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

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Introduction
Fentanyl is a controlled substance and has been categorized as a Schedule II drug under the “Controlled Substances Act” in the United States. Fentanyl and related compounds are synthetic opioids that are at least 100 times more potent than morphine. Their main therapeutic applications are intravenous or intramuscular analgesia and sedation and have been widely used for neuroleptic and surgical anesthetics at doses ranging from 2 – 50 µg/mL. However, the past five years have seen a significant increase in the trafficking and usage of synthetic opioids with a preference for fentanyl. Due to the highly addictive nature of fentanyl and its analogues, several communities worldwide are experiencing an epidemic of opioid-induced overdose, criminal activity, and job productivity. In addition to abuse of prescribed fentanyl and other opioids, many “underground” drug laboratories are synthesizing fentanyl analogues, such as alfentanil and buprenorphine, which have been designed to evade screening and prosecution by drug enforcement agencies. As the number of opioid deaths and drug overdoses increases, there is a growing need for analytical methods to quickly and accurately determine the concentrations of these drugs in biological samples.

Experimental
In this study, fentanyl and related analogues were extracted from urine using a mixed mode (C8/SCX) BioSPME fiber and subsequently analysed using an Ascentis Express Biphenyl column. The structures of fentanyl and its related analogues are shown in Figure 1. Quantitative analysis of the nine fentanyl analogs was performed by LC/MS/MS Analysis as shown in Figure 2. A calibration curve was established using 13C as internal standard. The fibre was conditioned in 1 mL of 50:50 water:50% ammonium hydroxide (200–30 min, 800 rpm agitation).

Extraction
A spiked urine sample was subjected to extraction using a mixed mode BioSPME fiber. The fiber was conditioned at 800 rpm in 1 mL of 50:50 water:50% ammonium hydroxide (200–30 min, 800 rpm agitation). The fiber was rinsed off with water (1 mL, 10 s, 800 rpm) prior to extraction. The fiber was immersed into the urine sample and extraction could proceed (1 mL, 30 min, 800 rpm) followed by a water rinse (1 mL, 10 µL, 500 rpm). The analytes were desorbed from the fiber using 90:10 methanol:water containing 0.1% formic acid and 0.1% ammonium hydroxide (200–100 min, 800 rpm agitation).

BioSPME
Biocompatible solid phase microextraction (BioSPME) is a variant of solid phase microextraction (SPME) in which the SPME fibers are coated with a non-swelling, biocompatible polymer. The benefit of this design is that it enables microextraction of biomacromolecules such as proteins and phospholipids but allows extraction of smaller analytes of interest. This coating enables the end user to directly extract analytes out of complex matrices without risk of protein interfering with diversive quantification of the analytes of interest. Using BioSPME eliminates many steps found in SPE methods and eliminates matrix effects often seen with dilute and shoot approaches.

The BioSPME technique only extracts the free portion of a drug within a biological sample; therefore, before sample quantitation can occur, a series of extracted standard curves were prepared. The calibration samples, which were spiked in syringe urine, were used to determine the average recovery of each analyte within the spiked samples.

Quantification and Recovery
The table below outlines the quantification results of the experiment for each analyte. Recoveries for each analyte ranged from 66.7% to 111.4%. All the analytes had recoveries greater than 70% at 0.05 ng/mL except for remifentanil and alfentanil. However, the lower recoveries may be an artifact of the experiment: stable label internal standards were not available for remifentanil and alfentanil. Having a matched internal standard for these two compounds would have improved the calculated recovery. Examination of the precision of the method also revealed a high degree of reproducibility as the percent relative standard deviation (%RSD) of the method was less than 10% for most analytes except for remifentanil and alfentanil. Again, the lower degree of precision for these two compounds could be due to the lack of an exact match internal standard.

Conclusion
The rise and spread of opioid abuse, especially fentanyl, is a growing concern in the healthcare industry. To better detect fentanyl and related analogues, a simple, three step extraction utilizing BioSPME fiber tips was developed for fast, reproducible detection. Linear responses from 0.05 ng/mL to 50 ng/mL were established for all analytes except for remifentanil which displayed a linear response from 1 ng/mL to 100 ng/mL. Limits of quantitation (LOQ) were demonstrated at 0.05 ng/mL for most compounds, except for remifentanil and alfentanil, which were at 2 ng/mL. The use of an Ascentis Express Biphenyl Fused-Core column yielded excellent separation of all nine analytes in less than three minutes with an overall run time of 5.1 min. Finally, due to the bio-compatible coating of the adsorbent, constituents of the matrix did not interfere with the extraction of the analytes. Combining the simple microextraction technique with the fast LC/MS/MS method, sub-ng/mL detection limits are possible for high-throughput analysis of urine samples using BioSPME.

Figure 1: Structures of fentanyl and its related analogues

Figure 2: LC/MS/MS results of the analysis of the nine fentanyl analogs on Ascentis Express Biphenyl column

Figure 3: Results of the experiment by comparing the “percent matrix effects” for each compound

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