Investigation of the Use of Deuterium and Oxygen in Illicit Fentanyl Analysis

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ABSTRACT

The Drug Enforcement Administration's (DEA) Fentanyl Signature Profiling Program (FSPP) currently analyzes illicit fentanyl samples seized from within the United States. The program utilizes impurity profiling along with stable isotope measurements to compare samples and discover unique scientific linkages that may not have been known to investigators. The method measures the carbon and nitrogen isotope ratios for illicit fentanyl and its immediate precursor, 4-ANPP. Through mass balance, the carbon isotopic signature of propionyl chloride is calculated. Despite the successful measurements of carbon and nitrogen isotope ratios, the measurements of deuterium and oxygen have not been feasible due to the high nitrogen content present in fentanyl. Traditional thermo-chemical elemental analysis isotope ratio mass spectrometry (TCEA/IRMS) methods have proven to be inaccurate for compounds containing a large proportion of nitrogen. New methods have been developed in recent years that include the use of chromium to circumvent this analytical issue. In an effort to improve the DEA FSPP's capabilities, a method to measure deuterium and oxygen isotope ratios via the chromium implementation was successfully developed. The method was initially evaluated with the analysis of representative samples from nine kilograms of illicit fentanyl (N=63). The pooled standard deviation of δ^{2}H for fentanyl and 4-ANPP were 1.32‰ and 1.54‰, respectively. The pooled standard deviation of δ^{18}O for fentanyl was 0.70‰. The complete isotopic profile of fentanyl and 4-ANPP were utilized to calculate the complete isotopic profile of propionyl chloride. The deuterium measurements were found to be reproducible for fentanyl and 4-ANPP which could provide further discrimination for linking samples; however, the variability found

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with deuterium in 4-ANPP may be a result of chemist technique. Oxygen isotope measurements in illicit fentanyl were found to be less useful due to a limited range of values and large reproducibility. Deuterium and oxygen isotope information may not be useful in linking exhibits of illicit fentanyl, however, the method may prove useful with carfentanil analyses.

Introduction

Since 2013, the abuse of fentanyl and fentanyl-related substances has resulted in thousands of overdoses and deaths. In an effort to address the current fentanyl crisis facing the United States, the Drug Enforcement Administration has developed the Fentanyl Signature Profiling Program (FSPP). The FSPP aims to make scientific linkages between illicit fentanyl seizures based on in-depth analyses. Linkages are made by comparing illicit fentanyl signature profiles comprised of data from multiple methodologies: gas chromatography with flame-ionization detection (GC/FID), nuclear magnetic resonance spectroscopy (NMR), gas chromatography mass spectrometry (GC/MS), and ^{13}C and ^{15}N isotope measurements of purified fentanyl and is immediate precursor, 4-ANPP. The linkages are utilized as investigative leads on seizures where linkages were unknown or only suspected. To date, the FSPP has established 96 linkages involving 192 cases and 380 exhibits from DEA seizures of more than 1100 kilograms of fentanyl.

Illicit fentanyl samples have very few organic impurities remaining, therefore, multiple methodologies must be utilized in order to gather as much information as possible in order to make a linkage. One key technique implemented currently is isotope ratio mass spectrometry (IRMS). IRMS is a popular instrumental technique utilized in many areas of research, including plant biosynthesis, climate reconstruction, and geosourcing of natural products such as emeralds and coffee. In forensic science, isotope measurements are routinely utilized for murder investigations, tracing the manufacturers of explosives, and determining the geographical origin of illicit drugs.

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The FSPP routinely isolates illicit fentanyl from seized exhibits through a series of cleanup procedures that may include solvent extractions, acid/base extractions, or preparatory column chromatography. A portion of the isolated fentanyl is then converted to its immediate precursor, 4-ANPP (Figure 1).

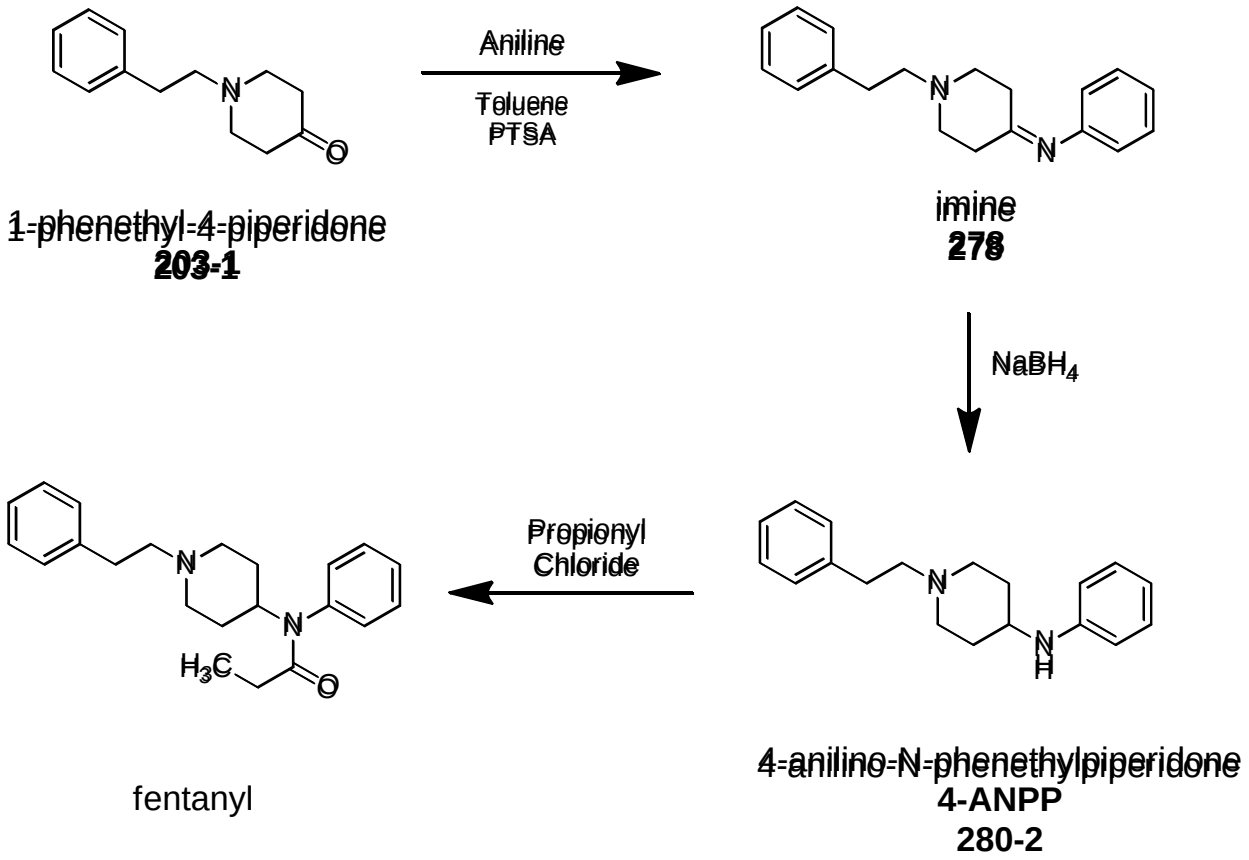

Figure 1. Synthetic scheme to fentanyl.

The ^{13}C and ^{15}N isotope content of the illicit fentanyl and 4-ANPP are then measured. Stable isotope measurements are expressed in the "delta" notation (δ). Delta is defined as:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right)$$

or also as:

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$$\delta\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R is the ratio of heavy to light isotopes (e.g., $^{13}C/^{12}C$). The delta notation is expressed in per mil (‰). Using mass balance, the ^{13}C signature of propionyl chloride is calculated (Eq. 1).

$$\delta^{13}C_{\text{propionyl chloride}} = ((\delta^{13}C_{\text{fentanyl}} + 1) - (\delta^{13}C_{\text{4-ANPP}} * 0.8636))/0.1364 \quad \text{Eq. 1}$$

The linkage process involves the use of data from multiple methodologies, however, isotopic profiles of the illicit fentanyl, 4-ANPP, and propionyl chloride are essential. While carbon and nitrogen isotope data are critical, measuring the isotopic contributions from ^{2}H and ^{18}O could prove to add even more discriminatory power. Currently, the DEA routinely measures $\delta^{2}H$ and $\delta^{18}O$ in cocaine and methamphetamine with a traditional column set-up. Fentanyl presents some difficulties with this method due to its higher nitrogen content, and a new instrumental method is required that utilizes chromium. A new method was developed and investigated for future implementation into FSPP. The data collected and outlook of this method are presented within.

Materials and Methods

Materials

All chemicals and solvents used were reagent grade or better and were obtained from Sigma-Aldrich.

Illicit Fentanyl

Nine kg of illicit fentanyl were seized in Baltimore, MD. Seven samples were removed from each of the nine kilograms (Figure 2) to result in 63 representative samples. Additionally, more than 340 illicit fentanyl exhibits previously analyzed by the FSPP were submitted for further isotope analyses.

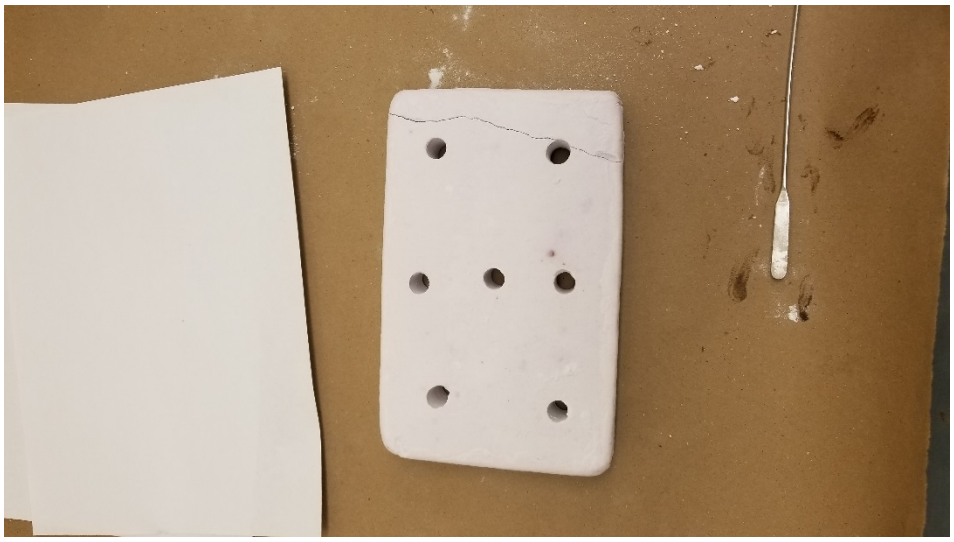

Figure 2. Photograph of one sampled kilogram to illustrate sampling pattern. Each of the nine kilograms were sampled in the same manner.

Gas Chromatography/Flame Ionization Detection (GC/FID)

Fentanyl quantitation was performed using an Agilent (Palo Alto, CA) Model 7890A gas chromatograph with flame ionization detection. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 µm) (Agilent, Santa Clara, CA) in constant flow mode at 43.5 cm/s of hydrogen carrier gas. The injection port and flame ionization detectors were maintained at 280^{o}C. The oven temperature was programmed as follows: Initial temperature, 250^{o}C; initial hold, 12.0 min; program rate, 25.0^{o}C/min; final temperature, 280^{o}C; final hold, 3.8 min. Nitrogen was used as the auxiliary make-up gas for the detector. Samples were injected (2 µL injection) in the split mode (21:1) by an Agilent 7683 Series Auto Injector. Illicit samples were accurately weighed (ca. 20 mg) into a 15-mL centrifuge tube, dissolved in 2 mL of water, sonicated for 15 min, rendered basic with 2 drops of concentrated NH_4OH, and extracted with 2 mL of $CHCl_3$ (containing 0.400 mg/mL of tetracosane). The organic phase was passed through a cotton-plugged pipet for injection.

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Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained using an Agilent 600MR-DD2 600 MHz NMR with a 5 mm OneNMR pulse field gradient probe. The sample temperature was maintained at 25°C. A standard Agilent proton pulse sequence was used (90 degree pulse, 45 second delay, 6 second acquisition time, 8 scans). Carfentanil citrate was dissolved in 1 mL deuterated chloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS, 0 ppm reference) with two drops of deuterated methanol (CD_3OD) to enhance solubility (Cambridge Isotopes, Tewksbury, MA). 1,4-BTMSB-d_4 (Wako Pure Chemical Industries, Saitama, Japan) was used as the quantitative internal standard. Carfentanil hydrochloride was dissolved in deuterated chloroform ($CDCl_3$) containing 0.03% v/v tetramethylsilane (TMS, 0 ppm reference) (Cambridge Isotopes).

Gas Chromatography/Mass Spectrometry (GC/MS)

Approximately 800 mg of fentanyl hydrochloride (or 50 mg equivalents of fentanyl) was weighed in a centrifuge tube. Ten mL of CH_2Cl_2 was added directly to the centrifuge tube, and the centrifuge tube was vigorously shaken and centrifuged. The organic layer was removed and filtered through a cotton-plugged pipet into a new centrifuge tube. One mL was removed for analysis via GC/MS. Gas chromatography/mass spectrometry (GC/MS) analyses were performed using an Agilent Model 5975C quadrupole mass-selective detector (MSD) interfaced with an Agilent 7890A gas chromatograph (GC). The MSD was operated in the electron ionization mode with an ionization potential of 70 eV, a scan range of 34-700 mass units, and at 1.34 scans/s. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 µm) in constant flow mode, at 36.5 cm/s of Helium. The GC oven was temperature programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; temperature program

rate, 6ºC/min to 300ºC; final hold, 5.6 min. The injector was operated in the split mode (22:1) and at a temperature of 280ºC. The auxiliary transfer line to the MSD was operated at 280ºC. Samples (*ca*. 5 mg/mL) were injected at a volume of 2 µL. Approximately 800 mg of fentanyl hydrochloride (or 50 mg equivalents of fentanyl) was weighed in a centrifuge tube. Ten mL of CH_2Cl_2 was added directly to the centrifuge tube, and the centrifuge tube was vigorously shaken and centrifuged. The organic layer was removed and filtered through a cotton-plugged pipet into a new centrifuge tube. One mL was removed for analysis via GC/MS.

Fentanyl Purification for Isotope Analyses

The CH_2Cl_2 containing fentanyl was dried down under air at 75°C. The fentanyl HCl residue was then dissolved in approximately 2 mL of water. Concentrated NH_4OH was added until basic (approximately 3 drops). Five mL of dry ether was added to the centrifuge tube, and the centrifuge tube was then vigorously shaken and centrifuged. Five mg of purified fentanyl base was then removed for isotope analyses. Approximately 1 mL of CH_2Cl_2 was added to the remaining portion of fentanyl base and allowed to evaporate over 24 hrs.

Conversion of Fentanyl to 4-ANPP

The remaining portion of fentanyl base for each sample was converted to the immediate precursor, 4-ANPP. Three hundred µL of 70% H_2SO_4 was added directly to the remaining fentanyl base in a centrifuge tube. The sample was then heated at 105°C for 24 hrs. After cooling, 5 mL of ether were added. The sample was shaken vigorously and centrifuged. The ether was discarded, and 5 mL of water were added followed by 50 µL of concentrated NH_4OH.

Five mL of ether were then added to the centrifuge tube, vigorously shaken, and centrifuged. The ether was then removed and filtered through a cotton-plugged pipet into a new centrifuge tube. The ether was dried under air at 75°C. The dried 4-ANPP residue was then weighed for isotope analyses.

Elemental Analyzer/Isotope Ratio Mass Spectrometry (EA/IRMS)

Carbon and nitrogen isotope ratio analyses were determined using an elemental analyzer (EA) (Costech Analytical Technologies Inc., Valencia, CA) interfaced with a Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., Bremen, Germany). Typically, 0.8-1.1 mg of fentanyl was accurately weighed into a tin capsule (Costech Analytical Technologies Inc.) and introduced into the EA using a Costech Zero-Blank autosampler (Costech Analytical Technologies Inc.). The EA reactor tubes were comprised of two quartz glass tubes filled with chromium (III) oxide/silvered cobaltous oxide and reduced copper, held at 1040°C and 640°C for combustion and reduction, respectively. A trap filled with magnesium perchlorate was used to remove water from generated combustion gases, and a post-reactor GC column was maintained at 65°C for separation of evolved N_2 and CO_2. Helium (99.999% purity, ARC3 Gasses, Richmond, VA) was used as the carrier gas, and the system head pressure was adjusted to achieve a measured flow of 90 mL/min. Data was acquired and processed using ISODAT 3.0 software (Thermo Fisher Scientific Inc., Bremen, Germany).

EA normalization and quality control materials consisted of internally calibrated atropine (TCI, St. Louis, Missouri) and cocaine base (DEA Laboratory, Dulles, Virginia). Sample sequences were bracketed by an internally calibrated atropine secondary standard, typically at intervals of one standard every seven samples. The atropine and cocaine secondary standards were calibrated

to primary isotopic standard materials IAEA-N-1, IAEA-N-2, USGS-25, LSVEC, and NBS-19 and corresponding scale normalized isotope values are expressed as $\delta^{13}C_{VPDB\text{-}LSVEC}$ for carbon and $\delta^{15}N_{AIR}$ for nitrogen. Overall system reproducibility of reference materials and replicate analyses of cocaine was consistently 0.1 ‰ and 0.2 ‰ or better for all EA-IRMS $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively.

Thermo-Chemical Elemental Analyzer/Isotope Ratio Mass Spectrometry (TCEA/IRMS)

Analysis of hydrogen isotope ratios was performed using a TCEA (Thermo-chemical elemental analyzer)-chromium reactor. The TCEA reactor was operated at 1300 °C with a flow rate of 110 mL/min helium, and separation of H_2 from other gases was completed by passing the raw effluent through a scrubber trap packed with ascarite and magnesium perchlorate followed by a 0.6 m 1/4" Molseive 5Å GC column held at 80 °C. Samples and reference materials were loaded in 3.2 x 4 mm silver capsules (Costech Analytical). Target masses for each material type were varied because of element composition differences, but the target amount was 9.7 µmol H with a 25% window.

Analysis of oxygen isotope ratios was performed using a TCEA reactor. The TCEA reactor was operated at 1400 °C with a flow rate of 110 mL/min Helium, and separation of H_2 from other gases was completed by passing the raw effluent through a scrubber trap (Ascarite/Magnesium percholorate) followed by a 1.6 meter ¼" Molseive 5Å GC column held at 80 ˚C. Both samples and reference materials were loaded in 3.2 x 4 mm silver capsules (Costech Analytical). Target masses for each material type varied because of element composition differences but the target amount was 0.41 µmol O with a 25% window.

Sample normalization reference materials for hydrogen were n-C24 and n-C28 (Table 1). QC reference materials were Stry1, Caf1, PEF-1, Lid2, and IAEA-600 (Table 2). Sample normalization reference materials for oxygen were UU-OH-5 and UU-OH-**7** (Table 1). QC reference materials were Stry1, Caf1, UU-OH-6, and IAEA-600 (Table 2). In addition to QC reference materials listed above, 1 in 10 of the samples was run in duplicate within an analytical sequence. The pooled standard deviation of the repeated samples of fentanyl are calculated through the first 4 sets where the table value is the pooled standard deviation (number of samples repeated) (Table 3).

	Certificate			
RM ID	**δ^2H (‰)**	**Uncertainty (1σ)**	**δ^{18}O (‰)**	**Uncertainty (1σ)**
n-C24	-35.69	3.14		
n-C28	-248.68	4.59		
UU-OH-5			+36.35	0.18
UU-OH-7			-2.78	0.38

Table 1. Isotope ratios of the normalization reference materials used in the analysis of the fentanyl and 4-ANPP samples.

	δ^2H (‰)			δ^{18}O (‰)			Certificate	
QC Materials	**Mean**	**Within**	**Between**	**Mean**	**Within**	**Between**	**δ^2H (‰)**	**δ^{18}O (‰)**
Stry1	-138.8	1.4	0.4	+16.63	0.37	0.25		
Caf1	+119.4	2.0	0.8	+27.67	0.58			
UU-OH-6				+26.06	0.57			
Lid2	-148.9	1.5	0.9					
IAEA-600	-155.4	1.6		-3.13	0.56		-156.1±1.3	-3.48±0.53
PEF-1	-99.6	1.8	0.2				-100.3±2.0	

Table 2. Mean and standard deviations of QC materials analyzed along with fentanyl and 4-ANPP samples in sets 1-4.

	Pooled Standard Deviation			
Material	**δ^2H (‰)**	***n***	**δ^{18}O (‰)**	***n***
Fentanyl	1.32	61	0.70	71
4-ANPP	1.54	56		

Table 3. Calculated and pooled standard deviations for repeated sample analyses of fentanyl and 4-ANPP.

Results and Discussion

Nine kilograms of fentanyl HCl were sampled in seven locations across each kilogram (N=63). Each sampling was analyzed in triplicate. The results from the fentanyl quantitation and isotope measurements are summarized in Table 4. The purity of fentanyl HCl ranged from 5.52% - 6.85%. Based on the measured isotopes of fentanyl and 4-ANPP, it was apparent kilogram "A" did not contain the same fentanyl as the remaining 8 kilograms in this seizure. Therefore, this kilogram was not included in any subsequent standard deviation calculations. As shown, the remaining eight kilograms contained the same fentanyl at approximately the same purities by weight. The standard deviations of each isotope measurement are within the method's pooled standard deviation during repeatability studies, indicating the method is suitable for laboratory analyses. However, there is much more variance within the 4-ANPP δ^2H values in comparison to those of the corresponding fentanyl measurements.

Kilo	Purity (%)	Fentanyl δ^{15}N (‰)	Fentanyl δ^{13}C (‰)	Fentanyl δ^{2}H (‰)	Fentanyl δ^{18}O (‰)	4-ANPP δ^{15}N (‰)	4-ANPP δ^{13}C (‰)	4-ANPP δ^{2}H (‰)
A	6.85	2.5	-27.6	-76.5	8.9	2.8	-27.2	-28.6
B	6.01	3.6	-28.0	-99.4	8.6	3.9	-27.6	-48.7
C	6.04	3.6	-28.0	-100.6	8.3	3.9	-27.6	-50.7
D	5.52	3.7	-28.0	-100.5	8.7	3.9	-27.6	-51.3
E	5.89	3.9	-28.0	-99.6	8.5	4.0	-27.6	-52.1
F	5.87	3.7	-28.0	-100.3	8.7	3.9	-27.6	-50.9
G	5.79	3.7	-28.0	-101.1	8.8	3.9	-27.6	-51.6
H	5.75	3.7	-28.0	-99.8	8.5	3.9	-27.6	-50.6
I	5.77	3.6	-28.0	-102.2	8.7	4.0	-27.6	-54.1
STD DEV	0.16	0.09	0.00	0.91	0.16	0.04	0.00	1.52

Table 4. Average purity and isotope measurements from a nine kilogram DEA exhibit. Each value is an average of triplicate measurements from seven separate locations per kilogram (N=63). The reported standard deviation does not include kilogram "A" as it was determined to be an outlier.

The isotope measurements completed for purified fentanyl and 4-ANPP allowed for the calculation of the isotopic signature of propionyl chloride with Equations 1-3.

$$\delta^2H_{\text{propionyl chloride}} = ((\delta^2H_{\text{fentanyl}}) - (\delta^2H_{\text{4-ANPP}} * 0.857))/0.143 \quad \text{Eq. 2}$$

$$\delta^{18}O_{\text{propionyl chloride}} = (\delta^{18}O_{\text{fentanyl}}) - 1.2 \quad \text{Eq. 3}$$

Evaluation of the isotopic content of propionyl chloride from these measurements (Table 5) indicates the calculated $\delta^{13}C$ and $\delta^{18}O$ fall within the acceptable standard deviation ranges for fentanyl and 4-ANPP. The calculated deuterium value falls outside of this range with a standard deviation of 5.90.

Kilo	Purity (%)	Fentanyl $\delta^{15}N$ (‰)	Fentanyl $\delta^{13}C$ (‰)	Fentanyl $\delta^{2}H$ (‰)	Fentanyl $\delta^{18}O$ (‰)	4-ANPP $\delta^{15}N$ (‰)	4-ANPP $\delta^{13}C$ (‰)	4-ANPP $\delta^{2}H$ (‰)	PC $\delta^{13}C$ (‰)	PC $\delta^{2}H$ (‰)	PC $\delta^{18}O$ (‰)
A	6.85	2.5	-27.6	-76.5	8.9	2.8	-27.2	-28.6	-22.6	-363.8	7.7
B	6.01	3.6	-28.0	-99.4	8.6	3.9	-27.6	-48.7	-23.1	-403.1	7.4
C	6.04	3.6	-28.0	-100.6	8.3	3.9	-27.6	-50.7	-23.2	-399.6	7.1
D	5.52	3.7	-28.0	-100.5	8.7	3.9	-27.6	-51.3	-23.1	-395.9	7.5
E	5.89	3.9	-28.0	-99.6	8.5	4.0	-27.6	-52.1	-23.2	-383.9	7.3
F	5.87	3.7	-28.0	-100.3	8.7	3.9	-27.6	-50.9	-23.5	-396.5	7.5
G	5.79	3.7	-28.0	-101.1	8.8	3.9	-27.6	-51.6	-23.1	-398.0	7.6
H	5.75	3.7	-28.0	-99.8	8.5	3.9	-27.6	-50.6	-22.9	-394.6	7.3
I	5.77	3.6	-28.0	-102.2	8.7	4.0	-27.6	-54.1	-22.9	-390.2	7.5
STD DEV	0.16	0.09	0.00	0.91	0.16	0.04	0.00	1.52	0.18	5.90	0.16

Table 5. Average purity, isotope measurements, and calculated isotope values of propionyl chloride (PC) from a nine kilogram DEA exhibit. Each value is an average of triplicate measurements.

By analyzing nine kilograms of fentanyl HCl from one seizure, it was possible to assess the developed TCEA/IRMS method as well as the current methodology employed by the FSPP for repeatability. Ultimately, it was important to test the developed method for incorporation into the process of identifying linkages between samples. Two sets of linked samples were examined in order to determine the suitability or need for deuterium and oxygen isotope analyses. The

isotopic measurements and calculated isotope values for propionyl chloride for Linkage 1 are presented in Table 6.

Sample	Purity (%)	Fentanyl δ^{15}N (‰)	Fentanyl δ^{13}C (‰)	Fentanyl δ^{2}H (‰)	Fentanyl δ^{18}O (‰)	4-ANPP δ^{15}N (‰)	4-ANPP δ^{13}C (‰)	4-ANPP δ^{2}H (‰)	PC δ^{13}C (‰)	PC δ^{2}H (‰)	PC δ^{18}O (‰)
04081A	5.21	3.9	-28.3	-115.8	8.0	4.0	-27.9	-62.2	-23.5	6.8	-437.2
04081B	5.31	3.9	-28.4	-115.9	8.6	4.1	-27.7	-66.9	-25.5	7.4	-409.9
04081C	5.30	3.9	-28.2	-115.5	8.8	4.0	-27.8	-71.5	-23.4	7.6	-379.7
04083B	5.31	3.9	-28.2	-116.1	8.7	4.0	-27.8	-71.4	-23.4	7.5	-384.2
04083C	5.19	3.6	-28.3	-114.9	8.2	4.0	-27.9	-67.8	-23.5	7.0	-396.9
STD DEV		0.13	0.08	0.49	0.35	0.04	0.08	3.83	0.92	0.35	23.13

Table 6. Fentanyl purity, isotope measurements, and calculated isotope values of propionyl chloride (PC) from a set of samples previously determined to be linked.

As expected based on the nine kilogram study, the four measured isotopes for fentanyl are well within the expected standard deviation limits. The δ^{15}N and δ^{13}C of 4-ANPP are also within these established limits. These measurements indicated the fentanyl in these exhibits was identical. However, the δ^{2}H of 4-ANPP exhibits a large variance in addition to the calculated isotopic value for ^{18}O and ^{13}C in propionyl chloride. A second linkage was examined further and presented in Table 7.

Sample	Purity (%)	Fentanyl δ^{15}N (‰)	Fentanyl δ^{13}C (‰)	Fentanyl δ^{2}H (‰)	Fentanyl δ^{18}O (‰)	4-ANPP δ^{15}N (‰)	4-ANPP δ^{13}C (‰)	4-ANPP δ^{2}H (‰)	PC δ^{13}C (‰)	PC δ^{2}H (‰)	PC δ^{18}O (‰)
02069A	6.0	-1.1	-29.6	-70.3	9.0	-0.6	-29.2	-42.3	-24.8	7.8	-238.1
02069B	5.9	-0.8	-29.5	-71.9	7.1	-0.7	-29.4	-47.1	-22.8	5.9	-220.6
02069C	6.0	-1.2	-29.5	-72.5	7.4	-0.8	-29.3	-47.3	-23.4	6.2	-224.1
03016A	6.5	-0.9	-29.5	-78.6	9.2	-0.6	-29.5	-45.5	-22.2	8.0	-277.1
03016B	6.0	-1.2	-29.5	-74.0	7.4	-0.9	-29.4	-44.1	-22.8	6.2	-253.3
03016C	5.7	-1.3	-29.5	-70.9	7.3	-0.8	-29.3	-49.3	-23.4	6.1	-200.2
03061A	6.2	-0.8	-29.5	-70.1	6.9	-0.6	-29.2	-46.0	-24.1	5.7	-214.5
03061B	6.3	-1.1	-29.5	-68.9	7.1	-0.6	-29.3	-45.2	-23.4	5.9	-210.6
03061C	5.8	-0.8	-29.5	-70.3	7.8	-0.4	-29.3	-46.2	-23.4	6.6	-215.3
03401A	4.3	-1.5	-29.5	-70.6	9.8	-1.7	-29.1	-51.2	-24.7	8.6	-186.9

03401B	4.7	-1.1	-29.6	-70.4	8.5	-1.0	-29.2	-51.2	-24.8	7.3	-185.3
STD DEV		0.23	0.04	2.67	0.99	0.34	0.11	2.79	0.88	0.99	27.45

Table 7. Fentanyl purity, isotope measurements, and calculated isotope values of propionyl chloride (PC) from a set of samples previously determined to be linked.

All measurements of $\delta^{15}N$ and $\delta^{13}C$ for fentanyl and 4-ANPP in these exhibits are within acceptable limits of standard deviation. The measurements of $\delta^{2}H$ in both fentanyl and 4-ANPP are outside of the acceptable limits, as well as the $\delta^{18}O$ of fentanyl. This variance is carried on to the calculation of the isotopes in propionyl chloride resulting in those values to also be outside of the method's acceptable standard deviation limits.

Conclusions

A new TCEA/IRMS method was developed to measure the ^{2}H and ^{18}O isotopes present in illicit fentanyl and its immediate precursor, 4-ANPP. The entire isotopic profile of propionyl chloride was calculated based on mass balance. The new method was utilized to evaluate the need for expanded isotope analyses by DEA's Fentanyl Signature Profiling Program in an effort to enhance the program's current capability in linking illicit fentanyl exhibits based on scientific data.

Reproducibility of individual fentanyl and 4-ANPP hydrogen isotope ratios were 1.32 and 1.54 permil, respectively, which is much smaller than the range of measured samples, 137 and 161 permil respectively. This should allow for differentiation between samples and calculation of the precursor propionyl chloride hydrogen isotope ratio. However, large variances were observed within deuterium measurements of 4-ANPP of previously linked exhibits. The variance wasn't

consistent; therefore, it may be a consequence of the chemists' technique when isolating pure fentanyl and converting it to 4-ANPP.

The reproducibility of individual fentanyl oxygen isotope ratio measurements are 0.70 permil compared to the total range of 26 permil. However, most of the samples have a range of oxygen isotope ratios of less than 5 permil. The limited range and relatively large reproducibility uncertainty may limit the usefulness of oxygen isotope ratio in differentiation between most samples. Additionally, the ^{18}O in propionyl chloride is dependent on the measurements made for fentanyl which further exaggerates any observed variances.

While this TCEA/IRMS method may not be helpful in making linkages for fentanyl, it may prove useful in carfentanil. Currently, FSPP typically only measures $\delta^{13}C$ in carfentanil due to its low concentrations in illicit samples. Adding additional isotope capabilities will enhance the FSPP's ability to examine carfentanil exhibits for linkages to seizures across the United States. TCEA/IRMS is particularly ideal for this scenario due to the need for very little bulk sample required.