APPLICATIONS

Utilizing a Rapid, Two-Step Method for the Clean-Up of Veterinary Drugs in Milk Using Strata®-X PRO Solid Phase Extraction (SPE)

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Overview

Analyzing trace veterinary drugs in milk is a growing area of concern meant to ensure the safety of food products. These drugs, which are used to treat animals, would ultimately be incorporated to humans by consumption of milk and pose health hazards. Any proposed solution needs to quickly and accurately quantify the residual drugs present in milk and related products before injection onto the LC column. When working with milk as a matrix, phospholipids from milk fat must be removed to reduce any ion suppression that could occur during LC-MS/MS analysis. To overcome these obstacles, Strata-X PRO, a solid phase extraction (SPE) product, offers a fast, two-step sample preparation method to remove phospholipids prior to MS analysis. This SPE product shows an improved solution over traditional protein precipitation methods and other types of SPE, due to clean up efficiency while maintaining a rapid and fast analysis time.

Materials and Methods

Reagents and Chemicals

Analytical reference standards were purchased from Cerilliant® Corporation (Round Rock, TX, and USA). All other chemicals were obtained from Sigma-Aldrich® (St. Louis, MO) and the analytes and internal standards were purchased from Cerilliant (Round Rock, TX). ALTA-DENA brand milk was used as a sample matrix for extraction. Ultrapure D.I. water was obtained from Sartorius® arium® comfort II, courtesy of Sartorious Corporation (Bohemia, NY).

SPE Protocol

Pre-treatment

To 1 mL of milk (spiked with analyte) add 3 mL of 0.2 % Formic acid in Acetonitrile/Methanol (90:10) and mix or vortex for 15-20 seconds. Centrifuge for 5 minutes at 10,000 RPM and collect supernatant.

Cartridge: Strata-X PRO 60 mg/3 mL
Part No.: 8B-S536-UBJ
Load: Pass the pre-treated sample through the SPE cartridge and collect
Dry: Evaporate the extract to dryness under a gentle stream of nitrogen at room temperature
Reconstitute: The dried sample in 1 mL of initial mobile phase (0.1 % Formic acid in Water/0.1 % Formic acid in Methanol (95:5)) spiked with deuterated internal standard.

LC Conditions for Chromatogram

Column: Kinetex® 2.6 µm Biphenyl 100 Å
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
SecurityGuard™ ULTRA: AJ0-9208
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol

Gradient:

Time (min)	%B
0	5
1.5	95
3	95
3.01	5
4.5	5

Flow Rate: 0.5 mL/min
Injection Volume: 5 µL
Temperature: 45 °C
Instrument: Agilent® 1260
Detector: SCIEX Triple Quad™ 4500 (ESI, +ve Ionization)

LC Conditions for Phospholipid Comparison

Column: Kinetex 2.6 µm 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
11.5	95
11.51	40
13	40

Flow Rate: 0.5 mL/min
Injection Volume: 2 µL
Temperature: 45 °C
Instrument: Agilent 1260
Detector: SCIEX Triple Quad 4500 (ESI, +ve Ionization)
Sample: Phospholipid (Retention time in minute)
1. Lyso PC (2.25), MRM transition 496.4/184.2
2. PC-1 (4.14), MRM transition 760.7/184.2
3. PC-2 (4.6), MRM transition 786.8/184.2

Results and Discussion

Table 1. % Recovery and CV of Veterinary Drugs (50 ng/mL) in Milk using Strata-X PRO SPE

Peak No.	Analyte Name	Retention Time (min)	% Recovery	% CV	Q1	Q3
1	Sulfaguanidine	1.48	46	5	215	156.1
2	Lincomycin	2.07	92	5	407.1	126
3	Sulfadiazine	2.19	38	7	251	156
4	Cephapirin	2.22	76	7	424	292.1
5	Sulfamerazine	2.32	44	5	265.1	155.8
6	Sulfamethoxazole	2.36	53	13	254.1	156.1
7	Sulfamethizole	2.36	45	8	271.1	92
8	Cefalexin	2.39	66	4	348.2	174.2
9	Sulfamethazine	2.44	59	13	279.1	186.1
10	Cortisone	2.72	83	8	361.2	163.2
11	Cortisol	2.73	95	6	363.4	120.9
12	β-methasone	2.76	97	3	393.4	355.2
13	Prednisolone	2.81	92	10	361.2	147.2

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Figure 1. Chromatogram of Veterinary Drugs in Milk Following Clean-up by Strata®-X PRO using a Kinetex® Biphenyl LC Column

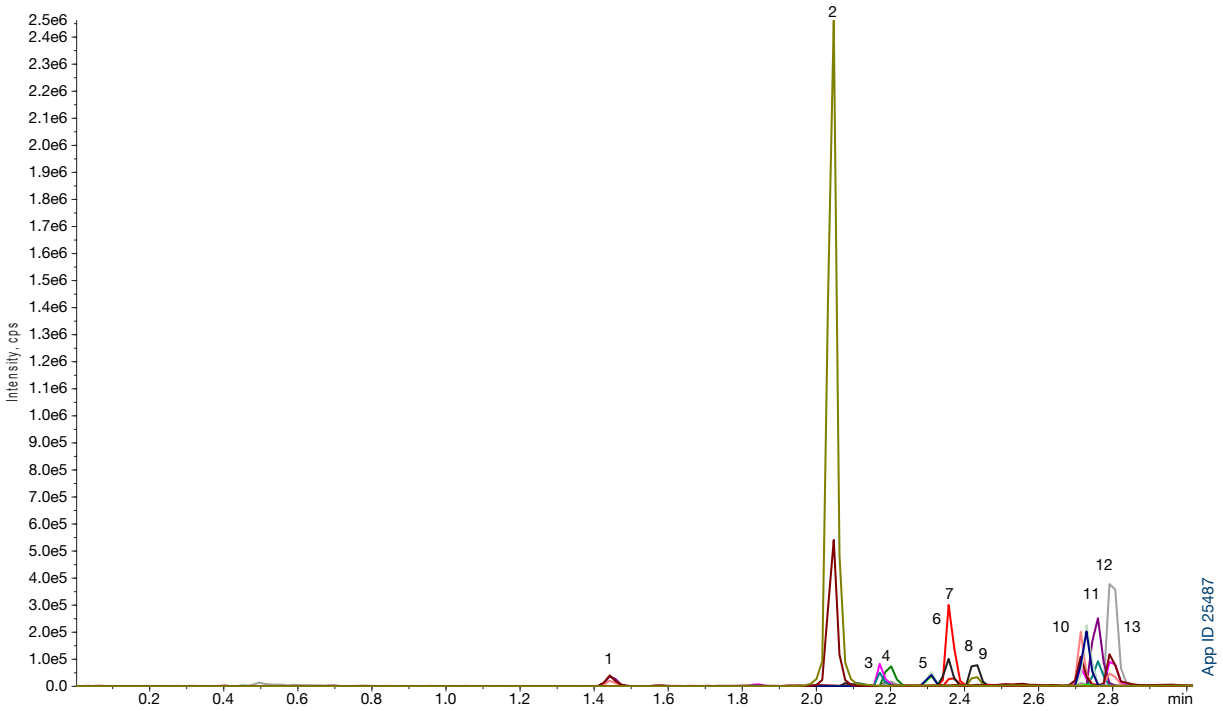
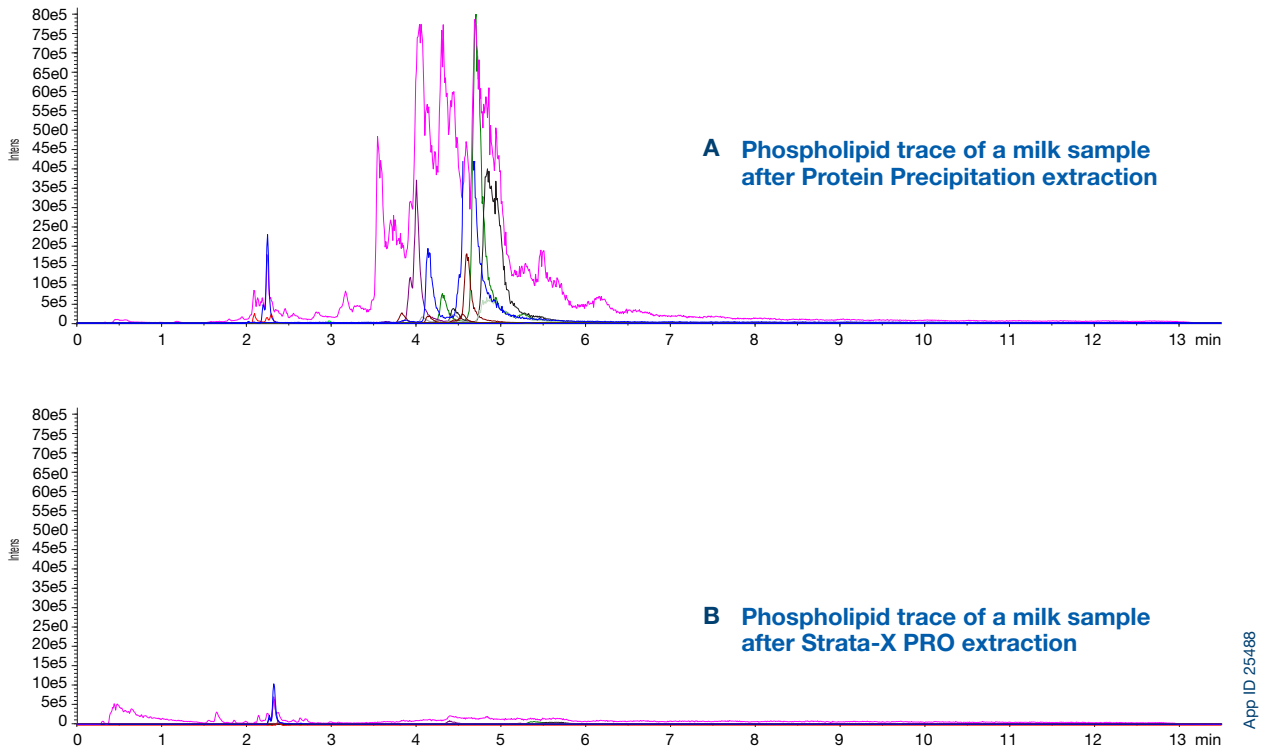


Figure 2. LC-MS/MS Total Ion Current (TIC) Comparison of Blank Milk Sample Processed with Standard Protein Precipitation (A) and Strata-X PRO SPE (B)



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In this technical note, Strata®-X PRO is compared to the protein precipitation method that is widely used for its simplicity, quick and minimal method development. In a parallel study, the TIC obtained from MS analysis, displays the high abundance of the phospholipids (**Fig. 2A**) in the sample extract from protein precipitation. The Strata-X PRO sample clean-up, on the other hand, depleted the majority of the phospholipids (**Fig. 2B**) resulting in absolute recovery of 13 analytes (**Fig. 1**) around 67% (**Table 1**) on the average. Failure to remove phospholipids in the sample results in matrix effect and eventually pre-mature LC column death due to continuous lipid build-up. Additionally, an increased instrument downtime and higher maintenance for the MS instrument is observed in the aftermath.

Conclusion

Though widely used, protein precipitation method co-extracts endogenous phospholipids that negatively affect chromatographic analysis resulting in skewed analytical data. A cleaner and yet a fast two-step method using Strata-X PRO, mitigates these effects, while ensuring the upkeep of the MS instrument. The analytical data elucidated in this study demonstrates effective removal of phospholipids and fats from milk matrix, maintaining the precision and accuracy of the assay, while Kinetex® Core-Shell LC columns were used in conjunction.

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	ea
	30 mg/well	8E-S536-TGA	ea
	60 mg/well	8E-S536-UGA	ea
96-Well Microelution Plate			
	2 mg/well	8M-S536-4GA	ea

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

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