

APPLICATIONS

Comparison of Antipsychotics Extracted from Human Plasma Using Microelution SPE

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Overview

This application describes a simple but effective sample preparation solution to increase productivity. Nine antipsychotics from human serum were extracted with ultra-sensitive Solid Phase Extraction (SPE) methods using Strata[®]-X-C Microelution 96-well plate and Waters[®] Oasis[®] MCX μ Elution 96-well plate. While using a cation-exchange polymeric SPE sorbent packed in 96-well plate in microelution format, labs are able to skip the dry-down step saving at least 30 minutes without negatively impacting the sensitivity of their analysis. The small volume of biological samples loaded onto the ion-exchange sorbent, undergoes a 100 % organic wash to result in a clean extract. The miniature elution volume capability of the packed 2 mg sorbent results in a 4x concentration enrichment of the nine basic antipsychotic drugs tested. This pre-concentration feature bypasses the need for dry down and reconstitution of the extracted samples before injection onto the LC-MS/MS. Possibility of analyte loss during dry-down is also eliminated for heat labile drugs. The reduction of sample processing time in a microelution format speeds up laboratory workflow by at least 50 %.

The nine antipsychotics were accurately quantified by LC-MS/MS using a Kinetex[®] 2.6 μ m PS C18 LC column. Kinetex has core-shell particle morphology that provides ultra-high column efficiency on any HPLC or UHPLC. The Kinetex PS C18 is a USP classified L1 column, that provides both a unique polar and hydrophobic selectivity and is 100 % aqueous stable.

Materials and Methods

Analytical reference standards and internal standards were purchased from Cerilliant Corporation (Round Rock, TX, USA). Human plasma was obtained from Bioreclamation/IVT[®] (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, NY).

SPE Protocol

96-Well Microelution Plate:	Strata-X-C Microelution Plate, 2 mg/well Oasis MCX μ Elution Plate, 2 mg/well
Part No.:	8M-S029-4GA (for Strata-X-C)
Condition:	200 μ L Methanol
Equilibrate:	200 μ L Water
Load:	400 μ L diluted human plasma (200 μ L plasma diluted 1:1 with 4 % Phosphoric acid in Water)
Wash 1:	200 μ L 2 % Formic acid in Water
Wash 2:	200 μ L Methanol
Elute:	2x 25 μ L 5 % Ammonium hydroxide in Acetonitrile/Methanol (60:40)
Spike IS:	Spike 50 μ L of internal standard in Methanol/Water (50:50) Sample was directly injected onto LC-MS/MS

LC Conditions

Column:	Kinetex 2.6 μ m PS C18	
Dimension:	50 x 2.1 mm	
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:	Time (min)	% B
	0	5
	3	95
	3.5	95
	3.51	5
	5	5
Flow Rate:	0.5 mL/min	
Injection Volume:	2 μ L	
Temperature:	Ambient	
LC Instrument:	Agilent [®] 1260	
Detector:	SCIEX [®] Triple Quad [™] 4500 (ESI, +ve Ionization)	

Results and Discussions

Figure 1. Representative Chromatogram of Extracted Plasma Sample Using Strata®-X-C 96-Well Microelution Plate

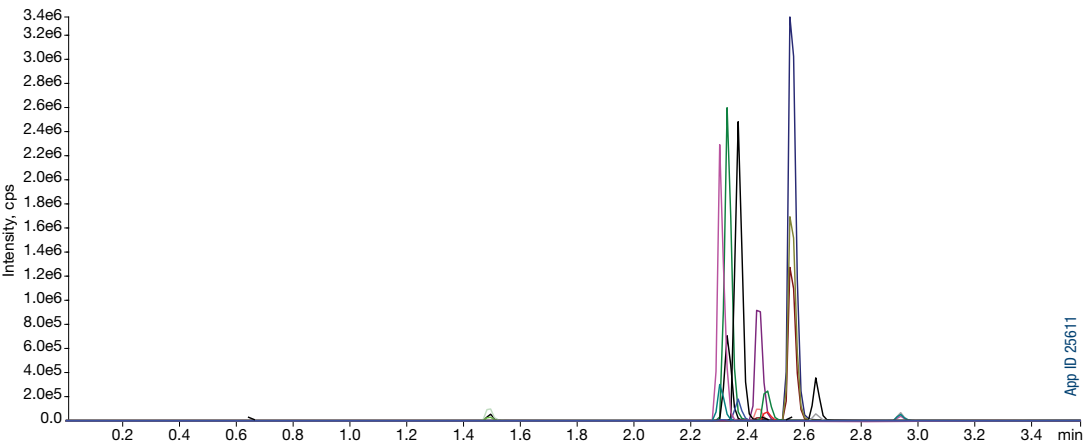


Figure 2. Representative Chromatogram of Extracted Plasma Sample Using Oasis® MCX 96-Well µElution Plate

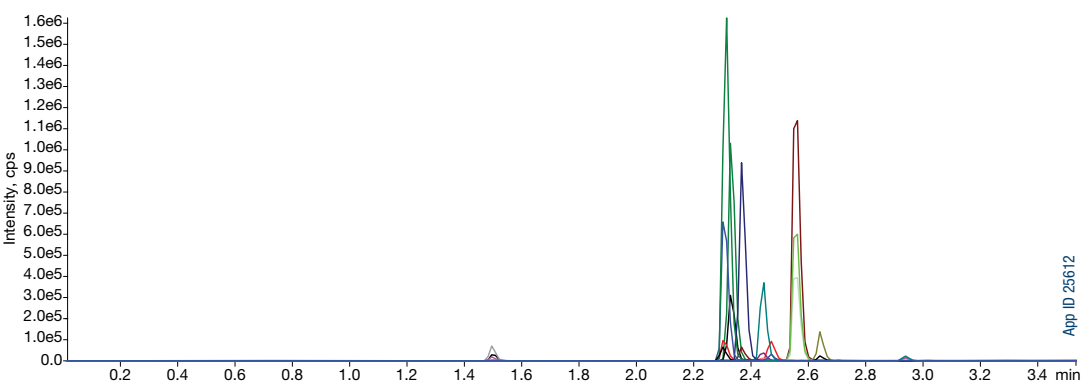


Table 1. % Absolute Recovery and CV for Extracted Plasma Samples

Antipsychotics	Spiked conc. (ng/mL)	RT (min)	Strata-X-C Microelution		Oasis MCX µElution	
			% Recovery	% CV (N=4)	% Recovery	% CV (N=4)
9-OH Risperidone	25	2.33	90	6.6	97	7.9
Olanzapine	25	1.53	98	6.9	101	8.6
Clozapine	25	2.47	95	4.0	98	6.6
Quetiapine	25	2.58	91	2.7	95	5.2
Norquetiapine	25	2.5	71	5.5	70	5.9
Risperidone	25	2.39	98	6.0	111	4.1
Norclozapine	25	2.36	92	7	106	12
Ziprasidone	25	2.67	61	10.7	68	6.8
Aripiprazole	25	2.96	65	6.0	79	0.7

The recommended LC conditions for the Kinetex[®] PS C18 LC column efficiently and sensitively resolved nine antipsychotics, which are basic in nature and closely related in chemical properties. The recovery values for Strata[®]-X-C averaged about 85% and 92% for Oasis[®] MCX respectively, with the % recovery for each analyte seen in **Table 1**. Strata-X-C displays predominantly consistent results with lower %CVs for more analytes compared to MCX. The parallel study of the above two cation-exchange sorbent shows very minor differences in recovery while microelution format was utilized. When observing **Figure 1 and 2** sensitivities can be compared of the two microelution formats being used with a Kinetex PS-C18 LC column showing the separation.

Conclusions

In a laboratory workflow, at least 50% of the total time spent in preparing and processing samples are from dry-down and reconstitution. The microelution format of the SPE increases the productivity of the sample prep process by eliminating the prolonged solvent evaporation and reconstitution, leaving it more simple and efficient. Our study under equal and controlled conditions demonstrated equivalent results for Phenomenex's Strata-X-C and Waters[®] Oasis MCX products in microelution format. The Kinetex PS C18 HPLC/UHPLC column's unique multi-modal selectivity and improved chromatographic performance when applied to the analysis of polar basic compounds. The unique selectivity and performance benefits of the Kinetex PS C18 were compared across certain antipsychotics to confirm consistent peak performance and selectivity.

Ordering Information

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)

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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More part numbers available online.
**Tab-less tubes available.

On-line Extraction Cartridge

Description	Part Number	Unit/Box
Strata-X-C on-line extraction cartridge, 20 x 2.0 mm	00M-S048-B0-CB	ea
Cartridge holder, 20 mm	CH0-5845	ea

Kinetex Core-Shell LC Columns

More part numbers available online.

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	AJ0-8951

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-YQ	00D-4780-YQ	00F-4780-YQ		AJ0-8950

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-EQ	00D-4780-EQ	00F-4780-EQ	00G-4780-EQ	AJ0-8949

for 4.6 mm ID

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#).



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