CHARACTERIZATION OF FENTANYL HEADSPACE

USING SOLID PHASE MICROEXTRACTION:

EFFECTS OF SAMPLING HEIGHT AND HUMIDITY

AT FIXED VOLUME AND TEMPERATURE

By

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Abstract: The rapid increase in illicit manufacturing of fentanyl and deaths due to fentanyl overdoses within the past few years has led to concerns for the safety of individuals unknowingly encountering it. In order to develop safer methods of field detection of fentanyl, understanding and observing fentanyl headspace is important. This study was conducted to determine the best sampling height for detecting fentanyl in fentanyl headspace. The headspace was sampled using solid phase microextraction (SPME) fibers at about 1 cm, 4 cm, 7 cm, and 9 cm from the fentanyl source. The samples were analyzed through manual injection of the SPME fibers on a gas chromatography – mass spectrometer (GC-MS). The relative abundance of fentanyl, 4-anilino-N-phenethyl-piperidine (4-ANPP), N-phenethyl-4-piperidone (NPP), and N-phenylpropanamide (NPPA) on these samples was measured. No 4-ANPP was detected throughout this study. It was determined that sampling at a further distance from the fentanyl source increased the amount of fentanyl and NPP detected, while distance had little effect on NPPA abundance. It was also determined that an increase in humidity increased the amount of fentanyl and NPP detected within the range of 30-45% humidity.

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CHAPTER I

INTRODUCTION

The prevalence of opioid-related drug overdoses has been rapidly rising since the introduction of opioids for prescription pain management. As more people are introduced to opioids, more people there are at risk of addiction. A rise in addiction would increase the number of people using opioids without a prescription or knowledge of appropriate dosage. Opioid overdose deaths have increased from 68,630 cases in 2020 to 80,411 cases in 2021, with synthetic opioids being involved in the majority of these cases [1]. The primary source of these synthetic opioid deaths is the drug fentanyl. Fentanyl was introduced in the 1960s as an intravenous anesthetic [2]. Since its introduction, the illegal manufacturing and sale of fentanyl has become increasingly prevalent. The rising presence and high potency of fentanyl have led to concerns about public safety. In particular, the safety of people who may unknowingly come into contact with it while in vapor phase such as law enforcement officers or first responders.

The potential danger of individuals being exposed to vapor-phase fentanyl is particularly concerning because the low dose of 2 mg can be lethal, with smaller amounts still causing negative symptoms in individuals who are opioid-naive [2]. Current field detection methods lack

ways to detect fentanyl accurately without close contact with the material itself. Developing methods for vapor detection of fentanyl would reduce this danger. A thorough understanding of fentanyl headspace would aid in developing vapor phase fentanyl detection. This research aims to investigate the best elevation point from fentanyl HCl powder for solid phase microextraction (SPME) fiber sampling in fentanyl headspace.

CHAPTER II

REVIEW OF LITERATURE

2.1 Fentanyl Dangers

Opioids are central nervous system (CNS) depressants known for their analgesic effects [3]. The painkilling effect is achieved by the drug binding to the mu, kappa, and delta opioid receptors, blocking the release of neurotransmitters. Impeding the neurotransmitters prevents the brain from processing painful sensations. The powerful pain-relieving ability of these drugs makes opioids useful for sedation during medical emergencies, recovery from surgical procedures, and chronic pain management such as patients with cancer or a terminal illness. While the analgesic effects of opioids are highly useful for treating cases of severe pain, overuse can have major negative effects. The expected drowsiness, delayed reflexes, and decreased blood pressure can escalate into respiratory depression, hypotension, seizure, coma, or even death [3]. If used in combination with other CNS depressants such as another opioid, alcohol, or benzodiazepines, opioids have an additive effect increasing the negative effects even further. Despite the negative effects opioids can have, the euphoria that can occur from removing the negative emotions accompanied by pain makes opioids extremely addictive.

The high level of addictiveness and increased access through legitimate and illicit means have made opioids a prevalent danger. According to the National Center for Drug Abuse Statistics, opioids were involved in about 72% of overdose-related deaths in 2019 [1]. Opioids are categorized as natural, semi-synthetic, and synthetic based on the materials used to manufacture the drug. The manufacture and use of synthetic opioids have risen and continue to increase exponentially in recent years, likely due to their higher potency as compared to natural opioids. This higher potency has also led to a higher lethality. From 2016 to 2021, synthetic opioids (not including methadone) were the most common drugs involved in overdose deaths [3]. In 2021, the Centers for Disease Control reported 70,601 drug overdose deaths involving synthetic opioids not including methadone [1]. A majority of these synthetic opioid cases involve illicitly manufactured fentanyl.

Fentanyl is a highly potent Schedule II synthetic opioid, being 50 to 100 times more potent than morphine, a natural opioid [4]. Illicitly manufactured, fentanyl is produced in powder or tablet form [2]. The product may contain other drugs in combination with fentanyl. Common mixtures include another CNS depressant like heroin or a stimulant such as cocaine. An analysis of 2021 fentanyl seizures by the Drug Enforcement Administration's Fentanyl Signature Profiling Program showed that the opioids fluoro-fentanyl, a fentanyl analog, and heroin were the most common secondary controlled substances combined with the fentanyl [5]. Of the seized tablets, about 44% contained at least 2 mg of fentanyl, which is the lethal dose of fentanyl. The seized powder samples had an average purity of 14.4% fentanyl, which would mean that a 1 kg sample of such a powder would contain about 72,000 lethal doses of fentanyl within it. Considering a majority of the seized powders from this 2021 analysis were 1 kg or larger, it lends credence to the concern for the safety of law enforcement officers and first responders who may be unknowingly exposed to fentanyl vapors. Fentanyl and its analogs are not only a danger due to their potency, but the lipophilicity allows the drugs to cross the blood-brain barrier quickly. Thus,

making fentanyl fast acting as well as potent. As the purity of samples has increased by 3% from 2020 to 2021, this concern is only likely to continue.

2.2 Detection of Fentanyl

The current methods of detecting fentanyl in the field are limited to immunoassay test strips, ion mobility spectrometry (IMS), and, in some cases, handheld Raman spectroscopy [6 – 8]. The test strips function by first dissolving the possible fentanyl sample in water, then placing the strip in the solution. After 5 minutes, the sample can be read as positive or negative depending on the number of lines on the strip. Current test strips are fairly sensitive with a lower limit of detection of $0.1 \mu g/ml$ with a false negative rate of 3.7% [6]. Unfortunately, these test strips lack quantitative results and are less successful when identifying the presence of fentanyl analogs. The strips have been shown to successfully detect only two fentanyl analogs, acetyl fentanyl, and furanyl fentanyl, but no others.

IMS and thermal desorption direct analysis in real-time mass spectrometry (DART-MS) are useful tools for detecting fentanyl and fentanyl analogs in a rapid and sensitive manner [7]. For IMS analysis, the samples are first dissolved in methanol and then dried on wipes. These wipes can be analyzed by the IMS for the presence of fentanyl and fentanyl analogs. This method of detection was found to be sensitive enough to detect fentanyl in a mixture with heroin down to 0.1% by weight of fentanyl [7]. Although it was less successful with fentanyl mixtures containing other substances, DART-MS samples are similarly prepared except with wipes coated in fiberglass. DART-MS showed itself to be successful in identifying the presence of fentanyl regardless of the cutting agents used [7]. Both IMS and DART-MS show promise as rapid and sensitive tools for detecting the presence of fentanyl from surface sampling, but still require direct

contact with the material. They also lack the ability to produce quantitative results, and DART-MS is not field-portable.

Surface-enhanced Raman Scattering detects target molecules by absorbing them on specific metals [8]. For Raman scattering, the fentanyl sample is dissolved in a water solution with gold nanoparticles. With this method, the creation of a calibration curve was successful, which indicates that quantitation of fentanyl samples is possible. The Raman spectrometer was still able to detect fentanyl in samples mixed with other substances such as heroin and glucose, but the quantitation of fentanyl was unsuccessful. While this method is sensitive, being able to detect fentanyl samples as low as $0.2\ \mu M$, it still requires direct handling of the substance for detection. Raman spectroscopy can indirectly test for the presence of a chemical compound using a laser probe, but this method has difficulty detecting fentanyl unless the samples have a high purity [6].

Of the current methods of fentanyl field detection, IMS and DART-MS limit contact with samples that may contain fentanyl the most. This limited contact is achieved by detection through sampling surfaces rather than directly sampling the powdered sample. Unfortunately, surface wiping still requires the person to be in close proximity to the sample prior to detection. This means that law enforcement or first responders could still come into contact with fentanyl by breathing in vapor phase fentanyl, or fentanyl in the air. Vapor detection of fentanyl would reduce the risk of unknowing contact with fentanyl. Potential methods of vapor detection are portable devices that can sample the air for fentanyl, or canines whose sensitive noses could detect its presence quickly. Both of these methods would require an understanding of how fentanyl acts in the vapor phase. So, an understanding of fentanyl headspace is necessary to develop safer in the field detection methods.

2.3 Current Characterization of Fentanyl Headspace

One emerging method of studying fentanyl headspace is through solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) [9-11]. Headspace analysis is a technique used to measure the abundance of volatile and semi-volatile compounds in the air [12]. The headspace is created by sealing the material being analyzed in an air-tight container, then allowing the material to volatilize and enter the gaseous phase. A sorbent material is exposed to the headspace to collect the compounds in the air, and then the material is desorbed, pulling the compounds off the surface to be analyzed.

SPME is a sampling method that can be coated in a chosen material that allows for high specificity when targeting specific analytes within the headspace [13]. The SPME device consists of a fiber coated with a sorbent material mounted inside a needle. The needle is inserted into a septum in the headspace container, while the headspace equilibrates, the volatile compounds enter the vapor phase. The fiber is then exposed from the needle into the headspace. There is an equilibrium between the fiber and the headspace, and exposure time is one factor that affects the amount of analytes extracted from the headspace. After extraction, the fiber is retracted into the needle and removed from the headspace container. The compounds can then be desorbed into an analytical instrument such as a GC-MS, releasing the compounds from the fiber to be analyzed. It has been found that the most successful SPME fiber for detecting fentanyl is a 100 µM silica fiber coated in polydimethylsiloxane (PDMS) exposed in a headspace between 30 - 60°C [14].

SPME fibers can be analyzed on a GC-MS using manual injection. Gas chromatography is a separation method that utilizes differing volatilities, polarity, and other chemical characteristics of a compound to separate it from a mixture [15]. An inert gas, such as helium, carries the compounds through the stationary phase of the column coating. The coating on the column along with the increasing temperature of the oven moves compounds through the column at different

rates. The compounds elute off the column at a specific retention time into the mass spectrometer. The mass spectrometer detector then ionizes the analyte using an ion source, usually electric ionization [15]. The ionization process causes the compound to fragment in patterns that are characteristic of that analyte. Ions are separated by mass-to-charge ratio (m/z) on the mass analyzer. The ion beams that emerge from the mass analyzer are detected, giving a mass spectrum that is unique to that compound. A highly sensitive GC-MS method has been developed and validated for the detection of fentanyl and some of its analogs in air and surface samples [16]. The limit of detection of fentanyl for air samples in the 2004 study was determined to be 0.4 ng of fentanyl. The method was also shown to detect alfentanil and sufentanil, common fentanyl analogs.

Headspace SPME samples analyzed on the GC-MS have detected a number of volatile compounds in fentanyl headspace [9 – 11]. The most abundant compounds detected, aside from fentanyl, are 4-anilino-N-phenethyl-piperidine (4-ANPP), N-phenethyl-4-piperidone (NPP), and N-phenylpropanamide (NPPA). 4-ANPP and NPP are precursors for the synthesis of fentanyl, so they are common impurities in fentanyl powder. NPPA is a common degradation compound of fentanyl. An initial study of fentanyl headspace found NPP and NPPA to be the main components of fentanyl headspace [9]. A study of the passive degradation of fentanyl found NPPA and styrene to be the most abundant compounds in the headspace [10]. Another degradation study identified the compounds NPPA, 1-phenethyl-4-propionyloxypiperidine, 4-ANPP, fentanyl, N-phenylacetamide, and NPP [11]. In this study, the abundance of these compounds was found to increase in the headspace as the fentanyl HCl powder degraded. The abundant detection of fentanyl, 4-ANPP, NPP, and NPPA makes them potential targets for vapor detectors.

2.4 Humidity and Vaporization of Fentanyl

Vaporization is the conversion of a compound from its solid or liquid phase to the vapor phase. Fentanyl has a low volatility or vaporization, so it does not easily enter the vapor phase and then diffuse through the air [17]. There are many factors, both environmental and particle properties, that can affect how well a powder material enters the air [18]. One environmental factor is humidity. Humidity is the measurement of the percentage of moisture in the air; the higher the humidity the more water is in the air. The relative humidity is known to affect the flowability, how tightly the particles stick to each other, of powders. The higher the flowability of a powder, the less that the powder moves. Flowability decreases with an increase in moisture content up to a certain critical water content, after which the flowability increases [19]. This means that below a certain relative humidity, the low flowability allows for more movement of the dry powder. Therefore, a lower flowability would make it easier for the powders to move around, perhaps even into the air. Flowability is also affected by particle properties such as morphology, size, size distribution, density, and surface area. All of these characteristics of the powder, along with environmental factors such as humidity and temperature, have a hand in the vaporization of fentanyl. There has been very little research done on the extent these characteristics affect vaporization. It has become apparent that humidity interferes with the vaporization, thus detection of fentanyl during headspace analysis, although the specifics of the interference are unknown as there is little known about the flowability of fentanyl [14].

2.5 Summary

Overall, the methods of detecting fentanyl in the field are limited, with most techniques requiring handling of the material in order to detect if it is fentanyl. Vapor detection of fentanyl would be a safer way of determining the presence of fentanyl because it wouldn't require law enforcement officers to be so close to potential sources of vapor phase fentanyl. Better headspace analysis would aid in the development of vapor detection methods. The focus of this research is to determine the optimal distance from the fentanyl source for SPME fiber sampling in the headspace, while monitoring humidity for its effect on vaporization.

CHAPTER III

METHODOLOGY

3.1 Safety Note

When handling bulk fentanyl, at least two individuals were always present. Appropriate personal

protective equipment (PPE) was worn. Naloxone (Narcan) was readily available with all

individuals informed on the appropriate administrative procedure. To minimize the risk of

exposure, bulk fentanyl was kept in a sealed container whenever possible.

3.2 Materials

One-quart unlined round paint cans with triple tite lid and securing clips were purchased from

Qorpak (Qorpak, Clinton, Pennsylvania). Rubber stoppers (Lot 0029647) were purchased from

ThermoScientific (ThermoFisher Scientific Inc., Waltham, MA). 100µm PDMS coated silica

solid phase microextraction (SPME) fibers and holders were purchased from Supelco (Supelco,

Inc. of Sigma-Aldrich Corp., Bellefonte, PA). Heated fentanyl HCl powder was synthesized at

Oklahoma State University Toxicology and Trace Laboratory (OSU-FTTL). Fentanyl standards

at a concentration of 1 mg/mL in methanol were purchased from Cerilliant (Cerilliant Corporation, Round Rock, TX, Lot: FC01071903). Fisherbrand Traceable thermometer/clock/humidity monitor (Model 11725843) was purchased from FisherScientific (ThermoFisher Scientific Inc., Waltham, MA). Thermco Accu-Safe Non-Mercury Laboratory Thermometer (Model 21099) was purchased from Thermco Products Inc. (Thermco Products Inc., Lafayette, NJ). VWR mini-incubator and VWR horizontal air flow oven were purchased from VWR (VWR International of Avantor, Randor, PA).

3.3 Instrumentation

An Agilent 7890A Gas Chromatograph (GC) paired with a 5975C mass selective detector (MSD) with a triple axis detector (Agilent Technologies, Inc., Santa Clara, CA) was used for instrumental analysis. The GC inlet was operated in splitless mode and set at 260°C. Chromatographic separation was achieved with a RXI-5ms capillary column (30 m x 250 um i.d. x 0.25 µm f.d.) from Resteck (Restek Corporation, Bellefonte, PA). Helium was the carrier gas, flowing at a constant rate of 19 mL/min at 15.967 psi. 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 for 1.5 minutes. The total run time was 7.5 min. The MS source was set to 230°C with a maximum of 250°C, and the quad was set to 150°C with a maximum of 200°C. The MS was operated with both scan and selected ion monitoring (SIM). The MS scanned for masses between 40 and 350. The SIM scanned for ions in three groups the first group was m/z 58, 146, and 245, the second group scanned for m/z 91 and 149, and the third group scanned for m/z 105 and 189. SPME injections were performed manually. For liquid injections, like standards, an Agilent 7693 autosampler was used to perform 1 µL injections.

3.4 Quality Control and Experiment Preparation

Blank and known positive injections of methanolic fentanyl standard at a concentration of $1\,\mu g/ml$ were performed to ensure the gas chromatography-mass spectrometer (GC-MS) was producing consistent results. Prior to experimentation, the instrument septum was changed, and the column was clipped. Before each headspace exposure, SPME fibers were analyzed on GS-MS to ascertain that the fibers were clean. Throughout experimentation, the humidity of the air where the headspace can was located was monitored. Humidity was noted each time the can was placed in the mini-incubator oven, fibers were exposed, and fibers were removed. The temperature of the oven was periodically checked to verify that it remained stable.

The headspace can was prepared by drilling four holes vertically along one side of the paint can using a drill bit. Holes in the side of the can were approximately 3 cm apart, with the lowest hole about 1.5 cm from the bottom. The edges were smoothed by using a 20 mm hollow punch to push through the hole, removing jagged pieces of metal and moving the rough edges inside the can. Pliers were used to bend any remaining pieces of metal against the interior of the paint can, reducing the chances of the edges of the holes catching on anything. A hole was also drilled into the lid of the paint can and the edges were similarly smoothed. The can and its lid were then sprayed with bleach and wiped down with Kimwipes. Similarly, they were cleaned with water and methanol. The can and its lid were then baked in the horizontal air flow oven at $200\,^{\circ}C$. Rubber septa were pressed into the created holes, providing an air-tight seal. Figure 1 provides a visual of the prepared headspace can.

Figure 1. Headspace can with five sampling points at the rubber septa.

3.5 Exposure Time

On an analytical balance, 250.6 mg of heated fentanyl HCl powder was weighed into a rust-resistant tin. The prepared headspace can was wiped down with a clean Kimwipe, and the lid of the rust-resistant tin with the measured fentanyl was carefully placed into the can with tongs. The lid of the headspace can was pressed closed and fastened with clips to ensure a strong seal. Five clean SPME fibers were removed from their holders, as seen in Figure 2. Four fibers were inserted in the bottom, low, middle, and high points of the can through the rubber septum. Pliers were used to hold the fiber steady while they were inserted to avoid bending or breaking the fibers. The headspace can was placed into the mini-incubator oven set to 30°C where the headspace was allowed to equilibrate. After 2 hours of equilibration, the fibers were exposed to the headspace. After two hours of exposure, the fiber at the bottom point was removed. The fiber was then assembled into a holder and analyzed on the GC-MS using the method described in Section 3.3: Instrumentation. The remaining fibers were removed at 4, 6, and 8 hours from the low, middle, and high points respectively. Each fiber was analyzed on the GC-MS after removal. Then a fifth fiber was placed at the top point of the can and exposed for 15 hours before

extraction. This process was replicated two days later, but with 346.8 mg of raw unheated fentanyl HCl powder as the source to increase signal.
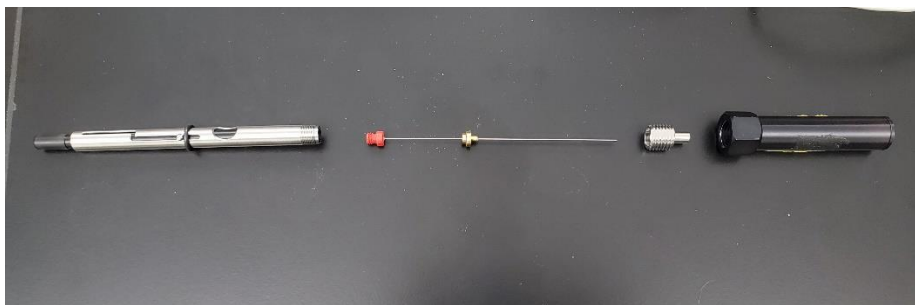

Figure 2. Disassembled red 100uM PDMS coated SPME fiber and holder.

3.6 Elevation Variation

The same 250 mg of heated fentanyl HCl powder, rust-resistant tin, and headspace can with 5 holes from the exposure time comparison were used. The mini-incubator oven was set to 50°C. The fentanyl was placed in the can and sealed closed. Five clean red 100μM SPME fibers were removed from their holders. The fibers were inserted into all five points. The headspace can was placed into the oven with as little agitation as possible and allowed to equilibrate for 2 hours before exposure. After 2 hours of exposure, all five fibers were removed from the can and assembled into holders. Fibers were analyzed on the GC-MS starting with the fiber inserted at the point closest to the fentanyl source and ending with the one furthest from the source. The fibers were analyzed in the order of bottom, low, middle, high, and then top.

After collecting the data from the first elevation comparison test, the lid of the headspace can was replaced with a lid without a hole drilled in it. Thus, the remaining sampling points were the bottom, low, middle, and high points. The new lid was cleaned by wiping it down with bleach, water, and then methanol. The lid was baked at 200°C in the horizontal air flow oven. The rubber septa were then secured with clear packaging tape to keep them secure, reducing the chance of air leaking out or into the headspace.

The heated fentanyl was sealed in the can using the new lid and secured with clips. The headspace can was placed in the mini-incubator oven set at 50°C. Clean 100 μM red SPME fibers were removed from the holders. After 2 hours, the four fibers were inserted into the rubber septa in the can with as little agitation of the can as possible. The fibers were exposed to the headspace for 2 hours and then removed. The fibers were assembled into holders and then analyzed using the GC-MS method described in Section 3.3: Instrumentation. The headspace can was unsealed under a vent hood and left overnight for the headspace to dissipate. This exposure process was repeated three more times in the following days.

3.7 Peak Selection and Data Analysis

The gas chromatograms and mass spectra from the GC-MS analysis were examined using Agilent's ChemStation software (ChemStation Software, Agilent Technologies, Santa Clara, CA). Peaks were identified by characteristic ions on the mass spectra and an appropriate retention time on the gas chromatogram. Fentanyl peaks were identified by the presence of ions, m/z 146, 189, and 245, and a retention time of about 6.505 minutes. 4-ANPP peaks were identified by the presence of ions, m/z 44, 146, and 189, and a retention time of about 5.675 minutes. NPP peaks were identified by the presence of ions, m/z 42 and 112, and a retention time of about 3.433 minutes. NPPA peaks were identified by the presence of ions, m/z 93 and 149, and a retention time of about 2.424 minutes. Figures 3-6 show extracted ion chromatograms for characteristic ions of each compound. The area of the identified peaks from the elevation variation tests with four points of measurement was measured. Integration of the identified fentanyl peaks was done manually as the fentanyl peaks were too small for automatic integration. Each peak was selected from base to base, as seen in Figure 7, to ensure the highest degree of accuracy during manual

integration. The areas of NPP and NPPA peaks were measured by automatic integration due to the larger size of the peaks.
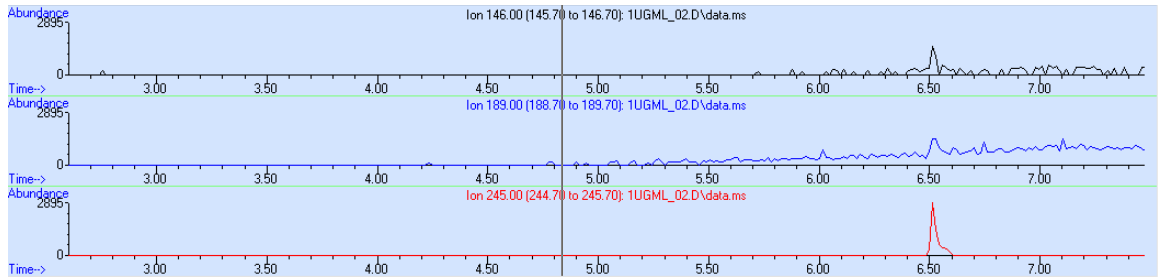

Figure 3. Extracted ion chromatogram of fentanyl standard showing all three characteristic fentanyl ions m/z 146, 189, 245.
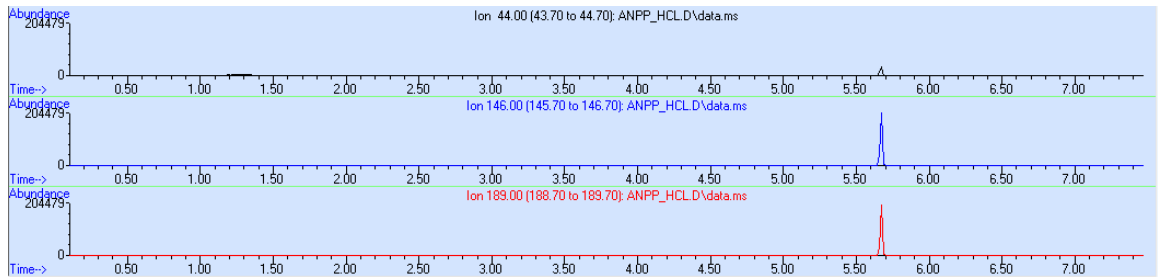

Figure 4. Extracted ion chromatogram of 4-ANPP standard showing all three characteristic 4-ANPP ions m/z 44, 146, and 189.
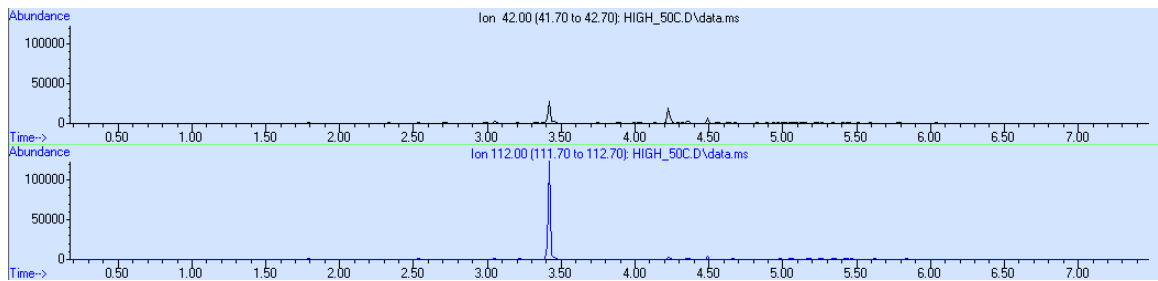

Figure 5. Extracted ion chromatogram of first high point sample from elevation comparison test showing both characteristic NPP ions m/z 42 and 112.
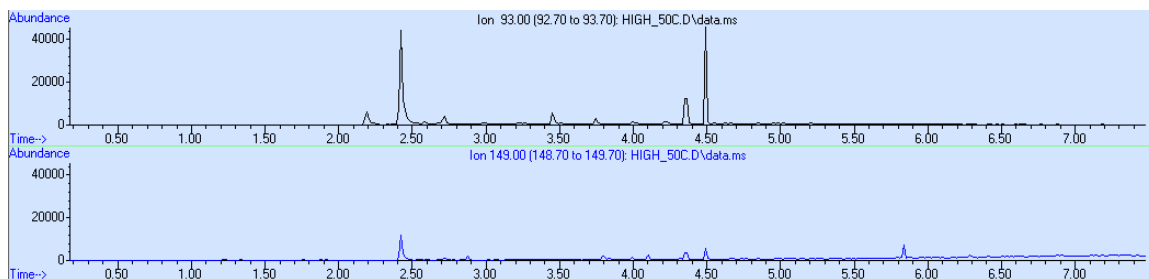
Figure 6. Extracted ion chromatogram of first high point sample from elevation comparison test showing both characteristic NPPA ions m/z 93 and 149.
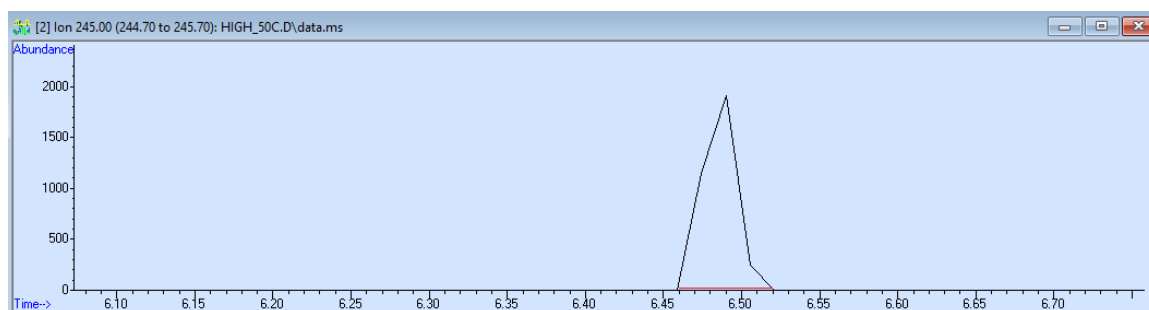

Figure 7. Extracted ion chromatogram of m/z 245 to isolate fentanyl peak and the red line spans base-to-base close to the bottom of the peak for a manual integration to be as accurate as possible.

3.8 Statistical Analysis

Statistical analyses of measured peak areas were done in GraphPad Prism (GraphPad Software, La Jolla, CA) and Microsoft Excel (Microsoft Office, Microsoft Corporation, Redmond, WA). A two-factor without replication ANOVA test was performed to determine if there was a statistically significant difference in abundance between the elevation levels of the fibers and if there was a statistically significant difference between each test.

CHAPTER IV

FINDINGS

4.1 Exposure Time

For the first exposure time test, fentanyl peaks were identified for every exposure time sample. The fentanyl peak from the 4-hour chromatogram was more prominent than the others. The difference in peak size can be seen in Figure 8. During the second exposure, fentanyl peaks were only identified for the 2- and 4-hour chromatograms. It should be noted that the rubber septum with the fiber exposed for 6 hours inserted into it was loosened while the fiber was removed. Thus, there was a slight loss of headspace at the 6-hour mark, which may have caused the fiber exposed for 8 hours to have a lower reading than if the seal had not been compromised. As the fibers that were exposed for 6 and 15 hours did not have identifiable fentanyl peaks, the loss of headspace was not likely to have been a major cause for the lack of an identifiable fentanyl peak from the 8-hour exposure. The overlay of the chromatograms at all five exposure times for the second test can be seen in Figure 9. The strongest exposure times for detecting fentanyl from both tests were found to be at 2 hours and 4 hours. The first exposure time test with the heated fentanyl HCl

powder had larger fentanyl peaks than the second exposure time test with the unheated fentanyl when there were peaks to compare, as depicted in Figure 10. Thus, the heated fentanyl HCl powder has a higher amount of fentanyl in the headspace than the unheated fentanyl HCl powder. The heated and unheated fentanyl HCl powders are different in that the heated fentanyl has moisture removed from it, indicating that the moisture in the unheated fentanyl may have been interfering with its vaporization.
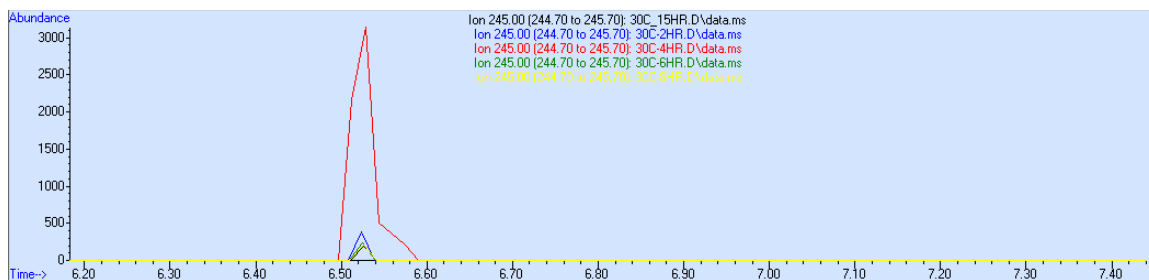

Figure 8. Overlay of extracted ion chromatogram of characteristic fentanyl ion m/z 245 for all five exposure times from the first exposure test. The large red peak is from the 4-hour exposure.
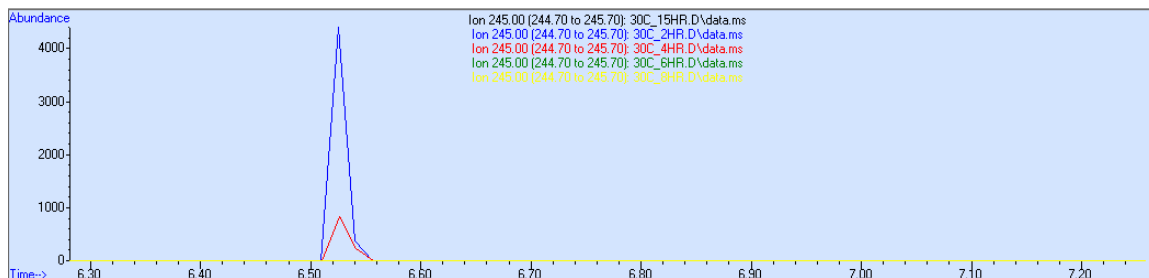

Figure 9. Overlay of extracted ion chromatogram of characteristic fentanyl ion m/z 245 for all five exposure times from the first exposure test. The large blue peak is from the 2-hour exposure.
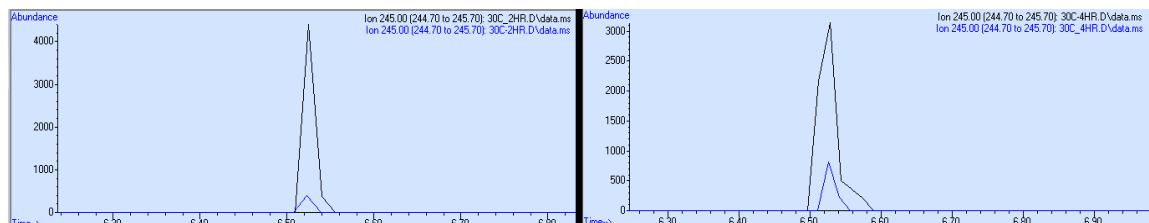

Figure 10. Overlay of extracted ion of characteristic fentanyl ion m/z 245 for both exposure tests, where the black peak is from the first exposure test and the blue peak is from the second exposure test. The left overlay is of the 2-hour exposure and the right overlay is of the 4-hour exposure.

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4.2 Elevation Variation

To increase the abundance of fentanyl in the headspace for better detection, the temperature of the oven was increased from the 30°C of the previous tests to 50°C. Packing tape was wrapped around the can to secure the rubber septum in place, avoiding the potential loss of headspace by a rubber septum from coming loose. The top sampling point in the lid of the headspace can was removed to increase the accuracy of the tests. One concern was that the hole drilled in the lid warped it and may compromise the integrity of the seal over time. Without a tight seal on the lid, headspace may be lost, and accurate data would not be able to be taken. The other concern was consistently inserting the fiber at the same length, due to the can being sealed before the fiber was inserted, it was difficult to ensure that the fiber was inserted the exact same amount each time. Thus, the top fiber may be inserted at varying elevations in the headspace. The top fiber could sample headspace lower than the high point or touch the fiber in the high point; either case would result in less accurate data. As the heated fentanyl HCl powder from the exposure time tests had a higher peak, the heated fentanyl was used in the elevation comparison tests. The 2-hour exposure was the earliest time fentanyl was detected, therefore a 2-hour fiber exposure was done for the elevation comparison tests.

4.2.1 Fentanyl

The areas of the identified fentanyl peaks varied widely between each test, as seen in Table 1, the highest value for each elevation point was found to be 4 - 13 times larger than the lowest quantitated area. This large variation in abundance from one test to the next makes it difficult to compare area quantity between tests. Figure 11 contains a graphical comparison of the average area for each elevation point, as the elevation point increases the area quantity also increases. This indicates that sampling the headspace for fentanyl further away from the source could

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increase the detection of fentanyl. After performing a two-way ANOVA, it was determined there was no statistically significant difference in the amount of fentanyl collected at the differing elevations ($p>0.01$), but there was a statistical difference between the different tests ($p<0.01$).

Table 1. Areas of the identified fentanyl peaks and the average peak area for each elevation point.

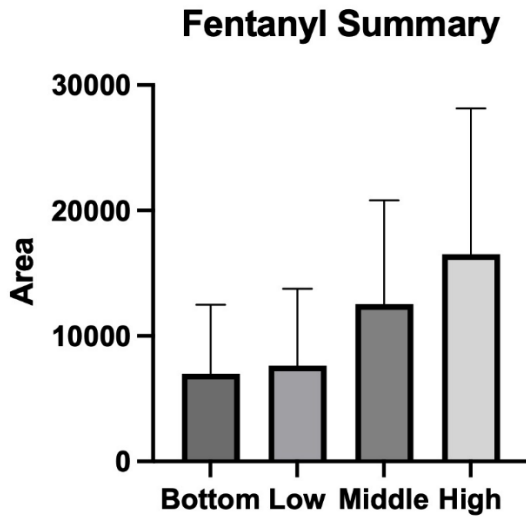|	Bottom	Low	Middle	High
Test 1	8348	15893	19669	25165
Test 2	4590	7485	5746	8341
Test 3	13914	5970	19717	27757
Test 4	1061	1157	4992	4788
Average	6978.25	7626.25	12531	16512.75

Figure 11. Graphical comparison of the average peak area of fentanyl for each elevation point.

4.2.2 4-ANPP

A 4-ANPP peak was not identified in any of the headspace samples taken during the elevation tests. The lack of 4-ANPP could be due to the degradation of fentanyl into other precursors, such as NPP.

4.2.3 NPP

The quantitated area of the identified NPP peaks can be seen in Table 2. Similar to fentanyl, the NPP detected between tests at the same elevation varies widely. Although, NPP has a much larger peak area than fentanyl, indicating a higher abundance of NPP in the headspace. NPP also shows a similar trend of the area of the peak increasing as the elevation increases, as depicted in Figure 12. After performing a two-way ANOVA, it was determined there was no statistically significant difference in the amount of NPP collected at the differing elevations ($p>0.01$), but there was a statistical difference between the different tests ($p<0.001$).

Table 2: Areas of the identified NPP peaks and the average peak area for each elevation point.

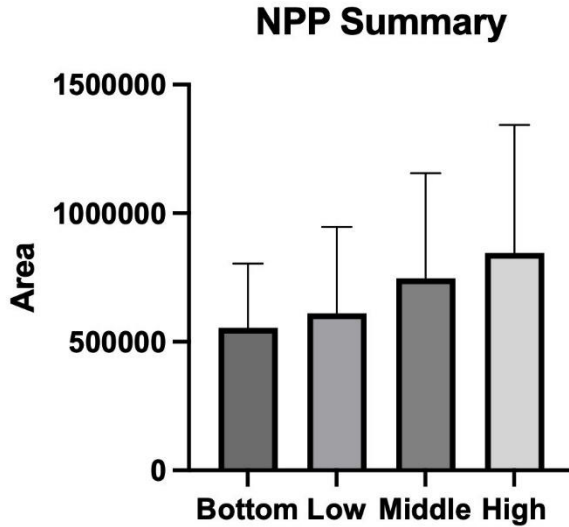|	**Bottom**	**Low**	**Middle**	**High**
Test 1	690739	981145	1137312	1422999
Test 2	564117	582288	647223	722666
Test 3	762782	707495	987609	998674
Test 4	200046	172685	217369	236761
Average	554421	610903.3	747378.3	845275

Figure 12. Graphical comparison of the average peak area of NPP for each elevation point.

4.2.4 NPPA

The integration of NPPA peaks were documented in Table 3. Figure 13 shows that the quantity of NPPA detected did not change much for the low, middle, and high points, while the bottom points had a lower area on average. The two-way ANOVA test that was performed indicated that the difference in elevation points as well as between tests was not significant ($p > 0.01$).

Table 3. Areas of the identified NPPA peaks and the average peak area for each elevation point.

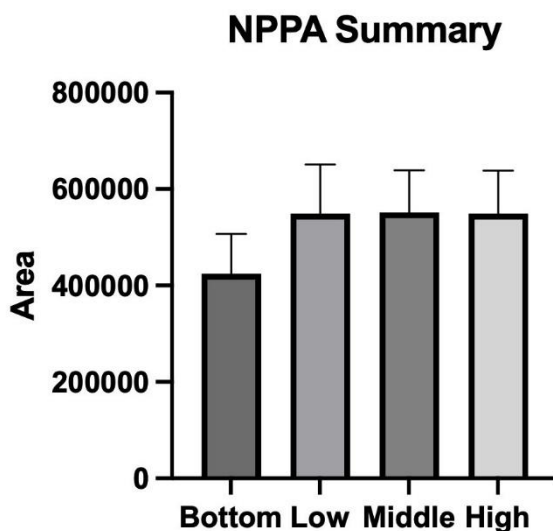|	Bottom	Low	Middle	High
Test 1	303377	663279	648750	665296
Test 2	451803	515570	498807	450801
Test 3	490372	0	599494	557623
Test 4	451436	469098	459510	521301
Average	424247	411986.8	551640.3	548755.3

Figure 13. Graphical comparison of the average peak area of NPPA for each elevation point.

4.3 Humidity

Throughout the elevation comparison tests, the humidity was monitored, Table 4 shows the humidity of the surrounding air outside the oven during each test. While the humidity did not change much throughout the day during the test, there was variation between days. The area of the peak at the elevation point was plotted against the average humidity. All four elevation points were plotted on the same graph so the change in area as the humidity changed could be observed together. The change in area as humidity changes can be observed in Figures 14-16 for each of the identified compounds. For fentanyl and NPP, there is a clear upwards trend of as humidity increases, the area quantity also increases for each of the elevation points. The two-way ANOVA test indicated that there was significance between the tests for fentanyl and NPP. The likely environmental factor for this variation is humidity. Thus, humidity was found to be a significant factor in the quantity of fentanyl and NPP in the headspace. For NPPA, there was also a positive trend in relating humidity and the area of the peak, but the difference was not found to be statistically significant. This indicates that NPPA was not as affected by humidity as the other compounds in the headspace.

Table 4. Recorded humidity at points throughout the test and the average humidity for each test.

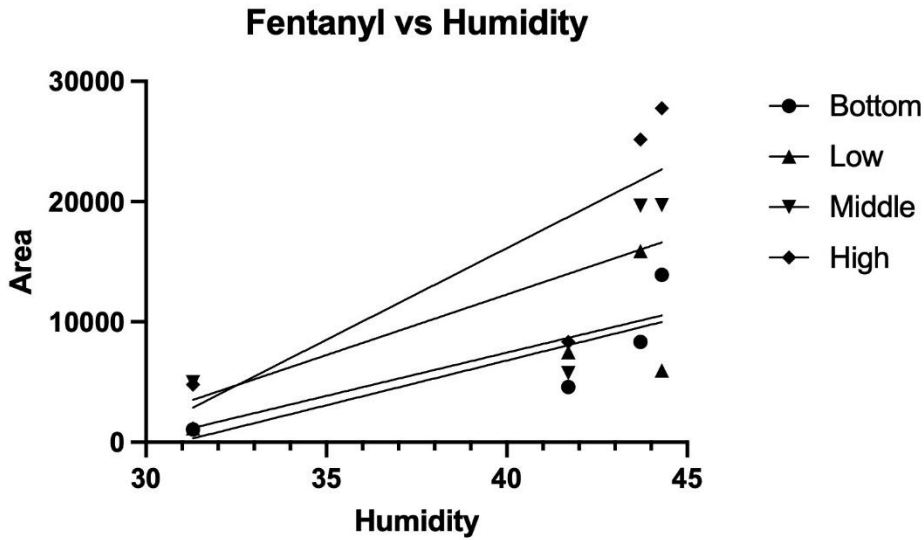|	Can in Oven	Fiber Exposed	Fiber Removed	Average
Test 1	43	45	43	43.66667
Test 2	45	42	38	41.66667
Test 3	45	45	43	44.33333
Test 4	33	31	30	31.33333

Figure 14. Graphical representation of change in fentanyl peak area as the humidity changes for each elevation point.
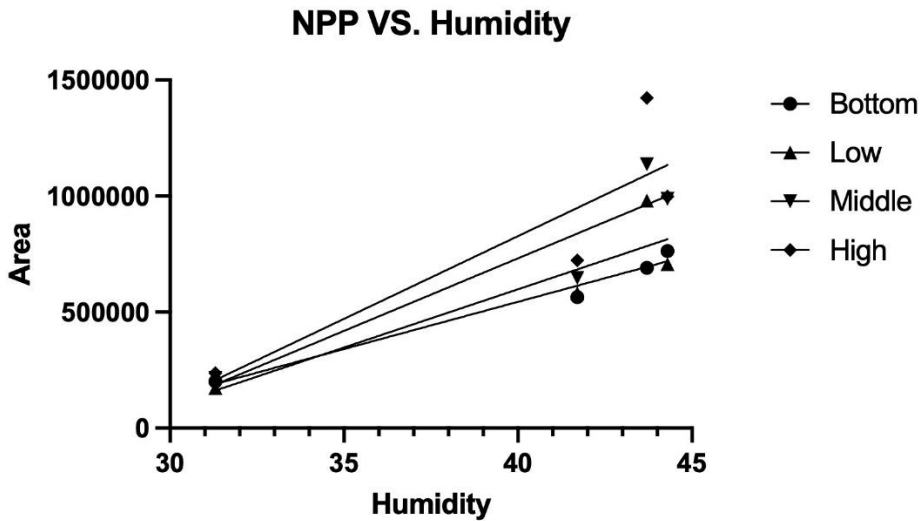

Figure 15. Graphical representation of change in NPP peak area as the humidity changes for each elevation point.
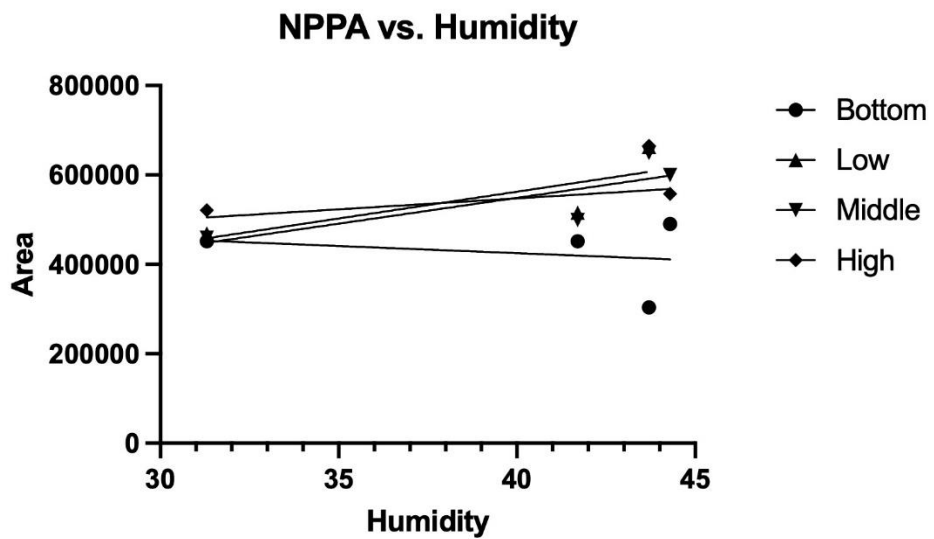
Figure 16. Graphical representation of change in NPPA peak area as the humidity changes for each elevation point.

CHAPTER V

CONCLUSION

The optimization of solid phase microextraction (SPME) sampling height in fentanyl headspace was performed by analyzing the same fentanyl headspace at four different elevations using gas chromatography-mass spectrometry. The optimal sampling height would be where the fentanyl would be most abundant, allowing for better detection in the headspace. First, the length of time for SPME exposure within the headspace set-up was determined by sampling the fentanyl headspace over a 15-hour period. Once headspace sampling conditions were determined, the relative abundance of fentanyl, NPP, and NPPA at different elevations was measured. It was found that the abundance of fentanyl and NPP increased as the distance from the fentanyl HCl powder increased. So, the SPME fiber sampling of the headspace at about a distance of 10 cm from the fentanyl source has the highest abundance for fentanyl and NPP. There was little variation in abundance across elevation or days for NPPA. The detection of fentanyl, NPP, and NPPA in the headspace makes them potential compounds for vapor detection. Further research could be done to determine if sampling further than 10 cm would continue to increase abundance, or if there is a certain point where abundance starts to decrease.

There was a significant difference in the abundance for fentanyl and NPP depending on the day the test was done. A potential contender for the environmental factor that is causing this abundance difference between each day is humidity. Measuring the average humidity of the day during the headspace analysis showed that as the humidity increased, so did the abundance of fentanyl and NPP within the range of 30 - 45% humidity. Studying fentanyl headspace in environmentally controlled containment would remove the interference of humidity.

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