Characterization of Fentanyl Analogues by Instrumental Analysis

by

Emily Ann Rue
B.S., University of New Haven, 2013

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Science in Forensic Science
in the Graduate College of the
University of Illinois at Chicago, 2018

Chicago, Illinois

Defense Committee:

Karl Larsen, Chair and Advisor
Richard van Breemen, Medicinal Chemistry and Pharmacognosy
Dejan Nikolic, Medicinal Chemistry and Pharmacognosy

ACKNOWLEDGMENTS

I could not achieve my level of success without a strong support group. First and foremost, I would like to thank my advisor, Dr. Karl Larsen for believing in me to take me on as a graduate student, for providing me with the tools necessary to conduct this research as well as his guidance and encouragement. This research wouldn't be possible without the support of my committee members, Dr. Richard van Breemen and Dr. Dejan Nikolic for their encouragement to pursue this research, their patience and stimulating conversations.

Besides my committee members, I would like to thank Brendan Heffron for his guidance and assistance with the GC-MS and LC-MS analysis. I would like to thank Dr. Aleksej Krunic and Brian David for their patience and aid with the NMR and IR analysis. Additionally, I'd like to thank Ethan Linstad who provided material as well as consultation for the NMR analysis. I would like to thank Dr. Petukhov and his lab members for providing me with an office to spend countless hours composing this thesis.

Lastly, I would like to thank all of my friends for believing in me, encouraging me, and providing me with a form of escape from my studies. Most notably, I would sincerely like to thank Atul Jain for his guidance, wisdom and overall his unconditional support and encouragement; from making sure my brain had a full dose of glucose to talking me down when stress levels reached an all-time high and nothing seemed possible, he was monumental in my perseverance to complete this project.

PREFACE

This isn't the conventional path to achieving a master's degree. I came to University of Illinois at Chicago to receive a doctorate in medicinal chemistry; along the way, I was encouraged to concurrently work towards this master's degree with the support of my PhD and Masters advisors, Richard van Breeman and Karl Larsen. Before coming to UIC I worked as a chemist in a prosecutor's office where I analyzed seized material for the presence or absence of controlled substances. At the time, it was odd to me that so many people would be in possession of controlled substances, but it was more impressive when repeat offenders reappeared. This is when I decided to go to graduate school to learn more about drugs, their interactions in the body, and everything imaginable from production to user's excretion. It was at that time that the current heroin epidemic began to emerge in America. Due to the heightened dangers of fentanyl and its role in the heroin epidemic, I wanted to perform research that was relevant to what our country is facing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SWGDRUG- Scientific Working Group for the Analysis of Seized Drugs

FDA- Food and Drug Administration

NPF- Non-pharmaceutical fentanyl

DEA- Drug Enforcement Administration

CDC- Centers for Disease Control and Prevention

HFTF- Heroin-Fentanyl Task Force

μ- mu

δ- delta

κ- kappa

NPP- N-Phenethyl-Piperidone

4-ANPP- 4-anilio-N-phenethyl-piperidone

CSA- United States Controlled Substances Act

GC-MS- Gas chromatography- mass spectrometry

LC-MS- Liquid chromatography- mass spectrometry

LC-MS/MS- Liquid chromatography- tandem mass spectrometry

FTIR- Fourier transform- infrared spectrometry

NMR- Nuclear magnetic resonance

IR- Infrared spectrometry

QTOF- Quadrupole time-of-flight

HPLC-MS- High performance liquid chromatography- mass spectrometry

μg/ μl: microgram per microliter

m- meter

mm- millimeter

μm- micrometer

ms- millisecond

mL/min- milliliter per minute

MRM- multiple reaction monitoring

SUMMARY

The purpose of this graduate thesis is to characterize isomeric fentanyl analogues utilizing instrumental analysis currently employed in forensic laboratories. Fentanyl analogues are a crucial part of the opioid epidemic that our country is facing; detection and identification of these compounds are the key to protecting law enforcement personnel, hospital personnel, and forensic scientists. This thesis utilized five different instruments for characterization purposes: gas chromatography-mass spectrometer, liquid chromatography-mass spectrometer (triple quadrupole and quadrupole time-of-flight), Fourier transform infrared spectrometer, and nuclear magnetic resonance. GC-MS, LC-MS, and IR are currently being used in forensic laboratories that is why these five instruments are being used to determine whether or not current analytical methods in forensic science are capable of distinguishing between isomeric forms. NMR is used to determine whether or not it should be incorporated into general forensic science practices.

The results show that analogues which are not isomers are easily detected by GC-MS, LC-MS, and FTIR, while positional isomers are best detected by chiral column separation and FTIR. Geometric isomers are detected by GC-MS, chiral column separation and FTIR. Therefore, current confirmatory tests which are set forth by SWGDRUG are capable of detecting fentanyl analogues including geometric and positional isomers. NMR is capable of identifying a specific isomeric form and should be more utilized in forensic laboratories.

Chapter 1: Introduction

1.1 Overview

Fentanyl (N-(1-(2-phenylethyl)-4-piperidinyl)-N-phenylpropanamide) is a stable core system of synthetic opioids, 50-100 times more potent than morphine (1). Currently, our country is facing an opioid epidemic where an increasing number of deaths have been associated with illicit fentanyl and its analogues in the United States (2). The lethal dose of fentanyl in most humans is 2 milligrams (Figure 1) (3).

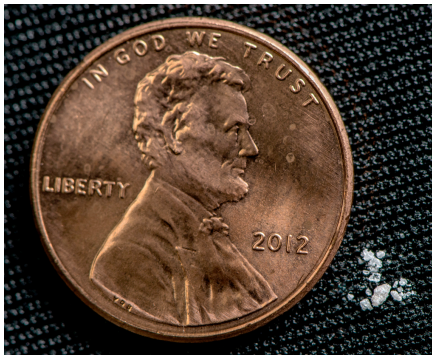

Figure 1: 2 milligrams of fentanyl, lethal dose in most humans, compared to United States penny (3)

Fentanyl was first synthesized in 1960 by Paul Janssen as a treatment for pain management (4). Since its birth, fentanyl has been seen all over the pharmaceutical market in various forms currently available as oral lozenges (Actiq®), effervescent buccal tablets (Fentora™), sublingual tablet (Abstral®), sublingual spray (Subsys™), nasal spray (Lazanda®), transdermal patches (Duragesic®) and intravenous (5). In 1972, the United States Food and Drug Administration (FDA) approved fentanyl as an intravenous anesthetic under the trade name Sublimaze® (6,7). In 1981, a year after fentanyl went off patent, there were the first reported cases of misuse and illicit use by clinicians (8,9). In the 1990s, the transdermal patch was

introduced in 1994, the FDA issued a warning regarding the dangers of fentanyl patches after reports of overdoses due to misuse (10). In the mid 2000s, there were a rise in overdose deaths from illicitly manufactured non-pharmaceutical fentanyl (NPF) (11,12). During the 2000s, the Drug Enforcement Administration (DEA) and Centers for Disease Control and Prevention (CDC) implemented a surveillance system which identified 1013 NPF-related deaths (13,14), the DEA was able to trace the production of these NPFs to a clandestine laboratory in Toluca, Mexico; this aided in the dissolution of the NPF outbreak (3). In 2010, there was a rise in counterfeit pills as well as heroin and cocaine being laced with NPF. Overdoses and in some cases, death, have been seen for users who are unaware of the potent additives (15,16). Today, opioids are the most significant contribution to overdose deaths in the United States in which, fentanyl and fentanyl analogues are the leading factor (17).

Since fentanyl was first synthesized, fentanyl analogues have been developed for medicinal and veterinary uses (18). The most common are sulfentanil, alfentanil, remifentanil, and carfentanil; of which, carfentanil is the only analogue that has been reported as being misused (19). In 1979, there were reports of overdoses as a result of "china white", which was later identified as α-methylfentanyl (20). In 1984, 3-methylfentanyl emerged, responsible for overdoses (21). In the mid to late 1980s more analogues were identified on the black market and contributed to heroin-laced deaths (22). In 2013, acetylfentanyl emerged and caused fatalities (23). In 2014, the DEA established the Heroin-Fentanyl Task Force (HFTF) consisting of multiple agencies working together to combat this opioid epidemic that is destroying our nation

(3). In 2015, the CDC published a public health advisory recommending more expansive toxicological analysis (24).

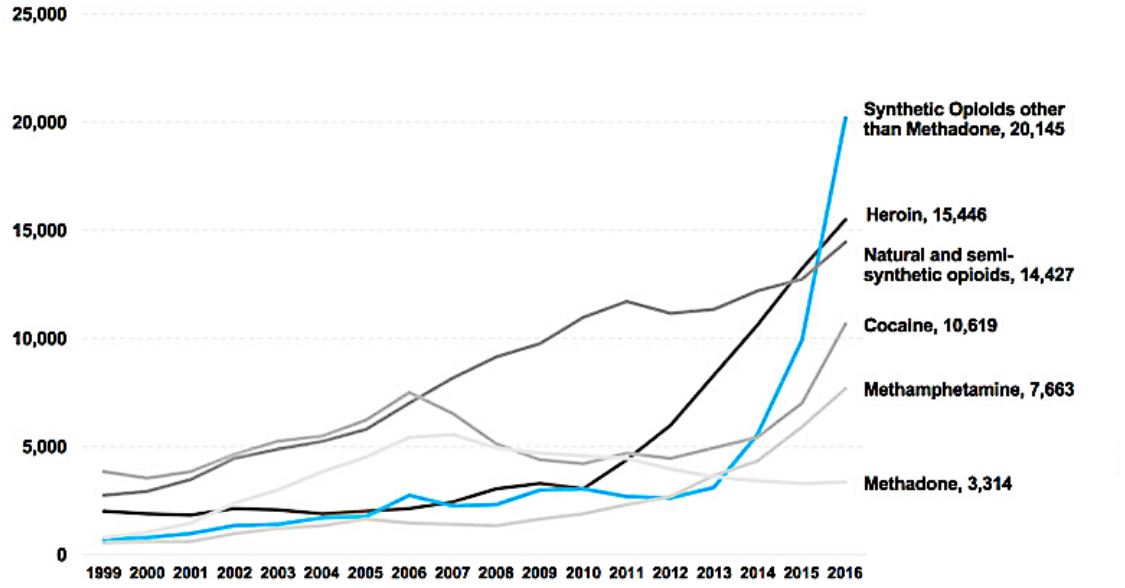

Figure 2: Drugs involved in U.S. overdose deaths, 2000 to 2016 (25)

Between 2000- 2015 (Figure 2), there has been an increase in fentanyl and fentanyl analogue overdose deaths, it was not until 2016 with the carfentanil outbreak that there was a sharp increase in deaths related to overdoses (26,27). In 2017, NYC Health Department warned people about cocaine-laced overdose deaths (28); while Georgia, releases a public safety alert warning people about the dangers of illicit synthetic opioids involving furanyl fentanyl (29). Currently, "grey death" has made headlines as the 'scariest' opioid death threat consisting of fentanyl and fentanyl-related substances being combined with heroin and other synthetic opioids; whatever the dealer has available. This makes "grey death" an imminent threat to the public and law enforcement personnel. Each batch will consist of different substances, this is where identification of fentanyl and its analogues are incredibly important (30).

1.2 Fentanyl Production

Fentanyl is a stable core, which doesn't dramatically change with the addition of chemical modifiers making this attractive for new drug synthesis (31). Fentanyl is highly lipid soluble which enables efficient blood-brain barrier transfer which corresponds to its high potency (32). Similar to other opioids, fentanyl is a full agonist highly selective at the μ-opioid receptor exhibiting little effect on the δ- and κ-opioid receptor (33–36). It has been shown that agonists at the μ-opioid receptor have higher analgesic potency while δ- antagonists can diminish the development of tolerance and dependence at the μ-opioid receptor (37–39). From a medical perspective, designing analogues could provide us with a more potent opioid without the negative side effects, which is ideal for anesthesia and pain management. From an illicit manufacturer's perspective, creating new analogues averts criminal possession (11). This is one of the reasons why our country is continually seeing new analogues on the street. Once an analogue is scheduled a new analogue is manufactured and no longer violating the law.

Fentanyl is cheap, easy to synthesize, and a quick google search will provide the step-by-step recipe for success. The following recipe for the homemade synthesis of fentanyl was found at opioids.com; however, many other websites including forums and blogs have the same recipe.

> *Underground Fentanyl Production:*
>
> *Synthesis is conducted at room temperature. The precursor, N-Phenethyl-Piperidone (NPP) is easily synthesized from piperidone and phenethyl-tosylate or phenethyl-bromide through a SN2 mechanism. NPP then reacts with aniline producing the imine derivative being reduce to 4-anilio-n-phenethyl-piperidone (4-ANPP). 4-ANPP then reacts with propionyl chloride giving the end product, fentanyl, which is then purified. Producing a 50-80% yield* (40).

For those who aren't good at synthesis, commentsection.com suggested purchasing NPP and ANPP from China by getting an illegal import license or "letter of no objection" from the government to purchase the restricted compounds from Cayman Chemical for "research" purposes. (41)

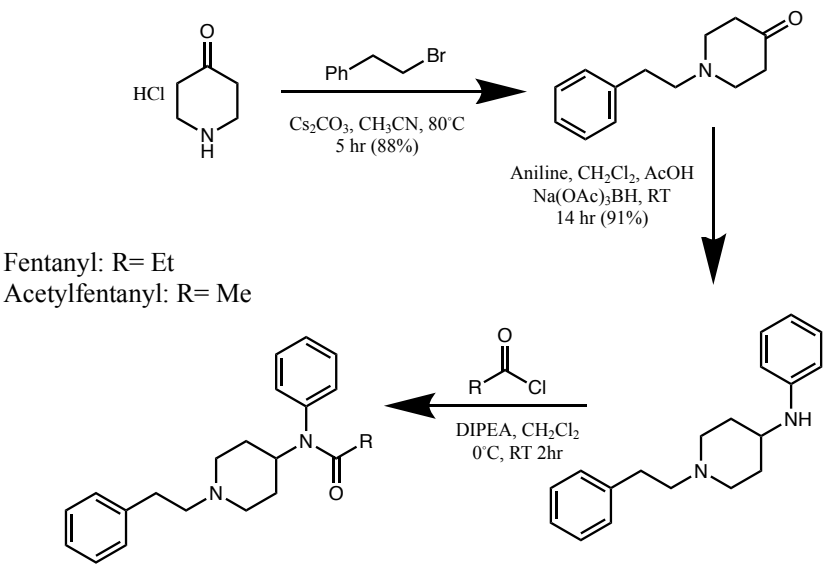

Figure 3: Synthesis of fentanyl and acetylthiofentanyl (42)

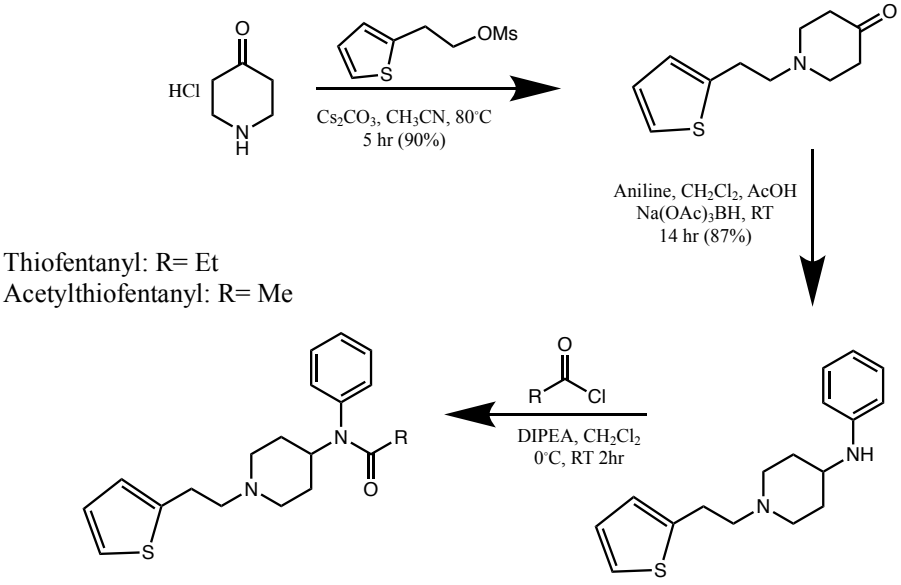

Figure 4: Synthesis of thiofentanyl and acetylthiofentanyl (42)

From a scientific approach, Valdez et al, synthesized fentanyl, acetlyfentanyl, thiofentanyl and acetylthiofentanyl utilizing three-step synthetic routes (Figure 3 and Figure 4). Their process was similar to the illicit production, however, they were more patient resulting in yields of 94-98% (42). Most commonly, different groups are added to the terminal carbonyl group, the piperdine ring, or either of the phenyl rings (43,44). Overall, this demonstrates how analogues can easily be synthesized (45,46).

1.3 Illicit Fentanyl

Fentanyl, originally developed for intravenous administration of an anesthetic, later was formulated for non-intravenous use such as oral transmucosal, intranasal and transdermal uses (7). Illicit fentanyl is a versatile white powder; administered orally, smoked, snorted, or injected (47). Fentanyl has been sold as opioid pills, heroin as well as an adulterant in heroin and cocaine samples (12,22,48,49). Street names include: apache, china white, china girl, dance fever, goodfella, friend, jackpot, murder 8, tango and cash, and TNT. Fentanyl is similar to other opioids, producing feelings of relaxation and euphoria as well as severe, prolonged respiratory depression, seizures, coma and hypertension leading to death (50).

In 2013, there were an alarming number of opioid overdoses and deaths where counterfeit pills, fentanyl, fentanyl analogues and synthetic opioids were the leading factors (13). Several factors have contributed to the proliferation of fentanyl and its analogues in the illicit drug market. These include ease of availability, profitability, and increased restrictions on prescription opioids. Fentanyl, fentanyl precursors, fentanyl analogues and other chemicals can be bought in multiple quantities online. Dark web access is not indexed by search engines and requires specific software or browsers. Due to the high level of encryption, illegal drug purchases can be

executed with encrypted currency transactions (51). Besides the dark web, some illegal drugs can be bought from other countries as well as the tools necessary to produce and manufacture illicit drug products (52,53). Synthetic opioids primarily enter the United States through China, where chemical and pharmaceutical industries weakly regulate laboratory supplies (53). Fentanyl has been used to mimic prescribed medication such as OxyContin (Figure 5) and Xanax (12). Due to the elevated potency 1 kilogram of fentanyl can be used to produce 1 million counterfeit pills producing 10- 20 million dollars in revenue (13). Although the DEA regulates things such as pill presses, they can be shipped unassembled, part by part from China (51). In response to the fentanyl crisis in the United States, China has responded by adding 116 synthetic chemicals, including 6 fentanyl products, to its controlled substances list. Although fentanyl precursors are not controlled, U.S. Secretary of State John Kerry asked for ANPP and NPP be added to the list of controlled chemicals under the 1988 U.N. Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, to which China would have to abide by as an original signatory (53).

Figure 5: (a) Seized fentanyl- heroin tablets, mimicking oxycodone (b) Oxycodone Hydrochloride 30 mg tablets, Mallinckrodt Pharmaceuticals (3)

Fentanyl analogues are most attractive to drug dealers, unfortunately, users and emergency personnel have to deal with the repercussions. Rhode Island performed a mixed method study where users voiced their concern about fentanyl and shared their methods to avoid it including: using trusted dealers, performing a test "bump", having a friend use first and observe their response, as well as snorting instead of injecting the substance (49). Despite the user's intentions to avoid this potent substance, it is being seen more widely mixed with heroin and being used to mimic other illicit substances. In the 2017 First Quarter Emerging Threat Report, DEA laboratory system identified 230 fentanyl, fentanyl analogues and other synthetic opioids within their seized evidence. Fentanyl accounted for 58% of the identified evidence, furanyl fentanyl accounted for 26%. Of the 58% of fentanyl identifications, 61% contained heroin (3). Currently, there are numerous fentanyl analogues in the market, most commonly 4-fluoroisobutryrl fentanyl, furanyl fentanyl, acryl fentanyl, acetyl fentanyl, carfentanil, and 3-methylfentanyl (54).

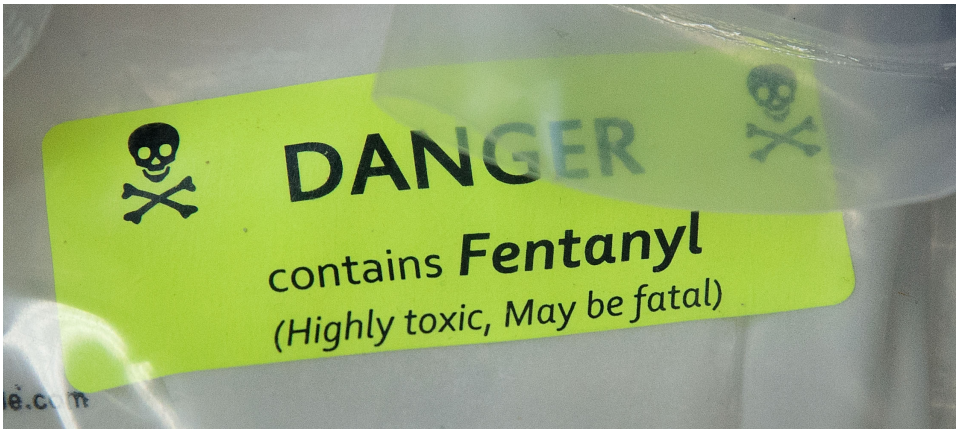

Figure 6: Seized fentanyl marked with warning label to exercise extreme caution while handling (55)

Fentanyl and its analogues are easily mistaken for cocaine, heroin and other illicit substances, but due to its hazardous nature this drug is more harmful to first responders, canine units, emergency personnel and forensic scientist (Figure 6); accidental inhalation can cause

dangerous health effects. Precautions should be taken when encountering anything that appears to be fentanyl. The onset of effects is rapid and may occur within minutes of exposure. Fentanyl can be absorbed through the skin making skin exposure extremely dangerous. The DEA has issued a warning urging all personnel to avoid contact in the field by not field testing suspected fentanyl (3).

1.4 Fentanyl laws

The United States Controlled Substances Act (CSA), Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970 sets forth regulations about the manufacture and distribution of narcotics, stimulants, depressants, and chemicals used in the illicit production of controlled substances (56). This act places controlled substances into 1 of 5 schedules (Table 1) based on the following criteria found in Section 201 (b) of the CSA (56,57):

- The drug's potential for abuse
- Scientific evidence of the drug's pharmacological effects
- Current scientific knowledge regarding the substance
- It's history and current pattern of abuse
- The scope, duration, and significance of abuse
- The risks to the public health
- The drug's psychic or physiological dependence liability
- Whether the substance is an immediate precursor to an already scheduled substance

In 1984, the CSA was amended to allow for the emergency scheduling, on a temporary basis, of substances that are an imminent hazard to public health. This applies to only substances

Table 1: DEA Scheduling Criteria		
Schedule	**Description**	**Examples**
I	Drugs, substances or chemicals are defined as drugs with no currently accepted medicinal use and a high potential for abuse	Heroin Lysergic acid diethylamide (LSD) Marijuana (cannabis) 3,4-Methylenedioxymethamphetamine (ecstasy) Methaqualone Peyote
II	Drugs, substances, or chemicals are defined as drugs with a high potential for abuse, with use potentially leading to severe psychological or physical dependence	Cocaine Methamphetamine Methadone Meperidine (Demerol) Oxycodone (Oxycontin) **Fentanyl** Adderall Ritalin
III	Drugs, substances, or chemicals are defined as drugs with a moderate to low potential for physical and psychological dependence. Abuse potential is less than Schedule I and II drugs but more than Schedule IV	Tylenol with codeine Ketamine Anabolic steriods Testosterone
IV	Drugs, substances, or chemicals are defined as drugs with a low potential for abuse and low risk of dependence	Xanax Darvon Valium Ativan Ambien Tramadol
V	Drugs, substances, or chemicals defined as drugs with lower potential for abuse than Schedule IV and consist of preparations containing limited quantities of certain narcotics	Robitussin AC Lyrica Parepectolin

with no medicinal benefit, placing the substance in Schedule I (57,58). In 1986, following the emerging fentanyl analogues, the Federal Analogue Act, a section of the United States Controlled Substances Act was passed. This allows any chemical "substantially similar" to a controlled substance listed in Schedule I or II to be treated as if it were also listed in those schedules, but only if intended for human consumption (59,60). This act has been difficult to enforce in the United States judicial system, due to the fact that it has to be proven that the analogue is substantially similar and intended for human consumption (61–64).

Fentanyl analogues that are for medicinal use are a Schedule II narcotic by the Controlled Substances Act (56). Over the years several fentanyl analogues have been scheduled (Table 2) starting in 1981 when alpha-methylfentanyl became a Schedule I narcotic. In 1986, 3-methylfentanyl became a schedule I narcotic. In 2015, the DEA announced the scheduling of acetylfentanyl as a Schedule I narcotic. In 2016, the DEA announced butyryl fentanyl and beta-

hydroxythiofentanyl as schedule I narcotics (65). On February 7, 2018, the DEA has emergency scheduled all illicit fentanyl analogues as Schedule I (66).

Table 2: Scheduled Fentanyl Analogues

Compound	DEA Schedule	DEA Number	Date Effective
Fentanyl	II	9801	
3-Methylfentanyl	I	9813	9/22/1986
3-Methylthiofentanyl	I	9833	5/29/1987
Acetyl fentanyl	I	9821	7/17/2015
Acetyl-alpha-methylfentanyl	I	9815	5/29/1987
Acryl Fentanyl	I	9811	7/14/2017
Alfentanil	II	9737	1/23/1987
Alpha-methyl fentanyl	I	9814	9/22/1981
Alpha-methylthiofentanyl	I	9832	5/29/1987
Beta-hydroxy-3-methylfentanyl	I	9831	1/8/1988
Beta-hydroxyfentanyl	I	9836	5/29/1987
Beta-hydroxythiofentanyl	I	9836	5/12/2016
Butyryl fentanyl	I	9822	5/12/2016
Carfentanil	II	9743	10/28/1988
Cyclopropyl fentanyl	I	9845	1/4/2018
Despropionyl Fentanyl (ANPP)	II	8333	
Furanyl fentanyl	I	9834	11/29/2016
Methoxyacetyl fentanyl	I	9825	10/26/2017
Ortho-fluorofentanyl	I	9816	10/26/2017
Para-fluorofentanyl	I	9812	5/29/1987
Para-fluoroisobutyryl Fentanyl	I	9824	5/3/2017
Remifentanil	II	9739	11/5/1996
Sufentanil	II	9740	5/25/1984
Tetrahydrofuranyl fentanyl	I	9843	10/26/2017
Thiafentanil	II	9729	8/26/2016
Thiofentanyl	I	9835	5/29/1987
Emergency Scheduled			
Cyclopentyl fentanyl	I	9847	2/1/2018
Isobutyryl fentanyl	I	9827	2/1/2018
Ocfentanil	I	9838	2/1/2018
Para-chloroisobutyryl fentanyl	I	9826	2/1/2018
Para-fluorobutyryl fentanyl	I	9823	2/1/2018
Para-methoxybutyryl fentanyl	I	9837	2/1/2018
Valeryl Fentanyl	I	9843	2/1/2018

*Temporary Scheduling

1.5 Research Goal

The goal of this research is to utilize instrumentation currently found in forensic laboratories to characterize fentanyl analogues which will help with the identification of specific analogue isomers. Previously, fentanyl derivatives were characterized by GC-MS, LC-MS,

NMR, and IR, however, in 1986, instruments had limited functionality (67). Here, 17 analogues were evaluated with gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high resolution-accurate mass liquid chromatography-tandem mass spectrometry (LC-MS/MS), Fourier transform infrared spectrometry (FTIR) and nuclear magnetic resonance (NMR). These methods are currently being used in the field of forensic science; however, isomers can cause issues for the correct detection of various isomeric forms (68,69). By utilizing all of these methods, the best course of action will be set forth to help facilitate the identification of isomers in forensic laboratories. This study focuses on the identification of fentanyl analogues in pure samples, however, this can be applied to toxicology testing. In a time when our country is facing this epidemic, this research will help law enforcement agencies, hospital personnel and forensic scientists by aiding in the proper identification of fentanyl analogues.

Chapter 2: Analytical Detection of Fentanyl Analogues

2.1 Overview

Analytical methods are widely used in forensic science (70,71). In relation to illicit drugs, research involves fentanyl analogues in biological matrices (72–75). A part of forensic science is drug chemistry, this area of forensic science deals with the identification of substances in non-biological matrices such as powders, capsules, and tablets (76). Identifying substances helps aid in police investigations as well as research purposes. When new illicit drugs enter the market, instrumentation helps identify these unknown substances; by knowing the identification it's possible to locate the manufacture(s), monitor trends in emerging drugs, and announce warnings for public health concerns.

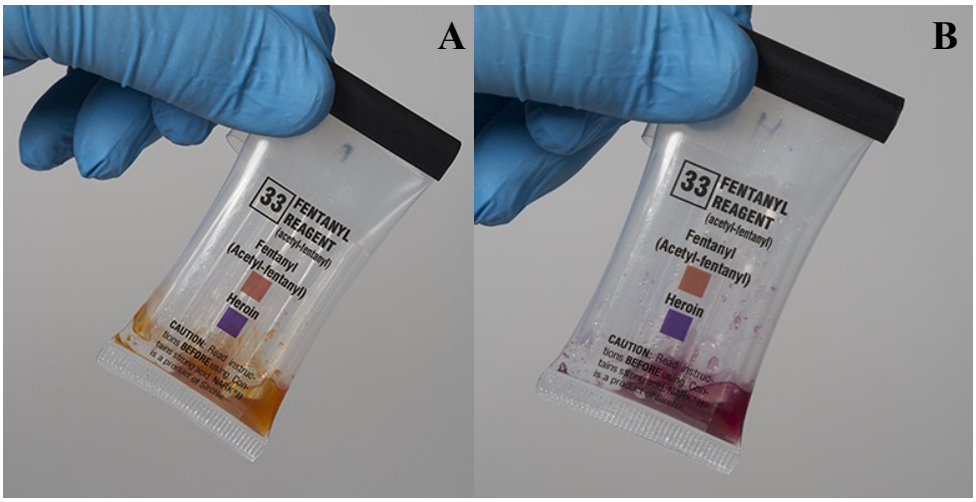

Figure 7: Marquis Field Test Kit (a) positive fentanyl test (b) positive heroin test (77)

Previously, presumptive field test kits could be used in the field to screen unknown compounds. For opioids, the marquis reagent (Figure 7) is used where a purple color is observed for heroin and an orange color appears for fentanyl and its analogues. Color tests are quick, inexpensive, portable, and require little training, making them ideal for use by law enforcement

personnel in the field. However, these tests aren't specific and aren't reliable for the positive identification of a substance (78). For confirmatory measures, instrumentation is used in the laboratory, which includes, but is not limited to gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and infrared spectrometry (IR) (79).

2.2 Gas Chromatography- Mass Spectrometry

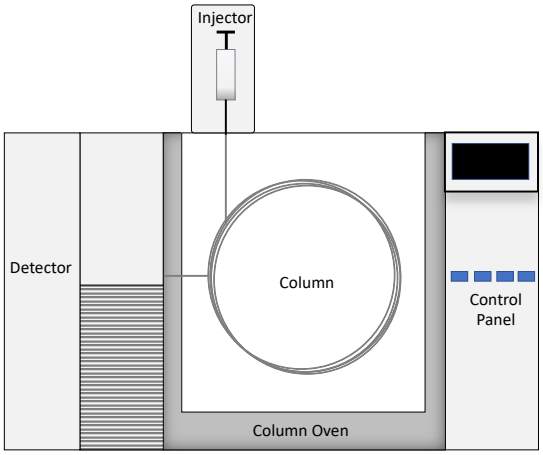

Figure 8: Schematic of GC-MS. Sample is injected into the column which sits in an oven, analytes come off the column at different temperatures then travel to the detector

GC-MS (Figure 8) is one of the most widely used instruments in forensic science utilized for human odor and decomposition products, controlled substances, toxicology, gunshot residue, explosives, fire debris, and trace evidence (80). Gas chromatography was developed in the 1950s as a separation technique for organic and inorganic volatile compounds based on the interaction of gaseous analytes in a gaseous mobile phase, interacting with the stationary phase within a column. Coupled to the gas chromatograph is a mass spectrometer, which is used to detect the analytes. GC-MS has an advantage over other techniques as being relatively inexpensive, easy to use, robust, and superior separation of thermally stable molecules (81,82).

2.3 Liquid Chromatography- Mass Spectrometry

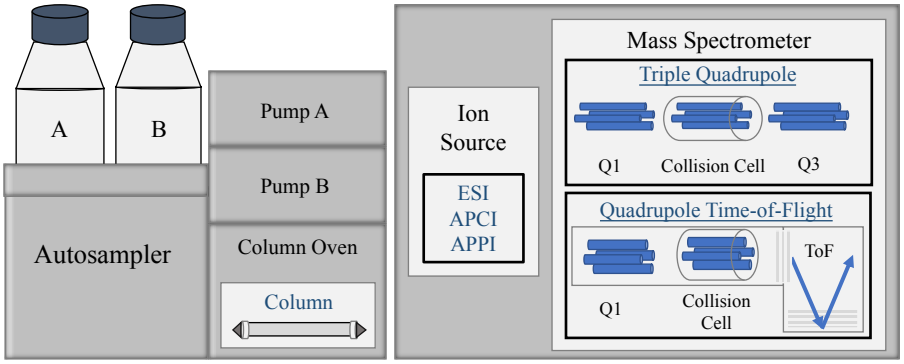

Figure 9: Schematic of LCMS, showing two mass spectrometers: triple quadrupole and quadrupole time-of-flight. Similar to GCMS, analytes come off the column at different gradients then travel to the detector

LC-MS (Figure 9) provides the opportunity for forensic scientist to analyze non-volatile, thermolabile and higher molecular weight analytes. In forensic science LC-MS is being used to detect compounds of chemical warfare, dyes and stains, explosives, toxicological samples, as well as perform high-throughput analysis (83,84). Liquid chromatography is the separation of analytes in a mobile phase interacting with the stationary phase within a column (85). LC is capable of being coupled to a mass spectrometer capable of detection utilizing single-stage MS and well as multi-stage MS/MS known as tandem mass spectrometry. While utilizing an MS detector, reference libraries can be used to accurately identify an unknown compound. In tandem mass spectrometry, the one advantage is the detection of accurate mass; dependent on the mass spectrometer used (86–88). LC-MS has evolved into a more user-friendly versatile instrument capable of specificity and sensitivity (85).

2.4 Fourier Transform Infrared Spectroscopy

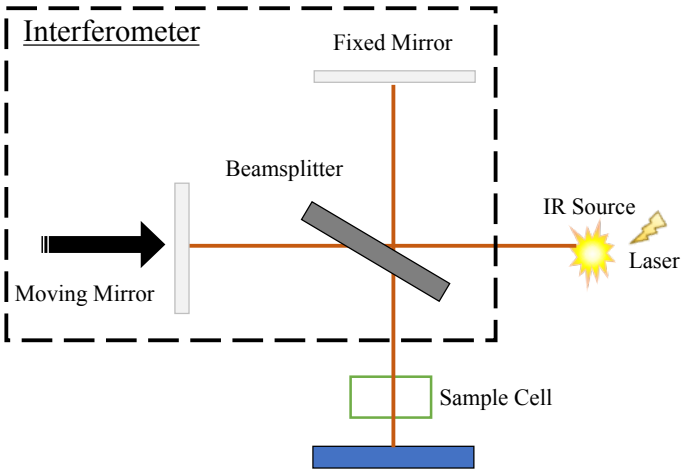

Figure 10: Schematic of FTIR mechanism. Infrared comes from source which passes the sample through the interferometer where the excitation/ relaxation is measured by the detector

Infrared spectroscopy is useful in forensic science because most of the evidence left at a crime scene are composed of organic compounds. IR is useful for paint, ink, sweat, fuels, hair and controlled substances. IR functions under the basis that atoms are made up of bonds which are capable of moving and absorbing energy. When a molecule is exposed to infrared energy functional groups within the molecule will absorb energy at different wavelengths producing a unique spectrum that gives a molecule's composition. Here, this study utilizes Fourier transform infrared spectrometry (FTIR) (Figure 10) where the beam splitter, splits the beam of light into two different paths of light where the interferometer is used to connect the two paths of lights into one. IR is user friendly, requires a small amount of sample, and is capable of being portable-making it ideal for analysis in the field (70).

2.5 Nuclear Magnetic Resonance

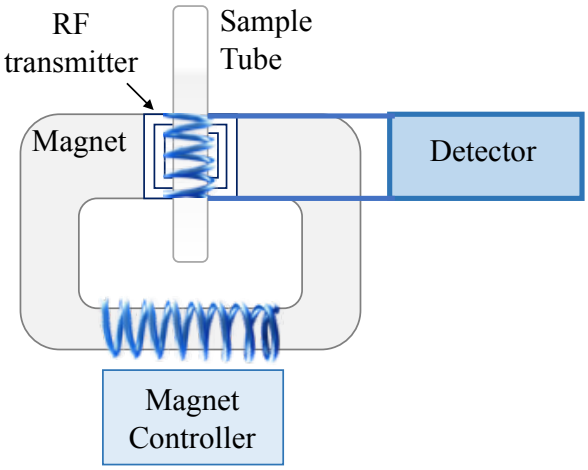

Figure 11: NMR mechanism. The sample is placed into the spinner which is held in a magnet field and hit with radio waves to encourage signal detection

The main use of NMR (Figure 11) in forensic science is the detection of controlled substances. NMR can be very useful in the identification of precursors and intermediates used to illicitly produce the substance. NMR is a technique that applies a magnetic field to nuclei that have spin, an energy transfer takes place at a wavelength that corresponds to radio frequencies. The spin is excited to the higher energy level during this transfer, when the spin returns to its base level, energy is emitted at the same frequency; this produces the NMR spectrum. NMR has the advantage over other instrumental methods by giving a specific fingerprint useful for structure elucidation. NMR is capable of identifying isomers which are more difficult with GC-MS and IR. The downside to NMR is the sample size; generally, 5-10 milligrams, which forensic scientists don't have when working with trace quantities or street samples, however, NMR can be invaluable at clandestine laboratory seizures. The instruments are very large, expensive, and contain high-power magnets which may cause issues for some users. (70)

2.6 Applications of Instrumentation

Little has been reported about the identification of fentanyl analogues as pure substances. More commonly, fentanyl analogue research concerns the detection of the substance and its metabolites in biological matrices as well as the pharmacodynamics and pharmacokinetic properties. In 1986, capillary GC-MS was used to detect fentanyl related compounds (89). In 1989, monomethylated fentanyl isomers were separated by GC/FTIR (90). In 2003, seized capsules and tablets containing para-fluorofentanyl were analyzed utilizing HPLC/UV (91). In 2012, capillary electrophoresis tandem mass spectrometry was used to detect trace levels of forensic derivatives (87) additionally, UHPLC-MS/MS was used to profile illicit fentanyl (92). In 2017, publications were focused on the identification of new fentanyl analogues that were found in seized evidence. Lui et al. was the first to characterize 2,2'-difluorofentanyl utilizing ultra-high performance LC-QTOF-MS, GC-MS, FTIR and NMR (93). In Poland, 4-fluorobutyrfentanyl was found in a seized e-cigarette, it was identified by GC-MS, HPLC-MS, NMR, FTIR (94). In Denmark, acrylfentanyl was found in seized capsules utilizing GC-MS, QTOF-MS, MALDI-Orbitrap-MS, NMR, and IR (95). Geometric isomers of 3-methylfentanyl were characterized by GC-MS, LC-MS, and NMR (96). In a comprehensive review about acryloylfentanyl, everything is discussed concerning the synthesis, pharmacodynamics as well as the analysis of the compound by NMR, FT-IR, GC-MS, QTOF-MS, and MALDI-MS (97). Leonard et al. utilized Raman spectroscopy on trace samples of fentanyl and carfentenil (98). In each of these publications, multiple instruments are used, this is the basis of forensic science and the basis of this research.

Chapter 3: Experimental

3.1 Standards

Fentanyl HCl (item no. 14719)(fentanyl), crotonyl fentanyl (item no. 22801)(crotonyl), cyclopropyl fentanyl (item no. 21739)(cyclopropyl), valeryl fentanyl (item no. 18934)(valeryl), isovaleryl fentanyl (item no. 22990)(isovaleryl), pivaloyl fentanyl (item no. 22991)(pivaloyl), *para*-methyl methoxyacetyl fentanyl (item no. 22979)(para), *meta*-methyl methoxyacetyl fentanyl (item no. 22978)(meta), *ortho*-methyl methoxyacetyl fentanyl (item no. 22977)(ortho), thiophene fentanyl (item no. 22802)(thiophene), furanyl fentanyl (item no. 18705)(furanyl), phenyl fentanyl (item no. 22551)(phenyl), cyclohexyl fentanyl (item no. 22390)(cylohexyl), (±) trans- 3-methyl fentanyl (item no. 9002482)(trans), (±) cis-3-methyl fentanyl (item no. 9002747)(cis), furanyl fentanyl 3-furancarboxamide isomer (item no. 21213)(furanyl isomer), furanyl fentanyl 3-furancarboxamide isomer-D_5 (item no. 21934)(furanyl isomer D_5) were purchased from Cayman Chemical (Ann Harbor, MI). These standards (Figure 12) were selected based on relationship to one another utilizing different moieties at different points along the backbone structure of fentanyl. It was taken into account which analogues have been seen on the street and those that have the potential of being found on the street. Throughout this study the following analogues were paired together for characterization purposes: cis and trans, crotonyl and cyclopropyl, cyclcohexyl and phenyl, furanyl, furanyl isomers and thiophene, para, meta and ortho, as well as valeryl, isovaleryl, and pivaloyl. Fentanyl was used for comparison and method development.

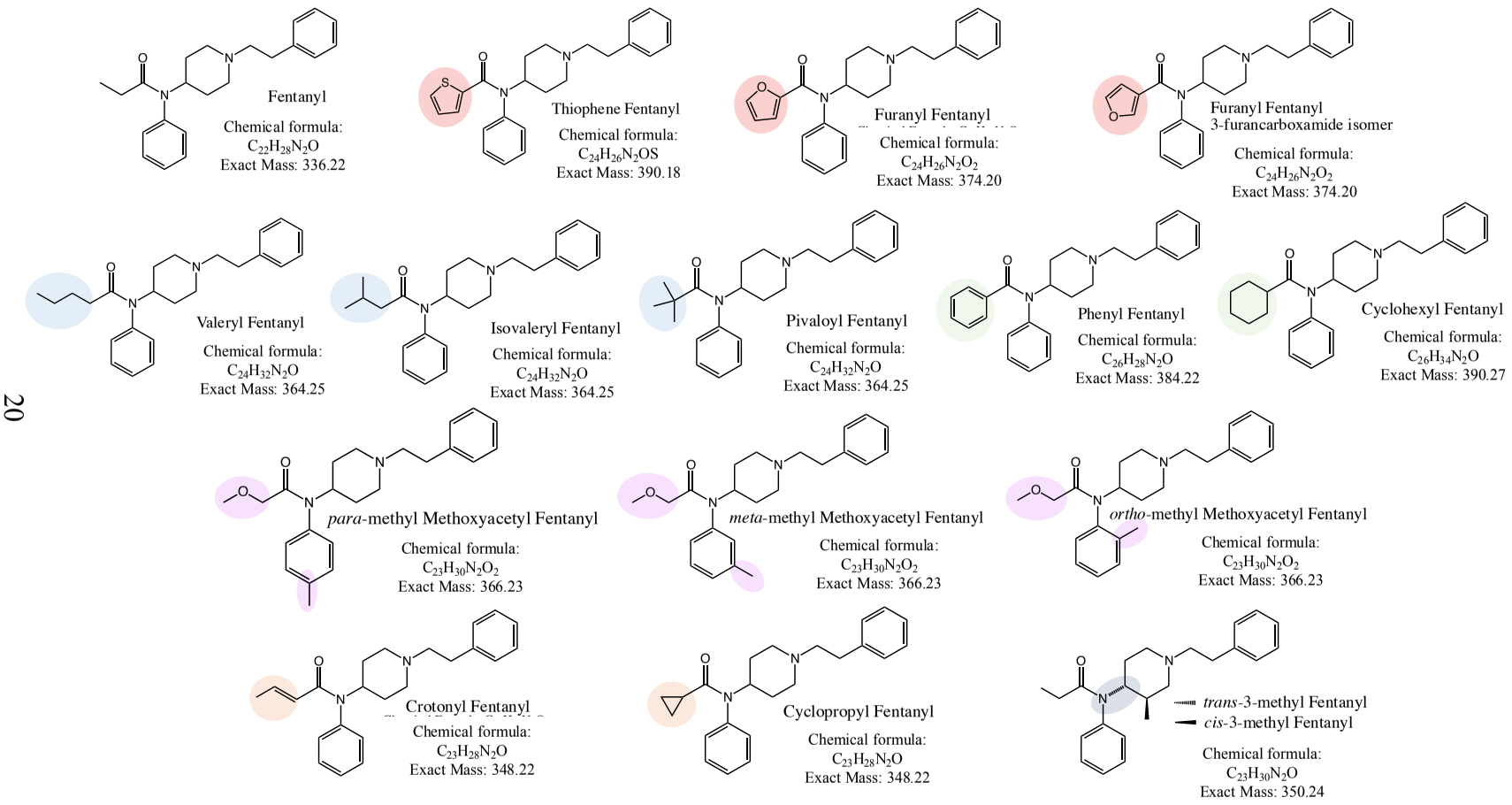

Figure 12: The seventeen standards used in this study

3.2 Gas Chromatography- Mass Spectrometry

Samples of 0.01 μg/μL were prepared in methanol. Standards were analyzed on an Agilent 6890 GC system coupled to an Agilent 5975 XL mass selective detector mass spectrometer. The column was a 15 m x .25 mm x .25 μm Agilent J&W DB-5ms. The oven temperature was programmed from 50 ˚C to 280 ˚C at a rate of 15 ˚C/min for standards: fentanyl, trans, meta, crotonyl, cycopropyl, valeryl, isovaleryl, pivaloyl. For standards: cis, ortho, para, cyclohexyl, phenyl, furanyl, thiophene, furanyl isomer, furanyl isomer d5 a new method was developed ramping the oven from 200 ˚C to 310 ˚C at a rate of 30 ˚C/min. Helium was used as a carrier gas at a linear flow rate of 1.0 mL/min. The mass spectra were obtained by electron-impact ionization under the following conditions: ionization voltage, 70 eV, ion source temperature, 230 ˚C, SCAN mode. Data were processed using Agilent ChemStation software.

3.3 Liquid Chromatography- Triple Quadrupole- Mass Spectrometry

3.3.1 Fentanyl Analogue Screening

All seventeen samples were analyzed on an Agilent 1500 series LC System coupled to an Agilent 6400 series triple quadrupole mass spectrometer. Data acquisition and processing was carried out using Agilent MassHunter QQQ Acquisition and Qualitative Analysis Software. The injection volume was 10 μL. The column was an Agilent Poroshell 120 EC-C18, 2.1 mm x 100 mm x 2.7 μm. The system employed mobile phase A, 0.2% formic acid in water and mobile phase B, 100% methanol with the following gradient: 90% A for 5 min, 0% A for 0.25 min, followed by 90% A hold for 0.1 min. The total run time was 7.5 min. The mass spectrometer was operated in positive ion mode using multiple reaction monitoring (MRM).

3.3.2 Chiral Column Separation

Some samples were analyzed on an Agilent 1500 Series LC System coupled to an Agilent 6400 series triple quadrupole mass spectrometer. Data acquisition and processing was developed by Agilent MassHunter QQQ Acquisition and Qualitative Analysis Software. The injection volume was 10 μL. The column was a Supelco Astec Chirobiotic V, 15 cm x 2.1 mm, 5μm. The system employed an isocratic gradient with methanol, 4% acetic acid, and 0.5% ammonium hydroxide solvent. The total run time was 20 min. The mass spectrometer was operated in positive ion mode using multiple reaction monitoring (MRM).

3.4 Liquid Chromatography- Quadrupole Time-of-Flight- Mass Spectrometry

Samples were analyzed on a Waters Alliance 2695 Separation Module coupled to a Waters Synapt G1 quadrupole time of-flight mass spectrometer. The separation was performed on YMC AQ 2 mm x 100 mm x 3 μm column. Mobile phase A consisted of 0.1% formic acid, mobile phase B consisted of acetonitrile. The following gradient was used: 85% A at time 0 ramped to 15% A at 8 minutes, ramped to 85% at 10 minutes. The flow rate was 0.2 mL/min. The injection volume was 2 μl, operating in positive ion v-mode. The desolvation gas flow was 300 L/hr and the cone gas flow was 20 L/hr. The nitrogen desolvation temperature was 220 ˚C while the source temperature was 100 ˚C. The capillary voltage of 3.6kV was used. Collision energy was 45eV. Data were processed using Waters MassLynx.

3.5 Fourier Transform Infrared Spectrometry

Samples were analyzed on a Thermo Scientific Nicolet 6700 Fourier Transform infrared spectrometer equipped with Smart iTR, an ultra-high performance attenuated total reflectance (ATR) diamond surface unit. The samples were placed onto the diamond crystal, the tip of the micrometer clamp was compressed onto the surface to allow adequate contact to block out interference light. A background blank was run prior to each sample. The background and sample were processed with 50 scans each and a resolution of 4 cm^{-1} to acquire the spectra. Data was acquired and processed using Thermo Electron OMINIC software.

3.6 Nuclear Magnetic Resonance

Samples were dried down using an Eppendorf Vacufuge Plus, samples were placed in test tubes and allowed to run for 3.5 hours to drive off the methanol present. A Schlenk line was used for 30 minutes as another measure to ensure all of the methanol was driven off. One dimensional NMR spectra (^{1}H NMR, ^{13}C NMR) were recorded on a Bruker 400 Hz NMR system. Proton NMR utilized 32 scans, while Carbon NMR utilized 12,000 scans. Valeryl, isovaleryl, and pivaloyl were dissolved in $CDCl_3$ and tetramethylsilane was used as the chemical shifts reference standard. The data were acquired using Bruker TopSpin and processed using Mestrenova software.

Chapter 4: Results

4.1 Gas Chromatography- Mass Spectrometry

All standards were analyzed and characterized utilizing a generic fentanyl method. Fentanyl, trans, meta, crotonyl, cyclopropyl, valery, isovaleryl, and pivaloyl were capable of utilizing this method, the other 9 standards displayed carry over. An optimized method was developed to identify the following 9 standards (cis, ortho, para, cyclohexyl, phenyl, furanyl, thiophene, furanyl isomers). Table 3, demonstrates the precursor ion and product ion ratios used to identify these standards. It is seen that there are differences in ratio abundances of the product ions within the various standard pairs, confirming the use of GC-MS to identify isomeric fentanyl analogues. Figure 13, displays the mass spectra of valeryl, isovalery, and pivaloyl. At first glance it would appear that the three samples are the same, showing similar fragments of *m/*z 273.1, 189.1, 146.1, 105.1, 57.1, however, examining the ratios of product ions relative to the most abundant precursor ion (*m/z* 273.1), it is seen that there are slight differences in the ratios, indicating different samples. Most notably, valeryl does not have fragment *m/z* 57.1 while pivaloyl has the highest abundance (73%) and isovaleryl has a minor abundance (16%). Para and meta have the same fragments, with similar ratios of product ratios, in this case, it would be best to utilize another instrument to differentiate between them. The same is true for crotonyl and cyclopropyl where *m/z* 69 is the most indicative difference between the two samples.

Table 3: GC-MS Data								
Compound	Fragment Ions							
	m/z	Abundance	*m/z*	Abundance	*m/z*	Abundance	*m/z*	Abundance
Fentanyl HCl	245.2	100	146.1	51	189.1	35	105.1	14
cis-3-Methyl Fentanyl	259.2	100	160.1	46	203.1	36	105.1	18
trans-3-Methyl Fentanyl	259.2	100	160.1	19	105.1	10	203.1	8
Valeryl Fentanyl	273.2	100	146.1	46	189.1	39	105.1	15
Isovaleryl Fentanyl	273.2	100	146.1	43	189.1	42	57.1	16
Pivaloyl Fentanyl	273.2	100	57.1	73	146.1	24	105.1	19
para-Methyl Methoxyacetyl Fentanyl	275.2	100	172.1	26	232.1	24	105.1	21
meta-Methyl Methoxyacetyl Fentanyl	275.2	100	172.1	23	232.1	20	105.1	15
ortho-Methyl Methoxyacetyl Fentanyl	275.2	100	232.1	29	172.1	26	105.1	20
Crotonyl Fentanyl	257.2	100	189.1	47	146.1	40	69.1	37
Cyclopropyl Fentanyl	257.2	100	189.1	46	146.1	40	69.0	22
Phenyl Fentanyl	105.0	100	293.2	76	77.1	30	250.1	13
Cyclohexyl Fentanyl	299.2	100	189.1	41	83.1	34	146.1	27
Thiophene Fentanyl	111.1	100	299.1	82	256.1	28	105.0	12
Furanyl Fentanyl	283.2	100	95.0	65	240.1	48	158.1	15
Furanyl Fentanyl 3-furancarboxamide	283.2	100	95.0	99	240.1	50	105.1	14
Furanyl Fentanyl 3-furancarboxamide D5	288.2	100	92.0	95	245.1	49	105.1	11

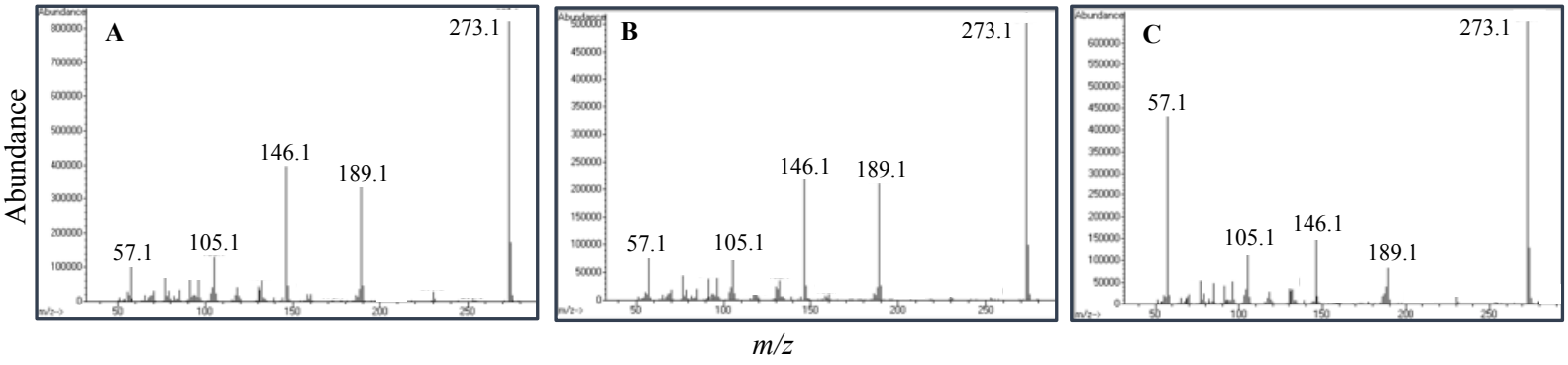

Figure 13: GC-MS spectra of (a) valeryl (b) isovaleryl (c) pivaloyl

4.2 Liquid Chromatography- Triple Quadrupole- Mass Spectrometry

All standards utilized a generic fentanyl method. The retention times, precursor and product ions were recorded (Table 4). Furanyl and thiophene, phenyl and cyclohexyl, as well as the furanyl isomers were easily separated. As expected, positional and geometric isomers were either poorly resolved or exhibited the same retention time, these

Table 4: Fentanyl Screening							
Compound	RT (min)	Precursor Ion	Product Ion 1	CE 1 (V)	Product Ion 2	CE 2 (V)	Fragment (V)
Fentanyl	2.64	337.2	188.1	24	105.1	44	145
cis-3-methyl Fentanyl	2.83	351.2	202.1	24	105.1	44	160
trans-3-methyl Fentanyl	2.81	351.2	202.2	24	105.1	44	140
Valeryl Fentanyl	3.07	365.3	188.1	24	105.1	48	155
Isovaleryl Fentanyl	3.08	365.3	188.1	20	105.1	48	140
Pivaloyl Fentanyl	3.07	365.3	188.1	24	105.1	48	155
para-Methyl Methoxyacetyl Fentanyl	2.65	367.2	188.1	24	105.1	48	150
meta-Methyl Methoxyacetyl Fentanyl	2.65	367.2	188.1	24	105.1	44	150
ortho-Methyl Methoxyacetyl Fentanyl	2.65	367.2	188.1	24	105.1	48	145
Crotonyl Fentanyl	2.78	349.2	188.1	24	105.1	44	145
Cyclopropyl Fentanyl	2.78	349.2	188.1	24	105.1	48	155
Phenyl Fentanyl	2.94	385.2	188.1	24	105.1	44	145
Cyclohexyl Fentanyl	3.26	391.3	188.1	28	83.1	36	165
Thiophene Fentanyl	2.94	391.2	188.1	24	105.1	48	150
Furanyl Fentanyl	2.75	375.2	188.1	24	105.1	48	150
Furanyl Fentanyl 3-furancarboxamide	2.83	375.2	188.1	24	105.1	48	150
Furanyl Fentanyl 3-furancarboxamide D5	3.08	380.2	188.1	24	105.1	48	160

samples were resolved on a chiral column (Table 5). The furanyl isomers displayed enough separation to not further utilize the chiral column. Although these compounds do not contain chiral centers, a chiral column was selected because it was available in the laboratory. Figure 14 shows the separation of valeryl (9.5 min), isovaleryl (9.7 min), and pivaloyl (8.6 min) utilizing the chiral column, here it is seen that there are differences in retention times.

Table 5: Chiral Column Separation	
Compound	RT (min)
cis-3-methyl Fentanyl	8.2
trans-3-methyl Fentanyl	6.7
Valeryl Fentanyl	9.5
Isovaleryl Fentanyl	9.7
Pivaloyl Fentanyl	8.6
para-methyl Fentanyl	12.6
meta-methyl Fentanyl	12.9
ortho-methyl Fentanyl	15.3
Crotonyl Fentanyl	11.5
Cyclopropyl Fentanyl	10.1

Table 6: Limit of Detection		
Compound	LOD	linear range
Fentanyl	100 fg	1ng - 1 fg
Furanyl Fentanyl	100 fg	1ng - 1 fg
cis-3-methyl fentanyl	10 fg	1ng - 1 fg

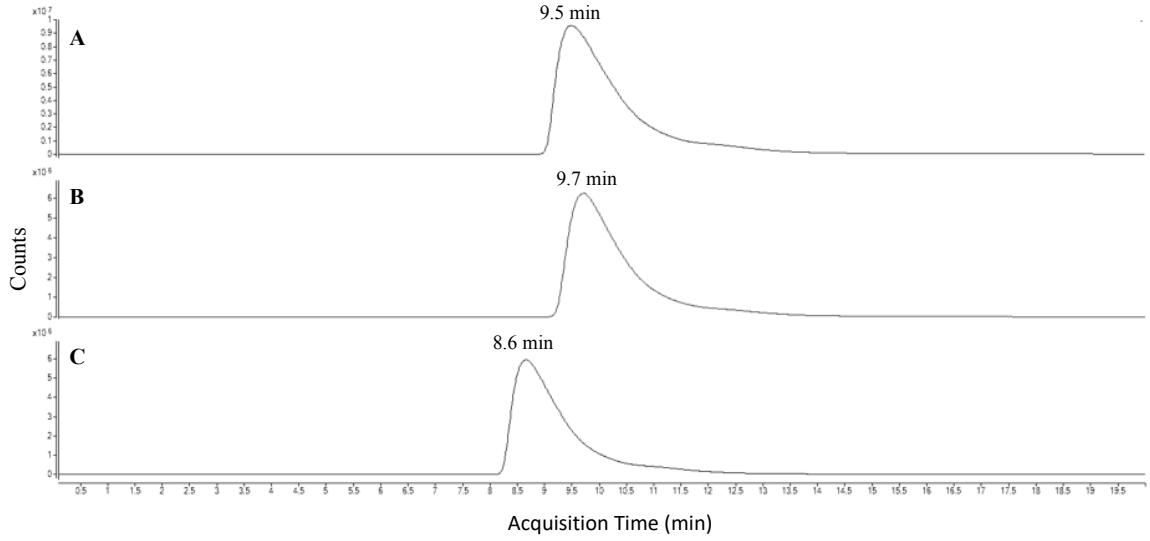

Figure 14: LC-MS chiral separation of (a) valeryl (b) isovaleryl (c) pivaloyl

Limit of detection (LOD) was determined for fentanyl, furanyl, and cis (Table 6). Only three standards were chosen because all analogues, having the same core system, would exhibit similar LODs, determined by their similar ionization. These three standards were chosen because they are being found on the street. Fentanyl's LOD is 100 fg, furanyl is 100 fg, and cis is 10 fg on column. As seen in the literature the level of detection is much lower that what has been seen in toxicological samples. Therefore, this method of detection is sensitive enough to detect fentanyl analogues in toxicological samples while easily differentiating between isomers (75,99).

4.3 Liquid Chromatography- Quadrupole Time-of-Flight- Mass Spectrometry

High resolution accurate mass measurement was taken on all standards this provided structure characterization. First, single MS acquisition was taken which provided the protonated molecule ($[M + H]^+$) then tandem MS/MS was acquired providing the fragments shown in Table 7. Figure 15 displays the spectra for valeryl, isovalerly, and pivaloyl; each sample has fragments of *m/z* 105, 188, 132, 146, and 117. Focusing on the *m/z* 115-120, you can see there is a

difference in the abundance of this minor fragments which could be used to help identify which isomer is present. Valeryl has a more abundant *m/*z 117 with a less abundant *m/*z 120, isovaleryl has *m/*z 117 and *m/*z 120 with similar abundances, and pivaloyl has *m/*z 117 and *m/*z 120 present in noise.

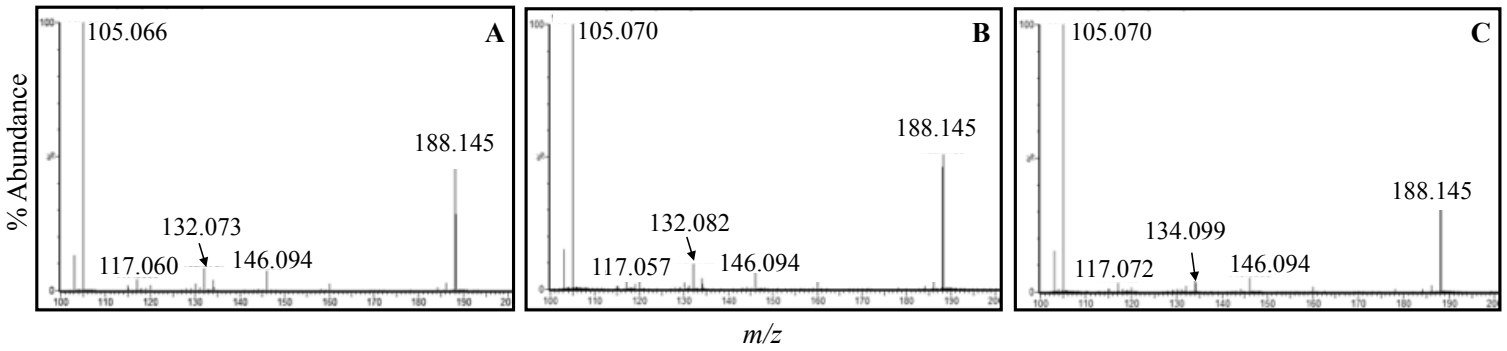

Figure 15: LC-QTOF-MS mass spectra (a) valeryl (b) isovaleryl (c) pivaloyl

Table 7: LC-QTOF-MS data

Compound	Product ion	Fragment ions				
Fentanyl	337.22	105.07	188.15	132.08	146.09	117.05
cis-3-methyl Fentanyl	351.22	105.07	202.15	132.07	146.09	117.06
trans-3-methyl Fentanyl	351.24	105.07	202.16	132.08	146.10	117.07
Valeryl Fentanyl	365.22	105.07	188.14	132.07	146.09	117.06
Isovaleryl Fentanyl	365.25	105.07	188.14	132.08	146.09	160.11
Pivaloyl Fentanyl	365.25	105.07	188.14	146.09	134.09	117.07
para-Methyl Methoxyacetyl Fentanyl	367.22	105.07	188.14	146.09	120.08	134.09
meta-Methyl Methoxyacetyl Fentanyl	367.23	105.07	188.14	146.09	120.08	
ortho-Methyl Methoxyacetyl Fentanyl	367.23	105.07	188.14	146.09	117.06	
Crotonyl Fentanyl	349.22	105.07	188.14	132.08	146.09	117.06
Cyclopropyl Fentanyl	349.22	105.06	188.14	132.08	146.09	117.06
Phenyl Fentanyl	385.21	105.06	188.14	146.09	180.08	
Cyclohexyl Fentanyl	391.25	105.06	188.14	146.09	134.09	160.10
Thiophene Fentanyl	391.18	105.07	188.14	110.99	149.02	134.10
Furanyl Fentanyl	375.20	105.07	188.14	146.09		
Furanyl Fentanyl 3-furancarboxamide	375.21	105.07	188.15	146.09	134.09	170.06
Furanyl Fentanyl 3-furancarboxamide D5	380.24	105.07	188.15	146.10	134.09	

Fentanyl analogues have the same core system with various substituents, the most common are found on the phenyl rings, the piperdine ring, or as an R group on the amide. The proposed molecules for the most common fragments are seen in Figure 16. Liu et al. proposed the fentanyl analogue fragmentation pathway seen in Figure 17. Out of the standards analyzed, they all displayed similar fragments of *m/z* 105, 188, 132, 146 with the noticeable exception of fragment *m/z* 202 from cis and trans. The fragment of 188 is from the cleavage between the piperdine ring and the *N*-phenyl-butanamide moiety while the fragment of 105 is a result of cleavage between the piperidine ring and the phenethyl moiety. Degradation of the piperdine ring results in the fragments of *m/z* 132, 134, and 146. 202 is from the addition of a methyl group on the piperdine ring which follows the same cleavage as 188.

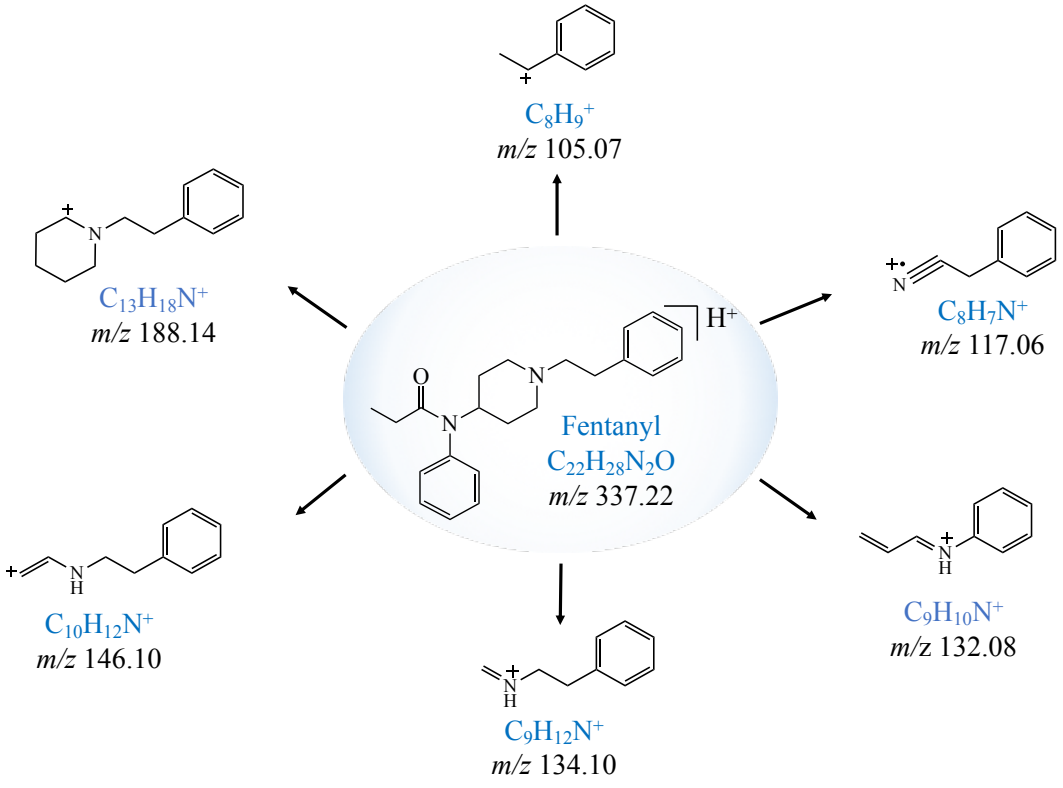

Figure 16: Common fragments of fentanyl analogues (93)

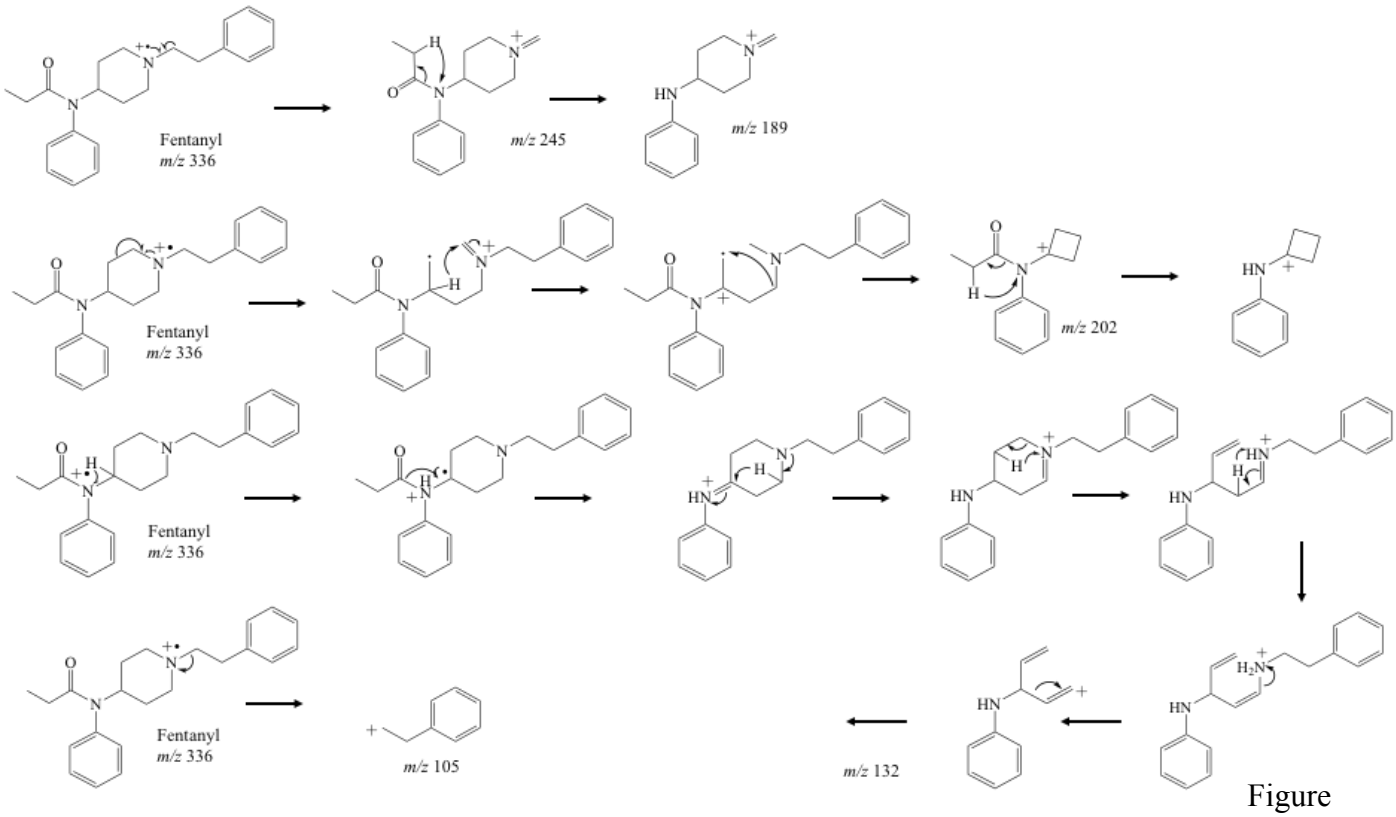

Figure 17: Proposed Fentanyl fragmentation pathway (93)

4.4 Fourier Transform Infrared Spectrometry

IR spectra were obtained for all standards (Figure 18) in the 500-4000 cm^{-1} region. For fentanyl (Figure 18a) the most prominent peak at 1650 is attributed to the amide. Broad peak around 3000, the pair of three peaks between 1600- 1475 and the strong peak at 700 cm^{-1} are attributed to the phenyl groups. Within the fingerprint regions: two peaks at 1400 and 1390 cm^{-1} are attributed to the CH_3 group while the peaks at 1270 and 1080 cm^{-1} are attributed to C-N stretch. For the remaining analogues, the noticeable differences between pairs are highlighted. Cis and trans (Figure 18b) displayed the least amount of difference, which is expected for geometric isomers. Crotonyl and cyclopropyl (Figure 18c) displayed an extra peak around 1600 cm^{-1} this is attributed to the alkene. The cyclohexyl group displays a peak at 2900 cm^{-1} and

phenyl displays a strong peak at 700 cm^{-1} for the three phenyl groups (Figure 18d). Furanyl (Figure 18e) has three peaks at 1600 cm^{-1}, this is indicative of fentanyl, also seen in the furanyl isomers (Figure 18f). In the deuterated isomer in addition to there being less hydrogen, the deuterium is heavier than hydrogen and causes the attached molecule to have a weaker absorbance, this is seen in the relative spectra. Isovalerly, valeryl, and pivaloyl displays peak differences in the 2900 and 700 regions, due to the differences in the terminal CH groups (Figure 18g). For meta, ortho, para (Figure 18h) the distinguishable peaks around 800-700 cm^{-1} are not as pronounced in these spectra, this is due to the meta director C-N bond. Generally, disubstituted benzene rings favor para and ortho position, with meta being less stable; since CN is meta directing, all three phases are unstable/ less stable causing the weak signal. Although, the spectra are similar, each standard is easily differentiated between one another; this holds true for the geometric isomers which are the most similar, potentially causing issues in detection.

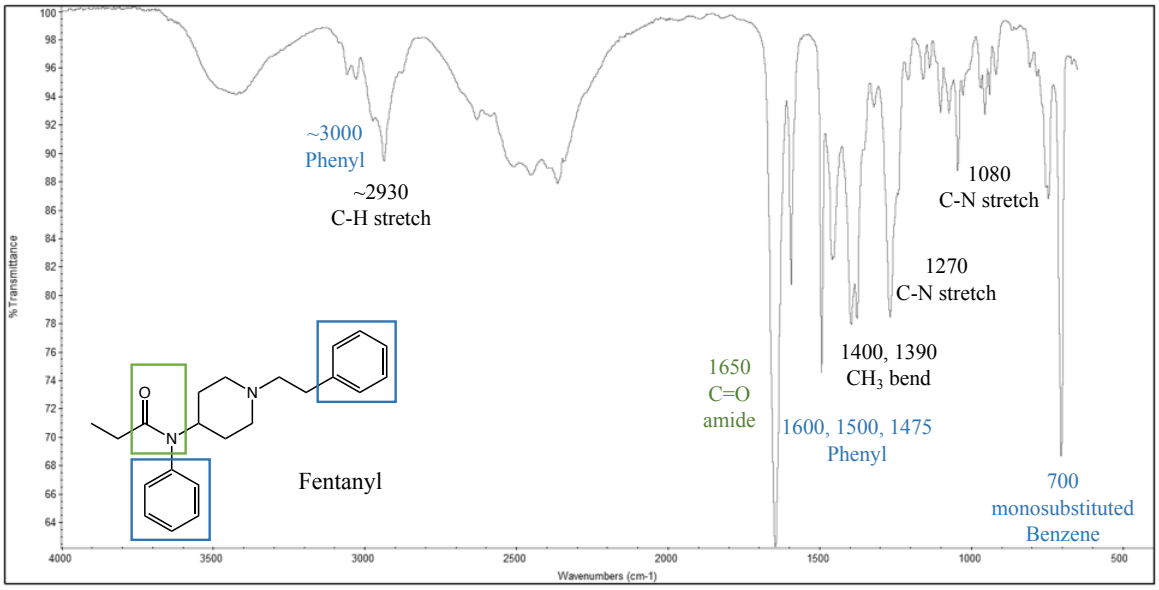

Figure 18a: FTIR spectrum of fentanyl

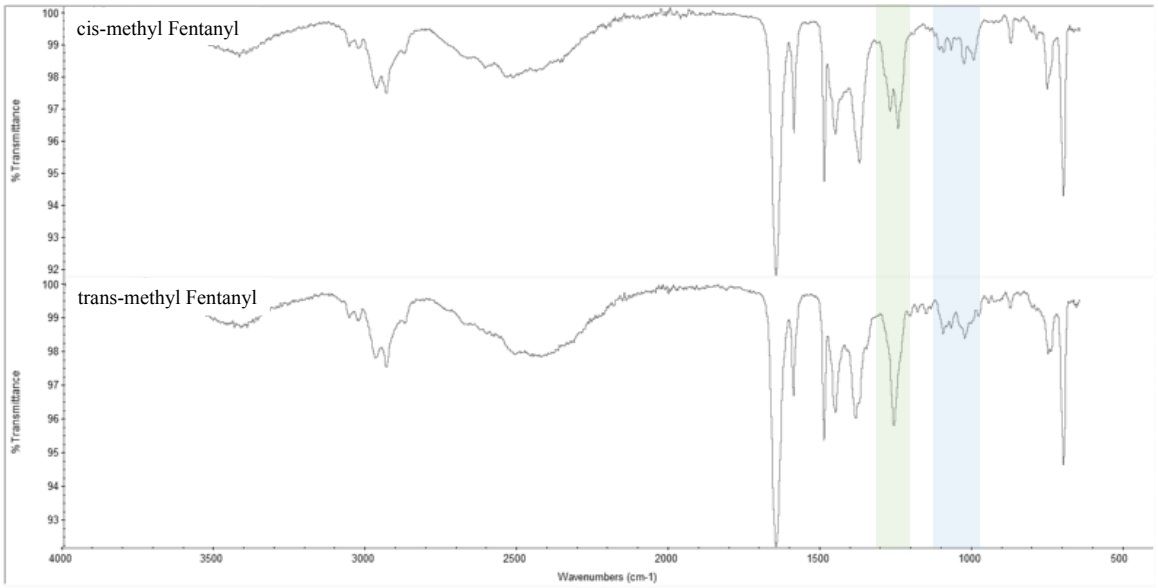

Figure 18b: FTIR spectra, cis and trans

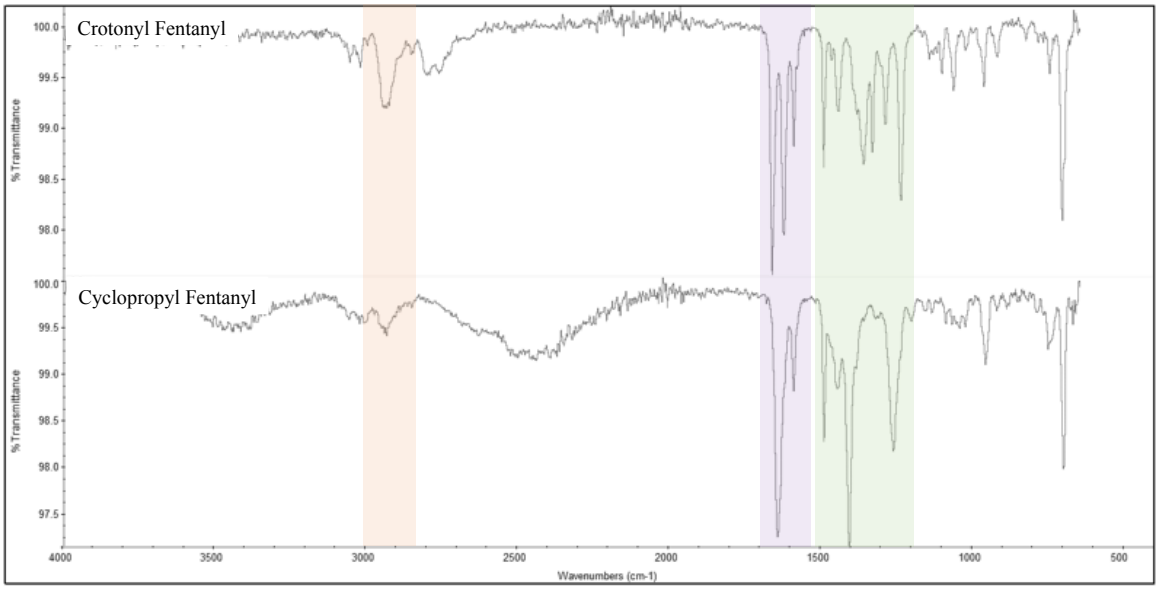

Figure 18c: FTIR spectra, crotonyl and cyclopropyl

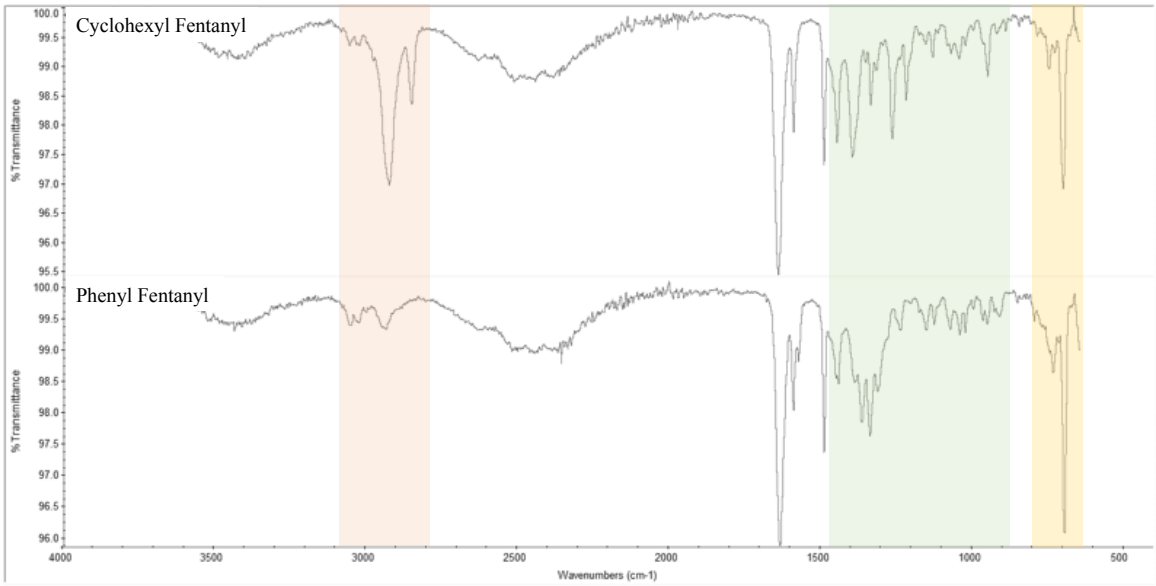

Figure 18d: FTIR spectra, cyclohexyl and phenyl

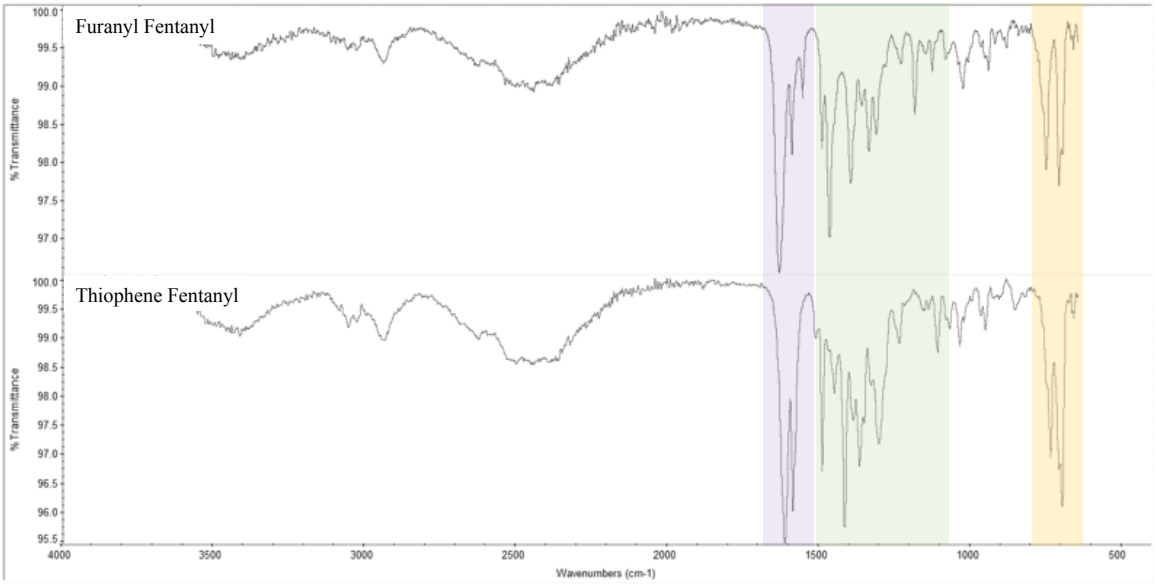

Figure 18e: FTIR spectra, furanyl and thiophene

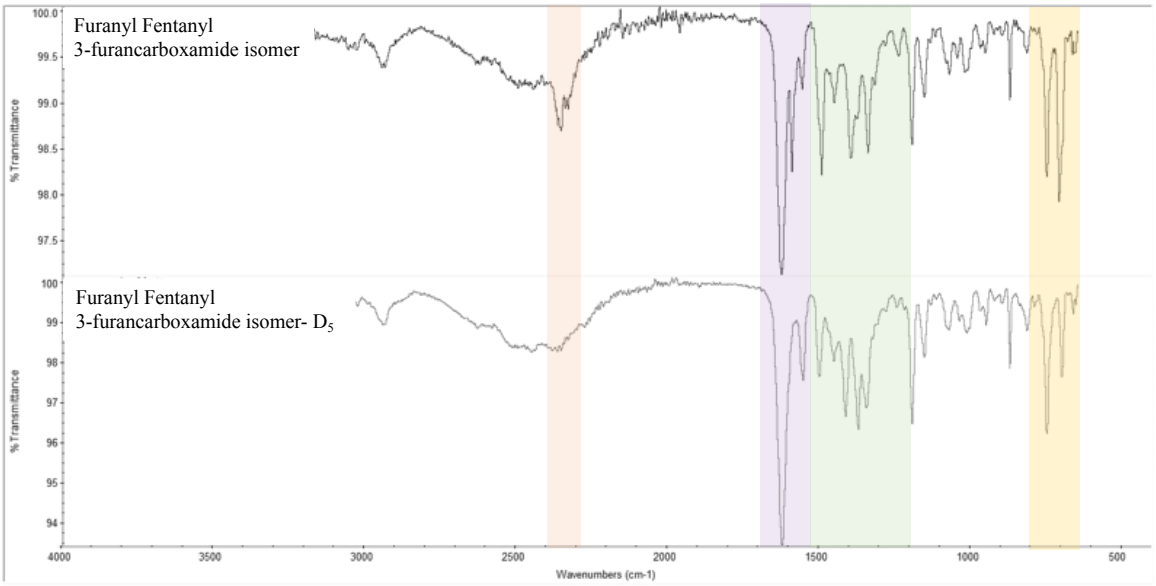

Figure 18f: FTIR spectra, furanyl isomers

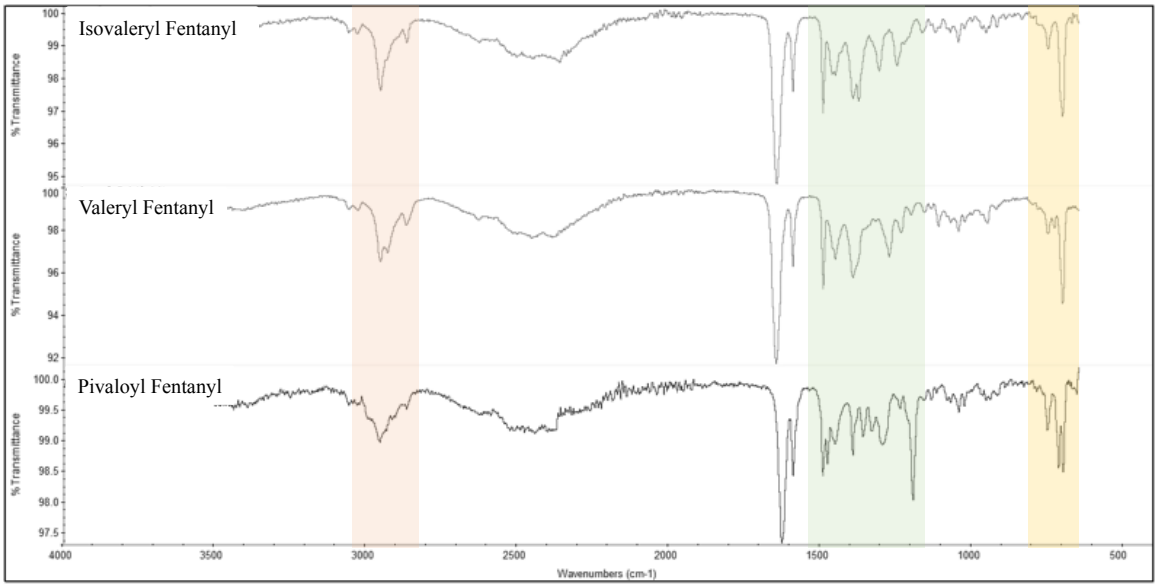

Figure 18g: FTIR spectra, isovaleryl, valeryl, and pivaloyl

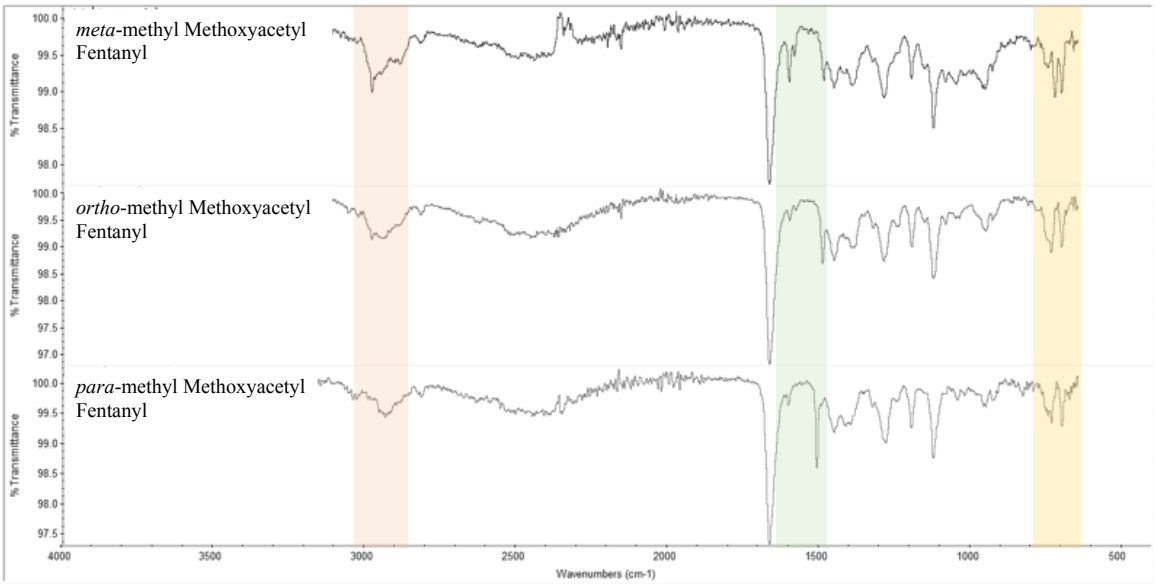

Figure 18h: FTIR spectra, meta, ortho, and para

4.5 Nuclear Magnetic Resonance

Proton and carbon NMR were obtained for valeryl, isovaleryl, and pivaloyl. The samples were dilute (~1 mg) therefore the peaks were overpowered by the solvent peak. The proton spectra (Figure 19) displayed a strong enough signal to view the differences in chemical shifts of each sample as well as splitting in valeryl and isovaleryl, however, the carbon spectra (Figure 21) was more challenging to acquire. Due to the dilute sample, the carbon spectra were not accurately acquired; carbons are missing and doesn't provide much information towards the identification of the compounds. However, proton NMR was able to give spectra suitable for interpretation.

Generally, in proton NMR you will see shift changes further down field for aldehyde and ketones, while further up field are the alkyl chains. In the proton NMR, there is a large solvent peak that interferes with the aromatic hydrogens (7-8ppm). The signal at 2-3 ppm corresponds to the piperdine ring where the nitrogen has an effect on

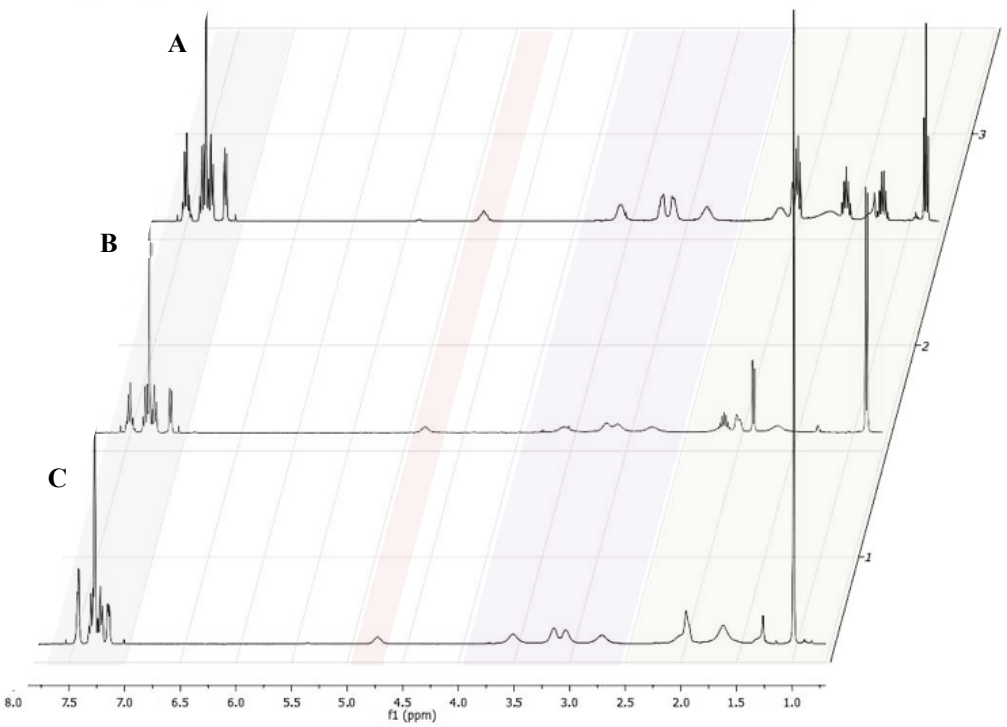

Figure 19: ^{1}H NMR spectra overlay of (a) valeryl (b) isovaleryl (c) pivaloyl. Aromatic region (7-8 ppm), piperdine region (2.5- 4 ppm), aliphatic region (0.5 – 2.5 ppm)

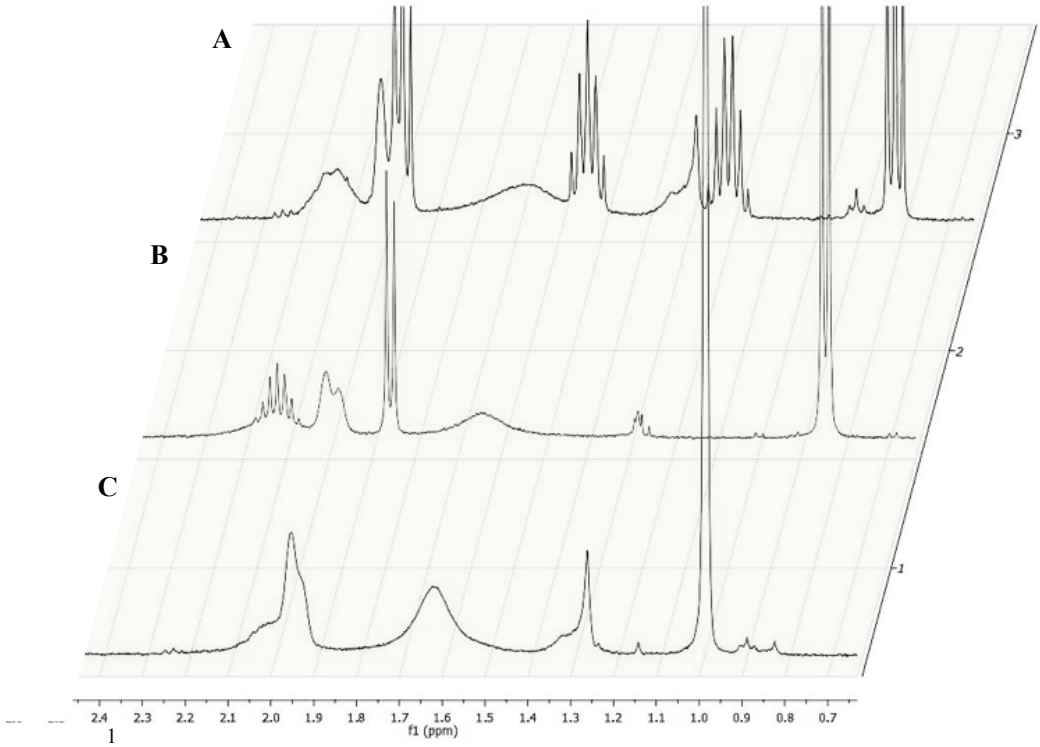

Figure 20: ^{1}H NMR spectra overlay of (a) valeryl (b) isovaleryl (c) pivaloyl in the aliphatic region (0.7-2.5 ppm)

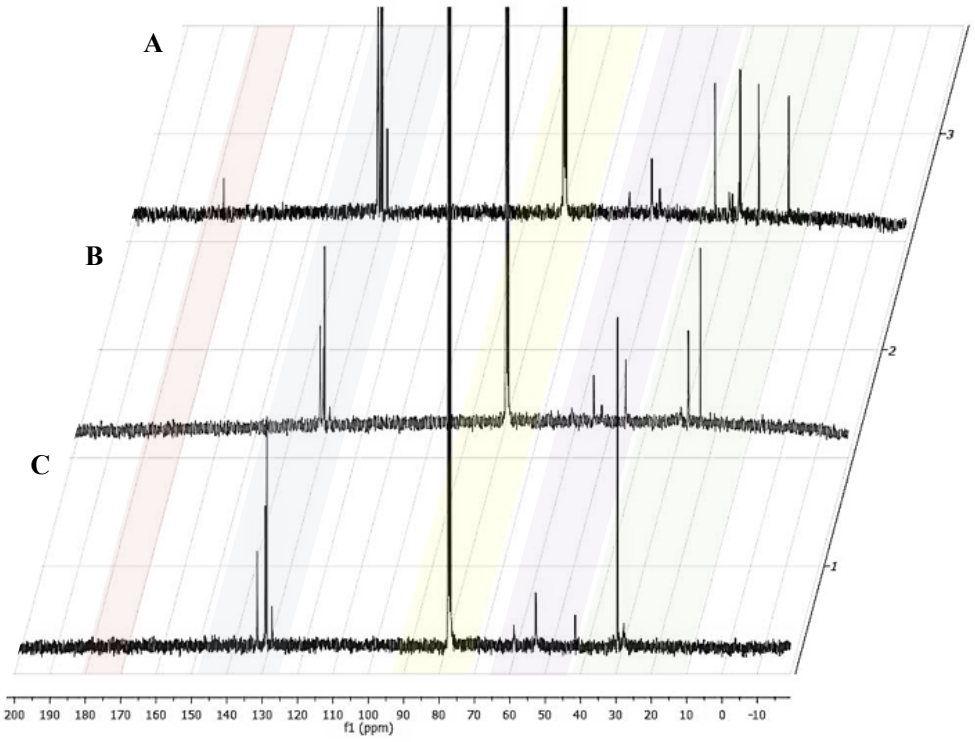

Figure 21: ^{13}C NMR Spectra of (a) valeryl (b) isovaleryl (c) pivaloyl amide region (170-180 ppm), aromatic region (125-140 ppm), solvent peak (77ppm), piperdine region (50-65ppm), aliphatic region (0-40ppm)

the hydrogen pulling them further down field. From 0 – 2.5 ppm there is the alkyl region. Figure 20 focuses on this region, there are differences between the three spectra, this is the main point of this analysis. The aliphatic region is observing the alkyl terminal region where the analogue varies between molecules. The spectra from 2.5- 14 ppm are expected to be the same since the fentanyl core is found in each of the molecules.

The carbon spectra were poorly acquired. The solvent peak (77 ppm) is highly abundant in all of the spectra, overpowering the carbon peaks found in these dilute samples. However, the carbonyl group (170-180 ppm) appears in valeryl spectra, while the other two samples are missing this. The aromatic region (125-140 ppm) are seen in each of the spectra. The piperdine carbons would be found in the 50- 65 ppm region while the aliphatic region is 0-40 ppm. While

looking at geometric isomers such as valeryl, isovaleryl, and pivaloyl, the carbon spectra will carry less importance over the hydrogen spectra. Generally, carbon is used to determine how many carbons are in the sample, of which, these samples have the same number of carbons. The hydrogens are more effected by their spatial relationship to one another and provide more information leading to the identification of isomers.

Chapter 5: Discussion and Conclusion

5.1 Summary of Results

All of the 17 standards were successfully analyzed with GC-MS, LC-MS, and FTIR. Valeryl, isovaleryl, pivaloyl fentanyl were further analyzed on NMR.

During the GC-MS analysis, increasing the initial temperature solved the issue of carryover, which was originally seen in our first run. Additionally, it shortened our overall run time which is always favorable.

LC-triple quadrupole was used to separate these standards. Separation of non-isomeric standards were achieved while isomers gave similar retention times. These compounds do not contain chiral carbons however, a chiral column was available in the laboratory therefore it was used to separate these isomers. Chiral columns are generally used for the separation of chiral molecules but work well with other isomers. Without the use of a chiral column the samples could have been separated by changing the solvent, changing the gradient, changing the pH of the buffer solution, or using modifiers. Additionally, UHPLC offers higher efficiency due to the smaller particles used in the column packing, this allows better separation of similar compounds and is recommended for use with positional isomers.

During the LC-QTOF study, different collision energies (30, 45, 60 eV) were used to determine which energy gave the most valuable fragments. It was determined that 30 eV only gave the major fragments, 60 eV gave too many minor fragments, while 45 eV gave major and minor fragments. Two different mass spectrometers were used, they both have their strengths and weaknesses, more notably QqQ has lower limits of detection, ideal for trace quantities and QTOF is more selective providing more fragmentation, which is beneficial when observing analogues.

During the FTIR study, initially a dilute sample (~1 ng) was attempted, the spectrum that was acquired that didn't show peaks indicative of IR spectra. It wasn't until a more concentrated sample (~100ng) was used, that the proper spectrum was acquired. Solid samples or concentrated samples in solution could have been used to give even better spectra, however, the characteristic differences between isomers within a pair were seen and therefore, a more concentrated sample was not used. Another way of producing better spectra would be increasing the number of scans, similar to NMR.

The NMR study utilized deuterated chloroform ($CDCl_3$) as the solvent. The most common solvents used are $CDCl_3$ and deuterated dimethyl sulfoxide (DMSO-d_6), both of these solvents interfered with peaks that would be seen on the spectra. $CDCl_3$ was used because it's interference is with the phenyl groups which all of the fentanyl analogues have in common. Acetone-d_6 was another option for the solvent and wouldn't display any interference with our spectra, however, acetone-d_6 is not commonly used in NMR studies, therefore the spectra that would have been acquired would seem irrelevant to other scientists. The sample size was dilute compared to conventional NMR testing, however, this better represents the amount of seized material forensic scientists will see in practice. Most importantly, a proton spectrum was easily acquired and provides information leading to structure characterization while ^{13}C NMR takes more of an effort.

Instrumentation was used extensively in this study. After reviewing all the data obtained from each study it appears that the spectra of fentanyl analogues are easily acquired on all instruments. Positional isomers are better detected with FTIR as a preliminary test while the chiral column best separated the compounds. For geometric isomers GC-MS can be used, however, as a secondary measure it should be followed with use of a chiral column or FTIR for

more accurate data. Finally, analogues that are structurally similar but don't have the same molecular weight are easily separated utilizing GC-MS, LC-MS, LCMS/MS, and FTIR. As previously mentioned, this study focused on pure solid-dose samples, this can also be used in toxicological studies for the identification of isomers in relation to analogues as well as metabolites.

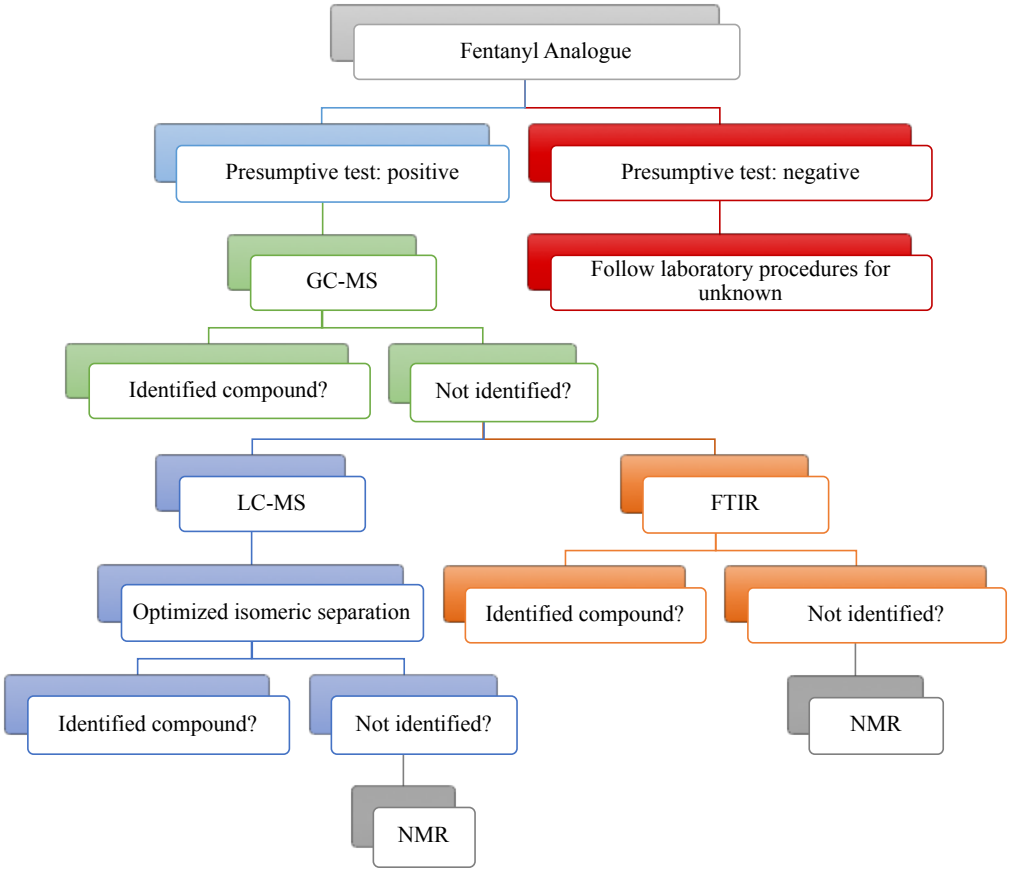

Figure 22: Suggested analytical testing procedure for fentanyl analogues

Forensic science is limited by money, time and sample size. FTIR is the cheapest instrument although it takes training and experience to properly identify compounds. GC-MS is the second cheapest instrument, it is user friendly and robust. LC-MS would be more expensive but useful for isomers and trace samples. NMR is the most expensive instrument and requires proper training. For these reasons, Figure 22 displays a suggested testing procedure for

laboratories analyzing samples with suspected fentanyl analogues. Starting with a presumptive color test, if this is positive then perform GC-MS analysis. GC-MS will be able to identify non-isomers, positional and geometric isomers. Para, meta, and ortho may be challenging; if your compound is identified as one of these, a secondary analysis should be performed. If GC-MS isn't able to identify your compound, either LC-MS or FTIR could be used next. LC-MS should have an optimized isomeric method utilizing special columns, buffers, modifiers or varying gradients to achieve isomeric separation capable of identifying positional and geometric isomers. FTIR is capable of differentiating between all isomers and non-isomers, however, samples that contain mixtures will cause issues therefore LC-MS would be better utilized. If LC-MS and/or FTIR are not capable of identifying the suspected analogue the questioned sample may be new to the illicit market, therefore NMR should be used for structure elucidation.

5.2 Future research

Further research can be done utilizing polarized light microscopy (PLM) to identify these analogues by morphology and optical properties of their crystal structures. This is widely used in forensic science and has the potential of being invaluable for optical isomers as a quick detection and identification method.

Further research could be done to improve this study, such as utilization of seized material to see the effects of other adulterants in street samples. This will help determine if adulterants are responsible for signal suppression or expression. Utilizing seized material, LC-QqQ-MS could be used to quantitate sample purity and further studies could be done on recovery, stability, matrix effects, and repeatability. Chiral column could be used on QTOF

which would prevent the need to utilize two different instruments while providing the chromatographic separation in retention time as well as high resolution accurate mass detection.

Much more could be done to improve the NMR study. A larger sample size, inserts for the NMR tube, more powerful magnet, as well as more scans could be utilized. NMR is usually run with 5-10 mg of material, the amount used was around 1 mg, producing a weak signal in relation to the solvent peak. For dilute samples a NMR tube insert can be purchased which allows the sample to be more concentrated providing a better spectrum. In this study a 400 Hz instrument was used, however, a 900 Hz instrument has a stronger magnet that could produce more defined coupling constants at the appropriate chemical shifts. The more scans that are acquired the better the spectra, therefore, if the samples were able to run for a longer time better spectra could be produced. Lastly, something that hasn't been mention so far, is the use of 2D NMR experiments, these are best used on samples where you don't have an idea of what the substance may be such is the case in seizure of clandestine laboratories where a large quantity of sample is available. 2D NMR experiments give structural information for structure elucidation studies.

5.3 Impact on community

Fentanyl analogues are a major component in today's heroin epidemic. Detecting these compounds are critical for the safety of law enforcement agencies, hospital personnel and forensic scientists. In forensic chemistry SWGDRUG outlines the guidelines for testing. Presumptive tests are run to give analysts an idea of what compound may be present; a confirmatory test is run to identify the compound. This study helps verify that the instrumental

testing being used in laboratories are capable of identifying analogues. The best methods of separating isomers which may be present is GC-MS, LC-MS chiral separation, and FTIR.

NMR displayed promising results despite the small sample size. Although it doesn't seem practical for forensic laboratories to purchase a NMR, law enforcement agencies have the opportunity to collaborate with academic institutions. A collaboration between university and police agencies has multiple benefits. Students will network with professionals in the field, develop their research skills, and gain firsthand experience working with seized material that they will one day encounter. Professionals will gain access to instrumentation needed, gain research experience, and offers the ability to mentor a student that is part of the newest generation of graduates.

Overall, the analytical testing performed in this study is readily available in forensic laboratories and confirms that the detection of fentanyl analogues is possible whether you have one of these instruments or all of them. Here, we outlined the differences between the five instruments, highlighting their strengths in detection of different isomeric fentanyl analogues. For laboratories that would like to utilize another instrument but don't have the funds to purchase it, it is recommended that a collaboration is established between the laboratory and an academic research institute. Taking everything into account, detection of these analogues was relatively easy; the instruments are user friendly and reliable, the materials used are commonly found in laboratories, the methods are currently used in practice, and advanced training is not necessary to perform this testing. Most importantly, the proper identification of isomeric analogues was executed; this assists lawyers in properly charging a defendant, helps hospital personnel revive patients and properly label cause of deaths, and assists the federal government in monitoring trends and emerging threats in our country.

CITED LITERATURE

1. Donner B, Zenz M, Tryba M, et al. Direct conversion from oral morphine to transdermal fentanyl: a multicenter study in patients with cancer pain. Pain. 1996;64(3):527–534.
2. Energy & Commerce. Combating the opioid crisis. 2017.
3. Drug Enforcement Administration. Fentanyl a briefing guide for first responders. 2017;
4. Stanley TH, Egan TD, Van Aken H. A tribute to Dr. Paul A. J. Janssen: entrepreneur extraordinaire, innovative scientist, and significant contributor to anesthesiology. Anesth Analg. 2008;106(2):451–462.
5. Drug Enforcement Administration. Fentanyl [Internet]. 2016. Available from: https://www.deadiversion.usdoj.gov/drug_chem_info/fentanyl.pdf
6. Sublimaze Injection® Product Information. CCDS. 2014. p. 1–13.
7. Stanley TH. The fentanyl story. J Pain. 2014;15(12):1215–1226.
8. Jungerman FS, Palhares Alves HN, Carmona MJC, et al. Anesthetic drug abuse by anesthesiologists. Rev Bras Anestesiol. 2012;62(3):375–386.
9. Wright EL, McGuiness T, Moneyham LD, et al. Opioid abuse among nurse anesthetists and anesthesiologists. AANA J. 2012;80(2):120–128.
10. Nelson L, Schwaner R. Transdermal fentanyl: pharmacology and toxicology. J Med Toxicol. 2009;5(4):230–241.
11. Fodale V, Mafrica F, Santamaria LB, et al. Fentanyl: is the fear justified? Expert Opin Drug Saf. 2008;7(3):213–217.
12. Prekupec MP, Mansky PA, Baumann MH. Misuse of novel synthetic opioids: a deadly new trend. J Addict Med. 2017;11(4):256–265.
13. Drug Enforcement Administration. Drugs of Abuse 2017 Edition: A DEA Resource

Guide. 2017;94. Available from: https://www.dea.gov/pr/multimedia-library/publications/drug_of_abuse.pdf

14. Centers for Disease Control and Prevention. Nonpharmaceutical fentanyl-related deaths -- - multiple states, April 2005--March 2007. MMWR: Morbidity & Mortality Weekly Report. 2008. p. 793–796.

15. DEA Intelligence Brief. Counterfeit prescription pills containing fentanyls: a global threat [Internet]. 2016. p. 1–9. Available from: https://www.dea.gov/docs/Counterfeit Prescription Pills.pdf

16. Paone D, Kunins H. Presence of fentanyl in cocaine contributing to increase in drug overdose deaths. New York City Department of Health and Hygiene. 2017. Advisory #10

17. Fentanyl accounted for the majority of opioid identifications in DEA drug evidence analyzed in the third quarter of 2017. Natl Drug Early Warn Syst. 2017;(5).

18. Bovill JG, Sebel PS, Stanley TH. Opioid analgesics in anesthesia: with special reference to their use in cardiovascular anesthesia. Anesthesiology. 1984;61:731–755.

19. Casale JF, Mallette JR, Guest EM. Analysis of illicit carfentanil: emergence of the death dragon. Forensic Chem. 2017;3:74–80.

20. Kram TC, Cooper DA, Allen AC. Behind the identification of china white. Anal Chem. 1981;53(12):1379–1386.

21. Hibbs J. An outbreak of designer drug-related deaths in Pennsylvania. J Am Med Assoc. 1991;265(8):1011–1013.

22. Ciccarone D, Ondocsin J, Mars SG. Heroin uncertainties: Exploring users' perceptions of fentanyl-adulterated and -substituted "heroin." Int J Drug Policy. 2017;46:146–155.

23. Rogers JS, Rehrer SJ, Hoot NR. Acetylfentanyl: an emerging drug of abuse. J Emerg

Med. 2016;50(3):433–436.

24. Influx of fentanyl-laced counterfeit pills and toxic fentanyl-related compounds further increases risk of fentanyl-related overdose and fatalities [Internet]. The Centers for Disease Control and Prevention. 2016. Available from: emergency.cdc.gov/han/han00395
25. National Institute on Drug Abuse. Drugs involved in overdose deaths [Internet]. CDC Wonder database. Available from: www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates
26. DEA issues carfentanil warning to police and public. Headquarter News. 2016.
27. National Institute on Drug Abuse. Drugs involved in overdose deaths [Internet]. CDC Wonder database. Available from: www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates
28. Health department warns New Yorkers about cocaine laced with fentanyl; occasional users at high risk of overdose. NYC Health. 2017.
29. GBI Issues Synthetic Opioids Alert. Press Release. 2017.
30. Murphy M. Emerging trend bulletin: potent new opioid/opiate compound known as "Grey Death." Gulf Coast HIDTA Investigative Support Network. 2017.
31. Bagley JR, Kudzma L V., Lalinde NL, et al. Evolution of the 4-anilidopiperidine class of opioid analgesics. Med Res Rev. 1991;11(4):403–436.
32. Haghighatnia Y, Balalaie S, Bijanzadeh HR. Designing and synthesis of novel amidated fentanyl analogs. Helv Chim Acta. 2012;95(5):818–824.
33. Waldhoer M, Bartlett SE, Whistler JL. Opioid Receptors. Annu Rev Biochem. 2004;73(1):953–990.
34. Mccurdy CR, Prisinzano TE. Opioid receptor ligands. In: Burger's Medicinal Chemistry

and Drug Discovery. John Wiley & Sons, inc; 2010. p. 569–736.

35. Subramanian G, Paterlini MG, Portoghese PS, et al. Molecular docking reveals a novel binding site model for fentanyl at the μ-opioid receptor. J Med Chem. 2000;43(3):381–391.

36. Dosen-Micovic L, Ivanovic M, Micovic V. Steric interactions and the activity of fentanyl analogs at the μ-opioid receptor. Bioorganic Med Chem. 2006;14(9):2887–2895.

37. Volpe D, Tobin G, Mellon R, et al. Uniform assessment and ranking of opioid mu receptor binding constants for selected opioid drugs. Regul Toxicol Pharmacol. 2011;59(3):385–390.

38. Law P, Wong YH, Loh HH. Molecular mechanisms and regulation of opioid receptor signaling. Annu Rev Pharmacol Toxicol. 2000;(40):389–430.

39. Vardanyan R, Cain J, Mowlazadeh Haghighi S, et al. Synthesis and investigation of mixed μ-opioid and δ-opioid agnoists as possible bivalent ligands for treatment of pain. J Heterocycl Chem. 2016;54:1228–1235.

40. Siegfried. Synthesis of fentanyl [Internet]. Available from: www.opioids.com/fentanyl/synthesis

41. @lexington_nyc. It's scary how easy it is to synthesize synthetic fentanyl. Conspiracy. 2017.

42. Valdez CA, Leif RN, Mayer BP. An efficient, optimized synthesis of fentanyl and related analogs. PLoS One. 2014;9(9):2–9.

43. Casy AF, Huckstep MR. Structure-activity studies of fentanyl. J Pharm Pharmacol. 1988;40(9):605–608.

44. Vardanyan RS, Hruby VJ. Fentanyl-related compounds and derivatives: current status and

future prospects for pharmaceutical applications. Futur Med Chem. 2014;6(4):385–412.

45. Micovic IV, Ivanovic MD, Vuckovic SM, et al. The synthesis and preliminary pharmacological evaluation of 4-methyl fentanyl. Bioorganic Med Chem Lett. 2000;10(17):2011–2014.

46. Bagley JR, Wilhelm JA. Synthesis and analysis of the opioid analgesic [^{14}C]-Fentanyl. J Label Compd Radiopharm. 1992;31(11):945–950.

47. Cicero TJ, Ellis MS, Kasper ZA. Increases in self-reported fentanyl use among a population entering drug treatment: the need for systematic surveillance of illicitly manufactured opioids. Drug Alcohol Depend. 2017;177:101-103

48. Boddiger D. Fentanyl-laced street drugs "kill hundreds". www.thelancet.com. 2006.

49. Carroll JJ, Marshall BDL, Rich JD, et al. Exposure to fentanyl-contaminated heroin and overdose risk among illicit opioid users in Rhode Island: a mixed methods study. Int J Drug Policy. 2017;46:136–45.

50. Pagliaro L, Pagliaro AM. Pagliaro's comprehensive guide to drugs and substances of abuse. 2nd ed. Tarleton Landis N, editor. Washington, DC; 2009. p.135-139

51. Quintana P, Ventura M, Grifell M, et al. The hidden web and the fentanyl problem: Detection of ocfentanil as an adulterant in heroin. Int J Drug Policy. 2017;40:78–83.

52. Van Hout MC, Hearne E. New psychoactive substances (NPS) on cryptomarket fora: an exploratory study of characteristics of forum activity between NPS buyers and vendors. Int J Drug Policy. 2017;40:102–10.

53. Connor SO, Analyst P. Fentanyl: China 's deadly export to the United States [Internet]. 2017;1–17. Available from: https://www.uscc.gov/sites/default/files/Research/USCC Staff Report_Fentanyl-China's Deadly Export to the United States020117.pdf

54. United Nations Office on Drugs and Crime. Fentanyl and its analogues - 50 years on. Global Smart Update [Internet]. 2017;17. Available from: https://www.unodc.org/documents/scientific/Global_SMART_Update_17_web.pdf

55. Mukherjee S. Why fentanyl terrifies the DEA and medical experts even more than other opioids [Internet]. Fortune. 2017. Available from: http://fortune.com/2017/06/07/overdose-public-health-fentanyl-dea/

56. Controlled Substances Act of 1970. United States Constitution. 1971;Title 21.

57. Schedules of controlled substances, 21 U.S.C. § 812 (c). 2011

58. Authority and criteria for classification of substances, 21 U.S.C. § 811 (h). 2006

59. Treatment of controlled substance analogues, 21 U.S.C. § 813. 2010.

60. Definitions, 21 U.S.C. § 802(32). 2002

61. United States v. Forbes. D. Colo. 1992;806 F. Supp. 232

62. Unites State v. Washam. 8th Cir. 2002;312 F. 3d 926

63. United States v. Roberts. 2nd Cir. 2004;363 F. 3d 118

64. United States v. Johnson. D. Nevada 2014.

65. Armenian P, Vo KT, Barr-Walker J,et al. Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review. Neuropharmacology. 2017;1–12.

66. U.S. Drug Enforcement Administration emergency schedules all illicit fentanyls in an effort to reduce overdose deaths. Headquarter News. 2018.

67. Cooper D, Jacob M, Allen A. Identification of fentanyl derivatives. J Forensic Sci. 1986;31(2):511–528.

68. Brandt SD, Kavanagh P V. Addressing the challenges in forensic drug chemistry. Drug Test Anal. 2017;9(3):342–346.

69. Skulska A, Ka£a M, Parczewski A. Fentanyl and its analogues in the forensic laboratory: medical and analytical problems. Probl Forensic Sci. 2004;59:127–142.

70. Barbara Stuart, editor. Molecular Spectroscopy. In: Forensic Analytical Techniques. 1st ed. John Wiley & Sons, Ltd; 2013. p. 69–111.

71. Marchei E, Pacifici R, Mannocchi G, et al. New synthetic opioids in biological and non-biological matrices: A review of current analytical methods. Trends Anal Chem. 2018;102:1–15.

72. Noble C, Dalsgaard PW, Johansen SS, et al. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MS^E mode and retrospective analysis of authentic forensic blood samples. Drug Test Anal. 2017;1-12.

73. Strano-Rossi S, Álvarez I, Tabernero MJ, et al. Determination of fentanyl, metabolite and analogs in urine by GC/MS. J Appl Toxicol. 2011;31:649-654.

74. Palaty J, Konforte D, Karakosta T, et al. Rapid identification of cyclopropyl fentanyl/crotonyl fentanyl in clinical urine specimens: a case study of clinical laboratory collaboration in Canada. 2018;53:164-167

75. Chatterton CN, Scott-Ham M. The distribution and redistribution of fentanyl and norfentanyl in post mortem samples. Forensic Sci Int. 2018;284:146–152.

76. Stuart BH. The Chemistry of Forensic Evidence. In: Forensic Analytical Techniques. 2013. p. 1–25.

77. NARK II Fentanyl Reagent. Sirchie. Available from: http://www.sirchie.com/nark20033-fentanyl-reagent.html#.WrLAcWaZPBI

78. Philp M, Fu S. A review of chemical “spot” tests: a presumptive illicit drug identification

technique. Drug Test Anal. 2018;10(1):95–108.

79. Drug Enforcement Administration. Scientific Working Group For The Analysis of Seized Drugs (SWGDRUG) Recommendations. 2016.

80. Kabir A, Furton KG. Applications of gas chromatography in forensic science. Gas Chromatography. 2012. p.563-604

81. Kolomnikov IG, Efremov AM, Tikhomirova TI, et al. Early stages in the history of gas chromatography. J Chromatogr A. 2018;1537:109–117.

82. Zavahir JS, Nolvachai Y, Marriott PJ. Molecular spectroscopy – Information rich detection for gas chromatography. Trends Anal Chem. 2018;99:47–65.

83. Wood M, Laloup M, Samyn N, et al. Recent applications of liquid chromatography-mass spectrometry in forensic science. J Chromatogr A. 2006;1130:3–15.

84. Oberacher H, Arnhard K. Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology. Trends Anal Chem. 2016;84:94–105.

85. Kumar PR, Dinesh SR, Rini R. LCMS-a review and a recent update. J Pharm Pharm Sci. 2016;5(5):377–391.

86. Laks S, Pelander A, Vuori E, et al. Analysis of street drugs in seized material without primary reference standards. Anal Chem. 2004;76(24):7375–7379.

87. Rittgen J, Pütz M, Zimmermann R. Identification of fentanyl derivatives at trace levels with nonaqueous capillary electrophoresis-electrospray-tandem mass spectrometry (MS^n, n = 2, 3): analytical method and forensic applications. Electrophoresis. 2012;33(11):1595–1605.

88. Remane D, Wissenbach DK, Peters FT. Recent advances of liquid chromatography–(tandem) mass spectrometry in clinical and forensic toxicology — an update. Clin

Biochem. 2016;49(13–14):1051–1071.

89. Moore JM, Allen AC, Cooper DA, et al. Determination of fentanyl and related compounds by capillary gas chromatography with electron capture detection. Anal Chem. 1986;58(8):1656–1660.

90. Suzuki S. Studies on fentanyl and related compounds: spectrometric discrimination of five monomethylated fentanyl isomers by gas chromatography/Fourier transform-infrared spectrometry. Forensic Sci Int. 1989;43(1):15–19.

91. De Boer D, Goemans WPJ, Ghezavat VR, et al. Seizure of illicitly produced para-fluorofentanyl: quantitative analysis of the content of capsules and tablets. J Pharm Biomed Anal. 2003;31(3):557–562.

92. Lurie IS, Berrier AL, Casale JF, et al. Profiling of illicit fentanyl using UHPLC-MS/MS. Forensic Sci Int. 2012;220:191–196.

93. Liu C, Li T, Han Y, et al. The identification and analytical characterization of 2,2'-difluorofentanyl. Drug Test Anal. 2017;1–7.

94. Rojkiewicz M, Majchrzak M, Celiński R, et al. Identification and physicochemical characterization of 4-fluorobutyrfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl)amino)butan-1-one, 4-FBF) in seized materials and post-mortem biological samples. Drug Test Anal. 2016;9:405–414.

95. Breindahl T, Kimergård A, Andreasen MF, et al. Identification of a new psychoactive substance in seized material: the synthetic opioid N-phenyl-N-[1-(2-phenethyl)piperidin-4-yl]prop-2-enamide (Acrylfentanyl). Drug Test Anal. 2017;9(3):415–422.

96. Kanamori T, Iwata YT, Segawa H, et al. Characterization and differentiation of geometric isomers of 3-methylfentanyl analogs by gas chromatography/mass spectrometry, liquid

chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. J Forensic Sci. 2017;62(6):1472–1478.

97. Ujváry I, Jorge R, Christie R, et al. Acryloylfentanyl, a recently emerged new psychoactive substance: a comprehensive review. Forensic Toxicol. 2017;35(2):232–243.

98. Leonard J, Haddad A, Green O, et al. SERS, Raman, and DFT analyses of fentanyl and carfentanil: toward detection of trace samples. J Raman Spectrosc. 2017;48(10):1323–1329.

99. Martucci HFH, Ingle EA, Hunter MD, et al. Distribution of furanyl fentanyl and 4-ANPP in an accidental acute death: a case report. Forensic Sci Int. 2017;283:e13–e17.

VITA

Education

Oregon State University — Corvallis, OR
Doctor of Philosophy in Pharmaceutical Sciences — Expected May 2020

University of Illinois at Chicago — Chicago, IL
Master of Science in Forensic Science, FEPAC accredited — Expected May 2018

University of New Haven — West Haven, CT
Bachelor of Science in Forensic Science, FEPAC accredited — May 2013
Minor in Chemistry

Research Experience

University of Illinois at Chicago — Chicago, IL
Graduate Student- Research Assistant — August 2015- present

Dissertation Advisor: Richard B. van Breemen, PhD

- Developed and optimized an ion mobility method to rapidly analyze procyanidins
- Performed qualitative analysis on procyanidins using ion mobility mass spectrometry and Maldi-ToF-ToF
- Performed quantitative analysis on psychosine samples in collaboration with Dr. Bongarzone's lab group
- Performed, maintained and operated analytical instruments such as quadrupole-time-of-flight mass spectrometer, triple quadrupole mass spectrometer, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometer

Thesis Advisor: A. Karl Larsen, PhD

- Analysis of isomeric fentanyl analogues utilizing instrumental analysis to identify unknowns
- Performed qualitative analysis on gas chromatography-mass spectrometer, liquid chromatography-QqQ- mass spectrometer, liquid chromatography- quadrupole- time-of-flight- mass spectrometer, Fourier transform infrared spectrometer, nuclear magnetic resonance instruments
- Properly handled and stored controlled substances

Mentor: Judy Bolton, PhD

- Treated MCF-7 cells with hops extract and measured induction of cytochrome P450 activity using EROD assay

Professional Experience

Hudson County Prosecutor's Office Jersey City, NJ

Chemist I Sept 2013- Aug 2015

- Analyzed evidence submitted by law enforcement agencies for the presence or absence of controlled dangerous substances such as marijuana, synthetic marijuana, cocaine, pharmaceutical tablets, heroin, fentanyl, phencyclidine, GHB, LSD, sublingual films, paraphernalia, etc
- Maintained chain of custody throughout handling, analysis, and evidence preservation
- Prepared standards for gas chromatography- mass spectrometer analysis
- Operated and maintained gas chromatography-mass spectrometer and stereoscopic microscope
- Prepared chemical mixtures for color reagents: Wagner's, Duquenois-Levine, Marquis, Modified Cobalt Thiocyanate
- Accurately documented findings on certified laboratory reports for use in court proceedings
- Successful completion of proficiency test from Collaborative Testing Services, Inc
- Maintained workspace cleanliness
- Analyzed 1495 cases
- Performed qualitative detection

Waterbury Connecticut Police Department Waterbury, CT

Forensic Intern Jan 2013- May 2013

- Assisted crime scene technicians in collecting, packaging, transporting, and documenting evidence in active investigations
- Fingerprinted the public for pistol permits, pardon applications, employment and DCF purposes
- Superglue fumed active evidence for the presence of fingerprints
- Performed presumptive testing for marijuana and cocaine using the Valtox Field Kit
- Swabbed active evidence for DNA that was sent to Connecticut's State lab for further analysis
- Used Canon EOS Rebel T3i digital camera to photograph active crime scenes and evidence
- Viewed live autopsies at the Office of the Chief Medical Examiner in Farmington, CT
- Created property sheets using a typewriter and evidence labels using New Evidence

Skills

Software:

- Shimadzu MaldiSolutions and LabSolutions
- Waters Masslynx
- Agilent ChemStation
- Microsoft Word, Excel, PowerPoint, and Project

Instrumentation:

- Shimadzu Matrix-Assisted Laser Desorption/Ionization- Time-of-flight- Time-of-flight Mass Spectrometer and Ultra High Pressure Liquid Chromatography- Triple Quadrupole Mass Spectrometer
- Waters Quadrupole- Ion Mobility- Time-of-flight Mass Spectrometer
- Agilent Gas Chromatography- Mass Spectrometer
- Polarized Light Microscopy (Olympus BH-2)

Training

Jun 2017 Ion Mobility Mass Spectrometry: an introduction to instrumentation, applications, and data analysis, ASMS, Indianapolis, IN

Nov 2014 Forensic Chemist Seminar, Drug Enforcement Administration, Dulles, VA

Publications

Emily A. Rue, Jan Glinksi, and Richard B. van Breemen. Ion mobility mass spectrometry as a rapid approach for the separation of procyanidins. *Manuscript in preparation.*

Michael S. Marshall, Benas Jakubauskas, Wil Bogue, Monika Stoskute, Zane Hauck, **Emily Rue**, Matthew Nichols, Lisa L. DiAntonio, Richard B. van Breemen, Jeffrey H. Kordower, Carlos A. Saavedra-Matiz, and Ernesto R. Bongarzone. Analysis of age-related changes in psychosine metabolism in the human brain. *PLOS ONE.* Accepted February 2018

Emily A. Rue, Michael D. Rush, and Richard B. van Breemen. Procyanidins: a comprehensive review encompassing structure elucidation via mass spectrometry. *Phytochemistry Review.* (2018) 17: 1-16

Shuai Wang, Tareisha L. Dunlap, Caitlin E. Howell, Obinna C. Mbachu, **Emily A. Rue**, Rasika Phansalkar, Shao-Nong Chen, Guido F. Pauli, Birgit M. Dietz, and Judy L. Bolton. Hop (Humulus lupulus L.) Extract and 6-Prenylnaringenin Induce P450 1A1 Catalyzed Estrogen 2-Hydroxylation. *Chemical Research Toxicology*. (2016) 29: 1142−1150

Oral Presentations

Emily A. Rue, Jan Glinski, and Richard B. van Breemen. Procyanidins: identification and analysis using ion mobility mass spectrometry. ASP Annual Conference. Portland, OR August 2017

Poster Presentations

Emily Rue, Brendan Heffron, Richard van Breemen, and Karl Larsen. Fentanyl Analogues: Instrumental Future of Illicit Drug Identification. No. 77. UIC Research Day. Chicago, IL. February 2018

Emily A. Rue and Richard B. van Breemen. Ion mobility mass spectrometry as a Rapid Approach for the Separation and Analysis of Procyanidins in Botanicals. AbbVie Analytical Research Symposium. North Chicago, IL. August 2017

Emily A. Rue and Richard B. van Breemen. Ion mobility mass spectrometry as a Rapid Approach for the Separation and Analysis of Procyanidins in Botanicals. No. 8. Chicago Mass Spec Day. Chicago, IL. July 2017

Emily A. Rue and Richard B. van Breemen. Ion mobility mass spectrometry as a Rapid Approach for the Separation and Analysis of Procyanidins in Botanicals. No. WP 391. ASMS annual conference. Indianapolis, IN. June 2017

Emily A. Rue and Richard B. van Breemen. Ion mobility mass spectrometry for the rapid identification of procyanidins in botanicals. No. 67. MIKI annual Meeting. Minneapolis, MN. March 2017

Emily A. Rue and Richard B. van Breemen. Ion mobility mass spectrometry for the rapid identification of procyanidins in botanicals. No. 69. UIC Research Day. Chicago, IL. February 2017

Conferences/ Meetings Attended

2018 UIC Research Day, Chicago, IL
2017 AbbVie Analytical Research Symposium, North Chicago, IL
2017 American Society of Pharmacognosy, Portland, OR
2017 Chicago Mass Spec Day, Chicago, IL
2017 American Society for Mass Spectrometry annual conference, Indianapolis, IN
2017 MIKI annual meeting, Minneapolis, MN
2017 Pittcon conference and exposition, Chicago, IL
2017 UIC Research Day, Chicago, IL
2016 MIKI annual meeting, Iowa City, IA
2016 UIC Research Day, Chicago, IL
2015 American Academy of Forensic Sciences annual meeting, Orlando, FL
2015 UIC Research Day, Chicago, IL

Professional Affiliation

American Academy of Forensic Sciences, member since 2015
American Chemical Society, member since 2015