LC/MS/MS Analysis of Fentanyl and Related Analogs Using Biocompatible Solid Phase Microextraction

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Indroduction

Fentanyl and its analogs are routinely used for pain management and anesthesia in the medical field. However, they also have a high rate of abuse in the USA. In recent years, these compounds have been linked to overdose fatalities.[1] Solid phase extraction (SPE) methods are commonly used for the determination of fentanyl and its related analogs from urine.[2] SPE methods typically involve multistep extractions (condition, equilibrate, load, wash, elute, evaporate, reconstitute) that can introduce sample preparation errors as well as analyte loss. SPE methods frequently involve an evaporation step prior to analysis to either concentrate the sample or to switch the solvent to be more compatible with the analytical technique. This evaporation step can be time consuming as well as introduce the potential for analyte loss for volatile or semi-volatile compounds.

In this study, Biocompatible Solid Phase Micro Extraction (BioSPME) fibers were used to determine the concentrations for fentanyl, acetyl fentanyl, sufentanil, remafentanil, norfentanyl, acetyl norfentanyl, alfentanil, butyryl fentanyl, and cis-3-methylfentanyl in urine samples. The Bio-SPME extraction eliminates the many steps in the sample preparation method including the evaporation step which reduces time of preparation and solvent use. Using the microextraction technique affords a fast, simple method for the quantitation at sub ng/mL levels for most of the compounds. Accuracy, precision, limits of quantitation, and matrix factors will be presented and discussed. BioSPME preparation followed by analysis on a biphenyl analytical column proved to be an accurate and precise method for the determination of fentanyl and some of its analogs with an analytical run time of less than six minutes

Process

BioSPME is an equilibrium extraction technique in which the analyte of interest partitions between the sample matrix and the extraction coating on a BioSPME device. The extraction coating contains functionalized silica particles that are embedded within a proprietary biocompatible binder (Figure 1). The role of this binder is to reduce or eliminate the extraction of matrix interferences during immersion, without reducing analyte extraction. This allows for the isolation of target analytes, while minimizing the presence of matrix, resulting in a highly sensitive microextraction technique.

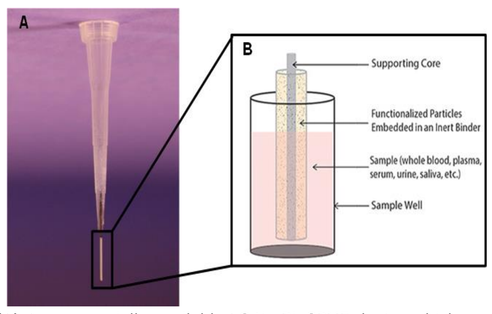

Figure 1. (A) A commercially available LC tip BioSPME device which consists of a coated fiber housed within a pipette tip. (B) A basic schematic of an extraction performed with a BioSPME fiber. The fiber is coated with functionalized particles that have been embedded within a proprietary binder. The binder allows the fiber to be placed directly within a biological fluid for sampling.

Mixed mode (C8/SCX) fibers were conditioned within 1 mL of 50:50 (MeOH/water) for 30 min with an agitation rate of 800 rpm. Followed by a water rinse for ~ 10 sec at 800 rpm.

Fibers were placed into 1 mL spiked urine samples and extracted for 30 min at 800 rpm.

Fibers were desorbed in 200 µL of 0.1% NH_4OH in 90 :10 MeOH:Water for 30 min at 800 rpm.

Final extracts analyzed via LC/MS/MS.

Analytical Method

column: Ascentis® Express Biphenyl, 5 cm x 2.1 mm, 2.7 µm
mobile phase:(A) 0.1% formic acid in water, (B)0.1% formic acid in MeOH
flow rate: 600 µL/min
column temp: 50 ºC
det.: MS/MS, ESI (+), MRM transitions (Table 1)
injection: 4 µL
gradient: 40%B to 50%B in 2 min, to 80%B in 1 min, hold 80%B for 1 min, to 40%B in0.1 min and hold at 40%B for 1.4 min
instrument: Agilent® 1290 Infinity II with Agilent 6460 QQQ

Table 1. MRM Transitions for Fentanyl and Related Analogs

Analyte	Precursor	Product	Frag (V)	CE (V)	RT(min)
Acetyl norfentanyl	219.1	84	98	16	0.79
Acetyl norfentanyl-13C	225.1	84	84	16	0.79
Norfentanyl	233.2	84	98	16	1.21
Norfentanyl-d5	238.2	84	98	16	1.21
Remafentanil	377.2	113	133	28	1.56
Acetyl fentanyl	323.2	188	128	20	1.9
Acetyl fentanyl-13C	329.4	188	138	24	1.9
Alfentanil	417.3	197	123	24	2.18
Fentanyl	337.2	188	118	24	2.26
Fentanyl-d5	342.3	188	133	24	2.26
Cis-3-methylfentanyl	351.2	202.1	133	24	2.5
Butyryl fentanyl	351.2	188	128	24	2.54
Sufentanil	387.2	238	133	16	2.68
Sufentanil-d5	392.2	238	108	16	2.68

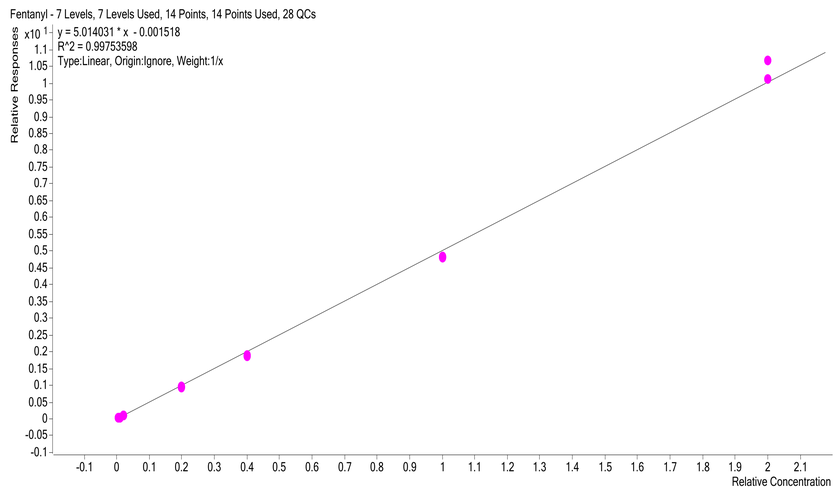

Figure 2 Representative calibration curve prepared in urine from 0.05 – 10 ng/mL.

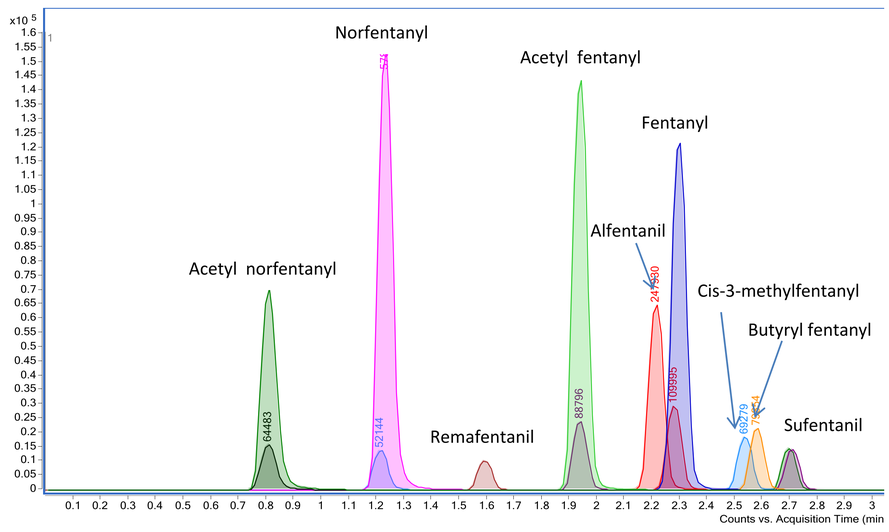

Figure 3 Representative chromatogram for Fentanyl and Related Analogs.

Results

- Recoveries ranged from 66.7% to 111%. All of the analytes had recoveries >70% at 0.05 ng/mL except for remafentanil and alfentanil.
- Precision was demonstrated with %RSD's less than 10% for most analytes except for remafentanil and alfentanil.
- The lower recoveries and higher variability for remafentanil and alfentanil could be attributed to the absence of an analyte specific stable label internal standard and lower MS sensitivity.

Table 2. Average Recoveries and %RSD values for spiked urine samples

Compound	0.05 ng/mL		0.1 ng/mL		1 ng/mL	
	Avg. % Rec	%RSD	Avg. % Rec	%RSD	Avg. % Rec	%RSD
Acetyl norfentanyl	83.9	7.6	90.1	6.1	98.5	1.6
Norfentanyl	78.5	2.5	86.1	2.9	99.2	0.8
Remafentanil*	BLQ	-	BLQ	-	111	18.9
Acetyl fentanyl	86.4	4.6	87.3	2.5	93.4	1.9
Alfentanil^	BLQ	-	66.7	14.7	78.2	10.6
Fentanyl	90.4	6.3	87.5	2.6	91.1	1.1
Butyryl fentanyl^	83.9	2.1	76.9	4.2	76.0	5.7
cis-3-methylfentanyl^	93.9	5.1	84.6	5.6	84.0	4.6
Sufentanil	95.3	6.1	88.6	4.9	84.8	2.6

BLQ = Below limit of quantitation

* used Acetyl fentanyl-13C as internal standard

^ used fentanyl-d5 as internal standard

- The effect the matrix had on the analyte response was also evaluated by spiking analyte into extracted urine blank samples and comparing the response to analyted spiked into the desorption solution.

$$\text{Matrix Effects} = \frac{\text{Analyte in presence of matrix}}{\text{Analyte in absence of matrix}} - 1 \times 100\%$$

- Matrix effects were calculated to be less than 10% for all of the analytes indicating that the extraction has eliminated most of the impact of the matrix on the analysis..

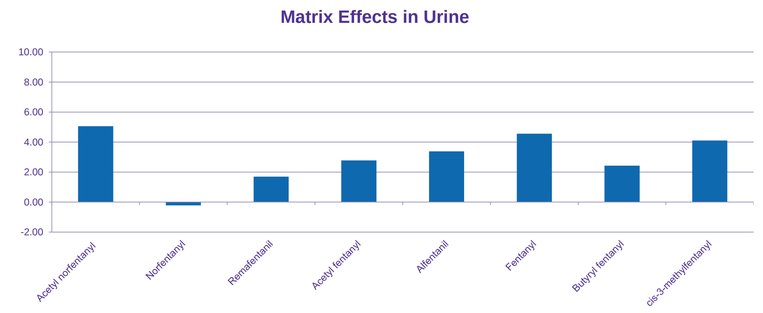

Figure 4 Matrix Effects for Fentanyl and Related Analogs in Urine

Summary

- A simple 3 step extraction utilizing BioSPME fiber tips was developed for fast reproducible detection of fentanyl and related analogs.
- Linear responses from 0.025 ng/mL to 10 ng/mL were established for all analytes except for remafentanil which linear responses ranged from 1 ng/mL to 10 ng/mL.
- Limits of quantitation were demonstrated at 0.05 ng/mL for most of the compounds, except for remafentanil and alfentanil, which were at 1 ng/mL.
- Using a biphenyl analytical column, all analytes were separated in less than 3 minutes with a total run time of 5.5 min.
- Sufficient sample clean up was demonstrated with matrix effects less than 10% for all of the analysis
- Combining the simple microextraction technique with the fast analytical separation, high throughput of urine samples is capable at sub ng/mL detection limits.

References

1. Mohr, A., Frisca, M., et. Al., (2016) Analysis of novel synthetic opioids U-47700, U-50488 and Furanyl Fentanyl by LC–MS/MS in postmortem casework, Journal of Analytical Toxicology, 40, 709-717
2. Shaner, R., Kaplan, P., et. Al., (2014) Comparison of two automated solid phase extractions for thedetection of ten fentanyl analogs and metabolites in human urine using liquid chromatography tandem mass spectrometry, Journal of Chromatography B, 962, 52-58

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