CHARACTERIZATION OF FENTANYL HEADSPACE USING SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

By

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Abstract: With an increased potency and widespread abuse in recent years, the unknowing exposure to fentanyl has become a growing public safety concern. Even though it has been one of the most frequently encountered opioids since 2013, efforts to characterize vapor phase fentanyl are few and far between. This research sought to help assess the public health impacts of vaporized fentanyl by characterizing fentanyl headspace and identifying factors that may cause variation. Using solid phase microextraction (SPME), vaporized fentanyl samples were collected with different fiber types, under varying temperatures, with varying humidity, and using silanized and untreated glassware. Once collected, these samples were analyzed using gas chromatography-mass spectrometry (GC-MS). It was determined that polydimethylsiloxane (PDMS) fibers were the most effective at detecting fentanyl in the headspace and that the detection of fentanyl is affected by the type of glassware in which the fentanyl is stored.

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CHAPTER I

INTRODUCTION

The prevalence of opioids in overdose deaths has been on the rise since opioid prescriptions became a regular practice for pain management in the 1990s. In 2018, opioids were involved in 46,802 overdose deaths (1), and in recent years, there has been a drastic increase in the abuse of synthetic opioids, such as fentanyl. In fact, of the 46,802 opioid-related overdose deaths in 2018, two-thirds of the cases involved a synthetic opioid (1). The high potency of synthetic opioids and the prevalence of fentanyl in drug seizures and overdoses in recent years raises questions about how fentanyl impacts the public safety for those who come in contact with it.

The unknowing exposure of individuals to vapor phase fentanyl is of large concern, given that two milligrams is considered lethal to an opioid-naïve individual by the United States Drug Enforcement Administration (DEA) (2). Though vapor phase fentanyl is not a new concept, there are no established methods to detect vapor phase fentanyl outside of controlled environments. As a result, research characterizing the prevalence of fentanyl in the air under variable conditions, and the quantities at which it exists is necessary to ensure public health and safety.

This research sought to fill this gap in the literature by using solid phase microextraction (SPME) fibers exposed to fentanyl-HCl powder in headspace vials and analyzing them by gas chromatography-mass spectrometry (GC-MS). The resulting data were used to answer the following research questions: 1.) Which SPME fiber composition is the best at characterizing fentanyl headspace? 2.) Does temperature impact the ability to detect fentanyl? 3.) Does variation in humidity impact fentanyl detection in the headspace? 4.) Do active sites in the fentanyl storage containers impact detection? The answers to these questions will provide a basis for determining the harm that vapor phase fentanyl has on public health and safety and can serve as a reference for future detection practices.

CHAPTER II

REVIEW OF LITERATURE

2.1 Fentanyl as an Opioid

Opioids, as a whole, are central nervous system (CNS) depressants. They are regularly utilized in the management of moderate to severe pain due to their analgesic properties. Opioids achieve analgesia by binding to opioid receptors (mu, delta, and kappa). By doing so, opioids are able to block the transmissions of painful stimuli by inhibiting the release of neurotransmitters at terminal nerve endings. Individuals then do not receive the signal for painful sensations and there is no subsequent negative emotional component with pain. In some cases, these effects can be euphoric (3).

Though the pain signal blocking effects of opioids are beneficial for pain management, their side effects can be unpleasant if not used correctly. Normal effects for opioids include drowsiness, sedation, delayed reflexes, analgesia, and decreased blood pressure (3), when they are used as prescribed. However, when used incorrectly, opioids have the potential to cause acute respiratory depression, resulting in death. The effects and danger of opioids can be exacerbated when used in combination with another CNS depressant. Examples of this behavior include combining multiple opioids, such as heroin and fentanyl, or by combining an opioid with a benzodiazepine and/or alcohol (5).

Opioids fall into three broad categories: natural, semisynthetic, and synthetic. Synthetic, or laboratory manufactured, opioids have become popular in recent years because of their increased potencies (3). However, because they are man-made, these drugs can be synthesized in clandestine, or illicit, laboratories and are prone to being cut with other harmful substances such as other opioids or cocaine. Fentanyl, in particular, is a synthetic opioid and has been one of the most frequently encountered synthetic opioids since 2013 (6). Similar to other opioids, fentanyl and its derivatives are useful analgesics, but they possess additional risks due to their faster onset of action and increased potency (7). For comparison, the potency of fentanyl is between 50 and 100 times greater than that of morphine, a natural opioid (8). Not only that, but fentanyl has several derivatives, namely sufentanil and carfentanil, that are substantially more potent than the parent drug (3), raising even more concern about the dangers associated with exposure to vapors. As for the faster onset of action, fentanyl is more lipophilic than many other opioids. As a result, it is able to cross the blood-brain barrier and act on the brain faster than other opioids, increasing its addiction potential (7).

2.2 Aerosolized Fentanyl

At large, aerosols are simply suspensions of tiny particles or droplets in the air (9). Aerosols are encountered daily, but their effects on an individual's health can vary drastically depending on the compound in question. Aerosolized fentanyl is not a new concept. In the 1990's, aerosolized fentanyl was introduced as a method of analgesia and shown to be useful in producing pain relief despite the pharmacokinetic variations between individuals (10). In fact, the inhalation of analgesics, like fentanyl, is particularly attractive in the hospital setting where intravenous access may be limited or dependent on

specialists (11). However, in recent years, there has been concern about the aerosolization of powdered fentanyl and the unknowing exposure of law enforcement and first responders in cases that involve overdose or illicit manufacturing. Not only that but following the 2002 attack on a Russian theatre in which an aerosolized fentanyl derivative was used as an incapacitating agent (12), there have been questions raised about the potential of aerosolized fentanyl to be used as a means of chemical warfare. As a result, it is important that there is a clear understanding of how fentanyl is aerosolized and the quantities in which it is getting into the air.

Aerosols can be created by several methods: forcing a liquid solution of a drug through small holes, dispersing them as dry powders, or generating them by thermal means (13). They raise special concerns because inhalation is one of the fastest routes of exposure, causing rapid onset of the drug after entering the system. In terms of the dangers that aerosolized fentanyl could present to law enforcement officers, the process of formulating dry powders that are able to aerosolize is complicated and generally requires substantial amounts of additives to facilitate dispersion (14). Currently, there is little research supporting the idea that fentanyl powder could be aerosolized upon disruption, but this sentiment indicates that the concern of this happening may be overemphasized by certain entities.

However, in one study, Manral et al., recognizes that fentanyl aerosols can be generated thermally by heating fentanyl at a high temperature for a very short period of time (13). When thermally aerosolized, fentanyl particles can degrade and form toxic byproducts. Researchers found that temperatures up to 350ºC resulted only in peaks corresponding to the parent compound when analyzed by GC-MS. When the temperature

was increased to 500ºC, two additional peaks were detected: N-phenethyl-1,2,5,6-tetrahydropyradine and N-phenylpropanamide. With subsequent temperature increase, the detected number of peaks increased, indicating not only the decomposition of fentanyl, but also the decomposition of the initial pyrolysis products. Additionally, hydrogen cyanide was produced when fentanyl underwent flash pyrolysis at 750ºC (13). These results indicate that fentanyl should not be heated above 350ºC under any conditions, especially in an attempt to create aerosolized particles.

At large, aerosolized fentanyl is a huge safety concern, however, it is important to first understand the dangers associated with gaseous or vapor phase fentanyl.

2.3 Vapor Detection

Vapors can enter the body through the eyes, nose, mouth, and skin. As a result, it is important that vapor phase fentanyl is detectable and can be characterized. At large, vaporization is the phase transition of a substance to a gas. These gaseous particles can then be sampled from an enclosed space. Headspace is any area in an enclosed space above a compound of interest, in this case fentanyl. After a given time, the solid will transition to the gas phase and both the headspace and solid will come to an equilibrium. Figure 1 depicts the equilibrium between the solid fentanyl and the headspace in a glass vial.

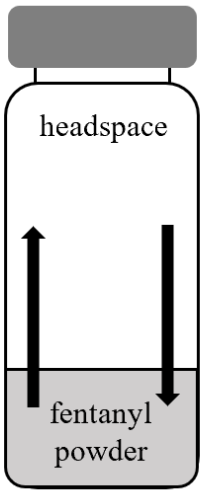

Figure 1. Graphical representation of headspace equilibrium

2.3.1 Field Detection

Methods of detection specific for vapor phase fentanyl are few and far between. Currently, colorimetric testing and, in some cases, handheld Raman testing, or canine detection, are available for the field testing of substances believed to be fentanyl (15, 16). However, these methods require drug handling and cannot detect any existing vapor phase fentanyl in the air or any that may have been vaporized during the presumptive tests. By detecting and quantifying fentanyl in a controlled setting, it can provide information about the risk associated with current presumptive testing methods and promote advancement in detecting vapor phase fentanyl in the field.

2.3.2 Solid Phase Microextraction

Several proposed methods of sample collection and analysis have been described regarding vapor phase fentanyl from powders. In one study concerned with the exhalation of intravenously administered fentanyl in a clinical setting, there is mention of the utilization of solid phase microextraction (SPME) in combination with GC-MS to

quantify the drug in breath. In this instance, gas samples were collected using an anesthetic circuit and then concentrated by adding and evaporating methanol. They were then sampled using a 65 μm DVB/PDMS fiber. The SPME fiber was exposed for 60 minutes at 85ºC and then allowed to desorb for 5 minutes in the GC-MS. They found that the concentration of fentanyl fluctuated with time and the peak concentration occurred 15-20 minutes after intravenous fentanyl administration. (17).

SPME is a manual sampling method that utilizes the presence of a fiber coating, or extraction phase, to bind compounds of interest. The extraction phase can be either a liquid or a solid and is housed in a protective needle. This needle is attached to a holder that operates like a syringe (18). Liquid, or polymer, and solid, or sorbent, coatings absorb the sample over an established extraction time and can then be inserted into a chromatographic instrument for desorption and analysis (18). The SPME fiber must come to an equilibrium with the headspace to obtain an accurate representation of the amount of vapor phase sample generated. The benefits of SPME are that is a solvent-free and non-destructive sampling method.

The coatings of SPME fibers vary in composition and thickness to optimize sampling over a range of molecular weights and polarities. PDMS coated fibers are considered liquid extraction phases and are non-polar (19). Solid modifiers, such as divinylbenzene (DVB) or carboxen (CAR) can be added to the PDMS fibers to help bind smaller or more volatile molecules. The smaller molecules are retained in the pores of the solid fiber coatings (20). However, modified fibers, such as the DVB/CAR/PDMS, will have less sample capacity due to the thinner coatings of each layer (19).

2.3.3 Whole Air Sampling

In addition to SPME sampling, whole air sampling is a beneficial practice because it does not rely on absorption and desorption of the sample from a fiber. As a result, the quantities observed from a sample of whole air provides a more accurate representation of the quantities of fentanyl that are in the vapor phase under a particular set of conditions. This is also because the introduction of a SPME fiber changes the equilibrium in the headspace due to the addition of binding sites and the need for a second equilibration in the presence of the fiber. The syringe, on the other hand is able to take up anything present in the headspace at the time of sampling. These whole air samples are drawn up using a gas tight syringe after being inserted into the headspace and then analyzed by GC-MS.

2.4 Gas Chromatography-Mass Spectrometry

Gas chromatography is a separation method that relies on the volatility of a compound to separate it from a mixture (21). It uses an inert gas, generally helium, as a mobile phase. The stationary phase is present as the coating on the column wall. Compounds in a sample will elute off of the column as a function of increasing temperature relative to their boiling point and vapor pressure. Upon injection into the instrument, the sample is heated until volatilized so that it is able to move through the system. The inert gas carries the sample into the column, a large, coiled tube usually made of fused silica, which is held inside of an oven. The oven is programable, allowing the temperature to be increased linearly, generally around 4 to 35°C per minute. After a

particular compound elutes from the GC column, the sample is introduced to the mass spectrometer by way of a transfer line.

The mass spectrometer acts as the detector for the GC. The ionization of the analyte is generally performed by electron ionization, in which the analyte vapor is bombarded by charged electrons while under vacuum. Most of the electrons will be scattered, but some will cause excitation and fragmentation of the analyte molecules into charged ions, allowing them to move through the MS. After production, ions are separated according to mass-to-charge (m/z) ratio on the mass analyzer. The most commonly used mass analyzer is the linear quadrupole which is constructed using four parallel hyperbolic rods placed in a radial array (21). Opposite rods are connected electrically, one pair to the positive end and the other pair to the negative end of a variable DC source. Variable radio frequency AC voltages, 180° out of phase are also applied to each pair of rods. Ions are accelerated into the space between the rods by a potential difference while AC and DC voltages are increased at the same time (22). Only the ions that have a limited range of m/z values and do not strike one of the rods are able to reach the transducer. The beam of ions that emerge from the mass analyzer are detected and converted to a usable signal.

The use of GC-MS for fentanyl detection is a technique that was validated well before its previously described analysis of SPME fibers. In 2004, Van Nimmen & Veulemans published literature presenting a highly sensitive GC-MS screening method for the simultaneous determination of nanogram levels of fentanyl and several of its analogs in air (23). In this experiment researchers collected air samples by using a filter sampler specifically designed for collecting inhalable particles. From there, the filters

were extracted, and the samples were transferred to vials. Upon analysis, fentanyl, sufentanil, and alfentanil were all well separated. Additionally, sensitivity was achieved utilizing a series of injections using the expected nanogram levels of each drug from an air sample (23). Overall, this study established new lower limits of detection for the drugs in question and proves that GC-MS is an effective method of detection for fentanyl in air samples.

2.5 Current Characterizations of Fentanyl Headspace

Current attempts at characterizing fentanyl headspace are limited to a single study performed by Vaughan et al. (24). This research uses SPME in combination with GC-MS to determine targets for identification of fentanyl in the vapor phase. Researchers placed 5 mg of pharmaceutical-grade fentanyl and 5 mg of fentanyl with 5 mg of various sugars in separate 20 mL headspace vials. The vials were equilibrated at 35°C for 30 minutes and sampled using a DVB/CAR/PDMS fiber that was exposed for 4 hours. Results indicate that known precursors, such as aniline and N-phenethyl-4-piperidone (NPP), were present in the vapor signatures, but this research was unsuccessful in identifying fentanyl as part of the vapor signature in the headspace.

2.6 Conclusion

Overall, there is a glaring lack of research regarding how fentanyl vapors are detected and the conditions under which they are formed. When considering its potency and the associated danger that fentanyl may present to those who are unknowingly exposed, it is important that methods are developed to detect, and eventually quantify, vapor phase fentanyl and its analogs. As previously mentioned, the presence of vapor

phase fentanyl is a growing issue, not only in terms of law enforcement and first responder exposure, but also in terms of its potential as a method of chemical warfare. However, we do not currently know the extent of this issue because, to date, there is no research focusing on the extent of fentanyl vapor is released from a dry powder when disturbed in an ambient environment or the quantities to which a person may be exposed.

The focus of this research is to characterize fentanyl headspace and quantify the vapor phase drug that is present under variable conditions. Fentanyl will be contained in headspace vials and sampled using PDMS and DVB/CAR/PDMS SPME fibers. The samples will be analyzed using GC-MS. This research aims to fill the gap in understanding of how and under what conditions vapor phase fentanyl is created. In turn, this will help to determine the risk associated with fentanyl encounters and aid in the development of detection methods for vapor phase fentanyl.

CHAPTER III

METHODOLOGY

3.1 Executive Summary

The purpose of this research was to understand the vaporization of fentanyl-HCl to ensure public health and safety. The research was conducted as four-part study, which included the comparison of multiple SPME fibers, a temperature comparison using a single optimized SPME fiber, a humidity variation, and a comparison using silanized and untreated headspace vials. In the fiber comparison, fresh fentanyl was heated to 60ºC and allowed to equilibrate for 7 minutes. Then each fiber was exposed three times in an alternate order, each for 15 minutes. For the temperature variation experiment, the same fentanyl was heated to 30, 40, 50, and 60ºC. A single fiber was exposed for 15 minutes to each temperature in triplicate after allowing the fentanyl to equilibrate for 30 minutes. Next, previously heated fentanyl from a different source was heated to 60ºC. Fentanyl in a 20 mL headspace vial, fentanyl in a 20 mL headspace vial with a 1 mL amber vial added, and fentanyl in a 20 mL headspace vial with an added amber vial approximately half full of water were each sampled in triplicate using a single SPME fiber. The fentanyl was allowed to equilibrate for 30 minutes, and the fiber was exposed for 15 minutes. For the final portion of experimentation, equal quantities of previously heated fentanyl from the former experiment were placed into two different headspace vials – one

untreated and one silanized. The fentanyl was heated to 60 ºC. The fentanyl in the untreated vial and the fentanyl in the silzanized vial were both sampled in triplicate using a single SPME fiber. Each vial was equilibrated for 30 minutes, and the fiber was exposed for 15 minutes. All samples were analyzed using GC-MS. They were then used to compare the amount of fentanyl that was volatilized into the headspace and bound to the SPME fibers.

3.2 Safety Note

Fentanyl was kept in sealed containers whenever possible to prevent exposure. Two individuals were present in any case involving the handling of bulk fentanyl. Naloxone (Narcan) was available at all times and all individuals involved had received training on proper administration.

3.3 Materials

Headspace vials were purchased from Environmental Sampling Supply (Environmental Sampling Supply, San Leandro, CA). The fentanyl-HCl powder used for the fiber comparison and the temperature variation was synthesized at the Oklahoma State University Forensic Toxicology and Trace Laboratory (OSU-FTTL). OSU-FTTL is a DEA registered entity for Schedule I-V controlled substances (fentanyl is Schedule II). The fentanyl-HCl powder used during the humidity variation was purchased from Synthcon (Synthcon, LLC, Colorado Springs, CO). Fentanyl standards at a concentration of 1 mg/mL in methanol were purchased from Cerilliant (Cerilliant Corporation, Round Rock, TX, Lot: FE12221601). Solid phase microextraction (SPME) fibers and holders were purchased from Supelco (Supelco, Inc. of Sigma-Aldrich Corp.,

Bellefonte, PA). A gas tight syringe (Model 81320) and needle (Model 90134) were purchased from Hamilton (Hamilton Co., Reno, NV). A heat block (Model 18823), 1 mL amber injection vials, LC-MS grade methanol, and 1,1,1,3,3,3-hexanemethyldisilazene (HMDS) were purchased from ThermoFisher (ThermoFisher Scientific Inc., Waltham, MA). VWR thermometer/clock/humidity monitor (Model 62344-734) and expanded-range thermometer (Model 61161-280) were both purchased from VWR (VWR International of Avantor, Randor, PA). Silanization was performed in a Napco Vacuum oven (Model 5861, National Appliance Co., Portland, OR) using 1,1,1,3,3,3-HMDS.

3.4 Instrumentation

An Agilent 7890A GC paired with a 5975C mass selective detector (MSD) (Agilent Technologies, Inc., Santa Clara, CA) was used for instrumental analysis. The GC inlet was operated in splitless mode and set at 250°C. Chromatographic separation was achieved with a RXI-5ms capillary column (30 m x 0.25 mm i.d. x 0.25 µm f.d.) from Resteck (Restek Corporation, Bellefonte, PA) and helium as a carrier gas, flowing at a constant rate of 1 mL/min. The GC oven temperature program started at 130°C, increased to 170°C at 40°C/min, and then increased to 320°C at 30°C/min. The oven was held at 320°C for 1.5 minutes for a total run time of 7.5 min. The MS interface was set at 230°C. The MS quad and source were set at 150°C and 230°C, respectively. The MS was operated in electron ionization mode at 70 eV with selected ion monitoring (SIM) for m/z 105, 146, 188, 189, 231, and 245. A 2.5-minute solvent delay was applied to the method. An Agilent 7693 autosampler was used to perform 1 µL injections of liquid samples, including the calibration curve; SPME and whole air sample injections were performed manually. For SPME, the fiber was exposed in the inlet for 5 minutes,

removed and soaked in methanol for 1.5 minutes, and then re-exposed in the inlet for the last 0.6 minutes of the method.

3.5 Quality Control

A weekly autotune and daily air and water checks were performed throughout the course of the experiment on the GC-MS. The parameters of the tunes were logged. Additionally, null, blank, and known positive injections of methanolic fentanyl standard at a concentration of 50 ng/mL were performed daily to ensure that the instrument was clean and producing consistent results. Each sample was actively logged as injected, and the instrument septum was changed approximately every 60 SPME injections or 120 ALS injections. Helium pressure was also logged daily. The room humidity was periodically monitored.

3.6 Instrument Limit of Detection and Experiment Preparation

The sensitivity of the instrument was determined by injecting 1, 10, and 50 ng/mL methanolic fentanyl standard solutions. The SPME fibers were assembled into holders. Figure 2 shows the reconstructed assembly for each of the three fibers. The red 100 µm and yellow 30 µm polydimethylsiloxane (PDMS) film SPME fibers were conditioned at 250ºC for 30 minutes, per the manufacturer's conditioning guidelines. The gray 50/30 µm divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) film SPME fiber was conditioned at 270ºC for 30 minutes per the manufacturer's conditioning guidelines. Fresh OSU-FTTL fentanyl-HCl powder (50 mg) was weighed out and placed into a 20 mL headspace vial.

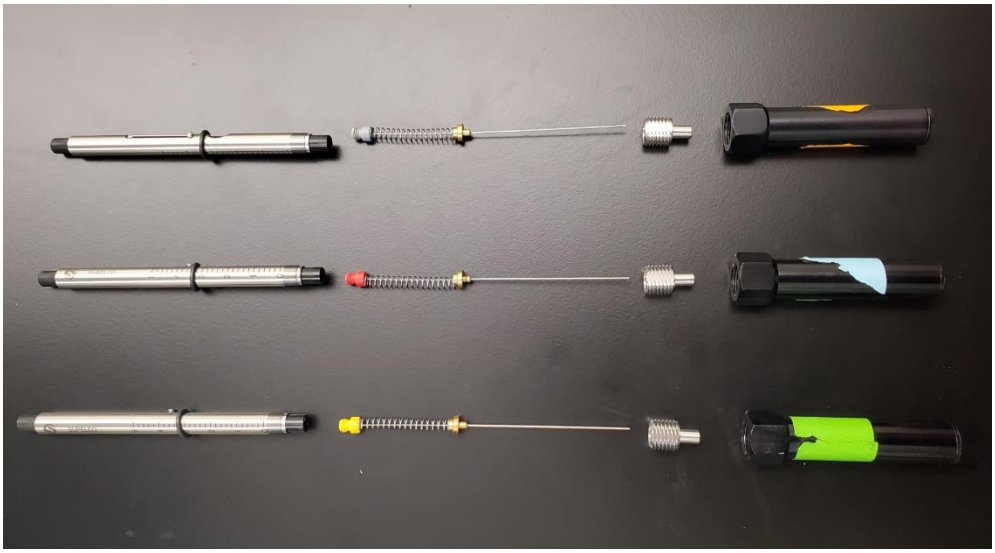

Figure 2. A deconstructed view of SPME fibers and holders*

*The fibers are from top to bottom as follows: Gray 50/30 µm DVB/CAR/PDMS, Red 100 µm PDMS, and Yellow 30 µm PDMS

3.7 Solid Phase Microextraction (SPME) Fiber Comparison

A thermometer probe was place into a heat block. The heat block was set to 60ºC. After reaching temperature, the 20 mL headspace vial containing 50 mg of fentanyl was placed into the heat block, as seen in Figure 3, and allowed to equilibrate for 7 minutes. The appropriate SPME fiber was then inserted into the top of the headspace vial and exposed for 15 minutes. Figure 4 shows the fiber exposure into the vial. Upon time completion, the fiber was removed from the vial and inserted into the instrument inlet and analyzed using GC-MS. The amount of time the fiber remained in the instrument is outlined in Section 3.4: Instrumentation.

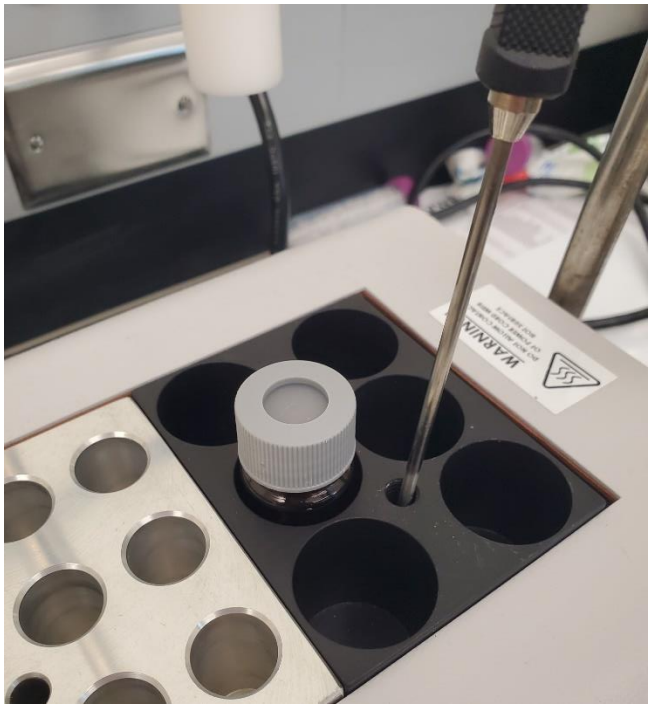

Figure 3. Heat block setup featuring fentanyl vial

Figure 4. SPME fiber exposed into the vial of fentanyl

For efficiency and to account for any change that may have occurred with the sample, the fibers were tested in an alternate order starting with the gray, then the red, and finishing with the yellow. Each fiber was exposed to the same fentanyl in the same headspace vial. In between samples, the headspace was given 7 minutes to re-equilibrate before the next fiber was inserted. This cycle of experimentation was repeated until triplicate samples had been taken for each fiber. It is important to note that the height of the SPME fiber holder and resulting depth of the fiber in the vial stayed consistent throughout experimentation. The vial remained in the heat block for the duration of experimentation. The triplicate data for the three fibers were collected in the same day.

3.8 Temperature Variation

The same 50 mg of fentanyl from the fiber comparison was used with the aforementioned setup. The temperature of the heat block was set to 30ºC. After reaching temperature, the vial of fentanyl was placed into the heat block and allowed to equilibrate for 30 minutes. Once equilibrated, the red SPME fiber was inserted into the vial and exposed for 15 minutes. The fibers were analyzed using GC-MS and removed from the instrument upon completion. At 30 ºC, quadruplet samples were collected. Only the red fiber was used.

After collecting four data points at 30 ºC, the vial of fentanyl was removed from the heat block. The heat block temperature was increased to 40 ºC and allowed to stabilize. Upon stabilization, the fentanyl was returned to its initial position on the heat block and allowed to equilibrate for 30 minutes. Following equilibration, the experimentation was carried out as previously described with a 15-minute red SPME

fiber exposure and GC-MS analysis. Quadruplet samples were collected at 40ºC. This procedure was repeated at 50 and 60ºC.

3.9 Humidity Variation

A new red fiber was conditioned at 250 ºC for 30 minutes, per the manufacturer's conditioning guidelines. Approximately 750 mg of previously heated Synthcon fentanyl-HCl powder was placed into a fresh 20 mL headspace vial. The heat block was set to 60ºC. After reaching temperature, the 20 mL headspace vial containing approximately 750 mg of fentanyl was placed into the heat block and allowed to equilibrate for 30 minutes. Once equilibrated, the red SPME fiber was inserted in the vial and exposed for 15 minutes. After 15 minutes the sample was analyzed using the GC-MS. This procedure was repeated three times.

After triplicate samples had been taken, the fentanyl was removed from the heat block, the cap was removed, and an empty 1 mL amber injection vial was carefully placed in the vial atop the fentanyl. The amber injection vial was added to the headspace vial to increase the surface area of exposed glass and assess the impact of the glass on the amount of vapor phase fentanyl in the headspace. The cap was returned to the vial and the vial containing the 750 mg of fentanyl and the 1 mL vial was returned to its previous position in the heat block. The headspace vial was equilibrated for 30 minutes. After 30 minutes, the red SPME fiber was inserted and exposed for 15 minutes. The sample was analyzed using GC-MS. The samples were taken in triplicate.

After three samples had been taken from the headspace vial containing the fentanyl and the empty 1 mL amber vial, the fentanyl was removed from the heat block.

The cap of the headspace vial was removed, and the empty amber injection vial was also removed. The 1 mL amber injection vial was filled approximately half full of water, and the vial was carefully placed back into the headspace vial and set atop the fentanyl. The addition of water to the 1 mL amber injection vial was a way to increase the humidity within the headspace vial and assess its impact on the amount of vapor phase fentanyl in the headspace. The cap of the headspace vial was replaced, and the fentanyl was returned to its position in the heat block. The vial was again equilibrated for 30 minutes before the red SPME fiber was inserted and exposed for 15 minutes. The sample was analyzed using the GC-MS. This procedure was repeated three times. The three conditions under which samples were collected are depicted in Figure 5.

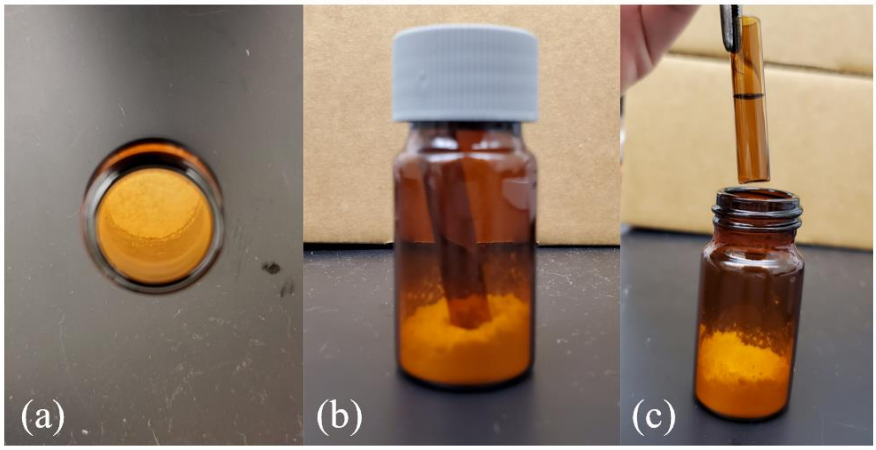

Figure 5. Depiction of the three sampling conditions in the humidity variation experiment where (a) is only the fentanyl in the 20 mL headspace vial, (b) is the fentanyl in the 20 mL headspace vial with an empty 1 mL amber vial, and (c) is the fentanyl in a 20 mL headspace vial with the 1 mL amber vial filled approximately halfway with water

3.10 Silanized v. Untreated Glass

From the previously heated 750 mg of Synthcon fentanyl-HCl, 250 mg were placed into an untreated 20 mL headspace vial and 250 mg were placed into a silanized

20 mL headspace vial. The heat block was set to 60ºC. After reaching temperature, the untreated 20 mL headspace vial containing fentanyl was placed into the heat block and allowed to equilibrate for 30 minutes. After 30 minutes, the red SPME fiber was inserted into the vial and exposed for 15 minutes. At the same time, the silanized 20 mL headspace vial containing fentanyl was placed into the heat block to equilibrate for 30 minutes. After 15 minutes, the fiber was removed from the untreated vial and analyzed by GC-MS. Upon analysis completion, the fiber was inserted into the silanized vial and exposed for 15 minutes. The fiber was then analyzed using GC-MS. This procedure was repeated until triplicate samples from each of the two vials had been collected.

3.11 Whole Air Sampling

Whole air samples were also collected throughout the previously described procedures using a 1 mL gas tight syringe. During the temperature variation portion of experimentation, 1 mL of whole air was collected from the 20 mL headspace vial containing 50 mg of OSU-FTTL fentanyl-HCl powder at each temperature increment, ranging from 30-60ºC. A whole air sample was also collected during the portion of the experiment involving the humidity variation. At that point, 1 mL of whole air was collected from the 20 mL headspace vial containing 750 mg of Synthcon fentanyl-HCl powder. Finally, a whole air sample was collected from the silanized headspace vial containing 250 mg of Synthcon fentanyl-HCl. For each of the whole air samples collected, the respective fentanyl was allowed to equilibrate in the heat block for 30 minutes prior to sampling. All samples were manually injected and analyzed using the GC-MS.

3.12 Calibration Curve

A calibration curve was prepared to quantify the amount of fentanyl introduced to the GC-MS based on peak areas. A 1 mg/mL fentanyl standard was used to perform serial dilutions. From the 1 mg/mL solution, 100 µL was used to generate a stock of 100 µg/mL with 900 µL of methanol. Next, 600 µL of the 100 µg/mL solution was used to generate a 10 µg/mL solution with 5400 µL (5.4 mL) of methanol. This 10 µg/mL solution was used as the starting concentration for preparing the curve. A total of 6 calibration points were made ranging from 50 ng/mL to 1500 ng/mL which is equivalent an on-column range of 0.050 ng to 1.5 ng in each 1 µL injection. Calibrators were prepared by using the previous calibration level and methanol, with the specified volumes necessary for a curve production as presented in Table 1. A single curve was run in triplicate to assess reproducibility and precision.

Table 1. Calibration curve sample preparation

Sample Name	10 ug/mL	1500 ng/mL	1000 ng/mL	500 ng/mL	250 ng/mL	100 ng/mL	Methanol (µL)	Total Volume (µL)	Remaining (µL)
1500 ng/mL	900 µL						5100	6000	2000
1000 ng/mL		4000 µL					2000	6000	3000
500 ng/mL			3000 µL				3000	6000	4500
250 ng/mL				1500 µL			1500	3000	2200
100 ng/mL					800 µL		1200	2000	1000
50 ng/mL						1000 µL	2000	2000	2000

3.13 Data Analysis and Peak Selection

Following the GC-MS analysis, the gas chromatograms and mass spectra were reviewed using Agilent's ChemStation software (ChemStation Software, Agilent Technologies, Santa Clara, CA). Peak areas were obtained using manual integration. It was necessary to manually integrate each peak as opposed to having the software perform

an automated integration. Figures 6 and 7 show the results of an automated integration and manual integration, respectively, on the same GC-MS chromatogram. The need for manual integration can be observed by the improper, large peak selection, the red line, on the chromatogram displaying the automated integration. Figure 8 displays an enlarged image of the manually integrated fentanyl-HCl peak (RT ~ 6.654 min) on the GC-MS chromatogram. As shown, each peak was carefully selected from base to base for each sample run. It should be noted that manual integration can vary by user and by individual selection to some extent, but by ensuring that each peak was selected from base to base, accuracy was increased. Additionally, the criteria for fentanyl peak acceptance were the presence of all three fentanyl ions, m/z 146, 189, and 245, at the proper retention time. Figure 9 shows an extracted ion chromatogram with all three fentanyl ions. Note, retention times did vary slightly (± 0.2 min) since SPME requires a manual sample injection.

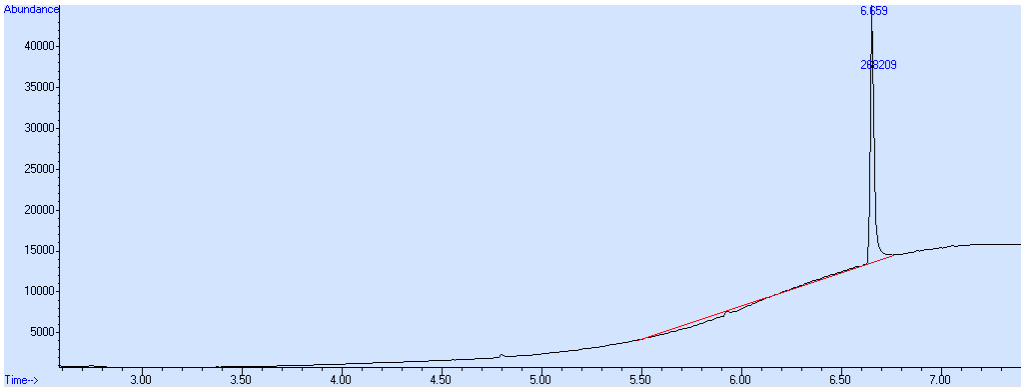

Figure 6. Fentanyl peak integrated using automated integration.
Note that the software inappropriately integrated more of the TIC than from base to base of the peak.

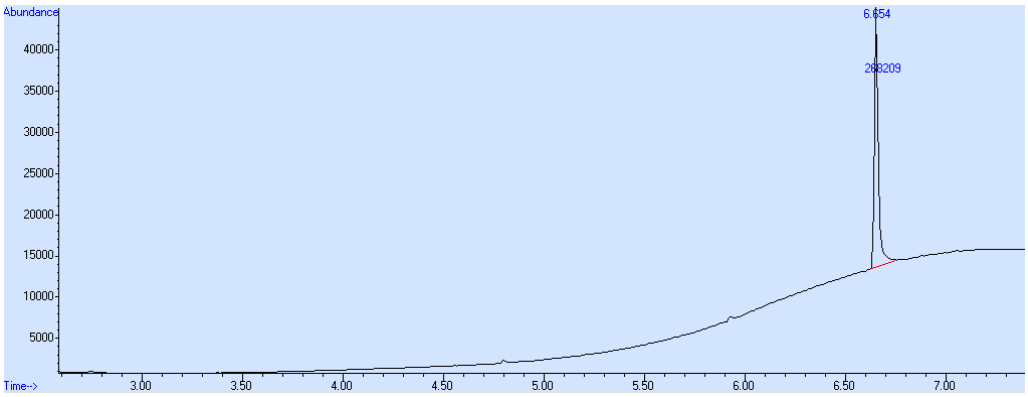

Figure 7. Fentanyl peak integrated using manual integration.
Note the proper base to base integration of the peak.

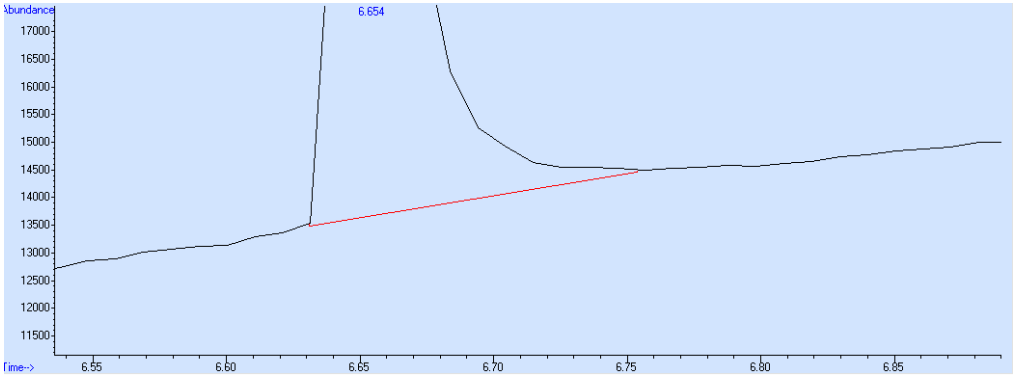

Figure 8. Enlarged image of a manually integrated fentanyl peak showing base to base peak selection

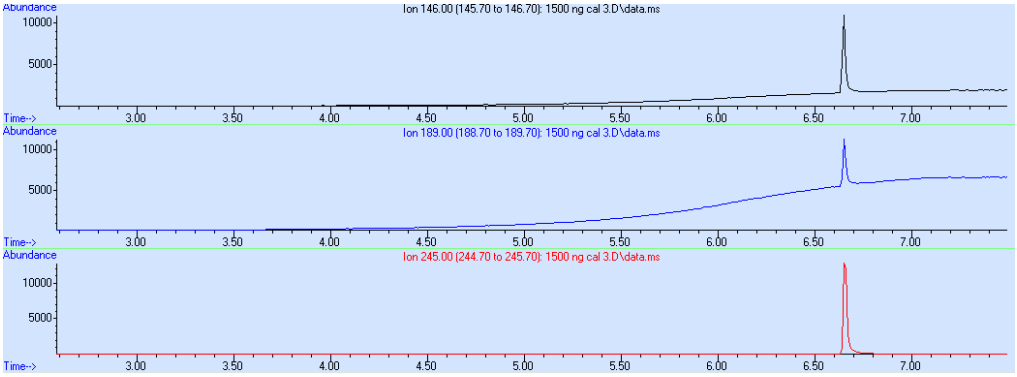

Figure 9. Extracted ion chromatogram showing the presence of all three fentanyl ions

3.14 Statistical Analysis

Statistical analyses of quantitated peak areas were done in GraphPad Prism 7.03 (GraphPad Software, La Jolla, CA) and Microsoft Excel (Microsoft Office, Microsoft Corporation, Redmond, WA). These analyses were comprised of one-way analysis of variance (ANOVA) tests with Tukey's multiple comparisons post hoc tests, and two-tailed t-tests to determine if any statistically significant differences were observed between each of the four data sets in question. The mass of fentanyl on the fiber from each sample was also calculated using a linear regression. All outliers were tested and removed using Grubbs' test in GraphPad Prism QuickCalcs (GraphPad Software, La Jolla, CA).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Limit of Detection

Prior to sampling with the fibers, the sensitivity of the instrument had to be determined. Fentanyl standards in methanol were run at concentrations of 1, 10, and 50 ng/mL. Fentanyl could be consistently positively identified at 50 ng/mL, therefore, that was the concentration utilized daily as the positive control, ensuring the limit of detection was achievable during each day of analysis.

4.2 SPME Fiber Comparison

The three-fiber comparison was performed at 60ºC because fentanyl was not detected until that point. The gray 50/30 µm DVB/CAR PDMS, red 100 µm PDMS, and yellow 30 µm PDMS fibers were tested in alternate order. Figure 10 presents a graphical representation of the peak areas of fentanyl collected using each of the three fibers.

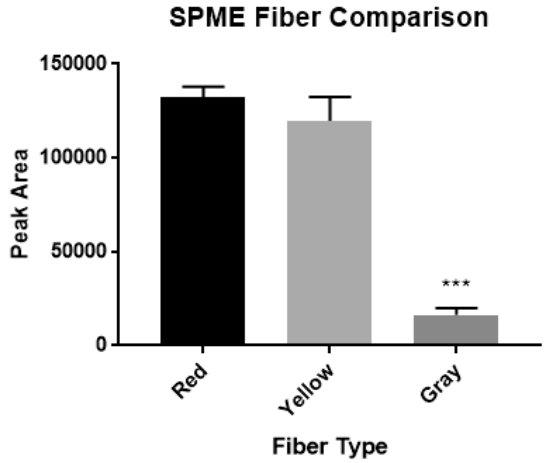

Figure 10. Graphical representation of the three-fiber comparison with error bars

***p-value <0.001 signifying an extreme statistical difference between the gray data set and the yellow and red data sets, N=3

Additionally, the concentration of fentanyl in the headspace from each sample was determined by using a linear regression from the calibration curve. The peak areas from each sample were plugged in to determine the amount of fentanyl on the SPME fiber in nanograms. Figure 11 shows the plotted calibration curve and the equation for the linear regression. Table 2 presents the average mass of fentanyl on the fiber by color.

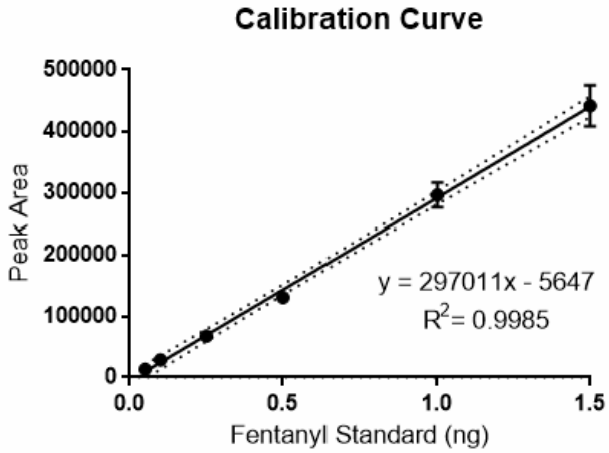

Figure 11. Calibration curve displaying the equation from the linear regression and the R^2 value

Table 2. Average mass of fentanyl on each fiber color

Fiber Color	Average Mass of Fentanyl (ng)	Standard Deviation (ng)
Gray***	0.0740	0.0120
Red	0.4649	0.0184
Yellow	0.4237	0.0351

Using an analysis of variance (ANOVA) and a post hoc multiple comparison test, it was determined that the gray fiber was statistically different from the yellow and red fibers (p-value <0.001), but the yellow and red fibers were not statistically different from one another. Therefore, either the yellow or red fiber would have been an appropriate choice for the remainder the experimentation. The difference in binding displayed by the red and yellow versus the gray fiber is likely a result of the polymer differences. The red and yellow fibers are made of 100 µm and 30 µm polydimethylsiloxane (PDMS) film, respectively. However, the gray fiber is coated with a 50/30 µm divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) film. Traditionally the DVB/CAR/PDMS film is better at binding compounds that are highly volatile and compounds with low molecular weights. Fentanyl-HCl is heavy and has a low vapor pressure. Additionally, DVB/CAR/PDMS fibers have lower sample capacity because the layers of each coating are thinner. As a result, it is not a surprise that the DVB/CAR/PDMS film rendered significantly lower results than that of the fibers coated with PDMS.

4.3 Temperature Variation Using the Red Fiber

After the SPME fiber comparison, it was realized that each time a sample was taken, the headspace was depleted, and adequate time had to be allowed for the

headspace to build back up in the vial. To achieve the best results, the time between exposures was extended from 7 minutes to 30 minutes for all the subsequent experimentation. As previously noted, there is no statistical difference between the data collected for the yellow and red fibers. However, the red fiber was used for all the following experimentation due to the slightly larger average peak areas observed. For the temperature variation portion, it was important to return to lower temperature because these temperatures are closer to the temperature that an individual would encounter in an ambient environment. As a result, collecting data at lower temperatures could be more helpful in influencing law enforcement and community safety. Additionally, while a small temperature variation experiment was determined prior to settling on 60 ^{o}C for the fiber comparison study, the various temperatures were not assessed with red fiber. Figure 12 presents a graphical representation of the samples taken with the red fiber at 30, 40, 50, and 60 ^{o}C. The mass of fentanyl on the fibers were also calculated using the peak areas at each of the temperatures as described previously. Table 3 presents the average mass of fentanyl on the fiber at each temperature point.

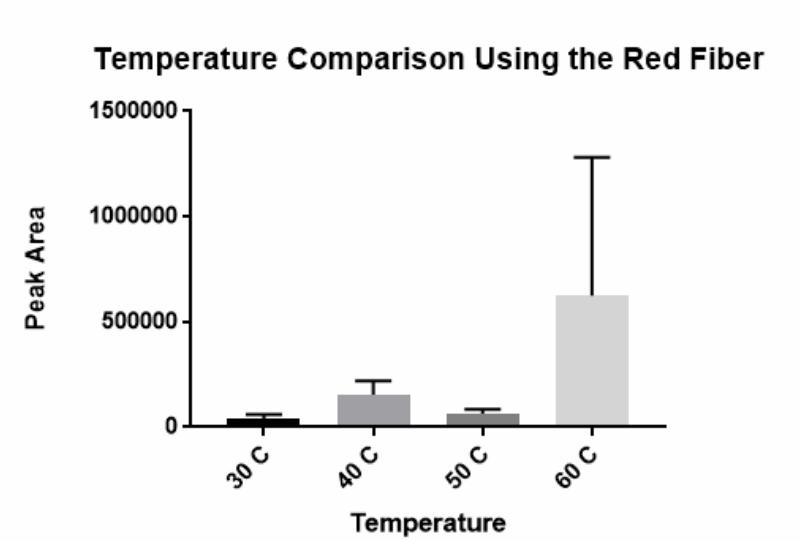

Figure 12. Graphical representation of the temperature comparison with error bars, N=4

Table 3. Average mass of fentanyl on the fiber at each temperature

Temperature (ºC)	Average Mass of Fentanyl (ng)	Standard Deviation (ng)
30	0.1577	0.0660
40	0.5386	0.2235
50	0.2355	0.0714
60	2.1254	0.5766

After performing an ANOVA and a post hoc multiple comparisons test, it was determined that there is no statistical difference between the data collected at any of the temperatures. Additionally, the error bars for the data sets are large, indicating substantial variation between the data points. This may have occurred for several reasons. First, SPME is an extremely variable sampling method due to its manual nature and the possibility of introducing human error. Next, there could have been a bulk fentanyl contamination which could cause the results to be skewed very high. Finally, agitation of the fentanyl can cause vaporization so some kind of agitation to the vial could be the cause of the large spread in results.

Aside from the large variation, the peak areas do trend upwards with temperature. This result is not unexpected because as the temperature increases, the vapor pressure also increases, causing the amount of sample entering the headspace to also increase.

4.4 Humidity Variation

Over the course of the experiment, the results of the SPME exposures varied from day to day. These variations were drastic, ranging from easily detectable fentanyl to none. Instrument issues could be ruled out because the daily air and water checks, as well as the positive control, were both consistent throughout experimentation. However,

it was noticed that humidity in the lab where the samples were being collected was subject to variation. Table 4 presents daily humidity. It was decided that water would be added to the headspace vial containing fentanyl in order to increase the humidity since it would be easier than attempting to decrease the humidity. Figure 13 presents a graphical representation of the peak areas of the fentanyl in the headspace vial, the headspace vial with an empty 1 mL amber injection vial, and the headspace vial with a 1 mL amber injection vial containing water. The peak areas from each sampling variation were used to calculate the mass of fentanyl on the fibers, as seen in Table 5.

Table 4. Daily recorded humidity*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Humidity (%)	40	32	21	24	41	44	44	40	30

*The three-fiber data was collected on Day 2, the temperature variation data was collected on Days 3 and 4, the humidity variation data on Day 8, and the samples comparing the untreated and silanized glass were collected on Day 9

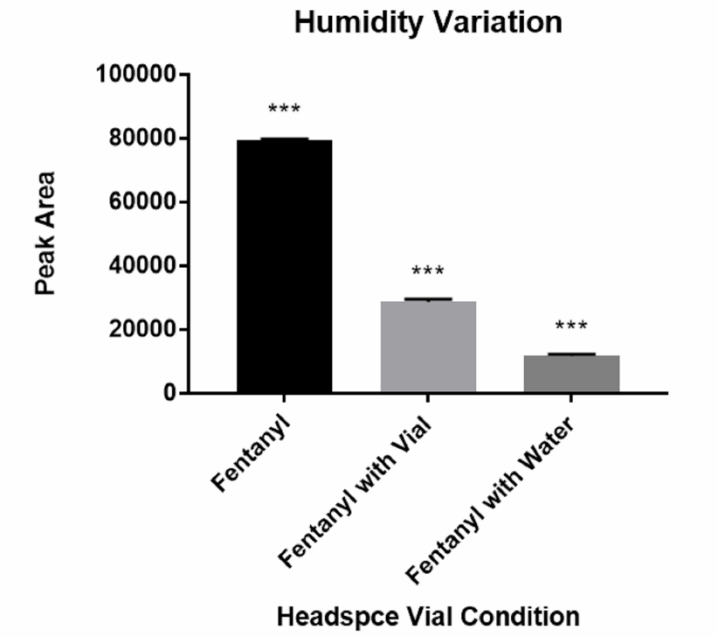

Figure 13. Peak areas of the sampling using only fentanyl, fentanyl with an empty amber vial, and fentanyl with an amber vial containing water, N=3

***p-value <0.001 signifying an extreme statistical difference between each of the data sets

Table 5. Average mass of fentanyl on the fiber under each of the three conditions

Condition	Average Mass of Fentanyl (ng)	Standard Deviation (ng)
Only Fentanyl***	0.2862	0.0013
Fentanyl with Empty Vial***	0.1158	0.0027
Fentanyl With Water***	0.0585	0.0019

The addition of the empty glass vial and the water into the fentanyl powder provides the most insight into its binding to the SPME fiber and the potential interferences. An ANOVA and a post hoc multiple comparisons test were performed on the three data sets. These analyses indicated significant difference between each of them. Since all three data sets are significantly different, it means that several factors could contribute to the binding a fentanyl to the SPME fiber or its presence in the headspace, thus accounting for the day-to-day variation in results. These results could be because the water in the vial is competing for binding on the fiber, suppressing the fentanyl binding, or that the glass vial has active sites that are allowing fentanyl to bind to the glass itself instead of the SPME fiber. However, the addition of the 1 mL amber injection vial may not be the only source of glass interference. After reviewing the headspace vials used for all three parts of the study, it was found that they were not silanized. In other words, the vials used for sampling are not treated to eliminate active sites. As a result, the variation from day to day could also be attributed to the glassware in which the fentanyl was heated in.

4.5 Untreated v. Silanized Glass

Based on concerns regarding the potential active site interferences from the glassware in which the fentanyl was being heated, comparing fentanyl contained in untreated vials and fentanyl contained in silanized vials was a necessity. In this case, "untreated" is used to refer to the glassware that was utilized throughout the previous experimentation. Figure 14 presents a graphical representation of the peak areas obtained from the sampling of the untreated and silanized vials. The peak areas from both scenarios were also used to calculate the mass of fentanyl on the fibers. Table 6 presents the average mass of fentanyl for the untreated and silanized vials.

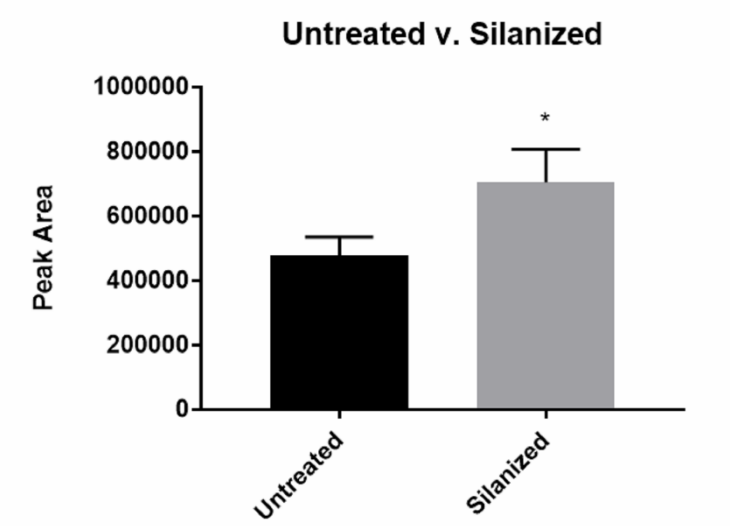

Figure 14. Graphical representation of the peak areas obtained from the fentanyl in the untreated glass and the silanized glass headspace vials, N=3

*p-value <0.05 signifying a statistical difference between each of the data sets

Table 6. Average mass of fentanyl on the fiber from the untreated and silanized glass headspace vials

Glass Condition	Average Mass of Fentanyl (ng)	Standard Deviation (ng)
Untreated	1.6322	0.1895
Silanized	2.3918	0.3440

A two-tailed t-test was used to compare the two data sets. The results indicated that there is a statistical difference between the peak areas obtained from the fentanyl in untreated vial and the silanized vial ($p<0.05$), confirming that variation in fentanyl detection can be attributed to the binding sites in the glassware in which the fentanyl was being heated.

4.6 Whole Air

Fentanyl was not detected in whole air samples taken from the headspace vials containing either the fresh 50 mg of OSU-FTTL fentanyl-HCl powder or the 750 mg of previously heated Synthcon fentanyl-HCl powder. Additionally, fentanyl was not detected in whole air samples taken from the silzanized headspace vial containing 250 mg of Synthcon fentanyl-HCl. Though humidity could still be a factor in the absence of fentanyl detection in whole air, the lack of fentanyl in whole air from the silanized headspace vial indicates that the SPME is likely successful because of its ability to concentrate the fentanyl before being analyzed by GC-MS, whereas whole air cannot.

CHAPTER V

CONCLUSIONS

The characterization of fentanyl headspace by gas chromatography-mass spectrometry was performed by comparing multiple SPME fibers over a range of temperatures, by introducing water to the inside of a headspace vial containing fentanyl, and by varying the containment vessels in which the fentanyl was being stored and sampled.

The goal of this study was to answer several questions: 1.) Which SPME fiber is the best at characterizing fentanyl headspace? 2.) Does temperature effect one's ability to detect fentanyl? 3.) Does variation in humidity affect fentanyl detection in the headspace? 4.) Do active sites in the fentanyl storage containers affect detection? As far as the viability of SPME fibers, the red and yellow polydimethylsiloxane fibers were statistically different than the gray divinylbenzene/carboxen/PDMS fiber indicating that PDMS is a better fiber coating for fentanyl sampling regardless of the thickness. The amount of fentanyl does trend upward with heat increase, but the sampling at different temperatures did not render statistically different results. The addition of water and glassware to the vial of fentanyl did render statistically different results from each other and from the fentanyl by itself, but it raised even more questions about the interference of binding sites on the glassware. Silanization of glassware did make a difference in the

detection of fentanyl, with silanized vials rendering significantly larger fentanyl quantities than untreated vials. Finally, whole air may not be a viable sampling method under the experimental conditions in this research.

Overall, very low quantities of fentanyl, significantly less than the lethal dose of two milligrams, are being vaporized under any of the conditions described above. In fact, it would take approximately 209 kg of fentanyl sitting out on a table at 60°C to produce 2 mg of fentanyl vapor. As a result, this indicates that the risk of the unknowing exposure to law enforcement or other members of the community may be lower than once believed. However, further research can be done to determine if and to what extent humidity has on the detection of vapor phase fentanyl.

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