Grant et al., J Forensic Toxicol Pharmacol 2019, 8:1

DOI: 10.37532/jftp.2019.8(1).164

Journal of Forensic Toxicology & Pharmacology

Short Communication A SCITECHNOL JOURNAL

Understanding the Method and Results from the Analysis of Fentanyl in Postmortem Blood Using Biocompatible Solid-Phase Microextraction

Grant CM[1*], Brettell TA[2], Land SD[1], Staretz ME[2], Fritch D[3] and Koenig N[3]

[1]*Forensic Pathology Associates, PA 18103, USA*

[2]*Department of Chemical and Physical Sciences, Cedar Crest College, PA 18104, USA*

[3]*Health Network Laboratories, PA 18109, USA*

[*]**Corresponding author:** Grant CM, Forensic Pathology Associates, Health Network Laboratories, PA, 18103, USA, Tel: +1(610)-402-8144; Fax: (610)-402-5637; E-mail: chandler.grant@healthnetworklabs.com

Received date: November 08, 2019; **Accepted date**: November 21, 2019; **Published date:** November 29, 2019

Abstract

The use of the Biocompatible Solid-Phase Microextraction (BioSPME) fiber in Grant et al. original work was to create an analysis method that is faster and simpler in identifying fentanyl in postmortem blood than current analysis methods. While the study was able to demonstrate that fentanyl was able to be absorbed onto the fiber and analyzed through the use of GC-MS and LC-MS-MS, the data reported was beyond the sensitivity for both instruments. The purpose of this communication is to provide more information regarding the methodology and sensitivity for both instruments and to reinterpret the data when comparing to the results from Health Network Laboratories. Based on the reinterpreted data, this current method was consistent with the results from Health Network Laboratories in identifying fentanyl in postmortem blood concentration equal to or greater than 21.4 ng/mL.

Keywords: BioSPME; Fentanyl; Postmortem; Toxicology; Blood

Introduction

Biocompatible solid-phase microextraction (BioSPME) is an in-vivo application that has been developed to be a simple, sensitive and effective technique that directly samples biological matrices without the inference of macromolecules [1-4] BioSPME is composed of a metal core tip containing a chemical sorbent, C18 or a strong cation exchange-C18, surrounded by a biocompatible polymer [5]. When directly injected into a biological matrix, such as postmortem blood, the BioSPME fiber would be able to absorb compounds of interest, like fentanyl, allowing for a clean extraction.

In the research conducted by Grant et al. [1], a C18 BioSPME method was created to screen and identify fentanyl in postmortem blood samples in hopes to generate a faster and simpler analysis method compared to current analysis methods. The data originally presented from the study [1] was interpreted beyond the sensitivity for both the GC-MS and LC-MS-MS. The purpose for this commentary is to reinterpret the data from the original study [1] by providing the sensitivity and more detailed methodology for both the GC-MS and LC-MS-MS used.

Methodology

GC-MS

A screening method was developed for fentanyl using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer. In order to determine the sensitivity when screening for fentanyl with the BioSPME method, the following calibrator solutions were prepared by diluting a 1 mg/mL methanolic fentanyl stock standard: 5, 10, 15, 25, 50, 75, and 100 μg/mL. A calibration curve was generated by analyzing calibrator solutions in triplicate. Figures of merit data, coefficient of determination (R^2), Limit of Detection (LOD), and Limit of Quantitation (LOQ) were obtained from the calibration curve. The fentanyl calibrator solutions produced a Linear Dynamic Range (LDR) from 5-100 μg/mL and with a regression (R^2) value of 0.9401. The LOD and LOQ for fentanyl were determined using the LINEST function in Excel. The LOD and LOQ values for the fentanyl calibrator solutions were 17.7 and 59.0 μg/mL, respectively.

LC-MS-MS

In order to determine the sensitivity using the Shimadzu LC-20AD Prominence system coupled with an AB Sciex 3200 QTRAP triple-quadrupole mass spectrometer, the following calibrator solutions were prepared by dilution with 0.1% formic acid in water of the 1 mg/mL stock fentanyl standard: 1, 5, 10, 50, 125, 250, and 500 ng/mL. Calibrator solutions were incorporated into bovine blood and extracted using the extraction method described by Grant et al. [1]. A calibration curve was generated by analyzing extracted samples in triplicate over two days using the C18 BioSPME fiber. Figures of merit data, coefficient of determination (R^2), Limit of Detection (LOD), and Limit of Quantitation (LOQ) were obtained from the calibration curve. The fentanyl extraction calibrator solutions produced a Linear Dynamic Range (LDR) from 1-500 ng/mL with an R^2 value of 0.9927 and 0.9994 for the 188 and 105 ions, respectively. The LOD and LOQ for fentanyl were determined using the LINEST function in Excel. The LOD and LOQ values for the extracted fentanyl using the C18 BioSPME fiber were 21.4 and 71.4 ng/mL for the 188 ion and 6.0 and 20.0 ng/mL for the 105 ion, respectively. In order to determine a positive fentanyl result, both the 105 and 188 ions needed to be present and the ion ratio was set at +/- 20%. Since both ions will be needed to identify fentanyl, the concentration cutoff was calculated to be 21.4 ng/mL.

Results and Discussion

Out of 43 cases analyzed, the Lehigh County Coroner's Office reported 15 postmortem cases containing fentanyl based on the results produced by Health Network Laboratories. Only 3 of the 15 cases reported had concentrations over 21.4 ng/mL (Table 1).

DOI: 10.37532/jftp.2019.8(1).164

Case number	Age/Sex	Brief history	BioSPME method		Health Network Laboratories LC-MS-MS method	
			GC/MS	LC-MS-MS	Fentanyl	Additional compounds
TS0008	23/Male	Found slumped in chair	Positive	Positive	Fentanyl 55.0 ng/mL	Norfentanyl 20.3 ng/mL, Naloxone
TS0017	46/Male	Found unresponsive in residence	*	Positive	Fentanyl 31.8 ng/mL	Morphine (total) 57 ng/mL, Norfentanyl 7.5 ng/mL
TS0031	25/Female	Found unresponsive, History of heroin abuse	Positive	Positive	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, Fluoxtine 71 ng/mL, Norfluoxetine 72 ng/mL, Dextromethorphan

Table 1: BioSPME extraction method results versus results obtained by Health Network Laboratories. (*Fentanyl screening was negative when using C18 BioSPME fiber but positive when using Mixed Mode BioSPME fiber.)

Health Network Laboratories LC-MS-MS method used a whole blood, plasma or serum sample which was extracted using Phenomenex Novum Supported Liquid Extraction MAX (SLE) 96 DWP format then analyzed using a Shimadzu Prominence HPLC with a Restek Ultra Biphenyl Column (50 mm × 2.1 mm, 5 μm) coupled with a Sciex 5500 QTRAP. LOD and LOQ for the Health Network Laboratories method are 0.5 ng/mL and 0.5 ng/mL, respectively.

Conclusion

The results obtained from the study [1] were compared to the results obtained by Health Network Laboratories. Based on the criteria set out for identifying fentanyl for this study, the BioSPME method was consistent with three Health Network Laboratories results in identifying fentanyl that was equal to or greater than 21.4 ng/mL.

References

1. Grant CM, Brettell TA, Land SD, Staretz ME (2019) Investigation into the analysis of fentanyl in postmortem blood using biocompatible solid-phase microextraction. J Forensic Toxicol Pharmacol 8: 1-8.
2. Kataoka H, Saito K (2011) Recent advances in SPME techniques in biomedical analysis. J Pharm Biomed Anal 54: 926-950.
3. 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.
4. 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.
5. Alam MD, Pawliszyn J (2018) Effect of binding components in complex sample matrices on recovery in direct immersion solid-phase microextraction: Friends or foe? Anal Chem 90: 2430-2433.