Improved Extraction of Pain Management and Illicit Drugs from Saliva using Popular Oral Fluid Collection Devices, Strata™-X-Drug B Solid Phase Extraction (SPE), and Kinetex® Core-Shell Phenyl-Hexyl HPLC/UHPLC Columns

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As the requirements for the analysis of controlled substances become ever greater, the need to make sampling quick, simple and reliable has also grown. The collection of oral fluid is gaining popularity due to the fact that it is not intrusive and it reduces the skill level requirement of the person taking the sample. Currently a consensus has not been reached on a device that should be used for sample collection. In this study we look at four different devices, and compare the results obtained for drugs varying in terms of hydrophobicity and their acidity/basicity.

Introduction

The testing of performance enhancing drugs and illegal substances in general has advanced dramatically over the last 10 years, thanks mainly to advances in instrumentation such as LC/MS/MS. This has allowed for more rapid screening of drugs such as opiates, amphetamines, benzodiazepines, and THC. Samples of blood and urine are routinely collected to establish compliance with legal limits or to monitor patient compliance.[1-3] Oral fluid sampling is now gaining popularity as it is a less invasive technique in terms of subject privacy meaning it can be conducted with less risk of sample adulteration or sample substitution. Additionally there is a decreased risk of infection compared to blood or urine sampling. There are an increasing number of sampling devices for oral fluids, some of which contain preservatives or stabilizing buffers. The choice of Oral Fluid Collector (OFC) can lead to differences in sample integrity and can potentially affect downstream analytical results.[4] This study was set up to compare a number of popular Oral Fluid Collection (OFC) devices and to develop a fast and effective solid phase extraction (SPE) method to allow for enhanced sample processing prior to LC/MS/MS analysis. The OFC devices contained different components that require adjustments on the load and wash steps of the general SPE protocol previously developed for other matrices. The probe compounds used for this work were a combination of acids, bases, and some neutral species. The hydrophobicity of these compounds (logP) ranged from 0.78 (oxymorphone) to 5.94 (Δ9-tetrahydrocannibinol). Following the SPE protocol HPLC was conducted using a high efficiency Kinetex 2.6 µm Phenyl-Hexyl core-shell column, utilizing an AB SCIEX API 5000™ detector for MS/MS analysis.

Experimental Conditions

SPE Conditions

Cartridge: Strata-X-Drug B, 30 mg/3 mL
Part No.: 8B-S128-TBJ

For SPE procedures for basic and acidic analytes see **Table 4**.

HPLC Conditions

Column: Kinetex 2.6 µm Core-Shell Phenyl-Hexyl
Dimensions: 50 x 4.6 mm
Part No.: 00B-4495-E0
Mobile Phase: A: 10 mM Ammonium formate
B: 0.1 % Formic Acid in Methanol

Gradient:	**Time (min)**	**B (%)**
	0	5
	4	100
	6	100

Flow Rate: 0.6 mL/min
Temperature: Ambient
Detection: API 5000 MS/MS (AB SCIEX)
Injection Volume: 1 µL
Instrument: Agilent® 1200

Alternative HPLC Conditions

Column: Kinetex 2.6 µm Core-Shell Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic Acid in Methanol

Gradient:	**Time (min)**	**B (%)**
	0	10
	2.5	100
	3.5	100
	3.51	10
	5.0	10

Flow Rate: 0.7 mL/min

Table1.
Analyte classes

Classes	Analyte
Benzodiazepines	Alprazolam
	Diazepam
	Flunitrazepam
	Flurazepam
	Lorazepam
	Oxymorphone
Opiates	Codeine
	Hydrocodone
	Morphine
THC & Metabolites	THC-COOH
	THC-OH
	THC
	AM694
	JWH-018
	JWH-018(5hp)
	JWH-073
	JWH-073(3hb)
Analgesics	Tramadol
	Buprenorphine
	Norbuprenorphine

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Table 2.
List of oral fluid collection (OFC) devices and their constituents

OFC Device	Preservative	Applicator	Collection Tube
Product O	Yes	Yes	Yes
Product Q	Yes	Yes	Yes
Product C	No	Yes	No
Product N	No	No	Yes

Table 3.
MS/MS conditions for acids

ID	Q1 Mass	Q3 Mass	DP (V)	CE (V)
THC-C00H	345.4	299.1	70	26
	345.2	327	55	29
AM694	436.1	231	50	39
	436.1	309.2	50	31
JWH 018	342.2	155.1	70	36
	342.2	214.2	70	31
JWH 018-(5hp)	358.3	155.2	70	31
	358.3	230.2	70	31
JWH 073	328.2	155.2	70	33
	328.2	200.2	70	31
JWH 073-(3hb)	344.2	155.2	70	29
THC-OH	331.4	193.1	100	33
	331.4	201.2	100	31
THC	315.2	193.2	60	35
	315.2	259.2	60	20
THC-D3	318	196.5	85	32
	318	123.1	90	41
JWH-073-D5	363.1	155.1	70	33

Table 4.
SPE procedures for basic and acidic analytes

	Basic Analytes	Acidic Analytes
Sample Pre-treatment	1. Place the applicator swab in 500 µL oral fluid, spiked with analyte mix 2. Place it in cartridge containing preservative buffer 3. Add 1 mL 1 % Formic acid / Methanol (1:1) 4. Add 1.5 mL 100 mM Sodium acetate buffer, pH 5.0 5. Transfer contents (after centrifugation) on the SPE cartridge	1. Place the applicator swab in 500 µL oral fluid, spiked with analyte mix 2. Place it in cartridge containing preservative buffer 3. Add 1 mL Acetonitrile / 100 mM Sodium acetate buffer, pH 5.0 (30:70) 4. Transfer contents (after centrifugation) on the SPE cartridge
Condition*	1 mL Methanol followed by 1 mL 100 mM Sodium acetate buffer, pH 5.0	1 mL Methanol followed by 1 mL Acetonitrile / 100 mM Sodium acetate buffer pH 5.0 (30:70)
Load	Pre-treated sample	Pre-treated sample
Wash 1	1 mL 100 mM Sodium acetate buffer, pH 5.0	1 mL 100 mM Sodium acetate buffer, pH 5.0
Wash 2	1 mL 30 % Methanol in water	1 mL Acetonitrile / 100 mM Sodium acetate buffer pH 5.0 (30:70)
Dry	5 min under 10-20" Hg vacuum	5 min under 10-20" Hg vacuum
Elute	2x 0.5 mL Methanol / Acetonitrile (1:1) + 5 % NH_4OH (28-30 %). Add 30 µL 0.1 % HCl in methanol.	2x 0.5 mL Ethylacetate / Isopropanol (85:15)
Dry Down	To dryness under N_2 (at 40-45 ˚C)	To dryness under N_2 (at 40-45 ˚C)
Reconstitute	500 µL of mobile phase (A/B, 85:15) spiked with deuterated internal standard	In 500 µL of mobile phase (A/B, 55:45) spiked with deuterated internal standard

* Strata-X-Drug sorbents do not require a condition/equilibration step. This step may be eliminated to provide further time and solvent savings.

Table 5.
Recovery of basic drugs from various OFC devices vs. SPE

Peak No.	Analyte	Product O	Product Q	Product C	Product N	No Device* (SPE only)
1	Morphine	89	79	76	82	84
2	Oxymorphone	89	81	76	89	84
3	Codeine	89	82	85	92	98
4	Hydrocodone	88	84	86	76	99
5	Tramadol	87	83	82	90	83
6	Norbuprenorphine	95	80	55	101	99
7	Buprenorphine	89	85	52	102	105
8	Flurazepam	89	88	80	92	93
9	Lorazepam	88	86	26	91	75
10	Flunitrazepam	79	89	30	95	75
11	Alprazolam	78	89	51	87	77
12	Diazepam	81	81	30	90	75
	% RSD range	0.8 - 8%	0.4 – 11%	4 – 13%	1.9 – 8%	0.6 – 11%

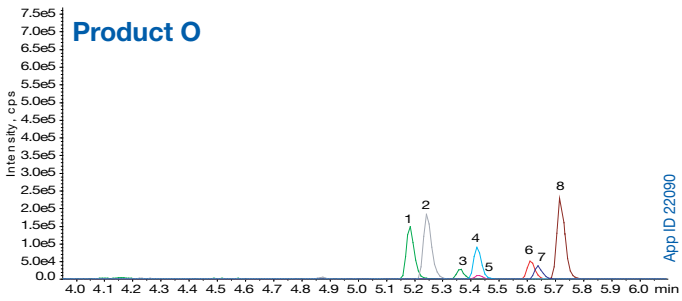
* Oral fluid passed through SPE cartridge without processing through the OFC device

Table 6.
Recovery of acidic drugs from various OFC devices vs. SPE

Peak No.	Analyte	Product O	Product Q	Product C	Product N	No Device* (SPE only)
1	JWH 073 (3hb)	56	74	4	68	74
2	JWH 018 (5hp)	58	73	3	70	78
3	THC-OH	38	55	10	65	81
4	AM694	50	70	3	73	80
5	THC-COOH	43	56	34	63	70
6	JWH 073	48	61	3	83	79
7	THC	25	37	3	64	83
8	JWH 018	42	58	2	81	87
	% RSD range	5 – 15%	2 – 10%	2 – 11%	2 – 11%	0.5 – 9%

* Oral fluid passed through SPE cartridge without processing through the OFC device

Figure 1.
Representative LC/MS/MS chromatograms of acidic drugs using the OFC devices



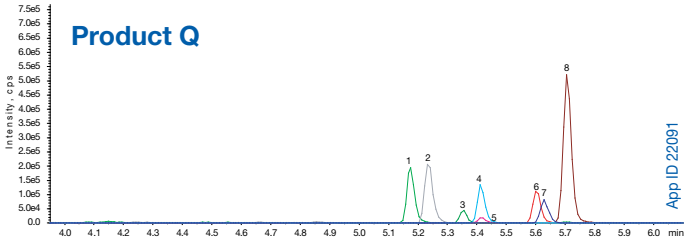
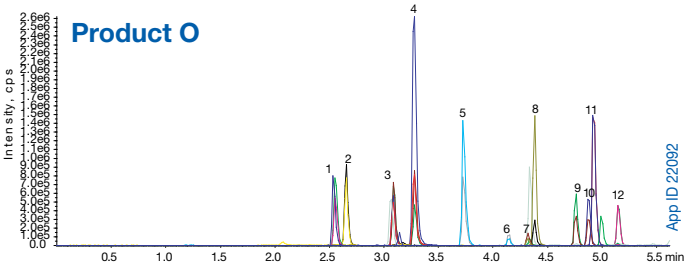
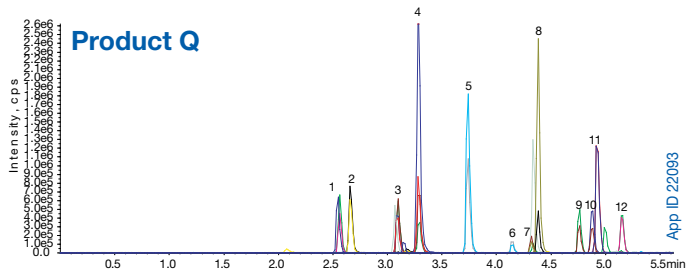


Figure 2.
Representative LC/MS/MS chromatograms of basic drugs using the OFC devices





Results and Discussion

Feasibility of the use of the OFC devices

- Based on recovery data and reproducibility, most OFC devices performed well for basic drugs (**Table 5**).
- Some acidic analytes displayed poor recovery data (**Table 6**) which could be attributed to the strong retention of the analyte to the applicator swab and/or other device component.
- Among the devices, Product C (no preservative buffer) and Product N (no buffer and no swab) are considered incomplete. With storage and sample collection being such critical issues, these two failed to meet the requirements of proper sample collection.
- Product N (device contains collection tube only) shows fair recovery for most of the compounds, but it is a mere reflection of performing SPE alone.
- Product Q shows the best performance when extracting these wide range test analytes from each component used in the device and yields a cleaner and ultrapure extract.

Conclusion

In this work, we present a viable and yet simple SPE method for the analysis of a wide range of drugs, using oral fluid collection as the matrix. Data generated for a number of collection devices have been compared to show their relative capacity and ease of use in the analysis and screening of the most commonly used illicit drugs.

References

1. Forensic Science International, vol. 219 (page 165-171), 2012
2. Forensic Science International, vol. 164 (page 126-130), 2006
3. Forensic Science International, vol. 227 (page 69-73), 20134.
4. Journal of Analytical toxicology vol 32 July/August 2008.

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Ordering Information

KINETEX® 2.6 µm COLUMNS (mm)							SecurityGuard™ ULTRA Cartridges‡		
Phases	**50 x 2.1**	**100 x 2.1**	**50 x 3.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**3/pk**	**3/pk**	**3/pk**
Phenyl-Hexyl	00B-4495-AN	00D-4495-AN	—	00B-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8788	AJ0-8781	AJ0-8774
Biphenyl	00B-4622-AN	00D-4622-AN	00B-4622-Y0	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9209	AJ0-9208	AJ0-9207
							for 2.1 mm ID	for 3.0 mm ID	for 4.6 mm ID

KINETEX 5 µm COLUMNS (mm)							SecurityGuard™ ULTRA Cartridges‡		
Phases	**50 x 2.1**	**50 x 3.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**	**3/pk**	**3/pk**
Phenyl-Hexyl	00B-4603-AN	—	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8788	AJ0-8781	AJ0-8774
Biphenyl	00B-4627-AN	00B-4627-Y0	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9209	AJ0-9208	AJ0-9207
							for 2.1 mm ID	for 3.0 mm ID	for 4.6 mm ID

‡SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000.

Strata™-X-Drug B SPE

Sorbent Mass	Part No.	Unit
Tube		
10 mg	8B-S128-AAK	1 mL (100/box)
10 mg	8L-S128-AAK†	1 mL (100/box)
30 mg	8B-S128-TAK	1 mL (100/box)
30 mg	8L-S128-TAK†	1 mL (100/box)
30 mg	8B-S128-TBJ	3 mL (50/box)
60 mg	8B-S128-UBJ	3 mL (50/box)
60 mg	8B-S128-UCH	6 mL (30/box)
60 mg	8B-S128-UCL	6 mL (200/bag)
Giga™ Tube		
100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate		
10 mg	8E-S128-AGB	2 Plates/Box
30 mg	8E-S128-TGB	2 Plates/Box
60 mg	8E-S128-UGB	2 Plates/Box

† Tab-less tube

Accessories

Collection Plates (deep well, polypropylene)		
AH0-7192	96-Well Collection Plate 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
Sealing Mats		
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk
Vacuum Manifolds		
AH0-6023*	SPE 12-Position Vacuum Manifold Set, for tubes	ea
AH0-6024*	SPE 24-Position Vacuum Manifold Set, for tubes	ea
AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea

*Manifolds include: Vacuum-tight glass chamber, vacuum gauge assembly, polypropylene lid with gasket, male and female luers and yellow end plugs, stopcock valves, collection rack assemblies, polypropylene needles, lid support legs. Waste container included with 12-positive manifold.

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