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Fast, Easy Solid Phase Extraction (SPE) Optimization for Prescribed Drugs Utilizing the Strata™-X Method Development Plate

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Introduction

Most of the work activity and operating cost in an analytical lab is spent in preparing and processing samples for injection. A simple and structured workflow can save time and cost. SPE provides the maximum cleanliness and sensitivity for LC-MS/MS, however, it has the longest method development time. Knowledge of analyte properties can streamline method development, but sometimes that information is not readily available and large panels of analytes can mean different optimal conditions for different compounds. To develop an efficient and structured approach, a Strata-X method development 96-well plate can be utilized. This method development plate is packed with 4 different Polymeric SPE sorbents. It allows for the screening of multiple conditions on multiple chemistries to determine best sorbent and starting extraction conditions.

In this technical note, we demonstrate the utilization of the Strata-X Method Development 96-well plate to determine the optimal extraction conditions for a panel of 47 drug analytes from multiple classes (**Table 1**). This was combined with a fast LC method using a Kinetex™ 2.6 µm Biphenyl LC column or a Luna™ Omega 3 µm Polar C18 LC column to resolve all target analytes and determine absolute % recovery and % CV. % recovery for the extracted samples was calculated as follows:

$$\%\,Recovery = \left(\frac{Pre-spiked\ serum\ analyte}{Post-spiked\ serum\ analyte} X\ 100\right)$$

% CV with N=4 was calculated for precision of each set of samples. For a more detailed explanation of the LC-MS/MS method, please refer to TN-1355 (Antipsychotics), TN-1356 (Antidepressants), and TN-1357 (Anticonvulsants).

Table 1. Analyte by Drug Class.

Analyte	Drug Class	Analyte	Drug Class
Olanzapine	Antipsychotic	Selegiline	Antidepressant
Norclozapine	Antipsychotic	Hydroxy bupropion	Antidepressant
Clozapine	Antipsychotic	Bupropion	Antidepressant
9-OH-Risperidone	Antipsychotic	Venlafaxine	Antidepressant
Haloperidol	Antipsychotic	Mirtazapine	Antidepressant
Risperidone	Antipsychotic	Citalopram	Antidepressant
Promethazine	Antipsychotic	N-Desmethyl-Doxepin	Antidepressant
Quetiapine	Antipsychotic	Doxepin	Antidepressant
Ziprasidone	Antipsychotic	Trazodone	Antidepressant
Dehydroaripiprazole	Antipsychotic	Norfluoxetine	Antidepressant
Chlorpromazine	Antipsychotic	Fluoxetine	Antidepressant
Fluphenazine	Antipsychotic	Amoxapine	Antidepressant
Aripiprazole	Antipsychotic	Desipramine	Antidepressant
Lurasidone	Antipsychotic	Imipramine	Antidepressant
Pregabalin	Anticonvulsant	Duloxetine	Antidepressant
Gabapentin	Anticonvulsant	Nortriptyline	Antidepressant
Levetiracetam	Anticonvulsant	Paroxetine	Antidepressant
Lamotrigine	Anticonvulsant	Amitriptyline	Antidepressant
Felbamate	Anticonvulsant	Trimipramine	Antidepressant
Lacosemide	Anticonvulsant	N-Desmethyl-Clomipramine	Antidepressant
Zonisamide	Anticonvulsant	Clomipramine	Antidepressant
Topiramate	Anticonvulsant	Sertraline	Antidepressant
Oxcarbazapine	Anticonvulsant		
Carbamazepine Epoxide	Anticonvulsant		
Carbamazepine	Anticonvulsant		

Sample Preparation

Screening Conditions

The Strata-X Method Development plate has 4 different sorbent chemistries: Strata-X, hydrophobic interaction SPE, Strata-X-C, mixed mode strong cation exchange, Strata-X-CW, mixed mode weak cation exchange, and Strata-X-AW, mixed mode weak anion exchange. Three different pre-treatment and elution conditions were used for each sorbent chemistry:

MD Plate Conditions		Load and Wash	Elution
NN	Neutral load and wash, neutral elution solvent	25 mM Ammonium Acetate, pH 6.9	Methanol / Acetonitrile (1:1, v/v)
AB	Acidic load and wash, basic elution solvent	25 mM Ammonium Formate, pH 4-5	Ethyl Acetate / Isopropanol / Ammonium Hydroxide (7:2:1, v/v/v)
BA	Basic load and wash, acidic elution solvent	25 mM Ammonium Bicarbonate, pH 9	1% Formic Acid in Methanol / Acetonitrile (1:1, v/v)

This resulted in 12 SPE method conditions that were evaluated. The Strata-X Method Development 96-well plate (Part No.: KS0-8209) was set up with the plate map in **Figure 1**. Human serum was spiked before SPE extraction with the appropriate drug class standard mix at a concentration of 1 ng/mL, following a general screening protocol:

Step	Description
Sample Pretreatment:	500 µL human serum was spiked with 1 ng/mL of the appropriate drug class standard mix and then diluted with the appropriate loading buffer for the specific condition (Neutral, Acidic, Basic).
Condition:	Strata-X SPE Method Development 96-well plate with 1 mL of Methanol.
Equilibrate:	1 mL Water.
Load:	1 mL of one of the following according to the specific condition: • Neutral Load: 25 mM Ammonium Acetate • Acidic Load: 25 mM Ammonium Formate, pH 4-5 adjusted • Basic Load: 25 mM Ammonium Bicarbonate, pH 9
Wash 1:	1 mL of one of the following according to the specific condition: • Neutral Wash: 25 mM Ammonium Acetate • Acidic Wash: 25 mM Ammonium Formate, pH 4-5 adjusted • Basic Wash: 25 mM Ammonium Bicarbonate, pH 9
Wash 2:	1 mL Methanol / Water (1:1, v/v).
Dry:	5-8 minutes at 20-25 in. Hg.
Elute:	2 aliquots of 300 µL of the following according to the specific condition: • Neutral Elution: Methanol / Acetonitrile (1:1, v/v) • Acidic Elution: Methanol / Acetonitrile (1:1, v/v) + 1 % Formic Acid • Basic Elution: Ethyl Acetate / Isopropanol / Ammonium Hydroxide (7:2:1, v/v/v)
Dry Down:	15-20 minutes at 40 °C under a gentle stream of Nitrogen.
Reconstitution:	500 µL on initial mobile phase spiked with 5 ng/mL Internal Standard mix.

Figure 1. Plate Map of Method Development Plate.

Strata-X			Strata-X-C			Strata-X-CW			Strata-X-AW		
NN	AB	BA	NN	AB	BA	NN	AB	BA	NN	AB	BA
Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank	Reagent Blank
Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank	Matrix Blank
Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum
Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum	Post-spiked Serum
Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum
Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum
Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum
Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum	Pre-spiked Serum

Results and Discussion

Antidepressants

The Strata™-X-C sorbent under the AB conditions gave the maximum % absolute recovery for the majority of the Antidepressants analyzed (**Table 2**). However, there were two exceptions: Bupropion and Selegiline. Bupropion is a basic analyte with a hydrophobic nature (LogP = 3.2) and only had a % recovery of 35 %. This could be due to Hydrogen bonding and formation of a 5-member ring on its sidechain. This could cause incomplete elution from the Strata-X-C sorbent. Selegiline had a % recovery of only 10 %. However, there was good recovery from all other sorbents under BA elution conditions for Selegiline: Strata-X 83 %, Strata-X-CW 86 %, and Strata-X-AW 92 %. This suggests that Selegiline is too retentive on the Strata-X-C sorbent and would require a stronger elution solvent, such as 5 % Ammonium Hydroxide in Methanol / Acetonitrile (1:1, v/v), to completely dislodge the analyte from the sorbent. The best sample preparation protocol for Antidepressants is shown in **Table 3**.

Table 2. Method Development Plate Results for Antidepressants.

Analyte	SPE Condition	% Recovery	%CV	Exceptions	SPE Condition	% Recovery	%CV	Comment
Amitriptyline	XC-AB	78	2.8					
Amoxapine	XC-AB	79	6					
Bupropion	XC-AB	35	30.5	YES	X-CW-BA	95	2.9	Too retentive on X-C. H bond formation possibly.
					X-BA	83	5.9	
Citaprolam	XC-AB	81	5.2					
Clomipramine	XC-AB	80	4.8					
Desipramine	XC-AB	77	7.1					
Doxepin	XC-AB	83	1.9					
Fluoxetine	XC-AB	78	7.5					
Hydroxybupropion	XC-AB	81	4.8					
Imipramine	XC-AB	78	7.8					
Mirtazapine	XC-AB	79	4.2					
N-Desmethyl-Clomipramine	XC-AB	73	5.5					
N-Desmethyl-Doxepin	XC-AB	80	5.5					
Nortriptyline	XC-AB	78	6.4					
Paroxetine	XC-AB	79	3.1					
Selegiline	XC-AB	10	65	YES	X-BA	83	4.8	All 3 sorbents except X-C worked.
					X-CW-BA	86	10.6	
					X-AW-BA	92	14.2	
Sertraline	XC-AB	94	10.9					
Trazodone	XC-AB	82	7.3					
Trimipramine	XC-AB	74	1.6					
Venlafaxine	XC-AB	84	10.9					
Norfluoxetine	XC-AB	82	10.1					
Duloxetine	XC-AB	112	14.5					

Table 3. Optimized Sample Preparation Protocol for Antidepressants Using the Strata-X-C Sorbent and the AB Elution Conditions.

Step	Description
Sample Pretreatment:	500 µL human serum was spiked with Antidepressant standard mix at a concentration of 1 ng/mL (except Duloxetine at 6 ng/mL) and then diluted with 1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Condition:	1 mL of Methanol.
Equilibrate:	1 mL Water.
Load:	About 1.5 mL of pre-treated sample.
Wash 1:	1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Wash 2:	1 mL Methanol / Water (1:1, v/v).
Dry:	5-8 minutes at 20-25 in. Hg.
Elute:	2 aliquots of 300 µL of Ethyl Acetate / Isopropanol / Ammonium Hydroxide (7:2:1, v/v/v).
Dry Down:	15-20 minutes at 40 °C under a gentle stream of Nitrogen.
Reconstitution:	500 µL on initial mobile phase spiked with 5 ng/mL Internal Standard mix (Venlafaxine-D_6, Amitriptyline-D_3, N-Desmethyl-Doxepin-D_3, Mirtazepine-D_3, Clomipramine-D_3, Hydroxybuprpion-D_6, Duloxetin-D_3).

Antipsychotics

The Strata™-X-CW sorbent under the AB conditions gave the maximum % absolute recovery for most of the Antipsychotics analyzed (**Table 4**). However, there was a single exception: Chlorpromazine. Chlorpromazine is a basic analyte with a hydrophobic nature (LogP = 4.5) and only had a % recovery of 59 % due to strong retention on the Strata-X-CW sorbent. There was much better recovery using the neutral Strata-X sorbent under the same AB elution conditions. A stronger elution solvent, such as 5 % Ammonium Hydroxide in Methanol / Acetonitrile (1:1, v/v), would aid in increasing the recovery of Chlorpromazine. The best sample preparation protocol for Antipsychotics on the Method Development plate using Strata-X-CW is shown in **Table 5**.

Table 4. Method Development Plate Results for Antidepressants.

Analyte	SPE Condition	% Recovery	%CV	Exceptions	SPE Condition	% Recovery	%CV	Comment
Ariprazole	X-CW-AB	85	2.9					
Chlorpromazine	X-CW-AB	59	15.8	Y	X-AB	80	13.6	Strong retention on X-CW.
Clozapine	X-CW-AB	91	5.5					
Dehydroariprazole	X-CW-AB	97	9.2					
Fluphenazine	X-CW-AB	95	22.6					
Haloperidol	X-CW-AB	91	1.5					
9-OH resperidone	X-CW-AB	97	12.3					
Lurasidone	X-CW-AB	95	9.2					
Norclozapine	X-CW-AB	90	10.3					
Olanzapine	X-CW-AB	80	8.9					
promethazine	X-CW-AB	97	14.4					
Quetiapine	X-CW-AB	91	14.5					
Risperidone	X-CW-AB	95	8.4					
Ziprasidone	X-CW-AB	85	7.9					

Table 5. Optimized Sample Preparation Protocol for Antipsychotics Using the Strata-X-CW Sorbent and the AB Elution Conditions.

Step	Description
Sample Pretreatment:	500 µL human serum was spiked with Antipsychotic standard mix at a concentration of 1 ng/mL (except Fluphenazine at 3 ng/mL) and then diluted with 1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Condition:	1 mL of Methanol.
Equilibrate:	1 mL Water.
Load:	About 1.5 mL of pre-treated sample.
Wash 1:	1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Wash 2:	1 mL Methanol / Water (1:1, v/v).
Dry:	5-8 minutes at 20-25 in. Hg.
Elute:	2 aliquots of 300 µL of Ethyl Acetate / Isopropanol / Ammonium Hydroxide (7:2:1, v/v/v).
Dry Down:	15-20 minutes at 40 °C under a gentle stream of Nitrogen.
Reconstitution:	500 µL on initial mobile phase spiked with 5 ng/mL Internal Standard mix (Lurasidone-D_8, Aripirazole-D_8, Fluphenazine-D_8, Olanzapine-D_8).

Anticonvulsants

The panel of Anticonvulsants analytes is complicated and diverse, composed of Zwitterions, sulfonamides, and neutral, acidic and basic compounds. There is a wide range of pKa values and polarity in this drug class, which makes developing a single method challenging. The best recovery for the majority of the analytes used the Strata™-X-CW sorbent under AB conditions (**Table 6**), however there were several deviations. Lacosamide, Felbamate, and Levetiracetam are neutral and very polar compounds with negative logP values. These compounds began eluting during the 50 % Methanol wash, lowering their overall recovery. Gabapentin and Pregabalin are Zwitterions, and both had about 50 % recovery on both cation exchange phases. A stronger elution solvent may help release the Zwitterions from the sorbent. Oxcarbazepine had 37 % recovery from Strata-X-CW under AB conditions but increased to 71 % under BA conditions. Zonisamide and Topiramate are neutral and relatively polar sulfonamides and had roughly 25-26 % recovery from the Strata-X-CW sorbent under AB conditions. Their recovery improved to 47 % and 83 % on Strata-X under NN conditions. This suggests an incomplete elution so a stronger elution solvent, such as 5 % Ammonium Hydroxide in Methanol / Acetonitrile (1:1, v/v), should improve recovery from the weak exchange (Strata-X-CW) sorbent. Since there is such diversity within this drug class, an optimized sample preparation protocol would be misleading, so a protocol that provides the best sample preparation conditions using the Method Development plate and screening protocol is presented in **Table 7**. Further method development would be required for an optimized method for this drug class using these results as a guide.

Table 6. Method Development Plate Results for Antidepressants.

Analyte	SPE Condition	% Recovery	%CV	Exceptions	SPE Condition	% Recovery	%CV	Comment
Carbamazepine	X-CW-AB	82	3.0					
Felbamate	X-CW-AB	31	17.6					Very polar, logP = -0.7 and neutral, eluting in wash 2
Gabapentin	X-CW-AB	50	8.9					Very polar, logP = -1.2 Zwitterion
Lacosemide	X-CW-AB	31	21.6					Behaving same as felbamate, no logP & pKa value found
Lamotrigine	X-CW-AB	76	7.6					
Levetiracetam	X-CW-AB	8	17.6					Very polar, logP = - 0.6 neutral, eluting in wash 2
Carbamazepine epoxide	X-CW-AB	96	2.9					
Oxcarbazpine	X-CW-AB	37	17.4	YES	X-CW BA	71	3.8	logP = 1.8, neutral. Better recovery using BA
Pregabalin	X-CW-AB	53	9	YES	X-C AB	53	9	Zwitterion. 50 % recovery with strong and weak cation exchange.
Zonisamide	X-CW-AB	25	17.1	YES	X NN	47	10	Strongly retained. H bond formation
Topiramate	X-CW-AB	26	54.1	YES	X NN	83	5.2	Poor recovery on all ion exchange sorbents suggests elution is incomplete on the SPE phases.

Table 7. Best Sample Preparation Protocol for Anticonvulsants Using the Strata-X-CW Sorbent and the AB Elution Conditions.

Step	Description
Sample Pretreatment:	500 µL human serum was spiked with Anticonvulsant standard mix at a concentration of 1 ng/mL and then diluted with 1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Condition:	1 mL of Methanol.
Equilibrate:	1 mL Water.
Load:	About 1.5 mL of pre-treated sample.
Wash 1:	1 mL of 25 mM Ammonium Formate, pH 4-5 adjusted.
Wash 2:	1 mL Methanol / Water (1:1, v/v).
Dry:	5-8 minutes at 20-25 in. Hg.
Elute:	2 aliquots of 300 µL of Ethyl Acetate / Isopropanol / Ammonium Hydroxide (7:2:1, v/v/v).
Dry Down:	15-20 minutes at 40 °C under a gentle stream of Nitrogen.
Reconstitution:	500 µL on initial mobile phase spiked with 5 ng/mL Internal Standard mix (Carbazepine-D_{10}, Gabapentin-D_{10}, Topiramate-D_{12}).

Conclusions

Sample prep method development is a difficult task for a large group of analytes. Drug analytes can have a lot of variability in functional groups and polarity from compound to compound, even within the same drug class. The Strata™-X Method Development plate for sample extraction can give insights into starting conditions for optimizing methods, but several factors must be considered before deciding on the "optimized" method: 1) All results for the Method Development plate should be considered when optimizing SPE methods. 2) Look at the analyte exceptions and which had the best results and use those insights to modify the extraction conditions. 3) Keep in mind that the "best" sorbent from the Method Development plate may not give the best overall SPE method. 4) The more diversity in analyte properties, the more concessions will have to be made. Here, we utilized the Strata-X Method Development plate on a panel of 47 different analytes across three drug classes to begin the optimization process of sample preparation and extraction.

SPE Ordering Information

Strata-X Method Development 96-Well Plate

Part No.	Description	Unit
KS0-8209	Strata-X, -X-C, -X-CW, and -X-AW 30 mg/well each	ea

Kinetex™ Ordering Information

2.6 µm Midbore™ Columns (mm)						SecurityGuard™ ULTRA Cartridges (mm)‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

for 3.0 mm ID

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

Luna™ Omega Ordering Information

3 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*/10pk
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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