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A Superior Sample Preparation of Comprehensive Drug Panel Analytes from Oral Fluid Collection Devices

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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

- *High recovery of multiple drug classes including amphetamines, benzodiazepines, opioids, drugs of abuse, barbiturates, and THC metabolites*
- *Great recovery of acidic, basic, and neutral compounds*
- *Near complete removal of excipients from collection device buffer solution*

Introduction

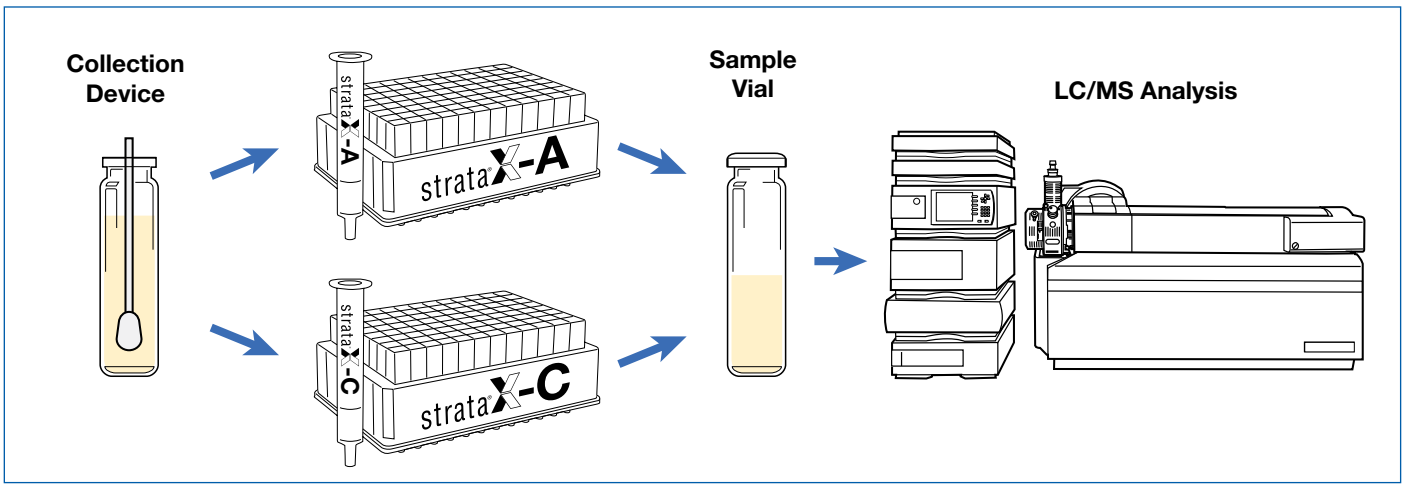
Drug testing in oral fluid has steadily gained popularity over other sample types such as urine and whole blood. One reason for this popularity is the easy non-intrusive sample collection procedure. Collection of oral fluid is especially suited for the road-side or work place drug screening where access to a proper medical facility or personnel may be limited. To address this increasing need, many companies are manufacturing oral fluid collection devices with a collection applicator and a preservative (or extraction) solution. The device buffer solution contains a number of excipients such as antibacterial agents and surfactants to prevent bacterial growth and increase the analytes stability during the sample transit to testing laboratories. The buffer solution poses challenges, such as ion suppression for LC/MS analysis. Here, we present a sample preparation procedure to significantly reduce the effects of the device buffer solution while maintaining a reproducible and consistent recovery of analytes.

Materials and Methods

Reagents and Chemicals

Analytical reference standards and human saliva were purchased from Cerilliant (Round Rock, TX, USA) and BioreclamationIVT (Chastertown, MD, USA) respectively. The Intercept i2® and Quantisal® oral fluid collection devices were obtained from OraSure Technologies, Inc. (Bethlehem, PA) and Immunanalysis Corporation (Pomona, CA). All other chemicals were obtained from the Sigma-Aldrich Company (St. Louis, MO). Water purification via Sartorius Arium® Comfort II (Goettinger, Germany)

Analysis Workflow



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Sample Preparation

Sample Collection

Oral fluid specimens (from both Quantisal® and Intercept i2® devices) were collected by placing the cellulose pad (on a plastic stick) orally until the indicator window turns blue. The saturated pad on the stick is then placed into the transport tube containing the buffer solution.

Sample Pretreatment

For Intercept i2 device	Remove plastic nipple at end of transport tube, place in centrifuge tube and centrifuge at 600 g for 15 min to collect the supernatant. Transfer 0.5 mL of it into a vial to perform SPE extraction as below.
For Quantisal collection device	Gently vortex the transport tube for 5-10 seconds before transferring 0.5 mL to a vial for SPE extraction. If solution is left to settle for 1-2 minutes, centrifugation is not necessary.

SPE Method

Step	Basic analyte extraction	Acidic analyte extraction
Product Name:	Strata®-X-C, 30 mg in 3 mL cartridge	Strata-X-A, 30 mg in 3 mL cartridge
Catalog Number:	8B-S029-TBJ	8B-S123-TBJ
Condition:	1 mL 100 % Methanol	1 mL 100 % Methanol
Equilibrate:	1 mL DI Water	1 mL DI Water
Load:	Combine 0.5 mL of pretreated sample with 1 mL 1 % Formic acid, mix/vortex 5-10 sec and load on Strata-X-C.	Combine 0.5 mL of pretreated sample with 1 mL 1 % Ammonium hydroxide, mix/vortex 5-10 sec and load on Strata-X-A.
Weak Wash:	1 mL DI Water	1 mL DI Water
Strong Wash:	1 mL 50:50 Acetone: Water	1 mL 50:50 Acetone : Water
Dry down:	3–4 minutes at maximum vacuum (15” Hg or higher)	3–4 minutes at maximum vacuum (15” Hg or higher)
Elute:	2 x 500 µL Methanol:Acetonitrile:Ammonium hydroxide (5:5:2)	2 x 500 µL Methanol:Acetonitrile:Formic acid (50:50:5)
Dry down:	Evaporate to dryness under gentle Nitrogen and 45-50 °C	Evaporate to dryness under gentle Nitrogen and 45-50 °C
Reconstitute:	With 125 µL initial MP	With 125 µL initial MP
	Combine into a single sample vial	

LC/MS Conditions

Positive ESI Panel

Column: Kinetex® 2.6 µm Biphenyl
Dimension: 50 x 3.0 mm
Part No.: 00B-4622-Y0
SecurityGuard™ Cartridge: AJ0-9208
SecurityGuard Holder: AJ0-9000
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol

Gradient:

Time (min)	%B
0.0	10
4.0	95
5.5	95
5.51	10
7.5	10

Flow Rate: 500 µL/min
Temperature: Ambient
Injection Volume: 10 µL
Detector: SCIEX API 5000™
Detection Mode: ESI+

Negative ESI Panel

Column: Kinetex 2.6 µm Biphenyl
Dimension: 50 x 3.0 mm
Part No.: 00B-4622-Y0
SecurityGuard Cartridge: AJ0-9208
SecurityGuard Holder: AJ0-9000
Mobile Phase: A: 10 mM Ammonium formate in Water
B: 100 % Methanol

Gradient:

Time (min)	%B
0.0	10
4.0	95
5.0	95
5.01	10
7.0	10

Flow Rate: 500 µL/min
Temperature: Ambient
Injection Volume: 10 µL
Detector: SCIEX API 5000
Detection Mode: ESI-

Results

Figure 1.
Representative TIC of ESI+ for Comprehensive Drug Panel Analytes

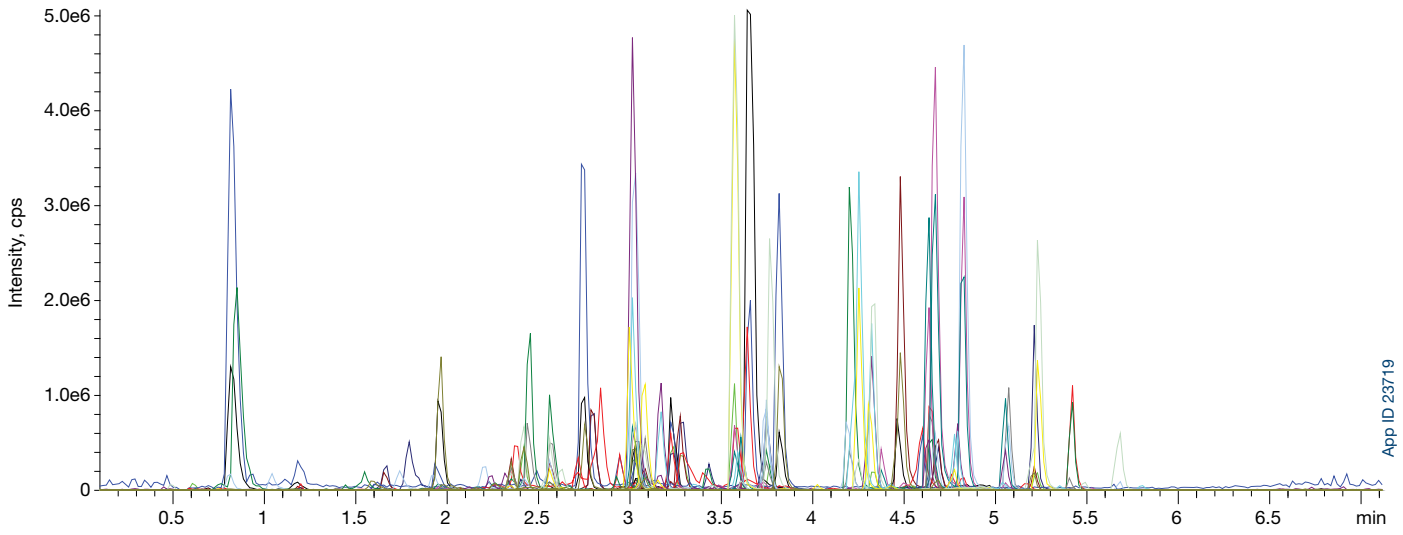

Figure 2.
Representative TIC of ESI- for Comprehensive Drug Panel Analytes

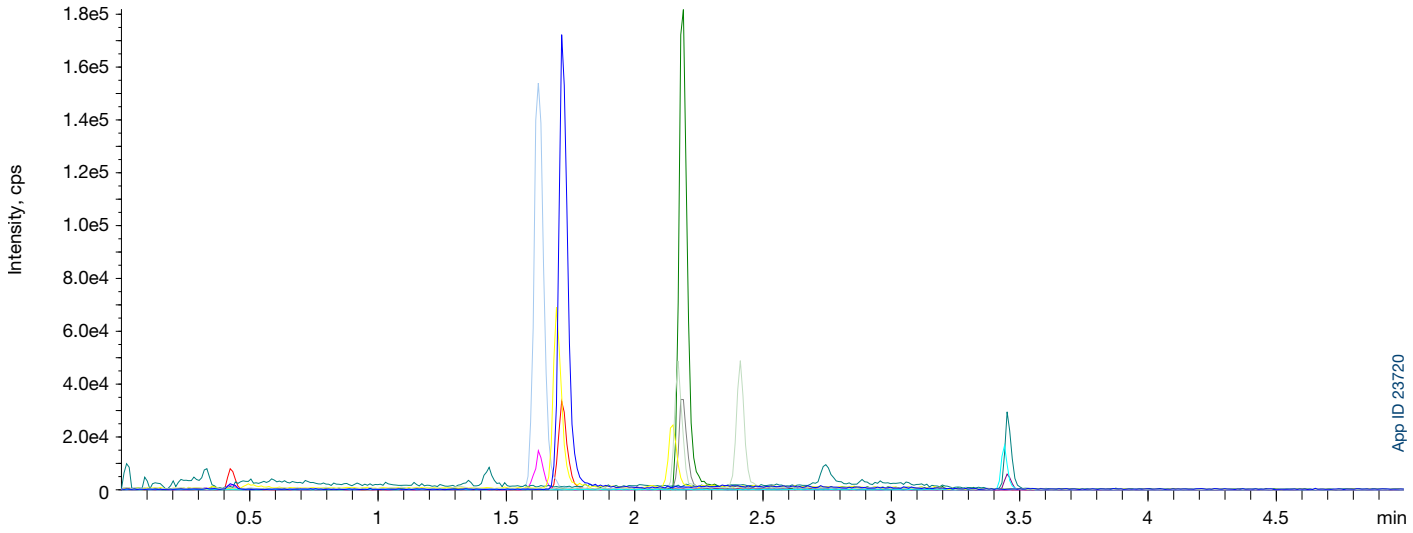

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Table 1.
Absolute recovery with extraction condition and ionization for comprehensive drug panel analytes from both devices (Acids on Strata®-X-A, Bases and Neutrals on Strata-X-C).

	Analyte	Extraction Cartridge	Ionization	Quantisal® %Rec (%CV)	Intercept i2® %Rec (% CV)
1	6-MAM	Strata-X-C	ESI+	67 (2.6)	79 (5.0)
2	7-Aminoclonazepam	Strata-X-C	ESI+	54 (6.6)	45 (2.8)
3	α-Hydroxyalprazolam	Strata-X-C	ESI+	54 (1.5)	69(8.9)
4	Alprazolam	Strata-X-C	ESI+	58 (5.0)	76 (7.1)
5	Amitriptyline	Strata-X-C	ESI+	66 (5.6)	59 (12.8)
6	Amphetamine	Strata-X-C	ESI+	65 (4.2)	60 (5.7)
7	Benzoylecgonine	Strata-X-C	ESI+	58 (0.9)	82 (4.9)
9	Citalopram	Strata-X-C	ESI+	58 (3.1)	104 (5.1)
10	Codeine	Strata-X-C	ESI+	66 (8.6)	86 (11.3)
11	Fentanyl	Strata-X-C	ESI+	56 (3.8)	84 (8.7)
12	Fluoxetine	Strata-X-C	ESI+	47 (2.7)	67 (12.0)
13	Gabapentin	Strata-X-C	ESI+	67 (5.8)	86 (6.0)
14	Hydrocodone	Strata-X-C	ESI+	57 (4.0)	68 (7.3)
15	Hydromorphone	Strata-X-C	ESI+	49 (8.0)	63 (3.6)
16	Imipramine	Strata-X-C	ESI+	57 (0.5)	76 (7.4)
17	Lorazepam	Strata-X-A	ESI+	64 (5.5)	89 (7.4)
18	MDMA	Strata-X-C	ESI+	54 (2.7)	72 (10.0)
19	Meperidine	Strata-X-C	ESI+	52 (2.7)	57 (7.3)
21	Methadone	Strata-X-C	ESI+	80 (1.8)	85 (1.2)
22	Methamphetamine	Strata-X-C	ESI+	66 (2.1)	73 (6.5)
23	Cotinine	Strata-X-C	ESI+	52 (7.7)	62 (9.9)
24	Diazepam	Strata-X-C	ESI+	54 (4.0)	75 (5.4)
25	EDDP	Strata-X-C	ESI+	46 (8.0)	67 (13.8)
26	Methylphenidate	Strata-X-C	ESI+	70(4.1)	72 (7.5)
27	Morphine	Strata-X-C	ESI+	64 (2.2)	82 (3.3)
28	Nordiazepam	Strata-X-C	ESI+	53 (2.4)	81 (5.5)
29	Norfentanyl	Strata-X-C	ESI+	58 (3.7)	86 (5.1)
30	Norhydrocodone	Strata-X-C	ESI+	57 (6.9)	76 (8.0)
31	Normorphine	Strata-X-C	ESI+	53 (18.3)	73 (15.0)
32	Noroxycodone	Strata-X-C	ESI+	52 (3.7)	67 (6.6)
33	Nortriptyline	Strata-X-C	ESI+	62 (13.4)	82 (5.4)
34	Methyltramadol	Strata-X-C	ESI+	74 (2.9)	89 (7.9)
35	Oxycodone	Strata-X-C	ESI+	55 (5.5)	65 (4.8)
36	Oxymorphone	Strata-X-C	ESI+	57 (5.8)	73 (9.5)
37	PCP	Strata-X-C	ESI+	65 (6.96)	87 (11.1)
38	Paroxetine	Strata-X-C	ESI+	53 (5.4)	73 (7.2)
39	Pregabalin	Strata-X-C	ESI+	73 (2.6)	86 (4.3)
40	Sertaline	Strata-X-C	ESI+	54 (17.4)	59 (2.6)
41	Tapentadol	Strata-X-C	ESI+	64 (5.3)	92 (2.7)
42	Temazepam	Strata-X-C	ESI+	50 (5.08)	54 (8.9)
43	Tramadol	Strata-X-C	ESI+	80 (4.4)	83 (3.3)
44	Zolpidem	Strata-X-C	ESI+	64 (2.4)	82 (4.3)
45	Zolpidem 4Carboxy	Strata-X-C	ESI+	60 (5.1)	82 (4.3)
46	Butalbital	Strata-X-A	ESI-	60 (1.2)	93 (1.5)
47	Secobarbital	Strata-X-A	ESI-	65 (8.1)	74 (9.5)
48	Phenobarbital	Strata-X-A	ESI-	68 (1.3)	77 (5.8)
49	THC-COOH	Strata-X-A	ESI-	80 (4.0)	63 (9.5)

Discussion

In this method, we sought to develop a clean extraction method with high recovery and minimal interference. One way to demonstrate the effectiveness of a sample preparation technique is to compare the total ion current of the extracted sample to the device buffer solution. We tested the buffer solutions from both collection devices well beyond the analyte window. A Q1 scan, performed on a SCIEX QTRAP® system, from 100 to 2,000 m/z revealed that in its untreated state both buffer solutions showed strong interference—specifically two clusters of peaks, one group in the mid-section of the chromatogram and another late-eluting group. On a closer examination, both groups of peaks displayed MS spectra consistent with homologues or polymeric species.

The intensity of interference peaks far exceeded the signal obtained from the injection of a 100 % Methanol, as shown in see **Figures 3** and **4**.

When comparing Q1 scan of neat solution to the extract from the method, interferences were successfully removed. There is strong evidence that removing buffer excipients improves the robustness of the LC/MS analysis. For further detail regarding our method development please find our MSACL 2016 poster at:

www.phenomenex.com/MSACLOralFluidPoster.

Figure 3.
Quantisal® representative LC/MS chromatogram of buffer solution and MS spectra of circled peaks. Blue trace: neat buffer solution, Green trace: Blank (Methanol), and Red trace: Extracted sample.

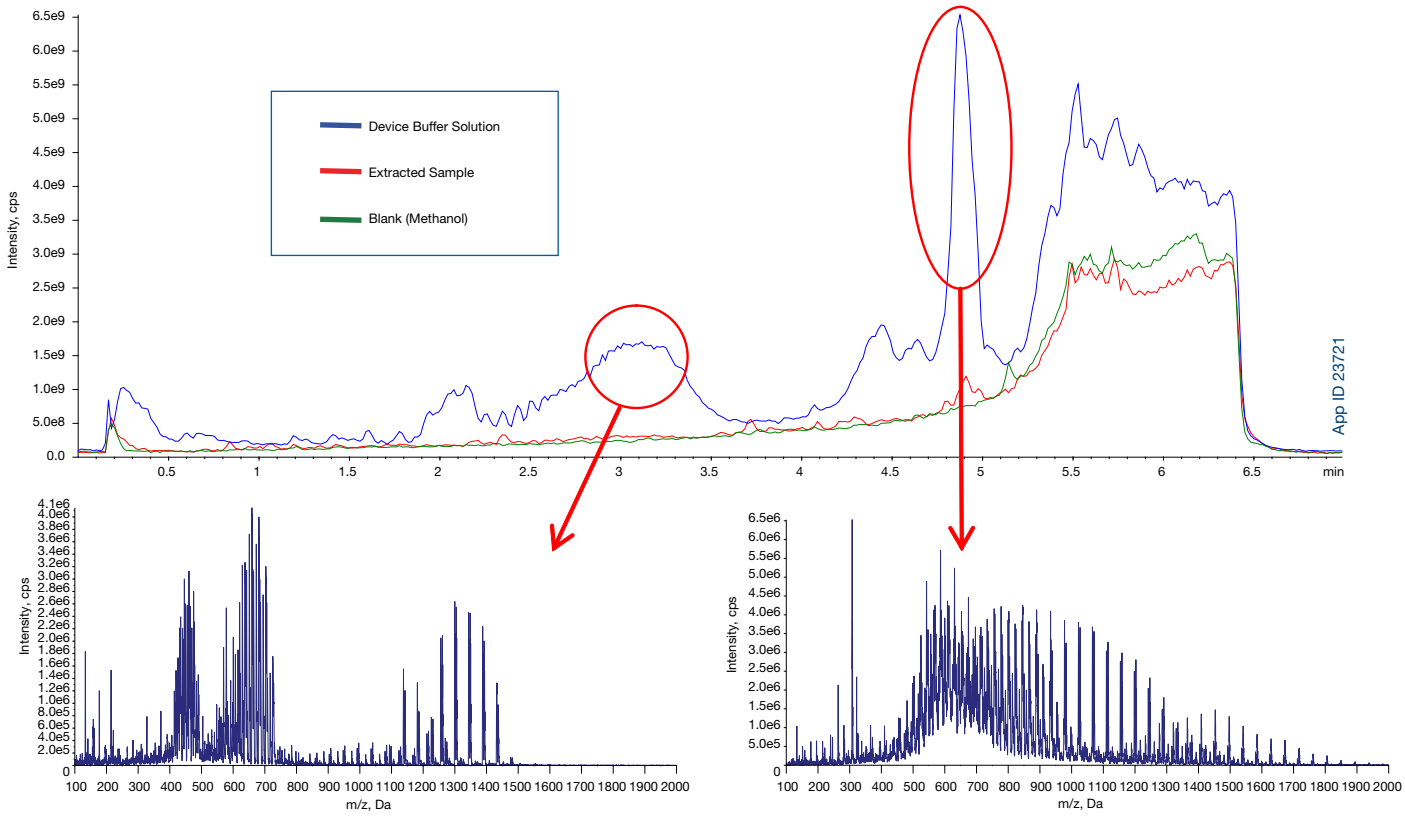

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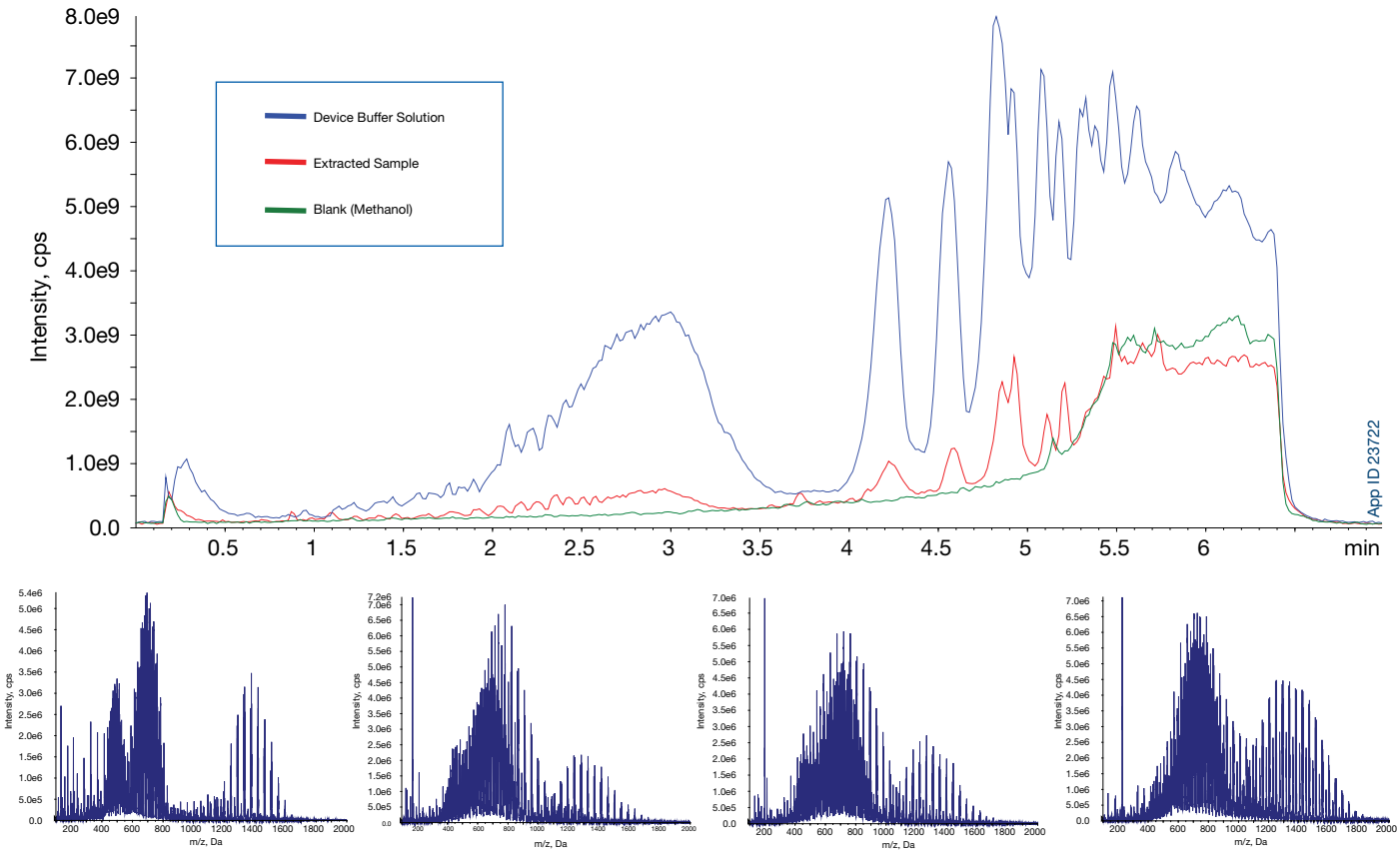
Figure 4.
Intercept i2® representative LC/MS chromatogram of buffer solution and MS spectra of circled peaks. Blue trace: neat buffer solution, Green trace: Blank (Methanol), and Red trace: Extracted sample.



This SPE procedure was applied to a comprehensive drug panel consisting of 49 analytes representing a wide range acidic, basic and neutral compounds. In order to achieve recovery of all analytes and sufficiently remove the excipients from the buffer solution an aggressive organic wash was required. The recovery of acidic analytes improved greatly by using Strata®-X-A (anion exchange) procedure (**Table 2**). Both extraction procedures together exhibited excellent recovery and reproducibility (4 replicates) for all probe compounds. The combination of cleanliness and recovery is the primary driver of a dual extraction method.

Table 2.
Parallel recovery of acidic analytes for Oral Fluid Devices using Strata-X-C and Strata-X-A methods

	Quantisol®		Intercept i2®	
Analyte	**Strata-X-C**	**Strata-X-A**	**Strata-X-C**	**Strata-X-A**
Lorazepam	11 %	64 %	13 %	89 %
Phenobarbital	4 %	68 %	0 %	77 %
Butalbital	3 %	60 %	0 %	93 %

Conclusion

To remove the harmful effect of excipients in the buffer solution, an aggressive organic wash was necessary. The result is a very clean extract where a majority of the excipients were removed. To maximize recovery of a comprehensive list of drugs we utilized the strength of 2 sample prep devices—Strata X-A and Strata X-C. This sample procedure can provide a consistent level of accuracy and precision.

Kinetex® Ordering Information

Kinetex Core-Shell HPLC/UHPLC 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

Strata®-X-C Ordering Information

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

**Tab-less tubes available. Contact Phenomenex for details.

Strata-X-A Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S123-TAK**	1 mL (100/box)
	30 mg	8B-S123-TBJ	3 mL (50/box)
	60 mg	8B-S123-UBJ	3 mL (50/box)
	100 mg	8B-S123-EBJ	3 mL (50/box)
	100 mg	8B-S123-ECH	6 mL (30/box)
	200 mg	8B-S123-FBJ	3 mL (50/box)
	200 mg	8B-S123-FCH	6 mL (30/box)
	500 mg	8B-S123-HBJ	3 mL (50/box)
	500 mg	8B-S123-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S123-HDG	12 mL (20/box)
	1 g	8B-S123-JDG	12 mL (20/box)
	1 g	8B-S123-JEG	20 mL (20/box)
	2 g	8B-S123-KEG	20 mL (20/box)
	5 g	8B-S123-LFF	60 mL (16/box)
96-Well Plate			
	10 mg	8E-S123-AGB	2 Plates/Box
	30 mg	8E-S123-TGB	2 Plates/Box
	60 mg	8E-S123-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S123-4GA	ea

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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