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RESEARCH PAPER

Comparative analysis of vapor profiles of fentalogs and illicit fentanyl

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Abstract

Availability of fentanyl is at a record high with 3138 kg of fentanyl and related substances being seized in 2019. Fentanyl's high toxicity makes a lethal dose for most mere milligrams. With such a high potency and a consistent rise of abuse, the chances of injury or death of frontline workers increase with every interaction. Development of a non-contact detection method for fentanyl would decrease the chances of a workplace mishap. To aid in the development of a non-contact detection method, target analytes in the vapor profile of fentanyl need to be identified. In order to achieve this goal, semi-quantitative headspace analysis of fentanyl analogs and confiscated fentanyl exhibits was accomplished using solid-phase microextraction and gas chromatography coupled with mass spectrometry (SPME-GC-MS). The vapor signatures of these samples were compared to a previously reported reference-grade fentanyl vapor signature to determine the target analyte(s) for fentanyl detection in the vapor phase. A total of 20 fentalogs and confiscated exhibits, with masses ranging from 2 to 19 mg, were sampled. N-Phenylpropanamide(NPPA) or N-phenethyl-4-piperidone(NPP) was identified as target analytes in 75% of these samples. This is a crucial component for the development of a non-contact detection method for fentanyl.

Keywords Fentanyl · Narcotics detection · SPME · Vapor detection · Fentanyl analogs

Introduction

The USA has been in a constant battle with the opioid epidemic since 1999, with a rise in synthetic opioid overdoses beginning in 2013 [1]. Synthetic opioids are defined as substances that are synthesized in a laboratory to target the brain in the same manner as naturally occurring opioids, such as morphine [2]. Currently in the USA, fentanyl is the most prevalent synthetic opioid, being available in both licit and illicit forms. Although approved for medical use, fentanyl exhibits a high potential for abuse. Thus, it is listed as a Schedule II synthetic opioid by the United States Controlled Substances Act [3]. In 2019, there were 100,378 confiscated fentanyl exhibits and 36,359 synthetic opioid deaths with majority caused by fentanyl or fentanyl-related substances [4, 5].

Fentanyl and related substances vary in potency, but generally have much greater potencies than morphine or heroin. For instance, fentanyl has been found to be 100 times more potent than morphine, while the most potent fentanyl analog, carfentanil, was found to be 10,000 times more potent than morphine [6]. Fentanyl-related substances, or fentalogs (fentanyl analogs), are structurally related analogs of fentanyl. Although a few fentalogs have legitimate uses, such as carfentanil being a large mammal tranquilizer, many have no known medical use and are listed as Schedule I by the U.S. Controlled Substances Act [3]. Contact with as little as 2 mg of fentanyl and 0.02 mg of carfentanil can result in an overdose. Due to the extreme potency and dangers of fentanyl and fentalogs, the USA has attempted to decrease the presence of these drugs by scheduling them; however, each fentalog must be scheduled individually. Unfortunately, by the time one fentalog has been scheduled, clandestine laboratories in China have already synthesized a new fentalog and began distribution into the USA [7]. In an effort to reduce distribution and overall risks, the U.S. Drug Enforcement Administration (DEA) placed a temporary emergency scheduling order of all illicit fentanyl-related substances [8]. In 2019, the National Forensic Laboratory Information System (NFLIS) reported 100,378 fentanyl and fentanyl-related confiscated exhibits [4]. Similarly, the

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2020 National Drug Threat Assessment by the DEA has noted that the availability and use of fentanyl and fentalogs have increased [4]. With the consistent threat of fentanyl being trafficked into the USA and its extreme potency, the safety of first responders and law enforcement personnel is constantly put at risk. The DEA recommends that any substance suspected to contain fentanyl should only be handled by trained personnel with proper personal protective equipment [3].

Currently, there are several techniques and methods to detect fentanyl in the field, such as ion mobility spectrometry (IMS) [9], colorimetric testing [10], nano-liquid chromatography-electron ionization-mass spectrometry (nLC-EI-MS) [11], direct analysis in real time with mass spectrometry (DART-MS), and Raman spectroscopy [10, 12]. Despite many of these techniques having high sensitivity and the ability to distinguish between fentanyl and related substances, they also possess a few disadvantages. Many of these detection methods are qualitative, subjective, and destructive to samples, and tend to be expensive. For example, DART-MS can be expensive, require a trained technician, and is traditionally laboratory based. Although colorimetric testing, nLC-EI-MS, and IMS are field portable, these techniques require contact or manipulation of a fentanyl sample, which put law enforcement and first responders at high risk to exposure. To circumvent exposure to fentanyl, Raman spectroscopy has been employed for in the field testing [10, 12]. While this technique is rapid and does not require contact with bulk sample, it is only capable of detecting bulk materials that are not visibly obscured [12, 13]. In this regard, a non-contact vapor detection technique would prove beneficial due to the capability of being field portable, non-destructive to the sample, and no contact with bulk sample; however, fentanyl is not likely to be detected in the vapor phase due to its low vapor pressure of $(5.9 \pm 4.7) \times 10^{-7}$ Pa at 25 °C [14]. In a recent publication, the main components in the headspace of reference-grade fentanyl were identified [15]. This information is fundamental in the development of a non-contact detection method of fentanyl. Detection target analytes can be determined from the main components found in the vapor profile of reference grade.

Herein, a comparative analysis of vapor profiles of confiscated (street-grade) fentanyl exhibits and several fentalogs was investigated to determine targets that can be utilized for examination of fentanyl and related substances in vapor phase. To accomplish this objective, a solid-phase microextraction (SPME) coupled with gas chromatography (GC-MS) [15] method was used to collect and determine the vapor signature of street-grade fentanyl and fentalogs. The headspace of fentanyl-related substances was extracted using SPME, while GC-MS was used to separate and detect volatile organic compounds (VOCs). The vapor profiles of street-grade fentanyl and fentalogs were compared to the vapor profile of reference-grade fentanyl [15] to determine if there were any common analytes that could be used as target analytes in the detection of fentanyl and related substances. The identification of target analytes in reference-grade fentanyl, street-grade fentanyl, and fentalogs will provide fundamental data for the development of a non-contact detection method in future work.

Materials and methods

Materials

N-Phenethyl-4-piperidone (NPP) was purchased from Sigma-Aldrich (St. Louis, MO), N-phenylpropanamide(NPPA) was purchased from Toronto Research Chemicals (Toronto, ON, Canada), and VOA sampling vials were purchased from Fisher Scientific (Pittsburgh, PA). Furanyl fentanyl, 4-anilino-N-phenethylpiperidine (4-ANPP), and acetyl fentanyl were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Fentanyl alkaloid was purchased from Mallinckrodt Pharmaceuticals (Dublin 15, Ireland), remifentanil HCl was purchased from PharmAgra Labs (Brevard, NC, USA), and carfentanil citrate was an in-house synthesis, with all three fentalogs provided by the Toxicology and Obscurants Division at Research and Technology Directorate at DEVCOM Chemical Biological Center. Street-grade samples labeled as P550020, P550036, P55037, P550064, P550071, P550080, and P550089 were provided by the Maryland State Police Forensic Sciences Division Laboratory. Confiscated samples labeled as BK002, BK003, BK005, BK009, BE003, BE004, and BE006 were provided by the DEA Special Testing and Research Laboratory. All chemicals and samples were solids and used as received without further purification.

Safety precautions for handling fentanyl and related substances

All handling of fentanyl and related substances was completed following a previously developed protocol [15]. All samples were handled by two trained personnel, where one was the handler and the other an observer. Both trained personnel wore lab coats with cuffed sleeves, nitrile gloves, goggles, and facemasks when handling solid fentanyl. With naloxone on-hand, the observer supervised while the handler transferred solid fentanyl or related substances to labeled VOA glass vials with properly fitted septa lids using a spatula in a well-ventilated hood. Once placed into VOA vials and sealed, the fentanyl and fentalog samples were not removed or manipulated during sampling, and all vials were kept closed. Alcohol wipes were used to clean vials after preparing samples to ensure that no opioid residue remained. Used wipes and gloves were disposed of as hazardous waste.

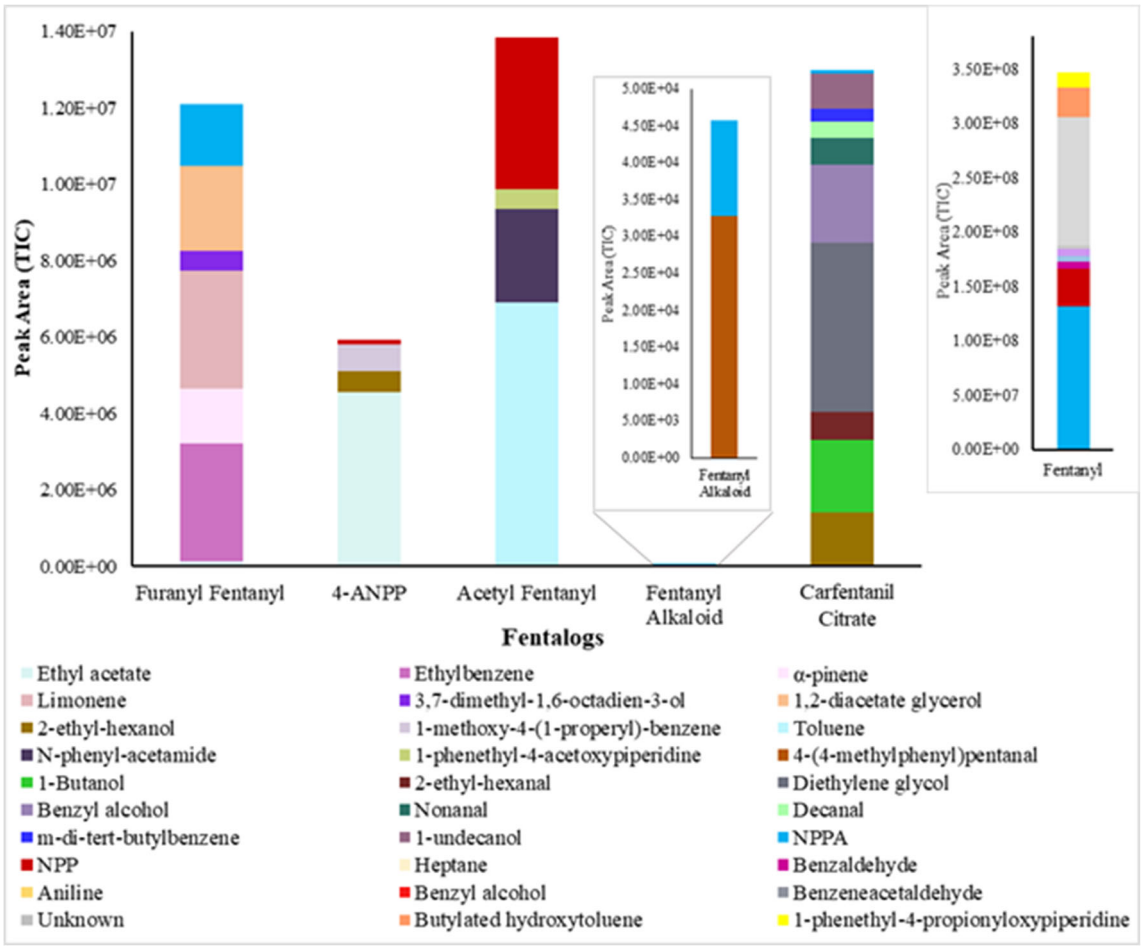


Fig. 1 Headspace analysis of common fentalogs. Reference-grade fentanyl headspace analysis is displayed in inset

Headspace sampling of fentanyl and related substances

The vapor signature of street-grade fentanyl and fentalogs, in powder form, was generated by placing approximately 5 mg of each respective substance in a 20-mL VOA sampling vial with septa. Using a previously developed SPME method for reference-grade fentanyl [15], the headspace of each sample was extracted. Briefly, the sample equilibrated at room temperature (approximately 21 °C ± 3°) for at least 24 h prior to sampling followed by heating at 35 °C for 30 min. Finally, the headspace of each sample was extracted at 35 °C by inserting a divinylbenzene/carboxen/polydimethylsiloxane SPME fiber into the septa of each sample for 4 h.

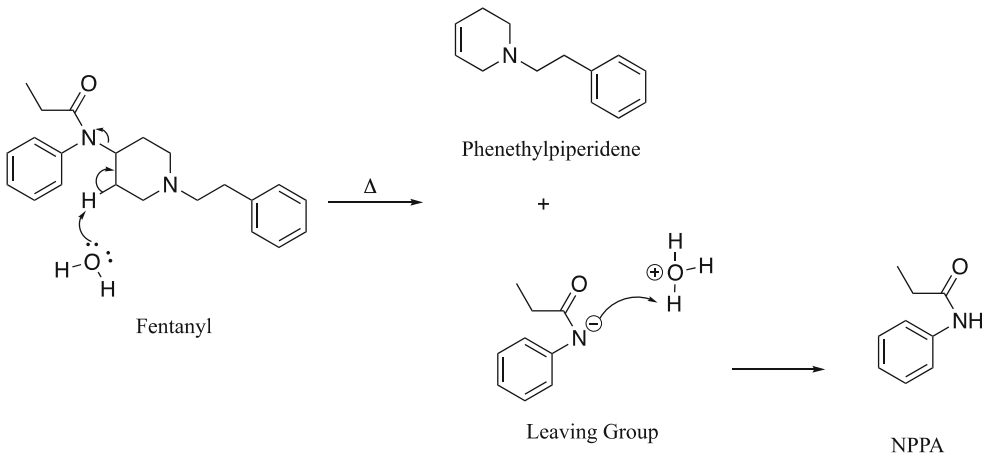
Headspace analysis of fentanyl and related substances

Volatile organic compounds (VOCs) found in the headspace of street-grade fentanyl and fentalogs were separated and detected using a 7890/5975 Agilent GC-MS (Agilent Technologies, Santa Clara, CA) equipped with a 15 m × 0.25 mm i.d. × 0.25 μm, Rtx-5MS column (Restek, Bellefonte, PA). Using a previously developed method [15], the analytes extracted from the headspace of each sample were desorbed from the SPME fiber at 260 °C with a 10:1 split ratio and a flowrate of 2 mL/min. The GC oven method was 40 °C for 30 s, ramped to 250 °C at 30 °C/min, and held for 30 s. The transfer line to the MS was 250 °C, with a 30-s solvent delay, and the mass scan range was *m/z* 40–300. Analytes of interest were confirmed using external standards. Calibration curves were used to calculate the mass of NPPA or NPP collected by SPME in the headspace of all samples.

Table 1 List of fentalogs and presence of potential targets

Sample	Presence of NPPA	Presence of NPP
Furanyl fentanyl	Y	N
4-ANPP	N	Y
Acetyl fentanyl	N	Y
Fentanyl alkaloid	Y	N
Carfentanil citrate	Y	N
Remifentanil HCl	N	N

Scheme 1 Reaction mechanism for the formation of NPPA from fentanyl



Results and discussion

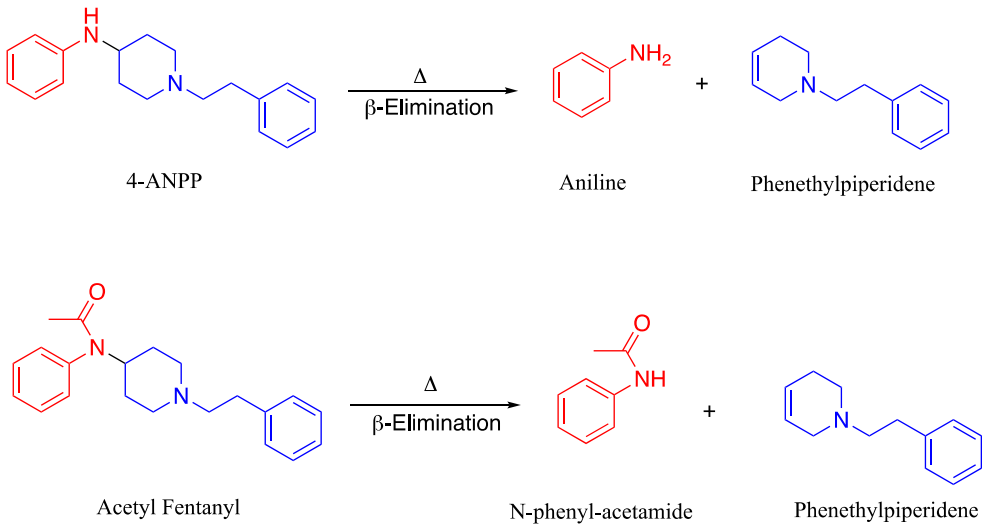
Headspace analysis of fentalogs

Recently, Vaughan et al. (2021) determined the vapor signature of reference-grade fentanyl [15]. In this study, several possible targets of interest were identified. NPPA and 1-phenethyl-4-propionyloxypiperidine were identified as common chemical markers in the synthetic routes of fentanyl [16], while NPP is a known precursor in the synthesis of fentanyl. An unknown compound was originally identified as a phenyl-substituted ethanone by the NIST library; however, after comparing retention times of external standards and referencing the SWGDRUG Mass Spectral Library, there were no rational matches [15]. Although the identity of the unknown compound was unable to be determined, it was of interest due to it being one of the most abundant VOCs in the headspace. To determine if any of these compounds could be used as target analytes in the detection of fentanyl and/or related substances in the vapor phase, the vapor signatures of several reference-grade fentalogs were investigated.

There are a variety of fentalogs available on the market, with most being Schedule I. In 2017, the DEA reported that of all confiscated fentanyl exhibits, 27% were identified as fentalogs or precursors [17]. We obtained furanyl fentanyl (7% of reported exhibits), 4-ANPP (1% of reported exhibits), acetyl fentanyl (2% of reported exhibits), carfentanil citrate (7% of reported exhibits) [17], fentanyl alkaloid, and remifentanil HCl. The headspace analysis of fentalogs was compared to the headspace analysis of reference-grade fentanyl [15] and can be seen in Fig. 1. Several semi-VOCs and VOCs were found in the headspaces of these fentalogs. Although all of these compounds are structurally related to fentanyl, each fentalog revealed its own unique vapor profile. As shown in Fig. 1, many of the VOCs present in the headspaces of the fentalogs were not seen in the headspace of reference-grade fentanyl. A complete list of all VOCs found in the headspace can be found in the Supplementary information (Table S1). However, two of the potential targets were found in five of the six fentalogs: NPPA and NPP.

Furanyl fentanyl, fentanyl alkaloid, and carfentanil citrate contained NPPA, while 4-ANPP and acetyl fentanyl

Scheme 2 Formation of degradants from fentalogs



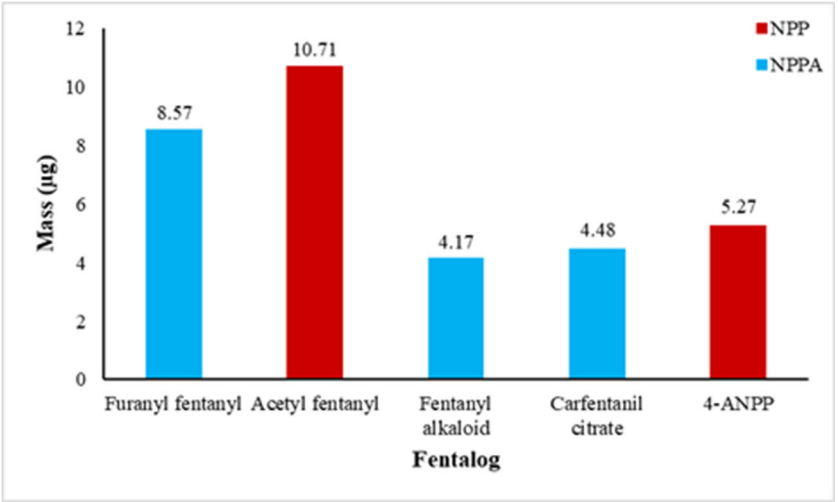


Fig. 2 Mass of NPP and NPPA found in five out of six fentalogs. *Mass is representative of the mass on the SPME fiber, not total mass

contained NPP (Table 1). There were no potential targets of interest found in the headspace of remifentanil HCl. As previously mentioned, NPPA is a chemical marker in the synthetic route of fentanyl, but it has also been identified as a known degradant of fentanyl forming through solvent-mediated thermal β-elimination [18, 19]. At an elevated temperature, water in the environment abstracts a proton from the β carbon of fentanyl. The electron pair from that C-H bond forms a new pi bond kicking off the leaving group and forming phenethylpiperidene. Lastly, the leaving group is protonated resulting in the formation of NPPA (Scheme 1). The structures of 4-ANPP and acetyl fentanyl would not allow for the formation of NPPA, but it would allow for the formation of aniline and N-phenyl-acetamide, respectively. Scheme 2 shows how their structures would fragment using the proposed β-elimination mechanism to form their degradants. Consequently, N-phenyl-acetamide was found in the headspace of acetyl fentanyl; however, we were unable to detect aniline in the headspace of 4-ANPP. This could be due to the amount of aniline present being outside the LOD of this technique, as well as the high vapor pressure of aniline resulting in evaporation in the headspace prior to sampling. The calculated mass of NPP and NPPA found in the headspace of 4-ANPP, acetyl fentanyl, furanyl fentanyl, fentanyl alkaloid, and carfentanil citrate, respectively, is shown in Fig. 2. It should be noted that this mass is representative of the mass adsorbed on the SPME fiber, not total mass of the sample. Thus far, NPP and NPPA prove to be promising target analytes for detection of fentanyl and related substances.

Headspace analysis of street-grade fentanyl

Detection of illicit fentanyl in the field is the ultimate goal. Therefore, to validate if NPP or NPPA can be used as target analytes for the detection of fentanyl, or related substances, a total of 14 confiscated (street-grade) fentanyl exhibits were sampled. Seven exhibits were provided by the Maryland State Police Forensic Sciences Division Laboratory and another seven provided by the DEA Special Testing and Research Laboratory. For both sets of confiscated exhibits, trained personnel traveled to respective laboratories to extract the headspace of confiscated samples onsite. A list of VOCs

Table 2 List of street-grade fentanyl samples from the Maryland State Police Forensic Sciences Division Laboratory

Sample	Identified substance(s)	Mass of sample in vial (mg)	Presence of NPPA	Presence of NPP
P550020	Heroin and parafluoroisobutyryl fentanyl, quinine	3	Y	N
P550036	Fentanyl (indication of heroin)	12	Y	N
P550037	Fentanyl, quinine, noscapine, acetaminophen	13	Y	N
P550064	Heroin and fentanyl, morphine, acetylcodeine, acetylmorphine	2	Y	N
P550071	U47700, fentanyl	12	N	N
P550080	Carfentanil	19	Y	N
P550089	Fentanyl	13	Y	N

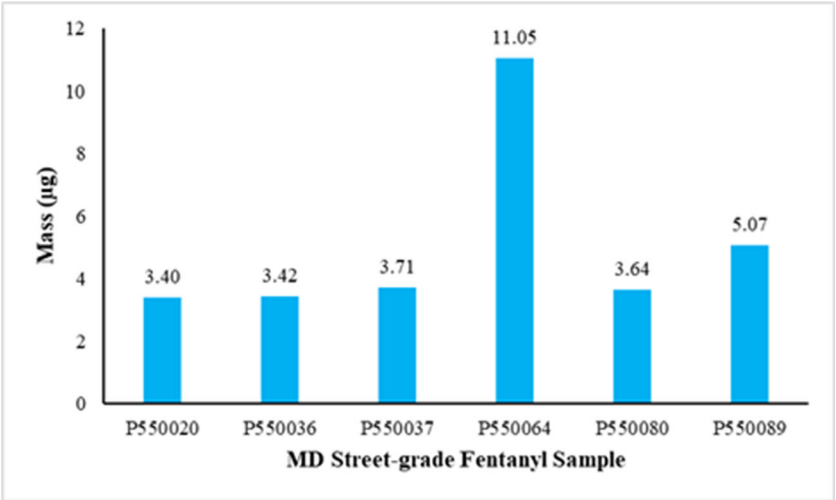


Fig. 3 Mass of NPPA found in the headspace of street-grade fentanyl from Maryland State Police Forensic Sciences Division Laboratory. *Mass is representative of the mass on the SPME fiber, not total mass

found in the headspaces of confiscated samples can be found in Tables S2 and S3.

Street-grade samples from the Maryland State Police Forensic Sciences Division Laboratory ranged in mass from 2 to 19 mg. Table 2 lists the sample number with identification of substances, corresponding mass, and presence of NPPA or NPP. Many of these street-grade fentanyl samples were adulterated with fentalogs, synthetic opioids, and naturally occurring opioids. Despite adulteration, six of the seven exhibits contained NPPA in the headspace; however, NPP was not detected in any of the samples. Similar to the fentalogs, mass of NPPA found in the headspace of each sample was calculated (Fig. 3). The total mass of sample in the vial did not have an effect on the amount of NPPA found in the headspace. In fact, sample P550064 was the smallest sample with a total mass of 2 mg and 11.05 μg NPPA found in the headspace, while sample P550080 was the largest sample with a total mass of 19 mg and 3.64 μg of NPPA found in the headspace. Sample P550071 was the only sample that did not contain a potential target of interest. This sample was identified as U47700, which is a synthetic opioid, with a small amount of fentanyl. It is possible that detection of NPP or NPPA in this sample was below the LOD of our technique.

Confiscated exhibits obtained from the DEA Special Testing and Research Laboratory consisted of seven adulterated fentanyl samples with respective masses of 5 mg. The fentanyl samples were found to contain sugars, opioids, and analgesics. The identified substances and their percentages within each sample were provided by the DEA (Table 3). As with the other set of street-grade samples, none was found to contain NPP. Conversely, NPPA was found in four out of the seven samples. Two of the three samples that did not contain NPPA or NPP were composed of less than 10% of fentanyl; therefore, lack of detection is likely due to NPP and NPPA being below LOD. The mass of NPPA extracted from the headspace of each sample was calculated and displayed in Fig. 4. Although there were varying percentages of fentanyl in the samples, the amount of NPPA found in each sample was consistent. Interestingly, the headspace of BK009 did not include NPPA, but did contain N-phenyl-acetamide(Table S3) due to the presence of acetyl fentanyl in the sample. As previously stated, N-phenyl-acetamide is deduced to be a degradant of acetyl fentanyl (Scheme 2). This may have resulted in competition between NPPA and N-phenyl-acetamide onto the SPME fiber, which could explain why NPPA was not seen in the headspace.

Table 3 List of street-grade fentanyl samples from DEA Special Testing and Research Laboratory

Sample	Identified substance(s)	Presence of NPPA	Presence of NPP
BK002	9.88% fentanyl and 87.5% inositol	Y	N
BK003	21% fentanyl and 74.8% mannitol	Y	N
BK005	16.48% fentanyl and 80.6% mannitol	Y	N
BK009	17.31% fentanyl, 13.3% acetyl fentanyl, and 68.5% lactose	N	N
BE003	5.52% fentanyl and 84.6% tramadol	N	N
BE004	7.02% fentanyl, 24.9% lactose, 24.2% inositol, and 19.6% tramadol	N	N
BE006	17.11% fentanyl, 39.1% mannitol, and 35.2% acetaminophen	Y	N

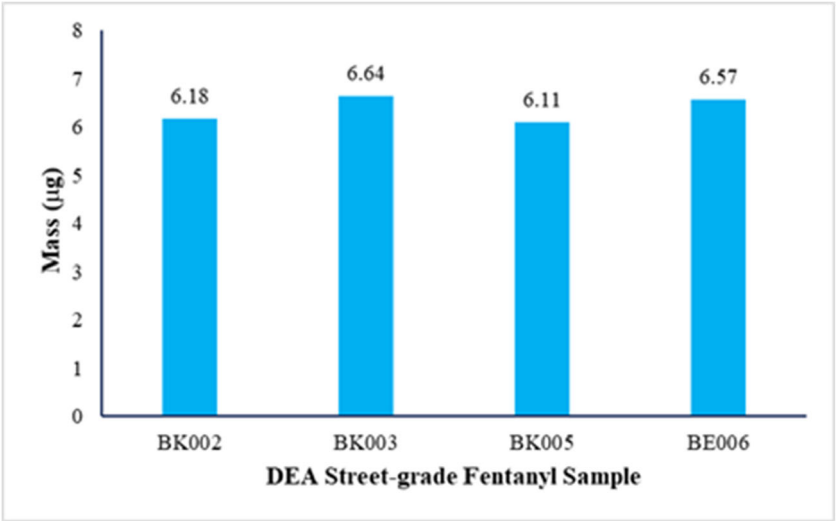


Fig. 4 Mass of NPPA found in the headspace of street-grade fentanyl samples from the DEA Special Testing and Research Laboratory. * Mass is representative of the mass on the SPME fiber, not total mass

The combined confiscated samples offer a map for how the headspace changes as fentanyl is dispersed into the USA clandestinely. The DEA fentanyl samples are confiscated prior to entering the USA and the Maryland State Police Forensic Sciences Division Laboratory samples are confiscated from those selling locally on the streets. Even with this range of fentanyl distribution, NPPA is shown to be a viable target for fentanyl detection, found in ten out of the fourteen street-grade samples. This could be due to the degradation of fentanyl generating NPPA at every stage of illicit distribution.

Conclusion

A comparative analysis of vapor profiles of fentalogs and confiscated (street-grade) fentanyl exhibits was completed. This analysis revealed two targets of interest for detection of fentanyl and/or related substances in the vapor phase: NPP and NPPA. NPPA is formed through the fragmentation of fentanyl through β-elimination, while NPP is a component of the synthetic route for fentanyl and many fentalogs. Out of the fourteen street-grade fentanyl exhibits, ten contained NPPA in the headspace while none contained NPP. A total of six fentalogs were sampled, with three containing NPPA and two containing NPP in the headspace. The lack of presence of NPPA in certain fentalogs is determined to be due to their structures not allowing for the formation of NPPA through β-elimination. From our previous study, it was found that reference-grade fentanyl contains both NPP and NPPA. Thus, it is believed that NPPA is a target for detection of fentanyl and related substances, while NPP is a target more specific to pure (unadulterated) substances. It was also found that N-phenyl-acetamide is a useful target for detection of acetyl fentanyl.

The ultimate goal of this research is the development of a non-contact method for the detection of fentanyl. With the vapor targets determined to be NPPA and NPP, future studies will focus on the development of an ion mobility spectrometry method for the detection of these analytes in the vapor mode. Further exploration on the formation of NPPA will be ascertained through fentanyl degradation studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-021-03670-4.

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Declarations

Conflict of interest The authors declare no competing interests.

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