RESTEK Pure Chromatography

The Analysis of Drugs of Abuse (DoA) and Novel Psychoactive Substances (NPS) in Oral Fluids by LC-MS/MS

Samantha Herbick, Alexander Sherman; Restek Corporation Bellefonte, PA

Introduction

Testing for drugs in biological matrices is an important part of forensic toxicology and workplace drug testing. The "gold standard" matrices that have been used for decades are typically blood and urine, however, the collection of these two matrices is invasive. Due to the ease of collection, oral fluid testing for drugs of abuse (DoA) has been gaining popularity. Despite its ease of collection, there are often issues with the buffer used in collection devices, such as difficulty removing surfactants and preservatives present in the device. These can cause matrix effect and poor analytical column lifetime. Achieving full recovery of analytes also presents a challenge due to varying techniques of extracting analytes from the sponge on the collection device. Often solid phase extraction (SPE), or lengthy extraction techniques are utilized, so establishing a workflow that uses a simple sample preparation paired with accurate and robust quantitation of the analytes is important for laboratories running these tests. The primary objective of this work is to demonstrate the analysis of DoA and novel psychoactive substances (NPS) in oral fluids by LC-MS/MS using a salt-assisted liquid-liquid extraction (SALLE).

Methods

Table 1: Analytical Method Conditions

Analytical Column:	Raptor Biphenyl 50 x 2.1 mm, 2.7 µm	
Guard Column:	Raptor Biphenyl Guard Column Cartridge 5 x 2.1 mm, 2.7 µm	
Mobile Phase A:	Water, 0.1% Formic Acid	
Mobile Phase B:	Methanol, 0.1% Formic Acid	
Flow:	0.5 mL/min	
Gradient:	Time (min)	%B
	0.00	15
	1.00	20
	2.00	20
	4.00	50
	6.00	60
	8.00	100
	9.00	100
	9.01	15
	10.00	STOP
Column Temp.:	40 °C	
Injection Volume:	5 µL	

Sample Preparation

Samples underwent a salt-assisted liquid-liquid extraction (SALLE) using a saturated sodium chloride solution. Utilizing this technique allowed for the analysis of a broad range of analytes from different classes and properties.

1 mL of synthetic oral fluid fortified with 100 µL of control was added to 3 mL of Quantisal buffer

100 µL of the oral fluid/buffer mixture was added to a microcentrifuge tube along with 20 µL of internal standard

Sample was vortexed for 10 seconds

100 µL of a saturated NaCl solution was added to the tube

Sample was vortexed for 10 seconds

280 µL of ACN was added to the sample

Sample was vortexed for 10 seconds

200 µL of the organic layer was aliquoted to a test tube and the sample was blown to dryness under nitrogen

Sample was reconstituted in 50 µL of 90:10 Mobile Phase A: Mobile Phase B and put onto the instrument for analysis

Recovery of Matrix

Total volume of a collection device with sample is typically 4 mL (3 mL of buffer and 1 mL of oral fluid). When performing extraction, it is important to recover as close to 4 mL as possible. The extraction process is challenging with most oral fluid kits because the sponge is difficult to fully empty. Due to the durability of the sponge, some techniques, such as manual compression are not feasible. Centrifugation should be used with careful manipulation of the sponge. Figures 1, 2, and 3 below show the sponge submerged in the buffer, the effects of using too high of a speed in the centrifuge, and how to manipulate the sponge for centrifugation, respectively.

Figure 1.

Figure 2.

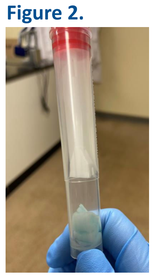


Figure 3.

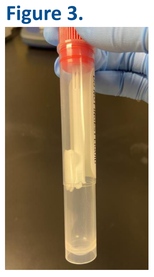


Figure 4 and 5 below show a visual comparison of the recovery of the manual compression technique and the centrifuge technique. In Figure 4, the value is close to 3 mL where Figure 5 shows almost full recovery.

Figure 4. Manual Compression

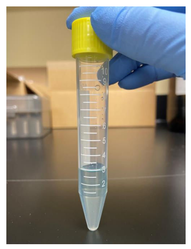
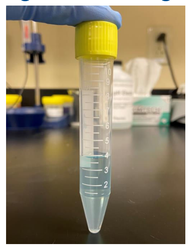
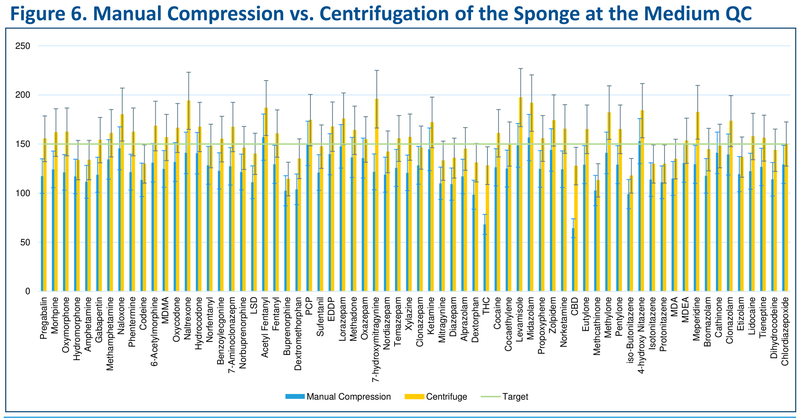


Figure 5. Centrifuge



In Figure 6 below, recovery was compared for a selection of DoA compounds using the manual compression method versus centrifuge method. Results show majority of samples from centrifuge method at or ± 15 % of the target value.

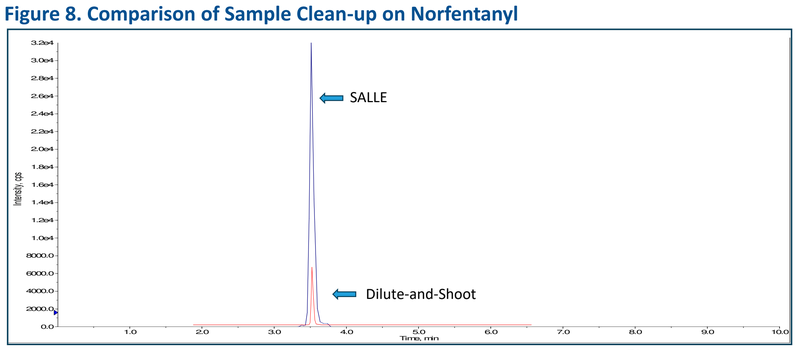
Figure 6. Manual Compression vs. Centrifugation of the Sponge at the Medium QC



Effects of Sample Clean Up

To demonstrate the effect that sample clean up has on oral fluid, samples were tested using a dilute-and-shoot method and compared to samples that had undergone the SALLE. In the example below (Figure 8) norfentanyl shows and increase of 4x the sensitivity with the SALLE approach compared to the dilute-and-shoot.

Figure 8. Comparison of Sample Clean-up on Norfentanyl



Chromatographic Separation

Chromatographic separation of all 68 analytes was achieved in a 10-minute cycle time. This includes separation of all 8 sets of isobars, achieving a resolution of 1.5 or higher, ensuring accurate quantitation of these analytes.

Figure 7. Chromatographic separation of all analytes.

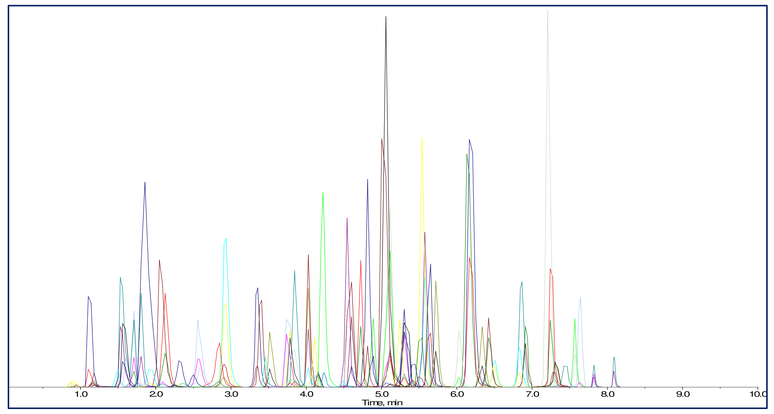


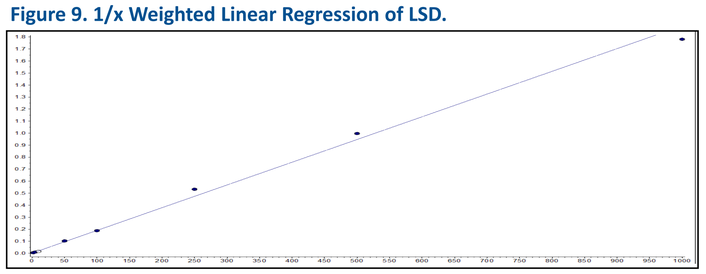
Table 2: Analytes and Retention Times (min).

Analyte	RT	Analyte	RT	Analyte	RT
Morphine	0.82	Norketamine	3.30	PCP	5.20
Pregabalin	0.88	Eutylone	3.39	Midazolam	5.22
Oxymorphone	0.92	Norfentanyl	3.55	Propoxyphene	5.33
Amphetamine	1.18	4-hydroxy Nitazene	3.70	Tianeptine	5.38
Hydromorphone	1.22	Pentylone	3.73	Protonitazene	5.49
Gabapentin	1.22	Dextrorphan	3.78	Sufentanil	5.62
Methcathinone	1.33	Xylazine	3.85	EDDP	5.62
MDA	1.50	Ketamine	3.90	Mitragynine	5.78
Methamphetamine	1.53	Benzoylecgonine	4.00	Iso-Butonitazene	6.10
Cathinone	1.65	Meperidine	4.03	Methadone	6.25
Phentermine	1.78	7-Aminoclonazepam	4.05	Lorazepam	6.35
Methylone	1.80	Cocaine	4.28	Oxazepam	6.40
Lidocaine	1.83	7-hydroxymitragynine	4.50	Clonazepam	6.50
Naloxone	1.90	LSD	4.60	Nordiazepam	6.80
Dihydrocodeine	2.09	Cocaethylene	4.60	Clonazolam	6.83
MDMA	2.15	Norbuprenorphine	4.65	Alprazolam	7.28
Codeine	2.22	Chlordiazepoxide	4.74	Temazepam	7.30
6-Acetylmorphine	2.38	Acetyl Fentanyl	4.75	Bromazolam	7.40
Levamisole	2.59	Zolpidem	4.80	Etizolam	7.52
Oxycodone	2.69	Fentanyl	5.15	Diazepam	7.59
Naltrexone	2.85	Dextromethorphan	5.17	THC	7.80
MDEA	2.92	Isotonitazene	5.18	Cannabidiol	8.00
Hydrocodone	2.98	Buprenorphine	5.20		

Quantitation of Analytes

Linearity: Using 1/x weighted linear regression, the analytes showed acceptable linearity with r^2 values of 0.99 or greater. An example of linearity can be seen in Figure 9 below.

Figure 9. 1/x Weighted Linear Regression of LSD.



Precision and Accuracy: Precision and accuracy analysis was performed over the course of multiple days. Method accuracy was demonstrated with recovery of ±15% of the nominal concentrations for all QC levels. The quantitative range for varied for all analytes based working limits of detection.

Conclusions

A panel of 68 DoA and NPS were analyzed in oral fluid using a SALLE sample preparation technique and LC-MS/MS. Results show this method demonstrates an accurate and robust solution for the analysis of these analytes. This method also offers a quick and efficient sample preparation, ensuring buffer surfactant removal, without the need for SPE or other tedious extraction techniques, leading to faster processing of samples in high through-put laboratories.