



Investigation into the Analysis of Fentanyl in Postmortem Blood using Biocompatible Solid-Phase Microextraction

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Abstract

Over recent years, the abuse of fentanyl and other opioids has become a slow motion mass disaster in the United States resulting in an increased number of drug-related deaths. Various postmortem biological samples are collected by forensic pathologists during autopsy and then sent to a toxicology laboratory to be analyzed for the presence of various compounds such as fentanyl. This process can be time consuming and results in a backlog, potentially hindering an investigation. A possible solution is Biocompatible Solid-Phase Microextraction (BioSPME) fibers. These fibers can be directly inserted into a biological matrix and absorb drug compounds without the interference of macromolecules that may be present allowing for a faster analysis time. An initial method has been developed to analyze fentanyl in postmortem blood using BioSPME followed by GC-MS and LC-MS-MS analysis. BioSPME fibers were conditioned, washed, directly inserted into postmortem blood, washed, filtered, desorbed into solution, dried down and reconstituted. The extracted samples were screened by GC-MS and subsequently analyzed by LC-MS-MS. GC-MS was performed using splitless injection on a Rxi-5Sil MS column (30.0 m × 0.25 mm, 0.25 μm) in the SIM mode. Samples were confirmed using an AB SCIEX™ 3200 QTRAP® triple quadrupole mass spectrometer with ElectroSpray Ionization (ESI) source in the positive ion mode. Liquid Chromatography was performed on a Shimadzu® LC system using an Ascentis® Express Biphenyl column (50 mm × 2.1 mm, 2.7 μm) with the weak mobile phase of 0.1%(v/v) formic acid in water and the strong mobile phase of 0.1%(v/v) formic acid in acetonitrile for an analysis time of seven min per sample. This method was developed using bovine blood and then applied to 43 postmortem blood samples provided by the Lehigh County Coroner's Office (Allentown, PA, USA).

Keywords

BioSPME; Biocompatible SPME; Forensic Toxicology; Postmortem; Blood; Fentanyl

Introduction

The opioid epidemic in the United States has become a slow motion mass disaster over recent years. Opioids are in a class of drugs known as narcotic analgesics, which includes drugs such as opium, opium derivatives, and semi-synthetic opioids, which can suppress an

individual's senses and can lead to pain relief. Many opioid drugs are prescribed by physicians because of their chemical nature to relieve or manage moderate-to-severe pain. One such opioid drug includes Duragesic®, a transdermal fentanyl patch, which is a synthetic drug approximately 100 times more potent than morphine [1]. Even when physicians prescribe multiple medications, such as opioids and benzodiazepines, patients may overdose due to the combined effects [2].

While some opioids may be prescribed by physicians, there has been an increase in the amount of counterfeit prescription opioids distributed throughout the U.S. drug markets. Due to opioids' potent and addictive nature, the misuse of prescription or illegal opioids has resulted in an increase in overdoses, opioid-related deaths, and even opioid-dependent individuals [3]. While many overdose related deaths have been reported to occur after the intake of a single opioid, there has been an increase in the amount of deaths that occur due to the combined action resulting from the intake of multiple compounds [4]. It has been observed that fentanyl (*N*-(1-(2-phenethyl)-4-piperidinyl)-*N*-phenyl-propanamide)), a Schedule II controlled substance, has been mixed with other commonly abused drugs, such as heroin and counterfeit prescription oxycodone [1,3].

The U.S. Department of Health and Human Services came out with a National Vital Statistics Report identifying specific drugs involved in drug-related deaths from 2011 through 2016. Within that six-year span, ten drugs within the opioid, benzodiazepines and stimulant drug classes remained consistent, fentanyl being one of them. Fentanyl was mentioned in approximately 1,600 drug-related deaths in 2011 and 2012 but jumped to approximately 18,335 drug-related deaths in 2016 [5]. The Philadelphia Field Division of the Drug Enforcement Administration (DEA) conducted an analysis of the abuse of illicit and pharmaceutical drugs in the state of Pennsylvania (PA). PA coroners and medical examiners reported 4,642 drug-related overdose deaths in 2016 and 5,456 drug-related overdose deaths in 2017 [6,7]. The presence of an opioid, whether illicit or prescription, was identified in 85% of the drug-related deaths for 2016. Of the opioid-related deaths, fentanyl and fentanyl-related substances had a 65% increase in drug-related deaths between 2015 and 2017 with 95% of the counties in PA reported the presence of fentanyl or fentanyl-related substances in decedents' toxicological results [6,7]. Specifically, the number of drug-related deaths that included acetyl fentanyl has increased in multiple southwestern counties of PA. Between January 2015 and February 2016, 41 drug-related deaths that included acetyl fentanyl were reported within Allegheny, Beaver, Butler and Lawrence Counties, which Allegheny County reporting 34 out of the 41 deaths [8]. In Massachusetts (MA), the opioid-related deaths increased 150% from 2012 to 2015. Barnstable, Bristol, and Plymouth were three counties in MA that were further investigated by the Massachusetts Department of Public Health and the Office of the Chief Medical Examiner because of their high opioid-related deaths, roughly 29.8-34.5 per 100,000 MA individuals in 2015. Approximately two thirds of the opioid-related deaths had fentanyl reported in the toxicological results [9]. The Miami-Dade County Medical Examiner Department (MDME) qualitatively identified illicit fentanyl and/or one or more fentanyl analogs in 375 out of 500 postmortem cases in 2015 to the end of 2016. Around 60% of those cases were also qualitatively positive for morphine/codeine/6-monoacetylmorphine (heroin) or

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Received: July 09, 2019 Accepted: July 24, 2019 Published: August 05, 2019

cocaine while 20% of those cases were qualitatively positive for both heroin and cocaine [10]. MDME was also able to develop and validate an analytical method using solid-phase extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS-MS) to quantify fentanyl, β -hydroxythiofentanyl, acetyl fentanyl, furanyl fentanyl, carfentanyl, butyryl fentanyl and para-fluoroisobutryl fentanyl in postmortem biological fluid [11].

Since being introduced in the early 1990's, solid-phase microextraction (SPME) has been widely used due to its simple and fast methodology for sample preparation and extraction. This method of extraction eliminates the need for excess extraction solvents [12]. SPME was developed to be either used in the laboratory setting or onsite in the field. An advantage of SPME is that the fiber is compatible with a separation/detection instrument, such as the gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS-MS). The general process of a SPME fiber includes the sample first coming into contact with the sorbent phase of the fiber. This incorporates sampling and preparation/extraction in just one step. The type of sorbent fiber material as well as the matrix can affect the extraction efficiency. The final step is to place the SPME fiber into the injection port of the instrument to allow for the sample introduction via desorption from the sorbent phase [13].

Extended toxicological analysis time is caused by backlog within the laboratory and lengthy procedures used to detect synthetic opioids and other emerging compounds. There is a need for new, easy and fast analysis methods in the field of forensic toxicology that will help in monitoring and identifying drugs and other compounds [14], which could be solved with the use of a SPME fiber. A variety of SPME methods have been utilized to analyze drugs, such as methamphetamine [15], venlafaxine [16], and tranexamic acid [17], in different biological matrices. These methods include the use of various SPME fiber coatings, headspace-SPME (HS-SPME), direct immersion-SPME (DI-SPME), in-tube SPME, and *in vivo* SPME [18].

Within recent years, the application of *in vivo* SPME has become an area of interest because of the ability to directly sample human and animal biological matrices with a SPME fiber without the removal of any of the biological matrix from the human or animal [18]. An issue that arises when conducting *in vivo* SPME analysis is macromolecules and other interferents adhering to the SPME fiber. Biocompatible SPME (BioSPME) fibers have been created to overcome these issues [19]. BioSPME has been designed to eliminate components of a biological sample and absorb the analytes of interest, minimize sample preparation and reduce the amount of sample and solvents needed. BioSPME is meant to be easy to use and a sensitive and effective technique [20]. Figure 1 shows a BioSPME fiber tippet (Supelco, Sigma-Aldrich). The BioSPME fiber contains a small metal core that is secured by a pipette tip. The metal core tip contains a sorbent of a particular chemistry, either C18 or mixed-mode (strong cation exchange-C18), which is covered by a protective biocompatible polymer. The BioSPME fiber can be inserted directly into a biological matrix of interest and will result in a clean extraction [21].

Forensic pathologists are responsible for collecting samples from deceased individuals for toxicological testing. These samples may include bile, vitreous humor, urine, central blood, peripheral blood, liver, gastric contents, brain, and kidney. After collection, these samples are then sent to a toxicology laboratory where the samples will be analyzed for prescription drugs, illicit drugs or poisons. A uniform toxicological test is typically performed on biological



Figure 1: Biocompatible Solid-Phase Microextraction (BioSPME) fiber tippet.

samples to analyze for the most common drugs that may be present as well as their concentration [22]. Toxicological analysis is important due to being able to document the role and prevalence of a drug in death investigation, as well as human performance and intoxication cases [23].

Current analysis of illicit substances for criminal investigation is time consuming, due to a backlog of cases, a lengthy analysis process or both. With the use of BioSPME fibers that can be directly inserted into any biological sample, the amount of time needed to analyze illicit substances should decrease. The objective of this study was to create and optimize a faster and simpler method than current analysis methods that can screen and identify fentanyl (Figure 2), which may be found in postmortem blood samples provided by the Lehigh County Coroner's Office (Allentown, PA, USA). With further refinement, the method has the potential to allow for the analysis of fentanyl to be faster and easier than current analytical methods used by forensic toxicologists by simply directly inserting the BioSPME fiber into the biological matrix.

Materials and Methods

Case samples

The Cedar Crest College Institutional Review Board (Allentown, PA, USA) approved the collection and analysis of postmortem blood samples for this study (IRB#2016-16).

A total of 43 casework samples were provided by the Lehigh County Coroner's Office (Allentown, PA, USA) between June 2016 and May 2017 for those cases where the next-of-kin of the decedent gave written and/or verbal consent to participate in this research. Blood samples were collected in a red top Vacutainer[®] blood tube, which contain no anticoagulants.

BioSPME fibers

BioSPME fibers were obtained from Supelco (Sigma-Aldrich). Both C18 and mixed-mode (strong cation exchange-C18) chemistry BioSPME fibers were analyzed. C18 BioSPME fibers were used

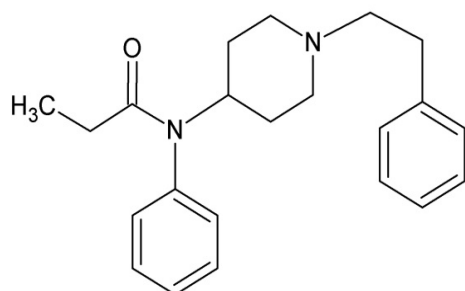


Figure 2: The chemical structure of fentanyl.

from lot#56592 while mixed-mode BioSPME fibers were used from lot#1964-91. Only data from the C18 BioSPME fibers are reported.

Chemicals and reagents

Performance Solution-12 (lot#FE042309-01), fentanyl (F-013; lot#FE04231502), and fentanyl-D5 (F-001; lot#FE05011502) analytical reference standards were purchased from Cerilliant (Round Rock, TX, USA). Sodium phosphate dibasic heptahydrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (28%) was purchased from Spectrum Chemical Manufacturing Corporation (Gardena, CA, USA). HPLC grade methanol, HPLC grade water, and LC-MS grade acetonitrile were purchased from EMD Millipore Corporation (Darmstadt, Germany). LC-MS grade formic acid was purchased from Thermo Scientific (Rockford, IL, USA). Bovine blood (lot#16G20126) was purchased from Lampire Biological Laboratories and (lot#B-B7133) from Quad Five. Eight other narcotic analgesics were analyzed during this study but only data for fentanyl are reported.

Extraction

When optimizing the extraction procedure, a 100 $\mu\text{g/mL}$ solution and 500 ng/mL solution was created by quantitatively pipetting from the original fentanyl drug standard solution and diluting with bovine blood to analyze using GC-MS and LC-MS-MS, respectively. A 100 ng/mL solution of fentanyl-D5 internal standard was used for LC-MS-MS analysis.

The case samples went through part of the extraction process at the Lehigh County Coroner's Office. The case samples were extracted as the experimental extraction method was being developed. The current extraction method consisted of conditioning the fibers for 20 min in a 50:50 HPLC grade methanol/HPLC grade water solution while agitating at 700 rpm on a ThermoFisher IKA MS 3 digital shaker. The fibers were washed in HPLC grade water for 10 seconds and then placed into 500 μL of a postmortem blood sample brought to a pH of 9.0 by using NH_4OH (28%), while agitating at 700 rpm. The fibers were removed from the blood sample, washed in 10 mM of sodium phosphate buffer (dibasic) for 10 s and then washed in water for 10 s at 700 rpm.

The fibers were then stored in glass test tubes and transported back to Cedar Crest College to continue the extraction process. Once back at Cedar Crest College, the fibers were placed into wells that contain 100 μL of HPLC grade methanol with 100 ng/mL of fentanyl-D5 internal standard to desorb any possible drug off of the fiber while agitating at 700 rpm. The samples were then filtered using a 1-mL disposable syringe and a 17 mm PVDF 0.2 μm syringe filter into 700

μL glass vials. The glass vials were dried at 40°C for 30 min under a gentle stream of nitrogen gas using a Zymark Turbovap. Samples that were analyzed using GC-MS were reconstituted with 40 μL of HPLC grade methanol and the samples that were analyzed using LC-MS-MS were reconstituted with 40 μL of 0.1%(v/v) formic acid in HPLC grade water.

Instrumentation

Gas chromatography was performed on an Agilent 7890A gas chromatograph equipped with an HP 7683B autosampler injector and a 30.0 m \times 0.25 mm, 0.25 μm Restek 13623 Rxi-5sil column. The gas chromatograph was then coupled to an Agilent 5975C mass spectrometer (Santa Clara, CA, USA). Gas chromatographic separation was performed after a 1 μL splitless injection with initial column flow of 1.0 mL/min and linear Helium gas velocity of 37 cm/s. The front inlet temperature was 275°C and the total flow was 54.0 mL/min. Temperature programming consisted of 50°C (2 min hold), 40°C (40°C/min) to 320°C (5.00 min hold) for a total run time of 13.75 min. Optimized parameters for GC-MS analysis are listed in Table 1. Results generated by the GC-MS were analyzed using SIM mode and examined using MSD ChemStation software. Liquid chromatography was performed on a Shimadzu LC-20 Prominence system equipped with two Shimadzu LC-20 AD Prominence liquid chromatography binary pumps, a Shimadzu DGO-20A3 Prominence degasser, and a Shimadzu SIL-20AC Prominence auto-sampler. A 50 mm \times 2.1 mm, 2.7 μm Ascentis® Express Biphenyl column (Supelco, Sigma-Aldrich) along with an Ascentis® Express Biphenyl guard column (Supelco, Sigma-Aldrich) was used for analysis of standards and postmortem blood samples. A binary mobile phase was used: the weak mobile phase (A) was 0.1%(v/v) formic acid in HPLC-grade water and the strong mobile phase (B) was 0.1%(v/v) formic acid in acetonitrile.

The flow rate was 0.3 mL/min. Pumps A and B were also purged before any experimental run to eliminate any cross contamination. The LC oven temperature was held constant at 30°C. The autosampler injection volume was set constant at 1 μL for each sample. To obtain optimal separation the following gradient was used: start with an autosampler delay of 1 min; 10% B to 20% B from 1.00 to 1.25 min; from 1.25 to 3.75 min linearly increase the concentration of B to 95%; hold the concentration of B at 95% to 5.00 min. The concentration of B decreased to 10% after completion of the data acquisition. The column then went through a washing step of 50:50 methanol: water solution and reequilibration for a total time of 10.5 min.

Mass spectrometric analysis of all samples was performed on an AB Sciex 3200 QTRAP triple-quadrupole mass spectrometer equipped with an electrospray ionization in positive-ion mode. Q1 and Q3 were both operated with unit resolution. The source temperature was

Table 1: Agilent 7890A GC and Agilent 5975C MS Conditions.

Column Type	Restek 13623 Rxi-5sil MS (30.0 m x 0.25 mm, 0.25 μm)
Initial Column Flow	1.0 mL/min
Linear Gas Velocity	37 cm/sec
Front Inlet Temperature	275°C
Front Inlet Mode	Splitless
Front Inlet Total Flow	54.0 mL/min
Carrier Gas Type	Helium
Temperature Programing	40°C (40°C/min) to 320°C (5.00 min hold) Total Run Time: 13.75 min
Autosampler	HP 7683B Injector
Injection Volume	1.0 μL

500°C and the ionization voltage was 5000 V. Fentanyl was quantified in Multiple Reaction Monitoring (MRM) mode with a dwell time of 50 ms. Optimized parameters for MS-MS analysis are listed in Table 2. The enhanced product ion data for fentanyl and fentanyl-D5 analyte are listed in Table 3. Results generated by the LC-MS-MS were analyzed using Analyst version 1.4.2 software.

Results and Discussion

BioSPME extraction method

The method consisted of conditioning the BioSPME fibers, directly injecting the BioSPME fibers into a blood sample, washing the fibers in two wash solutions, desorbing the fibers into solution, filtering the solution, drying down with Nitrogen gas, and reconstituting the sample. The extraction method developed during this study was applied to spiked bovine blood in an attempt to optimize the method as well as postmortem blood samples.

A blood matrix was chosen for this study due to laboratories typically conducting toxicology tests on postmortem blood samples as well as to test the C18 BioSPME fiber against a more complex biological matrix. Overall, this extraction method would take about 2 h from conditioning the BioSPME fiber to reconstituting the sample prior to analysis by GC-MS, which proves to be faster than solid-phase extraction (SPE) or other current analytical methods used.

It was necessary to add the wash steps after extraction, especially the 10 mM Na₂HPO₄ (dibasic) wash. Without this wash step, the blood matrix would adhere to the BioSPME fiber and hinder the ability of the fentanyl to desorb into the methanolic solution. The water wash step was able to remove any residual 10 mM Na₂HPO₄ that may have been on the fiber. This step may not be necessary but was used just as a precaution. Since both wash steps were for 10 s each, there was not a concern of fentanyl coming off the BioSPME fiber.

The desorbed solution was then filtered through a 17 mm 0.2 μm PVDF syringe filter. While filtering the sample was required to not damage the chromatographic columns, it did appear that there was a loss of solution when filtering. This loss could have affected the recovery of fentanyl and the amount of fentanyl being detected by the GC-MS and LC-MS-MS.

Casework samples

Casework samples were provided by the Lehigh County Coroner's Office (Allentown, PA, USA) as the extraction method was being developed. This allowed for more casework samples to be analyzed during the time of this study. In attempt to eliminate bias from the study, samples were obtained from any case where next-of-kin gave consent, written and/or verbal, to participate in the study. Postmortem blood samples had to be extracted at the Lehigh County Coroner's Office, which meant that materials from Cedar Crest College had to be transported to the coroner's office then back to Cedar Crest College. The two locations are within 4 miles of each other.

The extraction procedure was split into two procedures. Since the samples were not permitted to leave the Lehigh County Coroner's Office, conditioning, washing with water, adjusting the pH of the postmortem blood, directly injecting the BioSPME fibers into the sample, washing with 10 mM Na₂HPO₄, and washing with water was

done at the coroner's office. The BioSPME fibers were then transported back to Cedar Crest College where desorption, filtering, drying down, and reconstituting occurred. This process added time to the extraction procedure. Since the BioSPME fibers had to be transported back to Cedar Crest College, the tips would end up drying. The effect of transporting the BioSPME fibers on fentanyl recovery is unknown.

Data from a total of 43 Lehigh County Coroner's Office cases were analyzed by GC-MS and LC-MS-MS. Figure 3 is an example of the results from the GC-MS using the optimized SIM method using the C18 BioSPME fiber. This sample was reported by the Health Network Laboratories to contain 55 ng/mL of fentanyl. Casework samples were then analyzed using the optimized LC-MS-MS method. The chromatogram from corresponding casework sample example using this method is shown in Figure 4. Table 4 shows a comparison of the qualitative results from the BioSPME method to the qualitative and quantitative results obtained from Health Network Laboratories, which is the toxicology laboratory used by the Lehigh County Coroner's Office, for the cases that were positive for fentanyl. Out of the 43 cases, the Lehigh County Coroner's Office reported 14 of the cases to contain fentanyl based on results produced by Health Network Laboratories. Using the developed BioSPME method, 13 of the 14 cases containing fentanyl produced consistent results with Health Network Laboratories. For case TS0021, the fentanyl detected by Health Network Laboratories was reported to be below the LOD for the developed method explaining why fentanyl was not detected. In a total of 4 out of the 43 cases analyzed, the developed method detected fentanyl where the Health Network Laboratories did not. Health Network Laboratories reported that case TS0030 did detect a low concentration of fentanyl and case TS0040 did not detect fentanyl but both cases did have 4-ANPP and furanyl fentanyl present. 4-ANPP and furanyl fentanyl both possess the same precursor and product ions as fentanyl does, which can explain why the developed method detected fentanyl for both cases [24].

Table 2: Optimized MS-MS parameters for the determination of fentanyl.

Source Temperature (°C)	500
Ionization Voltage (V)	5000
Ion Source (GS1)	30
Ion Source (GS2)	45
Curtain Gas	40
CAD Gas	4
Decustering Potential	See Table 3
Entrance Potential (V)	10
Collision Energy	See Table 3
Collision Cell Exit Potential (V)	2.3

Table 3: Enhanced Product Ion information for fentanyl and fentanyl-D5 drug standards.

Drug	Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Decustering Potential (V)
Fentanyl	337	188, 105	31.5, 54	48
Fentanyl-D ₅	342	188, 105	32, 56	52

Table 4: BioSPME extraction method results versus results obtained by Health Network Laboratories.

Case Number	Age/Sex	Brief History	BioSPME Method		Health Network Laboratories Method	
			Fentanyl	Additional Compounds	Fentanyl	Additional Compounds
TS0002	40/Male	History of heroin, alcohol abuse and anxiety, found slumped in	Fentanyl	Morphine	Fentanyl 5.6 ng/mL	Ethanol 0.25% Morphine (total) 87 ng/mL, 6-monoacetylmorphine (total) 5 ng/mL
TS0003	51/Male	Found unresponsive in residence	Fentanyl	Codeine, Morphine, 6-monoacetylmorphine	Fentanyl 19.8 ng/mL	Alprazolam 23 ng/mL, Delta-9-THC 3.6 ng/mL, 11-Hydroxy-Delta-9-THC 1.2 ng/mL, Carboxy-Delta-9-THC 8.8 ng/mL, Codeine (total) 12 ng/mL, Morphine (total) 389 ng/mL, Doxepin 118 ng/mL, Desmethyldoxepin 68 ng/mL, Naloxone Metoprolol Diltiazem
TS0008	23/Male	Found slumped in chair	Fentanyl	No other compounds detected	Fentanyl 55.0 ng/mL	Norfentanyl 20.3 ng/mL Naloxone
TS0017	46/Male	Found unresponsive in residence	Fentanyl	No other compounds detected	Fentanyl 31.8 ng/mL	Morphine (total) 57 ng/mL Norfentanyl 7.5 ng/mL
TS0021	26/Female	Found unresponsive in residence	Not detected	No other compounds detected	Fentanyl 2.1 ng/mL	Codeine (total) 25 ng/mL Morphine (total) 681 ng/mL Gabapentin 0.5 µg/mL Citalopram/Escitalopram 490 ng/mL Naloxone
TS0022	57/Male	Possible substance toxicity	Fentanyl	No other compounds detected	Not detected	Acetaminophen 20 mcg/mL, Citalopram/Escitalopram 498 ng/mL, Mirtazepine 120 ng/mL
TS0023	22/Male	Suspected substance related death. History of IV drug abuse	Fentanyl	No other compounds detected	Not detected	Alprazolam 63 ng/mL, 7-aminoclonazepam 10 ng/mL, Morphine (total) 17 ng/mL, Gabapentin 21.4 µg/mL, Naloxone
TS0025	24/Male	Suspected substance abuse. Illicit paraphernalia at scene	Fentanyl	Morphine, 6-monoacetylmorphine	Fentanyl 4.7 ng/mL	Ethanol 0.03% Delta-9-THC 2.0 ng/mL, Carboxy-Delta-9-THC 20.2 ng/mL, Morphine (total) 67 ng/mL, Norfentanyl 0.8 ng/mL
TS0026	43/Male	Found unresponsive in residence. Pronounced in hospital	Fentanyl	No other compounds detected	Fentanyl 10.2 ng/mL	Ethanol 0.18% Diphenhydramine 201 ng/mL, Hydroxyzine Loperamide
TS0030	30/Male	Found unresponsive in residence	Fentanyl	No other compounds detected	Fentanyl 1.2 ng/mL	Amphetamine 103 ng/mL, Methamphetamine 662 ng/mL, Benzoylcegonine 277 ng/mL, Morphine (total) 47 ng/mL, Furanyl Fentanyl 1.6 ng/mL, 4-ANPP 3.6 ng/mL
TS0031	25/Female	Found unresponsive. History of heroin abuse	Fentanyl	Morphine, Oxycodone	Fentanyl 21.7 ng/mL	Morphine (total) 264 ng/mL, Hydromorphone (total) 5 ng/mL, Oxycodone (total) 50 ng/mL, Oxymorphone (total) 12 ng/mL, Gabapentin 61.0 µg/mL, Norfentanyl 0.6 ng/mL, Acetyl Fentanyl 3.7 ng/mL, Fluoxetine 71 ng/mL, Norfluoxetine 72 ng/mL, Dextromethorphan
TS0033	26/Male	Suspected substance related death	Fentanyl	No other compounds detected	Fentanyl 14.4 ng/mL	Pseudoephedrine 72 ng/mL Morphine (total) 93 ng/mL, Lamotrigine 0.9 mcg/mL, Gabapentin 4.1 µg/mL, Norfentanyl 4.1 ng/mL, Bupropion Hydroxybupropion
TS0034	54/Female	Found unresponsive in residence	Fentanyl	No other compounds detected	Fentanyl 4.0 ng/mL	Morphine (total) 108 ng/mL, Norfentanyl 2.6 ng/mL, Fluoxetine 1270 ng/mL, Norfluoxetine 875 ng/mL,
TS0036	27/Male	Possible substance abuse	Fentanyl	No other compounds detected	Fentanyl 11.3 ng/mL	Codeine (total) 5 ng/mL, Morphine (total) 155 ng/mL, 6-monoacetylmorphine (total) 3.7 ng/mL, Norfentanyl 1.3 ng/mL, Acetyl Fentanyl 0.5 ng/mL, Naloxone

TS0038	26/Male	Found unresponsive on couch by friends. Many drugs recovered from house, including heroin, ecstasy, methamphetamine, cocaine and marijuana	Fentanyl	Oxycodone	Not detected	Alprazolam 42 ng/mL, Phencyclidine 1.4 ng/mL, Cocaine 200 ng/mL, Benzoylcegonine 3056 ng/mL, Delta-9-THC 10.1 ng/mL, 11-Hydroxy-Delta-9-THC 2.4 ng/mL, Carboxy-Delta-9-THC 34.1 ng/mL, Oxycodone (total) 25 ng/mL, Naloxone Levamisole
TS0039	28/Male	Found unresponsive in bathroom. History of heroin use; syringe at scene	Fentanyl	No other compounds detected	Fentanyl 7.5 ng/mL	Morphine (total) 62 ng/mL, Lamotrigine 1.1 mcg/mL, Norfentanyl 0.5 ng/mL, Amitriptyline 62 ng/mL, Nortriptyline 77 ng/mL, Naloxone
TS0040	29/Male	Found unresponsive in residence	Fentanyl	Morphine, Oxycodone	Not detected	Diphenhydramine 214 ng/mL, Delta-9-THC 4.1 ng/mL, 11-Hydroxy-Delta-9-THC 2.2 ng/mL, Carboxy-Delta-9-THC 16.1 ng/mL, Oxycodone (total) 54 ng/mL, Oxymorphone (total) 16.0 ng/mL, Furanyl Fentanyl 5.9 ng/mL, 4-ANPP 13.7 ng/mL
TS0042	41/Male	Sudden unexpected death	Fentanyl	No other compounds detected	Not detected	Ethanol 0.06%
TS0043	27/Male	Suspected substance related death	Fentanyl	No other compounds detected	Fentanyl 12.9 ng/mL	Delta-9-THC 1.6 ng/mL, Norfentanyl 2.8 ng/mL

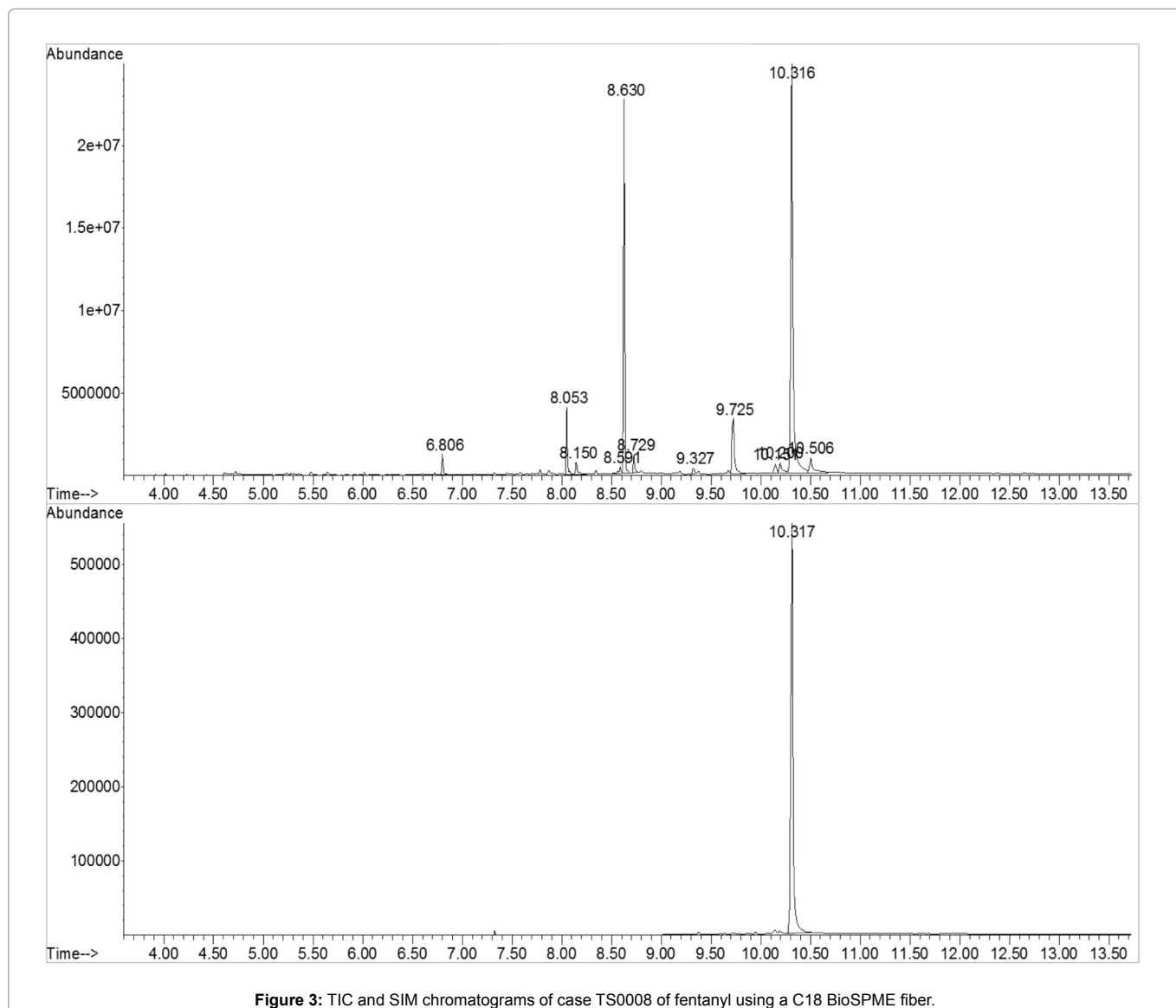


Figure 3: TIC and SIM chromatograms of case TS0008 of fentanyl using a C18 BioSPME fiber.

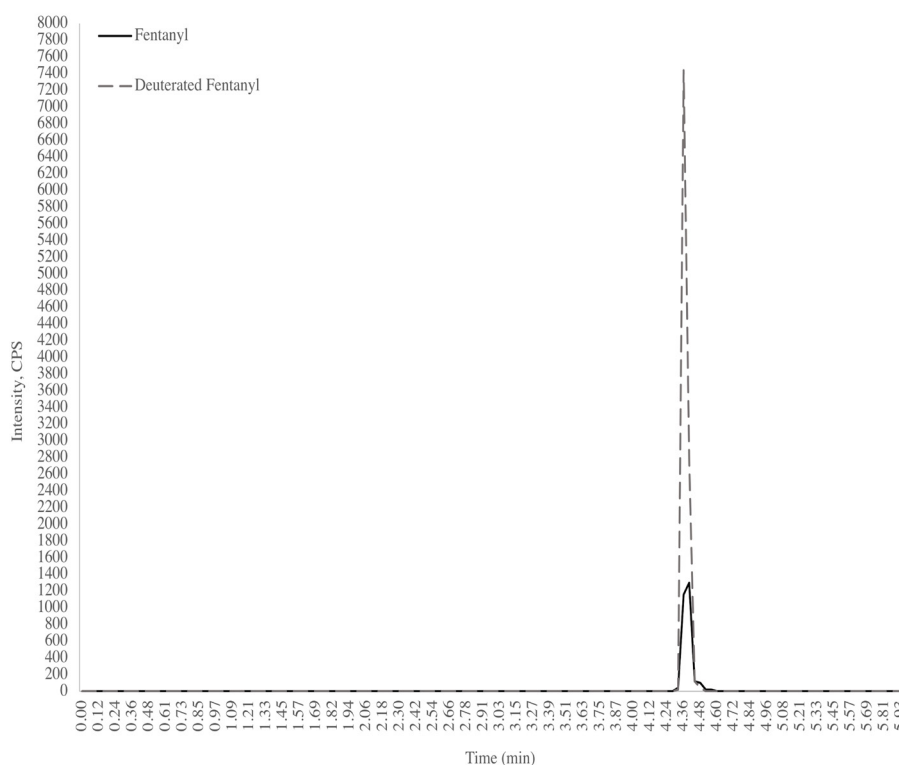


Figure 4: LC separation of fentanyl and fentanyl-D5 from case TS0008 using a C18 BioSPME fiber.

Conclusion

BioSPME fibers are simple to use in comparison to the traditional SPME fiber. The simple fiber and sample preparation allow for a faster extraction method of fentanyl from postmortem blood than traditional SPE methods with minimal extraction solvents.

A reliable and less costly procedure was developed for detecting fentanyl in postmortem blood. The final method included using BioSPME fiber tippets with C18 coating that provided an efficient extraction procedure followed by GC-MS screening and LC-MS-MS confirmation. The sensitivity and selectivity of the method were determined for the detection of fentanyl in blood. The limit of detection was sufficient to detect fentanyl in the postmortem blood of overdose victims. Authentic postmortem blood samples were provided for the study. The results demonstrated the validity and suitability of the method for the routine toxicological analysis of forensic postmortem blood samples for fentanyl. The applicability of GC-MS and LC-MS-MS in toxicology laboratories enables this method to have widespread use.

While there are more possible improvements that can be made when using BioSPME to detect opioids in postmortem blood samples, this study has shown that there is minimal required volume, sample preparation, as well as extraction solvents required to detect fentanyl in this type of matrix. The instrumentation along with the optimized analysis methods have allowed for a relative fast analysis time, which can cut down the timeframe from autopsy to toxicological result. The observations and results from this study have provided a step towards enhancing current toxicological methods using BioSPME.

Acknowledgement

This work was gratefully supported by the Forensic Science Program, Department of Chemical and Physical Sciences, Cedar Crest College, Allentown, PA. Research grant funding for 2016-2017 was awarded through Carol De Forest Student Research Grant given by the Northeastern Association of Forensic Scientists. The authors would like to thank Dr. Craig Aurand and Bob Shirey of Supelco, Sigma Aldrich, for providing the BioSPME fibers and fentanyl drug and deuterated fentanyl drug standards. The authors would also like to thank the Lehigh County Coroner's Office for providing the postmortem samples for this study and allowing the laboratory results to be included in this work.

References

1. Drugs of Abuse: A DEA Reference Guide. U.S. Department of Justice. 2017, pp: 38-41.
2. Sun EC, Dixit A, Humphreys K, Darnall BD, Baker LC, et al. (2017) Association between concurrent use of prescription opioids and benzodiazepines and overdose: Retrospective Analysis. *Brit Med J* 356.
3. Counterfeit prescription pills containing fentanyl: A Global Threat. DEA Intelligence Brief; 2016 Jul. Report No.: DEA-DCT-DIB-021-17.
4. Guerrieri D, Rapp E, Roman M, Druid H, Kronstrand R (2017) Postmortem and toxicological findings in a series of furanylfentanyl-Related Deaths. *J Anal Toxicol* 41: 242-239.
5. Hedegaard H, Bastain BA, Trinidad JP, Spencer M, Warner M (2018) Drugs most frequently involved in drug overdose deaths: United States, 2011-2016. *Natl Vital Stat Rep* 67: 1-14.
6. Analysis of drug-related overdose deaths in Pennsylvania (2016). DEA Intelligence Report; 2017 Jul. Report No.: DEA-PHL-DIR-034-16.

7. The opioid threat in Pennsylvania. DEA Intelligence Report; 2018 Sep. Report No.: DEA-PHL-DIR-036-18.
8. Dwyer JB, Janssen J, Luckasevic TM, Williams KE (2018) Report of increasing overdose deaths that include acetyl fentanyl in multiple counties of the southwestern region of the commonwealth of Pennsylvania in 2015-2016. *J Forensic Sci* 63: 195-200.
9. Somerville NJ, O'Donnell J, Gladden M, Zibbell JE, Green TC, et al. (2017) Characteristics of Fentanyl Overdose-Massachusetts, 2014-2016. *Morbidity and Mortality Weekly Report* 66: 382-386.
10. Shoff EN, Zaney ME, Kahl JH, Hime GW, Boland DM (2017) Qualitative identification of fentanyl analogs and other opioids in postmortem cases by UHPLC-Ion Trap-MSn. *J Anal Toxicol* 41: 484-492.
11. Kahl JH, Gonyea J, Humphrey SM, Hime GW, Boland DM (2018) Quantitative analysis of fentanyl and six fentanyl analogs in postmortem specimens by UHPLC-MS-MS. *J Anal Toxicol* 42: 570-580.
12. Novakova L, Vickova H (2009) A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation. *Anal Chim Acta* 656: 8-35.
13. Duan C, Shen Z, Wu D, Guan Y (2011) Recent developments in solid-phase microextraction for on-site sampling and sample preparation. *Analytical Chemistry* 30: 1568-1574.
14. Morrow JB, Roper-Miller JD, Catlin ML, Winokur AD, Cadwallader AB, et al. (2018) The Opioid Epidemic: Moving towards an integrated, holistic analytical response. *J Anal Toxicol* 43: 1-9.
15. Narapanyakul R, Tungtanuwat W, Yongpanich P, Sinchai T, Thong-ra-ar N, et al. (2014) Comparative study of postmortem blood, urine, and vitreous humor methamphetamine. *The Thai Journal of Pharmaceutical Science* 38: 5-13.
16. Mastrogianni O, Theodoridis G, Spagou K, Violante D, Henriques T, et al. (2012) Determination of venlafaxine in post-mortem whole blood by HS-SPME and GC-NPD. *Forensic Sci Int* 215: 105-109.
17. Bojko B, Vuckovic D, Cugjoe E, Hoque ME, Mirnaghi F, et al. (2011) Determination of tranexamic acid concentration by solid phase microextraction and liquid chromatography-tandem mass spectrometry: First step to in vivo analysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 879: 3781-3787.
18. Kataoka H, Saito K (2011) Recent advances in SPME techniques in biomedical analysis. *Journal of Pharmaceutical and Biomedical Analysis* 54: 926-950.
19. Mirnaghi FS, Pawliszyn J (2012) Reusable solid-phase microextraction coating for direct immersion whole-blood analysis and extracted blood spot sampling coupled with liquid chromatography-tandem mass spectrometry and direct analysis in real time-tandem mass spectrometry. *Anal Chem* 84: 8301-8309.
20. Alsenedi KA, Morrison C (2018) Determination of amphetamine-type stimulants (ATSs) and synthetic cathinones in urine using solid phase microextraction fibre tips and gas chromatography-mass spectrometry. *Anal Methods* 10: 1431-1440.
21. Alam MD, Pawliszyn J (2018) Effect of binding components in complex sample matrices on recovery in direct immersion solid-phase microextraction: friends or foe? *Analytical Chemistry* 90: 2430-2433.
22. Drummer OH (2007) Post-mortem toxicology. *Forensic Sci Int* 165: 199-203.
23. Fogarty MF, Papsun DM, Logan BK (2018) Analysis of fentanyl and 18 novel fentanyl analogs and metabolites by LC-MS-MS, and report of fatalities associated with methoxyacetylfentanyl and cyclopropylfentanyl. *Journal of Analytical Toxicology* 42: 592-604.
24. Stone PJW (2018) The detection and analytical confirmation of synthetic fentanyl analogues in human urine and serum using an Agilent Ultivo LC/TQ.

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