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Detection and characterization of pyromarkers of smoked drugs of abuse

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**DETECTION AND CHARACTERIZATION OF PYROMARKERS OF SMOKED
DRUGS OF ABUSE**

Rona Kiyomi Nishikawa

**Dissertation submitted to the Eberly College of Arts and Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
in
Chemistry**

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**Keywords: Fentanyl, Heroin, Pyrolysis, Pyromarkers, GC/MS
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Abstract

DETECTION AND CHARACTERIZATION OF PYROMARKERS OF SMOKED DRUGS OF ABUSE

Rona Kiyomi Nishikawa

Smoking or inhalation has increased in popularity as the choice route of administration of drugs of abuse amongst drug abusers. Combustion products associated with the smoking event (pyromarkers) of these smoked drugs of abuse can be useful in post mortem toxicological analysis both for investigative and public health applications. A method using analytical pyrolysis was developed to simulate the smoking process used for illicit drugs, which is significantly different than methods used for studies of conventional tobacco smoking. This method was used to identify possible markers associated smoked fentanyl and mixes of heroin and fentanyl. Thermocouple temperature probes were used to analyze the temperatures obtained through smoking the fentanyl transdermal patches (FTP) in means of the “chasing the dragon” technique. These temperatures were then applied to the pyrolysis gas chromatography (Py-GC/MS) to mimic these smoking conditions to determine pyromarkers that were produced. The presence of a trap on the pyrolysis unit allowed for aerobic conditions by exposing the pyromarkers to air. A second advantage of trapping is preconcentration of pyromarkers. The method enhanced the recovery and afforded a positive identification of the pyromarkers. The methods and models developed here can be extended to any smoked drug, including drugs of current concern such as cannabinomimetics and incense/bath salt compositions.

Dedication

This dissertation is dedicated to my parents, grandparents, sister, brother-in-law, niece, Uncle Jim, Aunty June, Aunty Georgia, cousins Kyle and Blaine, and Thomas. It was their endless love, support, encouragement, and endless boxes of care packages have undoubtedly provided me the foundation and motivation for me to succeed.

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List of Abbreviations, Symbols/Nomenclature

1-SPO – 1-Styryl-1H-pyridin-2-one
2-EECA – 2-Ethylhexyl ester cyclobutanecarboxylic acid
2-EEFA – 2-Ethylhexyl ester 2-furancarboxylic acid
2-EH – 2-Ethyl-1-hexenol
3-MAM – 3-O-Monoacetylmorphine
6-MAM – 6-O-Monoacetylmorphine, 6-Acetylmorphine
 μg – Microgram
 $\mu\text{mol/kg}$ – Micromole per kilogram
 μL – Microliter
 μm – Micrometer
 $^{\circ}\text{C}$ – Celsius
 $^{\circ}\text{C/min}$ – Celsius per minute
 $^{\circ}\text{C/mL}$ – Celsius per milliliter
 $^{\circ}\text{C/s}$ – Celsius per second
C-C – Carbon-carbon bond
 CH_3 – Methyl group
cm – Centimeter
C-N – Carbon-nitrogen bond
CNS – Central nervous system
Da – Dalton
DC – Direct current
DI – Deionized water
 ED_{50} – Effective median dose
EI – Electron impact
emf – Electromotive force
eV – Electron volt
FTP – Fentanyl transdermal patch
GC – Gas Chromatography
g/mol – Grams per mole
h – Hour
HCl – Hydrogen chloride
He – Helium
i.d. – Inner diameter
K – Kelvin
K/s – Kelvin per second
 LD_{50} – Dose that is lethal to 50% of the test subjects
 m^2 – Meter squared
m/z – Mass to charge ratio
min – Minute
mg – Milligram
mL – Milliliter
mm – Millimeter
mol – Mole
MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MS – Mass spectrometry
NIST – National Institute of Standards and Technology
NMR – Nuclear magnetic resonance
OTC – Over the counter
PEP – 1,2,3,6-Tetrahydro-1-(2-phenylethyl)pyridine
ppb – Parts per billion
ppm – Parts per million
Py-GC/MS – Pyrolysis Gas Chromatography Mass Spectrometry
RF – Radio frequency
RT – Retention time
s – Second
SRM – Standard reference material

Chapter 1: Introduction

1.1.0 Development of Research

Smoking or inhalation of drugs has become a popular route of administration amongst drug abusers. The physiological goal drug abusers see when smoking commonly smoked drugs of abuse, as listed in Table 1-1, is to obtain the sought after drug induced sensations, which can range from euphoric, to sexual, and analgesic effects.¹⁻³ Because smoked drugs are rapidly introduced into the bloodstream through absorption in the lungs, there is an increased risk of overdose and the potential production of toxic pyrolytic products that are not associated with other modes of administration. Thermal degradation of the parent drugs or other impurities can lead to the potential production of toxic pyrolytic products and can increase the risk of overdose. The drug pathway can also be heavily influenced by the anatomy and physiology of the lungs.

The term *pyromarkers* used here is any substance that is an indicator of a compound that undergoes pyrolysis. Pyrolysis is simply the breaking apart of chemical bonds by the use of thermal energy only, whereas combustion involve the exposure to oxygen to allow oxidation to take place.^{4,5} Analytical pyrolysis is the technique of studying molecules either by observing their behavior during pyrolysis or by studying the resulting molecular fragments that can infer much about the nature and identity of the original larger molecule.⁵ Detecting pyromarkers of smoked illicit drugs in human biological fluids can be used as a means of identifying individuals who are chronic users and abusers. The confirmatory methods that provide this information are widely used as tools for investigative purposes. The identification of the chemical composition of smoked drugs of abuse, in conjunction with enzymatic studies, can be used to identify potential unique pyromarkers in human biological matrices of those exposed. In addition, identification of these pyromarkers would

aid in the understanding of pharmacological and toxicological effects associated with exposure as a result of smoking drugs of abuse. In many instances, the drug abusers unknowingly expose themselves to something more toxic than the drug itself in which case the cause of death is not what might otherwise be assumed.

Although an intravenous injection produces the fastest rate of onset of toxic effects, smoking or inhalation is often preferred by users due to the fear of contracting AIDs, hepatitis, or other blood-borne infectious diseases from needle sharing.⁶ Other problems of infection include abscesses, collapsed veins, necrosis, sepsis, and endocarditis.⁷ Needle marks, such as bruising and scar tissue, can be avoided if smoking is used instead of injection. Smoking is not perceived to be as culturally deviant and drugs abusers associate intravenous injections with “hardcore users” due to extensive tobacco and marijuana smoking in many areas.⁸ An increase in the availability of some drugs of abuse has led to a decline in price and subsequent shift in the trend towards smoking drugs. Also, the willingness of many individuals to smoke drugs can play a major role in the emergence of drugs, such as the crack cocaine upsurge in the 1980s.⁸

The process of smoking may increase the risk of other hazardous effects caused by pyromarkers that are not associated with other modes of administration. Inhalation as the route of administration of a drug incorporates the anatomy and physiology of the lung that heavily influences the drug action. This is partly due to the large surface area of the lungs and extensive capillary network in close association with the alveoli with particles less than 1 μm in diameter.^{9,10} First, there is the absorption of the inhaled drug. There is an increased bioavailability when the fentanyl is inhaled, given that first-pass metabolism in the liver does not occur. The fast absorption from nasal mucosa and extensive lung capillaries results in an

immediate elevation of arterial blood drug concentration.⁶ Many drugs of abuse, particularly non-ionized lipophilic, drugs move rapidly from the alveolar spaces into the systemic circulation.⁸ The total distance between this alveolar space and plasma is estimated to be about 0.5 and 2.5 μm of cellular layers of the respiratory membrane.^{9,11,12} In addition, although absorption may take place over the entire respiratory tract, the largest surface area is presented by the alveolar epithelium with an estimated area of an adult is 35 m^2 during expiration and 100 m^2 in maximal inspiration.^{9,13,14} There is a relatively small total volume of blood in lungs (60-140 mL) that is spread over the large surface area, which results in a thin film of blood with a thickness of 8 μm that is similar to the diameter of pulmonary capillaries.^{9,12} This optimizes the process of absorption from the alveolar space into the plasma. When drugs are inhaled, they are delivered and distributed to target organs rapidly due to the high pulmonary capillary blood flow. Rapid delivery of drugs to the brain may also lead to other adverse effects, such as pulmonary disease or deleterious cardiovascular consequences.⁶ The high concentration of drug in the arterial blood can produce an intense psychoactive effect which can be qualitatively different from the drug's steady state effect.⁸ Other routes of administration do not typically experience the desired "rush" feeling felt by the abuser while smoking.

Secondly, uptake by the lungs allows the retention and short-term accumulation of the drug being smoked. This is due to the extensive involvement of extra- and/or intra-cellular binding to various proteins, receptors, and in some cases energy-dependent active transport.⁸ The lung becomes a reservoir for slow and persistent release of drug into the circulation and at high concentrations could result in local toxicity in the lungs.

Lastly, the lung is able to metabolize some drugs and endogenous chemicals by similar pathways as the liver.⁸ The ultimate goal of lung cells is to facilitate the elimination of lipophilic substances that would otherwise accumulate in cellular lipids.¹⁵ The concentration of drugs brought into contact with drug-metabolizing enzymes in the lungs is much greater than the liver due to the higher blood flow, even though the lung tissues have lower concentrations of drug-metabolizing enzymes than the liver.⁸ Therefore, metabolism by the lung can contribute substantially to total drug metabolism. Cytochrome P450 activity is primarily in nonciliated bronchial epithelial and Type 2 alveolar cells, which are most susceptible to injury as a result of metabolism of certain drugs and chemicals to reactive metabolites.⁸

In addition, the thermal degradation of the parent drug or other impurities can lead to the inhalation of toxic pyromarkers. An example of this, reported in the 1980s, is heroin leucoencephalopathy, a life-threatening condition of the brain that has occurred in individuals after smoking heroin.^{16,17} Heroin leucoencephalopathy is not associated with intravenous injection administration and no impurities present in the drug were identified as the cause. Pyromarkers of either the heroin or impurities present in the samples may have been the cause of this condition.^{16,17}

In all, the increased popularity of smoking drugs of abuse raises much concern about drugs that are currently being abused by other routes of administration. Consequently, little is known about whether side effects and toxicity would be increased by smoking. Table 1 provides a list of some of the most commonly smoked drugs and prescription drugs of abuse.^{1,2} These drugs listed on this table may be potential targets that can be evaluated using these methods developed here.

Analytical pyrolysis is the technique of studying molecules either by observing their behavior during pyrolysis or by studying the resulting molecular fragments.⁵ It has frequently been considered being a technique mainly applied to the analysis of natural and synthetic polymers in forms of paints, plastics, adhesives, etc.^{5,18} Recent work in our laboratory has demonstrated the utility of analytical pyrolysis for studying pyromarkers of smoked drugs of abuse for cocaine and methamphetamine.^{19,20} These results have been confirmed in literature of toxicological case samples of urine.^{21,22} The smoking process and conditions can be mimicked by interfacing a pyroprobe unit via gas chromatography coupled with mass spectrometry (Py-GC/MS).

The goal of this dissertation is a qualitative study of the pyrolysis of fentanyl and fentanyl transdermal patches (FTP) in the typical route of administration of inhalation through the method of “chasing the dragon” (discussed in further detail in Section 1.2.3). In addition, the pyrolysis of heroin mixed with fentanyl is also analyzed to determine significant pyromarkers.

With regard to smoking parameters, literature reviews have provided extensive data about manufactured tobacco cigarettes. Smoking machines have been used that take into account puffing and smoldering characteristics of the active process of smoking. Baker and Bishop have done extensive temperature analysis on manufactured tobacco cigarettes and have observed high temperatures of 605-770 °C.^{23,24} More discussion on manufactured tobacco smoking is discussed later in section 3.1.0. The pyrolysis of fentanyl was initially analyzed and modeled after these findings of the manufactured cigarettes. However, it was later determined that temperatures of pyrolysis were much lower by the use of a smoking box with temperature probes that was created to observe actual readings of smoking fentanyl.

This simple experiment indicated that manufactured tobacco cigarette smoking temperatures cannot be directly translated to drug abuse. These lower temperatures were then applied to further pyrolysis analysis.

Once the pyromarkers are determined, identifying metabolites formed by an *in vitro* breakdown of those compounds may lead to the discovery of a potential marker that could be used in human studies. Human lung and liver microsomes containing cytochrome P450 could be used for metabolic studies of the pyromarkers in the future.

Table 1-1. Common smoked drugs of abuse with the DEA drug schedule.^{2,25}

Substance: Category & Name	Examples of <i>Commercial and Street Names</i>	DEA Schedule
Tobacco		
Nicotine	Found in cigarettes, cigars, bidis, and smokeless tobacco (snuff, spit tobacco, chew)	
Cannabinoids		
Marijuana	weed, pot, dope, skunk, sinsemilla, ganja, blunt, dope, herb, Mary Jane, reefer, joint, green, trees, smoke, bud	I
Hashish	hash, hash oil, boom, hemp, chronic, gangster	I
Opioids		
Fentanyl and analogs	<i>Actiq, Duragesic, Sublimase</i> : china white, Apache, China girl, dance, fever, friend, goodfella, jackpot, murder 8, TNT, Tango and Cash	I, II
Heroin	<i>Diacetylmorphine</i> : brown sugar, skag, smack, horse dope, H, junk, skunk, white horse, China white; cheese (with OTC cold medicine and antihistamine)	I
Morphine	<i>Roxanol, Durgamorph</i> : Miss Emma, monkey, M	II, III
Opium	<i>Laudanum, paregoric</i> : big O, black stuff, block, gum, hop	II, III, V
Stimulants		
Cocaine	<i>Cocaine hydrochloride</i> : blow, candy, rock, C, Charlie, coke, crack, flake, snow, toot	II
Amphetamine	<i>Biphetamine, Dexedrine, Adderall</i> : bennies, speed, black beauties, crosses, hearts, LA turnaround, truck drivers, uppers	II
Methamphetamine	<i>Desoxyn</i> : chalk, fire, ice, meth	II
Methylphenidate	<i>Ritalin</i> : JIF, MPH, vitamin R	II
Dissociative Anesthetics		
Ketamine	<i>Ketalar SV</i> : cat Valium, Special K, vitamin K	III
Phencyclidine and analogs	<i>Phencyclidine</i> : angel dust, boat, hog, love boat, peace pill	I, II
Salvia divinorum	Salvia, Shepherdess's Herb, Maria Pastora, magic mint, Sally-D	
Hallucinogens		
Mescaline	peyote, cactus, buttons, mesc	I

1.2.0 Compounds of Interest

The compounds of interest in this study were fentanyl and heroin that are classified as opiates. Table 1-2 shows the structure and basic properties of both. Opiates is a term that refers to compounds that are not only derived from the opium poppy, *Papaver somniferum*, but also applies to compounds that are either pharmacologically similar or closely related structurally to morphine.³ Narcotic is also a term used and is similar to the opiates that mean a central nervous system (CNS) depressant that has a numbing action. The opium poppy is cultivated in temperate climates and is indigenous to Burma, Laos, and Thailand, commonly referred to during the last half of the 20th century as the Golden Triangle.²⁶ In addition to those three countries, much of the world's illicit supply of opium is cultivated in Afghanistan, Colombia, Mexico, and Pakistan, with Afghanistan being the largest producer.^{26,27} Opiates are typically divided into the naturally occurring, semi-synthetics and synthetics. Morphine and codeine are naturally occurring opiates. Semi-synthetics that are manufactured most easily by using starting materials that are themselves opiates are considered as hydrocodone, hydromorphone, oxycodone, oxymorphone, and heroin. Fentanyl is a synthetic opiate that may be prepared by synthesis from non-opiate precursors.

The mechanism of action of opiates begins with the opioid receptors within the brain, spinal cord, and other organs which structures bind natural, endogenous compounds known as endorphin peptide transmitters or beta endorphins.³ The endorphins bind to the opioid receptors that allow for the diminishing of the perception of pain throughout the pain pathway. Opiates enhance the opioid receptor response by stimulating these receptors in a manner similar to that induced by the natural compounds, endorphins.³ The binding of the receptor by the endogenous compound, beta endorphins, or the exogenous opiates inhibits the

release of a specific neurotransmitter or the substance P in the pain pathway. Opioid dependency is believed to be related to the opioid receptors existing within the limbic system. It is this part of the brain that is related to the drug dependence. It is this binding of the opiates within the limbic system that is presumably associated with the drug dependence that develops within the user.

The four kinds of opioid receptors are listed in the Table 1-3. The δ and μ receptors have the same functions, but are located in different regions. Receptor stimulation causes euphoria, analgesia, sedation, and respiratory depression. Those overdosed on opiates are in CNS depression with side effects ranging from lethargy to deep coma.³ Severe, acute overdose may result in death and is preceded by respiratory depression to the point of apnea, myocardial depression, rhabdomyolysis, seizures, and coma. The antidote of choice is naloxone (Narcan®), which is a pure opioid antagonist that has no actions that are additive to those of opiates.³ Naloxone displaces the opiate molecules from their receptor sites in the brain. Within 1 or 2 minutes respiration is dramatically improved with a dose of just 0.4 mg.³ Depending on the severity of overdose, naloxone is given repeatedly since it has a short half-life of about 60 min.³

Table 1-2. Properties and structure of fentanyl and heroin.^{28,29}

	Fentanyl	Heroin
Structure
Molecular Formula	$C_{22}H_{28}N_2O$	$C_{21}H_{23}NO_5$
Molecular Weight	336.5 g/mol ²⁸	369.4 g/mol
pKa	8.44	7.6
Solubility	Soluble in water and alcohol	Soluble in water, alcohol, and chloroform
Melting Temperature	83-84 °C	173 °C
Toxicity	2 mg	200 mg
Half-life, plasma	~3.7 h	Diamorphine ~3 min, Morphine ~2-3 h

Table 1-3. Opiate receptors.

Receptor	Location	Actions
Mu (μ)	Cerebral cortex, thalamus	Analgesia, respiratory depression dependence, euphoria, constipation
Delta (δ)	Frontal cortex, limbic system	Euphoria, analgesia, respiratory depression
Kappa (κ)	Spinal cord	Spinal analgesia, sedation, physical dependence, miosis
Sigma (σ)	Endoplasmic reticulum	Hallucinations, dysphoria, tachycardia, vasomotor stimulation

1.2.1 Fentanyl

Fentanyl is a lipophilic synthetic opiate used for anesthesia and analgesia. It was originally synthesized in Belgium in the late 1950s and patented by Janssen-Cilag International 1963.³⁰ It was introduced to the United States in the 1970s for the use in the induction of anesthesia and the relief of postoperative pain.³¹ Aside from clinical therapeutic pertinence, it is the euphoric effects of fentanyl that are sought after by drug users, making it increasingly popular as a choice drug of abuse.^{27,31-35} Fentanyl is approximately 50 to 100 times more potent than morphine and 30-50 times more potent than heroin depending on the physiological or behavioral endpoints being measured, the route of administration, and other factors.³⁶

Fentanyl may be administered by the transdermal route in a form of the FTP as an effective alternative to oral morphine in patients with stable opioid requirements.^{37,38} In particular, in patients who are not able to take oral medication, it provides a continuous delivery of fentanyl and is a less invasive alternative to subcutaneous opioid infusion.^{37,38} The form of fentanyl used in the FTPs is fentanyl citrate. The fentanyl citrate has a higher melting point of 153-154 °C, instead of 83-84 °C as noted in Table 1-2.³⁹ FTPs are often obtained through the black market, prescription fraud, and improper disposal. The reservoir FTPs has been in use since the early 1990s, but was associated with misuse and was consequently discontinued and phased out by most companies by 2009.^{31-33,38,40,41} Janssen-Cilag International developed the Durogesic® D Trans® developed the matrix FTPs (Figure 1-1B) after the initial reservoir FTPs (Figure 1-1A). To limit the potential of fentanyl abuse by the extraction of the fentanyl gel from the drug reservoir of the FTPs, an alternative generation of transdermal matrix FTP was developed where the active drug is dissolved in a

semi-solid polymer matrix. Other companies have developed different technologies of the matrix FTPs, such as Martifen (Nycomed Roskilde, Denmark), where fentanyl-containing dipropylene glycol droplets dispersed in a silicone matrix. The plastic matrix makes the patches far less suitable to transbuccal use and far more difficult to use illicitly than its gel filled counterpart.

There have been reports of abuse of reservoir FTPs by the following pathways: oral ingestion by either licking or chewing on the FTP;⁴² intravenous injection;⁴³ transdermal application of multiple patches cutaneously;⁴⁴ rectal insertion;⁴⁵ and inhalation by volatilization.⁴¹ One of the first reported cases of death by inhaled fentanyl was published by Mardquardt et al. in 1994.⁴¹ The fentanyl in a gel matrix was scraped off the drug reservoir of a FTP, heated on aluminum foil, and then inhaled. Another means of intoxication is by smoking illicitly manufactured fentanyl laced with heroin or cocaine, both of which are reported to have been prevalent in Delaware, Illinois, Maryland, Michigan, Missouri, New Jersey, and Pennsylvania.³⁶

Figure 1-1. Schematic representation of a reservoir type patch (A) and a matrix type patch (B).

Fentanyl is rapidly metabolized in the liver and intestinal catalyzed by CYP3A4.⁴⁶ Norfentanyl and despropionyl fentanyl are two commonly reported metabolites of fentanyl in the human body produced by piperidine N-dealkylation and amide hydrolysis, respectively.^{46,47} There is a significant overlap between therapeutic and toxic fentanyl concentrations in serum.³¹ Typical therapeutic and toxic fentanyl concentrations in serum are 1–3 ppb and >3 ppb, respectively.^{31,41,42} However, there are some reports of fentanyl deaths from as low as 2 ppb³³ and therapeutic concentration of 8-9 ppb perhaps due to gain in tolerance.³⁵ Tolerance simply refers to a phenomenon in which the exposure to a drug results in the diminution of an effect or the need for a higher dose to maintain an effect that is dependent on genetics and/or acquired tolerance.⁴⁸ Therapeutic concentrations can be even greater with respiratory assistance, since, like all opioids, fentanyl suppresses respiration.³³

Figure 1-2. Known fentanyl metabolites.

1.2.2 Heroin

Heroin (diacetylmorphine) was first synthesized and introduced into clinical practice over 125 years ago as a non-addictive treatment for soldiers since they had become physically dependent on morphine during the American Civil War.²⁶ In North America, heroin obtained is primarily produced illicitly in clandestine chemical laboratories located in Colombia, France and Italy.²⁶ In the 1980s, primary route of administration of heroin was through intravenous injection. However, the risk of transmitting HIV through shared needles and the increased availability of significantly purer form of heroin from 5% to over 90% the preferred route of administration had shifted to intranasal snorting and smoking.²⁶ Intranasal snorting of heroin can cause acute, potentially life-threatening exacerbation of asthma. With a short elimination half-life, heroin is not detectable in plasma after oral or rectal administration.

When heroin is orally ingested, it is rapidly deacetylated in the liver and GI tract and undergoes first-pass hepatic metabolism, to morphine.²⁶ Therefore, heroin offers no advantage over morphine and similarly, heroin offers no advantage over methadone in opiate-dependence maintenance programs.²⁶ Esterase enzymes that are present in the blood, the liver, and the brain rapidly converts heroin to 6-monacetylmorphine (6-MAM) and morphine (Figure 1-3).^{7,49-51}

Literature reports have suggested that deacetylation (hydrolysis) of 6-MAM to morphine is significantly impaired by the presence of high blood alcohol concentrations which has been postulated to contribute to the higher rate of heroin overdose deaths when alcohol intoxication is simultaneously present.²⁶ Administration through intravenous injection rapidly deacetylates heroin to 6-MAM with which is further deacetylated to

morphine and undergoes metabolism to morphine-3-glucuronide and morphine 6-O-glucuronide.²⁶ The elimination half-life of heroin after intravenous injection is 1.3 to 2.2 min.²⁶ Even in laboratory analysis, heroin hydrolyzes in aqueous solution to 3-O-monoacetylmorphine (3-MAM) and 6-MAM and morphine to a significant extent at room temperature.⁵²

The dangers of intravenous administration of heroin are increased due to many possible health complications that typically result from the bad quality of the product. The quality of the heroin can be limited by impurities, adulterants, diluents, and contamination with microorganisms.⁵³⁻⁵⁶ Inhalation of heroin therefore poses several advantages over intravenous administration besides blood-borne infectious diseases. Through inhalation, a given dose takes more time to consume that allows the abuser to have more control of the dosage and lessens the risk of drug overdose.⁷ In addition, the toxicity to systemic exposure to impurities and adulterants present in street heroin is less likely since there some will not be volatilized for inhalation exposure.⁷ Conversely, the heating of street heroin will cause degradation of components present that could be of more health hazardous than intravenous injection.

Figure 1-3. Heroin Metabolism.

When heroin is heated to produce vapors for inhalation, significant amounts of the drug is decomposed during the heating process. In 1985, Cook and Brine studied the pyromarkers of both heroin and heroin HCl using 20-41 mg of drug on a quartz boat and connected with dry and bubble traps to collect the pyromarkers. The traps were solvent washed and the collected pyromarkers were analyzed by a combination of proton and 13-carbon NMR and MS. It was determined that 6-MAM, N,6-O-diacetylnormorphine, and N,3-O,6-O-triacetylnormorphine were the major pyromarkers at 250 °C, with 3,4-diacetoxypheanthrene being the minor pyromarker found (Figure 1-4).⁵⁷⁻⁵⁹

Unlike fentanyl, the potency of heroin administration is lowered through the route of inhalation rather than injection. This can be evaluated by the effective median dose (ED₅₀). Heroin was somewhat less potent than by inhalation (ED₅₀ 1.9 µmol/kg) administration than intravenous injection (ED₅₀ of 0.69 µmol/kg).⁶⁰ In contrast, a similar compound like morphine has a higher potency by inhalation (ED₅₀ 0.77 µmol/kg) administration than intravenous injection (ED₅₀ 3.9 µmol/kg).⁶⁰ This is likely due to the fact that 6-MAM was found to be more potent at the µ-receptors than morphine. Although 6-MAM is a major pyromarker, it accounted for less than 5% of the recovered sample in one study,⁶⁰ whereas, heroin rapidly converts to 6-MAM in the blood stream during intravenous injection. The exposure to 6-MAM is higher during intravenous injection rather than inhalation. Inhalation exposure to heroin produced significantly more analgesic effects when volatilized at 250 °C than at 200 °C.⁶⁰

In 1987, Huizer evaluated the volatilization of heroin under a variety of conditions, particularly in the presence of various diluents often used in the smoking process.⁶¹ “Heroin pills” or “red pills” were seen as early as the 1920s in China, which were mixtures typically

of heroin, caffeine, a cinchona alkaloid (quinine cinchonine, or cinchonide), strychnine, and aspirin or salicylic acid mixed with starch, cane sugar or lactose, perfume and dye to color the pills.⁶² Today, typical diluent for heroin smoking are caffeine, strychnine, lactose, acetaminophen, and diphenhydramine.^{26,57,63-65} Studies have shown that caffeine enhanced the volatilization and changes the ratio of the pyromarkers with 6-MAM to be the most abundant.⁶⁶ Thus, the amount of heroin inhaled by smoking is strongly dependent on the presence of the diluents. Diluents can also influence the ratio of the pyromarkers.

Near the end of 2005 and early into 2006, the government and media reported an increase in the number of individuals who have overdosed on heroin that was laced with fentanyl as a diluent. The largest number of reported fatalities was in Chicago, in addition with Pittsburgh, St. Louis, and Camden, New Jersey reporting cases as well.⁶⁷ It was thought that these samples were sold by a network or gang that was producing tainted heroin; to counter this, they added fentanyl to improve the low quality heroin. The first time drugs were mixed with fentanyl were sold on the street was documented in Orange County, CA, in 1979.⁶⁸ As previously noted, the pyromarkers of heroin are known. However, the combination of fentanyl and heroin pyromarkers has not been studied and is evaluated here.

Figure 1-4. Known pyromarkers of heroin. * Denotes minor pyromarker.

1.2.3 Smoking Opioids by “Chasing the Dragon”

The coming of the method “chasing the dragon” is due in part to the smoking of heroin. The first report of heroin smoking originated in Shanghai, China, in the 1920s, where it then spread across much of Eastern Asia and to the United States over the next decade.⁶⁹ The smoking of heroin existed alongside the longer established use of opium both by swallowing and by smoking.⁶⁹ Early accounts of heroin smoking involved smoking heroin pills in which small china jar or porcelain vase into which a hole had been drilled and through which the heroin pill was heated gently so that the heroin smoker could inhale the fumes of the pill through a bamboo tube.^{62,69}

An early account of “chasing the dragon” is described in *Bulletin on Narcotics, 1958*. Cheap crude quality heroin that is typically unfit for injection, had a tendency to run into a single mass when heat was applied and the outsides became charred creating a heat barrier resulting in an incomplete combustion.⁷⁰ A barbiturate, commonly known at the time as “daai fan,” was sold with the crude heroin which prolonged the effects of the heroin and aided in the sublimation during heating.⁷¹ The refinement of the technique soon utilized a piece of foil, a straw, and cardboard. Both base powder and heroin placed half an inch apart in the crease of the foil where then the flame from the lit piece of cardboard is applied. The foil is then tilted back and forth whilst the flame is applied directly under the crease; at the same time the smoker directs the straw, which is held in the mouth over the foil and inhales the fumes emanating from the heated mixture.⁷⁰ To obtain the best effect the abuser chases the “smoky tail” of the liquid with the straw in mouth while moving the foil back and forth over the flame until nothing is left on the foil except for a black stain.⁷⁰ For this reason, the whole procedure was phrased as “chui lung” or “chasing the dragon.”⁷⁰

Today, websites and blogs showcase stories of individuals of utilizing this method for abusing fentanyl and FTPs.⁷²⁻⁷⁴ Prior to the dwindling manufacturing of drug reservoir FTPs, abusers would extract the fentanyl gel from the drug reservoir and placed it on a piece of aluminum foil, heated with a lighter until it sizzles, and then vapors are inhaled through a straw or pen casing. Matrix type FTPs were smoked similarly by placing pieces of the FTPs on foil, thus introducing the pyrolysis of plasticizers and adhesives that were incorporated in the manufacture of the FTPs.

1.3.0 Methods of Degradation

Pyrolysis is a process that breaks the chemical bonds of a compound using thermal energy. The products produced by pyrolysis are typically referred to as pyrolytic products. To study the pyromarkers of fentanyl, recent work in this laboratory has demonstrated the utility of analytical pyrolysis.^{75,76} Heating fentanyl beyond a certain temperature will allow for thermal degradation since its chemical bonds have a limited thermal stability. These chemical changes are similar to those occurring during the smoking process and high temperature conditions.

Analytical pyrolysis allows for reproducible control of parameters such as temperature, heating rate, and atmosphere. This enables researchers to study the fragmentation products that are characteristic of the parent compound.⁷⁷ Fragmentation is primarily based upon the bond strengths between each atom of a molecule, where weaker bonds are broken first. Thermal degradation reactions, given from most common to least, include eliminations, fragmentation, rearrangements, oxidations, reductions, substitutions, and additions (Figure 1-5).

(A) Eliminations

(B) Fragmentation

(C) Rearrangements

(D) Oxidations/Reductions

(E) Substitutions

(F) Additions

Figure 1-5. Scheme of common degradation mechanisms observed in pyrolysis.

Pyrolytic elimination is the most common amongst the above processes. Elimination does not always take place from the 1,2 positions, and depending on which atoms are involved in the elimination, these reactions are classified as α -eliminations, β -eliminations, 1,3-eliminations, etc.⁷⁸ Based on their mechanism, these eliminations have a radical E_i mechanism or in unusual cases an E_1 or E_2 mechanism.⁷⁸ Since pyrolytic elimination takes place with no other reagent present and often requires gas phase, the typical E_2 mechanism where a proton is pulled by a base is not common.⁷⁷

Pyrolytic α -eliminations involve two leaving groups connected to the same carbon (Figure 1-6A). Typically α -eliminations are encountered when the more common β -elimination is not possible. Pyrolytic β -eliminations are the most common type of elimination reactions that take place typically by an E_i mechanism with two groups lost from adjacent atoms (Figure 1-6B). If a double bond is present during an E_i type pyrolytic reaction the formation of a conjugate system is preferred if sterically possible. Otherwise, the orientation in the pyrolytic elimination is statistical and is determined by the number of β -hydrogens. Any newly formed double bond goes toward the least substituted carbon as governed by Hofmann's rule.⁷⁸

In addition, there are eliminations involving free radicals that are frequent at temperatures between 600 °C and 900 °C.⁷⁸ The initiation reaction typically takes place with a high probability at the bonds with a lower energy, whereas at higher temperatures, even bonds with higher energy are cleaved.⁷⁸ Figure 1-7 shows a generic elimination involving free radicals that includes the initiation, propagation, and termination step.

(A) α -Eliminations

(B) β -Eliminations

(C) 1,3-Eliminations

Figure 1-6. Pyrolytic Elimination Reactions.

Figure 1-7. Pyrolytic elimination reactions involving free radicals.

The nature of molecular fragments generated in pyrolysis is analogous to ion formation in electron impact mass spectrometry.⁷⁸ The difference is that during pyrolysis, there are no ions formed because the energies involved in the pyrolytic process are much lower than the required ion formation. Also, pyrolysis can be done on many molecules in condensed phase and can be performed at different temperatures.⁷⁸ Figure 1-6B is an example of a Grob fragmentation where one carbocation is the leaving group.

Rearrangements are very common in pyrolytic reactions following eliminations and fragmentation. Rearrangement reactions involve an atom or migration group moving from one place to another on the same molecule. Figure 1-6C is an example of a 1,2 shift that is a common rearrangement reaction. Rearrangement can also be observed in positions that are farther apart and usually involves a free radical being formed followed by a free radical rearrangement occurring between the two positions.⁷⁷

Oxidations, reductions, substitutions and additions more commonly follow an initial step that produces a elimination, fragmentation, or a rearrangement.⁷⁸ However, these reactions may sometimes be the first step in pyrolysis and are caused by higher reaction rates as a result of increased temperature. In general, all of the degradation products, as well as minor constituents and deviations can be simplified in three statements: 1) the mechanism behind degradation involves free radical processes and are initiated by breaking the weakest bond first; 2) pyromarkers are based on the stability of the free radicals involved and on the stabilities of the product molecules; 3) free radical stability follows the order of $3^\circ > 2^\circ > 1^\circ > \text{CH}_3$.⁵

To mimic realistic smoking conditions, pyrolysis must be performed in the presence of additional catalysts such as oxygen, water and hydrogen. This can be explained by

considering oxygen, which has the properties of a diradical, since in its ground state each of the two higher occupied molecular orbitals contain an unpaired electron. Figure 1-7 shows that diradical oxygen can rapidly react with many other radicals in a chain oxidation. Since a pyrolysis reaction typically generates free radicals, the formation of hydroxyperoxide is likely. Oxygen may also react at the elevated temperatures with the parent compound that is subject to pyrolysis.⁷⁸

The pyrolysis process can be more than the decomposition of one single molecular species, since the products of the initial decomposition may undergo further reaction under the influence of heat. Also, the initial process can be unique or more pyrolytic reactions occurring simultaneously can contribute to the generation of the pyromarkers. Even the cooling rate of the pyromarkers can influence the pyrolysis outcome.⁷⁸

Figure 1-8. Pyrolysis in the presence of oxygen.

1.4.0 Instrumentation

A thermocouple was used in this work for measuring temperatures associated with smoking FTPs by means of “chasing the dragon.” The pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) was used to mimic analytically the smoking conditions to analyze the products produced by application of different temperatures of heat.

1.4.1 Thermocouple

The thermocouple is one of the most common temperature transducers that essentially convert thermal energy to electric energy. It is the amount of electric energy that is produced, that can be used to measure the temperature, which is based on the continuous current that flows in the thermoelectric circuit. T. J. Seebeck discovered this thermoelectricity in 1821. It was first suggested that this could be used for high temperature measurement within five years of this discovery by A.C. Becquerel.⁷⁹ Seebeck’s work showed that a small electric current will flow in a close circuit composed of two dissimilar metallic conductors when their junctions are kept at different temperatures.⁸⁰ In Figure 8a, the current flows from the positive metal C with respect to metal B at the hot junction where temperature is measured. Under these conditions, the electromotive force (emf) is produced and is known as the Seebeck emf. It is the pair of conductors, or thermocouple elements, which constituted the thermoelectric circuit that is called a thermocouple.⁸⁰ If this circuit is broken at the center, the net open circuit voltage (the Seebeck voltage) is a function of the junction temperature and the composition of the two metals.⁸¹ All dissimilar metals exhibit this effect and for small changes in the temperature the Seebeck voltage is linearly proportional to temperature (eq. 1).

$$\Delta e_{AB} = \alpha \Delta T \quad (\text{eq. 1})$$

Where α is the Seebeck coefficient that is constant of proportionality, T is the temperature and e_{AB} is the Seebeck voltage.

The cold junction of the thermocouple is connected to a voltmeter and the leads of the voltmeter themselves create a new thermoelectric circuit (Figure 1-9B). Higher the temperature difference creates higher voltage. The right pair of metals is needed to measure the targeted temperatures of 20 K to 2000 K. There is seven “standardized thermocouples” with different alloy combinations that has internationally agreed reference tables for thermal emf vs. temperature with letter designations⁸². Base metal thermocouples are composed of inexpensive metals like nickel, iron, and copper. The thermocouple types, E, J, K, N, and T are among this group. These are the most commonly used type of thermocouple. Each leg of these different thermocouples is composed of a special alloy, which is usually referred to by their common names.

Figure 1-9. Schematic of A) the basic thermoelectric circuit and B) a practical circuit for thermocouple voltage measurements.

1.4.2 Py-GC/MS

The components of a pyroprobe unit consisted of a pyroprobe, trap, accessory, valve oven, and the transfer line (Figure 1-10). The pyroprobe is interfaced with a heated transfer line to a GC/MS with an electron ionization source and a single quadrupole mass analyzer. For most commercially available pyrolyzers, the sample size should be in the low microgram range to produce better results than larger ones.⁸³ The unit consist of three modes of operation that include: Clean, to remove residual material from the filament; Dry, to remove solvent from a sample deposit; and Run, for pyrolysis of the sample to be analyzed. Here, solid samples of approximately 10-100 μg are sandwiched between quartz wool inside the quartz tube. Liquid samples of approximately 10 μg are injected in the middle of the quartz wool plug inside the quartz tube and put through the dry method prior to pyrolysis. The quartz tube is inserted into the platinum coil filament of the pyroprobe and is capable of reaching temperatures of up to 1400 $^{\circ}\text{C}$ at rate of 0.01 $^{\circ}\text{C}/\text{min}$ to 2000 $^{\circ}\text{C}/\text{s}$.⁸⁴ The microprocessor of the unit controls the temperature of the filament by calculating the resistance of the filament at setpoint temperature and supplying the correct voltage to achieve that temperature.⁸⁴ The pyroprobe is inserted inside the heated chamber, known as the accessory region, of the pyrolysis unit on the left of Figure 1-10.

The unit can operate in both non-trap and trapping modes. The use of the trap allows the pyrolysis to be performed in a different atmosphere from the carrier gas.^{5,18,85} In trapping mode, there is a second heated zone connected to the valve oven, which there is an option to add a reactant gas, such as air, to the volatile sample in the trap region. Figure 1-11 is a representation of the pyroprobe temperature program in non-trapping mode and trapping mode. In trapping mode, the sample is swept into the trap that is shown with a dotted line.

As the pyroprobe is being heated by a controlled ramp rate inside the valve oven, the reactant gas carries the volatile components to the sorbent tube trap packed with 20:35 mesh Tenax-TA. The Tenax-TA is 2,6-diphenylene oxide that is commonly used for thermal desorption and is stable below 350 °C.⁸⁶ The combination of interface and trapping programs allows any volatile compounds to be vented during the interface program. The pyroprobe would then fire after the 8 port valve rotor had rotated to allow any pyrolysis and oxidation products to be collected on the trap. During the last minute of flow to the trap, the carrier gas is automatically changed to helium to remove the residual air from the trap before desorption of analytes to the GC-MS for continuous separation and detection using the transfer line. In general, the desorption temperature should be at least 20 °C above the boiling point of the adsorbed compounds, so the range of compounds which can be analyzed with thermal desorption is limited by the thermal stability of the sorbent.⁸⁶

Figure 1-11. Representation of pyroprobe temperature program in trapping mode, where T is temperature.

In the GC used in this research, the mobile phase is gaseous and the stationary phase is a tethered liquid inside of silicon capillaries. The partitioning of analytes between the stationary and mobile phases facilitates chromatographic separation. As the analytes move through the capillary, the analyte partitioning reaches equilibria. In chromatography, retention time of an analyte is a factor of dead time (time for the mobile phase to reach the detector) and time spent in the stationary phase.

The GC composed of the following: a gas supply (mobile phase); pressure regulators, gauges, and flow meters to control and maintain a constant flow rate; a sample injection chamber; a column contained in a thermostatted oven; and a detector that is networked to a data collection system.⁸⁷ For this research a quadrupole mass spectrometer was used as a detector, a standard detector available in forensic laboratories.

A mass spectrometer consists of three zones: an ionization region, a mass analyzer, and a detector. In this research, electron ionization (EI) was used to transition gas phase molecules to gas phase ions for mass spectrometric detection. Since GC and EI require the sample to be stable and volatile, coupling these two methods is ideal. The EI requires the use of an ion beam to react with and ionize analytes as they exit the column. It is classified as hard ionization which means fragmentation is usual and may be extensive. This can be advantageous by means that EI can provide the molecular mass and structural information of the molecule. The molecular ion M^+ may form, but generally this ion contains too much energy and fragments, therefore, under reproducible conditions (70 eV), EI spectra may be used to build searchable databases of fragmentation patterns. A large number of organic molecules that are less than 600 Da can be ionized using EI and well over 100,000 spectral library databases have been developed and accessed through most GC/MS software.⁸⁸

Limitations of using EI are the requirement of volatile and stable samples. Unstable compounds may over fragment to a point that the molecular ion cannot be determined from the mass spectrum that can return provide no structural information.

A quadrupole mass analyzer was used as the detector in this portion of the study. A quadrupole mass analyzer features four hyperbolic poles with oscillating applied DC and RF potentials. The RF signal influences the trajectory of light ions while the DC signal influences the trajectory of heavy ions. If an ion is destabilized by the oscillating signals, it will collide into the quadrupoles and lose charge. Stable ions travel through the quadrupole to the detector. The quadrupole mass analyzer may be scanned such that ions of varying mass to charge ratios arrive at the detector to form a spectrum. For detection, an electron multiplier was used. An electron multiplier is used to amplify signal (with a typical gain of 10^6 to 10^{11} electrons) prior to arrival of electrons at a Faraday cup.⁸⁸ A Faraday cup is grounded and as electrons strike a current is generated and measured. Identification solely based on mass spectral comparison using the MS database provided tentative identification of the analytes. Whilst, identification based on the database and standard reference materials (SRMs), where both retention time and MS spectra were compared, provides confirmatory identifications.

Py-GC/MS can provide both qualitative and quantitative information regarding pyromarkers. One common purpose for this technique is to determine the nature of the material that was pyrolyzed. This is possible either through the use of the composition of the pyrolytic products as a chromatographic pattern of the parent molecule, or from the similarity of the structure of fragment molecules from the pyrolytic product with that parent compound. Qualitative analysis in Py-GC/MS is based on the identification of various peaks in the

chromatogram with the help of MS database. The analysis can be limited to the identification of the main components in the pyrolytic products, or it may include minor components and sometimes trace compounds.

Py-GC/MS can be applied for quantitative purposes, although less common than qualitative analysis. Quantitative analysis is applied more frequently in polymer analysis, for example, in the determination of the amount of a specific polymer in a given complex matrix, such as a composite material, inorganic matrix, etc.^{78,89,90} Quantitative measurement of a pyrolysis product can be used successfully even for the analysis of other organic molecules.^{78,89} In order to have a reliable quantitative result by Py-GC/MS, an important requirement is the repeatability of the analysis. It has long been recognized that small samples, typically less than 1 mg, good heat transfer and rapid heating of the pyrolysis element are essential in achieving reproducibility.^{77,78,83,89} With same instrumental conditions and the same amount of sample, the reproducibility can be good with replicate analysis with relative standard deviations less than 1% frequently reported.^{78,83} Calibration is typically necessary by using internal standards, external standards, or a standard addition procedure.

A limitation to this process is that Py-GC-MS cannot completely mimic all factors of the smoking conditions such as humidity, airflow, and fluctuations in temperature. The pyrolysis unit can only perform and alter one temperature condition at a time. Even with these apparent limitations, pyromarkers produced by pyrolysis have been consistent with literature reports of smoked markers for cocaine and methamphetamine.^{21,22,91} Work produced by the Bell Research Group, including this research, has led to publications in the *Journal of Forensic Science* and the *Journal of Analytical Toxicology*, and both have been

cited in literature. Further research on this method will be used to study other smoked drugs such as Ritalin and other new designer drugs such as “bath salts” and synthetic cannabinoids.

Chapter 2: Application of the Py-GC/MS to Mimic the Smoking Conditions of Fentanyl and FTPs

2.1.0 Introduction

The method by which fentanyl is smoked can influence in the prominent pyromarkers observed. Methods used to smoke fentanyl include: glass pipes, cigarettes, and a method known as “chasing the dragon.” For over 30 years, Baker has been the pioneer on manufactured tobacco cigarette studies carrying out extensive analysis on temperature distribution and designed effects on tobacco pyromarkers. As recently as 2005, Baker and Bishop had used a similar pyrolysis unit to analyze non-volatile tobacco ingredients.^{23,24} Fentanyl smoking studies was therefore be based on what has been learned from manufactured tobacco cigarette studies, since cigarettes studies have been the forefront of smoking analysis due to its popularity tobacco over many years. Old dried gel drug reservoir FTPs or matrix type FTPs are smoked in a cigarette fashion, therefore the temperature distribution can be compared to temperatures which can reach from ambient to about 950 °C in the presence of varying concentrations of oxygen.⁹²

In general, Baker indicated that smoke from a tobacco cigarette is formed in two distinct zones of puffing and smoldering. The combustion zone takes place in the region of the lit cigarette where temperatures generally exceed 600 °C, called the coal.⁹³ From temperatures that can exceed ~900 °C with heating rates as high as 500 °C/s at the center of the coal in the presence of varying concentrations of oxygen such as during the puff of the cigarette.⁹⁴⁻⁹⁷ The second zone, immediately behind the coal, has temperatures below 600 °C and the tobacco is distilled and/or pyrolyzed to produce more chemically complex mixture of gases and vapor.⁹⁸ The generation of smoke is the result of a self-sustaining combustion cycle in which occurs puffing and smoldering conditions. During the puff, air is drawn in through the coal increasing the combustion rate and the coal temperature, with a typical puff

cycle of 2 s, can achieve heating rates as high as 500 K/s.⁹⁵ During the puff, the smoke generated during travels through the tobacco column. These mixtures of hot gases/vapors cool as they transfer down the column. As the distance from the coal to the filter reduces, the average temperature in the tobacco column increases, permitting more of the higher molecular weight compounds to remain in the vapor or aerosol phase.⁹⁵ The result is a more concentrated mainstream smoke further down the column as the tobacco is smoked.

During smoldering, combustion is maintained by convective flow around the burning zone that utilizes the natural airflow to carry the gases and vapors. Baker goes into further detail that incorporates distinction generation sites for side stream smoke. However, the author indicates that little change occurs in the oxygen concentration and temperature profiles of the side stream cigarette smoke, even during the puff.⁹⁹ By this, the authors demonstrated the independence of the combustion processes occurring at the surface and within the burning zone.

The intensity, duration and number of puffs, depth of inhalation, degree of mixing of smoke with air, and other factors influence the dose making smoking parameters complex to mimic.⁸ The absorption and bioavailability of inhaled drugs vary from person to person and potentially from smoke to smoke permitting the smoker considerable latitude in adjusting the dose to desired levels.⁸ Although these limitations are apparent and difficult to mimic using analytical pyrolysis, the products produced by pyrolysis of methamphetamines studied previously have been consistent with literature reports of smoked markers.^{20-22,91,100}

Although fentanyl produces the greatest analgesic effects is when it is volatilized at 300 °C, it is perceived that most drug abusers do not ascertain this temperature while smoking the drug.⁶⁰ Based on the reported average temperature during the rested smoldering

of coal for a manufactured tobacco cigarette^{93,95} and the recent successful pyrolysis analysis on cocaine and methamphetamine,²⁰ 750 °C was used to assess pyromarkers of fentanyl here. In addition, the pyrolysis unit is utilized in the trapping mode to afford collection of the pyromarkers and the use of a reactant gas as the carrier gas without introducing that gas to the GC. The reactant gas used here for analysis was helium and air for both anaerobic and aerobic conditions, respectively.

2.2.0 Materials and Methods

Pyridine, benzaldehyde, benzyl chloride, phenylacetaldehyde, (2-chloroethyl)benzene, propionanilide, and methanol were purchased from Sigma Aldrich (St. Louis, MO, USA). Fentanyl HCl was provided by the Department of Basic Pharmaceutical Sciences at West Virginia University. Norfentanyl oxalate was purchased from Cerilliant (Round Rock, TX, USA). Despropionyl fentanyl was purchased from Biomol (Plymouth Meeting, PA, USA). Unopened Duragesic® 75 µg/h reservoir FTPs were recovered from cases at the West Virginia Office of the Chief Medical Examiner.

2.2.1 Py-GC/MS

A CDS Analytical 5200 Pyroprobe was used in trapping mode to pyrolyze the samples under anaerobic and aerobic conditions using helium and air, respectively (CDS Analytical, Inc., Oxford, PA, USA). The sorbent tube traps were purchased from CDS Analytical, Inc. One was packed full with 20:35 mesh Tenax-TA and the other was one half-glass filled with glass beads and the other half filled with 20:35 mesh Tenax-TA. The pyroprobe was interfaced with a heated transfer line to a Perkin Elmer Clarus 500 GC/MS (PerkinElmer, Wellesley, MA, USA). The GC was equipped with an Equity-5 column (30 m, 0.25 mm, 0.25 µm film thickness, Supelco, Bellefonte, PA, USA). The MS was equipped

with an electron ionization source and quadrupole mass analyzer with a scanning range of m/z 50–600.

The components of the CDS pyroprobe unit consist of the pyroprobe, trap, accessory, valve oven, and transfer line (Figure 1-10). Solid sample analyses were performed by sandwiching 50-100 μg of fentanyl HCl between quartz wool inside the quartz tube. The quartz tube was inserted into the platinum coil filament of the pyroprobe. Each Duragesic® 75 mcg/h reservoir FTP contains 7.5 mg of fentanyl.¹⁰¹ Less than 6% of the gel matrix was extracted from the drug reservoir for each run (Figure 1-1A). The pyrolysis and GC conditions for solid fentanyl HCl and the gel from the reservoir of the patch are presented in Table 2-1. Solid fentanyl HCl and the FTP gel samples were analyzed using anaerobic and aerobic conditions. All samples were analyzed in triplicate to establish precision. Under the anaerobic conditions helium was used as the carrier gas in the pyroprobe and GC unit. For aerobic conditions, air was used as the reactant gas and helium was used as the carrier gas.

Figure 2-1 is a representation of the pyroprobe temperature program in trapping mode. As the pyroprobe is being heated, the reactant gas carries the volatile components to the sorbent trap. During the last minute of flow to the trap, the carrier gas is automatically changed to helium to remove the residual air from the trap before transferring the trap to the GC using the transfer line.

Table 2-1. Py-GC/MS parameters.

Pyroprobe Conditions	
Pyroprobe Temperatures	
Initial	50 °C, 1 s
Ramp Rate	20 °C/s
Final	750 °C, 10 s
Accessory Parameters	
Rest	50 °C
Initial	90 °C, 1 min
Ramp Rate	100 °C/min
Final	350 °C, 5 min
Trap Temperatures	
Reactant Gas	7.10 min
Rest	50 °C
Desorb	300 °C, 2 min
Iso Zones	
Transfer Line	305 °C
Valve Oven	305 °C
GC Conditions	
Injector Port	305 °C
Initial	70 °C
Ramp Rate	15 °C/min
Final	325 °C, 3 min hold

Figure 2-1. Schematic of a pyroprobe unit coupled to a GC/MS.

2.3.0 Results and Discussion

The pyromarkers of fentanyl HCl and FTPs under anaerobic and aerobic conditions are listed in Table 2-2. Pyromarkers were initially identified using the National Institute of Standards and Technology (NIST) MS library. Identification was confirmed by analysis of SRMs and comparison of retention times and mass spectra. Styrene and aniline were the only pyromarkers that were tentatively identified solely by NIST MS database. The percent areas of each pyrolytic product were normalized relative to the largest peak area in the chromatogram. Under all conditions, propionanilide resulted in the largest peak area in the chromatograms under both anaerobic and aerobic conditions.

Norfentanyl and despropionyl fentanyl are two commonly reported metabolites of fentanyl in the human body by piperidine N-dealkylation and amide hydrolysis, respectively.⁴⁶ Despropionyl fentanyl, aniline and phenylacetaldehyde were only observed under anaerobic conditions of the pyrolysis of fentanyl HCl. Norfentanyl and benzyl chloride were observed only in aerobic conditions of the pyrolysis of fentanyl HCl. Figure 2-2 displays commonality and differences in pyromarkers under the both anaerobic and aerobic conditions along with their structures.

Table 2-2. Pyromarkers of fentanyl and FTPs under anaerobic and aerobic conditions.

	Anaerobic	Area %	Aerobic	Area %
Fentanyl HCl	Pyridine	35.1	Pyridine	18.7
	Styrene	36.9	Styrene	17
	Benzaldehyde	1.1	Benzaldehyde	18.9
	Aniline	5.4	Benzyl chloride	8.8
	Phenylacetaldehyde	3.7	Phenylacetaldehyde	16.1
	(2-Chloroethyl)benzene	7.5	Propionanilide	100
	Propionanilide	100	Despropionyl fentanyl	1.1
	Despropionyl fentanyl	1.9	Fentanyl	3.1
	Fentanyl	6.9		
FTP	Pyridine	17.4	Propionanilide	100
	Styrene	39.8	Fentanyl	7.9
	Propionanilide	100		
	Fentanyl	41.6

Figure 2-2. Fentanyl pyromarkers comparisons in anaerobic and aerobic trapping conditions. *Denotes commonly reported metabolites.⁴⁶

2.3.1 Pyrolytic Degradation Pathways of Pyromarkers

Figure 2-3 shows the possible pyrolysis degradation pathways of fentanyl. The pyrolysis degradation mechanisms are typically free radical processes and are initiated by breaking the weakest bonds first.⁵ The compositions of these pyromarkers are typically based on the stability of the free radicals involved and the stabilities of the product molecules.⁵ The bond break at F1 of the carbonyl group at the amide site can result in the minor pyromarker of despropionyl fentanyl. Aniline is likely due to the less common α -elimination on two C-N bonds at F1 and F2 at the amide site. At temperatures above 700 °C, it has been reported that aniline starts decomposing with the formation of benzene, N₂, HCN, and other molecules including low levels of carbazol.⁸⁵ Cleaving at F1 and the C-N bond (F3) at the piperidine ring of fentanyl allowed for the formation of norfentanyl.

The formation of propionanilide is possibly a result of the cleavage of the C-N bond (F2) at the amide site where a β -elimination reaction occurred with the hydrogen of piperidine ring of fentanyl. The formation of pyridine can be explained by the initial formation of free radical during elimination cleaving at F2 and F3. The unsaturated piperidine ring may undergo dehydrogenation producing the pyridine ring.¹⁰²

Styrene, phenylacetylaldehyde, and (2-chloroethyl)benzene is due to the fragmentation at F3 producing a free radical. The phenyl group, which are sp^2 hybridized and more electronegative than sp^3 hybridized carbon atoms of alkyl groups, were not disrupted.¹⁰³ In the presence of free oxygen, slight differences can be observed. In its ground state, each of the two highest occupied molecular orbitals, which are degenerated, contain unpaired electrons, which allows ordinary oxygen the property of a diradical.⁸⁵ This can react with the pyromarker free radical producing phenylacetaldehyde. Since fentanyl HCl salt was used for

analysis, free radical halogenation can occur, which may explain the results of (2-chloroethyl)benzene.

The cleavage of the C-C bond (F4) next to the nitrogen at the piperidine ring of fentanyl likely caused the formation of benzaldehyde and benzyl chloride. It is feasible that these similar elimination reactions, as previously mentioned, produce free radicals that react with oxygen and chlorine to produce benzaldehyde and benzyl chloride, respectively.

Mass spectral fragmentation patterns were as expected, with one exception. Based on the abundance of propionanilide, it was thought that some form of phenyl-substituted pyridine might be observed such as 1,2,3,6-tetrahydro-1-(2-phenylethyl)pyridine (PEP) shown in Figure 2-3. This was of toxicological interest given structural similarities of PEP to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a known neurotoxin.¹⁰⁴⁻¹⁰⁶ Under the chromatographic conditions used, this compound, if present, would have been detected. However, none was observed in the total ion chromatograms. Using known spectral data,¹⁰⁷ a selected ion monitoring experiment was performed using all the same conditions except monitoring ions 187 (M+), 96, 91, 77, 65, and 42. The compound was not observed and it was hypothesized that a rearrangement occurred leading to the products that were observed.

Manral et. al. however, had observed PEP, along with propionanilide, at 500 °C using a direct injection and no reactant gas.¹⁰² Further pyrolysis at 750 °C showed increased degradation believed to be formed as a result of intramolecular rearrangements in the parent molecule.¹⁰² This is of toxicological importance in the observation by Manral et al. that at 750 °C phenylisocyanate may lead to the formation of hydrogen cyanide, which would be a large factor in the toxicity of smoked fentanyl.¹⁰²

Garg et al. also has observed this PEP through thermal degradation of the fentanyl residues and not the volatile vapors. It is explained the fentanyl is converted to PEP and propionanilide via solvent mediated thermal β -elimination, followed by N-oxidation, protonation, and rapid dehydration.¹⁰⁸ Garg et al. explained in detail how PEP is further degraded and reacted to form PEP salts and 1-styryl-1H-pyridin-2-one (1-SPO) detected in thermal degradation residues.

Figure 2-3. Possible pyrolysis degradation pathways of fentanyl.

Figure 2-4. Fentanyl pyromarkers from Manral et al.

2.3.2 Comparison of Fentanyl HCl and FTPs Under Anaerobic and Aerobic Conditions

A comparison of the pyrolysis of fentanyl HCl and FTPs under anaerobic conditions is shown in Figure 2-5. Fentanyl HCl and the FTP pyrolyzed under anaerobic conditions produce pyridine, styrene, propionanilide, and fentanyl. The peaks enclosed by the circle on the FTP chromatogram resulted from phthalates and longer chain alcohols. The remaining peaks in the FTP chromatogram could not be identified. Under aerobic conditions, propionanilide and fentanyl were observed in both the pyrolysis of fentanyl HCl and FTP (Figure 2-6). Again, phthalates and long chain alcohols were present throughout the FTP chromatogram hindering the identification of the well-defined peaks. Chlorine-containing pyromarkers obtained with the neat drug were not observed for the FTPs since fentanyl in the gel reservoir of the FTPs is in citrate salt form. This difference could potentially be useful as an investigative tool. Also, significant long chain alcohols and phthalates were seen and likely derived from the gel matrix of the drug reservoir. Area and peak height ratios were not reproducible for the pyrolysis of the FTPs due to difficulty in controlling the amount of gel in each sample introduction.

A trap that consisted of 50% glass beads (60:80) and 50% Tenax-TA (60:80) was employed in an attempt to address carryover problems. It was anticipated that some of the fentanyl would condense on the glass beads before reaching the Tenax-TA. Since the glass beads possess minimal sorbent characteristics, they should simply provide a surface area for condensation. The fentanyl should be stripped from the glass beads when the tube is heated. However, this trap configuration displayed poor desorption efficiency (Figure 2-7) compared to Tenax-TA alone. Therefore, a full Tenax-TA trap was employed for the remaining experiments.

A drawback of using the pyroprobe without a trap is poor chromatography of pyridine and styrene following pyrolysis of fentanyl. This is likely due to their volatile properties for the adsorption of the molecules and its physical adherences to the surface of the Tenax-TA that allow for the collection and efficient desorption. In addition, identification of some compounds was problematic without the trapping mode. No significant differences were seen beyond this observation. The trap enhanced the more volatile pyromarkers and afforded a positive identification in mass spectral comparison due to the improved chromatographic peak shape.

Figure 2-5. Fentanyl HCl and FTPs in anaerobic conditions.

Figure 2-6. Fentanyl HCl and FTPs in aerobic conditions.

Figure 2-7. FTP chromatogram using 50% Tenax-TA and 50% glass bead trap.

2.4.0 Conclusions

Propionanilide is a major pyromarker that was observed under both aerobic and anaerobic conditions in the pyrolysis of fentanyl HCl and FTPs. Aerobic and anaerobic conditions yielded slightly different products in the pyrolysis of fentanyl HCl. This study shows the possibility of differentiation between smoked fentanyl HCl salt and the FTPs.

Pyrolysis of fentanyl HCl and FTPs resulted in identification of several potential pyromarkers distinctive to smoked fentanyl and not seen as typical metabolites. The following was observed consistently in the pyrolysis of the fentanyl HCl under both the anaerobic and aerobic conditions: pyridine, styrene, benzaldehyde, phenylacetaldehyde, and propionanilide. Pyridine could be amenable to a simple headspace assay. When patches are smoked directly, a large suite of potential pyromarkers was identified. In this application, it may also be possible to identify what salt form of the drug was smoked based on the pyromarkers produced.

An assessment of actual accrued temperatures of smoking fentanyl patches is done in later chapters. This will focus on any changes and possible differences of pyromarkers at different temperatures reached. The next chapter will discuss the determination of these data points using thermocouple temperature analysis of smoking FTPs.

Chapter 3: Determination of the Temperature Characteristics of Smoking FTPs Using the “Smoking the Dragon” Method

3.1.0 Introduction

The previous chapter has discussed the pyromarkers at a temperature that was determined through the analysis of manufactured tobacco cigarettes. Here, the temperature analysis would need to be determined first to examine which pyromarkers are produced and most abundant between various smoking methods of FTPs. Temperature variance between methods of smoking drugs may lead to the production of different pyromarkers. Smoking methods can result in different temperature ramping rate and peak temperatures and therefore, a smoking method with higher peak temperature will further degrade pyromarkers than a lower temperature smoking method. As a result, this can produce adversely different effects in drug abuser since the dominant or more pronounced pyromarkers would change as a result of different smoking method used.

It is shown by Baker that the highest solid phase temperatures of ~900 °C, occurs in the periphery of the coal, whilst the gas phase temperature in the same region is 605-770 °C. Also, fentanyl smoking methods do not have a well defined/designed coal zone for smoldering like manufactured tobacco cigarettes. If fentanyl were to be smoked like a cigarette it would be hand-rolled and not uniform. One obvious difference in their burning characteristics is that hand-rolled cigarettes tend to go out unless puffed by the smoker, whereas a cigarette with tightly packed tobacco will smolders for about twenty minutes with no assistance from the smoker.¹⁰⁹ In most cases, drug abusers want to extinguish the burning of the drugs so as not to waste anything before the next hit. Also, it has been reported that the temperatures reached from “chasing the dragon” with heroin were between 200 °C and 300 °C, which is much lower than the tobacco cigarette studies.¹¹⁰

In the past, a number of temperature measurement methods have been used to determine the temperature distribution of tobacco cigarettes. These include thermocouples inserted into the cigarette, optical pyrometry, infrared photometry, and X-ray scanning of beads embedded in the tobacco, which melt at various temperatures.⁹⁵ Temperature distribution detection methods will be modeled on those described by Baker. In the analysis of tobacco cigarette temperature distribution, Baker employed two different methods of measurements, one for the solid phase (infrared system) and one for the gas phase (thermocouple system), and his results are generally accepted as being representative of the temperatures encountered inside a burning cigarette.¹¹¹ Although Laszlo et al. criticized that the thermocouple measurements for the solid phase inside a cigarette coal was inaccurate due to the poor thermal contact between the thermocouple junction and tobacco strands, Baker proved its insignificance.¹¹² The authors showed that occasionally (< 1%) one of the replicates values would be significantly different from the mean. This was presumably caused by an abnormal strand-junction contact, the thermocouple indicating a temperature dependence on both the gas and solid phases.¹¹²

Here a method was developed to determine the maximum temperature achieved and the rate of temperature increase by smoking FTPs using the common method of “chasing the dragon.” Bare thermocouple sensors were used for temperature analysis in a setup is furthered explained in this chapter. A box fabricated with Lexan® with an open top was constructed to shield airflow whilst under the fume hood. At the time of analysis, reservoir FTPs (Figure 1-1A) had entirely phased out of the pharmaceutical market due to the misuse and only matrix FTPs (Figure 1-1B) were available.

3.2.0 Materials and Methods

Duragesic® 100 mcg/h fentanyl transdermal system patches were obtained from McKesson (San Francisco, CA, USA). HPLC grade methanol was purchased from Fisher Science (Fair Lawn, NJ, USA).

3.2.1 Thermocouple

Temperature analysis was performed using a Type K, CHAL-010 thermocouple (Omega, Inc., Stamford, CT, USA). The Type K unsheathed fine gage thermocouples were 0.25 mm in diameter and 24 inches in length. The thermocouple sensors were composed solely of a Chromega® (nickel-Chromium) positive leg and an Alomega® (nickel-aluminum) negative leg that are beaded to create the thermocouple circuit. SMPW glass filled nylon Type K miniature connectors were used to connect the thermocouple sensors to the Datalogger. Temperature range for Type K alloys is -270 to 1372 °C.⁸¹

A HH506RA Dual Input, High Accuracy Datalogger/Thermometer with NIST traceable calibration with points was used (Omega, Inc., Stamford, CT, USA). The datalogger is a portable digital thermometer that can measure K, J, S, T, E, and N thermocouples with real-time data and data logging capabilities. A RS-232 cable was installed to connect the datalogger to the computer com port. Data acquisition was set to record the temperatures of the two thermocouples at 1 °C/s using the HH506RA software provided. Data obtained were in seconds for the time, temperature in Celsius for both T1 and T2, as well the as the temperature difference between T1 and T2 was recorded on the computer software. Data is then exported to Microsoft Excel worksheet.

3.2.2 Setup Diagram

Due to safety hazards associated with burning FTPs, the apparatus was positioned under a fume hood. A barrier was constructed to shield airflow from the fume hood so that the butane lighter could produce a steady laminar flame. Using Lexan®, a four-sided box shaped barrier was constructed according to the sketch shown as Figure 3-1. Slip-over panel piano hinges made of a polyvinyl carbon and polyester were used for ease of construction and manipulation.

This box here contained a ring stand to hold the FTP sample on a single layer of typical household aluminum foil and a sliding butane flame platform. Holes in the box were arranged so that the thermocouple sensors could go into the box and that the butane flame introducer could be positioned under the FTP sample.

Figure 3-1. Schematic of box set up with A) front angle view, B) front view, C) left view and D) right view.

The sample was heated in single layer of typical aluminum foil that was cut in a circle with a diameter of 6.5 cm and placed on a 6 cm in diameter ring on a stand. Due to safety features and the constant need for pressure to depress the gas release valve on typical cigarette lighters, a Microflame Torch Model B with only butane gas was used. A relaxed laminar flame allowed for an accurate simulation for the street practice of smoking FTPs by means of “chasing the dragon.” The tip of the flame was centered in the middle of the foil and was approximately 0.8 cm away from the underneath of the foil (Figure 3-2). The exact location of the flame was fixed as much as possible to eliminate variables of the analysis.

The pieces of the FTP were cut in 1.0 cm in width and 3.6 cm in length. The FTP piece was folded over thermocouple in channel port 1 on the datalogger (T1) and sandwiched in the middle of the FTP sample (Figure 3-3). The thermocouple in channel port 2 on the data logger (T2) rested on the very top of the sandwiched piece of FTP (Figure 3-3). Glass rods, electrical tape, and custom made glass rod holders were used to help position the thermocouple sensors accordingly.

For each FTP sample analysis the following events occurred: The butane release valve was rotated and a flint striker was used to create the spark to ignition. The butane lighter was then positioned on the platform within the designated markers. The door to the Lexan® box was closed and the fume hood sash was lowered. Data acquisition by the computer software was initiated and the flame was introduced by sliding the butane flame platform within the designated marking to insure fixed positioning after repeated runs. Once smoke was observed visually, the flame remained positioned for 10 s. After 10 s elapsed, the flame was removed by sliding the butane lighter platform back to its original location. Data

collection on the software remained approximately 30 s after flame was removed from the FTPs. Data from the software was then exported to Microsoft Excel worksheets.

Due to the cost of FTPs, the process was developed and optimized using typical office tape to mimic the FTPs. Here the markers for positioning were affixed into position using colored tape. Once a protocol was set, FTPs were used for a total of 16 trials.

Figure 3-2. “Chasing the dragon” temperature analysis setup inside the smoking box with two thermocouples and a dual input datalogger.

Figure 3-3. Close-up view of the T1 sandwiched in the middle and T2 rested on the top of the FTP sample on the foil.

3.3.0 Results and Discussion

The temperature results of the 16 trials in regards to the moment smoke was visually observed, maximum temperature obtained while burning, and the rate of temperature increase is summarized in Table 3-1. The full results of each trial run are located in Appendix A, where the asterisk denotes trial where the setup failed where the thermocouple sensors fell out of place during the burning process. The smoke temperature is the temperature at which smoke was visually observed emanating from the sample. These values were not consistent between trials as noted in Appendix A. This is most likely due to the fact that the heating of the FTP was not evenly distributed which is expected with the smoking method being studied. This factor is not critical in the scope of the present analysis; rather, determining the range of temperatures observed was crucial. Using this method, the maximum recorded temperature was 315.1 °C. This temperature is in rough agreement with that report for the heroin studies, but significantly lower for other fentanyl studies mentioned earlier.

The maximum temperature is the highest temperature obtained in the smoking process. This temperature was of importance in this study since the values used in Chapter 2 were of temperatures obtained in manufactured tobacco cigarettes. The temperature under cigarette smoldering conditions was hypothesized to be high relative to “chasing the dragon”. The temperatures recorded ranged from 214.1 - 441.6 °C, with most trials being between 250 °C and 300 °C (failed trials omitted).

The rate of temperature increase was assessed by the starting recorded room temperature of the FTPs to the maximum temperature obtained by the FTPs by heating. The rates were given as °C/s. These values ranged from 8.3 °C/s to 23.0 °C/s, with most of the

recorded values being around 20 °C/s. This value is the same rate of increase as the one used in the previous pyrolysis study in Chapter 2.

Figure 3-4 is a plot of T1 and T2 over time for Trial 8 analysis. This is just one example of the general trend of the temperature increase of each of the thermocouples. In this specific trial, it is observed that T1 has a faster rate of temperature increase and has a higher maximum temperature. In general, the trends vary slightly between trials, however, this is also not a critical factor of the overall scope of the analysis.

Table 3-1. Temperature data of smoking FTPs using the “chasing the dragon” method.

	Smoke Observed Temp.		Max. Temp.		Rate Increase	
	T1 (°C)	T2 (°C)	T1 (°C)	T2 (°C)	T1 (°C/s)	T2 (°C/s)
Max	315.1	308.6	441.6	397.7	28.0	24.9
Average	87.6	110.2	274.1	289.5	12.0	16.7

Figure 3-4. Comparison of T1 and T2 over time for Trial 8.

3.4.0 Conclusions

The temperature analysis was essential for determining realistic temperature estimations obtained during smoking of the FTPs by means of the “chasing the dragon” method. This experimentation allowed for a more accurate determination of parameters settings for the Py-GC/MS analysis of fentanyl rather than basing the parameters on manufactured tobacco cigarettes studies. Here it is noted that temperatures never exceeded 450 °C at either the smoke temperature or the maximum temperature readings. This is well below the 750 °C pyrolysis temperature used in Chapter 2 that was again based on manufactured tobacco cigarettes. The rate of temperature increase was approximately 20 °C/s for most of the trial runs in the experiment. Data obtained indicates that manufactured tobacco cigarette temperature is not applicable here under. The knowledge gained here will help determine if at lower temperatures different pyromarkers are present or even different pyromarkers is most dominant besides propionanilide noted in Chapter 2. The temperature parameters determined here is applied to the Py-GC/MS for the analysis of pyromarkers in both fentanyl and FTPs samples in the following chapter.

Chapter 4: Application of Temperatures Characteristics Obtained from Smoking FTPs By Means of “Chasing the Dragon” on the Py-GC/MS

4.1.0 Introduction

This chapter reflects the pyrolysis parameters that were obtained in the previous chapter that used thermocouples for temperatures analysis on smoking FTPs by means of the “chasing the dragon” method. The results from Chapter 3 indicated that the temperatures obtained by smoking FTPs by means of "chasing the dragon" did not reach to the 750 °C temperatures that were used in the previous study. Although 450 °C is the maximum temperature observed in the thermocouple temperature study, these lower increments of temperature were evaluated to see if 50 °C temperature variations made any differences.

As stated previously, Manral et al. analyzed the pyrolysis of fentanyl from temperature of 250 to 900 °C using a large sample size of 50 mg.¹⁰² It has long been recognized that small samples, typically less than 1 mg, good heat transfer and rapid heating of the pyrolysis element are essential in achieving reproducibility.^{89,113} The smaller the sample size, the more likely it is to pyrolyze in a uniform and reproducible way by having all of the sample material in close contact with the pyrolyzer to experience the same temperature at the same time.^{83,90}

Manral et al., also found only fentanyl and no pyromarkers observed up to 350 °C, therefore concluding that fentanyl was stable up to 350 °C. At 500 °C, propionanilide and PEP were the only pyromarkers observed. With this, the authors concluded that higher temperatures of 750 °C resulted in extensive degradation of not only the parent fentanyl compound itself, but also of the propionanilide and PEP. Manral et al. pyrolyzed propionanilide and PEP at 750 °C and determined 3-methylindone and phenylisocyanate as a result of intramolecular rearrangements (Figure 2-4).¹⁰² As stated before, the authors

ascertained that phenylisocyanate may lead to the formation of hydrogen cyanide, which would be a large factor in the toxicity of smoked fentanyl.¹⁰²

Data from this project does not support the idea that fentanyl is stable up to 350 °C as stated by Manral et al. and is evaluated at lower temperatures. Here six temperatures were evaluated from 200 to 450 °C in 50 °C increments on the Py-GC/MS. This is to observe any differences seen in regards to unique or prevalent pyromarkers present in the smoking process in comparison to the higher pyrolysis temperature previously studied.

4.2.0 Materials and Methods

Fentanyl and norfentanyl oxalate, both in methanol solvent were obtained from Cerilliant Corporation (Round Rock, TX, USA). Despropionyl fentanyl was purchased from Biomol (Plymouth Meeting, PA, USA). Duragesic® 100 mcg/h fentanyl transdermal system patches were obtained from McKesson (San Francisco, CA, USA). Pyridine, benzaldehyde, phenylacetaldehyde, propionanilide, and HPLC grade methanol were purchased from Sigma Aldrich (St. Louis, MO, USA).

4.2.1 Py-GC/MS

Here the same CDS Analytical 5200 Pyroprobe was used in trapping mode to pyrolyze the samples under aerobic conditions using air and a full 20:35 mesh Tenax-TA sorbent tube trap (CDS Analytical, Inc., Oxford, PA). However, instead of the Perkin Elmer GC/MS, a Shimadzu GC/MS QP2010S was used (Shimadzu Corporation, Kyoto, Japan). The GC was equipped with an Rxi®-5Sil MS with Integra-Guard® (30 m, 0.25 mm i.d., 0.50 µm film thickness, Restek, Bellefonte, PA, USA). The MS was equipped with an electron ionization source and quadrupole mass analyzer with a scanning range of m/z 50–600. The

interface and ion source temperatures were 300 °C and 250 °C, respectively. GC/MS parameters were slightly altered from previous analysis with a lower final GC oven temperature to minimize the capillary column bleeding and prolong the life of the column (Table 4-1).

The components and workings of the CDS analytical pyroprobe unit were explained previously in Section 2.2.1. Analysis was done only in aerobic conditions where air was used as the reactant gas and helium was used as the carrier gas. Table 4-1 lists the parameters for the Py-GC/MS at the 6 different pyrolysis temperatures for both the fentanyl standards and the FTPs. All samples were run in triplicate to determine repeatability of the method, at least qualitatively. With the sorbent tube trap, the desorb temperature is necessary to desorb the collected analytes and transfer them to the GC at the beginning of the run. In addition, a 250 °C desorption temperature was assessed in the 200 °C pyrolysis analysis run. This is to observe any pyromarkers that were not able to desorb at 200 °C.

With a quartz tube filled with a plug of quartz wool inside the filament of the pyrolysis probe, 10 µg of the fentanyl solution was deposited using a microliter syringe. The fentanyl in methanol solution was a concentration of 1000 ppm. The sample was then dried to remove some of the excess solvent by heating the filament to 50 °C for 30 s. The probe was then inserted into the pyroprobe unit for pyrolysis under the set conditions.

Table 4-1. Py-GC/MS parameters for the pyrolysis of fentanyl standards and FTPs.

[illegible]

For the FTPs, the backings were removed and the entire patch was folded over on itself on the adhesive side for ease of handling. Then small pieces of approximately 1 mm by 1 mm were cut resulting to a weight ranging from 200-300 μg . Each piece was then sandwiched between two plugs of quartz wool in the quartz sample tube. The sample tube was then inserted in the coiled filament of the probe and successively into the pyroprobe unit for pyrolysis under the set conditions.

4.3.0 Results and Discussion

The pyromarkers of the fentanyl standard and the FTPs listed in Table 4-2 and 4-3. Appendix B includes results of all pyromarkers observed for each trial run. These pyromarkers were initially identified using NIST MS library. Identification was confirmed by analysis of SRMs by the comparison of retention times and mass spectra for the following analytes: pyridine, benzaldehyde, phenylacetaldehyde, propionanilide, norfentanyl, despropionyl fentanyl, and fentanyl. The other pyromarkers observed were considered to be tentatively identified based solely by the NIST MS database. The percent areas of each pyromarker were normalized relative to the largest peak area in the chromatogram.

4.3.1 Pyromarkers of Fentanyl Standard

It is shown here that propionanilide is observed under all pyrolysis temperature conditions with the highest peak area, as low as 200 °C, for the pyrolysis of the fentanyl standard (Table 4-2). This indicates that fentanyl itself is not stable below 350 °C as found in literature and hence is not likely suitable for volatilization for inhalation administration as sought by Manral et al. At 200 °C, only pyridine and fentanyl was observed; however, with 250 °C for in desorption temperature, styrene, benzaldehyde, aniline, phenylacetaldehyde,

and propionanilide was observed in addition. This increase in 50 °C in temperature was necessary of these pyromarkers to desorb off of the Tenax-TA trap.

Since fentanyl HCl was not used in this analysis due to availability, chlorinated pyromarkers were not observed as seen for the pyrolysis at 750 °C in aerobic conditions (Table 2-2). The only pyromarker other than the chlorinated ones observed at 750 °C and not here under any of these temperatures was despropionyl fentanyl. This may have been due the smaller sample size used here in comparison to the previous studies where 50-100 µg of solid fentanyl HCl were used. Overall, pyridine, styrene, benzaldehyde, aniline, phenylacetaldehyde, propionanilide, and fentanyl were consistently detected at all six temperatures of pyrolysis.

The pyrolytic degradation pathways of pyridine, styrene, benzaldehyde, aniline, phenylacetaldehyde, and propionanilide likely underwent the same reactions as explained in section 2.3.1. The only difference observed was 3-methyl-pyridine at 450 °C. The formation of this product is not well understood. It is the likelihood that the piperidine ring free radical allows for dehydrogenation for the pyridine ring and sigmatropic rearrangement for a 1,5-migration of the methyl group.⁸⁵

Table 4-2. Pyromarkers of the pyrolysis of the fentanyl standard.

200 °C		250 °C desorb	250 °C		300 °C	
	% Area	% Area		% Area		% Area
1. Pyridine		24.0	1. Pyridine	20.8	1. Pyridine	56.5
2. Styrene		20.0	2. Styrene	21.8	2. Styrene	47.2
3. Benzaldehyde		12.2	3. Benzaldehyde	8.3	3. Benzaldehyde	26.5
4. Aniline		3.8	4. Aniline	1.0	4. Aniline	2.9
5. Phenylacetaldehyde		2.9	5. Phenylacetaldehyde	3.3	5. Phenylacetaldehyde	8.9
1,6. Propionanilide	100	100	6. Propionanilide	100	6. Propionanilide	100
2,7. Fentanyl	8.2	3.1	7. Fentanyl	12.4	7. Fentanyl	63.2
350 °C			400 °C		450 °C	
	% Area			% Area		% Area
1. Pyridine	58.5		1. Pyridine	46.2	1. Pyridine	42.2
2. Styrene	47.1		2. Styrene	38.6	2. 3-Methyl-pyridine	1.1
3. Benzaldehyde	24.5		3. Benzaldehyde	20.2	3. Styrene	32.3
4. Aniline	2.5		4. Aniline	1.5	4. Benzaldehyde	21.1
5. Phenylacetaldehyde	7.8		5. Phenylacetaldehyde	6.3	5. Aniline	3.8
6. Propionanilide	100		6. Propionanilide	100	6. Phenylacetaldehyde	4.3
7. Fentanyl	42.1		7. Fentanyl	65.5	7. Propionanilide	100
					8. Fentanyl	24.6

Figure 4-2 shows the comparison of the GC chromatograms of the pyrolysis of the fentanyl standard under all pyrolysis temperature conditions. The chromatographic peaks labeled with numbers corresponding to their identification listed on Table 4-2. Peaks that are not labeled could not be identified using the NIST MS database. It is seen here that the chromatography pattern is similar and is observed at all three trials at each temperature condition.

There is a noticeable difference in comparison of the GC chromatograms of 200 °C with 200 °C (Figure 4-1A) and 250 °C (Figure 4-1B) for desorption of pyromarkers off the Tenax-TA trap to the GC/MS for analysis. In all instances, propionanilide is observed with the highest peak area, as well as peak intensity, at RT ~9.329 min. As the temperature of pyrolysis increases, the intensity of the pyromarkers increases.

Figure 4-1. Chromatograms of the pyrolysis of fentanyl standard at 200-450 °C.

Figure 4-1. *Continued.*

4.3.2 Pyromarkers of FTPs

The pyromarkers observed under all temperature conditions for the pyrolysis of the FTPs are located in Table 4-3. The analytes reported in this table were observed consistently in all triplicate analyses. All observed pyromarkers, including those that were detected in all three replicates, for each trial run are located in Appendix B. The same pyromarkers observed for the pyrolysis of fentanyl standard under all six temperature conditions were detected in the pyrolysis of the FTPs at the under the same conditions with the exception of aniline. Aniline contained the lowest percent area for the fentanyl standard, and thus, not surprising that aniline is not observed in the pyrolysis of the FTPs.

Additional pyromarkers observed besides those see in Table 4-2 can be attributed to the other unknown material that is incorporated into the manufacturing of the FTPs. Again, these materials include plasticizers and adhesives to allow the patch to adhere to the skin of patients for dermal absorption. At the pyrolysis of 200 °C, both of the desorbing temperatures produced the same pyromarkers. This is likely due to the increase in sample size of the FTP in comparison to the fentanyl standard sample size deposited on the quartz wool.

It can be noted that from 200 °C to 300 °C, propionanilide has the greatest peak area. At 300 °C, 2-ethyl-1-hexanol (2-EH) increases in peak area until 400 °C, where it surpasses the peak area of propionanilide. 2-EH is considered to be not acutely toxic in regards to inhalation in rat studies and is a synthetic alcohol that is mainly used in the manufacture of ester plasticizer primarily to used in produce soft polyvinyl chloride.¹¹⁴ Although it is commonly found in the environment, it has the potential to be a pyromarker of importance for investigative purposes.

Table 4-3. Pyromarkers of FTPs from 200-450 °C.

		250 °C desorb				
200 °C	% Area	% Area	250 °C	% Area	300 °C	% Area
1. Pyridine	21.9	14.6	1. Pyridine	27.6	1. Pyridine	30.5
2. Styrene	25.9	23.0	2. Styrene	33.1	2. 3-Methylene-heptane	41.1
3. Benzaldehyde	11.8	8.0	3. Benzaldehyde	17.4	3. 3-Heptanone	7.8
4. Phenylacetaldehyde	9.1	7.2	4. Aniline	2.2	4. Styrene	31.6
5. Propionanilide	100	100	5. 3-Pyridinecarboxaldehyde	1.1	5. 2-Ethyl-2-hexenal	2.1
6. Fentanyl	2.0	16.2	6. Benzyl Alcohol	0.7	6. 2-Ethyl-hexanal	15.1
			7. Phenylacetaldehyde	7.3	7. Benzaldehyde	26.5
			8. 1-Phenyl-1,2-propanedione	1.5	8. 2-EH	57.7
			9. Benzenecarboxylic acid	1.2	9. Phenylacetaldehyde	10.1
			10. Propionanilide	100	10. 1-Phenyl-1,2-propanedione	2.0
			11. N-(2-phenylethyl)-formamide	2.0	11. 2-Ethylhexyl ester acetic acid	1.2
			12. Fentanyl	1.5	12. Benzenecarboxylic acid	3.4
					13. 2-Ethylhexyl ester 2-propenoic acid	6.6
					14. Propionanilide	100
					15. N-(2-phenylethyl)-formamide	2.4
					16. 2-EEFA	2.3
					17. Fentanyl	1.8

Table 4-3. Continued.

350 °C	% Area	400 °C	% Area	450 °C	% Area
1. Pyridine	32.0	1. Pyridine	23.2	1. Pyridine	23.5
2. 3-Methylene-heptane	36.1	2. 3-Methylene-heptane	50.0	2. 3-Methylene-heptane	65.9
3. 3-Heptanone	5.1	3. 3-Heptanone	5.6	3. 3-Heptanone	7.9
4. Styrene	34.2	4. Styrene	17.8	4. Styrene	13.5
5. 2-Ethyl-hexanal	10.8	5. 2-Ethyl-2-hexenal	3.2	5. 2-Ethyl-hexanal	12.6
6. Benzaldehyde	27.6	6. 2-Ethyl-hexanal	13.6	6. Benzaldehyde	26.1
7. 2-EH	55.8	7. Benzaldehyde	21.1	7. 2-EH	100
8. Benzyl Alcohol	1.8	8. 2-EH	100	8. Phenylacetaldehyde	4.2
9. Phenylacetaldehyde	8.3	9. Phenylacetaldehyde	5.0	9. 2-Ethylhexyl ester acetic acid	4.3
10. 2-Ethylhexyl ester acetic acid	1.1	10. 2-Ethylhexyl ester acetic acid	1.8	10. Benzenecarboxylic acid	3.4
11. Benzenecarboxylic acid	2.8	11. Benzenecarboxylic acid	2.7	11. 2-Ethylhexyl ester 2-propenoic acid	27.9
12. 2-Ethylhexyl ester 2-propenoic acid	4.8	12. 2-Ethylhexyl ester 2-propenoic acid	13.1	12. 2-Ethylhexyl methacrylate	3.1
13. Propionanilide	100	13. 2-Ethylhexyl methacrylate	1.6	13. n-Butyric acid 2-ethylhexyl ester	1.3
14. 2-EEFA	2.1	14. 2-EECA	1.8	14. 2-EECA	2.7
15. Fentanyl	1.7	15. Propionanilide	69.1	15. Propionanilide	63.0
		16. 2-Ethylhexyl ester pentanoic acid	3.8	16. 2-Ethylhexyl ester pentanoic acid	5.2
		17. 2-EEFA	3.8	17. 2-EEFA	1.9
		18. Fentanyl	2.7	18. Fentanyl	1.0

Figure 4-3 shows all the GC chromatograms of the pyrolysis of the FTPs under all pyrolytic temperature conditions. The peaks are labeled with numbers that coincide with the pyromarkers listed in Table 4-3. Peaks that were not labeled could not be identified through the NIST MS database. Again, most of these unidentifiable peaks were likely pyromarkers of the plasticizers of the FTPs since they are not observed in the fentanyl standard alone. For the 200 °C, both desorbing temperatures resulted in similar pyromarkers with higher peak intensity and areas for 250 °C (Figure 4-3A) than 200 °C (Figure 4-3B). Again, this higher temperature afforded more of the pyromarkers to desorb off of the Tenax-TA in the trap.

In Figure 4-3C, there is a magnified view of the chromatographic peaks for enhance viewing purposes. The pattern of the chromatograms is similar with greater intensity as the pyrolytic temperature increases. The increase in pyrolytic temperature produces an increase in number of peaks; however, most of those peaks were unidentifiable using the NIST MS database. At 350 °C and below, propionanilide dominates as having the largest peak area. Not until 400 °C and above, does 2-EH has the largest peak area and intensity in the chromatograms (Figure 4-3F and G).

Figure 4-2. Chromatograms of the pyrolysis of the FTPs at 200-450 °C.

Figure 4-2. Continued.

4.4.0 Conclusions

Overall, pyridine, styrene, benzaldehyde, phenylacetaldehyde, propionanilide, and fentanyl were consistently detected at all six temperatures of pyrolysis of both the fentanyl standard and the FTPs. These pyromarkers seen here were similar to that of the results under the pyrolytic conditions of 750 °C in section 2. Propionanilide has shown to have the highest peak area for both fentanyl standard and FTPs at all pyrolytic temperatures with exception to 400 °C and 450 °C for the FTPs analysis. At those temperature 2-EH increases to obtain the highest peak area and intensity. Although this plasticizer can be commonly found in the environment, it has the potential to be an additional marker for investigative purposes to link those who have smoked FTPs as mean of ingestion.

The increase in pyrolytic temperature for both the pyrolysis of the fentanyl standard and the FTPs increase the intensity of the pyromarkers detected. This increase in temperature has also allowed more chromatographic peak for the FTPs that were likely the inclusion of plasticizers, adhesives, and other materials that are incorporated into the manufacturing of the FTPs by the pharmaceutical company. Lastly, even as low as 200 °C for a pyrolytic temperature fentanyl is shown to degrade through elimination reaction to propionanilide. This clearly disputes the previously discussed literature references that stated fentanyl was stable up to 350 °C.

Chapter 5: Pyrolysis of Fentanyl and Heroin Mixtures

5.1.0 Introduction

Illicit drugs commonly contain other substances in addition to the active ingredient, which can have serious adverse health consequences or even cause premature death. In general, an “adulterant” typically refers to the pharmacologically active ingredients; “contaminant” refers to the by-products of the manufacturing process, and the “diluent” refers to inert substances.¹¹⁵ However, many have generalized the categories previously mentioned as adulterants. It has been seen that the adulterants used by drug dealers, drug users, and the general public include but not limited to mannitol, sugars, gravy powder, chalk, codeine, rat poison, ground glass, household cleaning products, and brick dust.¹¹⁵ Multiple combinations and variations of adulterants have been in illicit street samples of heroin. Some of the many reasons for the use of these adulterants in illicit drugs include to bulk, dilute, complement or even to enhance the effects of the drugs itself.¹¹⁵ Other explanations allude to unintentional addition as a result of manufacturing, productions, or storage techniques.

The quality assurance, sterile production, and accurate dosage administration cannot be upheld in the illicit manufacture in which drug sellers and users can only make inadequate assessments of the quality, purity, and chemical composition of any of the drugs they buy, sell, or use.¹¹⁶ Cases seen in recent years of heroin laced with fentanyl seem to enhance or mimic the illicit drug effects, either to give the impression of a better quality or mask the poor quality of the heroin obtained. This has been seen in other drugs, for examples: procaine and lidocaine have similar anaesthetic properties to cocaine; phenacetin has similar physical properties to cocaine; acetaminophen has similar bitter taste to (white) heroin; methylsulfonylmethane looks physically similar to methamphetamine; and caffeine has

similar (but milder) stimulant properties to cocaine, amphetamine, and ecstasy.¹¹⁵ Again, as mentioned earlier, caffeine in heroin has been observed to facilitate in volatilization of heroin.

As mentioned previously, near the end of 2005 and early into 2006, the government and media reported an increase in the number of individuals who have overdosed on heroin laced with fentanyl as a diluent. This fentanyl-laced heroin was a means of making up for the poor quality heroin. Given its toxicity drug dealers have trouble adjusting pure fentanyl into proper dosage concentrations. As a result, heroin users who knowingly or unknowingly use the substance containing fentanyl have difficulty determining how much to take to get their “high” and can mistakenly take lethal quantities of fentanyl.³⁶ Only a slight excess in the amount of fentanyl taken can be, and is often, fatal because the resulting level of respiratory depression is sufficient to cause the user to stop breathing.^{36,63,66} As previously noted, the pyromarkers of heroin are known. However, the combination of fentanyl and heroin pyromarkers has not been studied and is evaluated here. It was suspected that the pyrolysis of the combination of two different drugs had the potential to produce different pyromarkers not observed by pyrolysis as separate entities. This has been seen by the pyrolysis of methamphetamine mixed with tobacco.^{117,118}

Heroin base is preferred to heroin HCl, since the bases has a lower melting point of 173 °C than the HCl salt of 243-244 °C and because of its relative insensitivity to degradation.^{66,119} However, due to the availability, high cost, and time to obtain heroin base, heroin HCl was used here for analysis instead. Cook and Brine had observed heroin pyromarkers located in Figure 1-4 for both heroin base and heroin HCl at 250 °C.⁵⁷⁻⁵⁹

5.2.0 Materials and Methods

Fentanyl and 6-MAM standards were obtained from Cerilliant Corporation (Round Rock, TX, USA) and Sigma Aldrich (St. Louis, MO, USA). Heroin HCl standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol was purchased from Fisher Science (Fair Lawn, NJ, USA).

5.2.1 Py-GC/MS

The CDS Analytical 5200 Pyroprobe was used in trapping mode to pyrolyze the samples under aerobic conditions using air and a full 20:35 mesh Tenax-TA sorbent tube trap (CDS Analytical, Inc., Oxford, PA, USA). The pyrolysis unit was interfaced the Shimadzu GC/MS QP2010S. The GC was equipped with an Rxi®-5Sil MS with Integra-Guard® (30 m, 0.25 mm i.d., 0.50 μ m film thickness, Restek, Bellefonte, PA). The MS was equipped with an electron ionization source and quadrupole mass analyzer with a scanning range of m/z 50–600. The interface and ion source temperatures were 300 °C and 250 °C, respectively.

Analysis was done only in aerobic conditions where air was used as the reactant gas and helium was used as the carrier gas. All samples were run in triplicate for repeatability of method analysis. Table 5-1 lists the parameters for the Py-GC/MS at the 6 different pyrolysis temperatures for both the heroin standards and the heroin and fentanyl mixes. With the sorbent tube trap, a desorption temperature is necessary to desorb the collected analytes and transfer them to the GC at the beginning of the run. In addition, a 300 °C desorption temperature was determined to be necessary for to desorb the pyromarkers at 200 °C and 250 °C pyrolysis temperatures.

A sample of 1000 ppm solution was made for the pyrolysis of heroin alone. The 10 μg sample was deposited on a plug of quartz wool in the sample tube. In addition, three different samples mixes of heroin and fentanyl were prepared in ratios of 3:1, 1:1, and 1:3. Each sample mix was made from 1000 ppm stock solution of heroin and fentanyl. With a quartz tube filled with a plug of quartz wool inside the filament of the pyrolysis probe, 10 μL of the mixed solution was deposited using a microliter syringe. The sample was then dried to remove some of the excess solvent by heating the filament to 50 $^{\circ}\text{C}$ for 30 s. The probe was then inserted into the pyroprobe unit for pyrolysis under the set conditions.

Table 5-1. Py-GC/MS parameters for the pyrolysis of heroin standards and mixes.

Pyroprobe Conditions	200 °C	250 °C	300 °C	350 °C	400 °C	450 °C
Pyroprobe Temperatures						
Initial	50 °C, 1 s	50 °C, 1 s	50 °C, 1 s	50 °C, 1 s	50 °C, 1 s	50 °C, 1 s
Ramp Rate	20 °C/s	20 °C/s	20 °C/s	20 °C/s	20 °C/s	20 °C/s
Final	200 °C, 10 s	250 °C, 10 s	300 °C, 10 s	350 °C, 10 s	400 °C, 10 s	450 °C, 10 s
Accessory Parameters						
Rest	50 °C	50 °C	50 °C	50 °C	50 °C	50 °C
Initial	50 °C, 1 min	50 °C, 1 min	50 °C, 1 min	50 °C, 1 min	50 °C, 1 min	50 °C, 1 min
Ramp Rate	100 °C/min	100 °C/min	100 °C/min	100 °C/min	100 °C/min	100 °C/min
Final	200 °C, 5 min	250 °C, 5 min	300 °C, 5 min	300 °C, 5 min	300 °C, 5 min	300 °C, 5 min
Trap Temperatures						
Reactant Gas	6.50 min	7.00 min	7.50 min	7.50 min	7.50 min	7.50 min
Rest	50 °C	50 °C	50 °C	50 °C	50 °C	50 °C
Desorb	300 °C, 2 min	300 °C, 2 min	300 °C, 2 min	300 °C, 2 min	300 °C, 2 min	300 °C, 2 min
Iso Zones						
Transfer Line	300 °C	300 °C	300 °C	300 °C	300 °C	300 °C
Valve Oven	300 °C	300 °C	300 °C	300 °C	300 °C	300 °C
GC Conditions						
Injector Port	300 °C	300 °C	300 °C	300 °C	300 °C	300 °C
Initial	70 °C	70 °C	70 °C	70 °C	70 °C	70 °C
Ramp Rate	15 °C/min	15 °C/min	15 °C/min	15 °C/min	15 °C/min	15 °C/min
Final	300 °C, 4.67 min	300 °C, 4.67 min	300 °C, 4.67 min	300 °C, 4.67 min	300 °C, 4.67 min	300 °C, 4.67 min

5.3.0 Results and Discussion

Appendix C contains the chromatograms for the pyrolysis of the heroin standard and the results of identified pyromarkers of each of the heroin to fentanyl mixes. The pyromarkers of the heroin standard resulted in 6-MAM and heroin under all pyrolysis parameters. All pyromarkers were initially identified using NIST MS library. Identification was confirmed by analysis of SRMs and the comparison of retention times and mass spectra for the following analytes: pyridine, benzaldehyde, phenylacetaldehyde, propionanilide, norfentanyl, despropionyl fentanyl, fentanyl, 6-MAM, and heroin. The other pyromarkers observed were considered tentatively identified by NIST MS library search. The percent areas of each pyromarker were normalized relative to the largest peak area in the chromatogram. Under all temperature conditions, propionanilide resulted in the largest peak area in the chromatograms for both the fentanyl standard and FTPs.

The pyrolysis of heroin under all conditions resulted in the detection of heroin and 6-MAM (Appendix C). Unfortunately, heroin in the HCl salt form has a much higher melting point than the base form and did not produce any of the other pyromarkers with this method at the analyzed temperature that were previously reported in the literature. Nonetheless, it was interesting to note these results and how it would affect the pyromarkers with the addition of fentanyl.

Table 5-2, 5-3, and 5-4 show the results under all the pyrolytic conditions for the heroin to fentanyl mixes of 3:1, 1:1, and 1:3, respectively with a repeatability of $n=3$. All pyromarkers detected at each trial run as well as GC chromatograms are located in Appendix C. The pyromarkers that were common in all heroin to fentanyl mixes at all pyrolytic temperatures were the following: pyridine, styrene, benzaldehyde, phenylacetaldehyde,

propionanilide, 6-MAM, heroin, and fentanyl. Like the pyrolysis of the FTPs, aniline was not detected repeatedly in all trial runs.

5.3.1 Pyromarkers of the Pyrolysis of the 3:1 Heroin to Fentanyl Mix

For the 3:1 heroin to fentanyl mix, the only differences observed was the detection of norfentanyl at 200 °C and acetylbenzoyl at 250 °C (Table 5-2). It is unclear as to why the metabolite norfentanyl is observed here and not at any other pyrolytic temperature or other previous chapter. Also, at a 3:1 ratio, fentanyl is at its lowest concentration for analysis in comparison. The pathway for the formation of acetylbenzoyl is not clear as the formation could be likely similar to of phenylacetaldehyde from fentanyl or heroin which is due to the elimination reaction producing a free radical with a phenyl ring. The presence of oxygen allows the formation of acetylbenzoyl. This pyromarker has been detected in both the pyrolysis of the fentanyl standard and the FTPs; however it was not consistently observed in all three replicates (Appendix B).

5.3.2 Pyromarkers of the Pyrolysis of the 1:1 Heroin to Fentanyl Mix

For the 1:1 heroin to fentanyl mix, both norfentanyl and acetylbenzoyl was observed at 200 °C (Table 5-3). At 250 °C, acetylbenzoyl was similarly observed at 250 °C. In addition, at 400 °C, phthalic anhydride is observed. All other consistent reported pyromarkers were the same as mentioned.

5.3.3 Pyromarkers of the Pyrolysis of the 1:3 Heroin to Fentanyl Mix

For the 1:3 heroin to fentanyl mix, acetylbenzoyl is observed at 250 °C to 400 °C. With fentanyl having the higher concentration here, it is likely that that the formation of

acetylbenzoyl stems from the fentanyl component of the mix. Norfentanyl is observed at 400 °C, which is unusual given the previous results and trends.

Table 5-2. Pyromarkers of the pyrolysis of the 3:1 heroin to fentanyl mix.

200 °C	% Area	250 °C	% Area	300 °C	% Area
1. Pyridine	4.7	1. Pyridine	8.1	1. Pyridine	14.1
2. Styrene	3.6	2. Styrene	5.6	2. Styrene	9.2
3. Benzaldehyde	1.8	3. Benzaldehyde	3.8	3. Benzaldehyde	5.7
4. Phenylacetaldehyde	0.8	4. Phenylacetaldehyde	1.0	4. Phenylacetaldehyde	1.4
5. Propionanilide	29.1	5. Acetylbenzoyl	0.4	5. Propionanilide	31.5
6. Norfentanyl	0.2	6. Propionanilide	29.1	6. 6-MAM	9.5
7. 6-MAM	6.2	7. 6-MAM	8.6	7. Heroin	100
8. Heroin	100	8. Heroin	100	8. Fentanyl	19.0
9. Fentanyl	21.7	9. Fentanyl	21.8		
350 °C	% Area	400 °C	% Area	450 °C	% Area
1. Pyridine	18.3	1. Pyridine	18.7	1. Pyridine	22.1
2. Styrene	10.5	2. Styrene	12.2	2. Styrene	14.4
3. Benzaldehyde	8.3	3. Benzaldehyde	7.9	3. Benzaldehyde	7.8
4. Phenylacetaldehyde	1.8	4. Phenylacetaldehyde	1.7	4. Phenylacetaldehyde	2.3
5. Propionanilide	37.5	5. Propionanilide	35.4	5. Propionanilide	34.9
6. 6-MAM	4.0	6. 6-MAM	3.2	6. 6-MAM	17.0
7. Heroin	100	7. Heroin	100	7. Heroin	100
8. Fentanyl	11.7	8. Fentanyl	11.3	8. Fentanyl	95.3

Table 5-3. Pyromarkers of the pyrolysis of the 1:1 heroin to fentanyl mix.

200 °C		250 °C		300 °C	
	% Area		% Area		% Area
1. Pyridine	10.3	1. Pyridine	19.4	1. Pyridine	33.6
2. Styrene	9.1	2. Styrene	14.2	2. Styrene	22.4
3. Benzaldehyde	3.8	3. Benzaldehyde	7.1	3. Benzaldehyde	14.1
4. Phenylacetaldehyde	1.9	4. Phenylacetaldehyde	2.7	4. Phenylacetaldehyde	3.9
5. Acetylbenzoyl	0.5	5. Acetylbenzoyl	0.8	5. Propionanilide	92.5
6. Propionanilide	64.1	6. Propionanilide	71.5	6. 6-MAM	8.4
7. Norfentanyl	0.9	7. 6-MAM	8.1	7. Heroin	95.3
8. 6-MAM	7.5	8. Heroin	92.8	8. Fentanyl	99.2
9. Heroin	93.7	9. Fentanyl	98.2		
10. Fentanyl	96.7				
350 °C		400 °C		450 °C	
	% Area		% Area		% Area
1. Pyridine	33.5	1. Pyridine	42.0	1. Pyridine	48.0
2. Styrene	23.2	2. Styrene	24.8	2. Styrene	35.7
3. Benzaldehyde	14.0	3. Benzaldehyde	19.2	3. Benzaldehyde	22.0
4. Phenylacetaldehyde	3.8	4. Phenylacetaldehyde	4.4	4. Phenylacetaldehyde	5.5
5. Propionanilide	92.9	5. Phthalic anhydride	0.7	5. Propionanilide	69.1
6. 6-MAM	8.0	6. Propionanilide	100	6. 6-MAM	6.4
7. Heroin	82.9	7. 6-MAM	2.6	7. Heroin	49.4
8. Fentanyl	82.0	8. Heroin	58.1	8. Fentanyl	71.0
		9. Fentanyl	40.3		

Table 5-4. Pyromarkers of the pyrolysis of the 1:3 heroin to fentanyl mix.

200 °C		% Area	250 °C		% Area	300 °C		% Area
1.	Pyridine	12.5	1.	Pyridine	22.2	1.	Pyridine	45.4
2.	Styrene	11.5	2.	Styrene	16.3	2.	Styrene	24.3
3.	Benzaldehyde	5.1	3.	Benzaldehyde	9.4	3.	Benzaldehyde	20.2
4.	Phenylacetaldehyde	2.2	4.	Phenylacetaldehyde	2.7	4.	Phenylacetaldehyde	3.4
5.	Propionanilide	67.3	5.	Acetylbenzoyl	0.8	5.	Acetylbenzoyl	1.2
6.	6-MAM	4.2	6.	Propionanilide	73.7	6.	Propionanilide	67.7
7.	Heroin	18.0	7.	6-MAM	2.2	7.	6-MAM	1.5
8.	Fentanyl	100	8.	Heroin	20.4	8.	Heroin	16.0
			9.	Fentanyl	95.1	9.	Fentanyl	67.9
350 °C		% Area	400 °C		% Area	450 °C		% Area
1.	Pyridine	38.3	1.	Pyridine	43.4	1.	Pyridine	54.0
2.	Styrene	28.9	2.	Styrene	31.3	2.	Styrene	35.9
3.	Benzaldehyde	15.0	3.	Benzaldehyde	17.5	3.	Benzaldehyde	20.0
4.	Phenylacetaldehyde	4.5	4.	Phenylacetaldehyde	4.5	4.	Phenylacetaldehyde	6.1
5.	Acetylbenzoyl	1.4	5.	Acetylbenzoyl	1.4	5.	Propionanilide	70.7
6.	Propionanilide	96.9	6.	Propionanilide	96.9	6.	6-MAM	5.1
7.	6-MAM	1.0	7.	Norfentanyl	0.5	7.	Heroin	20.6
8.	Heroin	16.4	8.	6-MAM	0.7	8.	Fentanyl	63.7
9.	Fentanyl	82.3	9.	Heroin	15.6			
			10.	Fentanyl	55.1			

5.4.0 Conclusions

Overall, pyridine, styrene, benzaldehyde, phenylacetaldehyde, propionanilide, 6-MAM, heroin, and fentanyl were common in all heroin to fentanyl mixes at all pyrolytic temperatures. This is reasonable since only 6-MAM and heroin were observed for the pyrolysis of heroin HCl standard alone, despite pyromarkers reported in literature for the salt form of heroin. Acetylbenzoyl was more prevalent in these mixes under different pyrolytic temperatures rather than what was observed of solely the drug fentanyl, where it was not seen in all three replicates.

Future research should include the pyrolysis of these mixes using heroin base instead of the heroin HCl used. These methods and procedure could be used for such an analysis in which there is potential to observe the other pyromarkers of heroin and if it does interact with the form pyromarkers of fentanyl. Also, work could include the heroin and fentanyl mixes of both components in salt form and then converting the mixes in base form to pyrolyze. It would be interesting to observe changes that this process could have an effect of the pyromarkers detected.

Summary

Data obtained using the smoking box and thermocouple temperature probes clearly indicated that manufactured tobacco cigarette temperature studies were not applicable here. This simple study showed that the maximum temperature obtain while smoking FTPs under the “chasing the dragon” technique was approximately 450 °C in comparison to 750 °C. Nonetheless, similar pyromarkers were observed at both the lower and higher temperature of pyrolysis analysis. The literature suggests that fentanyl is stable up to 350 °C, however, it is clear here that fentanyl thermally degraded as low as 200 °C.

In all cases, the following pyromarkers were consistently observed under all conditions were fentanyl standard and FTPs: pyridine, styrene, benzaldehyde, phenylacetaldehyde, propionanilide, and fentanyl. Propionanilide was a major pyromarker that is prevalent under all conditions for both standards and FTPs, with the exception of the higher pyrolysis temperatures of FTPs where the plasticizer, 2-EH, had the highest peak area. Although this plasticizer can be commonly found in the environment, it has the potential to be an additional marker for investigative purposes to link those who have smoked FTPs as mean of ingestion.

For the heroin to fentanyl mixes, pyridine, styrene, benzaldehyde, phenylacetaldehyde, propionanilide, 6-MAM, heroin, and fentanyl were common in all pyrolytic temperatures and ratios. These show similar pyromarkers, with the addition of 6-MAM and heroin, to the standalone fentanyl analysis. Although not all literature pyromarkers were observed for heroin under this method, the stability and the combination of the pyromarkers that was seen here that can still be used for investigative purposes.

Propionanilide was generally observed to have the highest peak area for throughout this project. This pyromarker alone and/or the combination of the other pyromarkers have a

potential to be a marker in human biological samples to be indicative of the individual of having inhaled volatilized fentanyl.

Little is known regarding human toxicity of these observed pyromarkers. Pyridine, styrene, benzaldehyde, and phenylacetaldehyde are everywhere in the environment and the derivatives of propionanilide is a widely used hormone herbicide.¹²⁰ Typically, these at high concentrations pose as skin and eye irritants. The human oral lethal dose of pyridine is 0.5-5.0 g/kg.¹²¹ The LD₅₀ in rats for oral administration of benzaldehyde, phenylacetaldehyde, and propionanilide range between 1100-1500 mg/kg.¹²²⁻¹²⁴ The pyromarker with the lowest peak area for the pyrolysis of fentanyl standard was aniline, which has the lowest LD₅₀ for rats of 250 mg/kg.¹²⁵ It is not clear whether these pyromarkers would be considered more toxic than the drug itself, since little is known in regards to the human toxicity. In either case, it is not known whether these pyromarkers would metabolize in the human body to other compounds.

The work done here could be used to analyze other smoke drugs of abuse such as the newer designer drugs like bath salts, cannabinomimetics, Ritalin, etc. Temperature assessment using the smoking box and the temperature probe could provide precise temperature of the drug and choice of method to smoke them. Those parameters obtained can then be applied to the Py-GC/MS to mimic those smoking conditions and determine the pyromarkers produced.

Appendices

Appendix A: Thermocouple Data.

Trial	Smoke Observed Temp.		Max. Temp.		Rate Increase	
	T1 (°C)	T2 (°C)	T1 (°C)	T2 (°C)	T1 (°C/s)	T2 (°C/s)
1	69.7	128.5	248.6	286.5	13.2	15.4
2*	166.7	247.2	166.7	372.5	15.9	24.9
3	81.0	124.6	214.1	218.8	9.0	8.9
4*	74.8	53.9	146.6	108.3	4.5	4.0
5	315.1	308.6	345.7	357.9	20.2	21.0
6	39.2	38.1	441.6	267.4	28.0	17.6
7	35.3	36.2	289.4	376.1	16.7	23.6
8	49.4	34.8	379.5	289.0	23.8	17.8
9	33.8	63.0	268.1	256.9	14.5	13.8
10	86.0	157.3	244.9	288.2	10.6	14.6
11	45.6	65.0	320.6	387.0	17.6	22.8
12	76.6	208.1	311.9	397.7	14.5	23.4
13	69.2	66.9	222.8	224.2	8.3	8.8
14	35.0	32.4	246.7	282.1	12.5	16.3
15	22.4	22.3	254.4	225.2	12.9	11.9
16	201.4	177.0	283.8	293.7	17.4	22.7
Average	87.6	110.2	274.1	289.5	15.0	16.7

* Denotes trials where setup failed.

Appendix B: Extended data of the pyromarkers of the pyrolysis of fentanyl standard and FTPs.

Fentanyl Standards

200 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Propionanilide	9.377	362246	100	821486	100	1821474	100	100
Fentanyl	19.285	42245	11.7	69950	8.5	81259	4.5	8.2

200 °C with 250 °C desorb		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.080	5939578	27.6	5449021	23.2	4291684	21.1	24.0
Styrene	4.074	4500970	20.9	4352362	18.6	4145217	20.4	20.0
Benzaldehyde	4.694	2847604	13.2	2743127	11.7	2394119	11.8	12.2
Aniline	4.792	1035521	4.8	774770	3.3	697714	3.4	3.8
Phenylacetaldehyde	5.437	605213	2.8	739240	3.2	538169	2.6	2.9
1-Phenyl-1,2-propanedione	5.740	105415	0.5	183023	0.8			
Benzoic acid, methyl ester	5.932					92229	0.5	
Propionanilide	9.324	21512135	100	23447387	100	20351090	100	100
N-(2-phenylethyl)-formamide	9.617			153470	0.7			
Azobenzene	10.934	106871	0.5					
Fentanyl	19.264	37389	0.2	1460802	6.2	581155	2.9	3.1

250 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.261	3572919	21.9	2737378	20.7	2429237	19.8	20.8
Styrene	4.233	3794564	23.3	2837650	21.5	2544587	20.8	21.8
Benzaldehyde	4.837	1608732	9.9	1075989	8.1	832241	6.8	8.3
Aniline	4.931	256179	1.6	146084	1.1	24462	0.2	1.0
Phenylacetaldehyde	5.567	543428	3.3	398155	3.0	426109	3.5	3.3

1-Phenyl-1,2-propanedione	5.867	92413	0.6			51187	0.4	
Isothiocyanato-cyclohexane	7.625	195835	1.2					
Propionanilide	9.431	16315338	100	13211941	100	12249779	100	100
N-(2-phenylethyl)-formamide	9.731			49774	0.4			
Fentanyl	19.287	3618244	22.2	735800	5.6	1170893	9.6	12.4

300 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.142	4239862	34.6	3079778	78.4	2503003	56.6	56.5
Styrene	4.117	3307059	27.0	2540695	64.7	2216487	50.1	47.2
Benzaldehyde	4.727	1980298	16.2	1401793	35.7	1226698	27.7	26.5
Aniline	4.827	271332	2.2	135448	3.4	138588	3.1	2.9
Phenylacetaldehyde	5.465	557313	4.5	466656	11.9	450030	10.2	8.9
Phenylglyoxal	5.764			186939	4.8			
1-Phenyl-1,2-propanedione	5.788	141150	1.2			145839	3.3	
Propionanilide	9.334	12259111	100	3928913	100	4425958	100	100
N-(2-phenylethyl)-formamide	9.637	43471	0.4	46780	1.2			
Fentanyl	19.296	5747589	46.9	1927851	49.1	4150003	93.8	63.2

350 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.116	4845712	37.7	3668289	87.2	1809296	50.7	58.5
Styrene	4.103	3498986	27.3	3238319	77.0	1318662	36.9	47.1
Benzaldehyde	4.718	2299901	17.9	1452773	34.5	751686	21.1	24.5
Aniline	4.812	375804	2.9	128549	3.1	53675	1.5	2.5
Phenylacetaldehyde	5.457	583222	4.5	474141	11.3	273693	7.7	7.8
1-Phenyl-1,2-propanedione	5.783	147865	1.2					
Propionanilide	9.324	12838082	100	4206713	100	3569270	100	100
Fentanyl	19.285	1756961	13.7	1180791	28.1	3019079	84.6	42.1

400 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.117	6972996	31.4	5955515	45.7	4938345	61.3	46.2
3-Methyl-pyridine	3.846			141228	1.1	126585	1.6	
Styrene	4.103	5843127	26.4	4739549	36.4	4268022	53.0	38.6
Benzaldehyde	4.717	3983829	18.0	2362817	18.1	1965474	24.4	20.2
Aniline	4.814	247944	1.1	190130	1.5	165959	2.1	1.5
Benzonitrile	4.904					89583	1.1	
Benzyl Alcohol	5.329			78912	0.6	61182	0.8	
Phenylacetaldehyde	5.457	1005396	4.5	738430	5.7	688247	8.5	6.3
Phenylglyoxal	5.759			219988	1.7	187794	2.3	
1-Phenyl-1,2-propanedione	5.759	218822	1.0					
Phthalic anhydride	8.142			47812	0.4			
Propionanilide	9.324	22174011	100	13022329	100	8051227	100	100
N-(2-phenylethyl)-formamide	9.631			77094	0.6	81733	1.0	
Fentanyl	19.290	18562786	83.7	7209262	55.4	4626583	57.5	65.5

450 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.121	8614939	50.9	8597279	43.3	5059816	32.4	42.2
3-Methyl-pyridine	3.846	168320	1.0	134164	0.7	258169	1.7	1.1
Styrene	4.107	6376814	37.6	6542358	33.0	4121522	26.4	32.3
Benzaldehyde	4.719	4728881	27.9	4559441	23.0	1939635	12.4	21.1
Aniline	4.823	1105614	6.5	717335	3.6	198378	1.3	3.8
Benzonitrile	4.899			186140	0.9			
Benzyl Alcohol	5.330					82501	0.5	
Phenylacetaldehyde	5.459	756888	4.5	906590	4.6	616100	3.9	4.3
1-Phenyl-1,2-propanedione	5.761			286247	1.4	176427	1.1	

Phthalic anhydride	8.163			64465	0.3			
Propionanilide	9.330	16939476	100	19839327	100	15629638	100	100
N-(2-phenylethyl)-formamide	9.633			87799	0.4	100800	0.6	
Azobenzene	10.989	107059	0.6	70750	0.4			
Fentanyl	19.292	180978	1.1	1703020	8.6	10032023	64.2	24.6

FTPs

200 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.099	1174722	19.8	1584118	20.5	1120842	25.4	21.9
Styrene	4.089	1857401	31.3	1832391	23.7	999575	22.7	25.9
Benzaldehyde	4.697	757351	12.8	889564	11.5	485626	11.0	11.8
Phenylacetaldehyde	5.449	499583	8.4	819594	10.6	370116	8.4	9.1
Propionanilide	9.333	5929884	100	7716474	100	4408695	100	100
Fentanyl	19.262	127672	2.2	125871	1.6	101860	2.3	2.0

200 °C with 250 °C desorb	Trial #3	Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.033	453716	13.4	434242	14.5	669399	15.8	14.6
Styrene	4.049	915139	27.0	706582	23.6	782192	18.5	23.0
Benzaldehyde	4.672	248490	7.3	214076	7.2	400799	9.5	8.0
Phenylacetaldehyde	5.420	257017	7.6	197614	6.6	318023	7.5	7.2
Propionanilide	9.307	3387918	100	2993393	100	4234396	100	100
Fentanyl	19.268	524291	15.5	466113	15.6	746520	17.6	16.2

250 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Methyl formate	2.363					194764	1.6	
Pyridine	3.033	4645393	27.6	3677279	27.9	3368802	27.4	27.6

3-Methylene-heptane	3.216					424316	3.4	
Styrene	4.046	6249682	37.2	3880539	29.4	4024155	32.7	33.1
2-Ethyl-hexanal	4.485	177225	1.1			191364	1.6	
Benzaldehyde	4.667	2570474	15.3	2514348	19.1	2202299	17.9	17.4
Aniline	4.769	267022	1.6	381918	2.9	242562	2.0	2.2
3-Pyridinecarboxaldehyde	4.998	168249	1.0	147578	1.1	128754	1.0	1.1
2-EH	5.147					510698	4.2	
Benzyl Alcohol	5.284	137351	0.8	87079	0.7	87003	0.7	0.7
Phenylacetaldehyde	5.417	1469933	8.7	747348	5.7	916529	7.4	7.3
1-Phenyl-1,2-propanedione	5.722	193995	1.2	245588	1.9	177907	1.4	1.5
Benzenecarboxylic acid	6.381	184751	1.1	132061	1.0	180713	1.5	1.2
Propionanilide	9.317	16808919	100	13187542	100	12303657	100	100
N-(2-phenylethyl)-formamide	9.612	284173	1.7	294823	2.2	251358	2.0	2.0
Fentanyl	19.263	73189	0.4	197784	1.5	314725	2.6	1.5

300 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.021	4394098	35.4	3815696	26.4	4337389	29.7	30.5
3-Methylene-heptane	3.210	6534789	52.6	4276059	29.6	5975715	40.9	41.1
3-Heptanone	3.870	1645940	13.3	526918	3.7	953806	6.5	7.8
Styrene	4.036	4496873	36.2	3579109	24.8	4941010	33.8	31.6
2-Ethyl-2-hexenal	4.209	380592	3.1	201238	1.4	283967	1.9	2.1
2-Ethyl-hexanal	4.477	3084532	24.8	1305480	9.0	1677692	11.5	15.1
Benzaldehyde	4.662	4371825	35.2	3054965	21.2	3391789	23.2	26.5
Aniline	4.767	419414	3.4					
2-EH	5.142	8470103	68.2	6772988	46.9	8463971	57.9	57.7
Benzyl Alcohol	5.286			290241	2.0	357358	2.4	
Phenylacetaldehyde	5.413	1154824	9.3	1489212	10.3	1565208	10.7	10.1
1-Phenyl-1,2-propanedione	5.716	242203	2.0	288615	2.0	290961	2.0	2.0

2-Ethylhexyl ester acetic acid	6.278	176039	1.4	141742	1.0	182871	1.3	1.2
Benzenecarboxylic acid	6.376	459858	3.7	407890	2.8	520743	3.6	3.4
2-Ethylhexyl ester 2-propenoic acid	7.084	903269	7.3	754311	5.2	1044978	7.2	6.6
2-Ethylhexyl methacrylate	7.838	112829	0.9					
Phthalic anhydride	8.115	120630	1.0	106943	0.7			
Propionanilide	9.314	12412608	100	14435342	100	14614471	100	100
Acrylanilide	9.363	115729	0.9					
N-(2-phenylethyl)-formamide	9.611	288808	2.3	318006	2.2	377024	2.6	2.4
2-EEFA	10.290	382202	3.1	241536	1.7	315548	2.2	2.3
Fentanyl	19.267	20166	0.2	322358	2.2	423443	2.9	1.8

350 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.018	5007167	34.7	4617613	31.8	4375933	29.6	32.0
3-Methylene-heptane	3.212	5938456	41.2	4330641	29.8	5509295	37.2	36.1
3-Heptanone	3.867	680975	4.7	687879	4.7	872715	5.9	5.1
Styrene	4.035	5484007	38.0	5163947	35.5	4315403	29.2	34.2
2-Ethyl-2-hexenal	4.215	296156	2.1					
2-Ethyl-hexanal	4.471	1340437	9.3	1276284	8.8	2110434	14.3	10.8
Benzaldehyde	4.662	4096522	28.4	4034306	27.8	3944706	26.7	27.6
Aniline	4.763	398659	2.8	257240	1.8			
2-EH	5.141	9049021	62.8	6088817	41.9	9266297	62.6	55.8
Benzyl Alcohol	5.283	333796	2.3	278392	1.9	187403	1.3	1.8
Phenylacetaldehyde	5.412	1389825	9.6	1347572	9.3	896213	6.1	8.3
1-Phenyl-1,2-propanedione	5.716	290698	2.0	196429	1.4			
2-Ethylhexyl ester acetic acid	6.278	195482	1.4	141558	1.0	137994	0.9	1.1
Benzenecarboxylic acid	6.377	500283	3.5	331292	2.3	409448	2.8	2.8
2-Ethylhexyl ester 2-propenoic acid	7.084	918112	6.4	500482	3.4	692837	4.7	4.8
2-Ethylhexyl methacrylate	7.838	122416	0.8					

Propionanilide	9.316	14415944	100	14534791	100	14797242	100	100
Acrylanilide	9.363			133545	0.9	118115	0.8	
N-(2-phenylethyl)-formamide	9.612	329710	2.3			442899	3.0	
2-EEFA	10.290	361085	2.5	193354	1.3	378222	2.6	2.1
Fentanyl	19.267	219598	1.5	213646	1.5	289585	2.0	1.7

400 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.019	4075441	18.7	5375025	24.0	6757820	26.9	23.2
3-Methylene-heptane	3.208	10180024	46.7	11440491	51.0	13149918	52.4	50.0
3-Heptanone	3.867	1268850	5.8	1278998	5.7	1319572	5.3	5.6
Styrene	4.032	3403062	15.6	4004466	17.8	5023552	20.0	17.8
2-Ethyl-2-hexenal	4.207	742942	3.4	803378	3.6	669983	2.7	3.2
2-Ethyl-hexanal	4.473	2995224	13.7	3284885	14.6	3084095	12.3	13.6
Benzaldehyde	4.659	4107472	18.8	4787727	21.3	5808772	23.2	21.1
2-EH	5.141	21795820	100	22435959	100	25087109	100	100
Phenylacetaldehyde	5.410	939843	4.3	988776	4.4	1558080	6.2	5.0
2-Ethylhexyl ester acetic acid	6.276	345049	1.6	378476	1.7	564615	2.3	1.8
Benzenecarboxylic acid	6.382	588258	2.7	548783	2.4	707275	2.8	2.7
2-Ethylhexyl ester 2-propenoic acid	7.083	2804275	12.9	2713098	12.1	3613154	14.4	13.1
2-Ethylhexyl methacrylate	7.836	360879	1.7	365715	1.6	406327	1.6	1.6
2-EECA	8.609	408126	1.9	405280	1.8	466286	1.9	1.8
Butyl 2-ethylhexyl ester sulfurous acid	8.791					263892	1.1	
Propionanilide	9.319	13467368	61.8	14903654	66.4	19821574	79.0	69.1
2-Ethylhexyl ester pentanoic acid	9.637	934487	4.3	934811	4.2	763266	3.0	3.8
2-EEFA	10.288	813202	3.7	928862	4.1	899133	3.6	3.8
Fentanyl	19.264	1499527	6.9	78130	0.3	198013	0.8	2.7

450 °C		Trial #1	Trial #2	Trial #3	Average
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Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.017	9734424	21.2	7903034	21.9	6348045	27.3	23.5
3-Methylene-heptane	3.206	30031724	65.5	23002960	63.7	15959897	68.6	65.9
3-Heptanone	3.863	2965207	6.5	2171713	6.0	2595286	11.2	7.9
Styrene	4.029	5215870	11.4	4198655	11.6	4062135	17.5	13.5
2-Ethyl-hexanal	4.470	5077234	11.1	3883741	10.7	3723561	16.0	12.6
Benzaldehyde	4.654	10988711	24.0	9112778	25.2	6807454	29.2	26.1
2-EH	5.139	45832553	100	36134710	100	23274294	100	100
Phenylacetaldehyde	5.407	1873290	4.1	1352711	3.7	1110295	4.8	4.2
2-Ethylhexyl ester acetic acid	6.274	2075541	4.5	1514618	4.2	957883	4.1	4.3
Benzenecarboxylic acid	6.386	1696268	3.7	869219	2.4	922505	4.0	3.4
6-Methylheptyl ester 2-propenoic acid	7.079	12678473	27.7	10943290	30.3	6016383	25.8	27.9
2-Ethylhexyl methacrylate	7.832	1665919	3.6	927896	2.6	718823	3.1	3.1
n-Butyric acid 2-ethylhexyl ester	7.970	674654	1.5	423399	1.2	295891	1.3	1.3
2-EECA	8.605	1286234	2.8	1022424	2.8	547821	2.4	2.7
Propionanilide	9.317	26278124	57.3	21534669	59.6	16798918	72.2	63.0
2-Ethylhexyl ester pentanoic acid	9.633	2462353	5.4	1821194	5.0	1201459	5.2	5.2
2-EEFA	10.284	1042763	2.3	459647	1.3	481726	2.1	1.9
Fentanyl	19.256	227510	0.5	556264	1.5	213243	0.9	1.0

Appendix C: Extended data and GC Chromatograms of the pyromarkers of the pyrolysis of heroin and fentanyl mixes.

3:1 Heroin to Fentanyl Mix

200 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.082	427125	1.7	1025486	5.6	1340271	6.7	4.7
Styrene	4.073	353368	1.4	857860	4.7	936433	4.7	3.6
Benzaldehyde	4.692	174712	0.7	424453	2.3	473994	2.4	1.8
Aniline	4.784			58580	0.3	67187	0.3	
Phenylacetaldehyde	5.433	91625	0.4	159302	0.9	218861	1.1	0.8
Acetylbenzoyl	5.736			63068	0.3	75212	0.4	
Propionanilide	9.306	5094048	20.3	6047432	33.1	6798119	34.1	29.1
Norfentanyl	13.511	46558	0.2	25679	0.1	50073	0.3	0.2
6-MAM	17.509	1555767	6.2	1057359	5.8	1307222	6.6	6.2
Heroin	18.391	25139123	100	18289879	100	19926264	100	100
Fentanyl	19.275	5020428	20.0	4034237	22.1	4599114	23.1	21.7

250 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.087	852063	3.6	1025486	5.6	1726495	15.2	8.1
Styrene	4.077	699069	3.0	857860	4.7	1026517	9.0	5.6
Benzaldehyde	4.691	285818	1.2	424453	2.3	881822	7.8	3.8
Aniline	4.792			58580	0.3	122678	1.1	
Phenylacetaldehyde	5.432	124750	0.5	159302	0.9	165779	1.5	1.0
Acetylbenzoyl	5.734	52547	0.2	63068	0.3	68516	0.6	0.4
Propionanilide	9.300	4844623	20.5	6047432	33.1	3816356	33.6	29.1
6-MAM	17.505	3538088	15.0	1057359	5.8	578118	5.1	8.6
Heroin	18.383	23614437	100	18289879	100	11343491	100	100

Fentanyl	19.271	7353089	31.1	4034237	22.1	1381941	12.2	21.8
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300 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.088	1011904	5.8	2649647	15.5	2096698	21.0	14.1
Styrene	4.075	836633	4.8	1939014	11.3	1133958	11.4	9.2
Benzaldehyde	4.691	376413	2.2	978972	5.7	918998	9.2	5.7
Aniline	4.788			106457	0.6	92631	0.9	
Benzonitrile	4.875			106144	0.6			
Phenylacetaldehyde	5.429	133316	0.8	232943	1.4	193407	1.9	1.4
Acetylbenzoyl	5.737			99430	0.6			
Propionanilide	9.302	4859169	27.9	5044613	29.5	3702009	37.1	31.5
6-MAM	17.509	2382366	13.7	1892883	11.1	374971	3.8	9.5
Heroin	18.390	17391367	100	17113311	100	9972884	100	100
Fentanyl	19.270	3994111	23.0	3349170	19.6	1449919	14.5	19.0

350 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.089	974999	10.6	2403074	22.8	2499290	21.5	18.3
Styrene	4.076	651515	7.1	1358441	12.9	1342649	11.5	10.5
Benzaldehyde	4.692	557485	6.1	1014230	9.6	1068560	9.2	8.3
Aniline	4.79			122235	1.2	105475	0.9	
Benzonitrile	4.872			98579	0.9			
Phenylacetaldehyde	5.430	116738	1.3	219050	2.1	251754	2.2	1.8
Acetylbenzoyl	5.733			87890	0.8	93861	0.8	
Propionanilide	9.299	3512393	38.1	3931018	37.4	4314069	37.0	37.5
6-MAM	17.501	520099	5.6	277603	2.6	431403	3.7	4.0
Heroin	18.377	9213391	100	10518754	100	11649292	100	100
Fentanyl	19.268	951504	10.3	1294438	12.3	1446804	12.4	11.7

400 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.088	1508048	12.9	2619511	20.3	2246331	22.8	18.7
Styrene	4.077	1154659	9.9	1639437	12.7	1382670	14.1	12.2
Benzaldehyde	4.691	689837	5.9	1072694	8.3	927865	9.4	7.9
Aniline	4.784			132486	1.0			
Benzonitrile	4.871					94087	1.0	
Phenylacetaldehyde	5.43	178513	1.5	213573	1.7	188028	1.9	1.7
Acetylbenzoyl	5.732		0.0	75356	0.6	72266	0.7	
Phthalic anhydride	8.166	50878	0.4					
Propionanilide	9.299	5124945	43.8	4033329	31.3	3062012	31.1	35.4
6-MAM	17.502	325164	2.8	428586	3.3	351808	3.6	3.2
Heroin	18.379	11705004	100	12891518	100	9831045	100	100
Fentanyl	19.267	1328710	11.4	1532001	11.9	1035027	10.5	11.3

450 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.143	1524262	4.6	3530622	51.4	2348809	10.3	22.1
Styrene	4.12	1012630	3.1	2256962	32.9	1617539	7.1	14.4
Benzaldehyde	4.729	649691	2.0	1203888	17.5	898336	4.0	7.8
Phenylacetaldehyde	5.463	208919	0.6	343847	5.0	281550	1.2	2.3
Acetylbenzoyl	5.766			148537	2.2			
Propionanilide	9.325	7299502	22.1	2647695	38.6	9959385	43.9	34.9
Norfentanyl	13.521					107615	0.5	
6-MAM	17.528	2247466	6.8	842975	12.3	7267983	32.0	17.0
Heroin	18.403	32965334	100	6862895	100	22701106	100	100
Fentanyl	19.289	6856632	20.8	15439623	225.0	9091553	40.0	95.3

1:1 Heroin to Fentanyl Mix

200 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.084	1106715	6.3	1963516	10.8	2623180	13.8	10.3
Styrene	4.076	947187	5.4	1787957	9.8	2292694	12.1	9.1
Benzaldehyde	4.690	309520	1.8	722239	4.0	1087617	5.7	3.8
Aniline	4.784		0.0	77173	0.4	241858	1.3	
Phenylacetaldehyde	5.431	204072	1.2	397955	2.2	422599	2.2	1.9
Acetylbenzoyl	5.733	57874	0.3	97432	0.5	104884	0.6	0.5
Propionanilide	9.305	9509159	53.8	12917882	71.0	12826091	67.6	64.1
Norfentanyl	13.497	79533	0.5	183653	1.0	221110	1.2	0.9
6-MAM	17.494	1130945	6.4	1113491	6.1	1875776	9.9	7.5
Heroin	18.370	17673836	100	16565965	91.0	17067313	90.0	93.7
Fentanyl	19.264	15925706	90.1	18203484	100	18966113	100	96.7

250 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.091	1648630	8.4	3797643	26.2	4024882	23.8	19.4
Styrene	4.078	1363144	6.9	2595296	17.9	2987687	17.7	14.2
Benzaldehyde	4.692	415025	2.1	1505520	10.4	1491748	8.8	7.1
Aniline	4.791			147632	1.0			
Phenylacetaldehyde	5.432	250671	1.3	488198	3.4	567651	3.4	2.7
Acetylbenzoyl	5.730	74496	0.4	158243	1.1	174696	1.0	0.8
Isothiocyanato-cyclohexane	7.564	132391	0.7		0.0			
Propionanilide	9.304	10569080	53.6	12014280	82.8	13196551	78.0	71.5
Norfentanyl	13.495			124623	0.9	127016		
6-MAM	17.492	2452277	12.4	917676	6.3	921671	5.4	8.1
Heroin	18.370	19727933	100	13197787	91.0	14810783	87.5	92.8
Fentanyl	19.261	18635750	94.5	14506232	100	16917636	100	98.2

300 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.094	2158365	23.8	4672080	36.5	4662451	40.4	33.6
Styrene	4.081	1740787	19.2	2965312	23.2	2858251	24.8	22.4
Benzaldehyde	4.692	845912	9.3	2022480	15.8	1985057	17.2	14.1
Aniline	4.792					200051	1.7	
Benzonitrile	4.879			173656	1.4	155069	1.3	
Phenylacetaldehyde	5.432	293522	3.2	498044	3.9	533755	4.6	3.9
Acetylbenzoyl	5.731					183969	1.6	
Phthalic anhydride	8.115			59599	0.5	76991	0.7	
Propionanilide	9.304	8985753	99.0	10484422	82.0	11123282	96.5	92.5
Norfentanyl	13.499			98637	0.8	86796	0.8	
6-MAM	17.494	786149	8.7	1191441	9.3	840717	7.3	8.4
Heroin	18.369	9076491	100	11872286	92.9	10729043	93.1	95.3
Fentanyl	19.262	8866628	97.7	12783235	100	11529365	100	99.2

350 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.095	2256598	30.3	5187093	38.9	4787853	31.3	33.5
Styrene	4.079	1885619	25.3	3091721	23.2	3222107	21.1	23.2
Benzaldehyde	4.692	892174	12.0	2294462	17.2	1962840	12.8	14.0
Phenylacetaldehyde	5.431	257583	3.5	588078	4.4	546698	3.6	3.8
Acetylbenzoyl	5.734	81199	1.1			186201	1.2	
Phthalic anhydride	8.171	37321	0.5					
Propionanilide	9.304	7444606	100	13339386	100	12042474	78.8	92.9
Norfentanyl	13.498			126643	0.9	122799	0.8	
6-MAM	17.497	285379	3.8	986108	7.4	1956774	12.8	8.0
Heroin	18.372	5324947	71.5	12024124	90.1	13319359	87.1	82.9

Fentanyl	19.267	5053706	67.9	10429151	78.2	15290413	100	82.0
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400 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.091	2428066	31.0	5092072	51.2	5718865	43.8	42.0
Styrene	4.079	1744946	22.3	2600402	26.2	3391013	26.0	24.8
Benzaldehyde	4.691	1153720	14.7	2372587	23.9	2482115	19.0	19.2
Benzonitrile	4.878			214899	2.2			
Phenylacetaldehyde	5.430	296573	3.8	504254	5.1	585103	4.5	4.4
Acetylbenzoyl	5.734	90139	1.2			228259	1.7	
Phthalic anhydride	8.114	43148	0.6	82634	0.8	89964	0.7	0.7
Propionanilide	9.305	7826342	100	9939990	100	13053301	100	100
Norfentanyl	13.496					69996	0.5	
6-MAM	17.500	171985	2.2	203137	2.0	470712	3.6	2.6
Heroin	18.366	5949818	76.0	3968302	39.9	7603335	58.2	58.1
Fentanyl	19.261	3319345	42.4	2591005	26.1	6836278	52.4	40.3

450 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.138	3326437	39.8	915343	37.0	1665125	67.3	48.0
Styrene	4.115	2456511	29.4	609203	24.6	1310586	53.0	35.7
Benzaldehyde	4.725	1732791	20.7	286982	11.6	835978	33.8	22.0
Aniline	4.829	175212	2.1			75593	3.1	
Phenylacetaldehyde	5.462	297860	3.6	91692	3.7	230824	9.3	5.5
Acetylbenzoyl	5.786	99536	1.2					
Propionanilide	9.323	8360483	100	801917	32.4	1851205	74.9	69.1
6-MAM	17.523	51454	0.6	205569	8.3	253828	10.3	6.4
Heroin	18.395	3414052	40.8	996359	40.3	1661372	67.2	49.4
Fentanyl	19.289	1096374	13.1	2474783	100	2472778	100	71.0

1:3 Heroin to Fentanyl Mix

200 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.083	1461784	8.6	3521836	11.9	4717374	16.8	12.5
Styrene	4.075	1628668	9.6	3182182	10.8	3963862	14.1	11.5
Benzaldehyde	4.692	519299	3.1	1491855	5.0	2020698	7.2	5.1
Phenylacetaldehyde	5.430	291565	1.7	635000	2.1	802720	2.9	2.2
Acetylbenzoyl	5.856	63443	0.4					
Propionanilide	9.316	11067919	65.5	18923590	64.0	20335617	72.4	67.3
N-(2-phenylethyl)-formamide	9.606			57328	0.2	73871	0.3	
Norfentanyl	13.496			217260	0.7	215350	0.8	
6-MAM	17.480	389732	2.3	1394198	4.7	1605513	5.7	4.2
Heroin	18.339	2686647	15.9	5845676	19.8	5113120	18.2	18.0
Fentanyl	19.248	16902522	100	29558706	100	28069395	100	100

250 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.089	3218908	28.3	5212996	17.4	5848535	20.9	22.2
Styrene	4.077	2409551	21.2	3648197	12.2	4341288	15.5	16.3
Benzaldehyde	4.692	1380504	12.1	2189712	7.3	2424710	8.7	9.4
Aniline	4.793	358250	3.2	328143	1.1			
Phenylacetaldehyde	5.432	323627	2.8	684887	2.3	793078	2.8	2.7
Acetylbenzoyl	5.735	91005	0.8	197361	0.7	232550	0.8	0.8
Propionanilide	9.313	11368092	100	16872011	56.4	18090895	64.7	73.7
N-(2-phenylethyl)-formamide	9.605					67179	0.2	
Norfentanyl	13.493			155110	0.5	201272	0.7	
6-MAM	17.478	179332	1.6	606577	2.0	864436	3.1	2.2
Heroin	18.345	1896847	16.7	6761275	22.6	6134716	21.9	20.4
Fentanyl	19.246	9686494	85.2	29902873	100	27965102	100	95.1

300 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.093	3115480	9.9	8178317	100.0	6866035	26.4	45.4
Phenylethyne	3.933			70667	0.9			
Styrene	4.080	2212389	7.0	4018461	49.1	4383899	16.8	24.3
Benzaldehyde	4.693	1197178	3.8	3632648	44.4	3203154	12.3	20.2
Benzonitrile	4.872			326031	4.0			
Benzoxazole	5.217			69912	0.9			
Phenylacetaldehyde	5.431	452046	1.4	462080	5.7	816311	3.1	3.4
Acetylbenzoyl	5.733	140400	0.4	169009	2.1	249728	1.0	1.2
Phthalic anhydride	8.115					77435	0.3	
Propionanilide	9.309	12820902	40.8	7938451	97.1	16943450	65.1	67.7
Azobenzene	10.928			102838	1.3			
Norfentanyl	13.488					139117	0.5	
6-MAM	17.474	607716	1.9	28053	0.3	598445	2.3	1.5
Heroin	18.341	6514358	20.7	351007	4.3	5950385	22.8	16.0
Fentanyl	19.241	31397447	100	309065	3.8	26041394	100	67.9

350 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.091	3062943	32.4	7180368	41.5	7940002	40.9	38.3
Styrene	4.076	2490113	26.4	5217575	30.2	5885985	30.3	28.9
Benzaldehyde	4.689	1317977	13.9	2695383	15.6	3012354	15.5	15.0
Aniline	4.789	137722	1.5	304021	1.8			
Benzonitrile	4.875					225151	1.2	
Phenylacetaldehyde	5.427	389348	4.1	838200	4.8	898867	4.6	4.5
Acetylbenzoyl	5.729	99239	1.1	263501	1.5	305404	1.6	1.4
Phthalic anhydride	8.109			69247	0.4	80293	0.4	

Propionanilide	9.308	9448930	100	16789818	97.1	18195805	93.6	96.9
N-(2-phenylethyl)-formamide	9.601					63208	0.3	
Norfentanyl	13.499					95481	0.5	
6-MAM	17.491	9730	0.1	252186	1.5	275057	1.4	1.0
Heroin	18.358	693618	7.3	3584401	20.7	4092342	21.1	16.4
Fentanyl	19.263	4436447	47.0	17283755	100	19432079	100	82.3

400 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.093	5500189	38.6	8671796	51.9	6984427	39.8	43.4
Styrene	4.078	4839220	33.9	6011796	36.0	4182648	23.8	31.3
Benzaldehyde	4.690	2473203	17.3	3274332	19.6	2733848	15.6	17.5
Benzonitrile	4.879			215480	1.3			
Phenylacetaldehyde	5.428	597690	4.2	776247	4.6	794230	4.5	4.5
Acetylbenzoyl	5.729	150940	1.1	267496	1.6	279152	1.6	1.4
Phthalic anhydride	8.110			74009	0.4	73374	0.4	
Propionanilide	9.305	14266149	100	16701184	100	15899473	90.7	96.9
Norfentanyl	13.496	85155	0.6	59458	0.4	78561	0.4	0.5
6-MAM	17.489	9476	0.1	131203	0.8	208156	1.2	0.7
Heroin	18.358	553879	3.9	2672195	16.0	4733121	27.0	15.6
Fentanyl	19.260	3840375	26.9	6433219	38.5	17538159	100	55.1

450 °C		Trial #1		Trial #2		Trial #3		Average
Compound	RT (min)	Area	% Area	Area	% Area	Area	% Area	% Area
Pyridine	3.083	5007970	38.3	10501543	23.8	2463071	100	54.0
Styrene	4.084	4169439	31.9	7751148	17.6	1434261	58.2	35.9
Benzaldehyde	4.702	2152395	16.5	3486491	7.9	876852	35.6	20.0
Aniline	4.807					140136	5.7	
Phenylacetaldehyde	5.444	504240	3.9	1289766	2.9	287314	11.7	6.1

Acetylbenzoyl	5.777	136821	1.0					
Monoethyl ester phthalic acid	8.144			118386	0.3			
Propionanilide	9.322	13070145	100	9898687	22.5	2207919	89.6	70.7
Norfentanyl	13.520			190647	0.4	22319	0.9	
6-MAM	17.518	30395	0.2	3973140	9.0	150384	6.1	5.1
Heroin	18.387	673423	5.2	14981797	34.0	559021	22.7	20.6
Fentanyl	19.283	2966421	22.7	44091052	100	1686900	68.5	63.7

Pyrolysis of Heroin at 200 °C

Pyrolysis of Heroin at 450 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 200 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 250 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 300 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 350 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 400 °C

Pyrolysis of 3:1 Heroin to Fentanyl Mix at 450 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 200 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 250 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 300 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 350 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 400 °C

Pyrolysis of 1:1 Heroin to Fentanyl Mix at 450 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 200 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 250 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 300 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 350 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 400 °C

Pyrolysis of 1:3 Heroin to Fentanyl Mix at 450 °C

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