

APPLICATIONS

Extraction of Acidic, Basic, and Neutral Drugs from Human Plasma Comparing Solid Phase Extraction Cartridges

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Introduction

The goal of Solid Phase Extraction (SPE) is to assist in chromatographic analysis by removing impurities from sample matrix and to maximize analyte recovery.

The presence of matrix components in the sample can result in less sensitivity for the target analyte leading to irreproducible data. Other significant chromatographic issues such as high backpressure can result from unwanted impurity build up on the column, which can lead to premature column death. Therefore, determining the appropriate SPE product is a significant step for a successful analytical method.

In this technical note we compared the performance of the two polymeric, reversed phase SPE products, Strata[®]-X and Oasis[®] HLB in cartridge format, obtained from Phenomenex and Waters[®] respectively. We selected a wide range of analytes, comprising of acidic, basic, and neutral drugs that were extracted from human plasma samples. The results demonstrated differences in analyte recoveries that were minimal. A multi-modal Kinetex[®] PS C18 column was used for chromatographic analysis and for analytical detection a SCIEX 4500 Triple Quad MS instrument, in ESI, under positive ion-mode was employed.

Materials and Methods

Analytical reference standards and internal standards were purchased from Cerilliant[®] (Round Rock, TX, USA). Human plasma was obtained from BioreclamationIVT[™] (Westbury, NY). All other reagents and chemicals were purchased from Sigma-Aldrich[®] (St. Louis, MO). Ultrapure D.I. water was obtained from Sartorius[®] arium[®] comfort II, courtesy of Sartorius Corporation (Bohemia,

SPE Protocol

Cartridge:	Strata-X 30 mg/1 mL Oasis HLB 30 mg/1 mL
Part No.:	8B-S100-TAK (For Strata-X)
Condition:	1 mL Methanol
Equilibrate:	1 mL Water
Load:	1 mL diluted human plasma spiked with analyte (500 µL plasma diluted with 500 µL 2 % Phosphoric acid in Water)
Wash 1:	1 mL D.I. Water
Wash 2:	1 mL 20 % Methanol in Water
Dry cartridge:	2-3 minutes at 15-20" of Hg
Elute:	1 mL Acetonitrile/Methanol (50:50)
Dry Down:	To dryness under Nitrogen at 40 °C
Reconstitute:	0.5 mL initial mobile phase spiked with internal standard

LC Conditions

Column:	Kinetex 2.6 µm PS C18														
Dimensions:	50 x 2.1mm														
Part No.:	00B-4780-AN														
SecurityGuard™ ULTRA:	AJ0-8951														
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol														
Gradient:	<table> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>25</td> </tr> <tr> <td>0.5</td> <td>25</td> </tr> <tr> <td>1.5</td> <td>95</td> </tr> <tr> <td>3</td> <td>95</td> </tr> <tr> <td>3.01</td> <td>25</td> </tr> <tr> <td>5</td> <td>25</td> </tr> </tbody> </table>	Time (min)	% B	0	25	0.5	25	1.5	95	3	95	3.01	25	5	25
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0	25														
0.5	25														
1.5	95														
3	95														
3.01	25														
5	25														
Flow Rate	0.4 mL/min														
Injection Volume:	5 µL														
Temperature:	Ambient														
LC Instrument:	Agilent [®] 1260														
Detector:	SCIEX [®] Triple Quad [™] 4500 (ESI, +ve Ionization)														

Table 1.
% Absolute Recovery & % CV for Extracted Plasma Samples

Analytes	Nature of Analyte	Spiked conc. (ng/mL)	RT (min)	Strata-X % Recovery	Strata-X % CV (N=4)	Oasis HLB % Recovery	Oasis HLB % CV (N=4)
Acetaminophen	Neutral	50	0.89	50	1.8	70	6.9
Metoprolol	Basic	50	1.99	89	5.3	89	7.2
Naproxen	Acidic	50	2.85	95	2.0	92	2.6
Diclofenac	Acidic	50	2.99	88	4.1	88	3.6
Amitriptyline	Basic	50	2.55	89	1.3	91	0.9
Prednisone	Neutral	50	2.7	106	12.3	81	3.8
β-methasone	Neutral	50	2.8	91	7.3	90	2.9

Figure 1.
Representative Chromatogram of Plasma Extracted Sample Using Strata[®]-X SPE

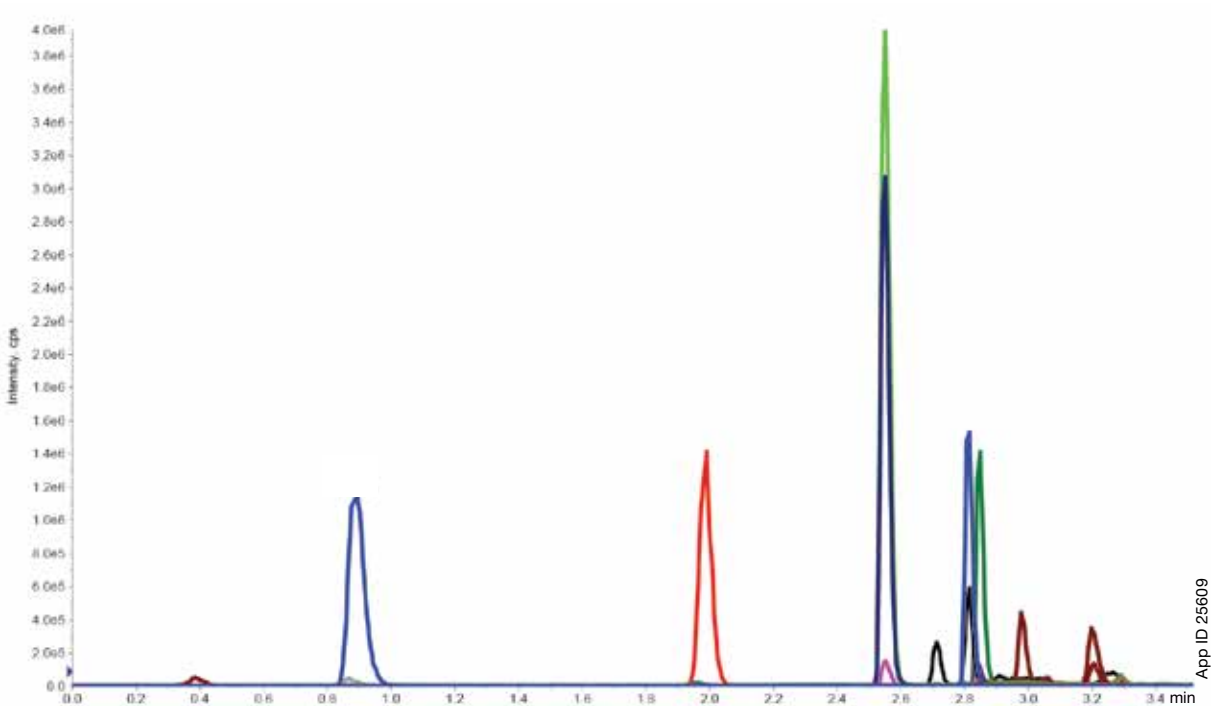
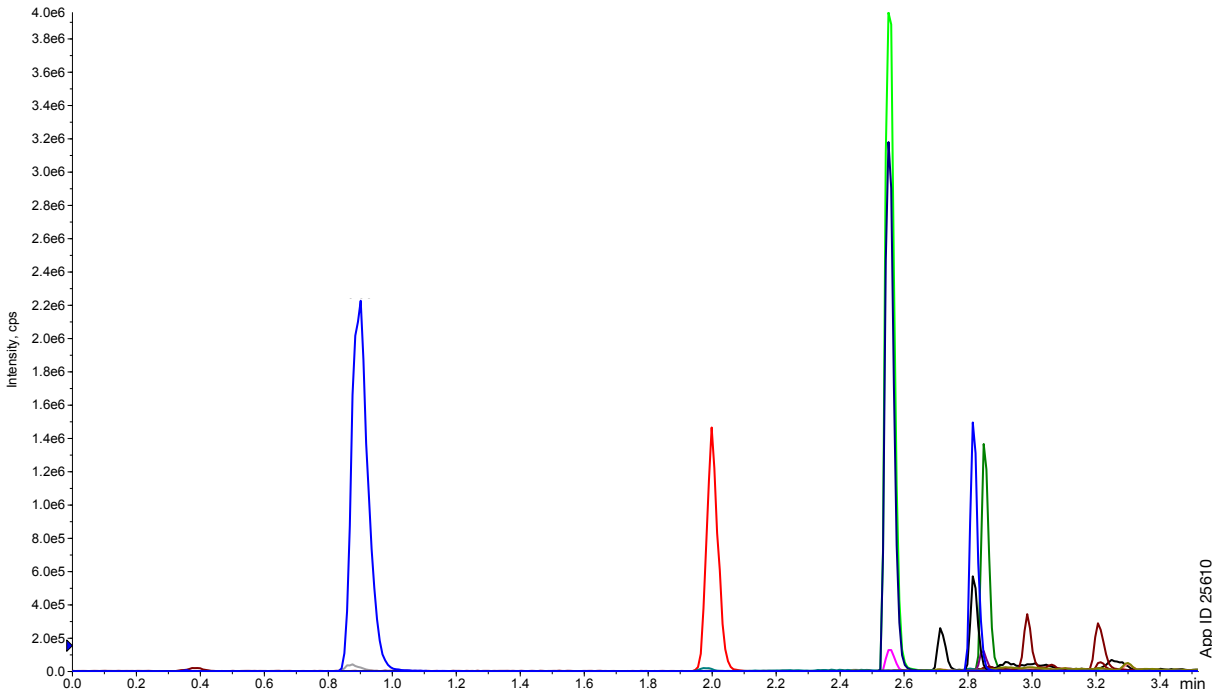


Figure 2.
Representative Chromatogram of Plasma Extracted Sample Using Oasis[®] HLB SPE




Results and Discussion

The SPE protocol was optimized for a 20 % methanol wash to compromise between an acceptable recovery and adequate matrix clean-up for the target analyte panel that displays a broad spectrum of polarity. The recovery for the 7 drugs on the average, came out 87 % and 86 % for Strata®-X and Oasis® HLB respectively. The developed LC conditions utilized a minute-long gradient from 25 % to 95 % methanol fortified with 0.1% formic acid while a Kinetex® PS C18 50 x 2.1 LC column was used. The 7 target drugs elute off the LC column, while the organic modifier was held at 95 % for 1.5 minutes (**Fig. 1** and **2**).

Ordering Information

Strata-X SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/box)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)

96-Well Plate

	10 mg	8E-S100-AGB	2 Plates/Box
	30 mg	8E-S100-TGB	2 Plates/Box
	60 mg	8E-S100-UGB	2 Plates/Box

96-Well Microelution Plate

	2 mg	8M-S100-4GA	ea
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More part numbers available online at www.phenomenex.com/StrataX

Kinetex Core-Shell LC Column

2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4780-AN	00B-4780-AN	00D-4780-AN	00F-4780-AN	AJO-8951 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
PS C18	00B-4780-YO	00D-4780-YO	00F-4780-YO	AJO-8950 for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
PS C18	00B-4780-E0	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJO-8949 for 4.6 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000.

Conclusion

This application shows absolute recovery data for 7 common acidic, basic, and neutral drugs on polymeric reversed phase SPE cartridges from Phenomenex and Waters® using human plasma samples. Strata-X provides comparable high and reliable recoveries (**Table 1**) for these 7 drug analytes. Although the % recovery for acetaminophen is lower, the variation coefficient (% CV) is much less, 1.8 % in comparison to 6.9 % on Oasis cartridge. The multi-modal chemistry of Kinetex PS C18 provided the desired selectivity with excellent retention and resolution for all 7 target drugs. The parallel study of the two polymeric phases Strata-X and Oasis HLB demonstrates comparable recoveries and % CV value for the chosen panel of drugs. The recovery study under equal and controlled conditions demonstrated similar performance capability of the two SPE chemistry.

APPLICATIONS

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Strata-X is patented by Phenomenex. U.S. Patent No. 7, 119, 145

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