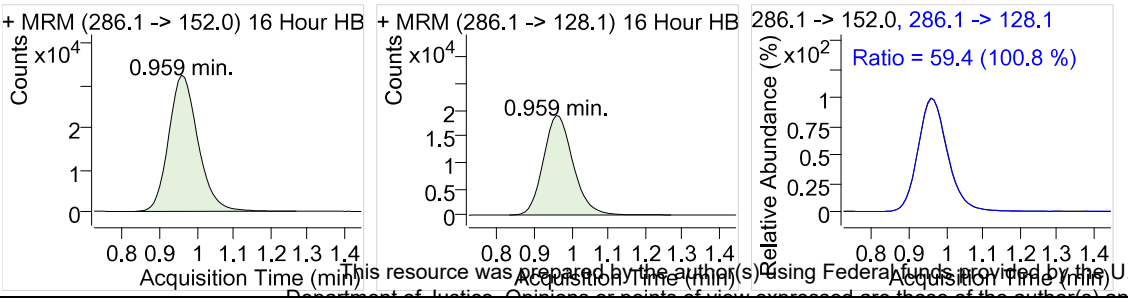
Normorphine



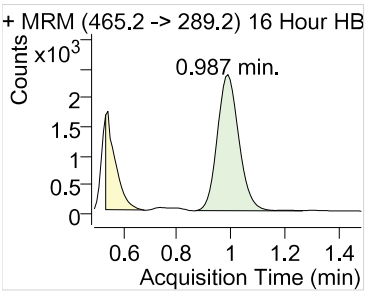
Morphine D6

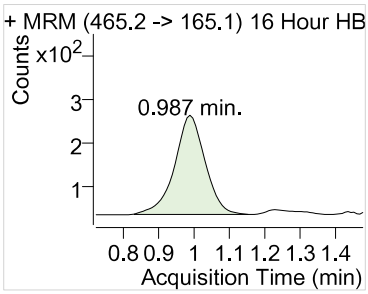


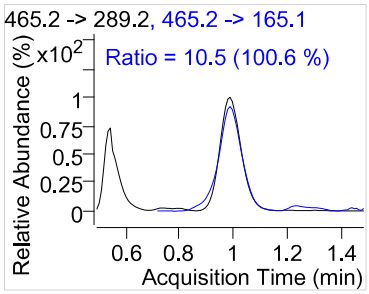
Morphine



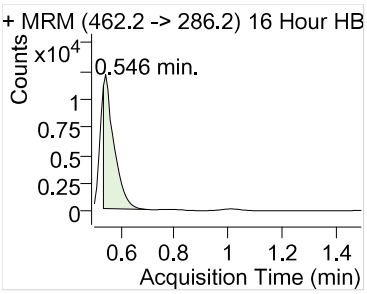
Morphine-6-B-D-Glucuronide D3

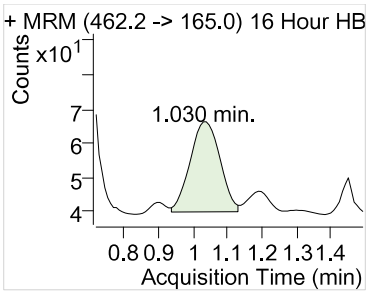


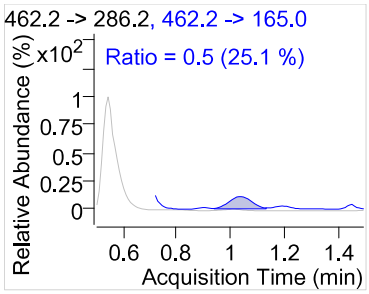




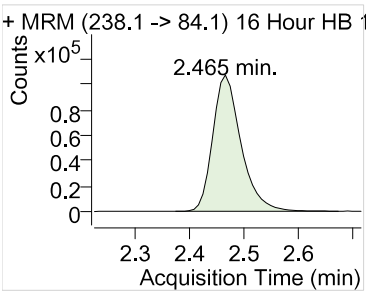
Morphine-6-B-D-Glucuronide



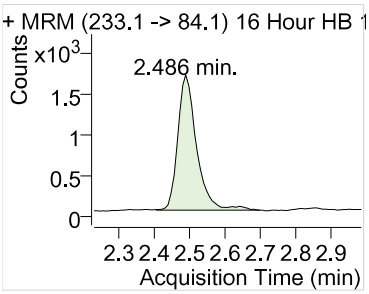


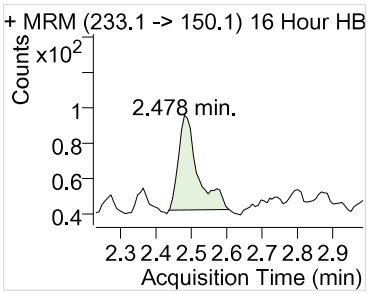


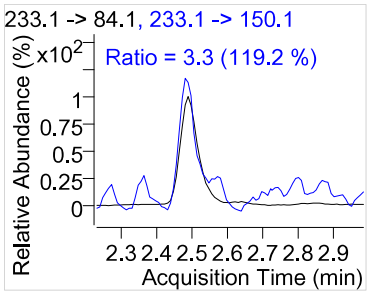
Norfentanyl D5



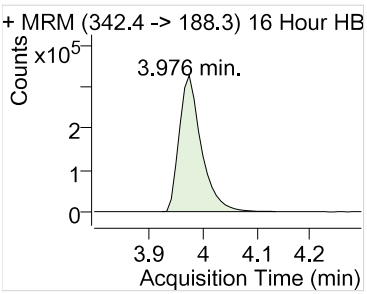
Norfentanyl

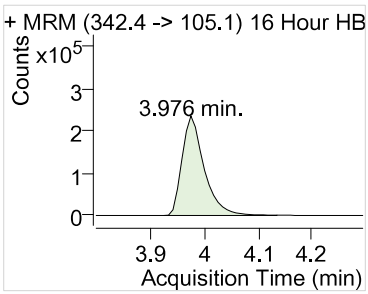


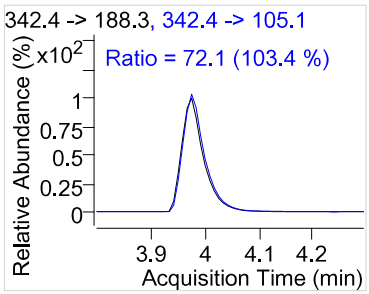




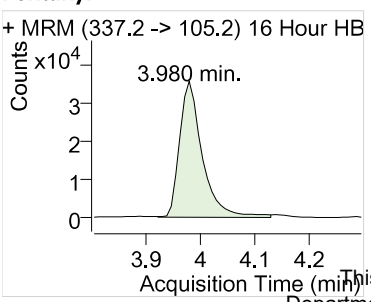
Fentanyl D5

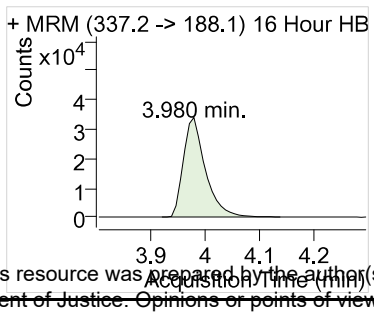


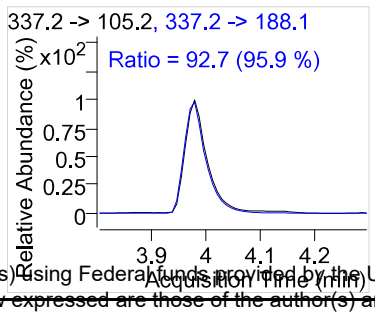




Fentanyl







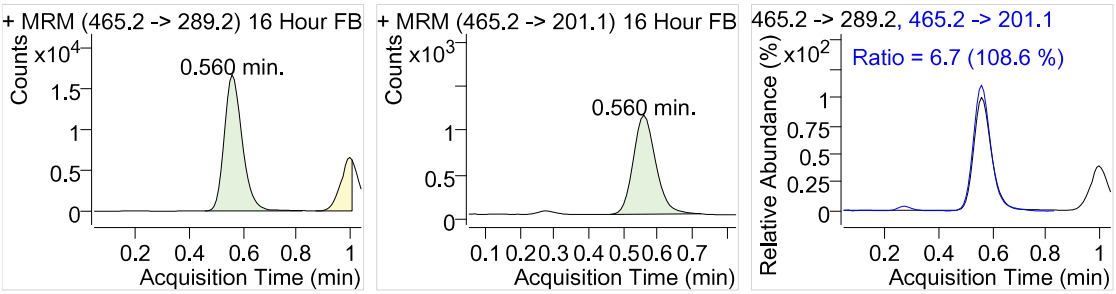
Quantitative Analysis Report

Agilent Technologies

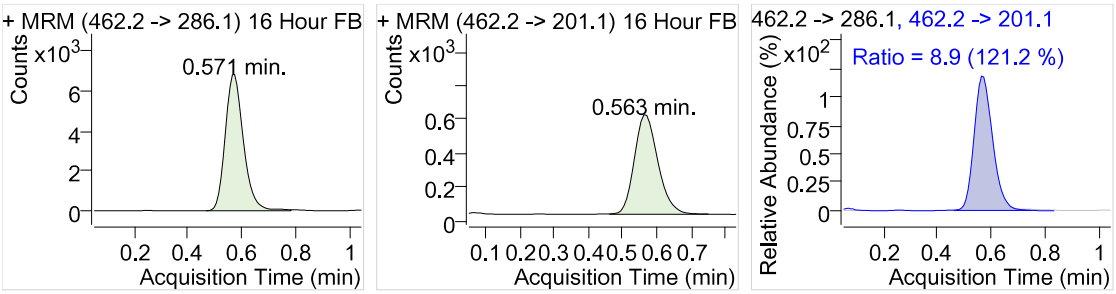
Sample Name:	:	16 Hour FB 3
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\16 Hour FB 3.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 3:37:10 PM
Dilution	:	14.1
Operator	:	
Sample Position	:	P1-D5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.56	76542			
	465.2 -> 201.1		5135	6.7	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.57	31540			819.4 ng/ml
	462.2 -> 201.1		2802	*8.9	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.95	74670			
	292.2 -> 128.2		12103	16.2	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.58	343			98.4 ng/ml
	272.1 -> 165.1		395	*115.3	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.95	74670			
	292.2 -> 128.2		12103	16.2	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.98	7630			487.5 ng/ml
	286.1 -> 128.1		5008	65.6	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	30888			
	465.2 -> 165.1		3013	9.8	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	23268			1145.3 ng/ml
	462.2 -> 165.0		25	*0.1	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.46	313338			
Norfentanyl	233.1 -> 84.1	2.49	706			6.0 ng/ml
	233.1 -> 150.1		32	*4.6	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	144249			
	342.4 -> 105.1		110664	76.7	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.97	887			4.2 ng/ml
	337.2 -> 188.1		1252	*141.2	77.3 - 116.0	

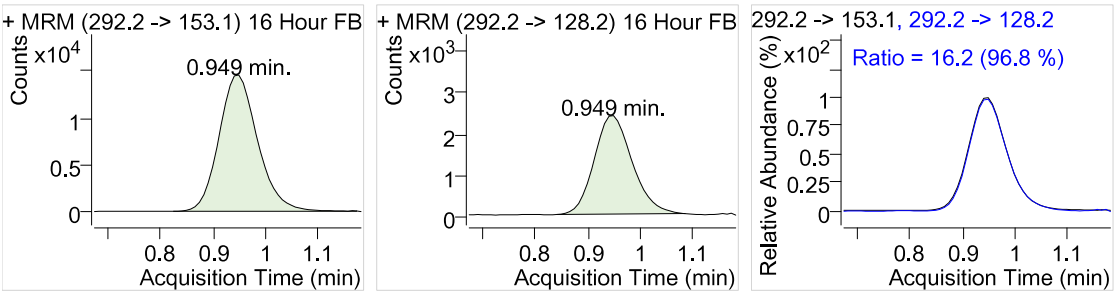
Morphine-3-B-D-Glucuronide D3



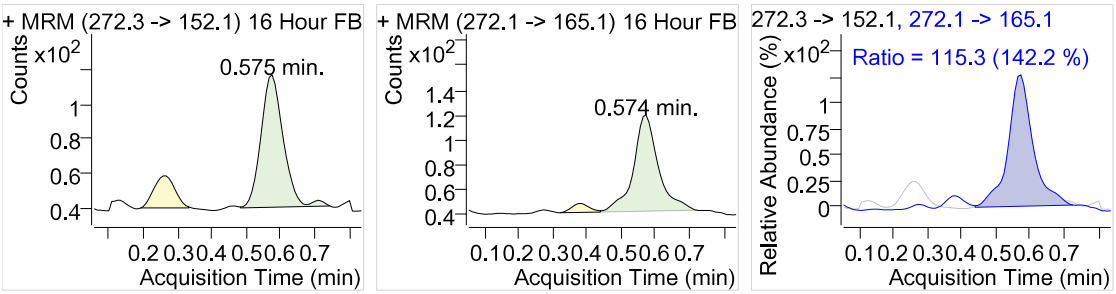
Morphine-3-B-D-Glucuronide



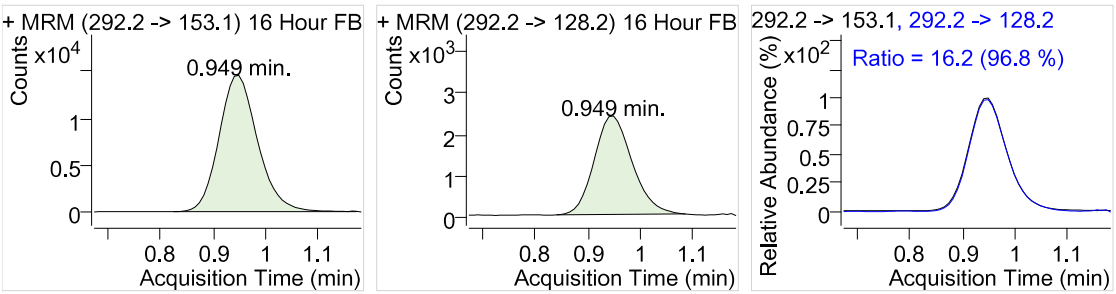
Morphine D6



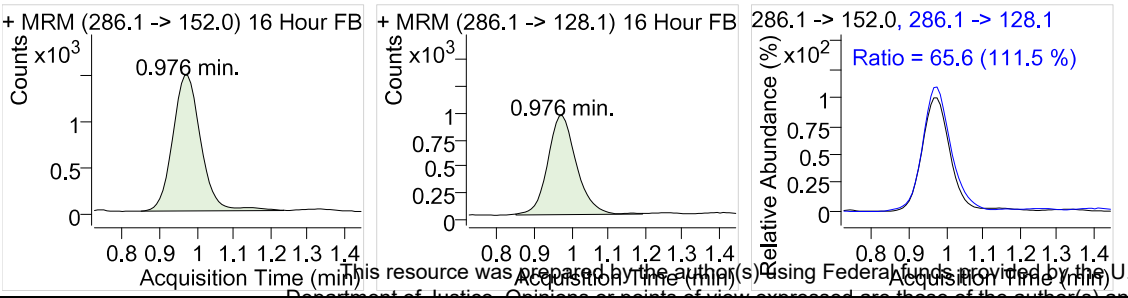
Normorphine



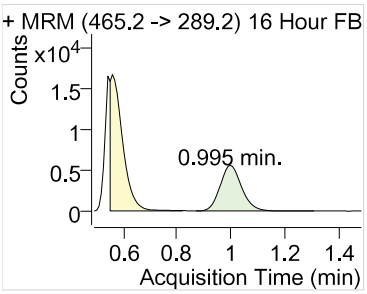
Morphine D6

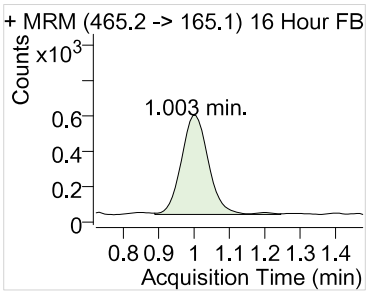


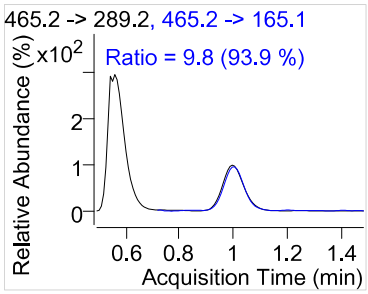
Morphine



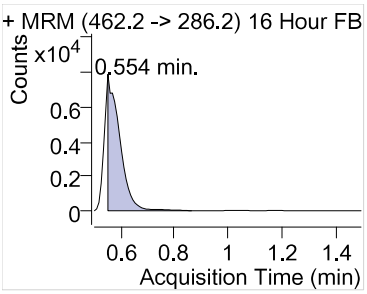
Morphine-6-B-D-Glucuronide D3

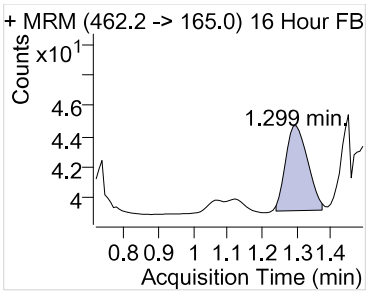


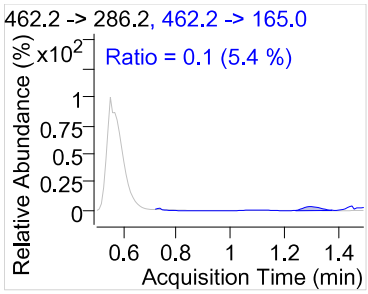




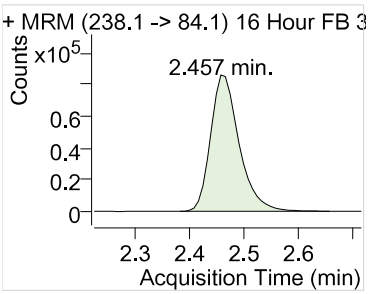
Morphine-6-B-D-Glucuronide



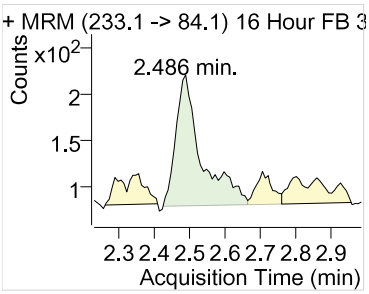


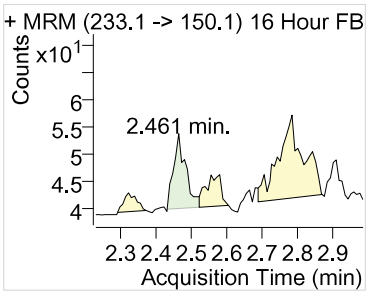


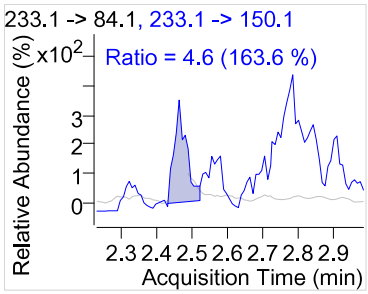
Norfentanyl D5



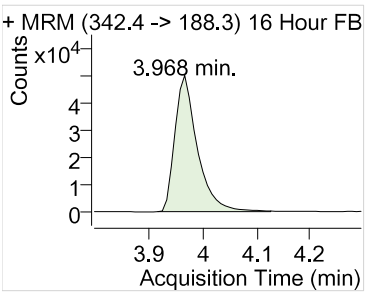
Norfentanyl

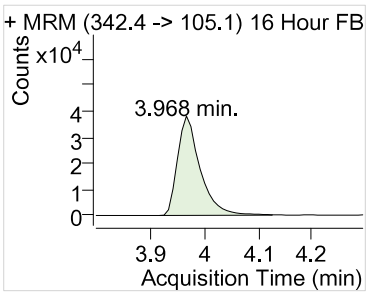


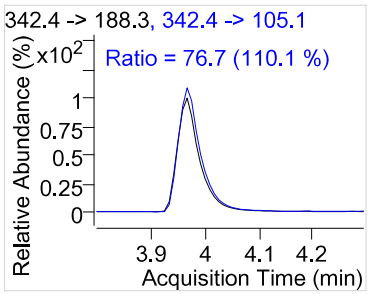




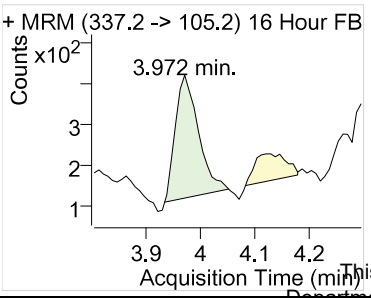
Fentanyl D5

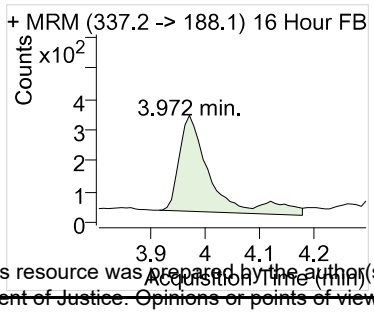


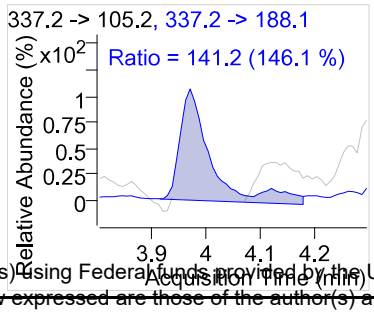




Fentanyl







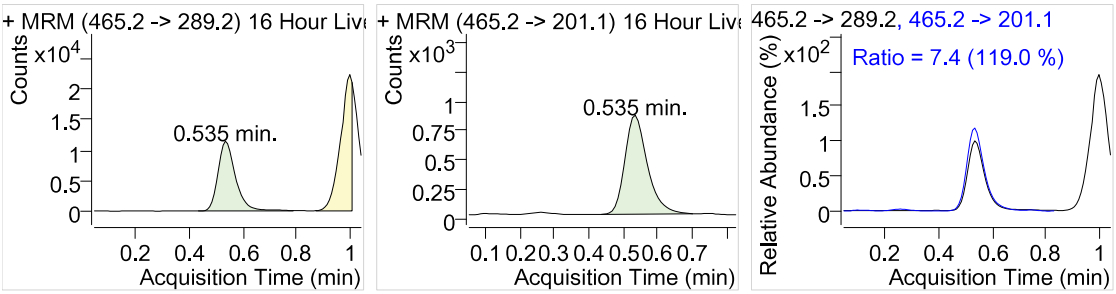
Quantitative Analysis Report

Agilent Technologies

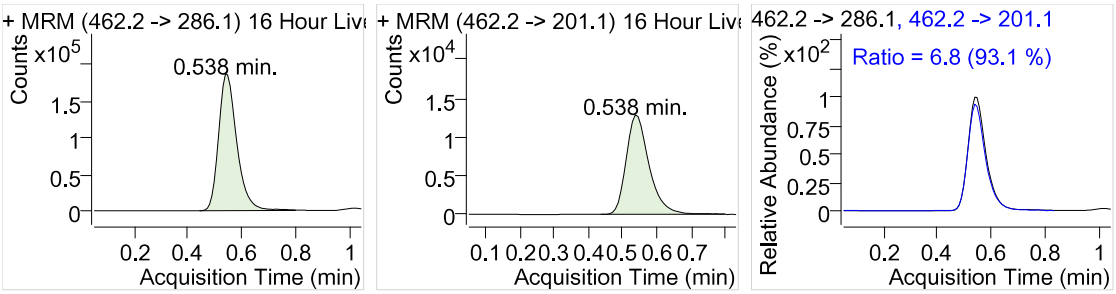
Sample Name:	:	16 Hour Liver 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\16 Hour Liver 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 3:38:33 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-D6

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	51708			
	465.2 -> 201.1		3803	7.4	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	871685			8842.1 ng/ml
	462.2 -> 201.1		59509	6.8	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.94	56523			
	292.2 -> 128.2		10066	17.8	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	403284			470.6 ng/ml
	272.1 -> 165.1		320737	79.5	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.94	56523			
	292.2 -> 128.2		10066	17.8	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.97	73662			1920.1 ng/ml
	286.1 -> 128.1		43616	59.2	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	104978			
	465.2 -> 165.1		10124	9.6	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	581677			2167.9 ng/ml
	462.2 -> 165.0		358	*0.1	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	350596			
Norfentanyl	233.1 -> 84.1	2.49	8906			2.7 ng/ml
	233.1 -> 150.1		406	*4.6	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	435853			
	342.4 -> 105.1		320184	73.5	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	28307			4.2 ng/ml
	337.2 -> 188.1		22668	80.1	77.3 - 116.0	

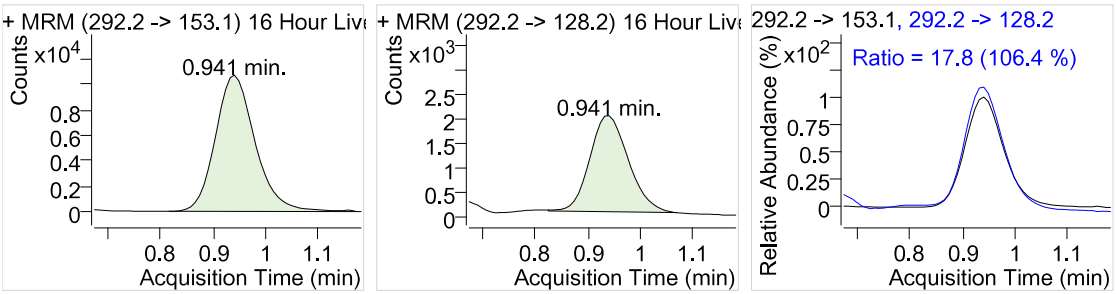
Morphine-3-B-D-Glucuronide D3



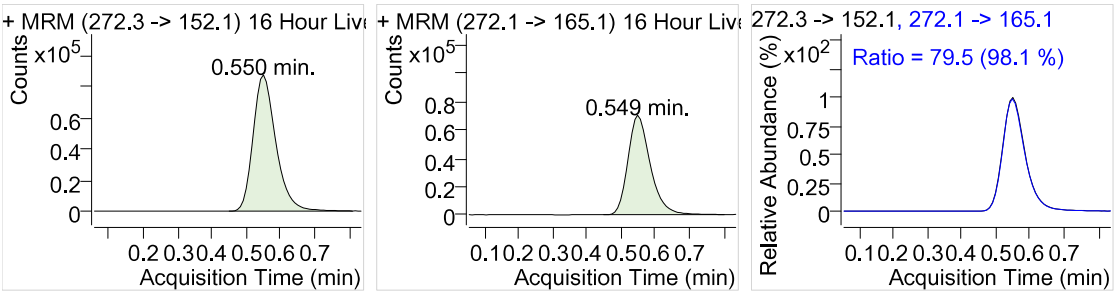
Morphine-3-B-D-Glucuronide



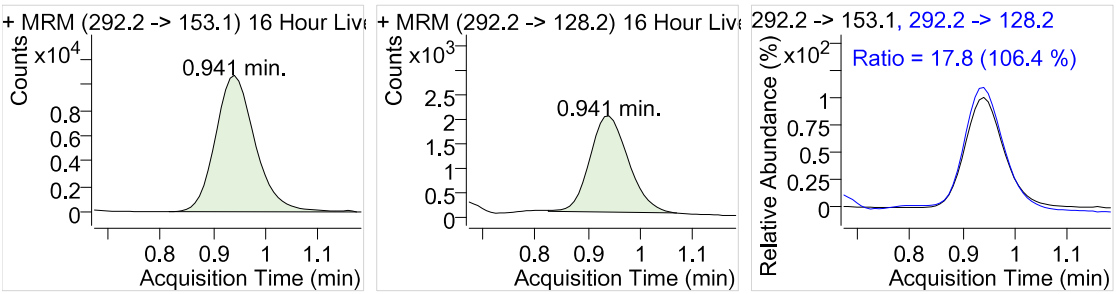
Morphine D6



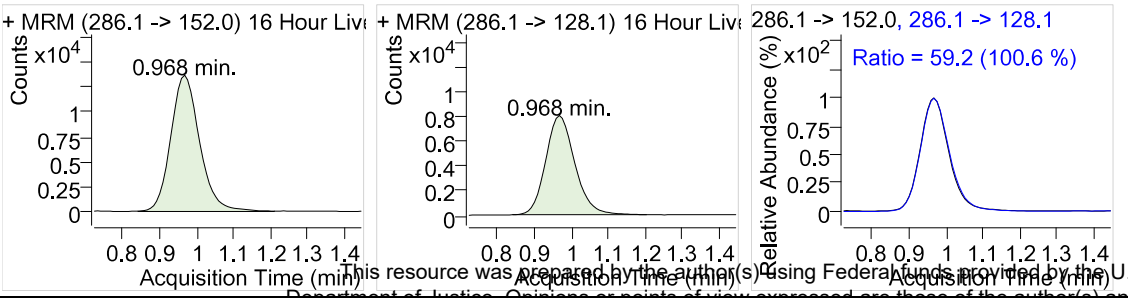
Normorphine



Morphine D6



Morphine



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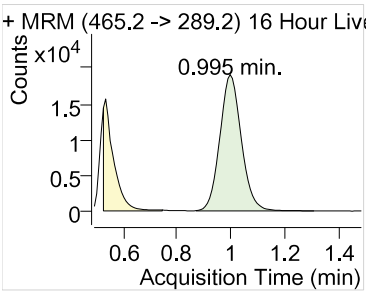
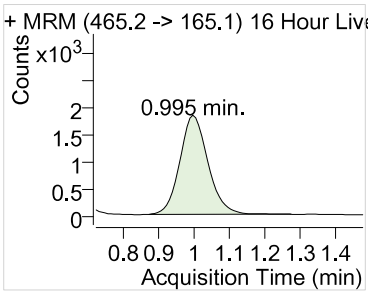
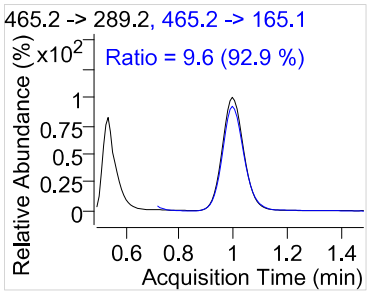
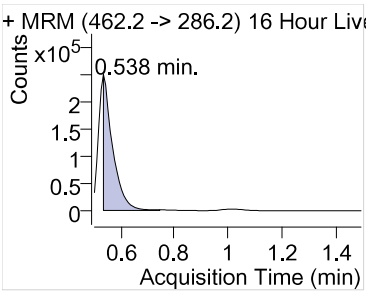
Morphine-6-B-D-Glucuronide D3

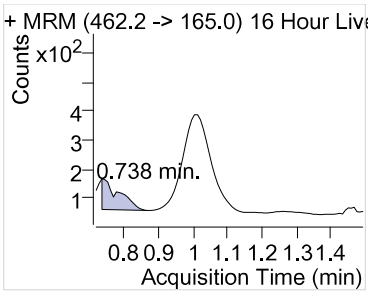


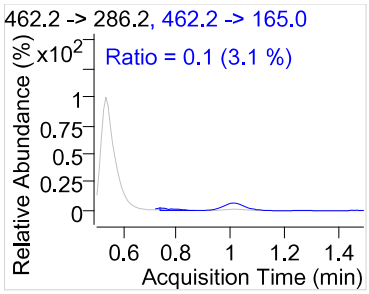




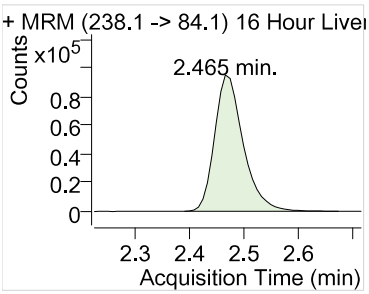
Morphine-6-B-D-Glucuronide



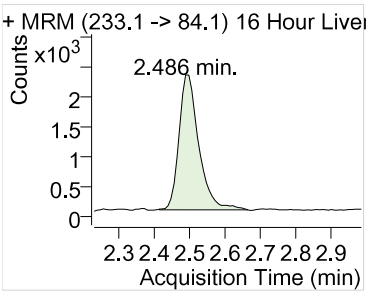


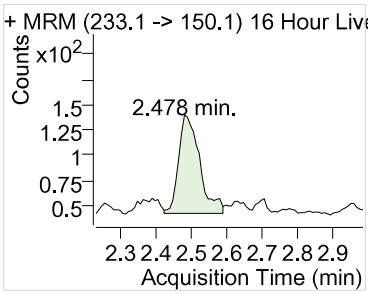


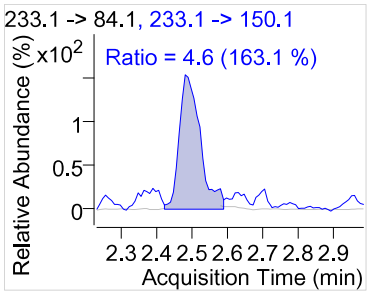
Norfentanyl D5



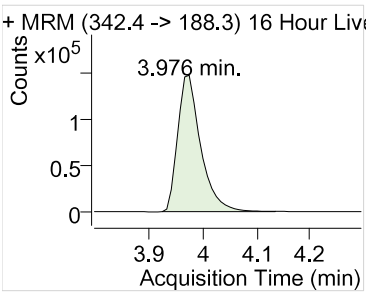
Norfentanyl

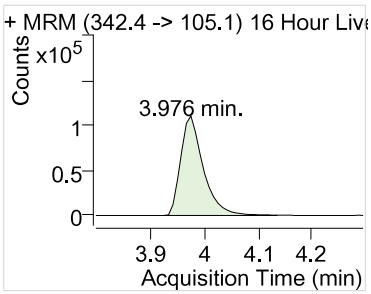


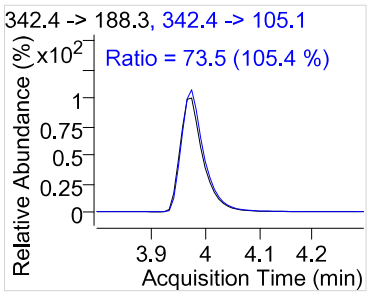




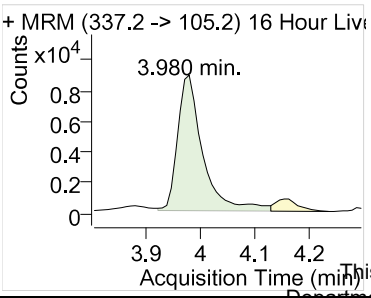
Fentanyl D5

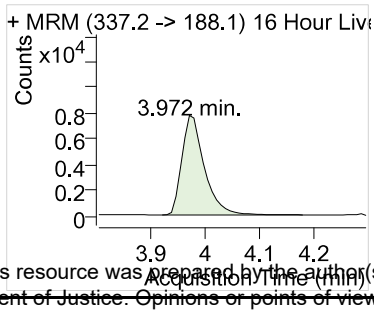


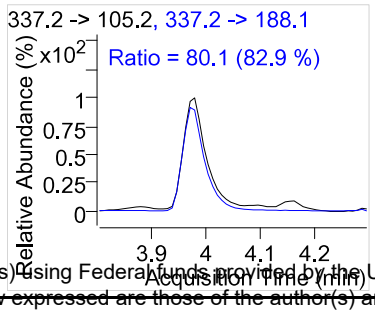




Fentanyl







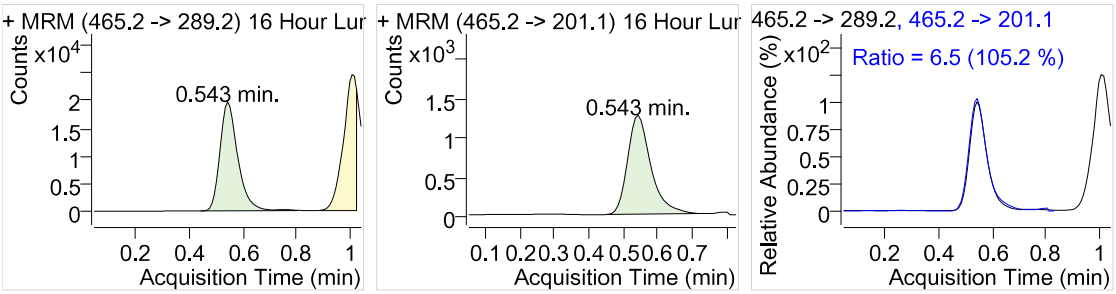
Quantitative Analysis Report

Agilent Technologies

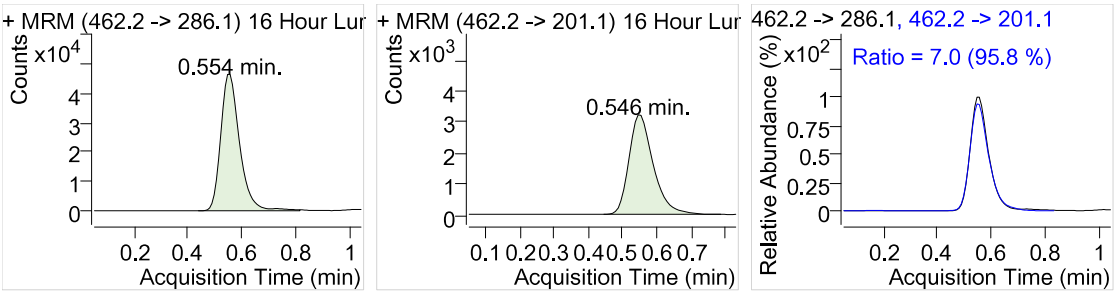
Sample Name:	:	16 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\16 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 4:16:17 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-E1

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	90208			
	465.2 -> 201.1		5867	6.5	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	223394			1222.9 ng/ml
	462.2 -> 201.1		15687	7.0	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.95	56037			
	292.2 -> 128.2		9021	16.1	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.56	51878			84.7 ng/ml
	272.1 -> 165.1		39888	76.9	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.95	56037			
	292.2 -> 128.2		9021	16.1	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.98	68821			1811.9 ng/ml
	286.1 -> 128.1		40338	58.6	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	118067			
	465.2 -> 165.1		11438	9.7	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	137019			482.3 ng/ml
	462.2 -> 165.0		480	*0.4	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	394819			
Norfentanyl	233.1 -> 84.1	2.49	2963			1.9 ng/ml
	233.1 -> 150.1		131	*4.4	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	980993			
	342.4 -> 105.1		713120	72.7	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	37984			2.9 ng/ml
	337.2 -> 188.1		35275	92.9	77.3 - 116.0	

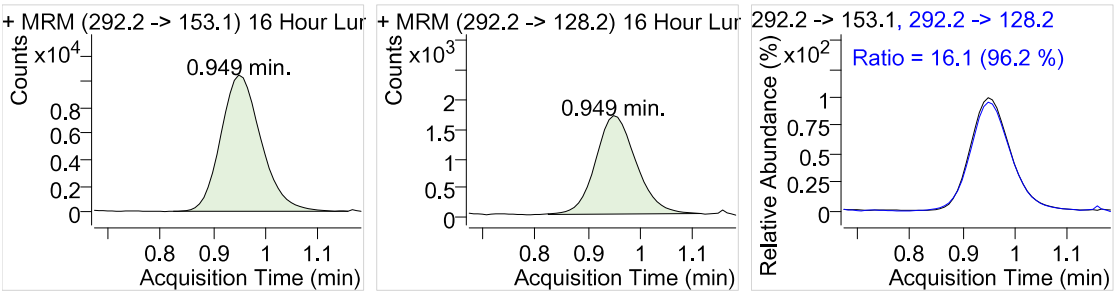
Morphine-3-B-D-Glucuronide D3



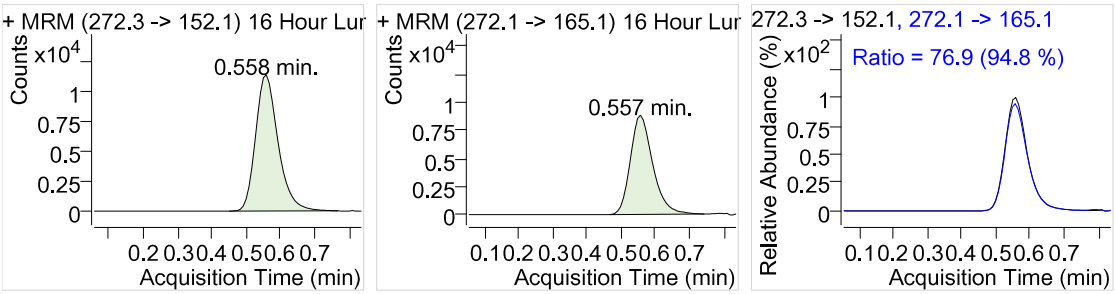
Morphine-3-B-D-Glucuronide



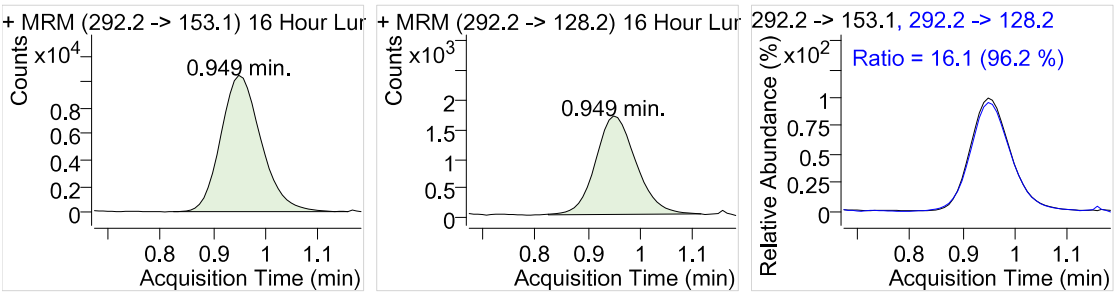
Morphine D6



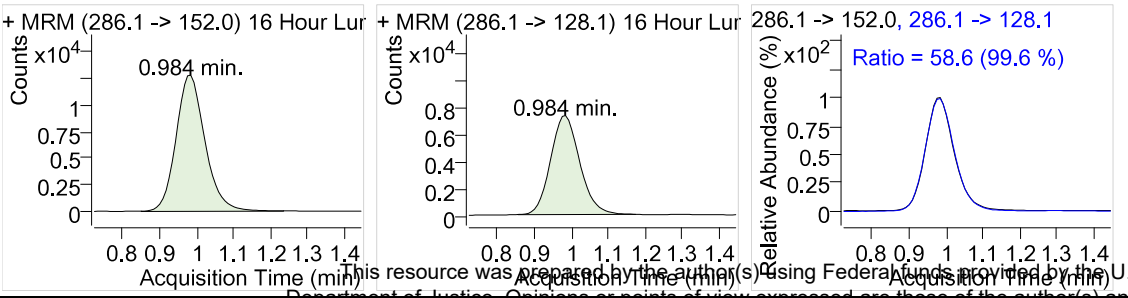
Normorphine



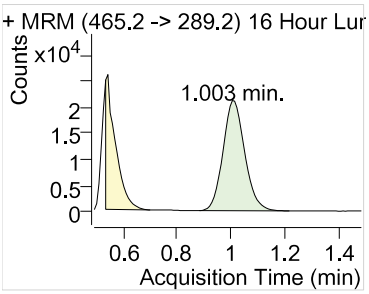
Morphine D6

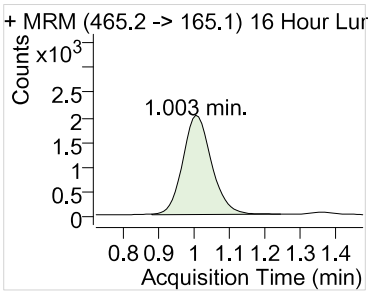


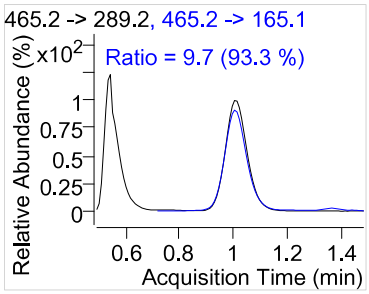
Morphine



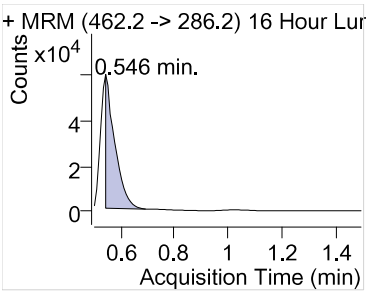
Morphine-6-B-D-Glucuronide D3

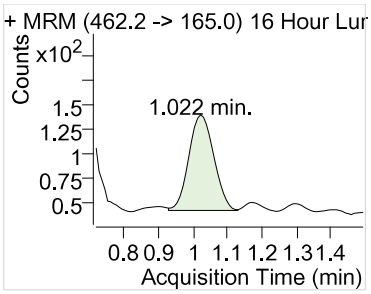


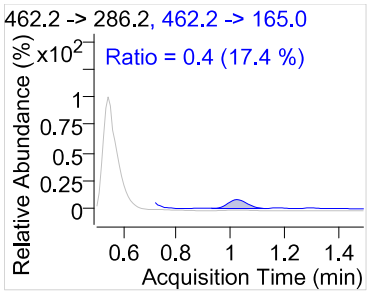




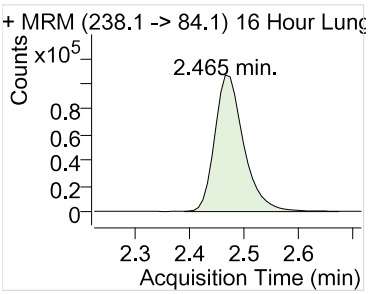
Morphine-6-B-D-Glucuronide



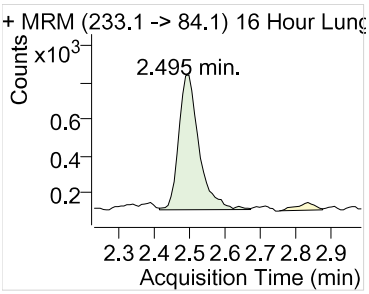


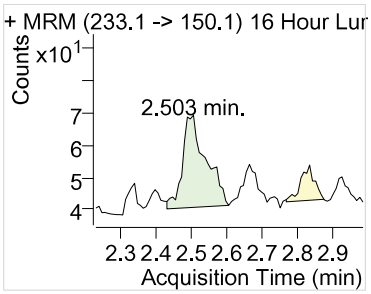


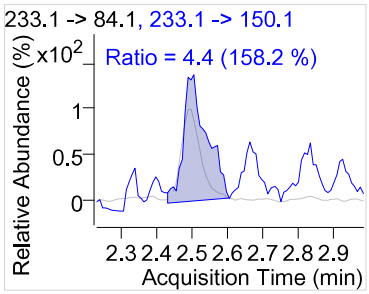
Norfentanyl D5



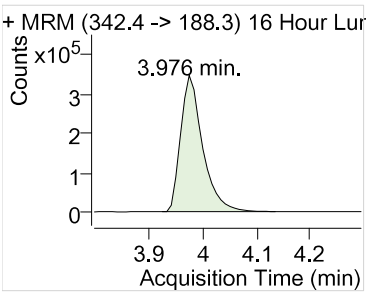
Norfentanyl

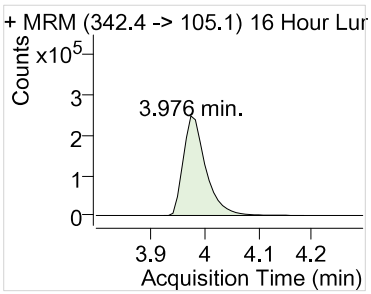


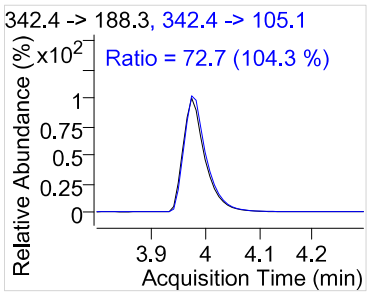




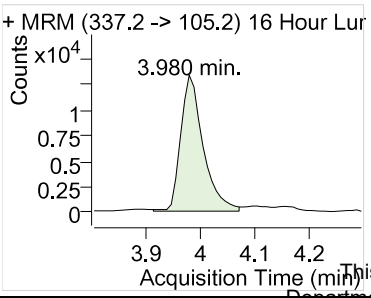
Fentanyl D5

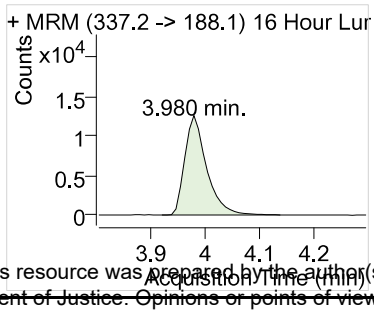


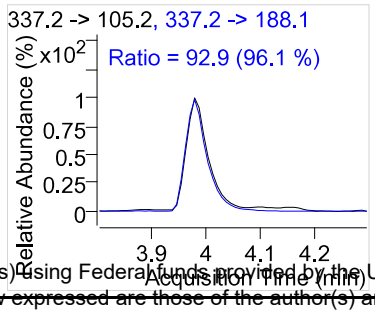




Fentanyl







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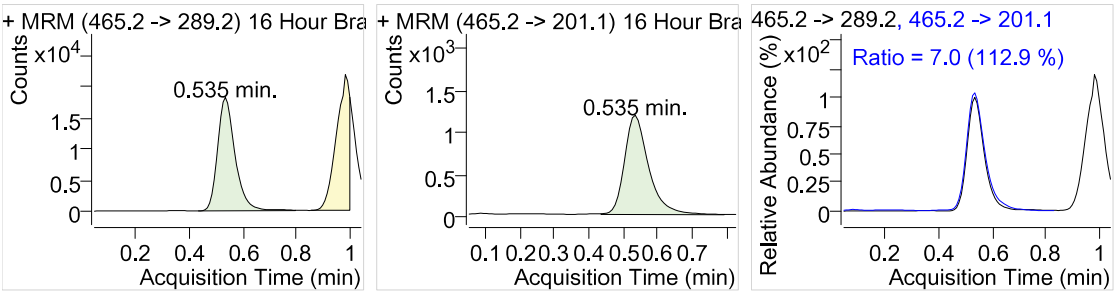
Quantitative Analysis Report

Agilent Technologies

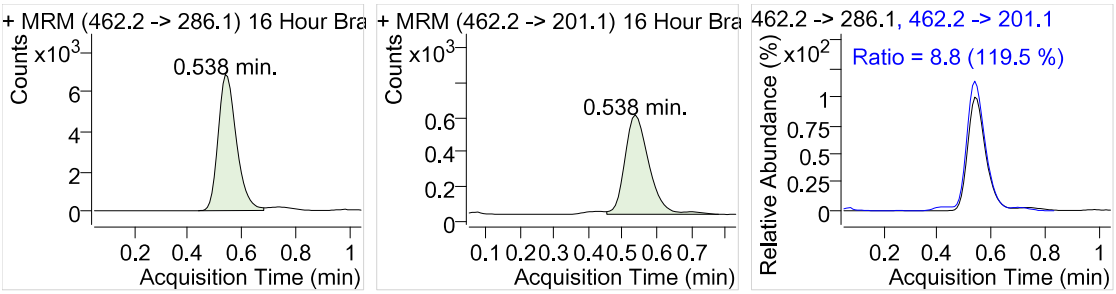
Sample Name:	:	16 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\16 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 4:25:41 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P1-E2

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	81990			
	465.2 -> 201.1		5722	7.0	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	32032			223.0 ng/ml
	462.2 -> 201.1		2805	8.8	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.93	68301			
	292.2 -> 128.2		11562	16.9	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	4502			31.7 ng/ml
	272.1 -> 165.1		3448	76.6	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.93	68301			
	292.2 -> 128.2		11562	16.9	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.96	19586			419.8 ng/ml
	286.1 -> 128.1		10680	54.5	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	105235			
	465.2 -> 165.1		10132	9.6	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	23274			120.2 ng/ml
	462.2 -> 165.0		65	*0.3	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	360775			
Norfentanyl	233.1 -> 84.1	2.49	1103			1.7 ng/ml
	233.1 -> 150.1		26	2.3	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	991029			
	342.4 -> 105.1		717849	72.4	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	46265			3.3 ng/ml
	337.2 -> 188.1		40890	88.4	77.3 - 116.0	

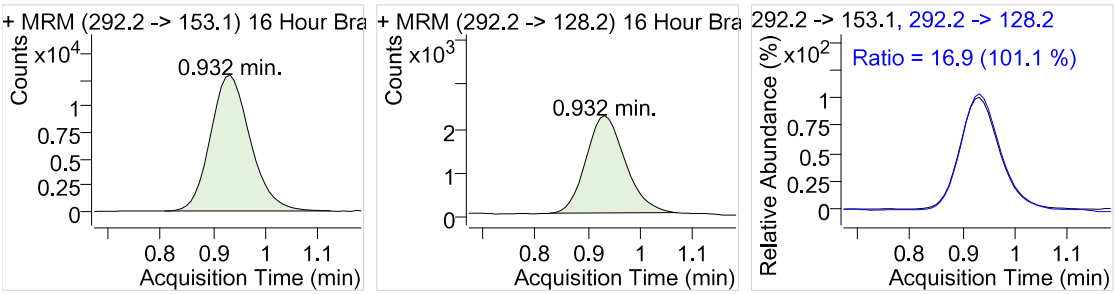
Morphine-3-B-D-Glucuronide D3



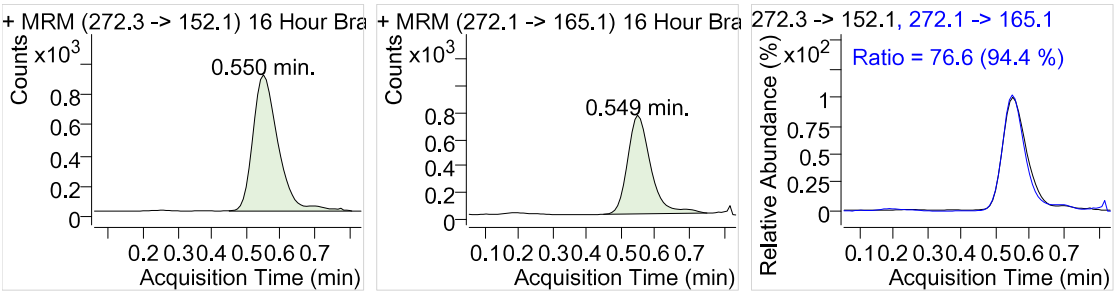
Morphine-3-B-D-Glucuronide



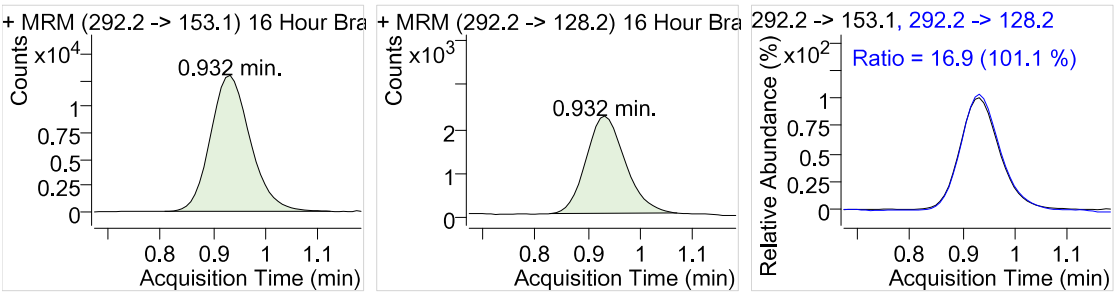
Morphine D6



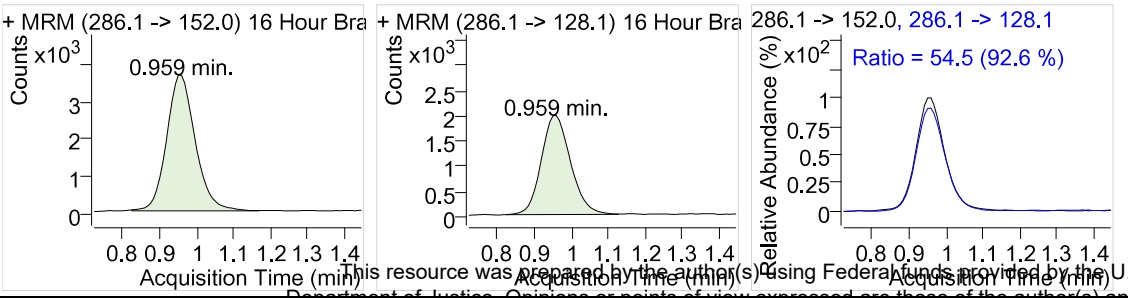
Normorphine



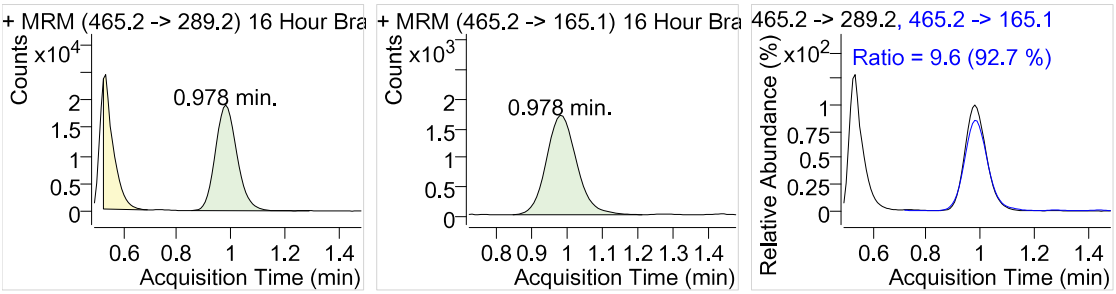
Morphine D6



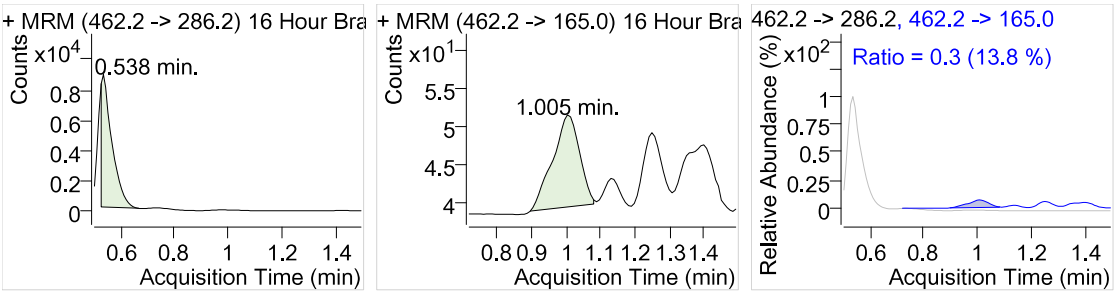
Morphine



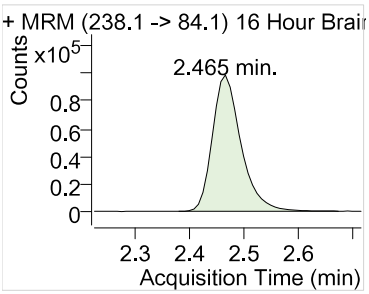
Morphine-6-B-D-Glucuronide D3



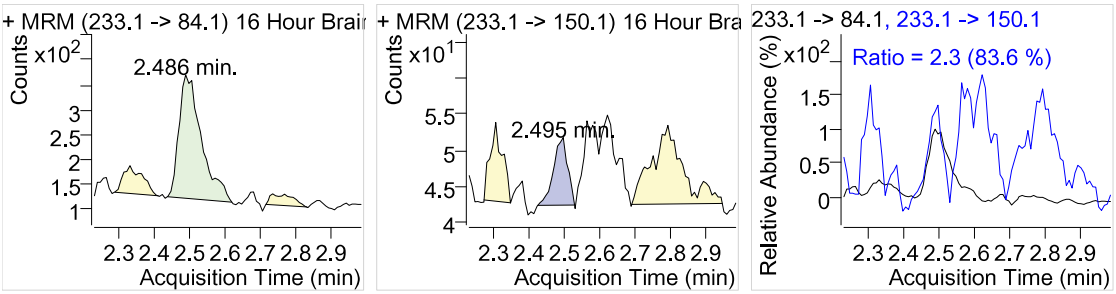
Morphine-6-B-D-Glucuronide



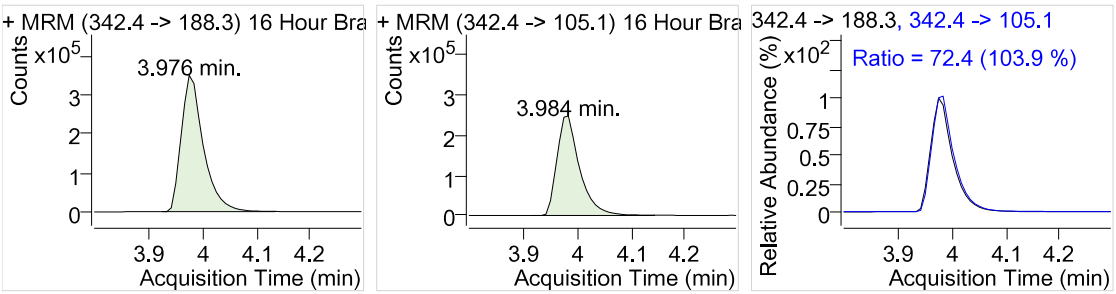
Norfentanyl D5



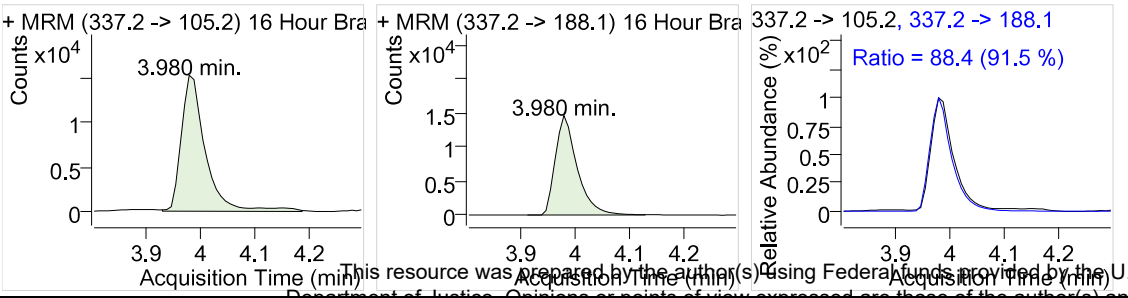
Norfentanyl



Fentanyl D5



Fentanyl



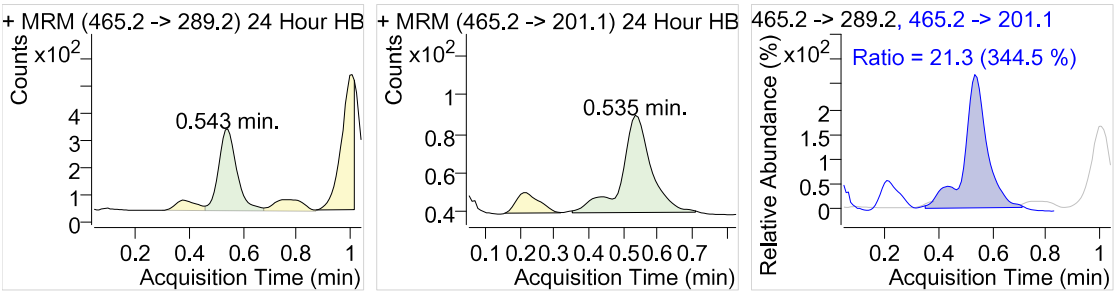
Quantitative Analysis Report

Agilent Technologies

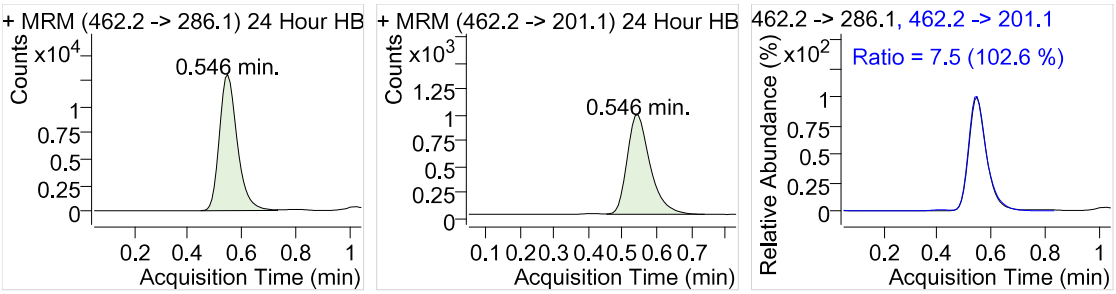
Sample Name:	:	24 Hour HB 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\24 Hour HB 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 5:39:32 PM
Dilution	:	0.9
Operator	:	
Sample Position	:	P1-E9

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	1410			
	465.2 -> 201.1		300	*21.3	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	59781			5158.0 ng/ml
	462.2 -> 201.1		4494	7.5	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.94	51431			
	292.2 -> 128.2		8703	16.9	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	173979			55.0 ng/ml
	272.1 -> 165.1		138635	79.7	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.94	51431			
	292.2 -> 128.2		8703	16.9	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.97	101175			664.9 ng/ml
	286.1 -> 128.1		60207	59.5	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	2410			
	465.2 -> 165.1		448	*18.6	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	41545			1545.4 ng/ml
	462.2 -> 165.0		374	*0.9	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	410661			
Norfentanyl	233.1 -> 84.1	2.49	14735			0.7 ng/ml
	233.1 -> 150.1		460	3.1	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	947639			
	342.4 -> 105.1		684932	72.3	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	88346			1.3 ng/ml
	337.2 -> 188.1		78093	88.4	77.3 - 116.0	

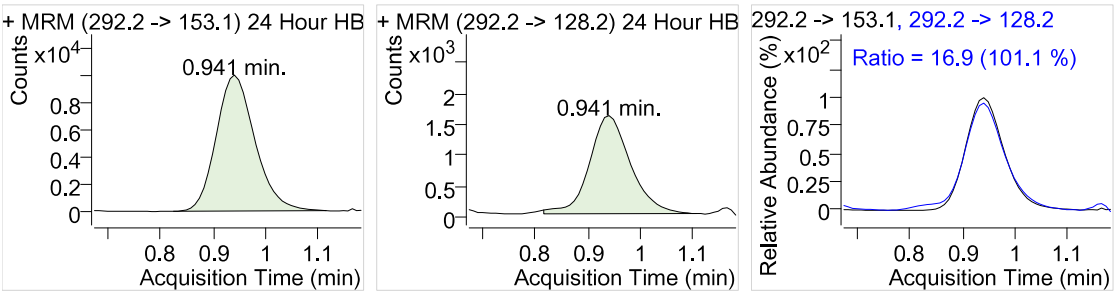
Morphine-3-B-D-Glucuronide D3



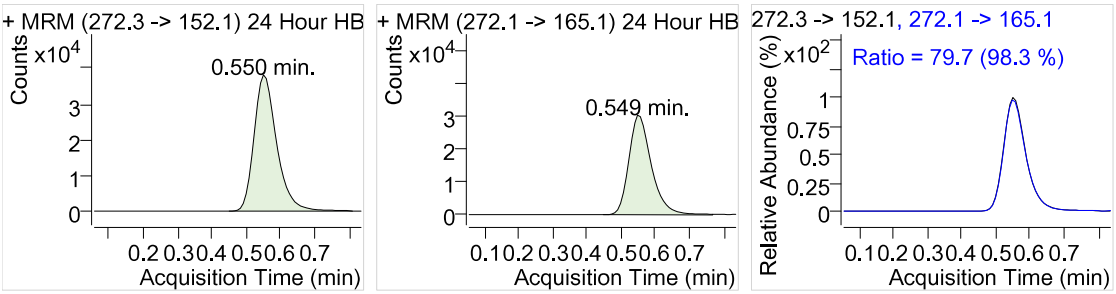
Morphine-3-B-D-Glucuronide



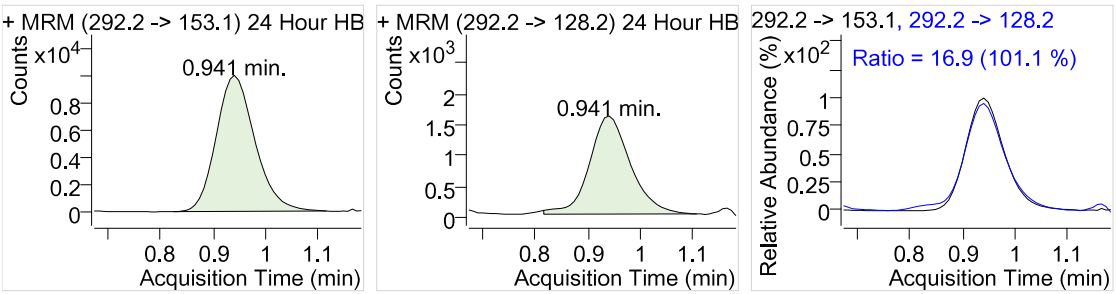
Morphine D6



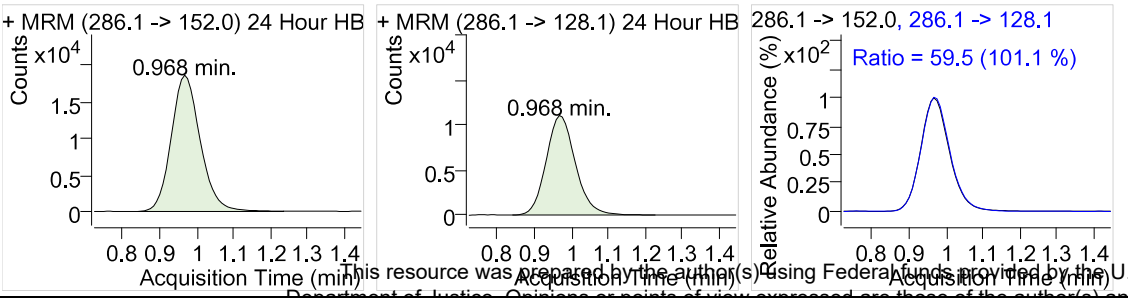
Normorphine



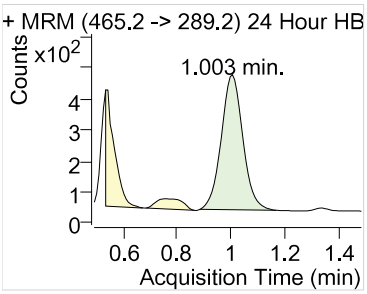
Morphine D6

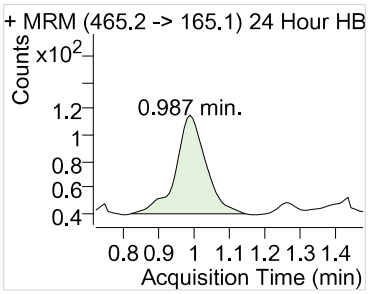


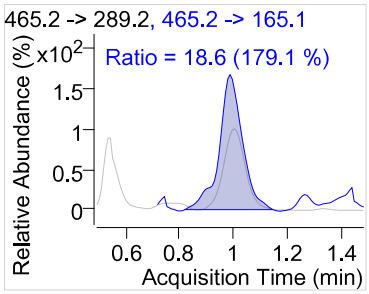
Morphine



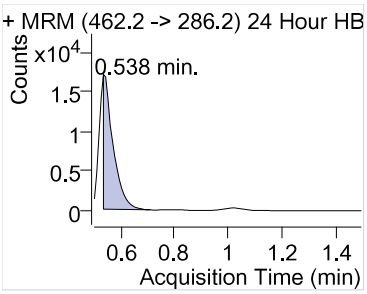
Morphine-6-B-D-Glucuronide D3

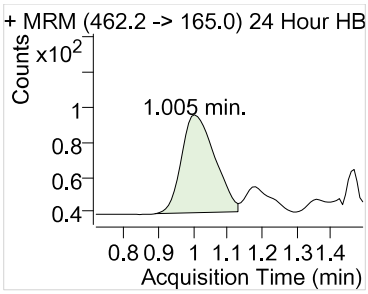


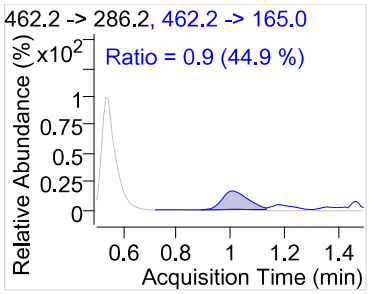




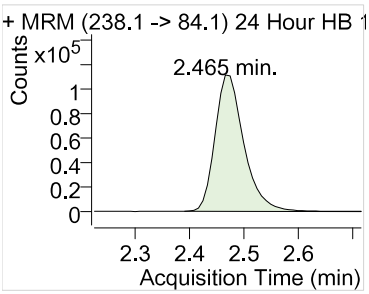
Morphine-6-B-D-Glucuronide



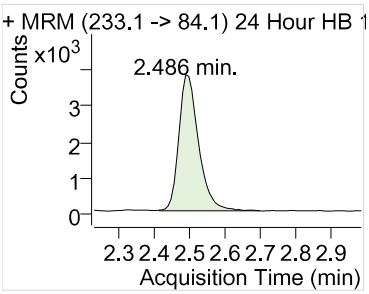


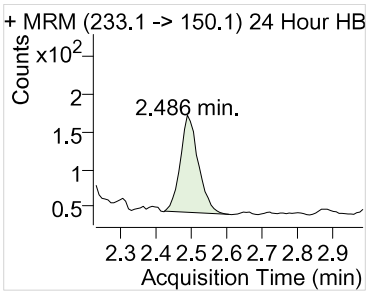


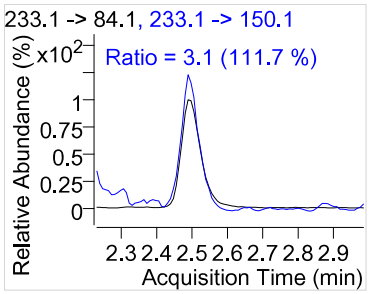
Norfentanyl D5



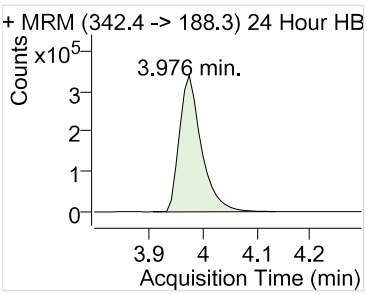
Norfentanyl

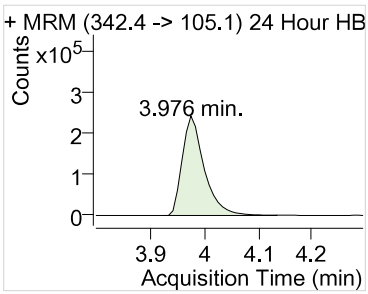


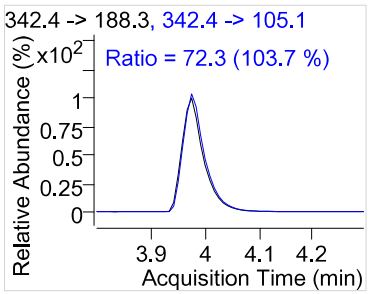




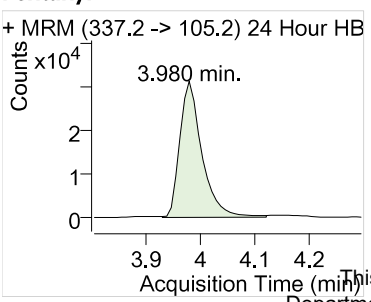
Fentanyl D5

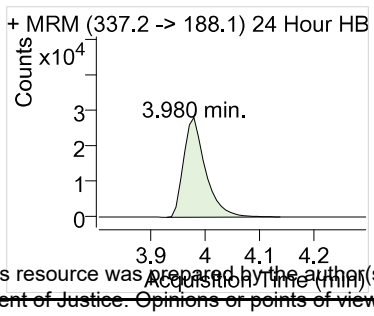


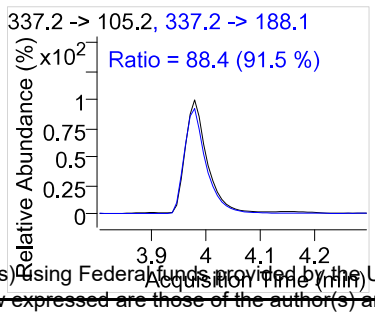




Fentanyl







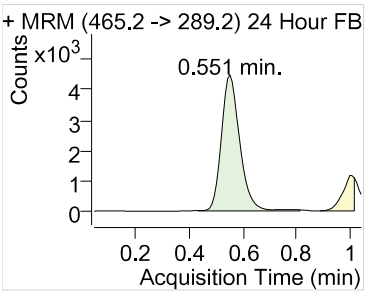
Quantitative Analysis Report

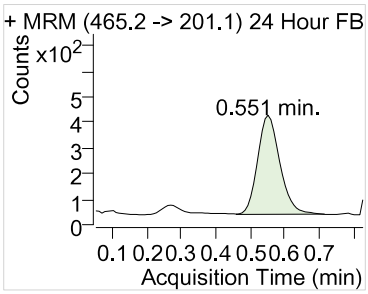
Agilent Technologies

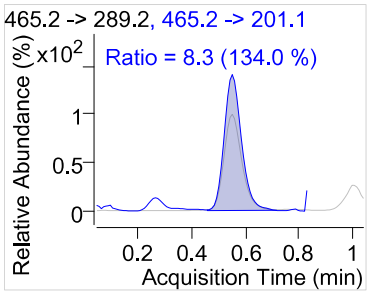
Sample Name:	:	24 Hour FB 2
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\24 Hour FB 2.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 6:17:11 PM
Dilution	:	13.2
Operator	:	
Sample Position	:	P1-F5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.55	21051			
	465.2 -> 201.1		1743	*8.3	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	274			172.5 ng/ml
	462.2 -> 201.1		72	*26.3	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.95	99490			
	292.2 -> 128.2		16562	16.6	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.56	479			92.0 ng/ml
	272.1 -> 165.1		396	82.6	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.95	99490			
	292.2 -> 128.2		16562	16.6	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.98	2324			57.5 ng/ml
	286.1 -> 128.1		977	*42.0	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.00	5355			
	465.2 -> 165.1		558	10.4	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.79	262			177.0 ng/ml
	462.2 -> 165.0		46	*17.6	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	424631			
Norfentanyl	233.1 -> 84.1	2.50	516			5.5 ng/ml
	233.1 -> 150.1		53	*10.4	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	944164			
	342.4 -> 105.1		701850	74.3	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.97	5216			3.9 ng/ml
	337.2 -> 188.1		4412	84.6	77.3 - 116.0	

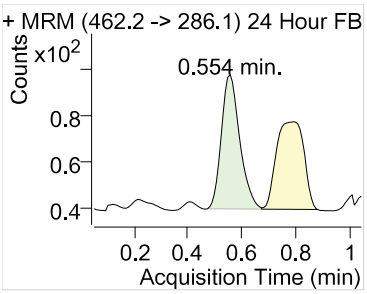
Morphine-3-B-D-Glucuronide D3

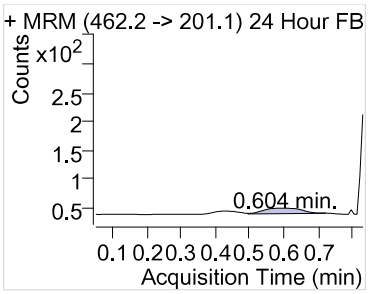


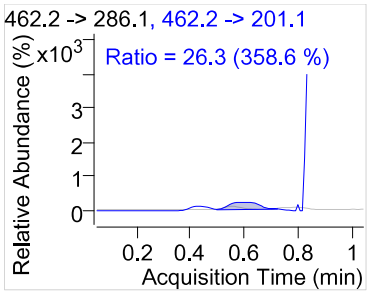




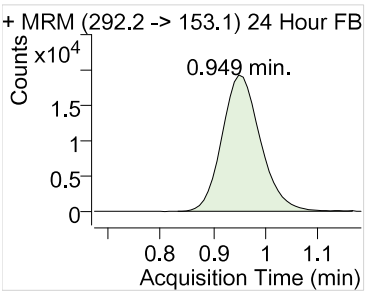
Morphine-3-B-D-Glucuronide

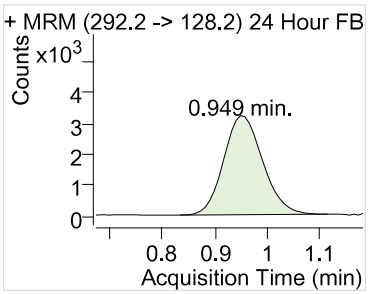


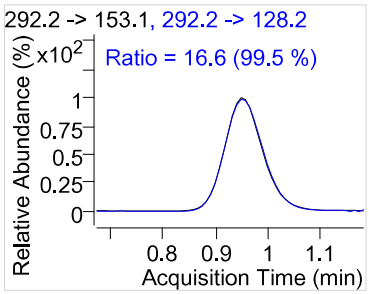




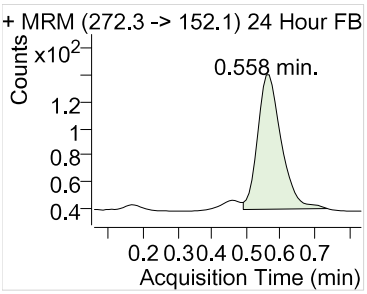
Morphine D6

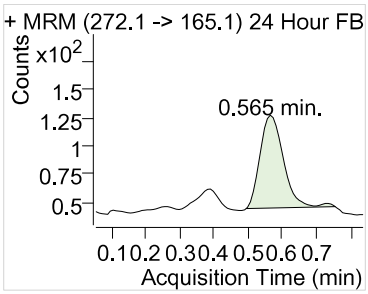


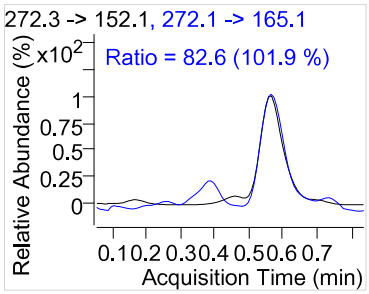




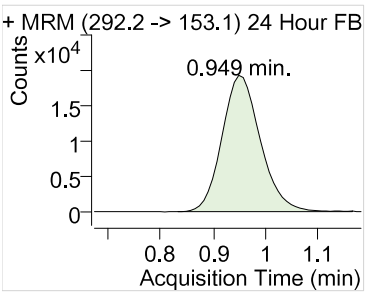
Normorphine

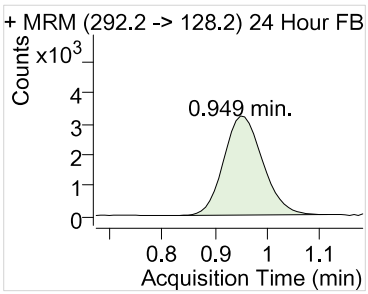


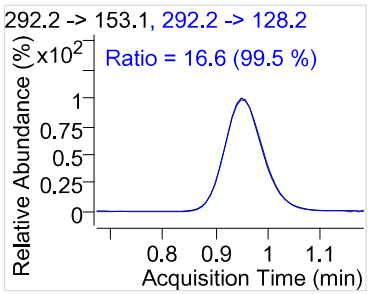




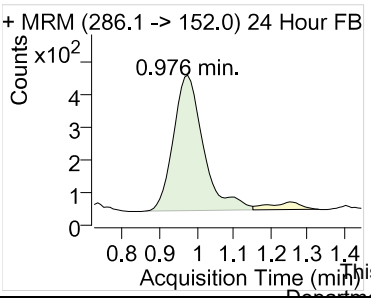
Morphine D6

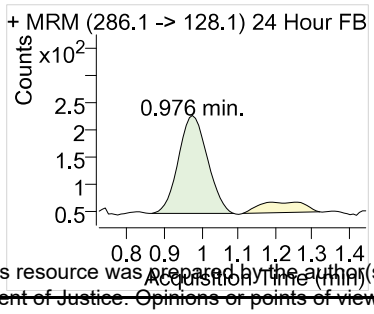


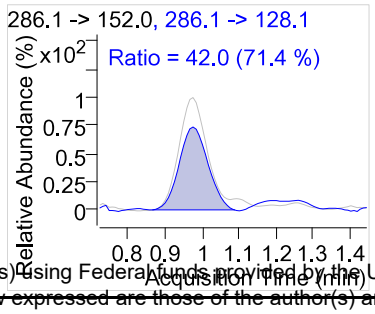




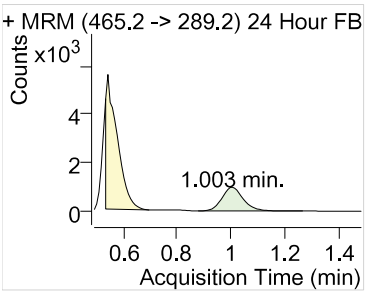
Morphine

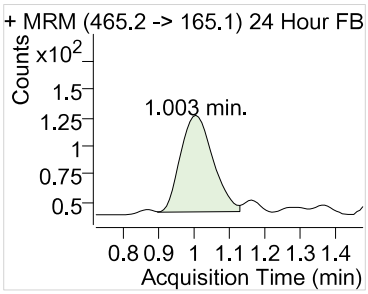


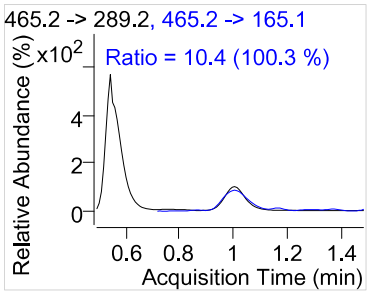




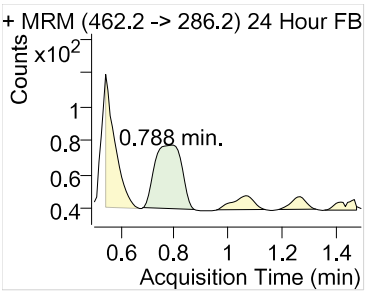
Morphine-6-B-D-Glucuronide D3

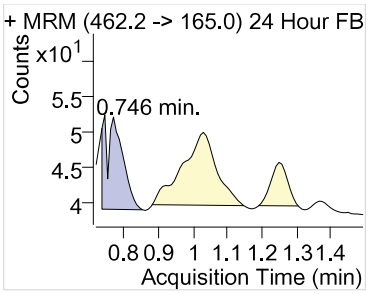


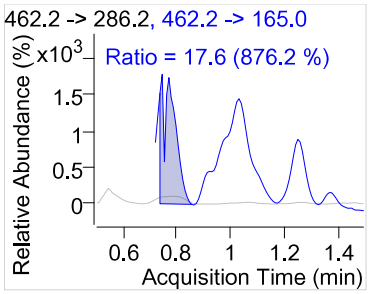




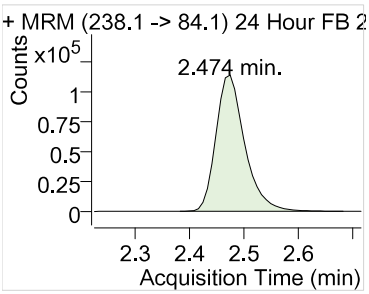
Morphine-6-B-D-Glucuronide



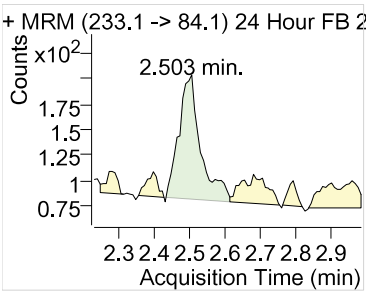


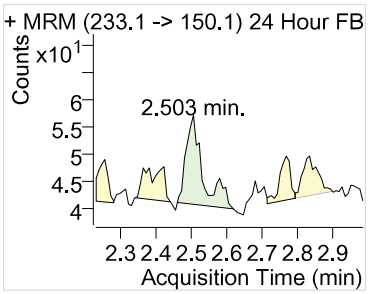


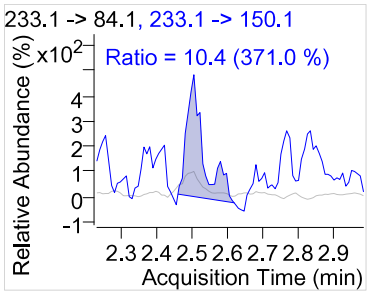
Norfentanyl D5



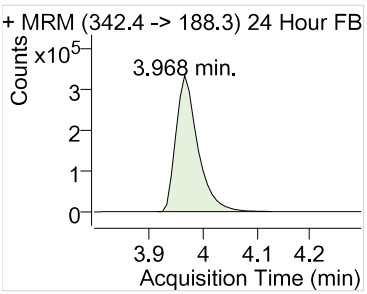
Norfentanyl

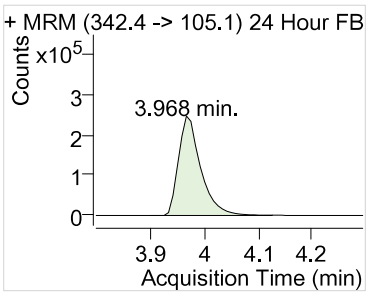


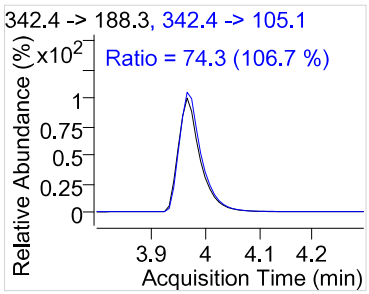




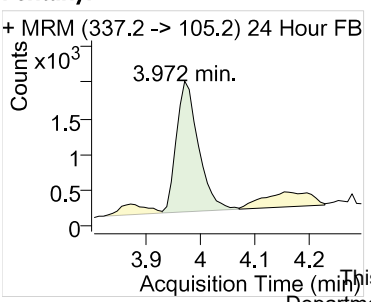
Fentanyl D5

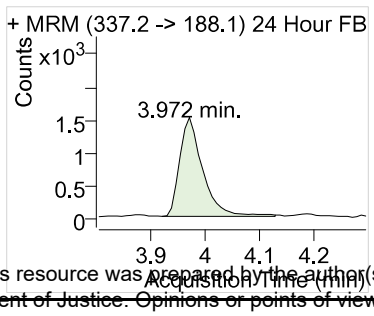


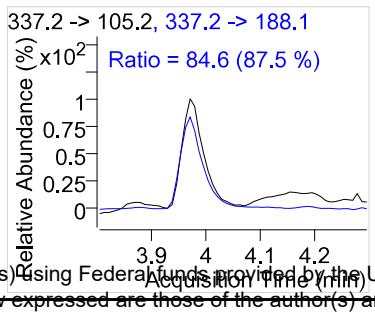




Fentanyl







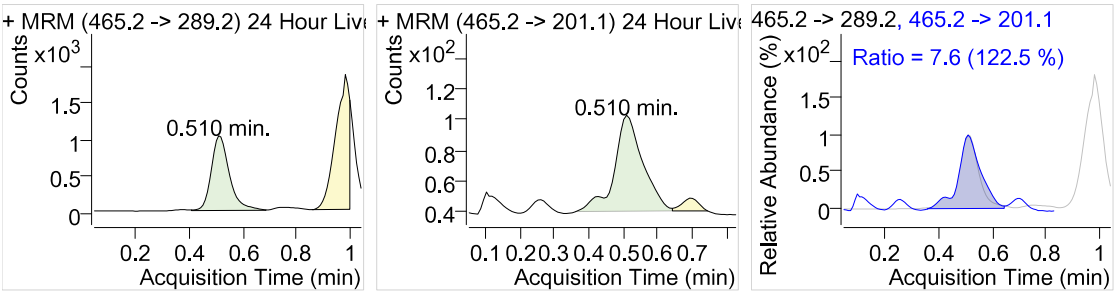
Quantitative Analysis Report

Agilent Technologies

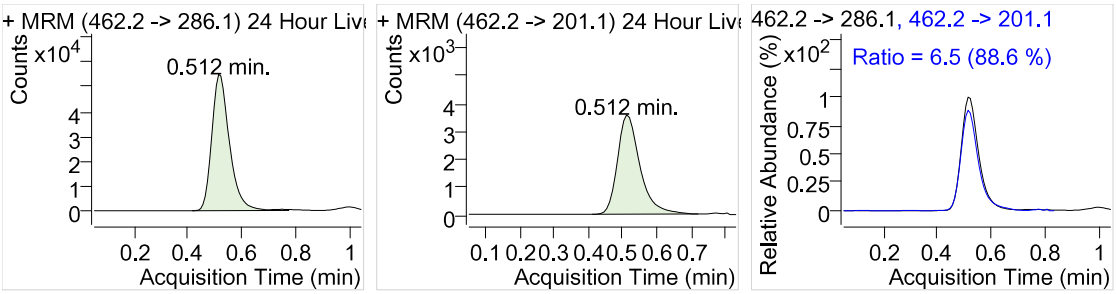
Sample Name: : 24 Hour Liver 1
Data File : C:\Users\jesgl\Dropbox\LCMSMS data\01062019\24 Hour Liver 1.d
Instrument : Nano Pump LC-MS(only)
Acq. Method : OPI(dynamic)trig.m
Acq. Date-Time : 1/6/2019 6:26:40 PM
Dilution : 4.0
Operator :
Sample Position : P1-F8

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	*0.51	4749			
	465.2 -> 201.1		359	*7.6	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	*0.51	250504			27585.8 ng/ml
	462.2 -> 201.1		16261	6.5	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.92	44184			
	292.2 -> 128.2		7923	17.9	13.4 - 20.1	
Normorphine	272.3 -> 152.1	*0.52	118824			193.6 ng/ml
	272.1 -> 165.1		91354	76.9	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.92	44184			
	292.2 -> 128.2		7923	17.9	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.94	59738			1990.2 ng/ml
	286.1 -> 128.1		38482	64.4	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.98	8780			
	465.2 -> 165.1		1168	*13.3	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	1.00	6764			332.0 ng/ml
	462.2 -> 165.0		1009	*14.9	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	388884			
Norfentanyl	233.1 -> 84.1	2.49	9733			2.7 ng/ml
	233.1 -> 150.1		411	*4.2	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.99	535494			
	342.4 -> 105.1		398473	74.4	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	4.00	32942			4.1 ng/ml
	337.2 -> 188.1		24244	*73.6	77.3 - 116.0	

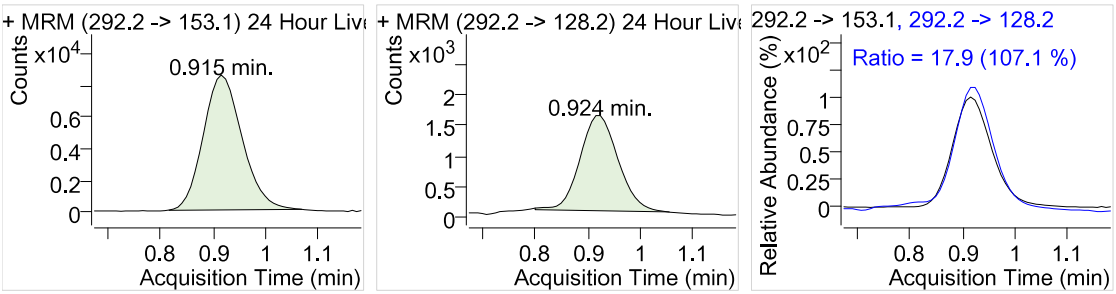
Morphine-3-B-D-Glucuronide D3



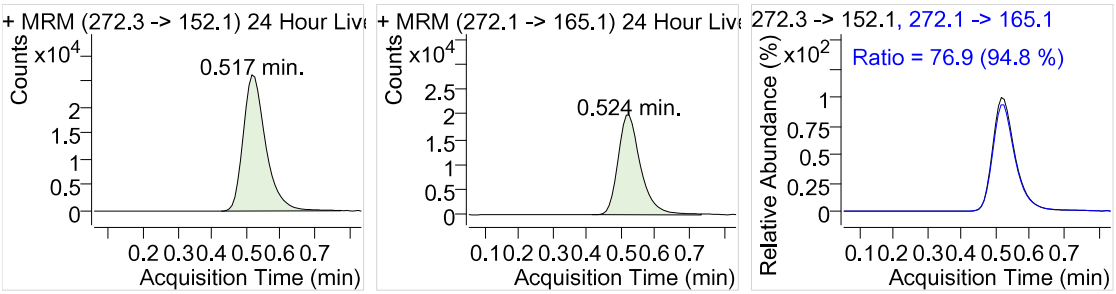
Morphine-3-B-D-Glucuronide



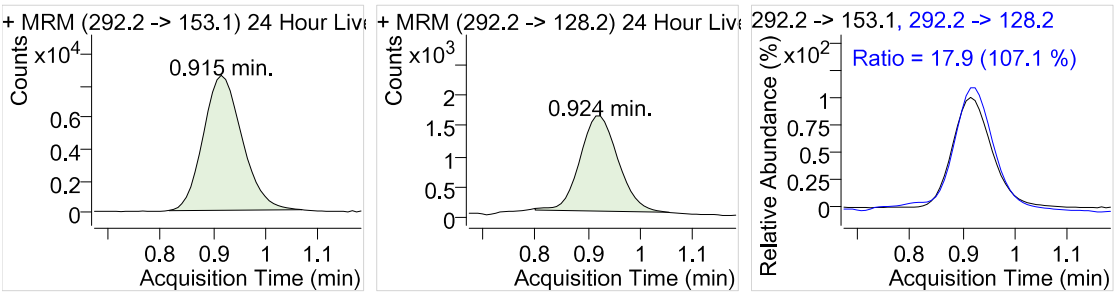
Morphine D6



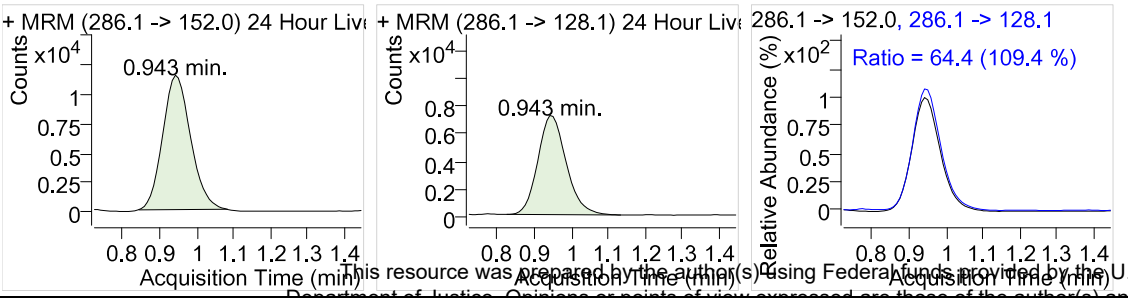
Normorphine



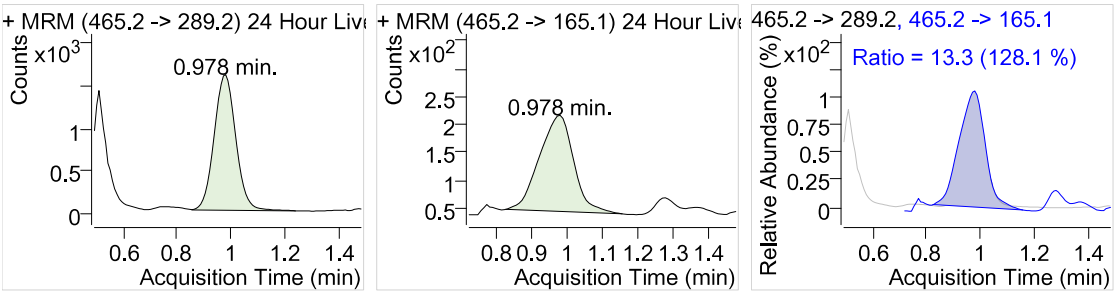
Morphine D6



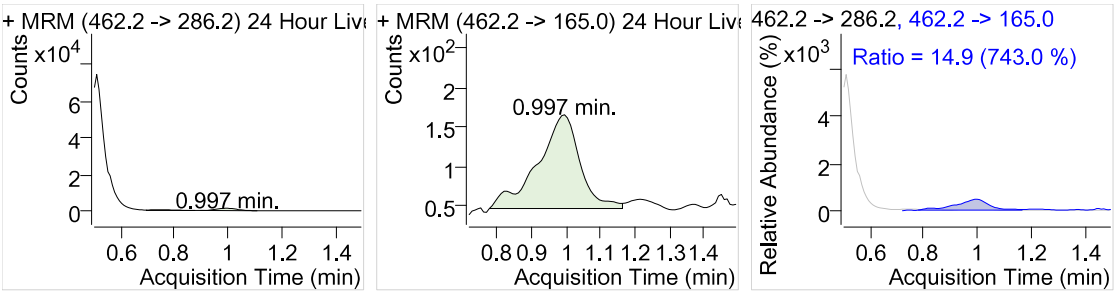
Morphine



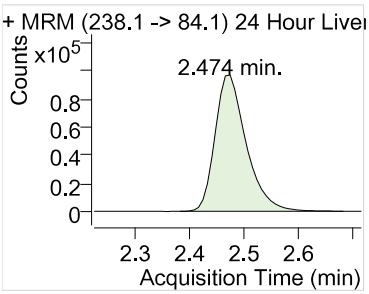
Morphine-6-B-D-Glucuronide D3



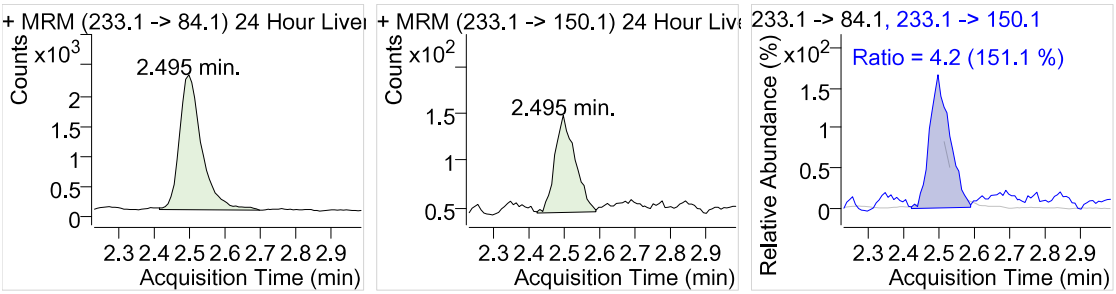
Morphine-6-B-D-Glucuronide



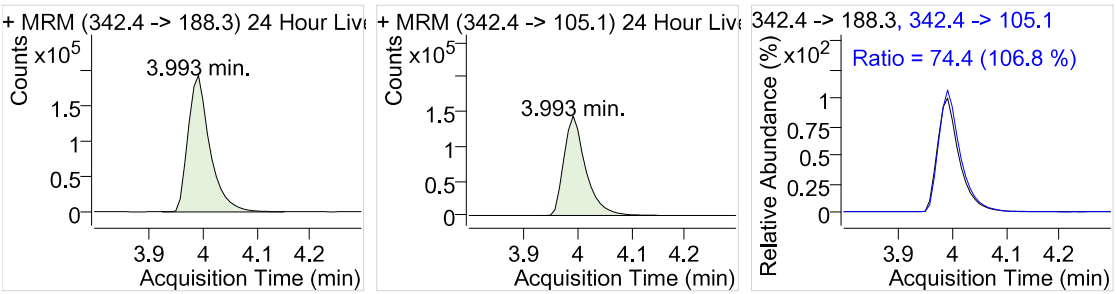
Norfentanyl D5



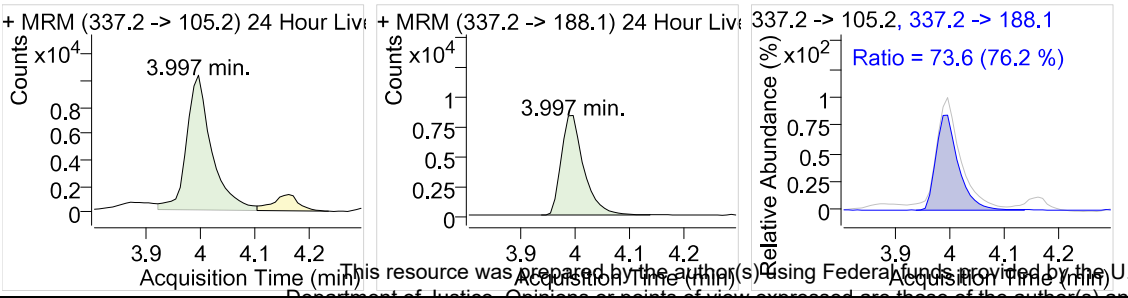
Norfentanyl



Fentanyl D5



Fentanyl



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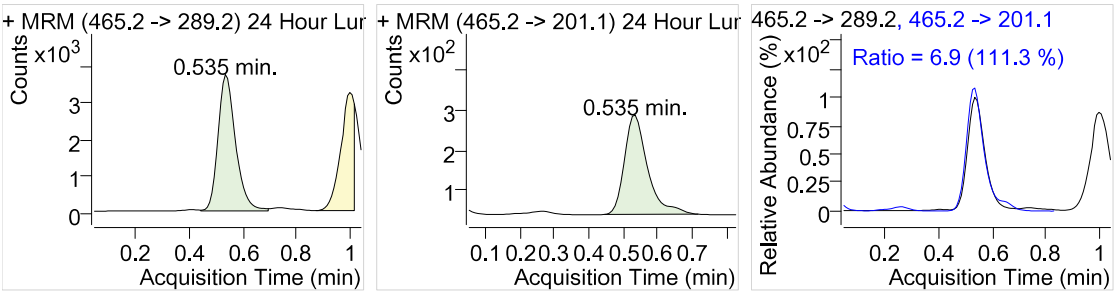
Quantitative Analysis Report

Agilent Technologies

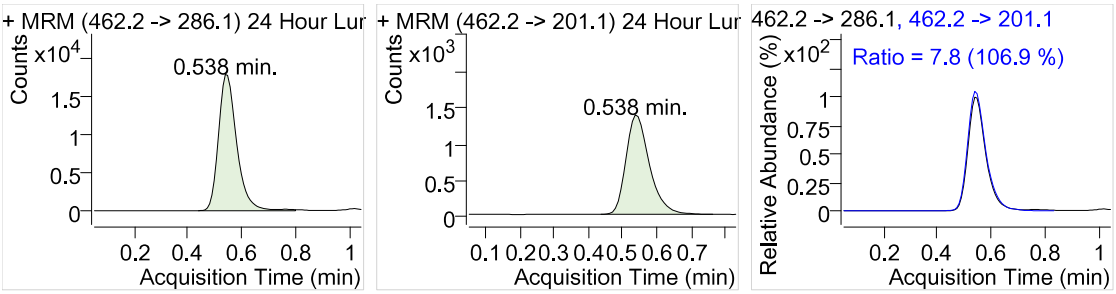
Sample Name:	:	24 Hour Lung 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\24 Hour Lung 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 6:56:25 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P2-A4

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.53	16879			
	465.2 -> 201.1		1161	6.9	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.54	82627			2543.0 ng/ml
	462.2 -> 201.1		6475	7.8	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.95	63132			
	292.2 -> 128.2		10962	17.4	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	57519			83.7 ng/ml
	272.1 -> 165.1		44872	78.0	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.95	63132			
	292.2 -> 128.2		10962	17.4	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.98	40566			954.0 ng/ml
	286.1 -> 128.1		26151	64.5	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	0.99	16351			
	465.2 -> 165.1		1813	11.1	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.54	52256			1266.1 ng/ml
	462.2 -> 165.0		58	*0.1	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	468586			
Norfentanyl	233.1 -> 84.1	2.49	5128			2.1 ng/ml
	233.1 -> 150.1		289	*5.6	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.97	1236166			
	342.4 -> 105.1		883038	71.4	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.97	32073			2.2 ng/ml
	337.2 -> 188.1		28367	88.4	77.3 - 116.0	

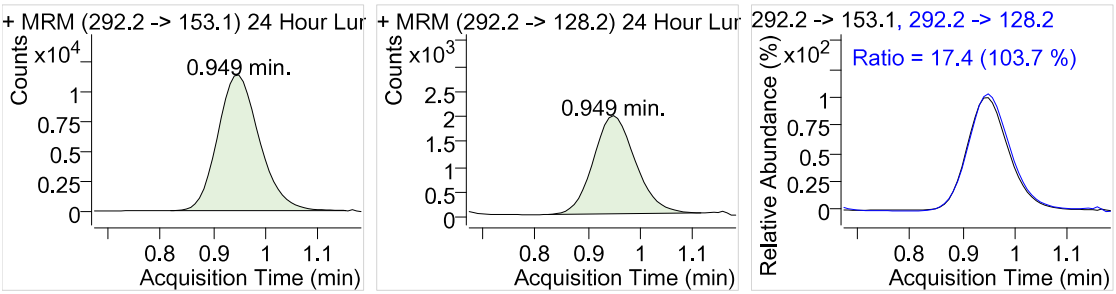
Morphine-3-B-D-Glucuronide D3



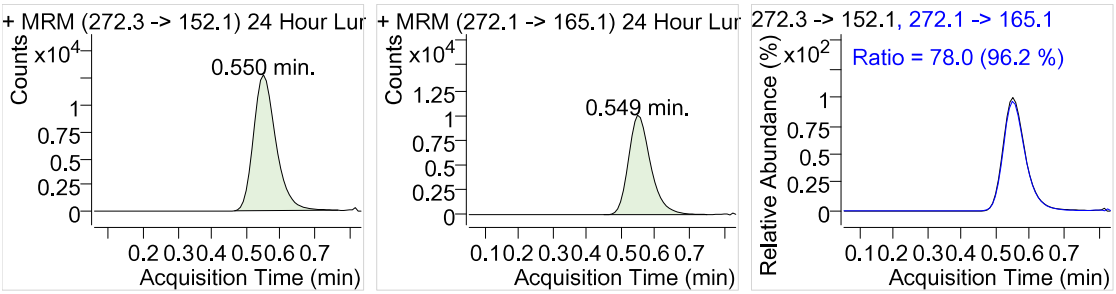
Morphine-3-B-D-Glucuronide



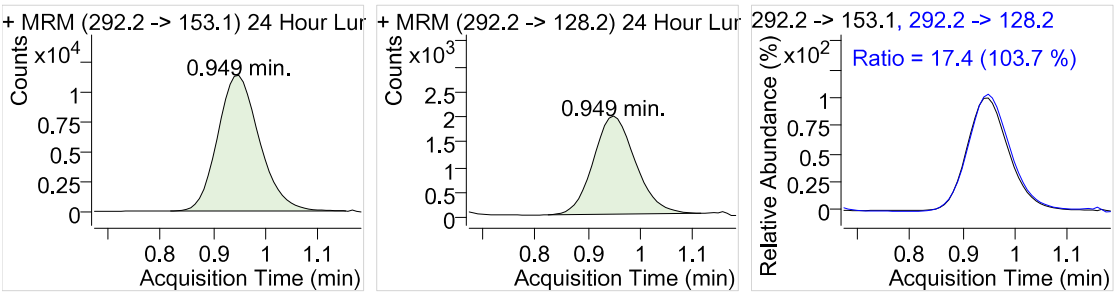
Morphine D6



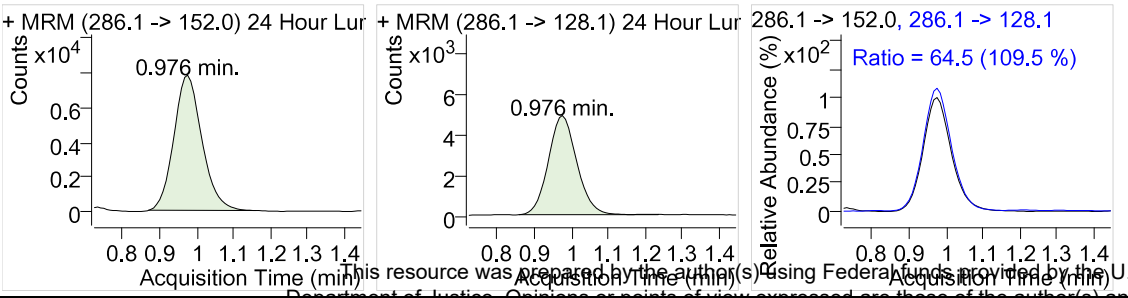
Normorphine



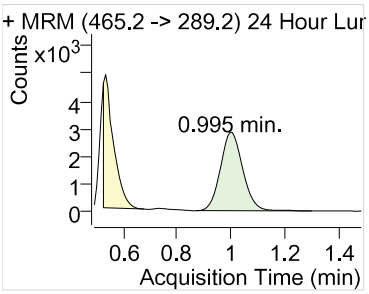
Morphine D6

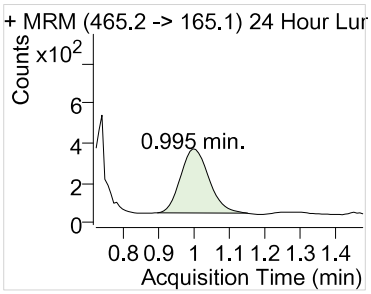


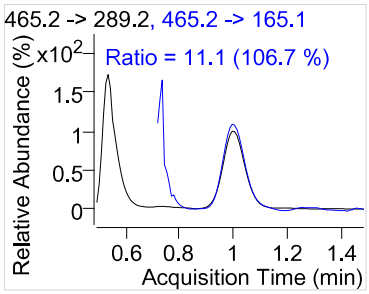
Morphine



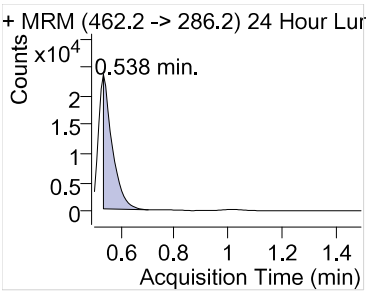
Morphine-6-B-D-Glucuronide D3

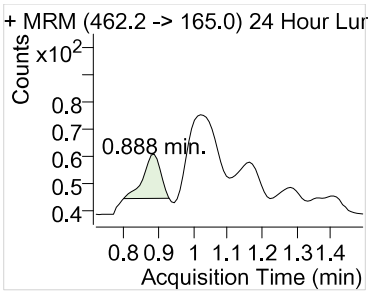


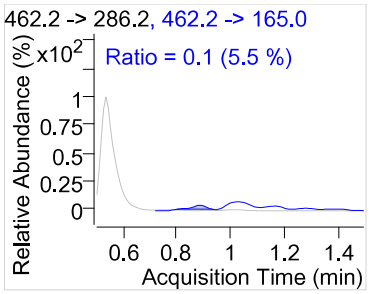




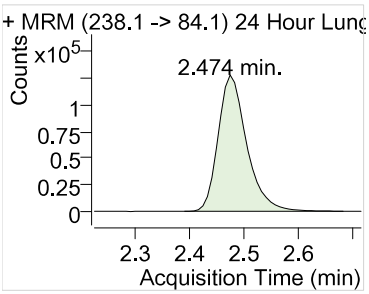
Morphine-6-B-D-Glucuronide







Norfentanyl D5



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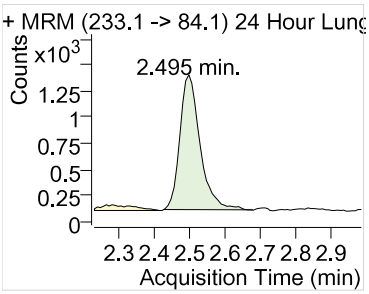

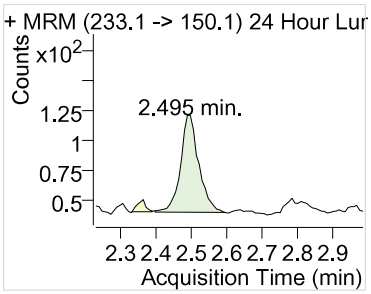

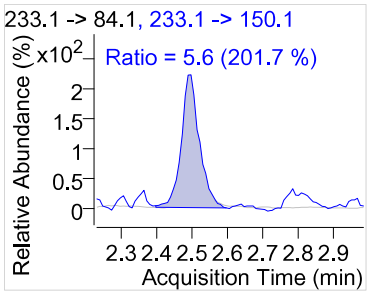

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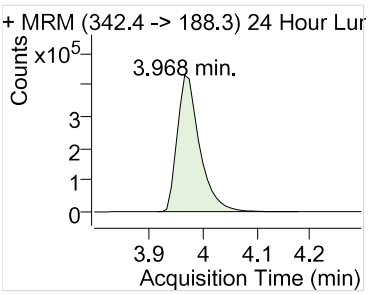

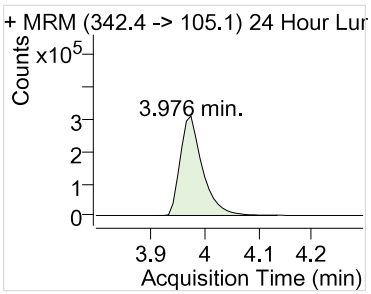

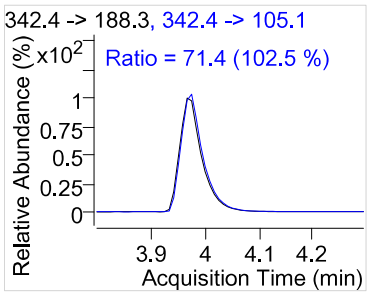

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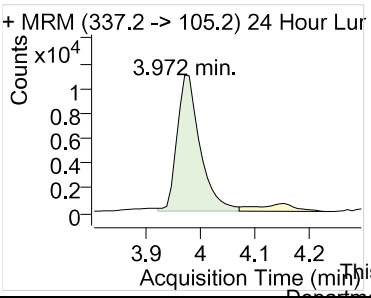
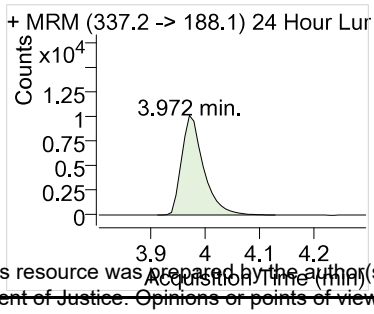
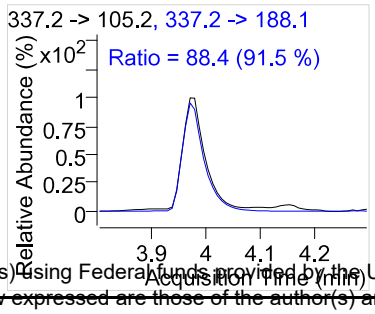






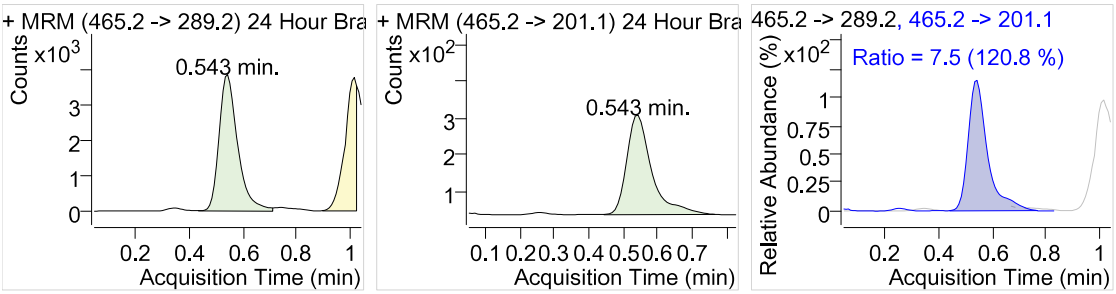
Quantitative Analysis Report

Agilent Technologies

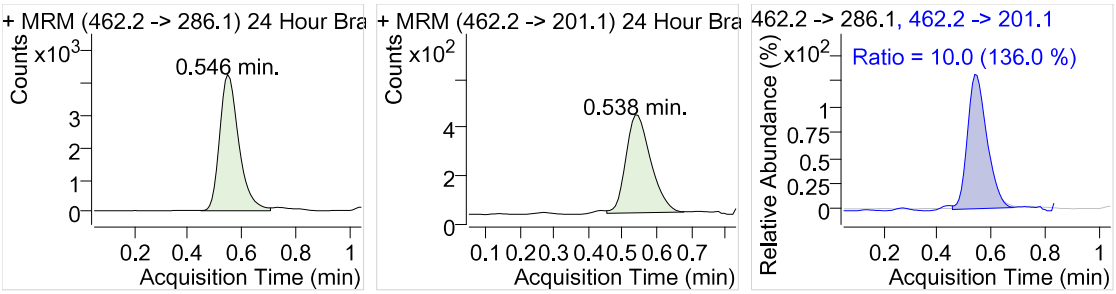
Sample Name:	:	24 Hour Brain 1
Data File	:	C:\Users\jesgl\Dropbox\LCMSMS data\01062019\24 Hour Brain 1.d
Instrument	:	Nano Pump LC-MS(only)
Acq. Method	:	OPI(dynamic)trig.m
Acq. Date-Time	:	1/6/2019 7:05:49 PM
Dilution	:	4.0
Operator	:	
Sample Position	:	P2-A5

Compound	Signal	RT	Response	QRatio	Limits	Conc.
Morphine-3-B-D-Glucuronide D3	465.2 -> 289.2	0.54	18253			
	465.2 -> 201.1		1363	*7.5	4.9 - 7.4	
Morphine-3-B-D-Glucuronide	462.2 -> 286.1	0.55	20686			567.2 ng/ml
	462.2 -> 201.1		2061	*10.0	5.9 - 8.8	
Morphine D6	292.2 -> 153.1	0.96	82201			
	292.2 -> 128.2		13749	16.7	13.4 - 20.1	
Normorphine	272.3 -> 152.1	0.55	4887			31.3 ng/ml
	272.1 -> 165.1		4270	87.4	64.9 - 97.3	
Morphine D6	292.2 -> 153.1	0.96	82201			
	292.2 -> 128.2		13749	16.7	13.4 - 20.1	
Morphine	286.1 -> 152.0	0.98	18129			319.2 ng/ml
	286.1 -> 128.1		11447	63.1	47.1 - 70.6	
Morphine-6-B-D-Glucuronide D3	465.2 -> 289.2	1.01	18726			
	465.2 -> 165.1		1885	10.1	8.3 - 12.5	
Morphine-6-B-D-Glucuronide	462.2 -> 286.2	*0.55	14098			325.2 ng/ml
	462.2 -> 165.0		22	*0.2	1.6 - 2.4	
Norfentanyl D5	238.1 -> 84.1	2.47	431292			
Norfentanyl	233.1 -> 84.1	2.49	1232			1.7 ng/ml
	233.1 -> 150.1		88	*7.1	2.2 - 3.4	
Fentanyl D5	342.4 -> 188.3	3.98	1210952			
	342.4 -> 105.1		875849	72.3	55.8 - 83.6	
Fentanyl	337.2 -> 105.2	3.98	35824			2.4 ng/ml
	337.2 -> 188.1		32408	90.5	77.3 - 116.0	

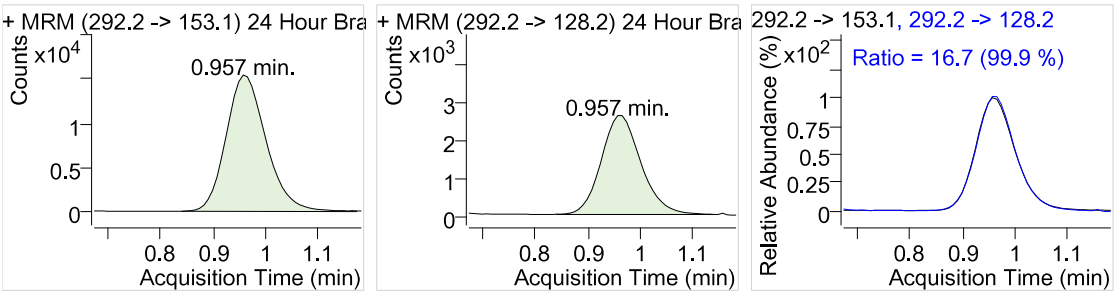
Morphine-3-B-D-Glucuronide D3



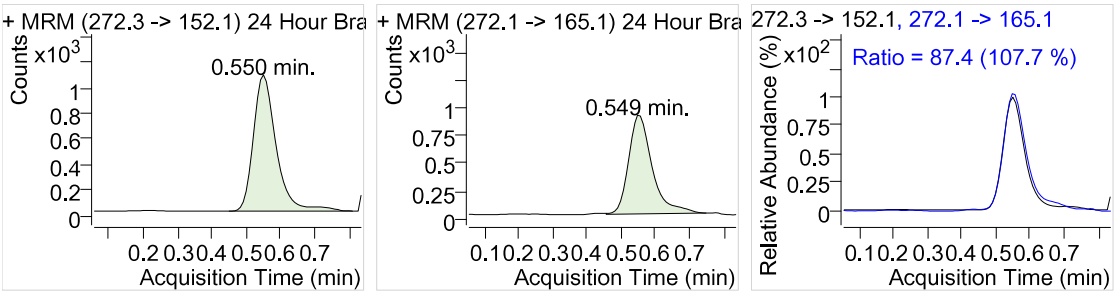
Morphine-3-B-D-Glucuronide



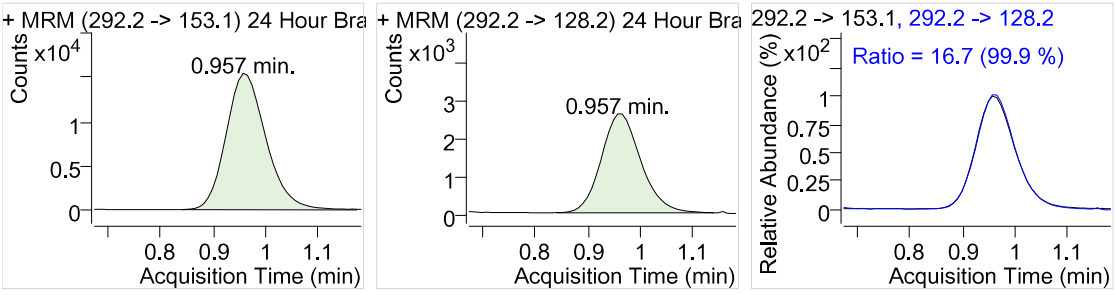
Morphine D6



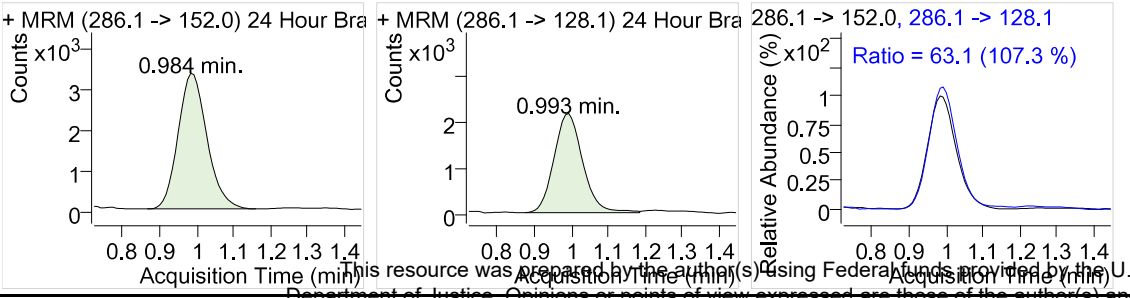
Normorphine



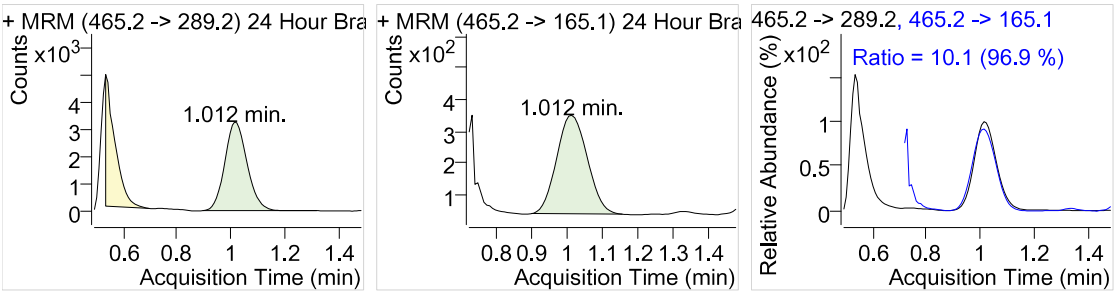
Morphine D6



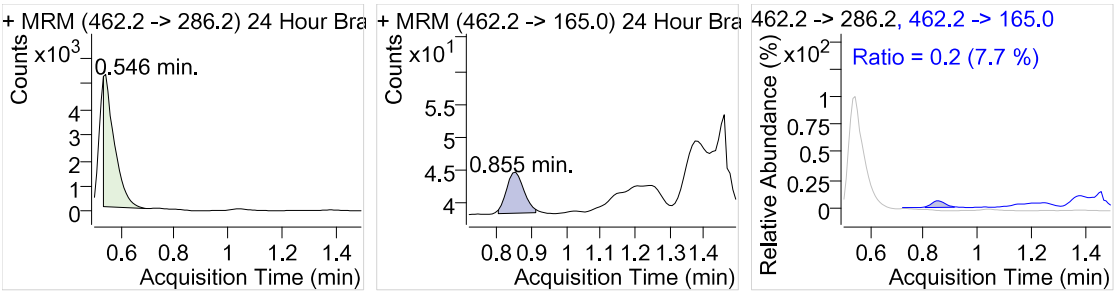
Morphine



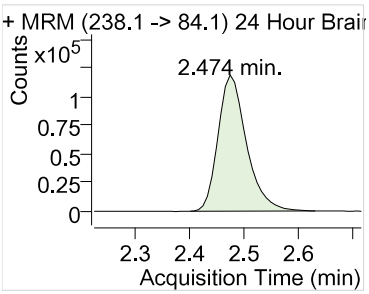
Morphine-6-B-D-Glucuronide D3



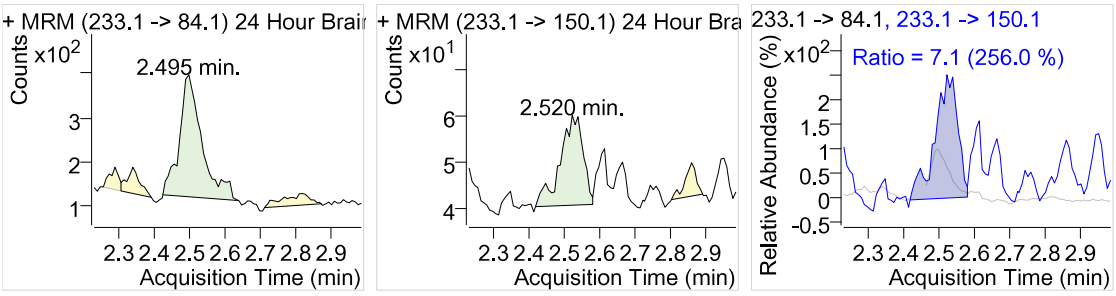
Morphine-6-B-D-Glucuronide



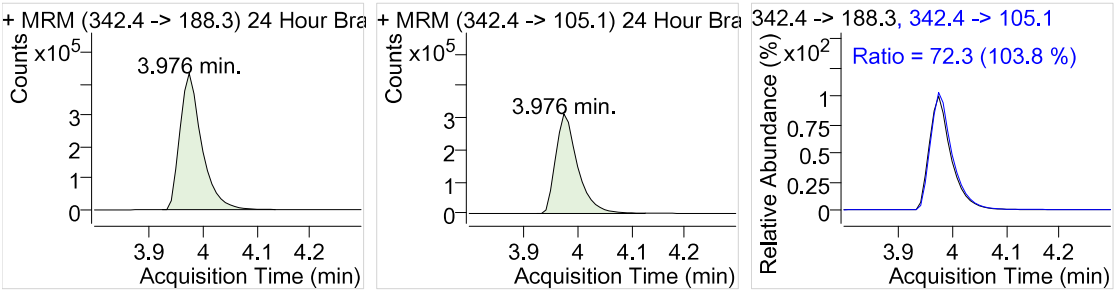
Norfentanyl D5



Norfentanyl



Fentanyl D5



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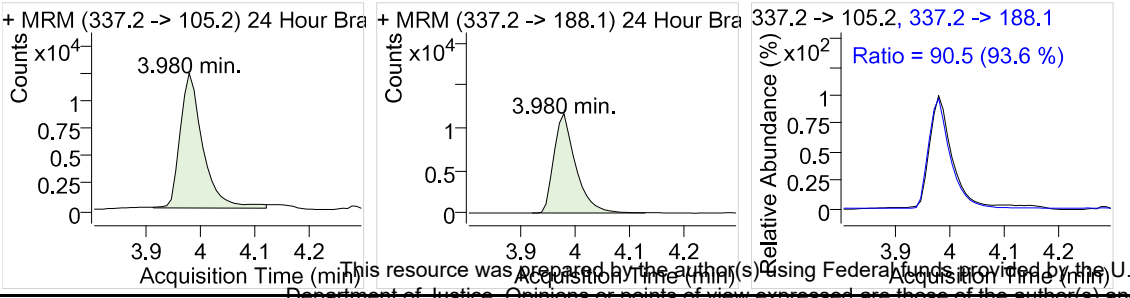

Appendix H

Individual Data Co-Administration of Morphine and Fentanyl Study Across all Postmortem Intervals and Matrices

Table of Contents

Abbreviations

BW: Body Weight

HB: Heart Blood

F: Female

FB: Femoral Blood

M: Male

MOR: Morphine

M3G: Morphine-3-Glucuronide

M6G: Morphine-6-Glucuronide

NM: Normorphine

PM: Postmortem

Table H-1: Individual data from Co-Administration Study for Heart Blood and Femoral Blood

Date	Injection Time	PM Interval	BW	Sex	M(HB)	NM(HB)	M3G(HB)	M(FB)	NM(FB)	M3G(FB)
11/10/2018	906	0	339	M	733	18	623	868	43	448
11/10/2018	913	0	327	M	1265	55	1944	1159	68	837
11/10/2018	917	0	191	F	895	12	2218	2113	42	1189
11/10/2018	920	0	183	F	2251	9	N/A	2149	59	657
1/4/2019	1035	0	284	M	964	43	1242	1049	49	434
1/4/2019	1039	0	285	F	1530	4	3027	603	36	697
1/4/2019	1041	0	286	F	2722	2	2154	1657	19	889
11/10/2018	724	8	356	M	2050	78	1494	1114	45	110
11/10/2018	734	8	317	M	701	17	227	889	38	136
11/10/2018	738	8	174	F	1216	13	933	1816	54	1024
11/10/2018	744	8	167	F	1252	15	584	2728	56	3155
1/4/2019	910	8	288	F	1522	10	1274			
1/4/2019	915	8	289	F	1028	15	694	765	95	342
11/10/2018	839	16	330	M	1282	69	2705	1099	40	325
11/10/2018	843	16	331	M	835	33	251	674	68	233
11/10/2018	851	16	180	F	1600	7	1706	3050	22	1496
11/10/2018	855	16	174	F	1434	5	3409	745	47	876
1/4/2019	956	16	290	M	2029	65	1267	491	133	312
1/4/2019	1000	16	291	M	833	31	529	174	47	64
1/4/2019	1005	16	292	F	1786	9	3097	487	98	819
1/4/2019	1011	16	293	F	1302	15	5223			
11/10/2018	704	24	294	M	147	5	628	7748	758	4638
1/4/2019	833	24	294	M	665	55	5158	57	92	173
1/4/2019	837	24	295	M	1078	50	704			
1/4/2019	851	24	296	F	951	5	9481			
1/4/2019	853	24	297	F	1747	9	2785			
11/10/2018	718	24	186	F	1999	35	509	1326	30	782
11/10/2018	714	24	197	F	702	11	37	613	77	649

Table H-2 : Individual Data from Co-Administration Study for Liver, Brain and Lung

Date	Injection Time	PM Interval	BW	Sex	M(Liver)	NM(Liver)	M3G(Liver)	M(Brain)	NM(Brain)	M3G(Brain)	M(Lung)	NM(Lung)	M3G(Lung)
11/10/2018	906	0	339	M	308	147	3271	171	39	135	1033	72	488
11/10/2018	913	0	327	M	435	366	7158	246	40	109	2336	129	785
11/10/2018	917	0	191	F	503	47	5998	238	39	137	1885	40	1569
11/10/2018	920	0	183	F	1100	44	10141	401	38	406	2343	41	3312
1/4/2019	1035	0	284	M	484	337	3995	134	16	73	1816	91	357
1/4/2019	1039	0	285	F	135	25	4593	1090	18	301	212	18	49
1/4/2019	1041	0	286	F	234	20	1708	151	16	44	1513	20	954
11/10/2018	724	8	356	M	1093	339	5633	266	25	179	1588	82	4784
11/10/2018	734	8	317	M	439	187	1731	229	25	127	957	46	343
11/10/2018	738	8	174	F	824	38	4738	244	24	191	1174	27	988
11/10/2018	744	8	167	F	1079	29	4048	259	24	198	1151	25	1898
1/4/2019	910	8	288	F	638	30	3790	222	28	125	1323	29	1193
1/4/2019	915	8	289	F	724	30	2440	134	28	106	966	29	499
11/10/2018	839	16	330	M	929	219	4797	324	25	128	1765	63	1348
11/10/2018	843	16	331	M	551	158	2082	236	25	89	540	55	329
11/10/2018	851	16	180	F	1415	29	5892	193	24	161	1595	26	962
11/10/2018	855	16	174	F	2698	29	7246	312	24	497	1967	26	2674
1/4/2019	956	16	290	M	1920	471	8842	420	32	223	1812	85	1223
1/4/2019	1000	16	291	M				181	30	199	1069	66	443
1/4/2019	1005	16	292	F	1273	39	7093	285	28	243	1956	32	914
1/4/2019	1011	16	293	F	1007	32	6112	212	28	341	1833	32	3629
11/10/2018	704	24	294	M	1372	27	894	241	24	35			
1/4/2019	833	24	294	M	1990	194	27586	319	31	567	954	84	2543
1/4/2019	837	24	295	M	866	130	16543	308	31	273	1243	66	1750
1/4/2019	851	24	296	F	528	31	8875	146	28	601	1138	31	3306
1/4/2019	853	24	297	F	1374	30	29448	365	28	1229	1866	32	2560
11/10/2018	718	24	186	F	3552	142	2555	8452	553	9482	559	135	1838
11/10/2018	714	24	197	F	8372	124	1232	210	24	84	877	24	214

Appendix I

Statistical Results Generated for Chapters 6 and 7 using One Way ANOVA and Two way ANOVA followed by Tukey's Post-Hoc Analysis as well as T-Test

Table of Contents

Abbreviations

HB: Heart Blood

F: Female

FB: Femoral Blood

M: Male

MOR: Morphine

M3G: Morphine-3-Glucuronide

M6G: Morphine-6-Glucuronide

NM: Normorphine

PM: Postmortem

One Way Analysis of Variance Saturday, September 08, 2018, 1:10:09 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.272)

Equal Variance Test: Passed (P = 0.651)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	1	1518.551	547.109	193.432
8.000	14	2	1234.340	443.413	128.002
24.000	14	2	1010.640	493.854	142.563
16.000	10	0	1250.232	357.062	112.913

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1247721.575	415907.192	1.954	0.137
Residual	38	8088308.467	212850.223		
Total	41	9336030.043			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.137).

Power of performed test with alpha = 0.050: 0.233

The power of the performed test (0.233) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:20:51 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.430)

Equal Variance Test: Passed (P = 0.095)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	5	4056.259	1371.945	685.973
8.000	14	7	1496.522	1090.372	412.122
24.000	14	11	926.441	996.752	575.475
16.000	10	6	344.228	255.342	127.671

Source of Variation	DF	SS	MS	F	P
Between Groups	3	31641637.464	10547212.488	9.869	<0.001
Residual	14	14962794.914	1068771.065		
Total	17	46604432.378			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.978

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **PM Interval**

Comparison	Diff of Means	p	q	P	P<0.050
0.000 vs. 16.000	3712.031	4	7.181	0.001	Yes
0.000 vs. 24.000	3129.819	4	5.606	0.007	Yes
0.000 vs. 8.000	2559.737	4	5.587	0.007	Yes
8.000 vs. 16.000	1152.294	4	2.515	0.324	No
8.000 vs. 24.000	570.082	4	1.130	0.854	Do Not Test
24.000 vs. 16.000	582.212	4	1.043	0.881	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Saturday, September 08, 2018, 1:23:08 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.381)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	1538.134	852.892	284.297
8.000	14	0	1251.541	546.276	145.998
24.000	14	0	1622.323	473.870	126.647
16.000	10	0	1408.235	686.889	217.213

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1054484.811	351494.937	0.896	0.451
Residual	43	16864369.323	392194.635		
Total	46	17918854.135			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.451).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:26:53 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.543)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	426.933	262.056	87.352
8.000	14	0	520.852	467.787	125.021
24.000	14	0	390.297	230.033	61.479
16.000	10	0	654.364	506.036	160.023

Source of Variation	DF	SS	MS	F	P
Between Groups	3	458180.099	152726.700	1.028	0.390
Residual	43	6386660.866	148526.997		
Total	46	6844840.965			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.390).

Power of performed test with alpha = 0.050: 0.054

The power of the performed test (0.054) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:28:58 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	3239.647	2141.984	713.995
8.000	14	0	1424.034	831.464	222.218
24.000	14	0	1281.779	1229.806	328.680
16.000	10	0	1435.270	1311.567	414.754

Source of Variation	DF	SS	MS	F	P
Between Groups	3	25503045.517	8501015.172	4.522	0.008
Residual	43	80835441.525	1879893.989		
Total	46	106338487.042			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.008).

Power of performed test with alpha = 0.050: 0.747

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **PM Interval**

Comparison	Diff of Means	p	q	P	P<0.050
0.000 vs. 24.000	1957.867	4	4.727	0.009	Yes
0.000 vs. 8.000	1815.612	4	4.383	0.017	Yes
0.000 vs. 16.000	1804.376	4	4.051	0.032	Yes
16.000 vs. 24.000	153.491	4	0.382	0.993	No
16.000 vs. 8.000	11.236	4	0.0280	1.000	Do Not Test
8.000 vs. 24.000	142.255	4	0.388	0.993	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Saturday, September 08, 2018, 1:19:49 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.138)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	1	2719.509	2395.627	846.982
8.000	14	2	1592.079	1164.627	336.199
24.000	14	2	1412.028	1096.985	316.672
16.000	10	0	2939.404	2771.027	876.276

Source of Variation	DF	SS	MS	F	P
Between Groups	3	18867951.281	6289317.094	1.739	0.175
Residual	38	137437588.931	3616778.656		
Total	41	156305540.212			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.175).

Power of performed test with alpha = 0.050: 0.187

The power of the performed test (0.187) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:22:20 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M3G(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.205)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	6	1370.803	294.496	170.027
8.000	14	9	1259.633	1009.174	451.316
24.000	14	11	913.566	986.633	569.633
16.000	10	3	218.609	165.585	62.585

Source of Variation	DF	SS	MS	F	P
Between Groups	3	4434159.694	1478053.231	3.254	0.054
Residual	14	6358580.248	454184.303		
Total	17	10792739.942			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.054).

Power of performed test with alpha = 0.050: 0.446

The power of the performed test (0.446) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:24:53 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M3G(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.417)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	6564.649	3040.431	1013.477
8.000	14	0	5572.170	2257.103	603.236
24.000	14	0	5744.188	3359.828	897.952
16.000	10	0	7222.415	4344.473	1373.843

Source of Variation	DF	SS	MS	F	P
Between Groups	3	20018146.982	6672715.661	0.628	0.601
Residual	43	456802168.374	10623306.241		
Total	46	476820315.357			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.601).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 15, 2018, 8:25:53 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.545)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	248.177	267.469	89.156
8.000	14	0	296.741	164.613	43.995
24.000	14	0	536.847	397.939	106.354
16.000	10	0	523.841	473.063	149.596

Source of Variation	DF	SS	MS	F	P
Between Groups	3	769778.544	256592.848	2.208	0.101
Residual	43	4997312.503	116216.570		
Total	46	5767091.047			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.101).

Power of performed test with alpha = 0.050: 0.293

The power of the performed test (0.293) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 15, 2018, 8:27:16 PM

Data source: Data 1 in Morphine_master

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.276)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	1668.315	1586.397	528.799
8.000	14	0	1188.168	869.130	232.285
24.000	14	0	1782.255	1242.022	331.944
16.000	10	0	884.791	849.708	268.701

Source of Variation	DF	SS	MS	F	P
Between Groups	3	5973602.745	1991200.915	1.515	0.224
Residual	43	56505355.076	1314078.025		
Total	46	62478957.821			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.224).

Power of performed test with alpha = 0.050: 0.143

The power of the performed test (0.143) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:13:23 PM

Data source: Data 1 in Morphine_master

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.418)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	1	58.615	66.691	23.579
8.000	14	2	38.870	27.895	8.053
24.000	14	3	57.899	61.467	18.533
16.000	10	1	106.820	158.412	52.804

Source of Variation	DF	SS	MS	F	P
Between Groups	3	24704.180	8234.727	1.065	0.376
Residual	36	278229.582	7728.599		
Total	39	302933.761			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.376).

Power of performed test with alpha = 0.050: 0.060

The power of the performed test (0.060) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:21:35 PM

Data source: Data 1 in Morphine_master

Dependent Variable: NM(FB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.368)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	5	50.686	21.639	10.819
8.000	14	7	62.102	47.042	17.780
24.000	14	11	83.862	80.736	46.613
16.000	10	4	191.059	156.661	63.957

Source of Variation	DF	SS	MS	F	P
Between Groups	3	70250.370	23416.790	2.491	0.097
Residual	16	150432.391	9402.024		
Total	19	220682.761			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.097).

Power of performed test with alpha = 0.050: 0.312

The power of the performed test (0.312) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 08, 2018, 1:24:13 PM

Data source: Data 1 in Morphine_master

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.537)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	585.554	800.767	266.922
8.000	14	0	312.839	486.841	130.114
24.000	14	0	339.890	388.589	103.855
16.000	10	0	609.568	990.114	313.102

Source of Variation	DF	SS	MS	F	P
Between Groups	3	844236.930	281412.310	0.637	0.595
Residual	43	18996966.028	441789.908		
Total	46	19841202.958			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.595).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 15, 2018, 8:26:33 PM

Data source: Data 1 in Morphine_master

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.431)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	0	154.277	205.639	68.546
8.000	14	1	78.122	75.844	21.035
24.000	14	1	78.951	61.909	17.170
16.000	10	0	108.909	134.665	42.585

Source of Variation	DF	SS	MS	F	P
Between Groups	3	39281.935	13093.978	0.871	0.464
Residual	41	616531.268	15037.348		
Total	44	655813.203			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.464).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, September 15, 2018, 8:24:59 PM

Data source: Data 1 in Morphine_master

Dependent Variable: NM(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.500)

Group Name	N	Missing	Mean	Std Dev	SEM
0.000	9	2	37.201	12.818	4.845
8.000	14	2	44.172	13.742	3.967
24.000	14	2	42.936	10.193	2.943
16.000	10	0	55.338	32.007	10.122

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1549.025	516.342	1.423	0.252
Residual	37	13426.358	362.875		
Total	40	14975.384			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.252).

Power of performed test with alpha = 0.050: 0.123

The power of the performed test (0.123) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Friday, March 15, 2019, 9:04:47 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: MOR

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	9	1	1518.551	547.109	193.432
Femoral Blood	4	0	4056.259	1371.945	685.973
Liver	9	0	1538.134	852.892	284.297
Brain	9	0	426.933	262.056	87.352
Lung	9	0	3239.647	2141.984	713.995

Source of Variation	DF	SS	MS	F	P
Between Groups	4	56589654.278	14147413.570	9.466	<0.001
Residual	34	50815536.518	1494574.603		
Total	38	107405190.796			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.998

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Femoral Blood vs. Brain	3629.326	5	6.987	<0.001	Yes
Femoral Blood vs. Heart Blood	2537.709	5	4.794	0.014	Yes
Femoral Blood vs. Liver	2518.126	5	4.847	0.013	Yes
Femoral Blood vs. Lung	816.613	5	1.572	0.799	No
Lung vs. Brain	2812.713	5	6.902	<0.001	Yes
Lung vs. Heart Blood	1721.096	5	4.097	0.048	Yes
Lung vs. Liver	1701.513	5	4.175	0.042	Yes
Liver vs. Brain	1111.200	5	2.727	0.323	No
Liver vs. Heart Blood	19.583	5	0.0466	1.000	Do Not Test
Heart Blood vs. Brain	1091.618	5	2.599	0.370	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:05:33 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: M3G

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	9	1	2719.509	2395.627	846.982
Femoral Blood	4	1	1370.803	294.496	170.027
Liver	9	0	6564.649	3040.431	1013.477
Brain	9	0	248.177	267.469	89.156
Lung	9	0	1668.315	1586.397	528.799

Source of Variation	DF	SS	MS	F	P
Between Groups	4	203401010.039	50850252.510	12.430	<0.001
Residual	33	135006001.721	4091090.961		
Total	37	338407011.761			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Brain	6316.472	5	9.369	<0.001	Yes
Liver vs. Femoral Blood	5193.847	5	5.447	0.004	Yes
Liver vs. Lung	4896.334	5	7.262	<0.001	Yes
Liver vs. Heart Blood	3845.140	5	5.533	0.004	Yes
Heart Blood vs. Brain	2471.332	5	3.556	0.112	No
Heart Blood vs. Femoral Blood	1348.706	5	1.393	0.860	Do Not Test
Heart Blood vs. Lung	1051.194	5	1.513	0.821	Do Not Test
Lung vs. Brain	1420.138	5	2.106	0.576	Do Not Test
Lung vs. Femoral Blood	297.513	5	0.312	0.999	Do Not Test
Femoral Blood vs. Brain	1122.626	5	1.177	0.919	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:05:10 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: NM

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	9	1	58.615	66.691	23.579
Femoral Blood	4	0	50.686	21.639	10.819
Liver	9	0	585.554	800.767	266.922
Brain	9	2	37.201	12.818	4.845
Lung	9	0	154.277	205.639	68.546

Source of Variation	DF	SS	MS	F	P
Between Groups	4	1790323.718	447580.930	2.603	0.054
Residual	32	5501651.695	171926.615		
Total	36	7291975.413			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.054).

Power of performed test with alpha = 0.050: 0.430

The power of the performed test (0.430) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Friday, March 15, 2019, 9:06:01 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: MOR

Normality Test (Shapiro-Wilk) Passed (P = 0.090)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	2	1234.340	443.413	128.002
Femoral Blood	8	1	1496.522	1090.372	412.122
Liver	14	0	1251.541	546.276	145.998
Brain	14	0	520.852	467.787	125.021
Lung	14	0	1424.034	831.464	222.218

Source of Variation	DF	SS	MS	F	P
Between Groups	4	7662952.873	1915738.218	4.290	0.004
Residual	56	25007695.853	446565.997		
Total	60	32670648.726			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.004).

Power of performed test with alpha = 0.050: 0.811

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Femoral Blood vs. Brain	975.670	5	4.460	0.021	Yes
Femoral Blood vs. Heart Blood	262.183	5	1.167	0.922	No
Femoral Blood vs. Liver	244.981	5	1.120	0.932	Do Not Test
Femoral Blood vs. Lung	72.488	5	0.331	0.999	Do Not Test
Lung vs. Brain	903.182	5	5.057	0.006	Yes
Lung vs. Heart Blood	189.694	5	1.020	0.951	Do Not Test
Lung vs. Liver	172.493	5	0.966	0.959	Do Not Test
Liver vs. Brain	730.689	5	4.091	0.042	Yes
Liver vs. Heart Blood	17.201	5	0.0925	1.000	Do Not Test
Heart Blood vs. Brain	713.488	5	3.838	0.065	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:07:35 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: M3G

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	2	1592.079	1164.627	336.199
Femoral Blood	8	3	1259.633	1009.174	451.316
Liver	14	0	5572.170	2257.103	603.236
Brain	14	0	296.741	164.613	43.995
Lung	14	0	1188.168	869.130	232.285

Source of Variation	DF	SS	MS	F	P
Between Groups	4	232573369.057	58143342.264	32.913	<0.001
Residual	54	95394614.282	1766566.931		
Total	58	327967983.339			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Brain	5275.429	5	14.851	<0.001	Yes
Liver vs. Lung	4384.002	5	12.342	<0.001	Yes
Liver vs. Femoral Blood	4312.536	5	8.808	<0.001	Yes
Liver vs. Heart Blood	3980.091	5	10.765	<0.001	Yes
Heart Blood vs. Brain	1295.338	5	3.503	0.111	No
Heart Blood vs. Lung	403.911	5	1.092	0.937	Do Not Test
Heart Blood vs. Femoral Blood	332.446	5	0.665	0.990	Do Not Test
Femoral Blood vs. Brain	962.893	5	1.967	0.636	Do Not Test
Femoral Blood vs. Lung	71.466	5	0.146	1.000	Do Not Test
Lung vs. Brain	891.427	5	2.509	0.399	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:06:38 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: NM

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	2	38.870	27.895	8.053
Femoral Blood	8	1	62.102	47.042	17.780
Liver	14	0	312.839	486.841	130.114
Brain	14	2	44.172	13.742	3.967
Lung	14	1	78.122	75.844	21.035

Source of Variation	DF	SS	MS	F	P
Between Groups	4	714555.724	178638.931	2.983	0.027
Residual	53	3174129.723	59889.240		
Total	57	3888685.447			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.027).

Power of performed test with alpha = 0.050: 0.552

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Heart Blood	273.969	5	4.024	0.047	Yes
Liver vs. Brain	268.667	5	3.947	0.054	No
Liver vs. Femoral Blood	250.737	5	3.130	0.191	Do Not Test
Liver vs. Lung	234.717	5	3.522	0.108	Do Not Test
Lung vs. Heart Blood	39.252	5	0.567	0.994	No
Lung vs. Brain	33.950	5	0.490	0.997	Do Not Test
Lung vs. Femoral Blood	16.020	5	0.197	1.000	Do Not Test
Femoral Blood vs. Heart Blood	23.232	5	0.282	1.000	Do Not Test
Femoral Blood vs. Brain	17.930	5	0.218	1.000	Do Not Test
Brain vs. Heart Blood	5.302	5	0.0751	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:08:13 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: MOR

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.111)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	10	0	1250.232	357.062	112.913
Femoral Blood	7	3	344.228	255.342	127.671
Liver	10	0	1408.235	686.889	217.213
Brain	10	0	654.364	506.036	160.023
Lung	10	0	1435.270	1311.567	414.754

Source of Variation	DF	SS	MS	F	P
Between Groups	4	6565760.834	1641440.209	2.739	0.042
Residual	39	23375911.295	599382.341		
Total	43	29941672.129			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.042).

Power of performed test with alpha = 0.050: 0.476

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Lung vs. Femoral Blood	1091.042	5	3.369	0.142	No
Lung vs. Brain	780.907	5	3.190	0.182	Do Not Test
Lung vs. Heart Blood	185.039	5	0.756	0.983	Do Not Test
Lung vs. Liver	27.035	5	0.110	1.000	Do Not Test
Liver vs. Femoral Blood	1064.007	5	3.285	0.159	Do Not Test
Liver vs. Brain	753.871	5	3.079	0.210	Do Not Test
Liver vs. Heart Blood	158.003	5	0.645	0.991	Do Not Test
Heart Blood vs. Femoral Blood	906.003	5	2.797	0.296	Do Not Test
Heart Blood vs. Brain	595.868	5	2.434	0.433	Do Not Test
Brain vs. Femoral Blood	310.135	5	0.958	0.960	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:09:16 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: M3G

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	10	0	2939.404	2771.027	876.276
Femoral Blood	7	0	218.609	165.585	62.585
Liver	10	0	7222.415	4344.473	1373.843
Brain	10	0	523.841	473.063	149.596
Lung	10	0	884.791	849.708	268.701

Source of Variation	DF	SS	MS	F	P
Between Groups	4	326515547.457	81628886.864	13.844	<0.001
Residual	42	247653942.387	5896522.438		
Total	46	574169489.844			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Femoral Blood	7003.806	5	8.277	<0.001	Yes
Liver vs. Brain	6698.574	5	8.723	<0.001	Yes
Liver vs. Lung	6337.624	5	8.253	<0.001	Yes
Liver vs. Heart Blood	4283.010	5	5.578	0.003	Yes
Heart Blood vs. Femoral Blood	2720.796	5	3.215	0.174	No
Heart Blood vs. Brain	2415.564	5	3.146	0.191	Do Not Test
Heart Blood vs. Lung	2054.613	5	2.676	0.337	Do Not Test
Lung vs. Femoral Blood	666.182	5	0.787	0.981	Do Not Test
Lung vs. Brain	360.950	5	0.470	0.997	Do Not Test
Brain vs. Femoral Blood	305.232	5	0.361	0.999	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:08:44 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: Nm

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.114)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	10	1	106.820	158.412	52.804
Femoral Blood	7	1	191.059	156.661	63.957
Liver	10	0	609.568	990.114	313.102
Brain	10	0	55.338	32.007	10.122
Lung	10	0	108.909	134.665	42.585

Source of Variation	DF	SS	MS	F	P
Between Groups	4	2032429.829	508107.457	2.181	0.089
Residual	40	9318832.426	232970.811		
Total	44	11351262.256			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.089).

Power of performed test with alpha = 0.050: 0.329

The power of the performed test (0.329) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Friday, March 15, 2019, 9:09:49 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: MOR

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	2	1010.640	493.854	142.563
Femoral Blood	4	1	926.441	996.752	575.475
Liver	14	0	1622.323	473.870	126.647
Brain	14	0	390.297	230.033	61.479
Lung	14	0	1281.779	1229.806	328.680

Source of Variation	DF	SS	MS	F	P
Between Groups	4	11470796.848	2867699.212	5.337	0.001
Residual	52	27938431.006	537277.519		
Total	56	39409227.855			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.001).

Power of performed test with alpha = 0.050: 0.917

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Brain	1232.026	5	6.289	<0.001	Yes
Liver vs. Femoral Blood	695.883	5	2.110	0.572	No
Liver vs. Heart Blood	611.683	5	3.000	0.227	Do Not Test
Liver vs. Lung	340.544	5	1.738	0.735	Do Not Test
Lung vs. Brain	891.483	5	4.551	0.018	Yes
Lung vs. Femoral Blood	355.339	5	1.078	0.940	Do Not Test
Lung vs. Heart Blood	271.139	5	1.330	0.880	Do Not Test
Heart Blood vs. Brain	620.343	5	3.042	0.215	No
Heart Blood vs. Femoral Blood	84.199	5	0.252	1.000	Do Not Test
Femoral Blood vs. Brain	536.144	5	1.626	0.779	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:10:43 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: M3G

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	2	1412.028	1096.985	316.672
Femoral Blood	4	1	913.566	986.633	569.633
Liver	14	0	5744.188	3359.828	897.952
Brain	14	0	536.847	397.939	106.354
Lung	14	0	1782.255	1242.022	331.944

Source of Variation	DF	SS	MS	F	P
Between Groups	4	228539350.258	57134837.565	16.143	<0.001
Residual	52	184046446.526	3539354.741		
Total	56	412585796.784			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Brain	5207.341	5	10.357	<0.001	Yes
Liver vs. Femoral Blood	4830.622	5	5.708	0.002	Yes
Liver vs. Heart Blood	4332.160	5	8.278	<0.001	Yes
Liver vs. Lung	3961.933	5	7.880	<0.001	Yes
Lung vs. Brain	1245.408	5	2.477	0.413	No
Lung vs. Femoral Blood	868.689	5	1.026	0.950	Do Not Test
Lung vs. Heart Blood	370.227	5	0.707	0.987	Do Not Test
Heart Blood vs. Brain	875.181	5	1.672	0.761	Do Not Test
Heart Blood vs. Femoral Blood	498.462	5	0.580	0.994	Do Not Test
Femoral Blood vs. Brain	376.719	5	0.445	0.998	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, March 15, 2019, 9:10:16 AM

Data source: Data 1 in Compare matrix at hour

Dependent Variable: NM

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
Heart Blood	14	3	57.899	61.467	18.533
Femoral Blood	4	1	83.862	80.736	46.613
Liver	14	0	339.890	388.589	103.855
Brain	14	2	42.936	10.193	2.943
Lung	14	1	78.951	61.909	17.170

Source of Variation	DF	SS	MS	F	P
Between Groups	4	803481.399	200870.350	4.678	0.003
Residual	48	2060971.588	42936.908		
Total	52	2864452.987			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.854

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Specimen Type**

Comparison	Diff of Means	p	q	P	P<0.050
Liver vs. Brain	296.954	5	5.152	0.006	Yes
Liver vs. Heart Blood	281.991	5	4.777	0.012	Yes
Liver vs. Lung	260.939	5	4.624	0.016	Yes
Liver vs. Femoral Blood	256.028	5	2.747	0.310	No
Femoral Blood vs. Brain	40.926	5	0.433	0.998	No
Femoral Blood vs. Heart Blood	25.963	5	0.272	1.000	Do Not Test
Femoral Blood vs. Lung	4.911	5	0.0523	1.000	Do Not Test
Lung vs. Brain	36.015	5	0.614	0.992	Do Not Test
Lung vs. Heart Blood	21.052	5	0.351	0.999	Do Not Test
Heart Blood vs. Brain	14.963	5	0.245	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Two Way Analysis of Variance Monday, March 04, 2019, 5:52:29 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.142)

Equal Variance Test: Passed (P = 0.488)

Source of Variation	DF	SS	MS	F	P
PM Interval	3	2058110.219	686036.740	2.692	0.054
Study	1	18638.703	18638.703	0.0731	0.788
PM Interval x Study	3	70244.917	23414.972	0.0919	0.964
Residual	64	16310986.194	254859.159		
Total	71	18515302.071	260778.902		

The difference in the mean values among the different levels of PM Interval is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Study. There is not a statistically significant difference (P = 0.054).

The difference in the mean values among the different levels of Study is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in PM Interval. There is not a statistically significant difference (P = 0.788).

The effect of different levels of PM Interval does not depend on what level of Study is present. There is not a statistically significant interaction between PM Interval and Study. (P = 0.964)

Power of performed test with alpha = 0.0500: for PM Interval : 0.413
Power of performed test with alpha = 0.0500: for Study : 0.0500
Power of performed test with alpha = 0.0500: for PM Interval x Study : 0.0500

Least square means for PM Interval :

Group	Mean	SEM
0.000	1499.296	130.639
8.000	1244.812	120.049
16.000	1318.922	119.732
24.000	1016.470	115.212

Least square means for Study :

Group	Mean	SEM
1.000	1253.441	78.986
2.000	1286.309	92.375

Least square means for PM Interval x Study :

Group	Mean	SEM
0.000 x 1.000	1518.551	178.486
0.000 x 2.000	1480.041	190.810
8.000 x 1.000	1234.340	145.734
8.000 x 2.000	1255.284	190.810

16.000 x 1.000	1250.232	159.643
16.000 x 2.000	1387.611	178.486
24.000 x 1.000	1010.640	145.734
24.000 x 2.000	1022.299	178.486

Two Way Analysis of Variance Monday, March 04, 2019, 5:53:22 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: M(FB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.441)

Source of Variation	DF	SS	MS	F	P
PM Interval	3	22412086.845	7470695.615	3.895	0.017
Study	1	763827.079	763827.079	0.398	0.532
PM Interval x Study	3	20813494.326	6937831.442	3.618	0.022
Residual	35	67124601.415	1917845.755		
Total	42	104116727.775	2478969.709		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of PM Interval depends on what level of Study is present. There is a statistically significant interaction between PM Interval and Study. (P = 0.022)

Power of performed test with alpha = 0.0500: for PM Interval : 0.638
Power of performed test with alpha = 0.0500: for Study : 0.0500
Power of performed test with alpha = 0.0500: for PM Interval x Study : 0.587

Least square means for PM Interval :

Group	Mean	SEM
0.000	2713.669	434.004
8.000	1371.129	385.233
16.000	652.081	434.004
24.000	1528.909	505.680

Least square means for Study :

Group	Mean	SEM
1.000	1705.863	342.069
2.000	1427.032	279.639

Least square means for PM Interval x Study :

Group	Mean	SEM
0.000 x 1.000	4056.259	692.432
0.000 x 2.000	1371.079	523.429
8.000 x 1.000	1496.522	523.429
8.000 x 2.000	1245.736	565.368
16.000 x 1.000	344.228	692.432
16.000 x 2.000	959.934	523.429
24.000 x 1.000	926.441	799.551
24.000 x 2.000	2131.377	619.330

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Study within 0**

Comparison	Diff of Means	p	q	P	P<0.05
1.000 vs. 2.000	2685.180	2	4.375	0.004	Yes

Comparisons for factor: **Study within 8**

Comparison	Diff of Means	p	q	P	P<0.05
1.000 vs. 2.000	250.786	2	0.460	0.747	No

Comparisons for factor: **Study within 16**

Comparison	Diff of Means	p	q	P	P<0.05
2.000 vs. 1.000	615.705	2	1.003	0.483	No

Comparisons for factor: **Study within 24**

Comparison	Diff of Means	p	q	P	P<0.05
2.000 vs. 1.000	1204.937	2	1.685	0.242	No

Comparisons for factor: **PM Interval within 1**

Comparison	Diff of Means	p	q	P	P<0.05
0.000 vs. 16.000	3712.031	4	5.361	0.003	Yes
0.000 vs. 24.000	3129.819	4	4.185	0.027	Yes
0.000 vs. 8.000	2559.737	4	4.170	0.028	Yes
8.000 vs. 16.000	1152.294	4	1.877	0.552	No
8.000 vs. 24.000	570.082	4	0.844	0.933	Do Not Test
24.000 vs. 16.000	582.212	4	0.778	0.946	Do Not Test

Comparisons for factor: **PM Interval within 2**

Comparison	Diff of Means	p	q	P	P<0.05
24.000 vs. 16.000	1171.444	4	2.043	0.481	No
24.000 vs. 8.000	885.641	4	1.494	0.718	Do Not Test
24.000 vs. 0.000	760.298	4	1.326	0.785	Do Not Test
0.000 vs. 16.000	411.146	4	0.785	0.945	Do Not Test
0.000 vs. 8.000	125.343	4	0.230	0.999	Do Not Test
8.000 vs. 16.000	285.802	4	0.525	0.982	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Two Way Analysis of Variance Monday, March 04, 2019, 6:32:32 AM

Data source: Data 1 in Comparision data

General Linear Model

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.073)

Source of Variation	DF	SS	MS	F	P
PM Interval	3	11696031.749	3898677.250	3.724	0.015
Study	1	1017999.694	1017999.694	0.972	0.328
PM Interval x Study	3	7383169.993	2461056.664	2.351	0.080
Residual	68	71184169.893	1046826.028		
Total	75	87793298.722	1170577.316		

The difference in the mean values among the different levels of PM Interval is greater than would be expected by chance after allowing for effects of differences in Study. There is a statistically significant difference (P = 0.015). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Study is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in PM Interval. There is not a statistically significant difference (P = 0.328).

The effect of different levels of PM Interval does not depend on what level of Study is present. There is not a statistically significant interaction between PM Interval and Study. (P = 0.080)

Power of performed test with alpha = 0.0500: for PM Interval : 0.634
Power of performed test with alpha = 0.0500: for Study : 0.0500
Power of performed test with alpha = 0.0500: for PM Interval x Study : 0.335

Least square means for PM Interval :

Group	Mean	SEM
0.000	997.631	257.808
8.000	990.208	236.812
16.000	1403.661	252.106
24.000	1948.141	226.730

Least square means for Study :

Group	Mean	SEM
1.000	1455.058	152.181
2.000	1214.762	190.311

Least square means for PM Interval x Study :

Group	Mean	SEM
0.000 x 1.000	1538.134	341.048
0.000 x 2.000	457.127	386.713
8.000 x 1.000	1251.541	273.447

8.000 x 2.000	728.875	386.713
16.000 x 1.000	1408.235	323.547
16.000 x 2.000	1399.087	386.713
24.000 x 1.000	1622.323	273.447
24.000 x 2.000	2273.959	361.736

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **PM Interval**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
24.000 vs. 8.000	957.933	4	4.132	0.024	Yes
24.000 vs. 0.000	950.511	4	3.915	0.036	Yes
24.000 vs. 16.000	544.480	4	2.271	0.382	No
16.000 vs. 8.000	413.453	4	1.690	0.632	No
16.000 vs. 0.000	406.030	4	1.592	0.675	Do Not Test
0.000 vs. 8.000	7.422	4	0.0300	1.000	Do Not Test

Comparisons for factor: **Study within 0**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	1081.006	2	2.965	0.040	Yes

Comparisons for factor: **Study within 8**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	522.666	2	1.561	0.274	No

Comparisons for factor: **Study within 16**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	9.148	2	0.0257	0.986	No

Comparisons for factor: **Study within 24**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
2.000 vs. 1.000	651.636	2	2.032	0.155	No

Comparisons for factor: **PM Interval within 1**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
24.000 vs. 8.000	370.782	4	1.356	0.773	No
24.000 vs. 16.000	214.088	4	0.715	0.958	Do Not Test
24.000 vs. 0.000	84.189	4	0.272	0.998	Do Not Test
0.000 vs. 8.000	286.592	4	0.927	0.913	Do Not Test
0.000 vs. 16.000	129.899	4	0.391	0.993	Do Not Test
16.000 vs. 8.000	156.694	4	0.523	0.983	Do Not Test

Comparisons for factor: **PM Interval within 2**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
24.000 vs. 0.000	1816.832	4	4.852	0.006	Yes
24.000 vs. 8.000	1545.084	4	4.126	0.024	Yes
24.000 vs. 16.000	874.872	4	2.337	0.357	No
16.000 vs. 0.000	941.960	4	2.436	0.320	No

16.000 vs. 8.000	670.212	4	1.733	0.613	Do Not Test
8.000 vs. 0.000	271.748	4	0.703	0.960	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Two Way Analysis of Variance Monday, March 04, 2019, 5:55:09 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: M(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.195)

Source of Variation	DF	SS	MS	F	P
PM Interval	3	4678399.853	1559466.618	1.648	0.186
Study	1	175287.611	175287.611	0.185	0.668
PM Interval x Study	3	7540946.830	2513648.943	2.656	0.055
Residual	69	65298572.181	946356.119		
Total	76	75483285.820	993201.129		

The difference in the mean values among the different levels of PM Interval is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Study. There is not a statistically significant difference (P = 0.186).

The difference in the mean values among the different levels of Study is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in PM Interval. There is not a statistically significant difference (P = 0.668).

The effect of different levels of PM Interval does not depend on what level of Study is present. There is not a statistically significant interaction between PM Interval and Study. (P = 0.055)

Power of performed test with alpha = 0.0500: for PM Interval : 0.174
Power of performed test with alpha = 0.0500: for Study : 0.0500
Power of performed test with alpha = 0.0500: for PM Interval x Study : 0.406

Least square means for PM Interval :

Group	Mean	SEM
0.000	387.015	245.125
8.000	372.773	225.161
16.000	462.396	230.722
24.000	967.714	215.576

Least square means for Study :

Group	Mean	SEM
1.000	498.111	144.694
2.000	596.838	178.006

Least square means for PM Interval x Study :

Group	Mean	SEM
0.000 x 1.000	426.933	324.269
0.000 x 2.000	347.097	367.687
8.000 x 1.000	520.852	259.994
8.000 x 2.000	224.694	367.687

16.000 x 1.000	654.364	307.629
16.000 x 2.000	270.428	343.940
24.000 x 1.000	390.297	259.994
24.000 x 2.000	1545.132	343.940

Two Way Analysis of Variance Monday, March 04, 2019, 6:30:24 AM

Data source: Data 1 in Comparision data

General Linear Model

Dependent Variable: M(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	DF	SS	MS	F	P
PM Interval	3	12875708.892	4291902.964	3.186	0.029
Study	1	2530448.759	2530448.759	1.879	0.175
PM Interval x Study	3	8952031.410	2984010.470	2.215	0.094
Residual	68	91598572.327	1347037.828		
Total	75	118738039.595	1583173.861		

The difference in the mean values among the different levels of PM Interval is greater than would be expected by chance after allowing for effects of differences in Study. There is a statistically significant difference (P = 0.029). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Study is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in PM Interval. There is not a statistically significant difference (P = 0.175).

The effect of different levels of PM Interval does not depend on what level of Study is present. There is not a statistically significant interaction between PM Interval and Study. (P = 0.094)

Power of performed test with alpha = 0.0500: for PM Interval : 0.525
Power of performed test with alpha = 0.0500: for Study : 0.142
Power of performed test with alpha = 0.0500: for PM Interval x Study : 0.303

Least square means for PM Interval :

Group	Mean	SEM
0.000	2415.458	292.449
8.000	1362.958	268.631
16.000	1501.275	275.265
24.000	1343.331	268.631

Least square means for Study :

Group	Mean	SEM
1.000	1845.183	172.628
2.000	1466.329	215.882

Least square means for PM Interval x Study :

Group	Mean	SEM
0.000 x 1.000	3239.647	386.873
0.000 x 2.000	1591.270	438.673
8.000 x 1.000	1424.034	310.189

8.000 x 2.000	1301.883	438.673
16.000 x 1.000	1435.270	367.020
16.000 x 2.000	1567.279	410.341
24.000 x 1.000	1281.779	310.189
24.000 x 2.000	1404.883	438.673

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **PM Interval**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
0.000 vs. 24.000	1072.127	4	3.818	0.043	Yes
0.000 vs. 8.000	1052.500	4	3.748	0.048	Yes
0.000 vs. 16.000	914.184	4	3.219	0.114	No
16.000 vs. 24.000	157.944	4	0.581	0.977	No
16.000 vs. 8.000	138.316	4	0.509	0.984	Do Not Test
8.000 vs. 24.000	19.627	4	0.0731	1.000	Do Not Test

Comparisons for factor: **Study within 0**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	1648.376	2	3.986	0.006	Yes

Comparisons for factor: **Study within 8**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	122.151	2	0.322	0.821	No

Comparisons for factor: **Study within 16**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
2.000 vs. 1.000	132.009	2	0.339	0.811	No

Comparisons for factor: **Study within 24**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
2.000 vs. 1.000	123.103	2	0.324	0.820	No

Comparisons for factor: **PM Interval within 1**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
0.000 vs. 24.000	1957.867	4	5.584	0.001	Yes
0.000 vs. 8.000	1815.612	4	5.178	0.003	Yes
0.000 vs. 16.000	1804.376	4	4.785	0.006	Yes
16.000 vs. 24.000	153.491	4	0.452	0.989	No
16.000 vs. 8.000	11.236	4	0.0331	1.000	Do Not Test
8.000 vs. 24.000	142.255	4	0.459	0.988	Do Not Test

Comparisons for factor: **PM Interval within 2**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
0.000 vs. 8.000	289.388	4	0.660	0.966	No
0.000 vs. 24.000	186.387	4	0.425	0.991	Do Not Test
0.000 vs. 16.000	23.991	4	0.0565	1.000	Do Not Test
16.000 vs. 8.000	265.396	4	0.625	0.971	Do Not Test

16.000 vs. 24.000	162.396	4	0.382	0.993	Do Not Test
24.000 vs. 8.000	103.000	4	0.235	0.998	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Saturday, April 13, 2019, 4:50:45 PM

Data source: Data 1 in Notebook1

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.672)

Equal Variance Test: Passed (P = 0.166)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	2	1292.891	542.671	121.345
M	25	3	1169.667	412.981	88.048

Source of Variation	DF	SS	MS	F	P
Between Groups	1	159072.401	159072.401	0.693	0.410
Residual	40	9176957.447	229423.936		
Total	41	9336029.849			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.410).

Power of performed test with alpha = 0.050: 0.047

The power of the performed test (0.047) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, April 13, 2019, 4:51:10 PM

Data source: Data 1 in Notebook1

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.186)

Equal Variance Test: Passed (P = 0.364)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	0	1625.745	687.395	146.553
M	25	0	1295.731	529.215	105.843

Source of Variation	DF	SS	MS	F	P
Between Groups	1	1274469.398	1274469.398	3.446	0.070
Residual	45	16644384.484	369875.211		
Total	46	17918853.882			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.070).

Power of performed test with alpha = 0.050: 0.318

The power of the performed test (0.318) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

One Way Analysis of Variance Saturday, April 13, 2019, 4:50:13 PM

Data source: Data 1 in Notebook1

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.463)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	2	2731.301	1871.502	418.481
M	25	3	1480.608	1869.180	398.510

Source of Variation	DF	SS	MS	F	P
Between Groups	1	16387185.129	16387185.129	4.685	0.036
Residual	40	139918356.468	3497958.912		
Total	41	156305541.597			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.036).

Power of performed test with alpha = 0.050: 0.454

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
F vs. M	1250.692	2	3.061	0.037	Yes

One Way Analysis of Variance Saturday, April 13, 2019, 4:52:35 PM

Data source: Data 1 in Notebook1

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	0	555.444	444.297	94.724
M	25	0	276.898	172.452	34.490

Source of Variation	DF	SS	MS	F	P
Between Groups	1	907943.120	907943.120	8.408	0.006
Residual	45	4859147.796	107981.062		
Total	46	5767090.916			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.006).

Power of performed test with alpha = 0.050: 0.770

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
F vs. M	278.546	2	4.101	0.006	Yes

One Way Analysis of Variance Saturday, April 13, 2019, 4:53:31 PM

Data source: Data 1 in Notebook1

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	0	1936.871	1316.023	280.577
M	25	0	913.500	759.749	151.950

Source of Variation	DF	SS	MS	F	P
Between Groups	1	12255496.271	12255496.271	10.981	0.002
Residual	45	50223461.526	1116076.923		
Total	46	62478957.797			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002).

Power of performed test with alpha = 0.050: 0.889

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
F vs. M	1023.371	2	4.686	0.002	Yes

One Way Analysis of Variance Saturday, April 13, 2019, 4:53:58 PM

Data source: Data 1 in Notebook1

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	4	19.262	10.376	2.446
M	25	3	99.405	106.350	22.674

Source of Variation	DF	SS	MS	F	P
Between Groups	1	63587.815	63587.815	10.096	0.003
Residual	38	239345.932	6298.577		
Total	39	302933.747			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.852

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
M vs. F	80.144	2	4.493	0.003	Yes

One Way Analysis of Variance Saturday, April 13, 2019, 4:54:35 PM

Data source: Data 1 in Notebook1

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	0	90.608	196.131	41.815
M	25	0	740.420	766.271	153.254

Source of Variation	DF	SS	MS	F	P
Between Groups	1	4941275.991	4941275.991	14.923	<0.001
Residual	45	14899926.775	331109.484		
Total	46	19841202.766			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.969

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
M vs. F	649.811	2	5.463	<0.001	Yes

One Way Analysis of Variance Saturday, April 13, 2019, 4:55:11 PM

Data source: Data 1 in Notebook1

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
F	22	2	41.539	8.794	1.967
M	25	0	147.550	148.531	29.706

Source of Variation	DF	SS	MS	F	P
Between Groups	1	124871.987	124871.987	10.113	0.003
Residual	43	530941.125	12347.468		
Total	44	655813.112			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003).

Power of performed test with alpha = 0.050: 0.856

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Sex**

Comparison	Diff of Means	p	q	P	P<0.050
M vs. F	106.012	2	4.497	0.003	Yes

T-test Friday, March 15, 2019, 1:56:52 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.429)

Equal Variance Test: Passed (P = 0.902)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	1694.961	543.368	271.684
M	5	1	1342.141	565.857	282.929

Difference 352.820

t = 0.899 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -606.984 to 1312.624

Two-tailed P-value = 0.403

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.403).

One-tailed P-value = 0.202

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.202).

Power of performed two-tailed test with alpha = 0.050: 0.119

The power of the performed test (0.119) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.199

The power of the performed test (0.199) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 1:58:12 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.797)

Equal Variance Test: Passed (P = 0.521)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	2103.398	929.786	464.893
M	5	0	1085.922	480.969	215.096

Difference 1017.476

t = 2.139 with 7 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -107.177 to 2142.129

Two-tailed P-value = 0.0697

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.070).

One-tailed P-value = 0.0349

The sample mean of group F exceeds the sample mean of group M by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group M is greater than or equal to the population mean of group F. (P = 0.035).

Power of performed two-tailed test with alpha = 0.050: 0.455

Power of performed one-tailed test with alpha = 0.050: 0.611

t-test Friday, March 15, 2019, 1:59:01 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.144)

Equal Variance Test: Passed (P = 0.304)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	4122.254	2746.669	1373.334
M	5	1	1316.764	774.402	387.201

Difference 2805.490

t = 1.966 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -685.947 to 6296.927

Two-tailed P-value = 0.0969

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.097).

One-tailed P-value = 0.0484

The sample mean of group F exceeds the sample mean of group M by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group M is greater than or equal to the population mean of group F. (P = 0.048).

Power of performed two-tailed test with alpha = 0.050: 0.380

Power of performed one-tailed test with alpha = 0.050: 0.538

t-test Friday, March 15, 2019, 2:00:13 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Passed (P = 0.086)

Equal Variance Test: Passed (P = 0.309)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	356.187	388.069	194.034
M	5	0	161.769	95.564	42.738

Difference 194.418

t = 1.097 with 7 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -224.542 to 613.378

Two-tailed P-value = 0.309

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.309).

One-tailed P-value = 0.154

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.154).

Power of performed two-tailed test with alpha = 0.050: 0.159

The power of the performed test (0.159) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.257

The power of the performed test (0.257) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:00:43 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.295)

Equal Variance Test: Passed (P = 0.371)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	2659.766	1968.106	984.053
M	5	0	875.155	599.061	267.908

Difference 1784.612

t = 1.948 with 7 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -381.703 to 3950.926

Two-tailed P-value = 0.0924

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.092).

One-tailed P-value = 0.0462

The sample mean of group F exceeds the sample mean of group M by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group M is greater than or equal to the population mean of group F. (P = 0.046).

Power of performed two-tailed test with alpha = 0.050: 0.391

Power of performed one-tailed test with alpha = 0.050: 0.544

t-test Friday, March 15, 2019, 2:01:12 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.847)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:01:12 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	0	14.847	5.640	25.715
M	5	1	109.943	29.795	165.748

Mann-Whitney U Statistic= 2.000

T = 12.000 n(small)= 4 n(big)= 4 P(est.)= 0.112 P(exact)= 0.114

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.114)

t-test Friday, March 15, 2019, 2:02:13 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.268)

Equal Variance Test: Passed (P = 0.075)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	60.882	49.092	24.546
M	5	0	1005.292	886.083	396.268

Difference -944.411

t = -2.099 with 7 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2008.120 to 119.299

Two-tailed P-value = 0.0739

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.074).

One-tailed P-value = 0.0370

The sample mean of group M exceeds the sample mean of group F by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group F is greater than or equal to the population mean of group M. (P = 0.037).

Power of performed two-tailed test with alpha = 0.050: 0.441

Power of performed one-tailed test with alpha = 0.050: 0.597

t-test Friday, March 15, 2019, 2:02:56 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.262)

Equal Variance Test: Passed (P = 0.069)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	43.833	10.710	5.355
M	5	0	242.633	250.065	111.832

Difference -198.800

t = -1.567 with 7 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -498.855 to 101.255

Two-tailed P-value = 0.161

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.161).

One-tailed P-value = 0.0806

The sample mean of group M does not exceed the sample mean of the group F by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group F is greater than or equal to the population mean of group M cannot be rejected. (P = 0.081).

Power of performed two-tailed test with alpha = 0.050: 0.274

The power of the performed test (0.274) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.409

The power of the performed test (0.409) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:04:11 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.485)

Equal Variance Test: Passed (P = 0.152)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	1	1391.938	551.463	225.134
M	7	1	1076.742	262.359	107.108

Difference 315.195

t = 1.264 with 10 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -240.310 to 870.701

Two-tailed P-value = 0.235

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.235).

One-tailed P-value = 0.117

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.117).

Power of performed two-tailed test with alpha = 0.050: 0.209

The power of the performed test (0.209) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.321

The power of the performed test (0.321) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:04:35 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.167)

Equal Variance Test: Passed (P = 0.213)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	0	1248.561	379.765	143.538
M	7	0	1254.522	708.752	267.883

Difference -5.961

t = -0.0196 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -668.135 to 656.213

Two-tailed P-value = 0.985

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.985).

One-tailed P-value = 0.492

The sample mean of group M does not exceed the sample mean of the group F by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group F is greater than or equal to the population mean of group M cannot be rejected. (P = 0.492).

Power of performed two-tailed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.052

The power of the performed test (0.052) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:04:56 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.305)

Equal Variance Test: Passed (P = 0.240)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	1	2340.177	1173.780	479.194
M	7	1	843.981	512.898	209.390

Difference 1496.197

t = 2.861 with 10 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 331.005 to 2661.389

Two-tailed P-value = 0.0169

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.017).

One-tailed P-value = 0.00846

The sample mean of group F exceeds the sample mean of group M by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group M is greater than or equal to the population mean of group F. (P = 0.008).

Power of performed two-tailed test with alpha = 0.050: 0.732

Power of performed one-tailed test with alpha = 0.050: 0.845

t-test Friday, March 15, 2019, 2:05:51 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Passed (P = 0.522)

Equal Variance Test: Passed (P = 0.495)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	0	413.251	144.086	54.459
M	7	0	180.231	79.224	29.944

Difference 233.020

t = 3.749 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 97.609 to 368.430

Two-tailed P-value = 0.00277

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.003).

One-tailed P-value = 0.00139

The sample mean of group F exceeds the sample mean of group M by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group M is greater than or equal to the population mean of group F. (P = 0.001).

Power of performed two-tailed test with alpha = 0.050: 0.930

Power of performed one-tailed test with alpha = 0.050: 0.970

t-test Friday, March 15, 2019, 2:07:22 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.782)

Equal Variance Test: Passed (P = 0.174)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	0	1552.070	976.504	369.084
M	7	0	824.265	611.655	231.184

Difference 727.805

t = 1.671 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -221.090 to 1676.699

Two-tailed P-value = 0.121

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.121).

One-tailed P-value = 0.0603

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.060).

Power of performed two-tailed test with alpha = 0.050: 0.337

The power of the performed test (0.337) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.473

The power of the performed test (0.473) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:07:58 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.341)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:07:58 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	1	17.071	14.993	35.862
M	7	1	51.464	29.050	78.231

Mann-Whitney U Statistic= 6.000

T = 27.000 n(small)= 6 n(big)= 6 P(est.)= 0.066 P(exact)= 0.065

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.065)

t-test Friday, March 15, 2019, 2:08:21 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:08:21 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	0	34.942	31.503	42.094
M	7	0	277.301	114.952	1070.183

Mann-Whitney U Statistic= 1.000

T = 29.000 n(small)= 7 n(big)= 7 P(est.)= 0.003 P(exact)= 0.001

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.001)

t-test Friday, March 15, 2019, 2:08:57 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:08:57 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	1	39.348	33.642	48.537
M	7	0	48.916	41.867	196.480

Mann-Whitney U Statistic= 7.000

T = 28.000 n(small)= 6 n(big)= 7 P(est.)= 0.054 P(exact)= 0.051

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.051)

t-test Friday, March 15, 2019, 2:09:48 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.420)

Equal Variance Test: Passed (P = 0.647)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	1127.091	433.506	216.753
M	6	0	1332.325	310.666	126.829

Difference -205.234

t = -0.879 with 8 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -743.563 to 333.095

Two-tailed P-value = 0.405

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.405).

One-tailed P-value = 0.202

The sample mean of group M does not exceed the sample mean of the group F by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group F is greater than or equal to the population mean of group M cannot be rejected. (P = 0.202).

Power of performed two-tailed test with alpha = 0.050: 0.122

The power of the performed test (0.122) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.201

The power of the performed test (0.201) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:10:11 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.135)

Equal Variance Test: Passed (P = 0.530)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	1543.470	944.385	472.193
M	6	0	1318.078	538.300	219.760

Difference 225.391

t = 0.486 with 8 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -843.395 to 1294.178

Two-tailed P-value = 0.640

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.640).

One-tailed P-value = 0.320

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.320).

Power of performed two-tailed test with alpha = 0.050: 0.072

The power of the performed test (0.072) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.115

The power of the performed test (0.115) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:11:13 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:11:13 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	0	2742.165	1174.960	5465.272
M	6	0	1864.496	529.189	4626.643

Mann-Whitney U Statistic= 8.000

T = 26.000 n(small)= 4 n(big)= 6 P(est.)= 0.456 P(exact)= 0.476

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.476)

t-test Friday, March 15, 2019, 2:11:39 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:11:39 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	0	388.410	241.297	1426.201
M	6	0	354.447	252.037	583.691

Mann-Whitney U Statistic= 11.000

T = 23.000 n(small)= 4 n(big)= 6 P(est.)= 0.915 P(exact)= 0.914

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.914)

t-test Friday, March 15, 2019, 2:12:08 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.119)

Equal Variance Test: Passed (P = 0.120)

Group Name	N	Missing	Mean	Std Dev	SEM
F	4	0	1285.062	1290.687	645.343
M	6	0	617.944	294.041	120.042

Difference 667.118

t = 1.254 with 8 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -559.208 to 1893.445

Two-tailed P-value = 0.245

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.245).

One-tailed P-value = 0.123

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.123).

Power of performed two-tailed test with alpha = 0.050: 0.198

The power of the performed test (0.198) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.310

The power of the performed test (0.310) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:12:36 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:12:36 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	1	28.326	28.267	35.841
M	6	0	65.334	38.932	238.477

Mann-Whitney U Statistic= 1.000

T = 7.000 n(small)= 3 n(big)= 6 P(est.)= 0.053 P(exact)= 0.048

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.048)

t-test Friday, March 15, 2019, 2:13:08 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:13:08 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	0	39.065	35.416	118.683
M	6	0	274.916	183.076	2387.252

Mann-Whitney U Statistic= 1.000

T = 11.000 n(small)= 4 n(big)= 6 P(est.)= 0.025 P(exact)= 0.019

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.019)

t-test Friday, March 15, 2019, 2:13:34 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:13:34 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	4	0	39.047	38.817	57.452
M	6	0	52.456	40.864	340.510

Mann-Whitney U Statistic= 4.000

T = 14.000 n(small)= 4 n(big)= 6 P(est.)= 0.110 P(exact)= 0.114

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.114)

t-test Friday, March 15, 2019, 2:14:04 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.309)

Equal Variance Test: Passed (P = 0.954)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	1	1036.330	531.949	217.167
M	7	1	984.950	502.004	204.942

Difference 51.381

t = 0.172 with 10 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -613.946 to 716.707

Two-tailed P-value = 0.867

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.867).

One-tailed P-value = 0.433

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.433).

Power of performed two-tailed test with alpha = 0.050: 0.053

The power of the performed test (0.053) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.069

The power of the performed test (0.069) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:14:26 PM

Data source: Data 1 in T test MVF

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.822)

Equal Variance Test: Passed (P = 0.239)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	0	1776.998	531.134	200.750
M	7	0	1467.648	385.493	145.703

Difference 309.350

t = 1.247 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -231.109 to 849.809

Two-tailed P-value = 0.236

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.236).

One-tailed P-value = 0.118

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.118).

Power of performed two-tailed test with alpha = 0.050: 0.210

The power of the performed test (0.210) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.320

The power of the performed test (0.320) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:14:46 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.584)

Equal Variance Test: Passed (P = 0.077)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	1	1931.012	1234.707	504.067
M	7	1	893.045	690.290	281.810

Difference 1037.968

t = 1.797 with 10 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -248.771 to 2324.707

Two-tailed P-value = 0.102

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.102).

One-tailed P-value = 0.0512

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.051).

Power of performed two-tailed test with alpha = 0.050: 0.369

The power of the performed test (0.369) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.512

The power of the performed test (0.512) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:15:10 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Brain)

Normality Test (Shapiro-Wilk) Passed (P = 0.534)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:15:10 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	0	775.285	235.508	1054.594
M	7	0	332.895	232.553	458.375

Mann-Whitney U Statistic= 13.000

T = 64.000 n(small)= 7 n(big)= 7 P(est.)= 0.160 P(exact)= 0.165

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.165)

t-test Friday, March 15, 2019, 2:15:28 PM

Data source: Data 1 in T test MVF

Dependent Variable: M3G(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.175)

Equal Variance Test: Passed (P = 0.941)

Group Name	N	Missing	Mean	Std Dev	SEM
F	7	0	2281.051	1181.487	446.560
M	7	0	1283.459	1168.718	441.734

Difference 997.593

t = 1.588 with 12 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -370.981 to 2366.166

Two-tailed P-value = 0.138

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.138).

One-tailed P-value = 0.0691

The sample mean of group F does not exceed the sample mean of the group M by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group M is greater than or equal to the population mean of group F cannot be rejected. (P = 0.069).

Power of performed two-tailed test with alpha = 0.050: 0.310

The power of the performed test (0.310) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.442

The power of the performed test (0.442) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 2:15:54 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:15:54 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	2	9.230	8.668	15.567
M	7	1	85.113	48.639	133.039

Mann-Whitney U Statistic= 0.000

T = 15.000 n(small)= 5 n(big)= 6 P(est.)= 0.008 P(exact)= 0.004

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.004)

t-test Friday, March 15, 2019, 2:16:43 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:16:43 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	0	37.965	35.435	45.712
M	7	0	375.093	174.090	937.760

Mann-Whitney U Statistic= 6.000

T = 34.000 n(small)= 7 n(big)= 7 P(est.)= 0.021 P(exact)= 0.017

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.017)

t-test Friday, March 15, 2019, 2:17:11 PM

Data source: Data 1 in T test MVF

Dependent Variable: NM(Lung)

Normality Test (Shapiro-Wilk) Passed (P = 0.292)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 2:17:11 PM

Data source: Data 1 in T test MVF

Group	N	Missing	Median	25%	75%
F	7	1	38.915	35.286	41.801
M	7	0	94.817	39.811	170.773

Mann-Whitney U Statistic= 4.000

T = 25.000 n(small)= 6 n(big)= 7 P(est.)= 0.018 P(exact)= 0.014

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.014)

t-test Friday, March 15, 2019, 3:22:46 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.084)

Equal Variance Test: Passed (P = 0.950)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1116.689	419.379	209.690
AM	4	0	1120.450	569.668	284.834

Difference -3.760

t = -0.0106 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -869.221 to 861.700

Two-tailed P-value = 0.992

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.992).

One-tailed P-value = 0.496

The sample mean of group AM does not exceed the sample mean of the group PM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group PM is greater than or equal to the population mean of group AM cannot be rejected. (P = 0.496).

Power of performed two-tailed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.051

The power of the performed test (0.051) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

T-test Friday, March 15, 2019, 3:24:05 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.417)

Equal Variance Test: Passed (P = 0.213)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	1	1976.339	1614.521	932.144
AM	4	0	1136.660	486.975	243.487

Difference 839.679

t = 1.010 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1297.502 to 2976.860

Two-tailed P-value = 0.359

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.359).

One-tailed P-value = 0.179

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.179).

Power of performed two-tailed test with alpha = 0.050: 0.132

The power of the performed test (0.132) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.221

The power of the performed test (0.221) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:25:13 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:25:13 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	0	1593.891	1397.611	2382.400
AM	4	0	633.820	556.372	1210.860

Mann-Whitney U Statistic= 1.000

T = 25.000 n(small)= 4 n(big)= 4 P(est.)= 0.061 P(exact)= 0.057

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.057)

t-test Friday, March 15, 2019, 3:23:31 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.539)

Equal Variance Test: Passed (P = 0.709)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1298.541	814.633	407.316
AM	4	0	1140.936	683.897	341.948

Difference 157.605

t = 0.296 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1143.718 to 1458.928

Two-tailed P-value = 0.777

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.777).

One-tailed P-value = 0.388

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.388).

Power of performed two-tailed test with alpha = 0.050: 0.057

The power of the performed test (0.057) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.084

The power of the performed test (0.084) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:24:51 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.305)

Equal Variance Test: Passed (P = 0.501)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	1	1137.838	1374.384	793.501
AM	4	2	1442.327	429.657	303.814

Difference -304.489

t = -0.290 with 3 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3643.296 to 3034.318

Two-tailed P-value = 0.791

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.791).

One-tailed P-value = 0.395

The sample mean of group AM does not exceed the sample mean of the group PM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group PM is greater than or equal to the population mean of group AM cannot be rejected. (P = 0.395).

Power of performed two-tailed test with alpha = 0.050: 0.055

The power of the performed test (0.055) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.079

The power of the performed test (0.079) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:26:04 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.894)

Equal Variance Test: Passed (P = 0.384)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	7481.708	361.186	180.593
AM	4	0	3983.808	700.656	350.328

Difference 3497.900

t = 8.875 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 2533.484 to 4462.317

Two-tailed P-value = 0.000114

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

One-tailed P-value = 0.0000570

The sample mean of group PM exceeds the sample mean of group AM by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM. (P = <0.001).

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

t-test Friday, March 15, 2019, 3:23:08 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.666)

Equal Variance Test: Passed (P = 0.351)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	37.321	23.635	11.818
AM	4	0	27.973	8.618	4.309

Difference 9.348

t = 0.743 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -21.431 to 40.127

Two-tailed P-value = 0.485

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.485).

One-tailed P-value = 0.243

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.243).

Power of performed two-tailed test with alpha = 0.050: 0.097

The power of the performed test (0.097) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.163

The power of the performed test (0.163) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:24:33 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.663)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:24:33 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	1	88.399	56.220	156.211
AM	4	0	36.072	21.225	43.117

Mann-Whitney U Statistic= 0.000

T = 18.000 n(small)= 3 n(big)= 4 P(est.)= 0.052 P(exact)= 0.057

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.057)

t-test Friday, March 15, 2019, 3:25:35 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.690)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:25:35 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	0	123.032	37.708	258.968
AM	4	0	67.238	34.740	111.098

Mann-Whitney U Statistic= 4.000

T = 22.000 n(small)= 4 n(big)= 4 P(est.)= 0.312 P(exact)= 0.343

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.343)

t-test Friday, March 15, 2019, 3:28:33 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.361)

Equal Variance Test: Passed (P = 0.367)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1161.453	390.196	195.098
AM	4	1	996.313	74.889	43.237

Difference 165.140

t = 0.707 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -435.503 to 765.783

Two-tailed P-value = 0.511

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.511).

One-tailed P-value = 0.256

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.256).

Power of performed two-tailed test with alpha = 0.050: 0.090

The power of the performed test (0.090) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.152

The power of the performed test (0.152) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:30:02 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.460)

Equal Variance Test: Passed (P = 0.820)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1066.339	260.922	130.461
AM	4	1	988.296	227.331	131.250

Difference 78.042

t = 0.412 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -408.923 to 565.008

Two-tailed P-value = 0.697

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.697).

One-tailed P-value = 0.349

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.349).

Power of performed two-tailed test with alpha = 0.050: 0.063

The power of the performed test (0.063) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.099

The power of the performed test (0.099) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:29:10 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.257)

Equal Variance Test: Passed (P = 0.201)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	3726.362	3861.300	1930.650
AM	4	1	851.209	301.374	173.998

Difference 2875.153

t = 1.256 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3008.933 to 8759.239

Two-tailed P-value = 0.265

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.265).

One-tailed P-value = 0.132

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.132).

Power of performed two-tailed test with alpha = 0.050: 0.177

The power of the performed test (0.177) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.289

The power of the performed test (0.289) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:29:39 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(FB)

Normality Test (Shapiro-Wilk) Passed (P = 0.334)

Equal Variance Test: Passed (P = 0.522)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	275.294	205.914	102.957
AM	4	1	143.029	60.493	34.925

Difference 132.265

t = 1.056 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -189.767 to 454.297

Two-tailed P-value = 0.339

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.339).

One-tailed P-value = 0.170

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.170).

Power of performed two-tailed test with alpha = 0.050: 0.139

The power of the performed test (0.139) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.233

The power of the performed test (0.233) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.500)

Equal Variance Test: Passed (P = 0.432)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	6770.192	1741.795	870.898
AM	4	1	2927.325	752.778	434.616

Difference 3842.868

t = 3.517 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 1033.900 to 6651.835

Two-tailed P-value = 0.0170

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.017).

One-tailed P-value = 0.00849

The sample mean of group PM exceeds the sample mean of group AM by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM. (P = 0.008).

Power of performed two-tailed test with alpha = 0.050: 0.801

Power of performed one-tailed test with alpha = 0.050: 0.912

t-test Friday, March 15, 2019, 3:28:49 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.107)

Equal Variance Test: Passed (P = 0.776)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	43.889	18.859	9.430
AM	4	1	40.916	16.416	9.478

Difference 2.973

t = 0.217 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -32.214 to 38.159

Two-tailed P-value = 0.837

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.837).

One-tailed P-value = 0.418

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.418).

Power of performed two-tailed test with alpha = 0.050: 0.054

The power of the performed test (0.054) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.073

The power of the performed test (0.073) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:30:23 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.373)

Equal Variance Test: Passed (P = 0.122)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	125.814	104.567	52.284
AM	4	1	166.614	138.815	80.145

Difference -40.800

t = -0.447 with 5 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -275.319 to 193.720

Two-tailed P-value = 0.673

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.673).

One-tailed P-value = 0.337

The sample mean of group AM does not exceed the sample mean of the group PM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group PM is greater than or equal to the population mean of group AM cannot be rejected. (P = 0.337).

Power of performed two-tailed test with alpha = 0.050: 0.066

The power of the performed test (0.066) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.105

The power of the performed test (0.105) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:31:14 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.721)

Equal Variance Test: Passed (P = 0.263)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1071.240	561.234	280.617
AM	4	0	581.720	220.330	110.165

Difference 489.520

t = 1.624 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -248.143 to 1227.182

Two-tailed P-value = 0.156

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.156).

One-tailed P-value = 0.0778

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.078).

Power of performed two-tailed test with alpha = 0.050: 0.278

The power of the performed test (0.278) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.419

The power of the performed test (0.419) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:32:18 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.754)

Equal Variance Test: Passed (P = 0.601)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	1510.147	698.178	349.089
AM	4	0	1640.054	383.479	191.739

Difference -129.908

t = -0.326 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1104.464 to 844.649

Two-tailed P-value = 0.755

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.755).

One-tailed P-value = 0.378

The sample mean of group AM does not exceed the sample mean of the group PM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group PM is greater than or equal to the population mean of group AM cannot be rejected. (P = 0.378).

Power of performed two-tailed test with alpha = 0.050: 0.059

The power of the performed test (0.059) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.088

The power of the performed test (0.088) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:31:56 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.832)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:31:56 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	0	612.583	488.310	1017.233
AM	4	0	1504.254	409.426	3241.176

Mann-Whitney U Statistic= 6.000

T = 16.000 n(small)= 4 n(big)= 4 P(est.)= 0.665 P(exact)= 0.686

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.686)

t-test Friday, March 15, 2019, 3:32:57 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: M3G(Liver)

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:32:57 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	0	4894.152	3799.556	12651.459
AM	4	0	6655.459	5570.964	7380.804

Mann-Whitney U Statistic= 4.000

T = 14.000 n(small)= 4 n(big)= 4 P(est.)= 0.312 P(exact)= 0.343

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.343)

t-test Friday, March 15, 2019, 3:31:35 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(HB)

Normality Test (Shapiro-Wilk) Passed (P = 0.555)

Equal Variance Test: Passed (P = 0.155)

Group Name	N	Missing	Mean	Std Dev	SEM
PM	4	0	50.481	45.868	22.934
AM	4	0	27.957	22.390	11.195

Difference 22.525

t = 0.883 with 6 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -39.922 to 84.971

Two-tailed P-value = 0.411

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.411).

One-tailed P-value = 0.206

The sample mean of group PM does not exceed the sample mean of the group AM by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of group AM is greater than or equal to the population mean of group PM cannot be rejected. (P = 0.206).

Power of performed two-tailed test with alpha = 0.050: 0.116

The power of the performed test (0.116) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

Power of performed one-tailed test with alpha = 0.050: 0.195

The power of the performed test (0.195) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

t-test Friday, March 15, 2019, 3:32:38 PM

Data source: Data 1 in ampm T TEST

Dependent Variable: NM(Liver)

Normality Test (Shapiro-Wilk) Passed (P = 0.436)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, March 15, 2019, 3:32:38 PM

Data source: Data 1 in ampm T TEST

Group	N	Missing	Median	25%	75%
PM	4	0	96.813	35.599	169.951
AM	4	0	186.533	41.378	363.158

Mann-Whitney U Statistic= 4.000

T = 14.000 n(small)= 4 n(big)= 4 P(est.)= 0.312 P(exact)= 0.343

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.343)