Direct Analysis in Real Time (DART®)-MS Analysis of Fentanyl and Related Analogues from Saliva Using Biocompatible Solid Phase Microextraction (BioSPME)

Author: Emily Barrey, Senior R&D Scientist, MilliporeSigma, Bellefonte, PA, USA

The extraction mechanism for Biocompatible Solid Phase Microextraction (BioSPME) combined with DART-MS was used to rapidly screen for fentanyl and some of its related analogues at ng/mL levels from human saliva. By employing a fast and accurate screening method, the number of samples that require confirmatory analysis can be reduced and laboratories can increase throughput and decrease costs associated with their drug screening programs. Improved sample preparation techniques, along with the use of stable labelled internal standards, allowed for reproducible and accurate quantitation of the compounds. Advantages over current methodologies with respect to the time of preparation, solvent usage and pre-concentration of the analytes to achieve screening detection limits were demonstrated.

Introduction

Fentanyl and its analogues are routinely used for pain management and anaesthesia 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 analogues in biological matrices like urine [2]. SPE methods typically involve multiple steps (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 one that is more compatible with the analytical technique. This evaporation step can be time consuming as well as introducing the potential for analyte loss of volatile or semi-volatile compounds.

The use of saliva for drug testing is currently on the rise due to less invasive and readiness of sample collection. Using saliva eliminates some of the issues related to urine collection that can be encountered, such as the patient's ability to produce specimen and also sample adulteration for non-supervised collections. In addition, using saliva can provide benefits over blood collection such as not having to stick the patient with a needle, as well as improving detection for compounds that tend to bind to plasma proteins.

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. For this study, the BioSPME device used was a pipette tip format, which consists of a coated fibre housed within a pipette tip (See Figure 1A). This format allows for the device to be easily manipulated via liquid handlers or robotics, and is therefore amenable to high throughput. The extraction coating consists of C18 functionalised silica particles that are embedded within a proprietary biocompatible binder (Figure 1B). The role of this proprietary binder is to reduce or eliminate the co-extraction of matrix interferences, without reducing analyte extraction. The binder acts as a shield preventing higher molecular weight species like proteins from being absorbed onto the fibre coating. This allows for the isolation of target analytes, while minimising the amount of matrix, resulting in a highly selective and sensitive microextraction technique. Since the binder also allows for the extraction step to be performed via direct immersion into the sample, it provides a sampling platform that can be used for direct analysis into the mass spectrometer.

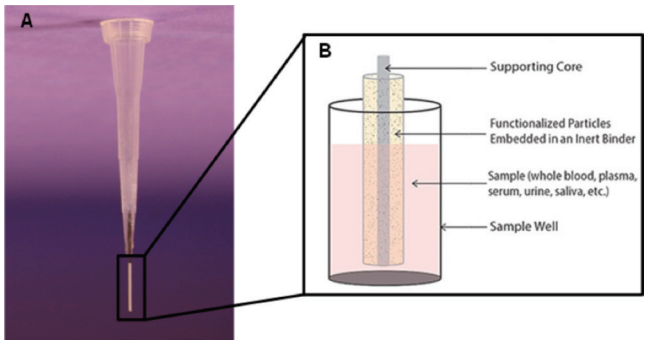

Figure 1. (A) A commercially available LC tip BioSPME device which consists of a coated fibre housed within a pipette tip. (B) A basic schematic of an extraction performed with a BioSPME fibre. The fibre is coated with functionalised particles that have been embedded within a proprietary binder. The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

Experimental

SPME LC Tips, C18 (Part No. 57234-U) and LiChrosolv® methanol were purchased from MilliporeSigma (Darmstadt, Germany). LC-MS grade water was obtained from a Milli-Q® Integral water purification system with a LC-Pak® polisher, also purchased from MilliporeSigma (Darmstadt, Germany). Certified analytical reference standards of fentanyl, fentanyl-d5, alfentanil, sufentanil,

sufentanil-d5, butyryl fentanyl, acetyl fentanyl, acetyl fentanyl-13C were purchased from Cerilliant® (Round Rock, TX). Blank human saliva was purchased from BioReclamation (NY).

BioSPME extraction procedure. SPME LC Tips were conditioned within 1 mL of 50:50 methanol for 30 min with 800 rpm agitation, followed by rinse in 1 mL of water for 10 seconds with agitation. The fibres were then placed in 1 mL of spiked saliva samples and agitated for 30 min at 800 rpm using an orbital shaker. The fibres were then rinsed with 1 mL of water for ~ 10 seconds with agitation and analysed directly on the DART-MS.

Blank human saliva was fortified with the analytes of interest over a concentration range of 50 to 2500 ng/mL in order to prepare a matrix matched calibration curve. In addition, six separate fibres were extracted in individual blank human saliva samples fortified at concentrations of 100 ng/mL, 500 ng/mL, and 1000 ng/mL. A 30 minute equilibration time was allowed after matrix spiking. For matrix effect evaluations, fibres were also extracted in blank saliva samples, blank water samples, and water samples fortified at 100 ng/mL (n=6).

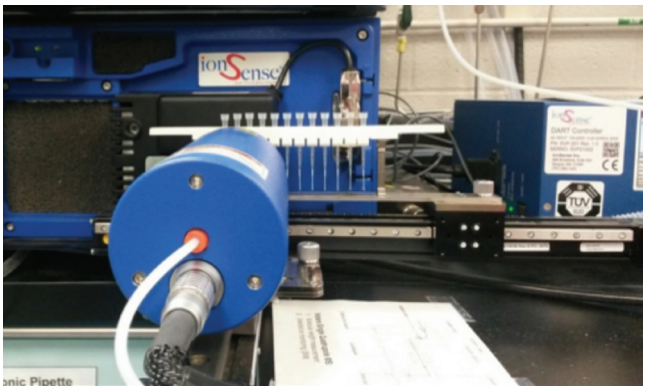

Figure 2. Schematic of DART-MS Set-up with BioSPME fibre tips.

DART®-MS analysis. Samples were analysed using an IonSense® DART®-SVP coupled to a Waters QDa® mass spectrometer. Figure 2 depicts the DART source interfaced to the QDa with the BioSPME fibres positioned for analysis. The MS source and compound dependent parameters are displayed in Tables 1 and 2.

Parameter	Setting
DART Temp. (°C)	300
Polarity	Positive
Cone (V)	15
Gas	Helium
Rail Speed (mm/sec)	0.3
Frequency (Hz)	20
Scan Type (SIM)	100-500 amu

Table 1. DART-MS source settings.

Analyte	EIC *(m/z)*
Fentanyl	337.4
Fentanyl-d_5	342.4
Alfentanil	417.4^
Sufentanil	387.3
Sufentanil-d_5	392.4
Butyryl fentanyl	351.4^
Acetyl fentanyl	323.3
Acetyl fentanyl-^{13}C	329.3

Table 2. Compound specific MS parameters.

Results and Discussion

After the extraction period, the fibres were passed in front of the DART source. The results for twelve of the fibres were stored in a single data file. Full scan data was collected and then extracted ion chromatograms were generated for each analyte and internal standard. An example data file with 12 fibre responses for butyryl fentanyl is shown in Figure 3.

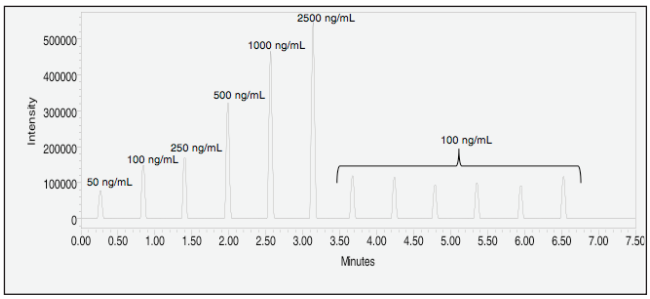

Figure 3. Extracted ion response for butyryl fentanyl in saliva

Calibration curves constructed from the extracted blank saliva samples that were fortified at concentrations ranging 50-2500 ng/mL were used to determine the average accuracy for measurement of each analyte for the 100 ng/mL spiked samples. Using linear regression, calibration curves demonstrated linearity from 50 – 2500 ng/mL in saliva with coeffecients of determination (R2) values greater than 0.985. An example calibration curve for butyryl fentanyl is given in Figure 4.

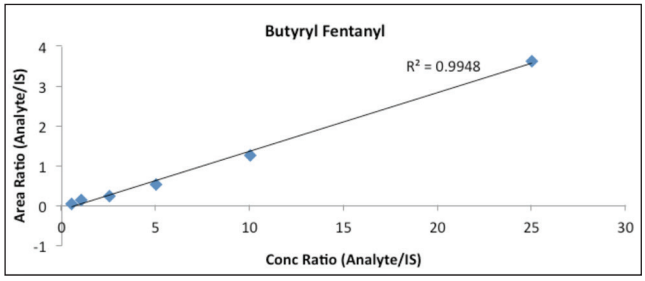

Figure 7. Calibration curve for butyryl fentanyl in saliva

The method demonstrated reproducible extraction efficiencies with accuracies ranging from 81-120% for all analytes and relative standard deviations (% RSD) ranging from 1.9-7.0%. Table 3 shows the average accuracies and % RSD's

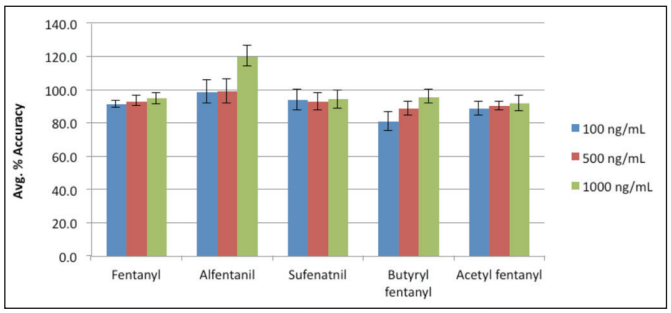

Table 3. Average measurement accuracies with %RSD from saliva samples

Limits of detection (LOD) and limits of quantitation (LOQ) were estimated by calculating the standard deviation of the response at 100 ng/mL and using 3x the value for LOD and 10x the value for LOQ. Estimated limits of detection and quantitation for the analytes in saliva are given in Table 4. These detection limits in saliva show the potential for this technique to be a quick and easy way to screen many samples, prior to using more extensive confirmatory methods on positive samples.

	LOD	LOQ
	(ng/mL)	(ng/mL)
Fentanyl	5	20
Alfentanil	20	70
Sufenatnil	15	50
Butyryl fentanyl	15	50
Acetyl fentanyl	10	40

Table 4. Estimated LOD and LOQ's for saliva

Typical mass spectra obtained from the blank saliva sample, blank water sample and fortified saliva samples are given in Figures 9-10. Protonated molecular ions (M+H) (see Table 2) can be identified in the spectra in Figure 10. Additional m/z peaks at 116.0, 195.1, 271.2, and 430.3 are also present. These masses are present in the blank saliva sample but not in the blank water sample; indicating that they are small molecular weight compounds derived from the saliva matrix. The identity of these interferences was not determined as part of this study. However, the intensity of these masses was significantly higher in the blank saliva sample compared to the fortified saliva sample. The source of this enhancement effect has not been determined, but one possible cause could be that the target analytes have a higher affinity for the fibre than the matrix constituents and therefore take up the binding sites, preventing the matrix interferences from binding to the fibre.

In addition to the mass spectra, analyte responses from fortified saliva samples were compared to fortified water samples (Figure 11). The analyte responses in the saliva samples were found to be significantly higher than the responses in water. The increases in signal in the saliva samples could have several possible causes.

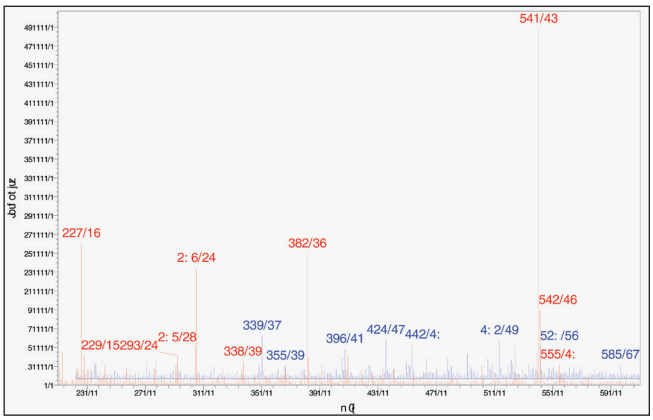

Figure 9. Mass spectra for blank saliva (red) and blank water (blue) after extraction

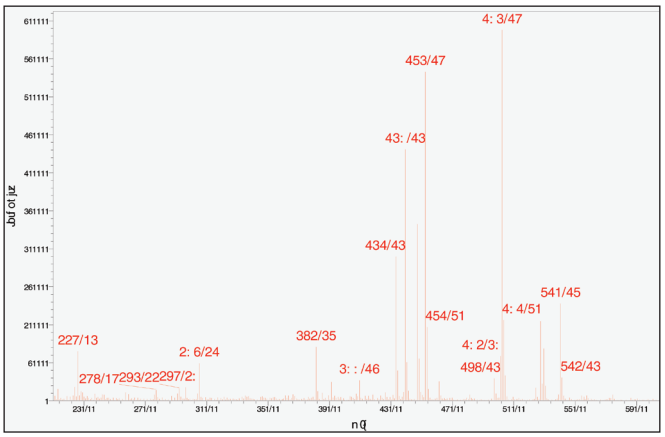

Figure 10. Mass spectra obtained from 50 ng/mL fortified saliva sample after extraction

The presence of some of the matrix constituents could possibly be increasing the ionisation of the analytes, which would increase their MS signal compared to water, which has none of these constituents present. Another potential explanation is that the saliva matrix could be shifting the extraction equilibrium, which would cause the analytes to have higher affinities to the C18 phase on the fibre resulting in higher analyte concentrations on the fibre. A third proposed scenario is that, in the absence of matrix, the analytes in the water samples are binding more tightly to the phase on the fibre; and thus are less readily desorbed into the MS from the DART ionisation process. To determine if one or more of these scenarios are contributing to the results observed, further investigation is warranted.

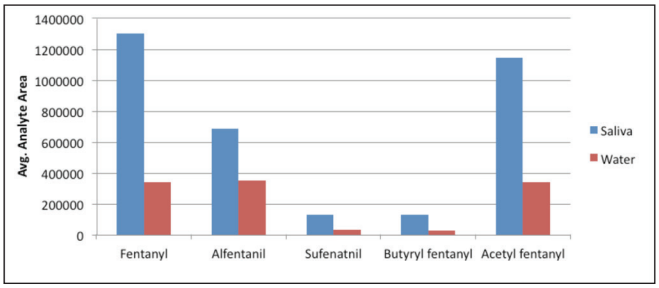

Figure 11. Analyte responses from fortified saliva and water samples

Conclusions

The method developed in this study for screening saliva samples presents an alternative to current methods with a number of significant benefits. Taking saliva samples presents an easier, non-invasive sampling procedure which benefits the patient. A BioSPME extraction followed by DART-MS analysis resulted in acceptable measurement accuracies at 100, 500, and 1000 ng/mL for fentanyl and some of its related analogue compounds. By eliminating steps that are necessary in some of the more extensive SPE procedures (i.e. elution and evaporation), the BioSPME method obtained reproducible and accurate results in less time. While not an exhaustive extraction technique, BioSPME still enables concentration of the analyte onto the fibre; thus lowering the detection limits that can sometimes be an obstacle with dilute and shoot methods. Incorporation of direct MS analysis with the DART-QDa system, enabled analytical results in seconds, and with no solvent usage as compared to HPLC analyses. Combining the simple BioSPME extraction procedure with DART-MS produced a fast, reproducible screening method for high throughput analysis of saliva samples.

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