

APPLICATIONS

Fast and Efficient Extraction of Barbiturates from Serum Using a Strata[®]-X PRO SPE Cartridge

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Overview

In this application, barbiturates are extracted from serum using the fast and efficient Strata-X PRO Solid Phase Extraction (SPE) method. This protocol saves time by reducing a traditional 6 step SPE method down to 3 quick and easy steps. With Strata-X PRO, the serum matrix is cleaned up in fewer steps while resulting in high recoveries and low variation between samples. Standards were spiked into serum at 50 µg/mL concentration with recoveries averaging 97%. **Figure 1** displays the chromatogram for the separation of barbiturates. To further demonstrate the resulting cleanliness of samples extracted with Strata-X PRO, the developed 3 step SPE process was compared with a standard protein precipitation step. The phospholipid comparison between Strata-X PRO and protein precipitation method is observed in **Figure 2**.

LC Conditions

Column: Kinetex[®] 2.6 µm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: [00B-4622-Y0](#)
SecurityGuard™ ULTRA: [AJ0-9208](#)
Mobile Phase: A: 10 mM Ammonium formate
 B: Methanol
Gradient:

Time (min)	% B
0	50
0.2	50
2.5	95
4	95
4.1	50
6	50

Flow Rate: 0.6 mL/min
Column Temperature: 25 °C
Injection Volume: 10 µL
Instrument: Agilent[®] 1260
Detector: MS/MS SCIEX Triple Quad™ 4500

SPE Protocol

Cartridge: Strata-X PRO, 30 mg/3 mL

Part No.: [8B-S536-TBJ](#)

Load: 400 µL Human Serum/1% Formic acid in Water (1:1)

Wash: 600 µL 5% Methanol in Water

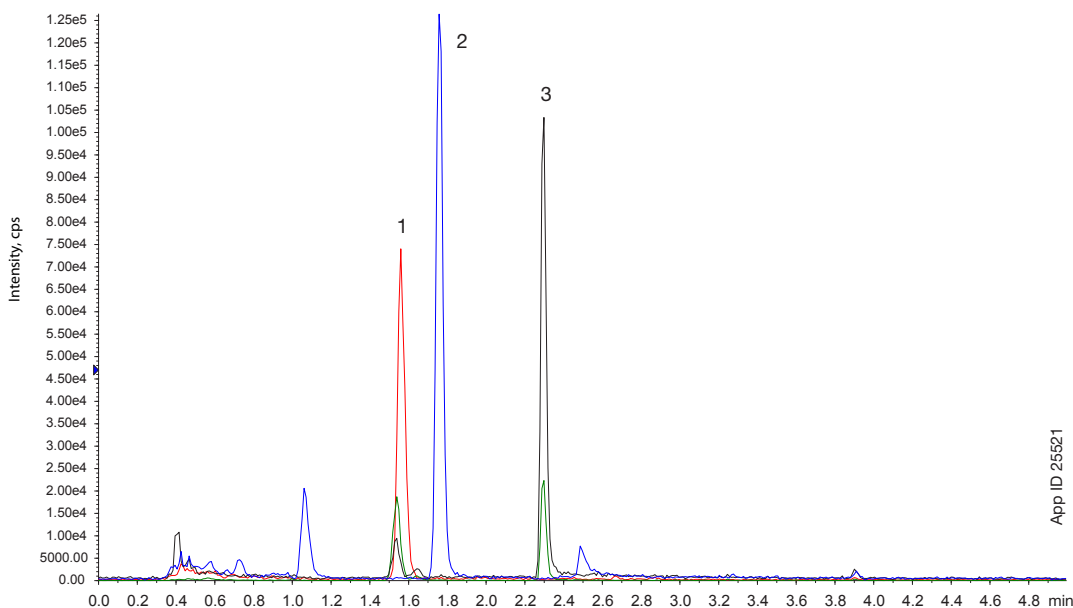
Elute: 600 µL 0.1% Formic acid Acetonitrile in Methanol (90:10)

Dry Down: Under a gentle stream of Nitrogen at 40 °C to dryness

Reconstitute: 200 µL 10 mM Ammonium formate/Methanol (1:1)

Analyte	RT (Time)	Recovery	% CV	Q1	Q3
1. Phenobarbital	1.56	91	3.4	231.1	42.1
2. Butalbital	1.76	103	2.3	223.1	42.1
3. Secobarbital	2.3	98	2.1	237	42.1

Figure 1.
Separation of Barbiturates from Plasma



App ID 25521

LC Conditions for Phospholipid Removal

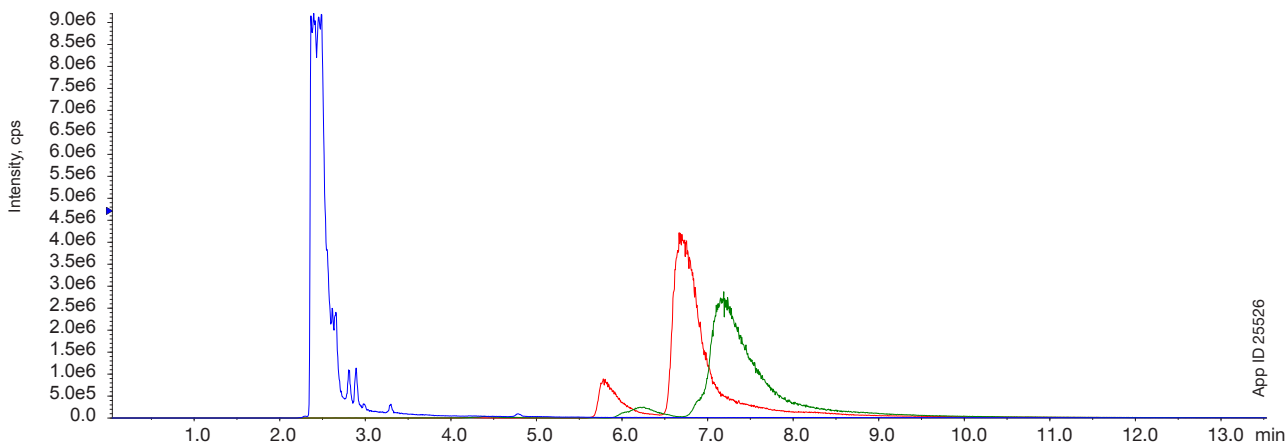
Column: Kinetex[®] 2.6µm C18
Dimensions: 50 x 2.1 mm
Part No.: [00B-4462-AN](#)
SecurityGuard™ ULTRA: [AJ0-8782](#)
Mobile Phase: A: 0.1 % Formic acid in Water
 B: 0.1 % Formic acid in Methanol
Gradient:

Time (min)	% B
0	40
0.5	95
15.5	95
15.51	40
17	40

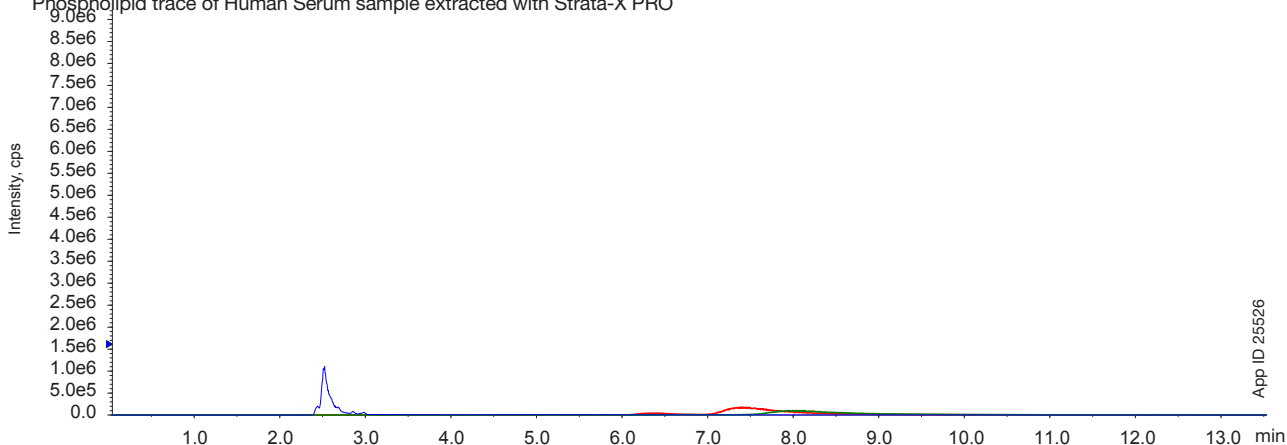
Flow Rate: 0.4 mL/min
Column Temperature: 30 °C
Injection Volume: 5 µL
Instrument: Agilent[®] 1260
Detector: MS/MS SCIEX Triple Quad™ 4500
Sample: Phospholipid (retention time in minute)
 1. Lyso PC (2.4), MRM transition 496.4/184.2
 2. PC-1 (6.7), MRM transition 760.7/184.2
 3. PC-2 (7.2), MRM transition 786.8/184.2

Figure 2. LC-MS/MS total ion current (TIC) comparison of blank plasma processed with standard protein precipitation (A) and Strata[®]-X PRO SPE (B)

A. Phospholipid trace of Human Serum sample extracted by Protein Precipitation




B. Phospholipid trace of Human Serum sample extracted with Strata-X PRO



Ordering Information

Strata[®]-X PRO SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	10 mg	8B-S536-AAK	1 mL (100/box)
	30 mg	8B-S536-TAK	1 mL (100/box)
	30 mg	8B-S536-TBJ	3 mL (50/box)
	60 mg	8B-S536-UBJ	3 mL (50/box)
	200 mg	8B-S536-FBJ	3 mL (50/box)
	100 mg	8B-S536-ECH	6 mL (30/box)
	200 mg	8B-S536-FCH	6 mL (30/box)
	500 mg	8B-S536-HCH	6 mL (30/box)

96-Well Plate

	10 mg/well	8E-S536-AGA	1 plate/box
	30 mg/well	8E-S536-TGA	1 plate/box
	60 mg/well	8E-S536-UGA	1 plate/box

96-Well Microelution Plate

	2 mg/well	8M-S536-4GA	1 plate/box
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Presston[™] 1000 Positive Pressure Manifold

Part No.	Description
AH1-7033	Presston 1000 Positive Pressure Manifold, 96-Well Plate



Phenomenex warrants the Presston 1000 will be free of defects in materials and workmanship under normal installation, use, and maintenance for a period of 12 months following delivery. Please visit www.phenomenex.com/Presstonwarranty for complete warranty information.

Kinetex[®] Core-Shell LC Columns

2.6 µm Minibore Columns (mm)						SecurityGuard [™] ULTRA Cartridges [‡]
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	–	00D-4622-AN	00F-4622-AN	AJ0-9209
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
						for 2.1 mm ID

2.6 µm MidBore [™] Columns (mm)						SecurityGuard ULTRA Cartridges [‡]
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	–	00B-4622-Y0	–	00D-4622-Y0	00F-4622-Y0	AJ0-9208
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
						for 3.0 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges [‡]
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	–	00B-4622-E0	–	00D-4622-E0	00F-4622-E0	AJ0-9207
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
						for 4.6 mm ID

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

APPLICATIONS



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