APPLICATIONS

Automated Sample Preparation for a Comprehensive Drug Research Panel from Oral Fluid Using Quantisal® Device

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Overview

- Automation of clean extraction, high recovery oral fluid sample preparation method
- Sample prep with no human interaction or supervision
- High precision and accuracy for low and high QCs

Introduction

There is a growing interest in oral fluid testing over other test matrices such as urine and blood because it is non-intrusive, convenient, and observable – making adulteration or substitution difficult. In our previous technical note (www.phenomenex.com/TN100) we developed a unique sample preparation procedure that encompasses a wide range of illicit and pain management drugs that result in a very clean extract with high recovery.[1,2] Here, we extend our scope in automating the procedure by utilizing a liquid handler. The speed of data analysis has improved significantly in the past decade due to increased detection capabilities of mass spectrometry coupled with liquid chromatography. Despite these advances, the biggest time constraint in sample processing is the manual process around sample preparation. One survey attributes 61% of an analytical chemist's time is spent on sample processing[3]. Automated liquid handling can increase lab productivity and circumvents human error.

Materials and Methods

Reagents and Chemicals

Analytical reference standards and human saliva were purchased from Cerilliant® Corporation (Round Rock, TX) and BioreclamationIVT® (Chestertown, MD). The Quantisal oral fluid collection devices were obtained from Immunalysis Corporation (Pomona, CA). All other chemicals were obtained from the Sigma-Aldrich Company (St. Louis, MO). D.I. water via Sartorius® arium® Comfort II, courtesy of Sartorius Corporation (Bohemia, NY). Liquid Handling via Tecan Freedom EVO® 100. (San Jose, CA)

Methods

Sample collection

1.0 mL of saliva was pipetted onto the application tip of the oral fluid collection device. The saturated pad was then placed into the transport tube containing the buffer solution.

Sample pretreatment

The Quantisal applicator tip that absorbed about 1 mL of oral fluid was transferred to the transport tube containing the preservative buffer and left for 1 to 2 hours. The transport tube was placed directly on the automation platform. Liquid handler pipetted 0.5 mL from the top of the sample, to avoid transfer of debris onto the SPE cartridge.

Sample Prep Automation

The method, found on page 2, can be amenable to many different automation platforms. We selected single carrier configuration with inserts that process up to eight samples in parallel with industry standard 3 mL cartridges. This selection accommodates loading of 40 SPE cartridges simultaneously. Because all wash solvents used in the extraction steps for both acidic and basic compounds were the same, automation allowed for simultaneous extraction. For the elution solvent, the liquid handler loaded two elution solvent separately for the cationic and anionic sorbent media respectively. The duration and amount of positive pressure application to the extracted cartridges are all software controlled. Calibrators for the 7-point linearity curve were prepared by serial dilution of the spiked oral fluid. The curve spanned a total of seven concentration levels. The QC samples for extraction were prepared at two concentration levels. Addition of acidic (methanolic) solution to the eluted samples were necessary to prevent loss of free bases in the dry down step.

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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
Part No.:	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.
Wash 1:	1 mL DI Water	1 mL DI Water
Wash 2:	1 mL Acetone/Water (50:50)	1 mL Acetone/Water (50:50)
Dry Cartridge:	2-3 minutes under positive pressure	2-3 minutes under positive pressure
Elute:	2 x 500 µL Methanol/Acetonitrile/ 28-30 % Ammonium Hydroxide (5:5:2)	2 x 500 µL Methanol/Acetonitrile/ Conc Formic acid (50:50:5)
Optional Addition*:	30 µL of 50 mM HCl/Methanol	–
Dry down:	Evaporate to dryness under gentle stream N_2 at 45-50 °C	Evaporate to dryness under gentle stream of N_2 at 45-50 °C
Reconstitute:	With 125 µL initial mobile phase	With 125 µL initial mobile phase
	Combine both fractions into a single autosampler vial	

*to help prevent the loss of free bases during evaporation

LC/MS Conditions

The LC/MS/MS method utilized a Kinetex® Biphenyl 2.6 µm, 50 x 3.0 mm column (Part No.:00B-4622-Y0) with a simple mobile phase consisting of 0.1 % formic acid in water and methanol. A fast LC gradient resulted in total run time of 5 min. The detection was carried out on a SCIEX API 5000™ equipped with ESI source. For basic compounds the MS was operated under positive polarity and in a separate injection, all acidic compounds (except lorazepam) were analyzed in negative polarity.

Positive ESI Panel

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
SecurityGuard™ Ultra Cartridge: AJ0-9208
SecurityGuard Ultra Holder: AJ0-9000
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	95
	5.01	10
	7.5	10

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

Negative ESI Panel

Column: Kinetex 2.6 µm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
Guard Cartridge: AJ0-9208
Guard Holder: AJ0-9000
Mobile Phase: A: 0.1 mM Ammonium formate in Water
B: 100 % Methanol

Gradient:	**Time (min)**	**% B**
	0	10
	4	95
	5	95
	5.01	10
	7	10

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

Table 1.
Precision, accuracy and linear regression data for analytes

Analyte	R^2	QC1 (ng/mL)	% Accuracy (QC1)	% CV (QC1)	QC2 (ng/mL)	% Accuracy (QC2)	% CV (QC2)
6-MAM	0.9987	2.5	107.2	15.7	8	85.9	7.6
7-Aminoclonazepam	0.9981	25	96.74	8.77	80	90.45	7.8
α-Hydroxyalprazolam	0.9971	25	92.84	12.49	80	91.78	10.28
Alprazolam	0.9995	25	98.39	14.06	80	93.4	5.5
Amitriptyline	0.9976	12.5	100.76	9.38	40	103.53	8.75
Amphetamine	0.9996	25	93.74	15.18	80	85.25	11.15
Benzoylecgonine	0.9978	25	93.95	10.27	80	88.24	14.55
Buprenorphine	0.9952	5	90.33	11.31	16	92.66	6.92
Citalopram	0.9992	12.5	89.47	4.18	40	95.41	5.38
Codeine	0.9992	25	95.05	12.27	80	92.15	16.05
Diazepam	0.9952	25	90.87	9.4	80	86.49	7.8
Fentanyl	0.9965	2.5	95.93	6.48	8	98.47	3.95
Fluoxetine	0.9952	25	93.98	4.83	80	89.29	5.7
Gabapentin	0.9946	25	92.21	13.38	80	97.81	10.66
Hydrocodone	0.9952	25	97.84	8.4	80	92.83	10.26
Hydromorphone	0.9975	25	99.73	7.7	80	90.15	0.86
Imipramine	0.9994	12.5	95.36	6.07	40	94.11	7.06
Lorazepam	0.9973	25	111.67	9.5	80	85.03	3.57
MDMA	0.9983	25	90.14	4.15	80	88.12	7.46
Meperidine	0.9959	25	99.74	6.56	80	99.49	8.2
Methadone	0.9993	25	95.93	7	80	88.25	7.33
Methamphetamine	0.9959	25	101.3	7.8	80	85.42	11.56
Methylphenidate	0.9991	2.5	97.78	12.07	8	90.79	9.1
Morphine	0.9995	25	105.74	8.5	80	100.87	7.9
Norbuprenorphine	0.9961	5	99.81	3.76	16	92.06	6.48
Nordiazepam	0.999	25	94.38	5.14	80	89.54	6.07
Norfentanyl	0.9998	2.5	107.31	11.47	8	88.97	2.67
Norhydrocodone	0.998	25	95.71	8.55	80	87.82	13.4
Noroxycodone	0.9988	25	107.26	6.26	80	94.07	6.85
Normorphine	0.9969	25	102.69	14.4	80	93.35	9.23
Nortriptyline	0.997	12.5	109.43	6.9	40	90.08	7.17
O-Desmethyltramadol	0.9991	25	104.9	3.15	80	95.5	11.2
Oxycodone	0.9972	25	94.88	4.66	80	87.75	10.59
Oxymorphone	0.9977	12.5	97.02	5.09	40	96.31	7.19
Paroxetine	0.9977	12.5	97.02	5.09	40	96.31	7.19
PCP	0.9974	12.5	96.77	4.58	40	100.83	3.04
Pregabalin	0.9992	25	96.2	10.98	80	103.13	5.11
Sertraline	0.9972	12.5	93.91	6.09	40	93.83	4.75
Tramadol	0.9973	25	101.84	4.49	80	103.45	7.92
Tapentadol	0.9978	25	95.16	8.07	80	93.96	8.48
Zolpidem	0.9987	5	93.27	7.1	16	91.19	6.8
Zolpidem-p-carboxylic	0.999	5	94.35	4.04	16	95.71	13.1
Butalbital	0.9992	25	96.3	11.18	80	90.81	3.96
Phenobarbital	0.9978	25	101.16	7.89	80	102.15	3.01
Secobarbital	0.9995	25	99	5.58	80	96.79	2.64
THC-COOH	0.9992	12.5	104.28	6.17	40	112.24	4.97

Figure 1.
Calibration curve of extracted samples representing dynamic range of morphine (1-300ng/mL); R=0.9996.

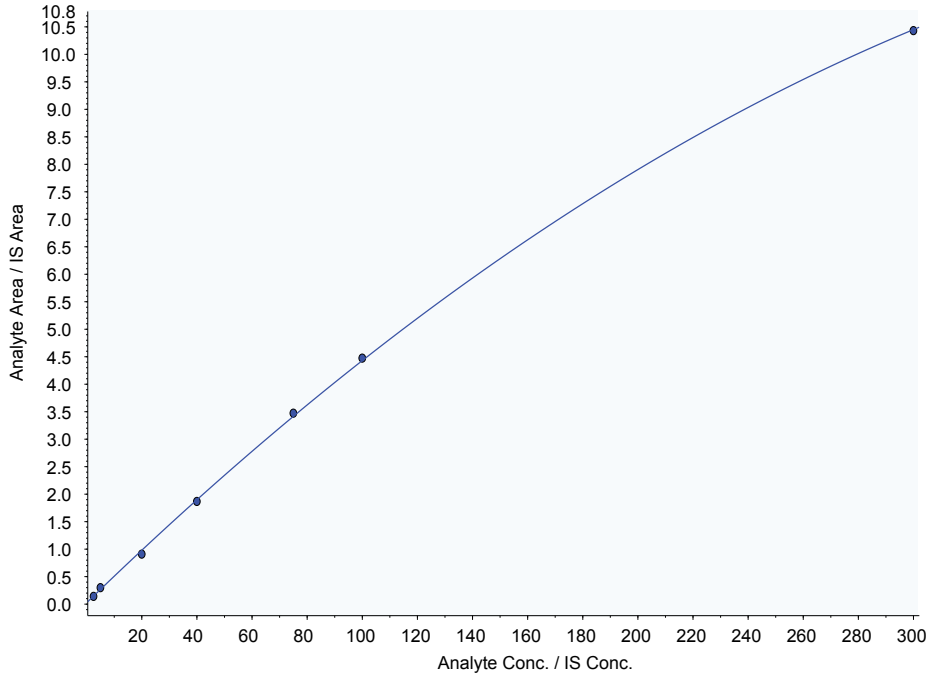


Figure 2.
Calibration curve of extracted samples representing dynamic range of 6 MAM (0.1-30ng/mL); R=0.9987.

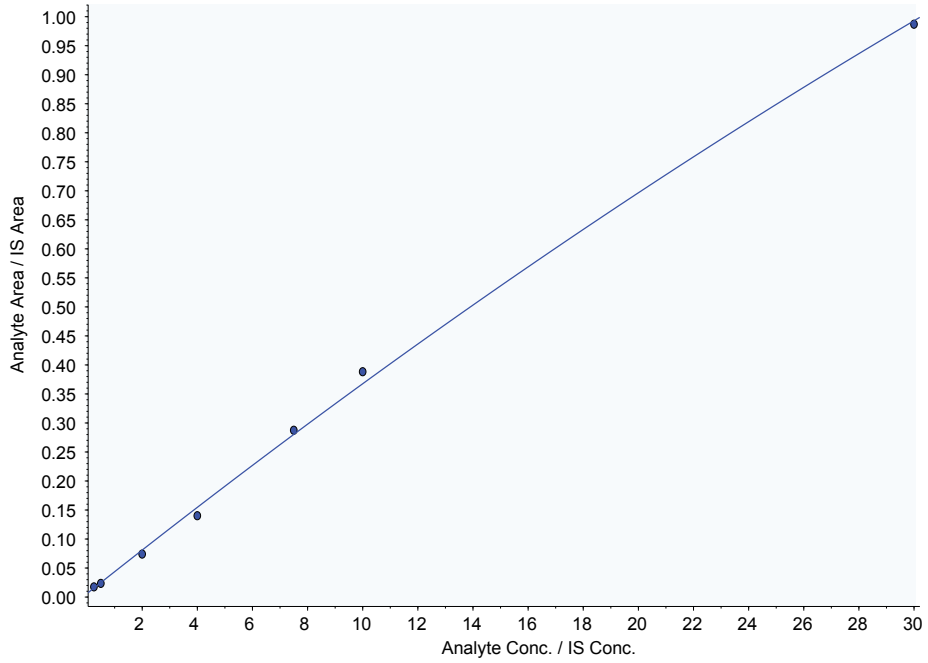


Figure 3.
Calibration curve of extracted samples representing dynamic range of zolpidem (0.5-60 ng/mL); R=0.9987.

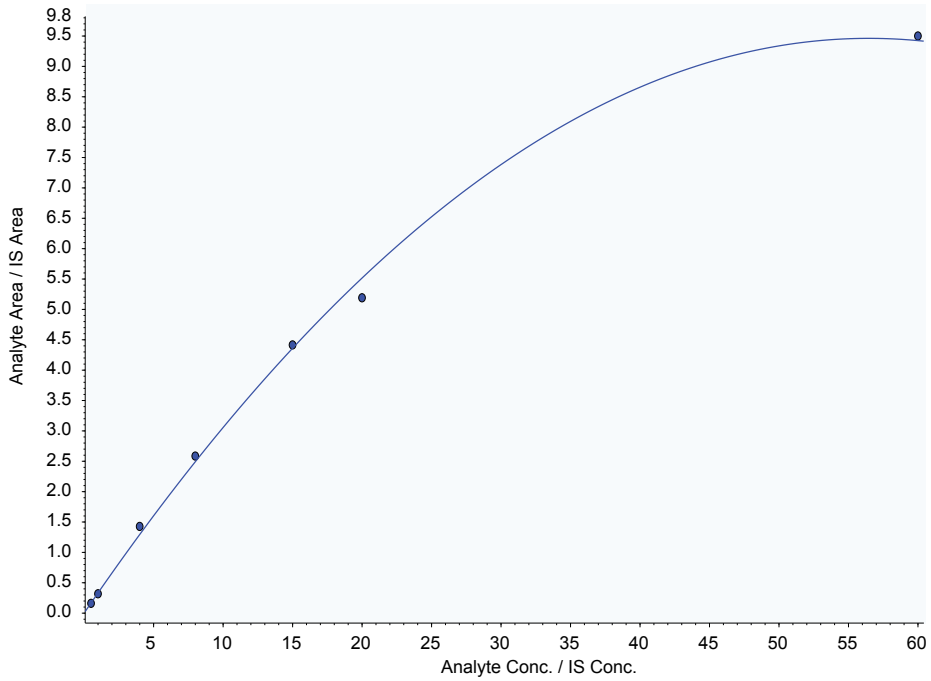


Figure 4.
Calibration curve of extracted samples representing dynamic range of norfentanyl (0.25-30 ng/mL); R=0.9998.

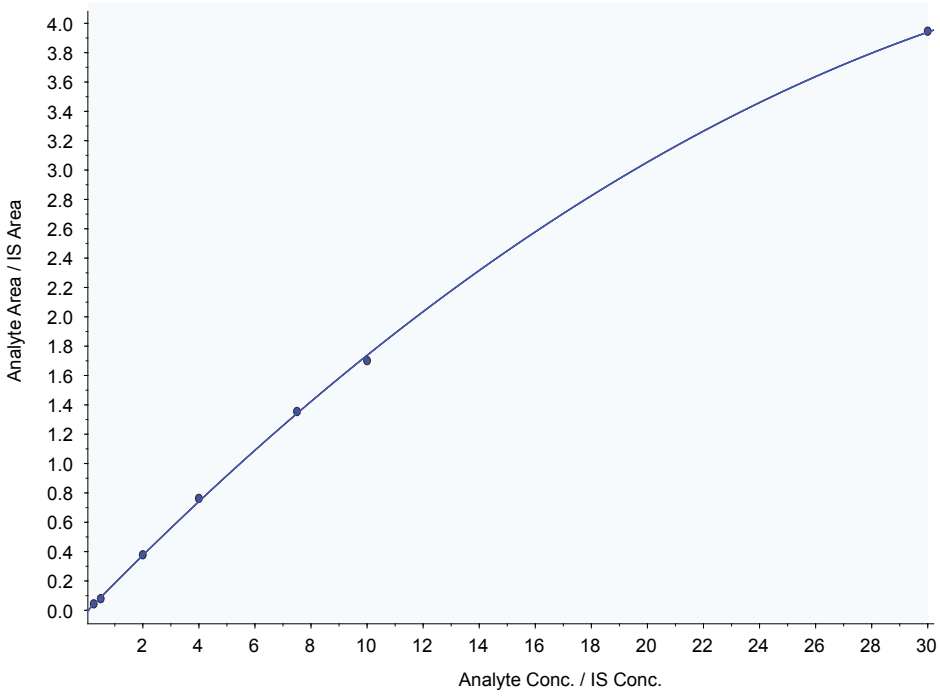


Figure 5.
Calibration curve of extracted samples representing dynamic range of butalbital (1-300 ng/mL); R=0.9992.

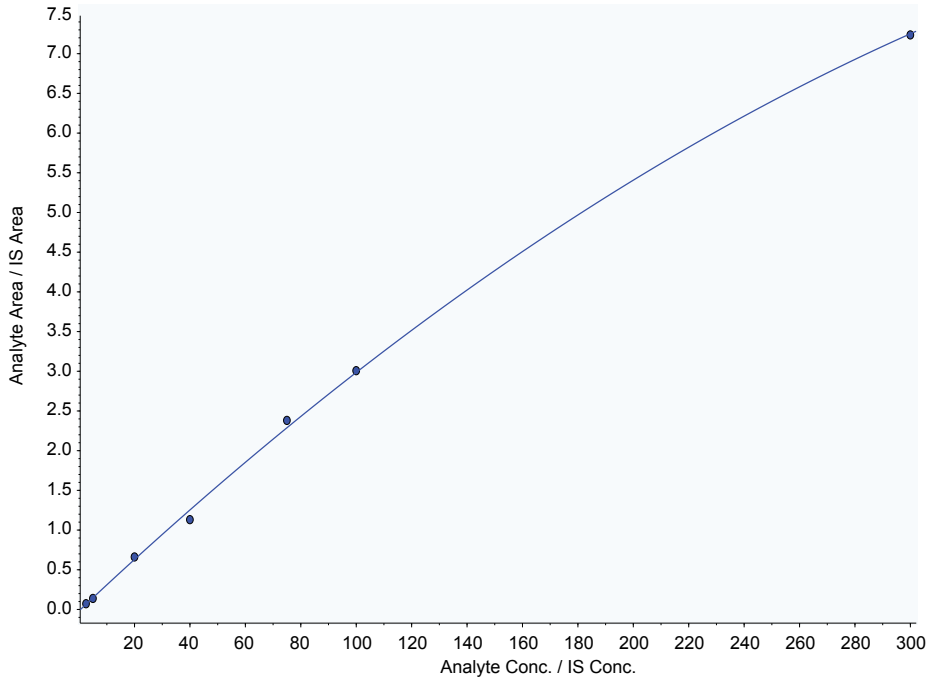
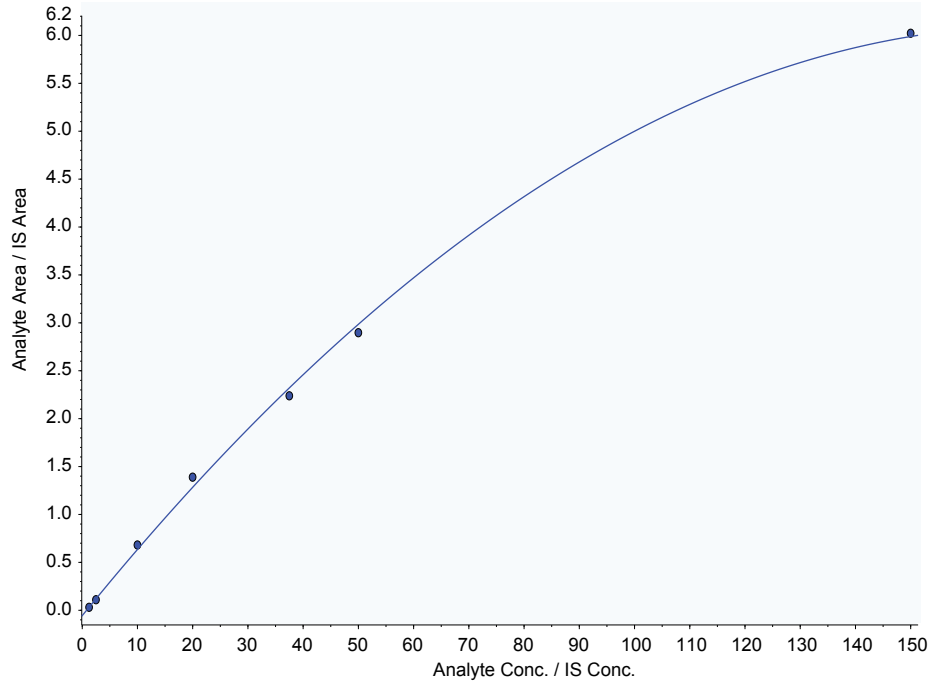


Figure 6.
Calibration curve of extracted samples representing dynamic range of THC-COOH (0.5 ng/mL-150 ng/mL); R=0.9990.



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Results and Discussion

Calibration curves for extracted samples in this study covered a range from 0.1 ng/mL to 300 ng/mL (**Figures 1-6**). At least five points per analyte were used for calibration curve. Beyond 300 ng/mL we encountered saturation of the MS for most of the analytes. Several analytes, zolpidem, norfentanyl, butalbital and THC-COOH (**Figures 3-6**), displayed non-linear curve. A quadratic calibration curve with 1/x weighting was applied to all analytes in this assay. **Table 1** shows, the correlation coefficient value (R) in all cases were greater than 0.995. The precision and accuracy for low and high QCs spanned from 3-15 % and 85-112 % respectively for all four replicates at each concentration level.

Conclusion

Oral fluid is a complex matrix and LC/MS/MS analysis requires good recovery and a clean extract. Automated sample prep on a liquid handling robot helps rapidly increase throughput and reduce human error. This procedure provides good dynamic calibration range with good precision and accuracy with little human intervention.

References:

1. S. Huq, S. Sadjadi, L. Snow; "A Superior Sample Preparation of Comprehensive Drug Panel Analytes from Oral Fluid Collection Devices; Phenomenex TN-0100
2. S. Sadjadi, S. Huq, L. Snow; "An Investigation into Removing the Excipients from Select Oral Fluids Collection Devices by SPE and LC/MS Detection;" Mass Spec Application for Clinical Laboratory Conference, 2016
3. LC|GC Editors. "Overview of Sample Preparation" LC|GC November 01, 2015 Volume 33, Issue 11 (pg 46-51). Accessed on Sept 12, 2016 from http://www.chromatographyonline.com/overview-sample-preparation.

Ordering Information

Kinetex®

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

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

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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