RESTEK Pure Chromatography

Comparison of Sample Preparation Techniques for the Analysis of Drugs of Abuse in Oral Fluids

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Introduction

Drug testing 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 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 present in the device. These can cause matrix effect and poor analytical column lifetime. 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 objective of this work is to demonstrate the analysis of drugs of abuse in oral fluids by LC-MS/MS and comparing different sample preparation techniques: salt-assisted liquid-liquid extraction (SALLE) and supported liquid extraction (SLE) to dilute-and-shoot.

Materials and Method

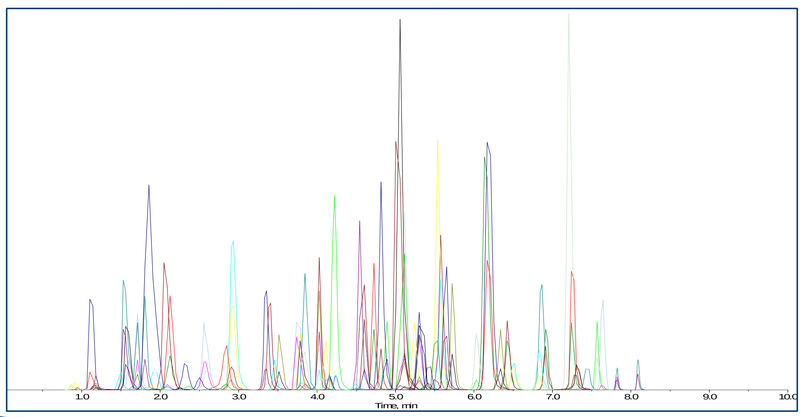
Table 1: Analytical Method Conditions

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	

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
Cathinone	0.94	4-hydroxy Nitazene	3.70	Tianeptine	5.38
Amphetamine	1.18	Pentylone	3.73	Protonitazene	5.49
Hydromorphone	1.22	Dextrorphan	3.78	Sufentanil	5.62
Gabapentin	1.22	Xylazine	3.85	EDDP	5.62
Methcathinone	1.33	Ketamine	3.90	Mitragynine	5.78
MDA	1.50	Benzoylecgonine	4.00	Iso-Butonitazene	6.10
Methamphetamine	1.53	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	Cannabidiol	7.85
MDEA	2.92	Isotonitazene	5.18	THC	8.10
Hydrocodone	2.98	Buprenorphine	5.20		

Figure 1. Chromatographic Analysis of All Analytes.



Sample Preparation

To demonstrate the effect that sample clean up has on oral fluid, samples were tested using a dilute-and-shoot and compared to samples that had undergone SALLE and SLE. Workflows for both SALLE and SLE can be found in Figure 2 and Figure 3 below. Both workflows were optimized for this specific set of analytes based on polarity and analyte properties.

Figure 2. SALLE Workflow for Oral Fluids.

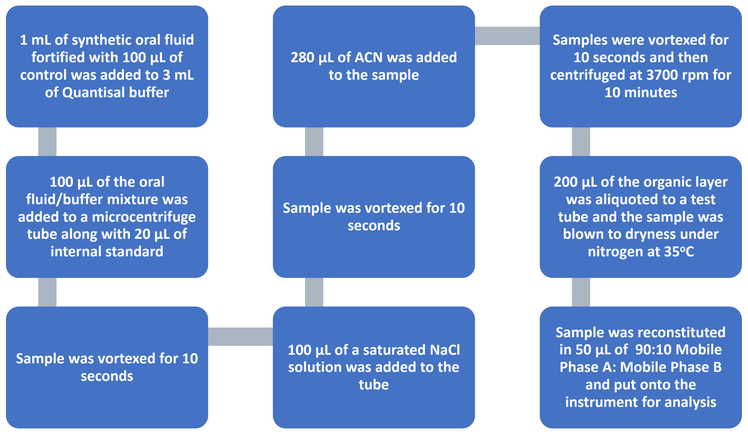
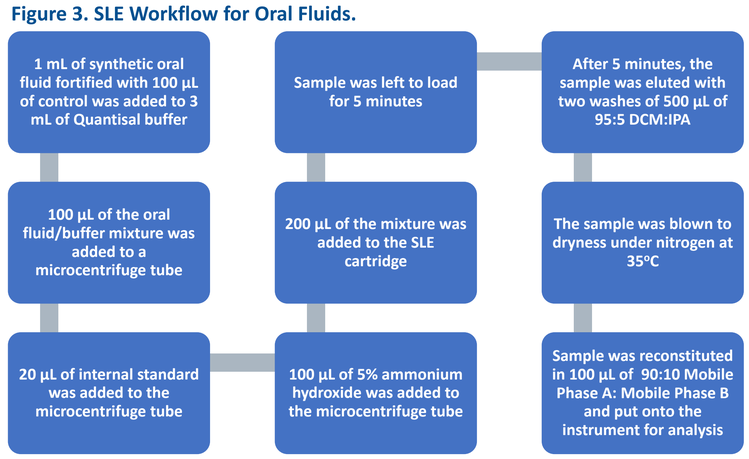


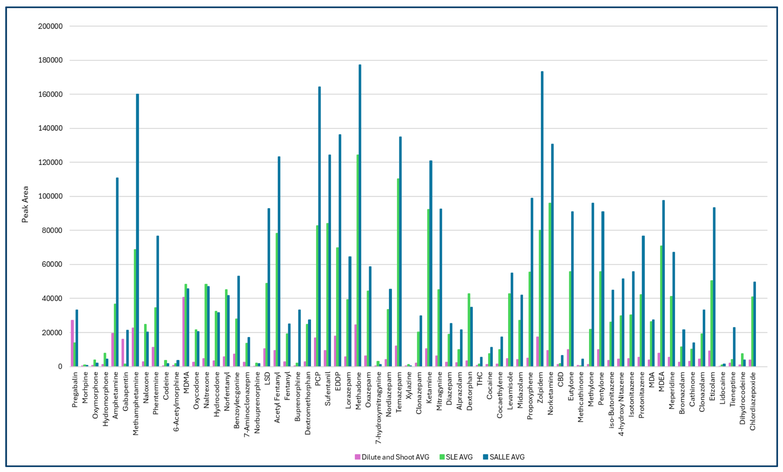
Figure 3. SLE Workflow for Oral Fluids.



Effects of Sample Clean-Up

To directly compare each sample preparation technique, Low QC (10 ng/mL) samples were prepared using each of the sample preparation methods. Peak area for each analyte was collected and compared for each sample preparation technique. The analyte's response to the different techniques was greatly influenced by the analyte's properties. Overall, 100% of the analytes showed a vast improvement in recovery when using either SLE or SALLE as compared to dilute-and-shoot. These results can be found in Figure 4.

Figure 4. Comparison of Sample Preparation on Full List of Analytes.



Effects of Sample Clean-Up Cont.

Depending on analyte properties (polarity, pKa, etc.), some analytes responded better to either SLE or SALLE. Mitragynine (Kratom) showed a much better response when using SALLE over SLE; however, norfentanyl and other opiates showed better recovery when using SLE. These results can be found in Figure 5 and Figure 6, respectively.

Figure 5. Comparison of Sample Clean-up Techniques on Mitragynine.

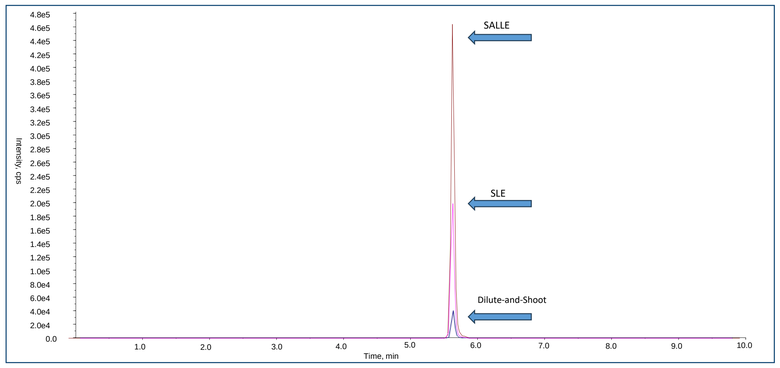
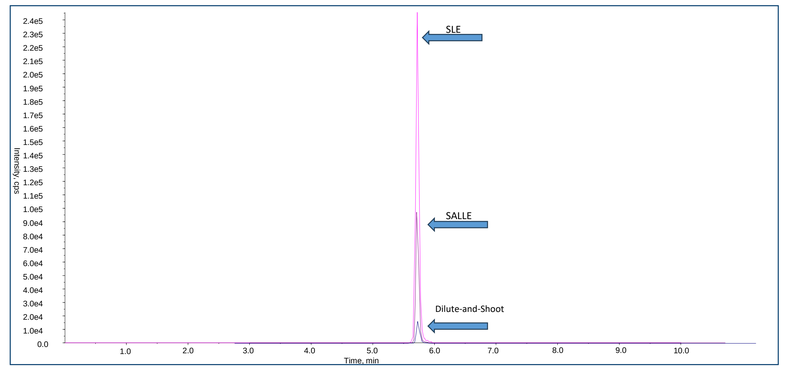


Figure 6. Comparison of Sample Clean-up Techniques on Norfentanyl.



Quantitation of Analytes

Chromatographic analysis of all 68 analytes was achieved in a 10- minute cycle time (Figure 1). This includes separation of all 8 sets of isobars, achieving a resolution of 1.5 or higher, ensuring accurate quantitation of these analytes.

Linearity: Using 1/x weighted linear regression, the analytes showed acceptable linearity with r^2 values of 0.99 or greater.

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 varied for all analytes based working limits of detection.

Conclusions

- Analysis of 68 DoA and NPS in oral fluids in 10-minute cycle time with resolution of 8 sets of isobars
- Accurate quantitation of analytes
- Comparison of sample preparation techniques on a broad list of analytes
- SALLE works better for full list of analytes
- Specific SLE workflow works better for opiates
- Both sample preparation techniques show increased recovery compared to dilute-and-shoot

References

1. Valente, Ines Maria, et. al. Another glimpse over the salting-out assisted liquid-liquid extraction in acetonitrile/water mixtures. *Journal of Chromatography A.* 2013, 1308, 58-62.
2. Majors, Ronald E. Salting-Out Liquid-Liquid Extraction (SALLE). *LCGC International.* 2009. 27, 7. 526-533.