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 clinical therapeutic applications are intravenous or intramuscular analgesia and sedation and have been widely used for preanesthetic analgesia and surgical anesthesia at doses ranging from 2 – 10 ng/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 overdoses, criminal activity, and loss productivity. In addition to abuse of prescribed fentanyl and other opioids, many "underground" drug laboratories are synthesizing illicit analogues of fentanyl, such as acetyl fentanyl and butyryl fentanyl, which have been designed to evade screening and prosecution by drug enforcement agencies. As the number of opioid drugs and deaths 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 BioSPME fiber and subsequently analysed using an Ascentis Express Biphenyl column. The structures of fentanyl and its related analogs are shown in Figure 1.

Extraction

The extraction of these structures reveals that all these compounds have several sites of delocalized π electrons either through the benzene ring or centered around the amide functional group. The Ascentis Express Biphenyl column incorporates ligands with biphenyl moieties which are also rich in π electrons. Therefore, π-π stacking can occur between the apolar and the hydrophilic groups enabling an interaction between the compounds and the stationary phase. In addition, the planar structure of the biphenyl ligand enables the column to determine similar analyte analogs, allowing for increased resolution between structurally similar compounds.

Separation

A spiked urine sample was subjected to extraction with a mixed mode BioSPME fiber. The fiber was conditioned in 100:50 methanol/water (1 ml, 10 min, 800 rpm agitation). The fiber was rinsed off with water (1 ml, 10, 800 rpm) prior to extraction. The fiber was immersed into the urine sample and extraction could proceed (1 ml, 30, 800 rpm) followed by a water rinse (1 ml, 10, 800 rpm). The analytes were desorbed from the fiber using 90:10 methanol:water containing 1% formic acid/1% ammonium hydroxide (200 µl, 30, 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-solvent, biocompatible polymer. The benefit of this design 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 divergent quantitation of the analyses 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 for each analyte. These calibration samples, which were spiked in synthetic urine, were used to determine the average recovery of each analyte within the spiked samples.

LC/MS/MS Analysis of Fentanyl and Related Compounds

Due to the aromatic nature of the analytes of interest, an Ascentis Express Biphenyl column was employed for the separation of the nine fentanyl analogs. The chromatogram below shows the LC/MS/MS results of the analysis. The Ascentis Express Biphenyl column provided good resolution of the nine fentanyl analogs which allowed for accurate quantification of the analytes.

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%. 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-related analogs, 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 analogues except for remifentanil which displayed a linear response from 1 ng/mL to 10 ng/mL. Limits of quantitation (LOQ) were demonstrated at 0.05 ng/mL for most compounds, except for remifentanil and alfentanil, which were at 1 ng/mL. The use of an Ascentis Express Biphenyl Fused-Core column yielded excellent separation of all nine analogues in less than three minutes with an overall run time of 5.3 min. Finally, due to the biocompatible coating of the absorbent, constituents of the matrix did not interfere with the extraction. For this reason, the matrix was not pre-cleaned prior to extraction. Additionally, the BioSPME method, sub-ng/mL detection limits are possible for high throughput analysis of urine samples using BioSPME.