Analysis of fentanyls in silt sediment using electron ionization gas chromatography-mass spectrometry

C. A. Valdez, S. Hok, A. K. Vu

December 11, 2024

Analytical Letters

“Determination of synthetic opioids belonging to the fentanyl class in silt using electron ionization gas chromatography-mass spectrometry (GC-MS).”

Carlos A. Valdez[1-4,*], Derrick C. Kaseman[2,3,5], David S. Cho[2, 3], Saphon Hok[2, 3], Alexander K. Vu[2, 3]

[1]Biosciences and Biotechnology Division, [2]Forensic Science Center, [3]Physical and Life Sciences Directorate, [4]Global Security Directorate, and the [5]Materials Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA, 94550, USA.

*Corresponding author:
Dr. Carlos A. Valdez
Physical and Life Sciences Directorate,
Biosciences and Biotechnology Division, and the
Forensic Science Center, L-090
7000 East Avenue, Livermore, CA 94550, USA
Email: valdez11@llnl.gov
Phone: +1 925 423 1804
ORCID: 0000-0002-8641-9077 (C. A. V.)
0000-0003-2076-1264 (D. C. K.)
0000-0003-4154-0364 (D. S. C.)
0000-0002-8545-3628 (S. H.)
0000-0001-7326-9180 (A. K. V.)

Abstract

From a drug enforcement perspective, the discovery of fentanyl or its main degradation product norfentanyl in soil matrices strongly insinuates the current or past existence of a clandestine laboratory engaged in the illegal production of this synthetic opioid. In this work, an extraction protocol from silt sediment of fentanyl and three analogs: acetylfentanyl, thiofentanyl and acetylthiofentanyl, spiked at two concentrations each and separately (at ~1 and ~10 μg/g), is described. Silt was chosen for testing the approach as it is a notoriously difficult matrix to extract and/or derivatize analytes for GC-MS analysis, due to its high water content and ability to shield analytes from derivatization agents. In addition, the identity of the fentanyls preliminarily identified by electron ionization gas chromatography-mass spectrometry (EI-GC-MS) analysis, can be corroborated by reacting each opioid in the silt's extract with 2,2,2-trichloroethoxycarbonyl chloride (Troc-Cl). The reaction between Troc-Cl and each opioid generates two unique products that can be pieced together to retrospectively identify the original opioid using EI-GC-MS therefore serving as a corroborating tool for known opioids as well as an initial structure elucidation step for new, unknown fentanyl analogs. The findings described in this work will serve to provide an additional avenue for detecting fentanyls in the environment and henceforth yielding information for chemical forensics purposes.

Keywords: fentanyl, acetylfentanyl, synthetic opioids, chloroformate, silt, gas chromatography-mass spectrometry (GC-MS).

Introduction

No expert or visionary in the fields of medicine and medicinal chemistry over 60 years ago would have predicted that fentanyl would become one of the most effective and widely used painkillers in the medical field (Stanley 2014). Synthesized by Paul Adriaan Janssen in 1960 and arising from systematic structure-activity relationship (SAR) studies on the pethidine scaffold, fentanyl proved to be more than just an unrivaled drug for the elimination of pain in millions of patients in the clinic (Janssen and Eddy 1960; Janssen 1962) (Figure 1). Further studies revealing its faster anesthetic onset coupled to its minimal adverse effects on the cardiovascular system of patients suffering from chronic pain from certain diseases states like cancer have made it a preferred alternative over the gold standard for the management of pain, namely morphine (Mercadante et al. 2010; Chen and Ashburn 2015; Vithlani and Baranidharan 2010) (Figure 1). Unfortunately, aside from all their medical benefits, particularly during perioperative surgical procedures, fentanyl and analogs thereof have found themselves at the center of the global opioid pandemic due to their dangerously addictive nature and lethal effects at low doses (Lovrecic et al. 2019; Pardo et al. 2021).

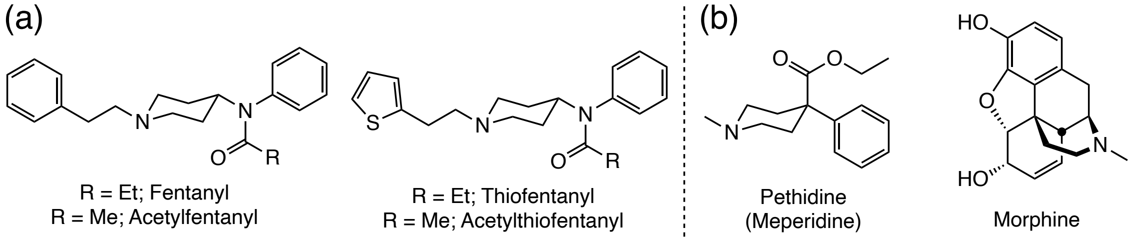

FIGURE 1 Chemical structures of the synthetic opioids employed in this study: fentanyl, acetylfentanyl, thiofentanyl and acetylthiofentanyl; (b) structure of pethidine that served as the starting SAR platform for the eventual discovery of fentanyl by Janssen and the gold standard in the management of pain in the clinic, morphine.

What heightens the fear of the current pandemic is the fact that common recreational drugs such as ecstasy are unscrupulously laced with fentanyl and other more powerful analogs to enhance or prolong their euphoric effects often resulting in death via accidental overdoses (Gladden, Martinez and Seth 2016; Lee et al. 2016; Rodda et al. 2017). Since the fentanyl pandemic began, government drug enforcing agencies have attempted to control access to the drug with very limited success. One of the main reasons for this is the clandestine, simple production of the opioid using chemicals that are still not under tight regulation following online methods (Siegfried 2023) as well as published scientific protocols (Gupta et al. 2005; Valdez, Leif, and Mayer 2014; Walz and Hsu 2019). It is this combination of ease of production coupled to their lethal effects in extremely low concentrations (e.g., fentanyl's LD_{50} rat ~ 3.05 mg/kg (Leen and Jurrlink 2019)), that have prompted research agencies worldwide to develop better methods for their detection (Valdez et al. 2018a; Valdez 2020a) and to discover new, more powerful antidotes and/or medical countermeasures to treat affected individuals and provide protection to first-responders (Yeung et al. 2020). As current methods for the treatment of fentanyl poisoning are generally successful (e.g., Naloxone) these need to be administered in a timely manner due to the fast-acting nature of the opioid (France et al. 2021). Due to the modular nature of the synthetic routes employed in their construction, analogs with a variety of physical properties and potencies can be produced. One such example that was not in the radar of drug enforcement authorities until it was identified as the causative agent in ten overdose fatalities in Rhode Island during 2013 is acetylfentanyl (Ogilvie et al. 2013; Lozier et al. 2015; Fort et al. 2016). Acetylfentanyl is structurally similar to fentanyl with the only difference lying in its amido group, where the acyl group in acetylfentanyl is the acetyl group while in fentanyl is the propionyl group (Figure 1a). Acetylfentanyl exhibits a potency ~15 times more than that of morphine and despite this feature, it is not used as an alternative to morphine or fentanyl in the clinic (Katselou et al. 2016; Armenian et al. 2018). Two other fentanyls that have become part of the growing list of fentanyl analogs but without any known impact to public health are thiofentanyl and acetylthiofentanyl (Figure 1a). Thiofentanyl and thioacetylfentanyl are unique structurally due to their 2-thiophenyethyl side chain that differs from the phenylethyl side chain found in fentanyl and acetylfentanyl.

The analysis and detection of fentanyl and related opioids has been largely covered by gas chromatography-mass spectrometry (GC-MS) (Valdez 2020a) and liquid chromatography-mass spectrometry (LC-MS) (Moody et al. 2018; Wei and Su, 2022). Both analytical techniques have been used separately or in tandem in the forensic analysis of biological samples originating from overdose decedents and survivors (Petersen et al. 2016; Danaceau et al. 2020). In addition to this, their combined use in the field of chemical forensics as leading techniques for the identification of chemical attribution signatures (CAS) in different synthetic pathways of fentanyl production is noteworthy and have become important tools in this field (Mayer et al. 2016; Mayer et al. 2018; Mörén et al. 2019; Ovenden et al. 2021; Toske et al. 2023).

Most analyses to date have involved cases where fentanyl is identified in biological matrices or identified by directly testing seized material. Work in the fields of forensic chemistry and forensic data collection for field intelligence purposes has been established for quite some time and this concept has been applied to the analysis, detection and correct identification of controlled substances in soil samples (Ehleringer et al. 2000; Mallette et al. 2016). This is obviously important as the detection of drugs, particularly synthetic opioids, in soil insinuates the latent or past presence of a clandestine production center (Valdez, Rosales and Leif 2023a). Previous work in our laboratory has made use of electron-ionization gas chromatography-mass spectrometry (EI-GC-MS) in the analysis and detection of fentanyls from a soil rich in organic content as well as one possessing high clay content (Valdez et al. 2023b). While the approach involves the effective extraction of the synthetic opioid from soils, it is the subsequent chemical modification of the fentanyl using 2,2,2-trichloroethoxycarbonyl chloride (Troc-Cl) that provides another layer of sample analysis and corroboration for this approach. The reaction between Troc-Cl and the fentanyls provides two unique products whose identities are dependent on the nature of the original fentanyl, and that can be used to deduce the structure of the original opioid (Figure 2). This approach has proven useful in retrospectively confirming the presence and identity of fentanyls in various matrices including urine and plasma samples (Valdez et al. 2022a). Silt is a sediment with a complex, non-uniform composition of mica, feldspar, quartz and a small percentage of clay minerals, all these components suspended or charged with water. As such, silt is a known difficult soil matrix to deal with due to its physical form consisting of fine, crystalline silicon oxide particles that can trap analytes in their interior and thus blocking any access to extracting solvents and/or derivatizing agents (Xuan, Blassengale, and Wang 2008; Xu, Wu, and Chang 2009). Furthermore, reactive sites at these silicon oxide particulates often results in decreased analyte derivatization due to their scavenging effects on electrophilic reagents. The approach described in this work uses EI-GC-MS and although the technique's sensitivity is significantly lower to the one that can be attained using LC-MS, it is introduced as a complementary analytical tool in the analysis and detection of fentanyls.

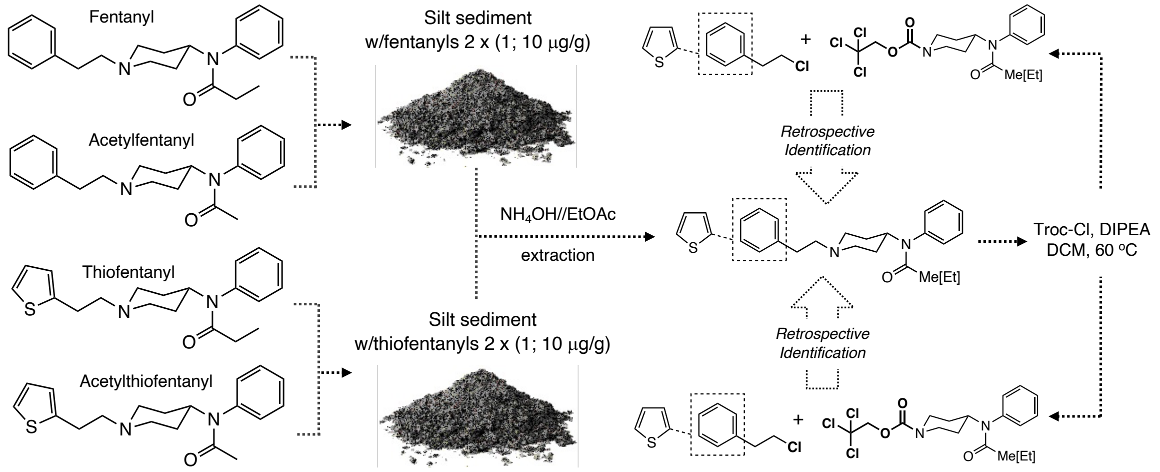

FIGURE 2 Chemical structures of the fentanyls used in this work and outline of the protocol using silt sediment. The extraction protocol employs basic NH_4OH to generate the opioid in its free base form, followed by extraction of this species into an organic layer (ethyl acetate). After concentration, reaction of the residue with Troc-Cl in DCM produces and alkyl chloride and a Troc-nor-fentanyl as predictable products that can be used to retrospectively identify the original fentanyl.

Materials and methods

Chemicals and reagents

All chemicals were purchased from commercial suppliers and used as received. Anhydrous dichloromethane and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO.). 2,2,2-Trichloroethoxycarbonyl chloride (Troc-Cl) was purchased from Alfa Aesar (Tewksbury, MA). Sodium carbonate and anhydrous sodium sulfate were purchased from Acros Organics (Westchester, PA.). Autosampler vials and glass inserts were purchased from Agilent Technologies (Santa Clara, CA.). Silt sediment was obtained from the EPA soil standards in the Forensic Science Center (FSC) at LLNL. Fentanyl, acetylfentanyl, thiofentanyl and acetylthiofentanyl were synthesized using established protocols. Troc-norfentanyl and Troc-noracetylfentanyl were synthesized as previously described. Ammonium hydroxide, Wheaton scintillation vials (20-mL capacity) and glass vials (4-mL capacity) were purchased from VWR (Radnor, PA). Thin layer chromatography (TLC) was used to monitor the coupling reactions after aliquot work up using Agela Technologies glass back MF_{254} TLC plates (pH ~ 5) and detection accomplished with UV light (λ = 254 nm) in conjunction with development of color with ceric ammonium molybdate (CAM) and iodine vapor. The Troc-labeled standards were purified by flash column chromatography using a Biotage Isolera purification system using Biotage Sfär silica high-capacity duo cartridges (10 G).

Preparation of the fentanyl-, acetylfentanyl-, thiofentanyl- and acetylthiofentanyl-spiked silt sediments

Cautionary note: The synthesis of fentanyl and related opioids should be executed by specially trained personnel and always involve the use of the appropriate personal protective equipment (PPE) that includes lab coat, nitrile gloves underneath butyl gloves, face shield and eye protection. All the synthetic steps leading to the formation of the final opioid should take place in a well-ventilated chemical fume hood for maximum shielding from the chemicals. In addition, Narcan (Naloxone HCl) antidote should always be present nearby for rapid use when these synthetic and analytical standard preparations are taking place.

Separate stock solutions (10 mL) of each fentanyl at 1 mg/mL each in dichloromethane (DCM) were prepared in 20-mL glass scintillation vials. The stock solutions (0.1 mL) were further diluted to 10 mL with DCM to yield a 10 µg/mL stock solutions that were kept under refrigeration (~5-7 ºC) and only taken out when preparing the final stock solutions (1 and 0.1 µg/mL) used for sediment spiking. For the preparation of the 10 ppm spiked soil, silt sediment (300 mg) was treated with 3 mL of the 1 ppm fentanyl solution, while the 1 ppm spiked soil was prepared by treating the soil (300 mg) with 3 mL of the 0.1 ppm solution. The soil suspensions in DCM were evaporated carefully in a rotary evaporator.

Extraction of silt and reaction with Troc-Cl

Fentanyl-spiked silt sediment (300 mg) was treated with 1 M ammonium hydroxide (pH = 11.2, 3 mL) in a 20 mL glass scintillation vial. After the particulates settled to the bottom of the vial, the mixture was extracted with ethyl acetate (3 x 1 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and transferred to a 4 mL glass vial. The volume of collected organic phase was evaporated to dryness from the original (~3 mL) starting volume. The residue was re-dissolved in DCM (200 μL) and analyzed by EI-GC-MS to assess the efficiency of the opioid extraction step. After the analysis, the DCM (200 μL) was treated sequentially with Troc-Cl (50 μL), sodium carbonate (2 mg) and heated to 60 °C for 4 hours. The reaction mixture was then analyzed by EI-GC-MS.

Mass Spectrometry Analysis

A 6890 Agilent GC with a 5975 MS detector equipped with a split/splitless injector was used for all analyses as previously described (Valdez, Leif, and Alcaraz 2016; Valdez et al. 2018a). The GC column used for the analyses was an Agilent HP-5ms UI capillary column (30 m × 0.25 mm id × 0.25 μm film thickness). Ultra-high purity helium, at 0.8 mL/min, served as the carrier gas.

The inlet was operated in pulsed splitless mode (25 psi for 1 minute, followed by a 50 mL/min purge flow). The injector temperature was set at 250 °C and the injection volume used was 1 μL. The oven temperature program was as follows: 40 °C, held for 3 minutes, increased at 8 °C/min to 300 °C and held for 3 minutes. The MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. Electron impact (EI) was used with an ionization energy of 70 eV. The MS was operated to scan from *m/z* = 29 to 600 in 0.4 seconds with a solvent delay of 3.5 minutes.

Results and discussion

In parallel fashion to efforts from our group and others in the area of identifying CAS that can serve as useful tools in the determination of the synthetic route used to construct a given fentanyl sample, the development of a method for the efficient identification of these opioids in various soil matrices has also been an area of concurrent research efforts. The approach described in this work addresses an important gap in the field of chemical forensics and intelligence collection regarding synthetic opioids. It consists of a two-step process with the first one involving an extraction step of an opioid followed by its specific and predictive chemical modification in the second one. The efficiency of the extraction step is crucial as it efficiently sets the stage for the second step of the methodology where the chemical modification of the opioid using chloroformate chemistry takes place. Although detection of the extracted fentanyl can already be accomplished using a combination of analytical techniques such as the extraordinarily sensitive LC-MS and the internal spectral library-containing GC-MS, both techniques will face major difficulties when encountering an unknown, newly emergent fentanyl. It is in these cases that further analyses must be undertaken to determine and to corroborate the structure of these unknown and in some instances equally toxic synthetic opioids.

Methods for the extraction of fentanyl and other members of this family of opioids generally involve the initial basification of the matrix under study whether this one is biological or environmental. The basification of the matrix is usually accomplished with a basic buffer (usually pH > 10) in order to ensure that the opioid will be in its free basic form. Regarding the extraction of fentanyl from biological matrices, several organic solvents have been used with the most common one being 1-chlorobutane (Foerster and Mason 1974) however, preliminary screening of organic solvents in our hands settled for using ethyl acetate as our extraction solvent. To this end, the silt sediment we chose for our studies, was spiked with four fentanyls (fentanyl, acetylfentanyl, thiofentanyl and acetylthiofentanyl) separately at two concentrations per opioid, a high (10 µg/g) and a low (1 µg/g). During the initial soil extraction step, ammonium hydroxide (pH = 11.2) was chosen as an extractant along with ethyl acetate as the pH of the silt sediment used was determined to be ~5.5. The ethyl acetate extracts were evaporated to yield residues that were reacted with Troc-Cl. This reaction provides four different products from four fentanyls used, 2-(2-chloroethyl)thiophene (2-CET), (2-chloroethyl)benzene (2-CEB), Troc-norfentanyl and Troc-noracetylfentanyl (Figure 2).

Table 1. Retention indices and retention times for Troc-norfentanyl and Troc-noracetylfentanyl in silt sediment.

	Troc-noracetylfentanyl	Troc-norfentanyl
Retention index (RI)	2784.5	2837.6
Retention time (min)	33.41	33.91

Results for the extraction efficiencies of all four fentanyls spiked separately in the silt sediment at both, the high and low concentrations, are presented in Figures 3a and 3d respectively. Starting with the extraction of fentanyl and acetylfentanyl, it can be observed that for the high concentration samples, both opioids were extracted with high efficiency ranging for ~73-81% for fentanyl and ~70-80% for acetylfentanyl. With equal degree of success, the extraction efficiencies for these opioids in the low concentration samples were ~59-71% for fentanyl and ~61-74% for acetylfentanyl. The high recovery of the opioids during these extractions is crucial in this type of analysis since it provides them in detectable quantities for their subsequent reaction with Troc-Cl.

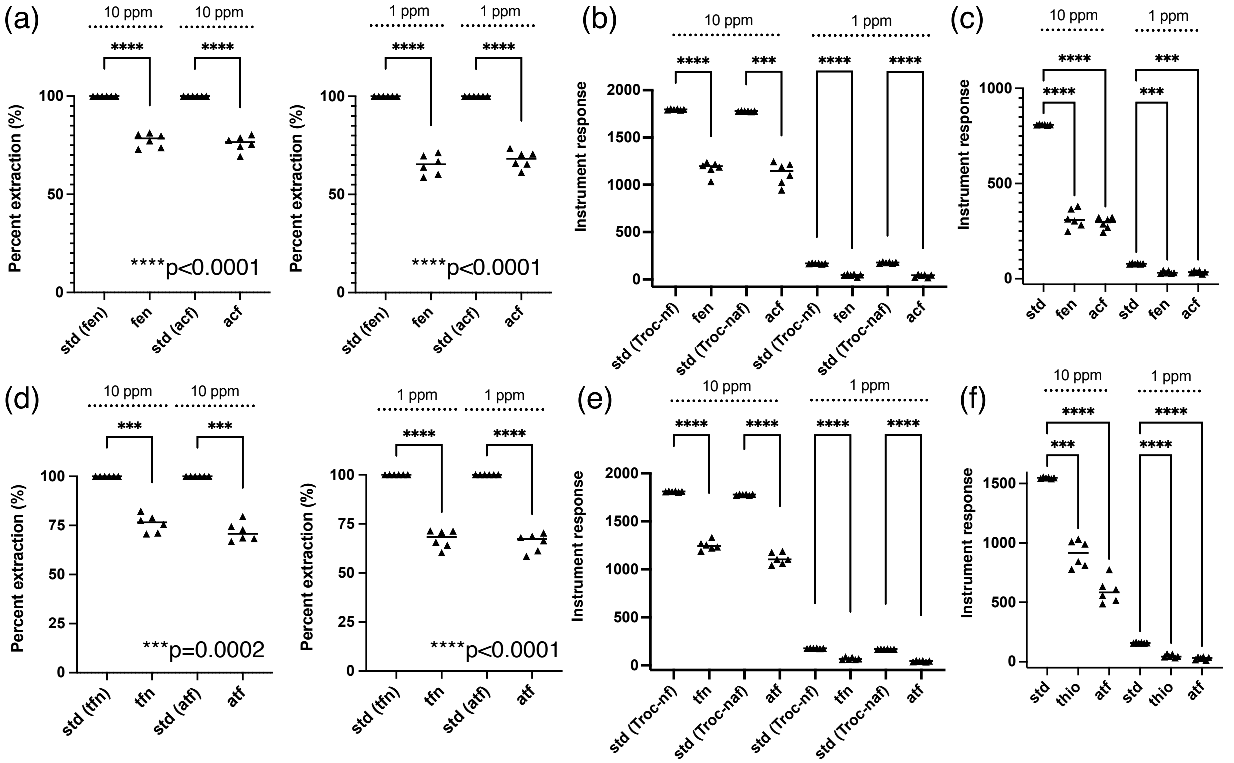

FIGURE 3 GC-MS analysis fentanyl (fen), acetylfentanyl (acf), thiofentanyl (tfn) and acetylthiofentanyl (atf) in silt sediment and their reaction with Troc-Cl when spiked at two concentrations each (10 and 1 µg/g (ppm)): (a) Extraction of fentanyl and acetylfentanyl along with standard solutions at both concentrations for comparison, statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****$p<0.0001$. All data are displayed as mean ± SEM; (b) average ($n=6$) peak areas (+/- the standard deviation) for Troc-norfentanyl (Troc-nf) and Troc-noracetylfentanyl (Troc-naf) from reaction between the extracts and Troc-Cl along with standard solutions

of 1 and 10 ppm concentrations, statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****p<0.0001, ***p=0.0004; (c) average (n=6) peak areas (+/- the standard deviation) for 2-(chloroethyl)benzene (2-CEB) from reaction between the extracts and Troc-Cl along with standard solutions of 10 and 1 ppm concentrations of 2-CEB (std.), statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****p<0.0001, ***p=0.0003 and ***p=0.0004; (d) Extraction of thiofentanyl and acetylthiofentanyl along with standard solutions at both concentrations for comparison, statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****p<0.0001, ***p=0.0002, all data are displayed as mean ± SEM; (e) average (n=6) peak areas (+/- the standard deviation) for Troc-norfentanyl (Troc-nf) and Troc-noracetylfentanyl (Troc-naf) from reaction between the extracts and Troc-Cl along with standard solutions of 10 and 1 ppm concentrations, statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****p<0.0001; (f) average (n=6) peak areas (+/- the standard deviation) for 2-(chloroethyl)thiophene (2-CET) from reaction between the extracts and Troc-Cl along with standard solutions of 10 and 1 ppm concentrations of 2-CET (std.), statistical analysis using one-way ANOVA followed by tukey's multiple comparison's test analysis revealed significance at a level of ****p<0.0001, ***p=0.0002, ***p=0.0003.

Therefore, the collected extract for each sample were evaporated to dryness using a gentle nitrogen stream, the resulting residue was then resuspended in DCM and heated to 60 ^{o}C with Troc-Cl and sodium carbonate for 4 hours. All the replicates were analyzed individually for the predicted by-products from each opioid. Thus, fentanyl and acetylfentanyl produce the same (2-chloroethyl)benzene (2-CEB) product, however, they can be differentiated from each other by the nature of the second product of the reaction, namely Troc-norfentanyl and Troc-noracetylfentanyl. All these products were detected for both high and low-concentration spiked silt samples for both opioids. It can be observed in Figure 3 that the reaction efficiencies between fentanyl and acetylfentanyl with Troc-Cl are very similar, leading to comparable concentrations of both the Troc-norfentanyl and Troc-noracetylfentanyl (Fig. 3b) as well as for 2-CEB (Fig. 3c). For the Troc-norfentanyl and Troc-noracetylfentanyl, comparison of their concentration at which they were generated from the 10 ppm spiked sample, relative to their authentic standards at 10 ppm shows that the reaction provides ~60-70% of the material from either opioid (denoted std (Troc-nf) and std (Troc-naf) in Fig. 3b). Several factors can affect the complete efficiency of the reaction such as time, temperature and obviously the matrix and its components which are not present when the reaction is carried out on the pure opioid without any other interferences. In contrast, the reaction was found to provide both Troc-labeled norfentanyls in ~50-60% for the low concentration samples (spiked at 1 ppm for each opioid). Interestingly, the estimated efficiency for the formation of the second by-product 2-CEB is lower (Fig. 3c) based on calculations made using an authentic standard of 2-CEB (denoted std. in Fig. 3c). An important facet of this protocol is that both products can be detected at the lower concentration values for both opioids which makes it an attractive option for the analyses of these substances. Regarding the second set of opioids, thiofentanyl and acetylthiofentanyl, their reaction with Troc-Cl yields Troc-norfentanyl and Troc-noracetylfentanyl (Fig. 3e) and 2(2-chloroethyl)thiophene (2-CET) (Fig. 3f). For the Troc-norfentanyl and Troc-noracetylfentanyl, comparison of their concentration at which they were generated from the 10 ppm spiked sample, relative to their authentic standards at 10 ppm shows that the reaction provides ~55-70% of the material from either opioid (denoted std (Troc-nf) and std (Troc-naf) in Fig. 3e). In contrast, the reaction was found to provide both Troc-labeled norfentanyls in ~45-58% for the low concentration samples (spiked at 1 ppm for each opioid). Interestingly, the estimated efficiency for the formation of the second by-product 2-CET is lower (Fig. 3f) based on calculations made using an authentic standard of 2-CET (denoted std. in Fig. 3f).

Starting with fentanyl and the spiked silt sample at high concentration (i.e., ~10 μg/g), both products, 2-CEB and Troc-norfentanyl, can be detected in the total ion chromatogram (TIC) without the need of using a single ion extraction (SIE) mode (Figure 4a). Thus, the product arising from the side chain of the opioid, 2-CEB, exhibits a retention time of RT = 12.04 min., while the one arising from the piperidine portion of the opioid, Troc-norfentanyl features a retention time of RT = 33.91 min. (Figure 4a). The first product, 2-CEB, remained an easy to detect analyte with a well-defined peak that yielded a clean mass spectrum for comparison and matching to the instrument's internal NIST library (R. Match = 941 (84%)). For the unambiguous identification of the Troc-norfentanyl, this one was compared to the authentic standard synthesized for this purpose. The mass spectrum for 2-CEB is simple and its two main features are the base peak (m/z = 91) arising from the tropylium radical cation and the molecular ion peak (m/z = 140) showing the distinct isotopic pattern for a mono-chlorinated species (Figure 4b). Despite its low intensity (25% of the base peak), the molecular ion peak for 2-CEB can be observed and this is the case in subsequent opioids where this side chain is featured even in the low concentration samples (i.e., ~1 μg/g). On the other hand, the mass spectrum for Troc-norfentanyl is more complex than 2-CEB and there are several mass fragments that can be discussed. Starting with the molecular ion peak (m/z = 406) displaying its unique isotopic pattern originating from the trichloroethoxycarbonyl (Troc) tag that is now part of the norfentanyl molecule (Figure 4c and inset). The molecular ion peak, although observable, constitutes ~8.6% of the intensity of the base peak. The implications of this low intensity become apparent on the low concentration samples as it will be described below where the molecular ion peak can barely be observed (*vide infra*). The base peak for Troc-norfentanyl is m/z = 149, which is attributed to the loss of the substituent at the 4-position to generate a $[C_9H_{11}NO]^{\cdot+}$ fragment. The loss of this fragment is a dominant fragmentation pattern as it is also observed for the Troc-noracetylfentanyl which exhibits a base peak at m/z = 135. Another peak of interest is the one resulting from the loss of the substituent at the 4-position of Troc-norfentanyl to generate a Troc-piperidine containing fragment with a m/z = 259 (~28% of the base peak's intensity) and displaying a similar isotopic pattern to the molecular ion peak (i.e., Troc tag) (Figure 4c). This fragment is important for two reasons, the first one is that it is a unique ion that can be traced directly to the Troc-norfentanyl molecule, and the second one is that it is the most abundant fragment that contains the Troc tag that enabled us to use it for estimating the method's limit of detection (LOD) as well as its lower limit of quantitation (LLOQ) for this unique compound arising from the starting fentanyl. The base peak (m/z = 149) unfortunately could not be used as it is a commonly encountered fragment in mass spectrometric analyses due to its association with ubiquitous phthalate-based impurities.

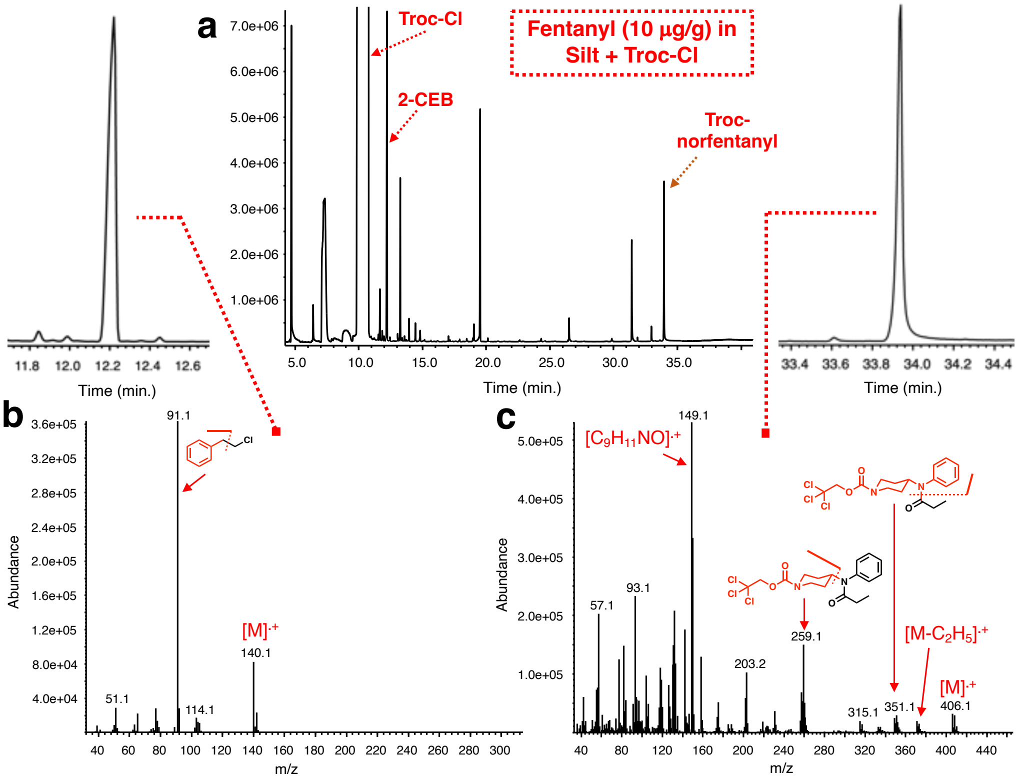

FIGURE 4 Reaction between Troc-Cl and the silt extract (fentanyl spiked ~ 10 µg/g) for additional corroboration for the presence of fentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)benzene (2-CEB) and Troc-norfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CEB (NIST match score = 941) and (c) mass spectrum for Troc-norfentanyl showing the molecular ion peak as well as the classic chlorine isotopic pattern associated with this material (m/z = 406).

The low concentration samples naturally presented some challenges arising from the fact that both analytes arising from the reaction between the fentanyl and Troc-Cl were present at low levels.

Gratifyingly, interferences arising from organic material in the silt and breakdown products from the Troc-Cl reagent did not co-elute thereby masking their signals (Figure 5a). For the low concentration samples, the identification process involved using the single ion mode extraction using the base peak for both products of the reactions, that is m/z = 149 for Troc-norfentanyl and m/z = 91 for 2-CEB (Figure 5b). Although as noted above with the high concentration sample, the SIE process using the m/z = 149 ion can in fact bring up the ubiquitous phthalate impurities that may be present and co-eluting with the Troc-norfentanyl compound, it is very unlikely that this will be the case specially when the SIE process can be complemented using other unique mass fragments for the material. The mass spectrum for 2-CEB at 1 μg/g can be accessed fairly simple using background extraction of the peak and its identity can be quickly determined using the instrument's internal library (R-match = 889) (Figure 5b). In the case of Troc-norfentanyl, the SIE process although providing an isolated peak, its mass spectra is not very clear and plagued with peaks arising from other co-eluting components that do not normally present a problem in the high concentration sample. Even though some key fragments can be identified, their intensity is not enough for a confident confirmation of the material (Figure 5c) in which case the use of a standard, for addition to the sample and/or retention time comparison, is highly recommended. Inspection of the mass spectrum reveals that the molecular ion peak (m/z = 406), along with its isotopic pattern, can be gleaned and delineated to some extent. This same outcome is encountered when other fragments such as m/z = 349 and m/z = 378 representing acyl group and chloride losses respectively are analyzed. Again, the m/z = 259 is a still visible in the mass spectrum, a very diagnostic fragment originating from the loss of the N-acylaniline moiety and could be used in conjunction with the other fragments to initially assess the nature of this product. As stated previously, these fragments along with the side by side retention time analysis with the standard, should be used to fully confirm the Troc-norfentanyl.

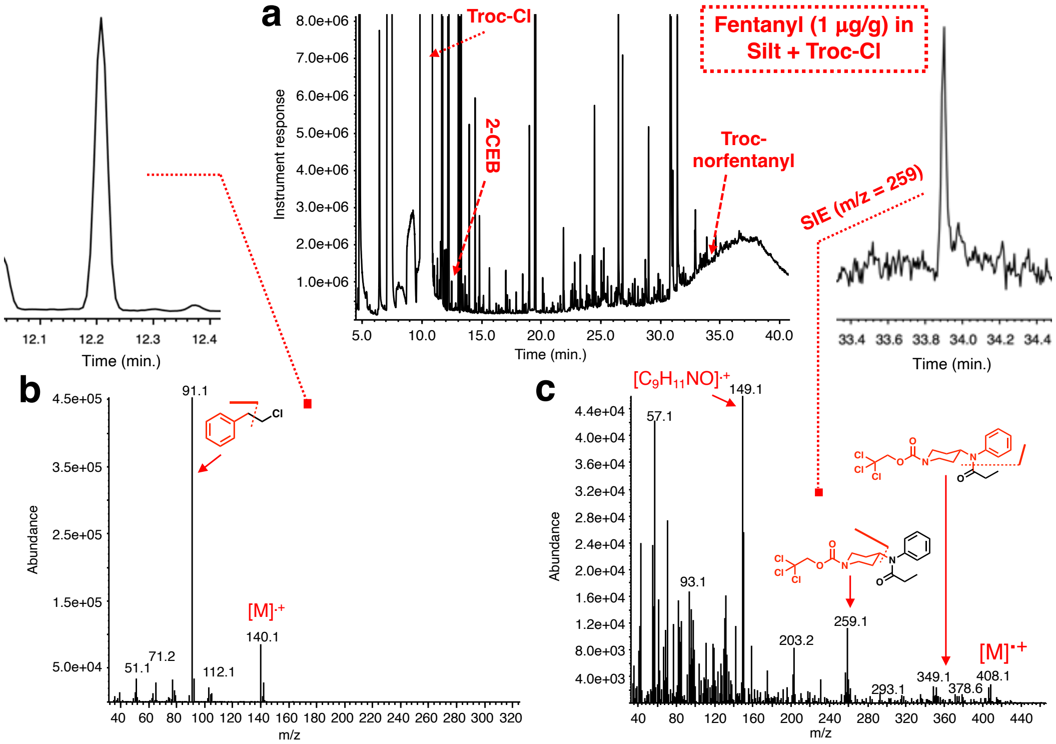

FIGURE 5 Reaction between Troc-Cl and the silt extract (fentanyl spiked ~ 1 µg/g) for additional corroboration for the presence of fentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)benzene (2-CEB) and Troc-norfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CEB (NIST match score = 943) and (c) mass spectrum of Troc-norfentanyl.

When acetylfentanyl is spiked at the high concentration (i.e., ~10 µg/g), both products arising from the reaction, 2-CEB and Troc-noracetylfentanyl, can be seen in the TIC without the need of using the SIE mode (Figure 6a). Therefore, the product arising from the side chain of the opioid, 2-CEB, can be detected (RT = 12.04 min.), as well as the one arising from the piperidine portion of the opioid, Troc-noracetylfentanyl (RT = 33.41 min.) (Figure 6a). The first product, 2-CEB, remained an easy to detect analyte with a well-defined peak that yielded a clean mass spectrum for comparison and matching to the instrument's internal NIST library (R-match = 903 (92%)). With regards to the Troc-norfentanyl, the authentic standard synthesized for this purpose was used to aid in its unambiguous identification. As observed in the fentanyl case, the mass spectrum for 2-CEB is simple and easily identifiable with its peak (m/z = 91) and its molecular ion peak (m/z = 140) (Figure 6b). On the other hand, the mass spectrum for Troc-noracetylfentanyl is more complex and there are several fragments that can be discussed. The first peak to discuss is the molecular ion peak (m/z = 392), that displays the Troc's isotopic pattern. The base peak for Troc-noracetylfentanyl is m/z = 149, attributed to the loss of the substituent at the 4-position to generate a $[C_8H_8NO]^{\cdot+}$ fragment. The loss of this fragment is a dominant fragmentation pattern giving rise to the base peak at m/z = 135. Another peak is the one resulting from the loss of the substituent at the 4-position of Troc-noracetylfentanyl to generate a Troc-containing fragment with a m/z = 259 and displaying a similar isotopic pattern to the molecular ion peak (Figure 6c).

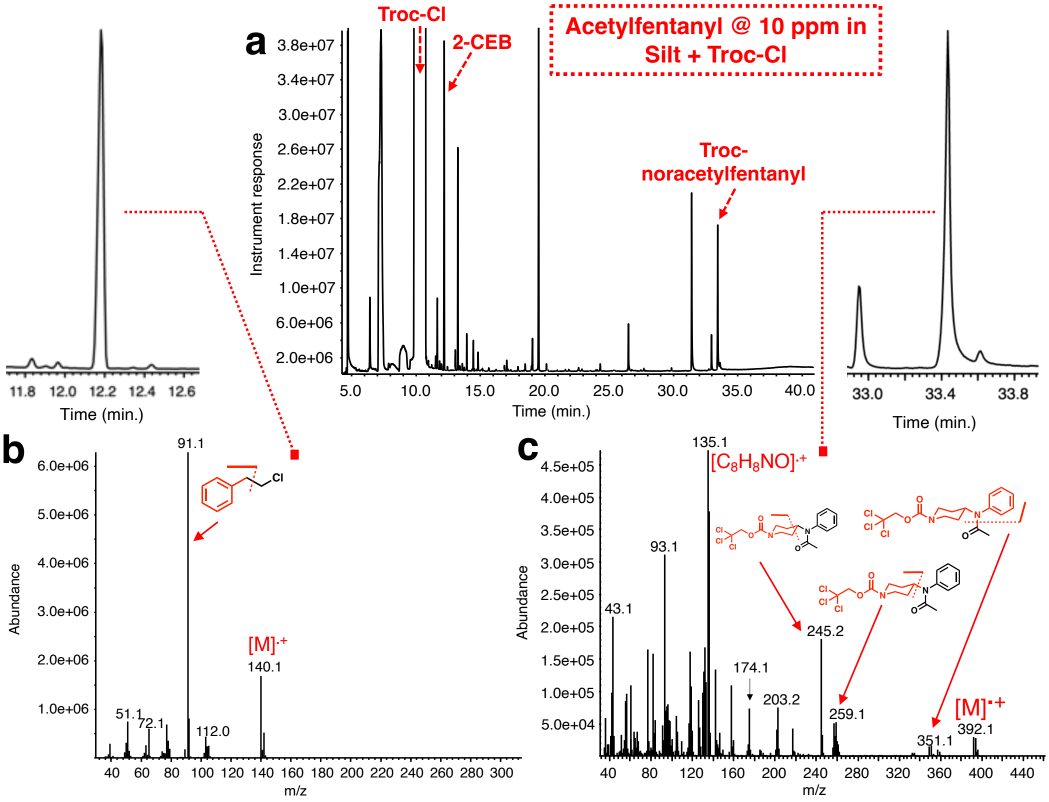

FIGURE 6 Reaction between Troc-Cl and the silt extract (acetylfentanyl spiked ~ 10 µg/g) for additional corroboration for the presence of acetylfentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)benzene (2-CEB) and Troc-noracetylfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CEB (NIST match score = 932) and (c) mass spectrum for Troc-noracetylfentanyl showing the molecular ion peak as well as the classic chlorine isotopic pattern associated with this material (m/z = 392).

Again, and as expected, the low concentration samples provided us with some challenges mainly arising from their presence at such low levels, but luckily for us interferences arising from organics present in the silt and breakdown products from the Troc-Cl, these did not co-elute or masked their signals (Figure 7a). For the low concentration samples, the identification process involved using the SIE mode using the base peak for both products of the reactions, so m/z = 91 for 2-CEB (R-match = 939 (85%)) and m/z = 135 for Troc-noracetylfentanyl (Figures 7b and 7c).

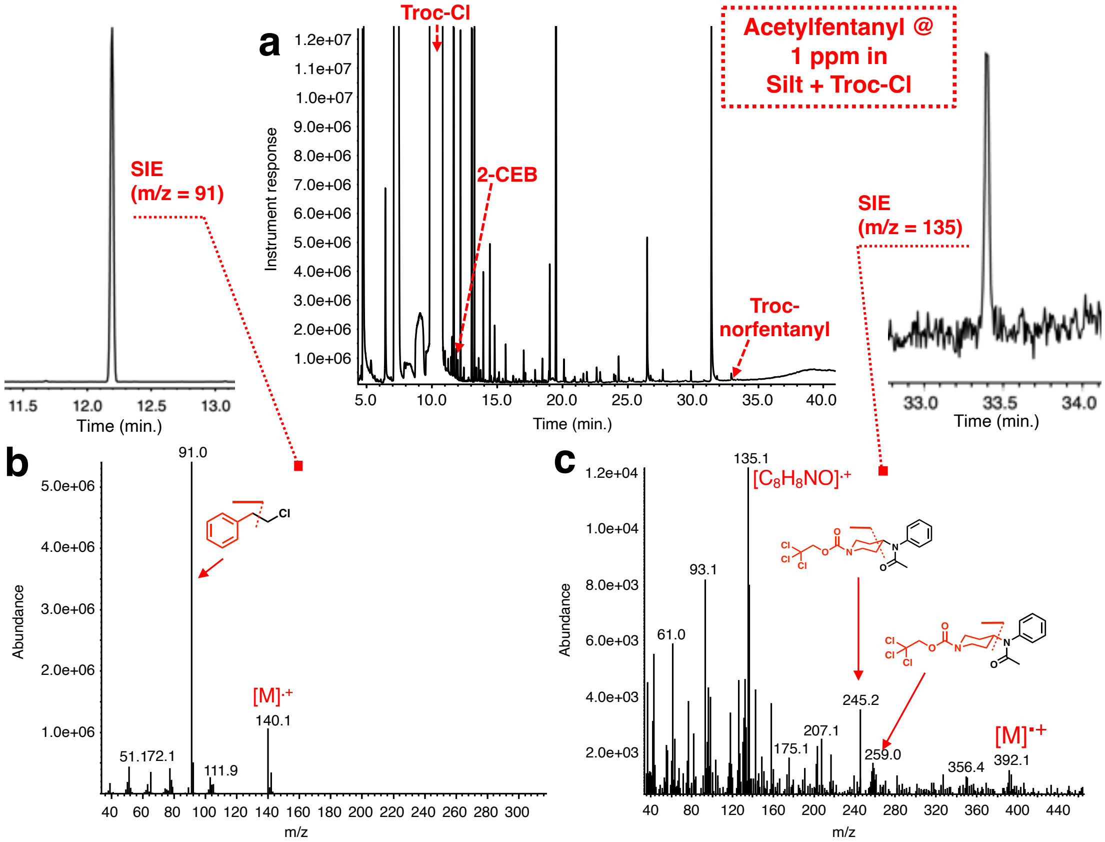

FIGURE 7 Reaction between Troc-Cl and the silt extract (acetylfentanyl spiked ~ 1 µg/g) for additional corroboration for the presence of acetylfentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)benzene (2-CEB) and Troc-noracetylfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CEB (NIST match score = 939) and (c) mass spectrum of Troc-noracetylfentanyl.

Regarding thiofentanyl, when the silt was spiked with this opioid at a high concentration (i.e., ~10 µg/g), one can see that both products, 2-(2-chloroethyl)-thiophene (2-CET) and Troc-norfentanyl, can be detected in the TIC without the need of using the SIE mode (Figure 8a). Thus, the product arising from the side chain of the opioid, 2-CET, can be detected at a retention time of RT = 12.12 min., while Troc-norfentanyl can be observed again at RT = 33.91 min. (Figure 8a). The first product, 2-CET, remained an easy to detect analyte with a well-defined peak that yielded a clean mass spectrum for comparison and matching to the instrument's internal NIST library (R-match = 919 (82%)). The mass spectrum for 2-CET is simple and its two main features are the base peak (m/z = 97) arising from loss of a chloromethyl fragment of the side chain and the molecular ion peak (m/z = 146) showing the distinct isotopic pattern for a mono-chlorinated species (Figure 8b). In a similar manner for 2-CEB, 2-CET's molecular ion is ~23% of the intensity of the base peak and both can be used in the detection of this species as it is observed well in the high and low concentration samples. The mass spectrum for Troc-noracetylfentanyl is more complex than 2-CET and at this high concentration, many fragments can be identified and matched to the authentic standard (Figure 8c).

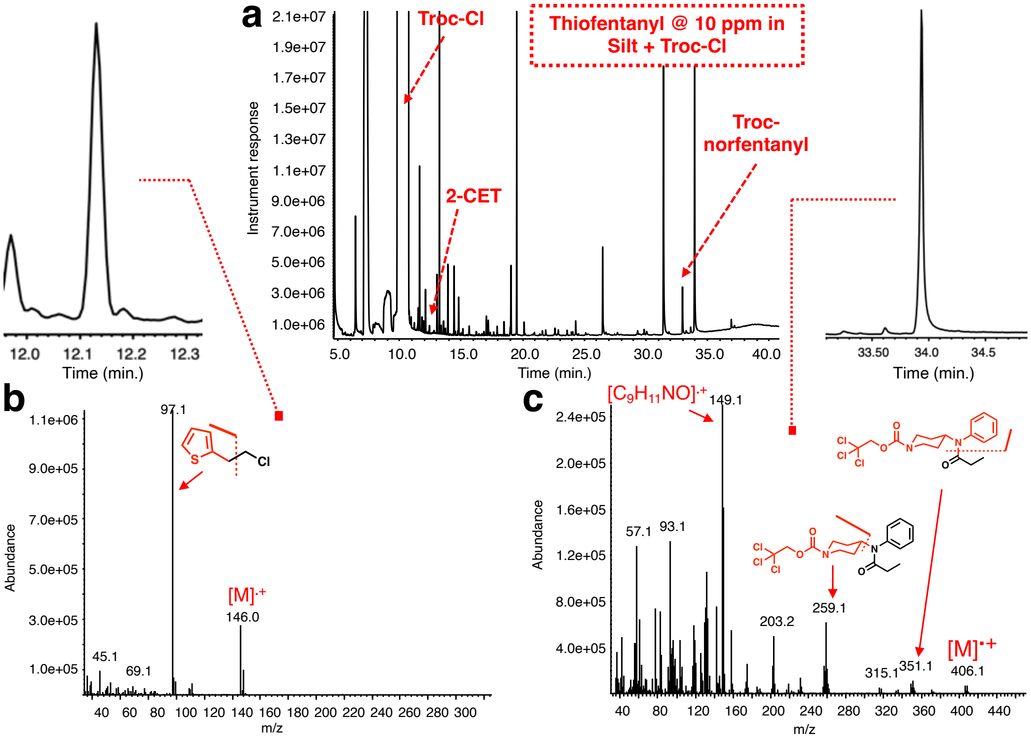

FIGURE 8 Reaction between Troc-Cl and the silt extract (thiofentanyl spiked ~ 10 µg/g) for additional corroboration for the presence of thiofentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)thiophene (2-CET) and Troc-norfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CET (NIST match score = 919) and (c) mass spectrum for Troc-norfentanyl showing the molecular ion peak as well as the classic chlorine isotopic pattern associated with this material (m/z = 406).

The low concentration samples provided us with some challenges mainly arising from the fact that the two analytes were present at such low levels, but fortunately interferences arising from organics present in the silt and breakdown products from the Troc-Cl did not co-elute or masked their signals (Figure 9a). For the low concentration samples, the identification process involved using the SIE mode using the base peak for both products of the reactions, so m/z = 97 for 2-CET (R-match = 922 (88%)) and m/z = 135 for Troc-noracetylfentanyl (Figures 9b and 9c).

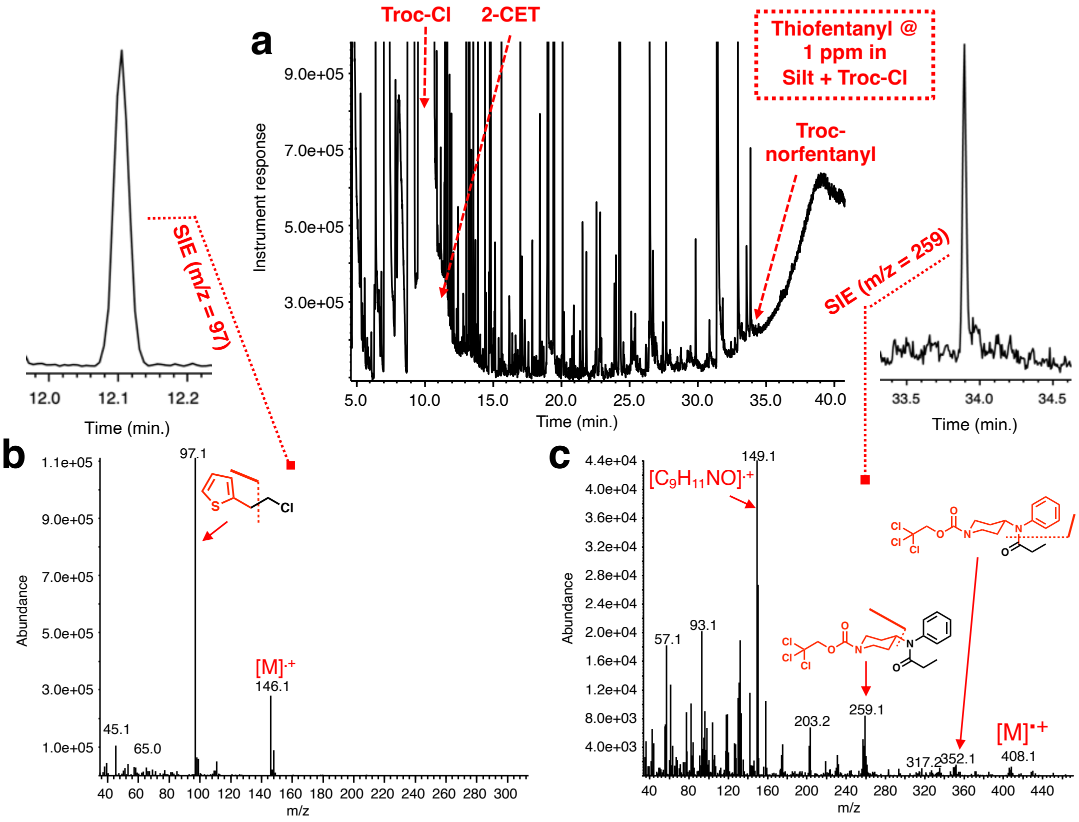

FIGURE 9 Reaction between Troc-Cl and the silt extract (thiofentanyl spiked ~ 1 μg/g) for additional corroboration for the presence of thiofentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)thiophene (2-CET) and Troc-norfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CET (NIST match score = 922) and (c) mass spectrum of Troc-norfentanyl.

Regarding the spiked silt sample at high concentration (i.e., ~10 μg/g) with acetylthiofentanyl, both products can be observed in the TIC (i.e., 2-CET and Troc-norfentanyl) without the need of using the SIE mode (Figure 10a). Thus, the product arising from the side chain of the opioid, 2-CET, can be detected at a retention time of RT = 12.04 min., while the one arising from Troc-noracetylfentanyl can be observed at RT = 33.41 min. (Figure 10a). The first product, 2-CET, remained an easy to detect analyte with a well-defined peak that yielded a clean mass spectrum for comparison and matching to the instrument's internal NIST library (R-match = 970) (Figure 10b). For the unambiguous identification of the Troc-noracetylfentanyl, again this one was compared to the authentic standard synthesized for this purpose (Figure 10c).

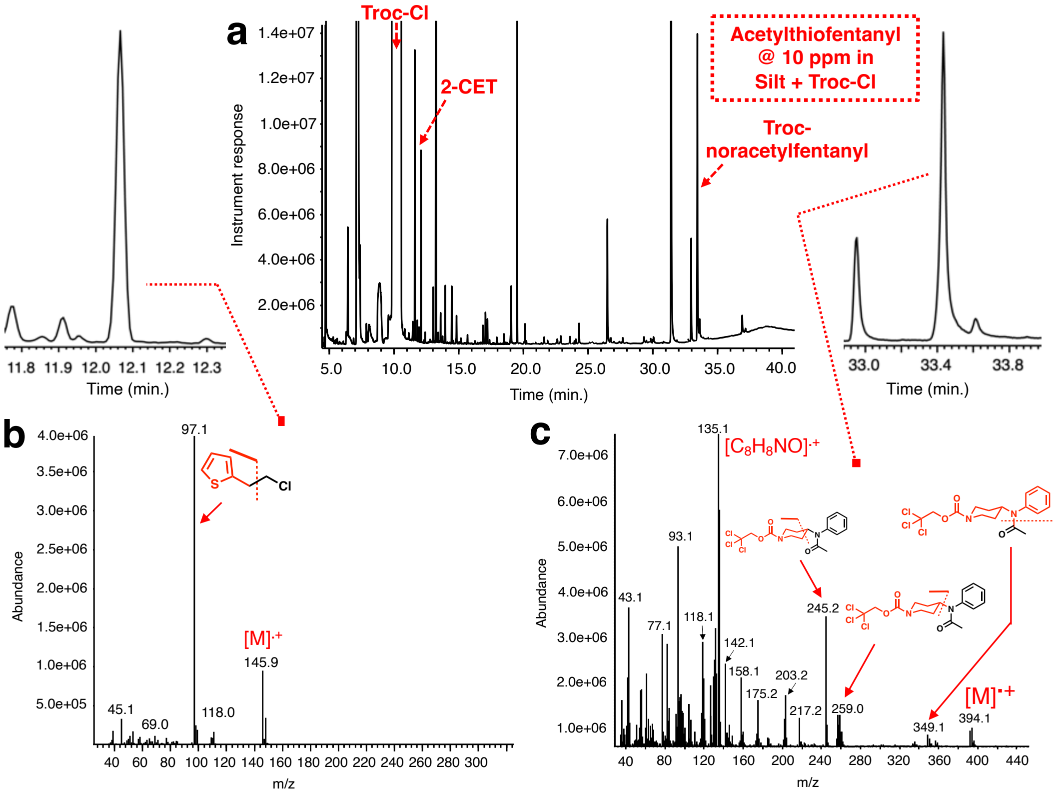

FIGURE 10 Reaction between Troc-Cl and the silt extract (acetylthiofentanyl spiked ~ 10 μg/g) for additional corroboration for the presence of acetylthiofentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)thiophene (2-CET) and Troc-noracetylfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CET (NIST match score = 970) and (c) mass spectrum for Troc-noracetylfentanyl showing the

molecular ion peak as well as the classic chlorine isotopic pattern associated with this material (m/z = 406).

The low concentration samples provided us with some challenges as expected, however, the signals arising from the components of the reaction can still be identified in the TIC (Figure 11a). For the low concentration samples, the identification process involved using the SIE mode using the base peak for both products of the reactions, so m/z = 97 for 2-CET and m/z = 135 for Troc-noracetylfentanyl and (Figures 11b and 11c).

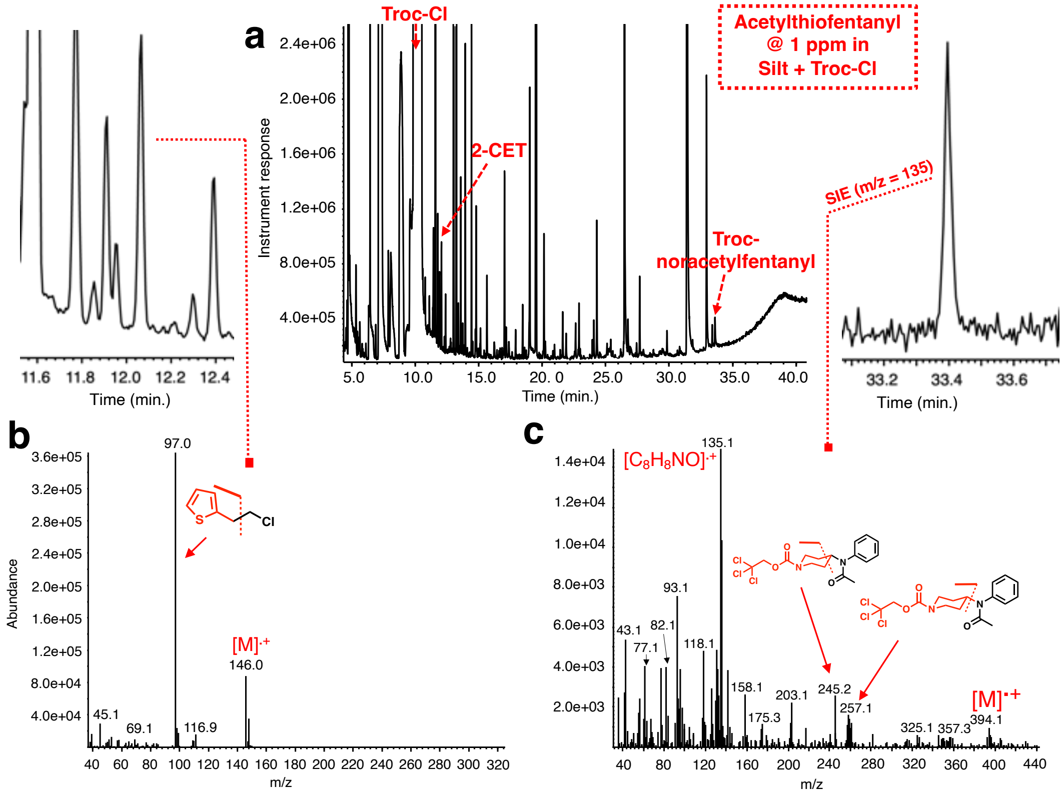

FIGURE 11 Reaction between Troc-Cl and the silt extract (acetylthiofentanyl spiked ~ 1 μg/g) for additional corroboration for the presence of acetylthiofentanyl. (a) full total ion chromatogram (TIC) with the location of the two products from the reaction, (2-chloroethyl)thiophene (2-CET) and Troc-noracetylfentanyl indicated by dashed arrows and expanded on either side of the TIC; (b) mass spectrum for 2-CET (match score = 957) and (c) mass spectrum of Troc-noracetylfentanyl.

The results obtained in these studies highlight the feasibility of using chloroformate chemistry to interrogate a fentanyl sample whether this one is in pure, isolated form or present in a given matrix such as silt. Silt is a difficult soil to carry out direct derivatizations due to its water content that in most cases serves to hydrolyze the derivatization agent before this one can act on the analytes of interest. To this end, the analyst relies on employing excess quantities of the derivatization agent, or as it is described in this work, a prior extraction of the analytes of interest can be performed before derivatization can be done. In the overall plan of analysis of a given sample, the analytical laboratory will have to assess both lines of analyses, direct derivatization and the two-step extraction followed by derivatization keeping in mind that those extractions will also undergo analysis not only by GC-MS but other techniques, more notably LC-MS. Below is Table 2 that summarizes the major fragments for each one of the four products that arise from the reaction between the fentanyls studied herein and Troc-Cl.

Table 2. Notable ions in the mass spectra for all four products arising from the reaction of the opioids with Troc-Cl. Abbreviations: Troc-NF: Troc-norfentanyl, Troc-NAF: Troc-noracetylfentanyl, 2-CEB: 2-chloroethylbenzene, 2-CET: 2-chloroethylthiophene. *Fragments that are part of larger set of peaks due to the isotopic pattern caused by the three chlorine atoms in them, these represent the highest peak of the pattern.

	Troc-NF	Troc-NAF	2-CEB	2-CET
Molecular ion	406.1 $[M]^{+\cdot}$	392.1 $[M]^{+\cdot}$	140.1 $[M]^{+\cdot}$	146.0 $[M]^{+\cdot}$
Fragments	*377.1 $[M-C_2H_5]^+$ *349.0 $[M-C_3H_5O]^+$ *259.0 $[M-C_9H_{10}NO]^+$ 149.0 $[C_9H_{10}NO]^+$	*349.0 $[M-C_3H_5O]^+$ *259.0 $[M-C_8H_8NO]^+$ *245.2 $[M-C_9H_9NO]^+$ 135.0 $[C_8H_8NO]^+$	91.1 $[C_7H_7]^+$	97.7 $[C_5H_5S]^+$

Summary and conclusions

A method involving the extraction of fentanyl, acetylfentanyl, thiofentanyl and acetylthiofentanyl followed by their confirmation using trichloroethoxycarbonylation in silt sediment has been accomplished. Each fentanyl was spiked in the sediment at two concentrations (1 and 10 μg/g) and extracted using ethyl acetate and aqueous NH_4OH (pH ~ 11.2). After the extraction, residues containing each fentanyl were reacted with Troc-Cl at 60 ^{o}C to generate two unique and predictable products from each opioid that can be used to retrospectively confirm their presence and identity using EI-GC-MS. The products were 2-CEB and 2-CET, and the Troc-containing products, Troc-norfentanyl and Troc-noracetylfentanyl. The method's limit of detection (MDL/LOD) for Troc-norfentanyl and Troc-noracetylfentanyl were estimated to be 18.8 ng/mL and 21.1 ng/mL. In addition, the method's limit of quantitation (LOQ) for Troc-norfentanyl and Troc-noracetylfentanyl were determined to be 56.4 ng/mL and 63.4 ng/mL respectively. As detection of these opioids is important in the environment when gathering intelligence information on potential locations for their clandestine, illegal production, the methodology presented herein should significantly aid in the detection and unambiguous confirmation of these lethal substances in the environment.

Auspices statement: This work was performed under the auspices of the U. S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Acknowledgements

The authors would like to thank Dr. Carolyn J. Koester and Mr. Armando Alcaraz for critical reading of the manuscript and insightful discussions about the GC-MS analyses described in this paper.

References

Armenian, P., K. T. Vo, J. Barr-Walker, and K. L. Lynch. 2018. Fentanyl, Fentanyl Analogs and Novel Synthetic Opioids: A Comprehensive Review. *Neuropharmacology* 134:121-132. doi:https://doi.org/10.1016/j.neuropharm.2017.10.016

Chen, A., and M. A. Ashburn. 2015. Cardiac Effects of Opioid Therapy. *Pain Medicine* 16:S27-S31. doi:https://doi.org/10.1111/pme.12915

Danaceau, J. P., M. Wood, M. Ehlers, and T. G. Rosano. 2020. Analysis of 17 fentanyls in plasma and blood by UPLC-MS/MS with interpretation of findings in surgical and postmortem casework. *Clinical Mass Spectrometry* 18:38-47. doi:https://doi.org/10.1016/j.clinms.2020.10.003

Ehleringer, J. R., J. F. Casale, M. J. Lott, and V. L. Ford. 2000. Tracing the geographical origin of cocaine. *Nature* 408:311-312. doi:https://doi.org/10.1038/35042680

Foerster, E. H. and M. F. Mason. 1974. Preliminary Studies on the Use of n-Butyl Chloride as an Extractant in a Drug Screening Procedure. *Journal of Forensic Sciences* 19:155-162.

Fort, C., B. Curtis, C. Nichols, and C. Niblo. 2016. Acetylfentanyl Toxicity: Two Case Reports. *Journal of Analytical Toxicology* 40:754-757. doi:https://doi.org/10.1093/jat/bkw068

France, C. P., Ahern, G., Averick, S., Enright, H. A., Esmaeli-Azad, B., Federico, A., Gerak, L., Husbands, S. M., Kolber, B., Lau, E. Y., Lao, V., Maguire, D. R., Malfatti, M. A., Martinez, G., Mayer, B. P., Pravetoni, M., Sahibzada, N., Skolnick, P., Snyder, E. Y., Tomycz, N., Valdez, C. A., Zapf, J. 2021. Countermeasures for Preventing and Treating Opioid Overdose. *Clinical Pharmacology and Therapy* 109:578-590. doi:https://doi.org/10.1002/cpt.2098

Gladden, R. M., P. Martinez, and P. Seth. 2016. Fentanyl Law Enforcement Submission and Increases in Synthetic Opioid-Involved Overdose Deaths - 27 States, 2013-2014. *MMWR*

Morbidity and Mortality Weekly Report 65:837-843. doi:http://dx.doi.org/10.15585/mmwr.mm6533a2

Gupta, P. K., K. Ganesan, A. Pande, and C. P. Malhotra. 2005. A convenient one pot synthesis of fentanyl. *Journal of Chemical Research* 7:452-453. doi:https://doi.org/10.3184/030823405774309078

Janssen, P. A. J. and N. B. Eddy. 1960. Compounds Related to Pethidine-IV. New General Chemical Methods of Increasing the Analgesic Activity of Pethidine. *Journal of Medicinal Chemistry* 2:31-45. doi:https://doi.org/10.1021/jm50008a003

Janssen, P. A. J. 1962. A Review of the Chemical Features Associated with Strong Morphine-Like Activity. *British Journal of Anaesthesia* 34:260-268. doi:https://www.bjanaesthesia.org/article/S0007-0912(17)40203-0/pdf

Katselou, M., I. Papoutsis, P. Nikolaou, C. Spiliopoulou, and S. Athanaselis. 2016. Old Opioids, New Concerns: The Case of Acetylfentanyl. *Forensic Toxicology* 34:201-212. doi:https://doi.org/10.1007/s11419-016-0310-4

Lee, D., C. W. Chronister, W. A. Broussard, S. R. Utley-Bobak, D. L. Schultz, R. S. Vega, and B. A. Goldberger. 2016. Illicit Fentanyl-Related Fatalities in Florida: Toxicological Findings. *Journal of Analytical Toxicology* 40:588-594. doi:https://doi.org/10.1093/jat/bkw087

Leen, J. L. S., and D. N. Jurrlink. 2019. Carfentanil: a narrative review of its pharmacology and public health concerns. *Canadian Journal of Anesthesia* 66:414-421. doi:https://doi.org/10.1007/s12630-019-01310-1

Lovrecic, B., M. Lovrecic, B. Gabrovec, M. Carli, M. Pacini, A. G. I. Maremmani, and I. Maremmani. 2019. Non-Medical Use of Novel Synthetic Opioids: A New Challenge to Public Health. *International Journal of Environmental Research and Public Health* 16:177. doi:https://doi.org/10.3390/ijerph16020177

Lozier, M. J., M. Boyd, C. Stanley, L. Ogilvie, E. King, C. Martin, and L. Lewis. 2015. Acetyl Fentanyl, a Novel Fentanyl Analog, Causes 14 Overdose Deaths in Rhode Island, March-May 2013. *Journal of Medical Toxicology* 11:208-217. doi:https://doi.org/10.1007/s13181-015-0477-9

Mallette, J. R., J. F. Casale, J. Jordan, D. R. Morello, and P. M. Beyer. 2016. Geographically Sourcing Cocaine's Origin - Delineation of the Nineteen Major Coca Growing Regions in South America. *Scientific Reports* 6:23520. doi:https://doi.org/10.1038/srep23520

Mayer, B. P., A. J. DeHope, D. A. Mew, P. E. Spackman, and A. M. Williams. 2016. Chemical Attribution of Fentanyl Using Multivariate Statistical Analysis of Orthogonal Mass Spectral Data. *Analytical Chemistry* 88:4303-4310. doi:https://doi.org/10.1021/acs.analchem.5b04434

Mayer, B. P., C. A. Valdez, A. J. DeHope, P. E. Spackman, and A. M. Williams. 2018. Statistical Analysis of the Chemical Attribution Signatures of 3-Methylfentanyl and Its Methods of Production. *Talanta* 186:645-654. doi:https://doi.org/10.1016/j.talanta.2018.02.026

Mercadante, S., G. Porzio, P. Ferrera, F. Aielli, C. Adile, C. Ficorella. 2010. Low doses of transdermal fentanyl in opioid-naive patients with cancer pain. Current Medical Research and Opinion 26:2765-2768. doi:https://doi.org/10.1185/03007995.2010.532545

Moody, M. T., S. Diaz, P. Shah, D. Papsun, and B. K. Logan. 2018. Analysis of fentanyl analogs and novel synthetic opioids in blood, serum/plasma, and urine in forensic casework. *Drug Testing and Analysis* 10:1358-1367. doi:https://doi.org/10.1002/dta.2393

Mörén, L., J. Qvarnström, M. Engqvist, R. Afshin-Sander, X. Wu, J. Dahlén, C. Löfberg, A. Larsson, and A. Östin. 2019. Attribution of Fentanyl Analogue Synthesis Route by Multivariable Data Analysis of Orthogonal Mass Spectral Data. *Talanta* 203:122-130. doi:https://doi.org/10.1016/j.talanta.2019.05.025

Ogilvie, L., C. Stanley, L. Lewis, M. Boyd, and M. Lozier. 2013. Notes from the Field: Acetyl Fentanyl Overdose fatalities-Rhode Island, March-May 2013. *MMWR Morbidity and Mortality Weekly Report* 62:703-704.

Ovenden, S. P. B., L. J. McDowall, H. E. McKeown, N. W. McGill, O. A. H. Jones, J. R. Pearson, M. Petricevic, M. L. Rogers, T. J. Rook, J. Williams, R. L. Webster et al. 2021. Investigating the Chemical Impurity Profiles of Fentanyl Preparations and Precursors to Identify Chemical Attribution Signatures for Synthetic Method Attribution. *Forensic Science International* 321:110742. doi:https://doi.org/10.1016/j.forsciint.2021.110742

Pardo, B., J. Taylor, J. Caulkins, P. Reuter, and B. Kilmer. 2021. The Dawn of a New Synthetic Opioid Era: The Need for Innovative Interventions. *Addiction* 116:1304-1312. doi:https://doi.org/10.1111/add.15222

Peterson, A. B., R. M. Gladden, C. Delcher, C., E. Spies, A. Garcia-Williams, A., Y. Wang, J. Halpin, J. Zibbell, C. L. McCarty, J. DeFiore-Hyrmer, M. DiOrio, et al. 2016. Increases in Fentanyl-Related Overdose Deaths - Florida and Ohio, 2013-2015. *MMWR Morbidity and Mortality Weekly Report* 65:844-849.

Rodda, L. N., J. L. Pilgrim, M. Di Rago, K. Crump, D. Gerostamoulos, and O. H. Drummer. 2017. A Cluster of Fentanyl-Laced Heroin Deaths in 2015 in Melbourne, Australia. *Journal of Analytical Toxicology* 41:318-324. doi:https://doi.org/10.1093/jat/bkx013

Siegfried. Synthesis of Fentanyl. 2014. https://erowid.org/archive/rhodium/chemistry/fentanyl.html (accessed July 3, 2023).

Stanley, T. H. 2014. The fentanyl story. *Journal of Pain* 15:1215-1226. doi:https://doi.org/10.1016/j.jpain.2014.08.010

Toske, S. G., J. R. Mitchell, J. M. Myslinski, A. J. Walz, D. B. Guthrie, E. M. Guest, C. A. Corbett, and E. D. Lockhart. 2023. Organic impurity profiling of fentanyl samples associated with recent clandestine laboratory methods. *Journal of Forensic Sciences* 68:1-14. doi:https://doi.org/10.1111/1556-4029.15281

Valdez, C. A., R. N. Leif, and B. P. Mayer. 2014. An Efficient, Optimized Synthesis of Fentanyl and Related Analogs. *PLoS ONE* 9, e108250. doi:https://doi.org/10.1371/journal.pone.0108250

Valdez, C. A., R. N. Leif, and A. Alcaraz. 2016. Effective methylation of phosphonic acids related to chemical warfare agents mediated by trimethyloxonium tetrafluoroborate for their qualitative detection and identification by gas chromatography-mass spectrometry. *Analytica Chimica Acta* 933:134-143. doi:https://doi.org/10.1016/j.aca.2016.05.034

Valdez, C. A., R. N. Leif, S. Hok, and B. R. Hart. 2018a. Analysis of Chemical Warfare Agents by Gas Chromatography-Mass Spectrometry: Methods for their Direct Detection and Derivatization Approaches for the Analysis of their Degradation Products. *Reviews in Analytical Chemistry* 37:1-26. doi:https://doi.org/10.1515/revac-2017-0007

Valdez, C. A., M. K. Marchioretto, R. N. Leif and S. Hok. 2018b. Efficient derivatization of methylphosphonic and aminoethylsulfonic acids related to nerve agents simultaneously via methylation employing trimethyloxonium tetrafluoroborate for their detection and identification in soils by EI-GC-MS and GC-FPD. *Forensic Science International* 288:159-168. doi:https://doi.org/10.1016/j.forsciint.2018.04.041

Valdez, C. A. 2022a. Gas Chromatography-Mass Spectrometry Analysis of Synthetic Opioids Belonging to the Fentanyl Class: A Review. *Critical Reviews in Analytical Chemistry* 52 (8):1938-1968. doi:https://doi.org/10.1080/10408347.2021.1927668.

Valdez, C. A., R. N. Leif, T. H. Corzett and M. L. Dreyer. 2022b. Analysis, identification and confirmation of synthetic opioids using chloroformate chemistry: Retrospective detection of fentanyl and acetylfentanyl in urine and plasma samples by EI-GC-MS and HR-LC-MS. *PLoS ONE* 17 (11):e0275931. doi:https://doi.org/10.1371/journal.pone.0275931.

Valdez, C. A., J. A. Rosales and R. N. Leif. 2023a. Determination of fentanyl and acetylfentanyl in soil in their intact form and orthogonal corroboration of their presence by EI-GC-MS using chloroformate chemistry. *Forensic Chemistry* 34:100504. doi:https://doi.org/10.1016/j.forc.2023.100504

Valdez, C. A., J. A. Rosales, A. K. Vu and R. N. Leif. 2023b. Detection and Confirmation of Fentanyls in High Clay-Content Soil by Electron Ionization Gas Chromatography-Mass Spectrometry. *Journal of Forensic Sciences* 68:2138-2152. doi:https://doi.org/10.1111/1556-4029.15354

Vithlani, R. H., Baranidharan, G., 2010. Transdermal Opioids for Cancer Pain Management. *Reviews in Pain* 4:8-13. doi:https://doi.org/10.1177/204946371000400

Walz, A. J. and F. L. Hsu. 2017. An Operationally Simple Synthesis of Fentanyl Citrate. *Organic Preparations and Procedures International* 49 (5):467-470. doi:https://doi.org/10.1080/00304948.2017.1374129

Wei, Q. and F. H. Su. 2022. Determination of Nine Fentanyl Drugs in Hair Samples by GC-MS/MS and LC-MS/MS. *ACS Omega* 7(23):19176-19182. doi:https://doi.org/10.1021/acsomega.2c00087

Xu, J., L. Wu., A. C. Chang. 2009. Degradation and absorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. *Chemosphere* 77:1299-1305. https://doi.org/10.1016/j.chemosphere.2009.09.063

Xuan, R., A. A. Blassengale & Q. Wang. 2008. Degradation of Estrogenic Hormones in a Silt Loam Soil. *Journal of Agriculture and Food Chemistry* 56:9152-9158. doi:https://doi.org/10.1021/jf8016942

Yeung, D. T., K. J. Bough, J. R. Harper and G. E. Platoff. 2020. National Institutes of Health (NIH) Executive Meeting Summary: Developing Medical Countermeasures to Rescue Opioid-

Induced Respiratory Depression (a Trans-Agency Scientific Meeting)-August 6/7, 2019. Journal of Medical Toxicology 16(1):87-105. doi:https://doi.org/10.1007/s13181-019-00750-x