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LC-MS/MS-based Quantification of 47 Therapeutic Drug Monitoring Compounds in Serum: A Simple Sample Preparation Strategy for Efficient Analysis

Babak Sheikhpoor[2], Stephanie Marin, PhD[2], Scott Krepich[1], Karl Oetjen, PhD[1], and Bryan Tackett, PhD[2]

[1]AB SCIEX, LLC, 500 Old Connecticut Path, Framingham, MA 01701 USA

[2]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods are considered the gold standard for therapeutic drug monitoring (TDM) of medications. LC-MS/MS provides several advantages for TDM, including high sensitivity, selectivity, and the ability to analyze multiple drugs in a single sample. Typically, serum or whole blood is used as the standard matrix for therapeutic drug monitoring, with serum preferred due to its ease of handling compared to whole blood. However, serum is a complex biological matrix containing various endogenous compounds that can interfere with analysis. These matrix effects can affect the accuracy and precision of drug concentration measurements, often requiring the use of complex sample preparation methods.

A crucial step in analyzing these bioanalytical samples is the removal of phospholipids. Phospholipids naturally occur in blood samples and can cause issues when injected into the LC-MS/MS system. They can accumulate on LC columns, leading to a shorter lifespan for the columns and buildup in the mass spectrometer, ultimately reducing instrument performance over time. This requires samples to be prepared using protein precipitation or solid phase extraction (SPE) prior to analysis. However, protein precipitation alone does not remove phospholipids, and SPE methods require multiple steps, which increase turnaround time and labor costs. To address this, Phree™ phospholipid removal (PLR) can be used as a fast and effective solution to simultaneously remove proteins and phospholipids, resulting in a clean sample suitable for reconstitution and subsequent LC-MS/MS analysis of the target analytes.

In this technical note, we demonstrate a fast and accurate method for the LC-MS/MS analysis of 47 drug analytes from multiple drug classes (**Table 1**) using a single-step Phree PLR sample preparation to remove proteins and phospholipids. This is combined with a fast LC method using a Kinetex 2.6 µm Biphenyl LC column to resolve all target analytes and a SCIEX QTRAP 6500+ system for MS/MS analysis. Additionally, the phospholipid content of prepared Phree-extracted samples was compared to protein precipitation alone to assess removal success.

Sample Preparation

A total of 47 TDM compounds were spiked into blank serum at concentrations of 1 ng/mL, 5 ng/mL, and 50 ng/mL. Then, 100 µL aliquots were added to protein precipitation plates with an oleophobic membrane and a sorbent to selectively remove phospholipids (Phree PLR, Part No.: 8E-S133-TGB). Protein precipitation began upon the addition of 300 µL Acetonitrile, followed by gentle shaking for 5 minutes before elution and collection of the filtrate on a Preston positive pressure manifold. Control samples were prepared at a concentration of 50 ng/mL in blank serum, and 100 µL aliquots were protein precipitated with the addition of 300 µL Acetonitrile in a centrifuge tube. The supernatant was then filtered into autosampler vials through a 0.2 µm filter.

LC Conditions

Column: Kinetex™ 2.6 µm Biphenyl
Dimensions: 100 x 2.1 mm
Part No.: 00D-4622-AN
Mobile Phase: A: 5 mM Ammonium Formate + 0.1 % Formic Acid in Water
B: 5 mM Ammonium Formate + 0.1 % Formic Acid in Methanol / Acetonitrile (50:50, v/v)

Gradient:

Time (min)	%B
0	30
3.0	50
5.0	100
7.0	100
7.1	10
9.5	10

Flow Rate: 0.4 mL/min
Injection Volume: 2 µL
Temperature: 40 °C
LC System: Shimadzu® LC-30AD
Detection: MS/MS
Detector: SCIEX® QTRAP® 6500+

MS Conditions

Ion Source: Turbo Spray IonDrive
Polarity: Positive
IS: 2500 V
GS1: 55 psi
GS2: 65 psi
CAD: 10
CUR: 35 psi
Temperature: 450 °C

Table 1. Analytes by Drug Class.

Analyte	Drug Class	Analyte	Drug Class
Carbamazepine	anticonvulsant	Aripiprazole	antipsychotic
Felbamate	anticonvulsant	Chlorpromazine	antipsychotic
Gabapentin	anticonvulsant	Lurasidone	antipsychotic
Lacosamide	anticonvulsant	Olanzapine	antipsychotic
Lamotrigine	anticonvulsant	Promethazine	antipsychotic
Levetiracetam	anticonvulsant	Quetiapine	antipsychotic
Oxcarbazepine	anticonvulsant	Risperidone	antipsychotic
Pregabalin	anticonvulsant	Clozapine	antipsychotic
Topiramate	anticonvulsant	Fluphenazine	antipsychotic
Zonisamide	anticonvulsant	Haloperidol	antipsychotic
Carbamazepine epoxide	anticonvulsant metabolite	Ziprasidone	antipsychotic
Selegiline	antidepessant (MAO-B inhibitor)	Dehydroaripiprazole	antipsychotic metabolite
Bupropion	antidepressant	Norclozapine	antipsychotic metabolite
Trazodone	antidepressant	9-OH-Risperidone	antipsychotic metabolite
Mirtazapine	antidepressant (NaSSA)	Amitriptyline	antidepressant tricyclic (TCA)
Duloxetine	antidepressant (SNRI)	Amoxapine	antidepressant tricyclic (TCA)
Citalopram	antidepressant (SSRI)	Clomipramine	antidepressant tricyclic (TCA)
Fluoxetine	antidepressant (SSRI)	Desipramine	antidepressant tricyclic (TCA)
Paroxetine	antidepressant (SSRI)	Doxepin	antidepressant tricyclic (TCA)
Sertraline	antidepressant (SSRI)	Imipramine	antidepressant tricyclic (TCA)
Venlafaxine	antidepressant (SSRI)	Trimipramine	antidepressant tricyclic (TCA)
Hydroxybupropion	antidepressant metabolite	Nortriptyline	antidepressant tricyclic (TCA) metabolite
Norfluoxetine	antidepressant metabolite (SSRI)	N-Desmethyl-Clomipramine	antidepressant tricyclic (TCA) metabolite
		N-Desmethyl-Doxepin	antidepressant tricyclic (TCA) metabolite

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Table 2. Analyte Transitions, MS Parameters, and Retention Times.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	DP (V)	EP (V)	CE (V)	CXP (V)
Phospholipid CE 5	184	184.01	0	40	10	5	10
Phospholipid CE 1	184	184.02	0	40	10	15	10
Amitriptyline 1	278.1	233.1	4.62	70	10	23	12
Amitriptyline 2	278.1	91	4.62	70	10	27	7
Amoxapine 1	314.032	271	4.17	131	10	33	16
Amoxapine 2	314032	193.1	4.17	131	10	59	14
Amoxapine 3	314.032	164.1	4.17	131	10	85	12
Aripiprazole 1	448.048	285.1	4.81	131	10	37	18
Aripiprazole 2	448.048	287.1	4.81	131	10	33	14
Aripiprazole 3	448.048	176.1	4.81	131	10	41	12
Bupropion 1	241.2	132.1	2.60	56	10	37	10
Bupropion 2	241.2	131.1	2.60	56	10	51	10
Carbamazepine 1	237.1	194.1	3.96	85	10	27	10
Carbamazepine 2	237.1	193.1	3.96	85	10	45	10
Carbamazepine epoxide 1	253.1	180.1	3.22	50	10	39	10
Carbamazepine epoxide 2	253.1	210.1	3.22	50	10	19	10
Chlorpromazine 1	319.032	86	4.84	41	10	25	10
Chlorpromazine 2	319.032	214.1	4.84	41	10	57	14
Chlorpromazine 3	319.032	152.1	4.84	41	10	97	10
Citalopram 1	325.1	109	3.65	80	10	38	8
Citalopram 2	325.1	262.1	3.65	80	10	26	12
Clomipramine 1	315.1	86.1	4.90	26	10	23	12
Clomipramine 2	315.1	58.1	4.90	26	10	67	16
Clozapine 1	327.065	270.1	3.31	131	10	33	16
Clozapine 2	327.065	192.1	3.33	131	10	57	12
Clozapine 3	327.065	164.1	3.33	131	10	99	10
Dehydroaripiprazole 1	447.068	286.1	4.64	126	10	33	24
Dehydroaripiprazole 2	447.068	285.1	4.64	126	10	33	10
Dehydroaripiprazole 3	477.068	98	4.64	126	10	53	14
Desipramine 1	267.1	72	4.36	70	10	30	8
Desipramine 2	267.1	193	4.36	70	10	50	12
Doxepin 1	280.1	107	3.87	70	10	28	10
Doxepin 2	280.1	115	3.87	70	10	60	10
Duloxetine 1	298.1	154	4.53	40	10	9	12
Duloxetine 2	298.1	44	4.53	40	10	30	6
Felbamate 1	239.087	178.1	2.26	31	10	11	12
Felbamate 2	239.087	117	2.26	31	10	23	6
Fluoxetine 1	310.1	44	4.26	80	10	40	8
Fluoxetine 2	310.1	148	4.26	80	10	12	12
Fluphenazine 1	439.2	171	4.97	105	10	36	12
Fluphenazine 2	439.2	143	4.99	105	10	40	10
Gabapentin 1	172.1	137	1.59	50	10	20	10
Gabapentin 2	172.1	95	1.59	50	10	30	8
Haloperidol 1	376.1	165	3.81	61	10	35	12
Haloperidol 2	376.1	123	3.81	61	10	55	10
Hydroxybupropion 1	257.2	132.1	2.21	61	10	39	12
Hydroxybupropion 2	257.2	131.1	2.19	61	10	63	12
9-OH-Risperidone 1	427.2	207.2	2.84	91	10	38	16
9-OH-Risperidone 2	427.2	110.1	2.84	91	10	60	8
Imipramine 1	281.1	86	4.45	70	10	21	6
Imipramine 2	281.1	58	4.45	70	10	51	6
Lacosamide 1	251.113	108	2.15	41	10	13	48
Lacosamide 2	251.113	91	2.15	41	10	33	12
Lacosamide 3	251.113	116.1	2.15	41	10	23	14
Lamotrigine 1	256.1	211.1	2.11	80	10	59	12
Lamotrigine 2	256.1	157	2.11	80	10	45	12

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	DP (V)	EP (V)	CE (V)	CXP (V)
Levetiracetam 1	171.054	126.1	1.53	26	10	21	14
Levetiracetam 2	171.054	154.1	1.53	26	10	11	20
Levetiracetam 3	171.054	98.2	1.53	26	10	33	8
Lurasidone 1	493.201	166.1	5.24	86	10	53	10
Lurasidone 2	493.201	177.1	5.24	86	10	55	14
Lurasidone 3	493.201	120	5.24	86	10	83	16
Mirtazapine 1	266.1	195.2	2.64	61	10	36	16
Mirtazapine 2	266.1	209.2	2.64	61	10	32	16
N-Desmethyl-Clomipramine 1	301.1	72	4.84	75	10	20	8
N-Desmethyl-Clomipramine 2	301.1	242.1	4.84	75	10	32	16
Norclozapine 1	313.035	192.1	3.03	86	10	55	12
Norclozapine 2	313.035	270.1	3.03	86	10	33	18
Norclozapine 3	313.035	164	3.03	86	10	95	18
N-Desmethyl-Doxepin 1	266.1	107	3.76	70	10	29	8
N -Desmethyl-Doxepin 2	266.1	235.1	3.76	70	10	21	10
Norfluoxetine 1	296.1	134.1	4.17	20	10	10	10
Norfluoxetine 2	296.1	30	4.19	20	10	50	10
Nortriptyline 1	264.1	233.1	4.53	60	10	17	14
Nortriptyline 2	264.1	117.1	4.53	60	10	25	10
Olanzapine 1	313.1	256.1	1.66	70	10	32	14
Olanzapine 2	313.1	198	1.66	70	10	50	12
Oxcarbazepine 1	253.059	236	3.27	71	10	19	20
Oxcarbazepine 2	253.059	180.1	3.27	71	10	43	10
Oxcarbazepine 3	253.059	208	3.27	71	10	27	12
Paroxetine 1	330.079	192.1	4.36	86	10	29	12
Paroxetine 2	330.079	109	4.36	86	10	77	18
Paroxetine 3	330.079	135	4.36	86	10	51	8
Pregabalin 1	160.1	55	1.52	50	10	30	6
Pregabalin 2	160.1	97	1.52	50	10	20	8
Promethazine 1	285.072	86.1	4.21	66	10	21	40
Promethazine 2	285.072	198	4.21	66	10	31	12
Promethazine 4	285.072	154.1	4.21	66	10	63	10
Quetiapine 1	384.1	253.1	3.83	80	10	33	14
Quetiapine 2	384.1	221.1	3.83	80	10	50	14
Risperidone 1	411.2	191.1	3.20	100	10	40	12
Risperidone 2	411.2	110	3.20	100	10	70	8
Selegiline 1	188.083	91	2.09	61	10	29	18
Selegiline 2	188.083	119	2.09	61	10	17	6
Sertraline 1	306.1	159	4.82	40	10	36	12
Sertraline 2	306.1	275.1	4.82	40	10	18	14
Topiramate 1	340.002	264	2.99	66	10	13	10
Topiramate 2	340.002	184.1	3.01	66	10	19	12
Topiramate 3	340.002	282.1	3.01	66	10	9	20
Trazodone 1	372.2	176	3.31	26	10	34	10
Trazodone 2	372.2	148.1	3.31	26	10	41	10
Trimipramine 1	295.145	100.1	4.68	46	10	23	14
Trimipramine 2	295.145	193.1	4.68	46	10	57	12
Trimipramine 3	295.145	208.1	4.68	46	10	35	12
Venlafaxine 1	278.145	260.2	2.73	41	10	17	14
Venlafaxine 2	278.145	121.1	2.73	41	10	39	8
Venlafaxine 3	278.145	147.1	2.73	41	10	33	10
Ziprasidone 1	412.029	194	3.83	101	10	39	12
Ziprasidone 2	413.029	130.1	3.85	101	10	99	8
Ziprasidone 3	413.029	159.1	3.83	101	10	55	12
Zonisamide 1	212.954	132	2.32	106	10	21	14
Zonisamide 4	212.954	104	2.32	106	10	31	16

Results and Discussion

In this study, 11 anticonvulsants, 22 antidepressants, and 14 antipsychotics were spiked into serum and extracted using a Phree™ PLR extraction before being analyzed on a SCIEX® QTRAP® 6500+ system. The total run time was 6 minutes, and all compounds exhibited good peak shape (**Figure 1**). The Phree PLR extraction successfully removed phospholipids from serum samples when compared to protein precipitation alone (**Figure 2**). This resulted in reduced ion suppression and improved detection of the target analytes. External calibration curves without internal standards were constructed using a regression model of 1/x, showing good linearity with R^2 values ≥ 0.991 for all analytes.

Percent recovery values and average concentrations for all analytes are summarized in **Table 3**. Percent recovery was between 70 % and 130 % for all compounds, except for Dehydroaripiprazole and Mirtazapine, which fell below 70 %. Average concentrations for extraction replicates (n=3) were calculated based on 1 ng/mL, 5 ng/mL, and 50 ng/mL spikes (**Figures 3**, **4**, and **5**). Mirtazapine was the only compound that had consistently low calculated concentrations compared to the amount in the spiked samples. The addition of internal standards and a calibration curve using a response factor would improve concentration accuracy.

Figure 1. Extracted Ion Chromatogram (XIC) Showing That All Compounds Exhibited Good Peak Shape.

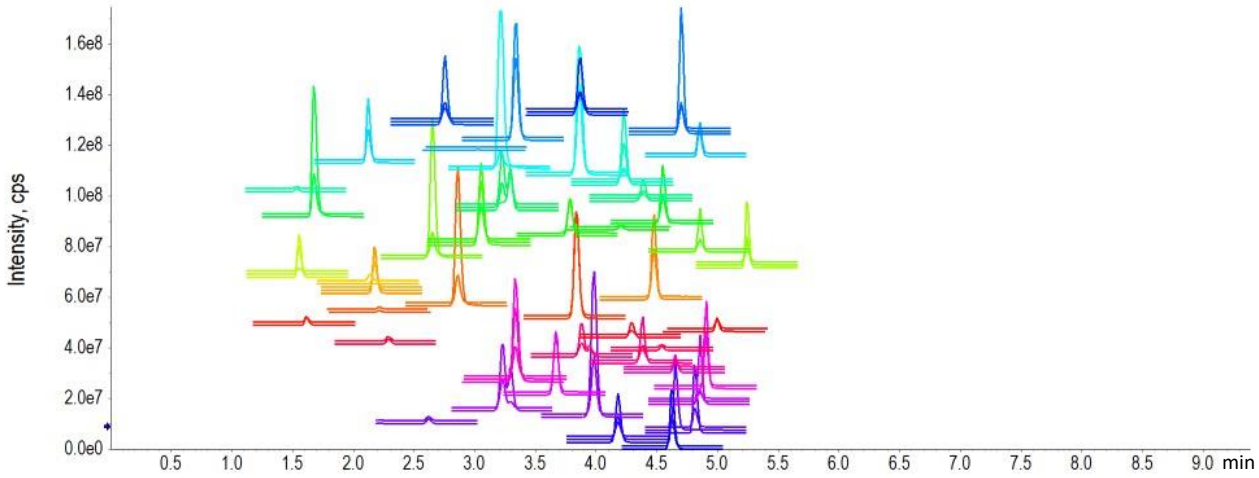

Figure 2. Comparison of Phospholipid Removal Methods.

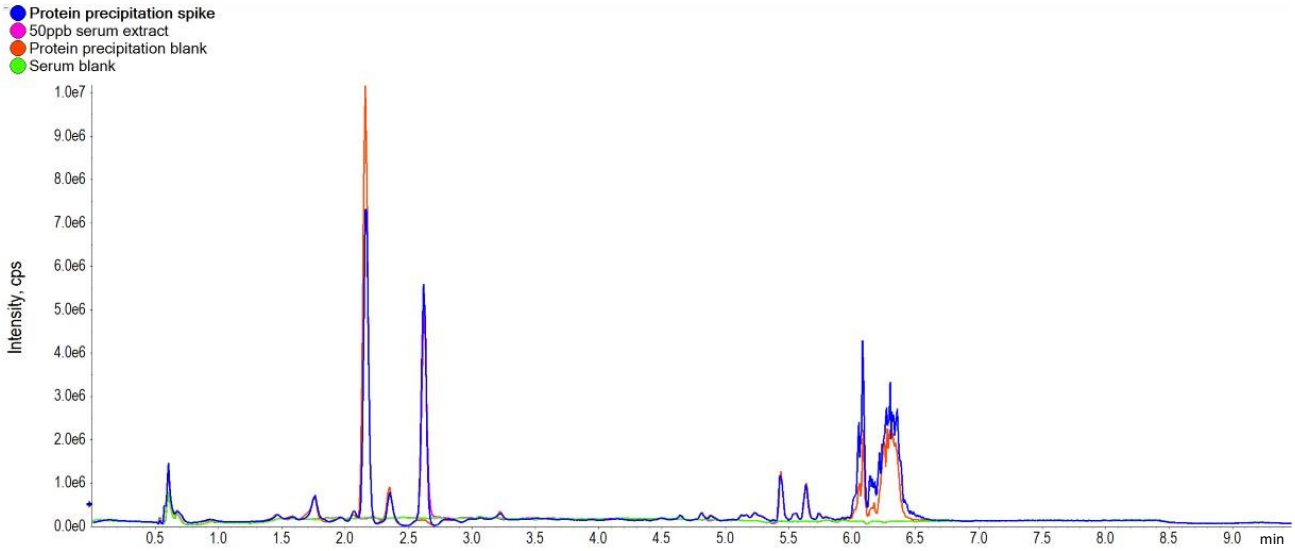

Figure 3 . Average Concentration of 47 Drug Analytes in Serum with a 1 ng/mL Spike.

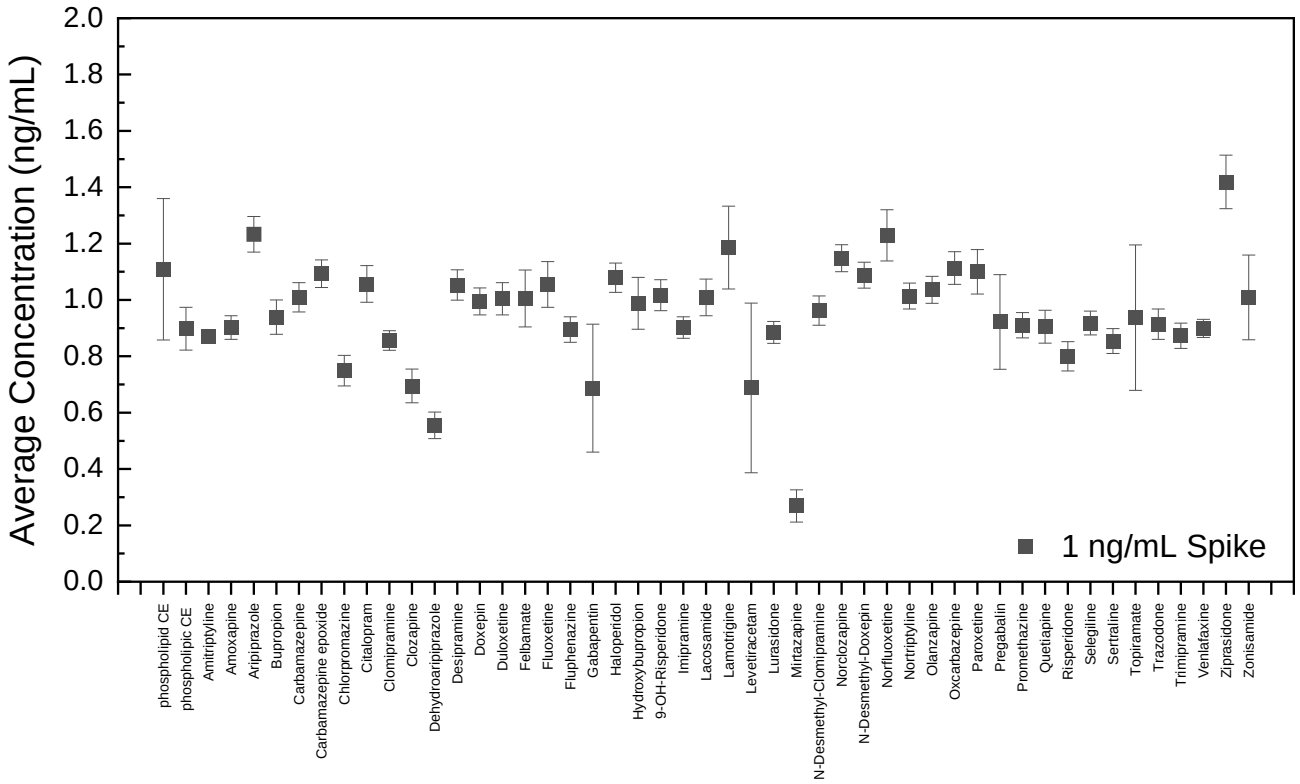

Figure 4 . Average Concentration of 47 Drug Analytes in Serum with a 5 ng/mL Spike.

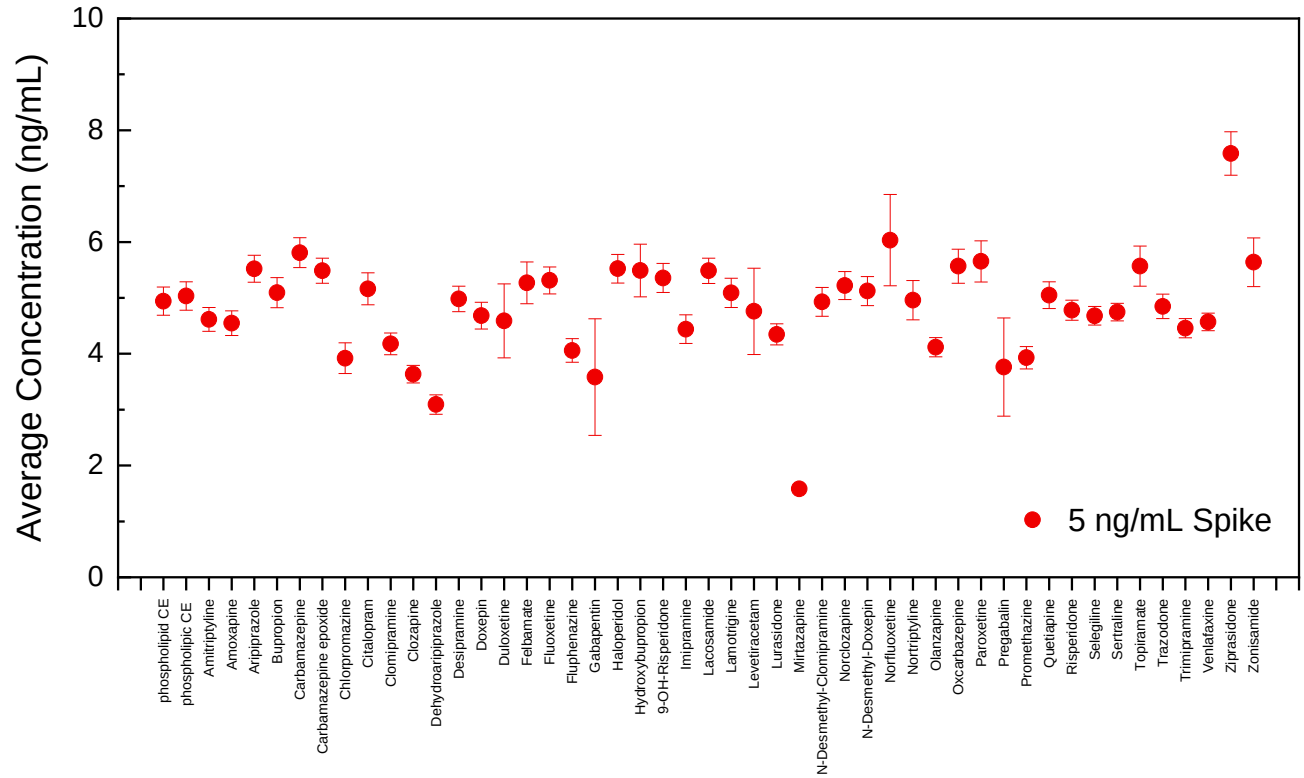

Figure 5 . Average Concentration of 47 Drug Analytes in Serum with a 50 ng/mL Spike.

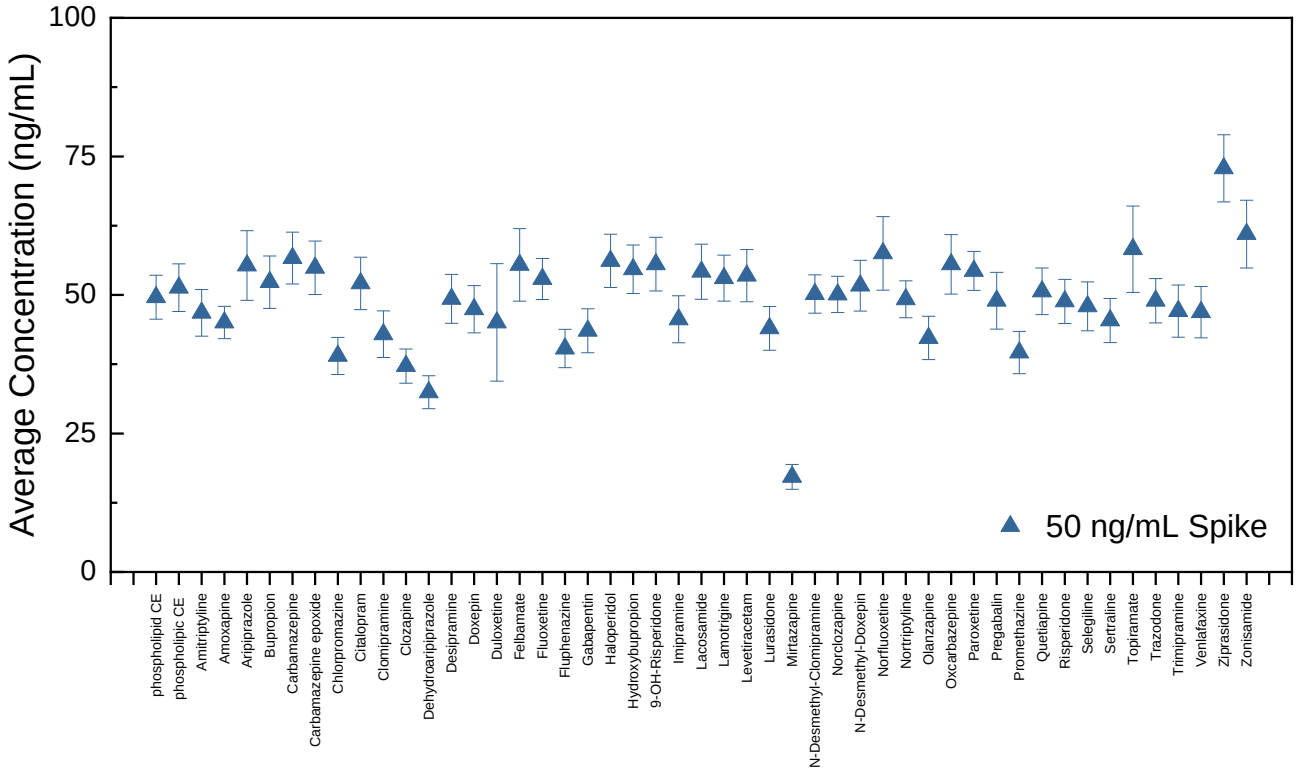

Figure 6 . Recovery of 47 Drug Analytes Across all Spiked Concentrations.

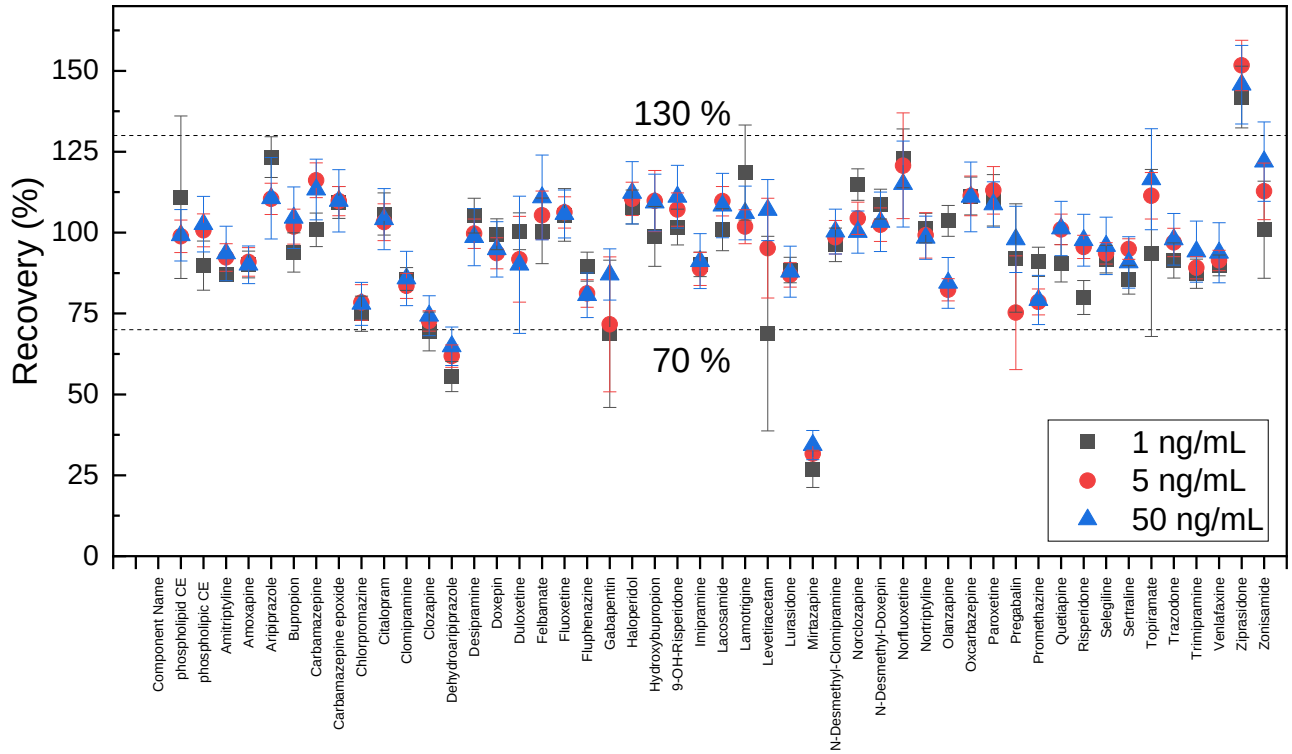

Table 3 . Average Concentration and % Recovery of 47 Drug Analytes at 1 ng/mL, 5 ng/mL, and 50 ng/mL Spike Concentrations.

	1 ng/mL		5 ng/mL		50 ng/mL	
Component Name	Average Concentration	% Recovery	Average Concentration	% Recovery	Average Concentration	% Recovery
phospholipid CE	1.1	110.9	4.9	98.8	49.6	99.2
phospholipic CE	0.9	89.8	5.0	100.7	51.3	102.6
Amitriptyline	0.9	87.0	4.6	92.3	46.8	93.6
Amoxapine	0.9	90.2	4.5	90.9	45.0	90.1
Aripiprazole	1.2	123.3	5.5	110.4	55.3	110.6
Bupropion	0.9	93.9	5.1	101.9	52.3	104.6
Carbamazepine	1.0	100.9	5.8	116.2	56.7	113.3
Carbamazepine epoxide	1.1	109.3	5.5	109.7	54.9	109.8
Chlorpromazine	0.7	74.9	3.9	78.4	39.0	78.0
Citalopram	1.1	105.7	5.2	103.2	52.1	104.2
Clomipramine	0.9	85.6	4.2	83.5	42.9	85.8
Clozapine	0.7	69.5	3.6	72.7	37.2	74.3
Dehydroaripiprazole	0.6	55.5	3.1	61.8	32.4	64.9
Desipramine	1.1	105.3	5.0	99.7	49.3	98.6
Doxepin	1.0	99.5	4.7	93.6	47.4	94.8
Duloxetine	1.0	100.4	4.6	91.8	45.0	90.1
Felbamate	1.0	100.5	5.3	105.4	55.4	110.9
Fluoxetine	1.1	105.5	5.3	106.3	52.9	105.7
Fluphenazine	0.9	89.5	4.1	81.2	40.3	80.7
Gabapentin	0.7	68.7	3.6	71.7	43.5	87.0
Haloperidol	1.1	107.9	5.5	110.4	56.2	112.3
Hydroxybupropion	1.0	98.8	5.5	109.8	54.6	109.3
9-OH-Risperidone	1.0	101.7	5.4	107.1	55.5	111.1
Imipramine	0.9	90.2	4.4	88.8	45.6	91.2
Lacosamide	1.0	100.9	5.5	109.7	54.2	108.4
Lamotrigine	1.2	118.6	5.1	101.8	53.0	106.1
Levetiracetam	0.7	68.8	4.8	95.2	53.5	107.0
Lurasidone	0.9	88.5	4.3	86.9	44.0	87.9
Mirtazapine	0.3	26.9	1.6	31.6	17.2	34.3
N-Desmethyl-Clomipramine	1.0	96.2	4.9	98.6	50.2	100.3
Norclozapine	1.1	114.8	5.2	104.4	50.1	100.2
N-Desmethyl-Doxepin	1.1	108.8	5.1	102.4	51.7	103.3
Norfluoxetine	1.2	122.9	6.0	120.7	57.5	115.0
Nortriptyline	1.0	101.4	5.0	99.2	49.2	98.4
Olanzapine	1.0	103.6	4.1	82.3	42.2	84.5
Oxcarbazepine	1.1	111.3	5.6	111.3	55.5	111.0
Paroxetine	1.1	110.0	5.7	113.1	54.3	108.7
Pregabalin	0.9	92.2	3.8	75.3	49.0	97.9
Promethazine	0.9	91.0	3.9	78.6	39.6	79.2
Quetiapine	0.9	90.5	5.0	101.0	50.6	101.3
Risperidone	0.8	80.0	4.8	95.6	48.8	97.6
Selegiline	0.9	91.8	4.7	93.6	48.0	95.9
Sertraline	0.9	85.4	4.7	94.9	45.4	90.8
Topiramate	0.9	93.7	5.6	111.4	58.2	116.5
Trazodone	0.9	91.4	4.8	97.0	49.0	97.9
Trimipramine	0.9	87.3	4.5	89.1	47.1	94.1
Venlafaxine	0.9	89.9	4.6	91.4	46.9	93.8
Ziprasidone	1.4	141.9	7.6	151.7	72.9	145.7
Zonisamide	1.0	100.9	5.6	112.8	61.0	122.0

Conclusions

The Phree™ PLR product combines the simplicity of protein precipitation with the selectivity of SPE, and it provided the selective elimination of most phospholipids while efficiently eluting the analyte of interest. Finally, the use of a Kinetex™ 2.6 µm Biphenyl LC column, in conjunction with the sensitivity and robustness of the QTRAP 6500+ system from SCIEX, enabled fast sample preparation and analysis for this large panel of analytes.

Column Ordering Information

Kinetex™ 2.6 µm Minibore Columns (mm)							SecurityGuard™ ULTRA Cartridges*
Phases	**20 x 2.1**	**30 x 2.1**	**50 x 2.1**	**75 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	00M-4725-AN	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
PS C18	—	00A-4780-AN	00B-4780-AN	—	00D-4780-AN	00F-4780-AN	AJ0-8951
Polar C18	—	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJ0-9532
Biphenyl	00M-4622-AN	00A-4622-AN	00B-4622-AN	—	00D-4622-AN	00F-4622-AN	AJ0-9209
XB-C18	—	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00M-4462-AN	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
C8	—	00A-4497-AN	00B-4497-AN	00C-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
HILIC	—	00A-4461-AN	00B-4461-AN	00C-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786
Phenyl-Hexyl	—	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
F5	—	00A-4723-AN	00B-4723-AN	—	00D-4723-AN	00F-4723-AN	AJ0-9322

*SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000 — for 2.1 mm ID

SPE Ordering Information

Phree™ Phospholipid Removal Products

Part No.	Description	Unit
8B-S133-TAK	Phree Phospholipid Removal Tabbed 1 mL Tubes	100/pk
8E-S133-TGB	Phree Phospholipid Removal 96-Well Plates	2/pk

Collection Plates

Description	Unit	Part No.
350 µL/well 96-Square Well Conical V-bottom Collection Plate	50/pk	AH0-7192
1 mL/well 96-Square Well Conical V-bottom Collection Plate	50/pk	AH0-7193
1 mL/well 96-Round Well Round Bottom 7 mm Collection Plate	50/pk	AH1-7025
2 mL/well 96-Square Well Conical V-bottom Collection Plate	50/pk	AH0-7194
2 mL/well 96-Round Well Round Bottom 8 mm Collection Plate	50/pk	AH0-8636
1.2 mL/well 96-Round Well Round Bottom Collection Plate	50/pk	AH0-9332
0.5 mL/well 96-Round Well V-Bottom, 7 mm Collection Plate, Sterile	50/pk	AH0-9333
0.5 mL/well 96-Round Well Conical Bottom 7 mm Collection Plate	50/pk	AH0-9341
2 mL/well Low-Bind 96-Round Well Conical Bottom (deep well, polypropylene, glass lined) Collection Plate	120/pk	AH1-7036

Sealing Mats

Description	Unit	Part No.
Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk	AH0-8597
Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk	AH0-8598
Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk	AH0-8631
Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk	AH0-8632
Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk	AH0-8633
Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk	AH0-8634
Sealing Tape Pad	10/p	AH0-7362

Need a different column size or sample preparation format?

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Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

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