

APPLICATIONS

Faster Extraction of a Comprehensive Drug Research Panel from Oral Fluid

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When not in the lab, Sean enjoys just about anything involving the outdoors: hiking, climbing, surfing, etc. He is especially at home in the mountains, being an avid skier and motorcyclist.

Overview

- Single cartridge extraction from oral fluid
- Good recovery for most analytes of interest

Introduction

Among various biological test matrices, oral fluid collection is emerging as the most preferred and economic over other matrices, such as serum and urine. Oral fluid, not only ensures the lowest chance of adulteration or substitution, but simplifies specimen collection process. Manufacturers are responding to the demand by introducing various oral fluid collection devices that include a collection applicator and a preservative solution (or device buffer solution).

The device's buffer solution contains many excipients such as antibacterial agents and surfactants to increase the analytes stability during the sample transit to testing laboratories. In addition, a blue dye for volume adequacy is used to indicate that the correct amount of oral fluid is present in the device.

The buffer solution poses challenges, such as ion suppression for LC-MS analysis and possible buildup on the mass spec if not removed from the samples before injection on mass spectrometer. In this technical note, we demonstrate a single cartridge solution targeted toward a comprehensive drug research panel that yields reproducible and consistent recovery while minimizing the detrimental effects of the device buffer solution.

Materials and Methods

Reagents and Chemicals

Analytical reference standards, internal standards and human saliva were purchased from Cerilliant[®] Corporation (Round Rock, TX, USA) and BioreclamationIVT (Chastertown, MD, USA) respectively. The Intercept i2[®] Oral fluid collection device was obtained from Orasure Technologies, Inc. (Bethlehem, PA). All other chemicals, were obtained from Sigma-Aldrich[®] (St. Louis, MO). Ultrapure DI water was obtained from Sartorius arium[®] comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

Experimental Conditions

Sample Pre-treatment

Transfer 1 mL oral fluid collected on an applicator tip in its preservative buffer. Leave it for at least 2 hours. Remove plastic nipple at end of transport tube, place in centrifuge tube and centrifuge at 600 g for 15 min. Remove 0.5 mL of supernatant and combine with 1 mL 1 % Formic acid in DI Water, mix/vortex 5-10 secs to load on Strata[®]-X-CW 30mg 96-Well Plate.

Solid Phase Extraction (SPE) Protocol

96-Well Plate:	Strata-X-CW 33 µm Polymeric Weak Cation, 30 mg / well
Part No.:	8E-S035-TGB
Condition:	1 mL of Methanol
Equilibrate:	1 mL of DI Water
Load:	1.5 mL Pre-treated sample
Wash 1:	1 mL 1 % Formic acid in DI Water
Wash 2:	1 mL DI Water
Dry:	5 to 10 minutes at max vacuum (or positive pressure)
Elute:	2x 500 µL Methylene chloride/Isopropanol/30 % Ammonium hydroxide (80:18:2)
Dry down:	Evaporate eluate to dryness @ 45-50 °C under a gentle stream of Nitrogen
Reconstitute:	200 µL of mobile phase A (0.1 % Formic acid in Water)

LC Conditions (ESI+)

Column:	Kinetex [®] 2.6 µm Biphenyl 100 Å	
Dimension:	50 x 3.0 mm	
Part No.:	00B-4622-Y0	
Recommended Guard:	SecurityGuard [™] ULTRA Biphenyl	
Guard Part No.:	AJ0-9208	
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol	
Gradient:	Time (min)	%B
	0	10
	4	95
	5.5	95
	5.51	10
	7	10
Flow Rate:	0.5 µL/min	
Temperature:	Ambient	
Injection Volume:	5 µL	
Instrument:	Agilent [®] 1260 LC	
Detector:	MS/MS (SCIEX API 5000 [™]) ESI+	

LC Conditions (ESI-)

Column:	Kinetex 2.6 µm Biphenyl 100 Å	
Dimension:	50 x 3.0 mm	
Part No.:	00B-4622-Y0	
Recommended Guard:	SecurityGuard ULTRA Biphenyl	
Guard Part No.:	AJ0-9208	
Mobile Phase:	A: 10 mM Ammonium formate B: Methanol	
Gradient:	Time (min)	%B
	0	50
	0.2	50
	2.5	95
	4	95
	4.1	50
	5	50
Flow Rate:	0.6 µL/min	
Temperature:	Ambient	
Injection Volume:	10 µL	
Instrument:	Agilent 1260 LC	
Detector:	MS/MS (SCIEX API 5000) ESI-	



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Table 1.
Percent Recovery for All Analytes in a Comprehensive Drug Research Panel

Analyte panel	% Absolute recovery	% CV (N=4)	Ionization
6-MAM	79	7.2	ESI+
α-Hydroxyalprazolam	88	6.8	ESI+
Alprazolam	79	7.7	ESI+
Amitriptyline	57	11.6	ESI+
Amphetamine	94	9.8	ESI+
Benzoyllecgonine	89	3.9	ESI+
Buprenorphine	63	5.5	ESI+
Carisoprodol	95	4.8	ESI+
Citalopram	81	3	ESI+
Cocaine	84	11.2	ESI+
Codeine	89	5	ESI+
Diazepam	74	5.7	ESI+
EDDP	72	5.7	ESI+
Fentanyl	74	4.8	ESI+
Fluoxetine	42	7.7	ESI+
Hydrocodone	102	10	ESI+
Hydromorphone	98	6.6	ESI+
Imipramine	64	10.5	ESI+
Lorazepam	69	3.8	ESI+
MDMA	67	16.1	ESI+
Meperidine	84	8.9	ESI+
Mephedrone	84	8.9	ESI+
Meprobamate	92	13	ESI+
Methadone	72	6.6	ESI+
Methamphetamine	81	5.4	ESI+
Morphine	56	15.5	ESI+
Naloxone	83	6.7	ESI+
Norbuprenorphine	87	11	ESI+
Nordiazepam	75	5.5	ESI+
Norfentanyl	85	7	ESI+
Norhydrocodone	89	3	ESI+
Noroxycodone	85	4.6	ESI+
Nortriptyline	48	1.6	ESI+
Oxycodone	84	7.1	ESI+
Oxymorphone	89	17.1	ESI+
Paroxetine	42	8.8	ESI+
PCP	84	3.6	ESI+
Ritalinic Acid	47	19.9	ESI+
Tapentadol	83	7.5	ESI+
Temazepam	86	2.8	ESI+
Tramadol	87	0.8	ESI+
Zolpidem	64	1	ESI+
Butalbital	71	6	ESI-
Secobarbital	82	5	ESI-
Phenobarbital	83	3.4	ESI-
THC-COOH	53	5.7	ESI-

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Figure 1.
Representative TIC of Comprehensive Drug Research Panel Run under ESI+

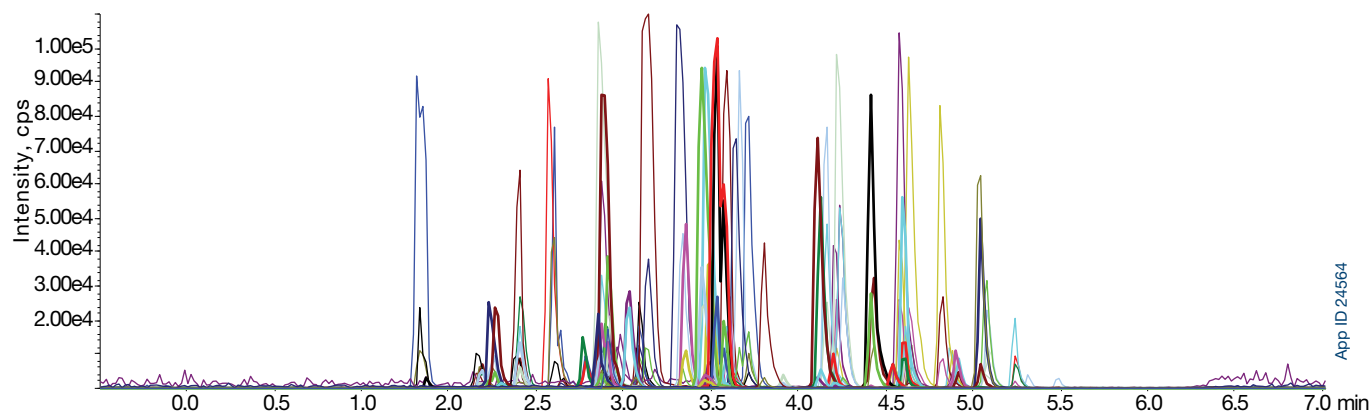
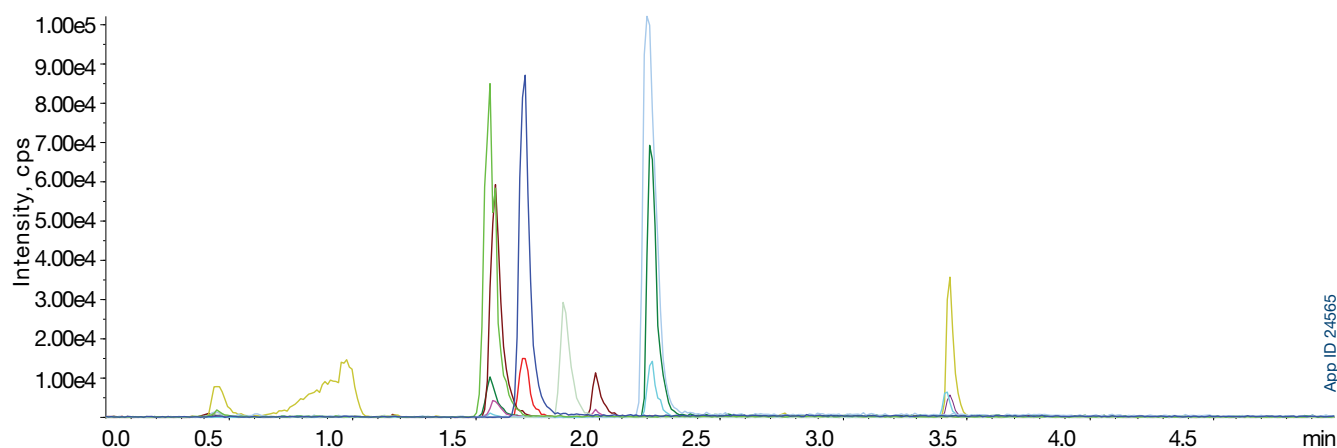


Figure 2.
Representative TIC of Comprehensive Drug Research Panel Run under ESI-



Discussion

A Kinetex[®] 2.6 μ m Biphenyl 50 x 3.0mm LC column was selected for analysis under both positive and negative ionization (as appropriate) for the comprehensive drug research panel consisting of 46 analytes analyzed in under 4 and 5 minute run time respectively (**Figure 1, 2**). The absolute recoveries obtained for the analytes on the average are more than 77% (**Table 1**). The developed method is a generic approach, focused toward 'one method fits all' strategy. No attempt was made to apply a strong organic wash to clean the unwanted matrix impurities. However, the extraction conditions were designed to capture the buffer and other excipients from the matrix, and at the same time, dislodge the analyte from the SPE phase in one simple elution step. The elution condition is selective toward retention of all unwanted impurities on the SPE chemistry that suffice the blue indicator dye

(from applicator tip) as well. While the developed method is not as selective as our previous technical note (which utilized both Strata[®]-X-A and Strata-X-C) the main objective of this method was to simplify the sample prep, emphasizing time-savings and associated cost.¹

Conclusion

In this technical note, we demonstrated a fast oral fluid extraction with good recovery for most analytes in a large research panel. The recommended sample prep is a single method that virtually needs no method development.

References

1. Huq, S.; Sadjadi, S.; "A Superior Sample Preparation of Comprehensive Drug Panel Analytes from Oral Fluid Collection Devices; TN-0100, Phenomenex, Inc.



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Ordering Information

Kinetex[®] Core-Shell LC Columns

2.6 µm Minibore Columns		SecurityGuard [™] ULTRA Cartridges*
Phase	50 x 3.0 mm	3/pk
Biphenyl	00B-4622-Y0	AJ0-9208

*SecurityGuard ULTRA Cartridges require holder; Part No.: AJ0-9000

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Strata[®]-X-C Solid Phase Extraction

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S029-TAK**	1 mL (100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ**	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)

Giga[™] Tube

	500 mg	8B-S029-HDG	12 mL (20/box)
	1 g	8B-S029-JDG	12 mL (20/box)
	1 g	8B-S029-JEG	20 mL (20/box)
	2 g	8B-S029-KEG	20 mL (20/box)
	5 g	8B-S029-LFF	60 mL (16/box)

96-Well Plate

	10 mg	8E-S029-AGB	2 Plates/Box
	30 mg	8E-S029-TGB	2 Plates/Box
	60 mg	8E-S029-UGB	2 Plates/Box

96-Well Microelution Plate

	2 mg	8M-S029-4GA	ea
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**Tab-less tubes available. Contact Phenomenex for details.

Presston[™] 100 Positive Pressure Manifold

Part No.	Description
AHO-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate
AHO-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly
AHO-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly
AHO-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly

Presston 100 Tube Adapter Kits (for AHO-9334)

Part No.	Description
AHO-9344	1 mL Tube Adapter Kit
AHO-9345	3 mL Tube Adapter Kit
AHO-9346	6 mL Tube Adapter Kit



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