

APPLICATION

Per- and Polyfluorinated Alkyl Substances (PFAS) from Milk, Eggs, Butter, Cheese, and Fish using QuEChERS, SPE, and LC-MS/MS

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Scott enjoys surfing and eating. He is crazy about chromatography, because his mom is really into CSI and thinks that is what he does.

Introduction

Per- and polyfluorinated alkyl substances (PFAS), are a group of synthetic fluorinated carbon polymers used on various commercial products, such as fire-fighting foams, non-stick surfaces, and food packaging. These have been detected throughout the global environment, food products, even human plasma. PFAS are associated with various adverse health effects, they are bio-accumulative, ubiquitous, and their analysis level requirements are very low, due to an expected lifetime of exposure.

In the following method we explore sample preparation techniques using a QuEChERS extraction for dairy and fish samples to achieve low ppb sensitivities, and a subsequent solid phase extraction (SPE) for further sample clean-up and ppt sensitivities. In addition, a range of buffers were explored to maximize ionization efficiency, mass spec sensitivity, and maintaining selectivity throughout the wide aliphatic range, including branched isomers.

The method described is a fast (4-minute gradient) and effective LC-MS/MS methodology. Using a Luna[®] Omega PS C18, a novel C18 column chemistry with a positive surface charge for the determination of PFAS at a 0.05-1000 ppb range in dairy and fish samples, a QuEChERS extraction for accurate 1 ppb recoveries in matrix, and secondary solid phase extraction clean-up for further accurate 0.1 ppb recoveries in matrix.

Experimental Conditions

Sample Preparation

Step 1. QuEChERS Extraction and dSPE Protocol

roQ Extraction Kit (Part No. KS0-8910)

- Take 1 g homogenized sample and add into 50 mL centrifuge tube
 - * Spike samples with IS/surrogate and/or analytical spike as appropriate
- Add 10mL Acetonitrile and 10mL Water
- Add salts in salt packets (4 g MgSO₄ + 1g NaCl salts)

Caution: Exothermic Reaction

Note: Salt pack will clump (vortexing is not violent enough to break up clumps), specifically shake the sample violently until homogenous and then vortex for 30 seconds.
- Centrifuge until there is distinct separation between Acetonitrile, Water, and Solids layers
- Transfer 200 µL to autosampler vial for LC-MS/MS analysis, or remove 1 mL clean Acetonitrile and proceed to dSPE Protocol

roQ QuEChERS dSPE Kit (Part No. KS0-8920)

- Transfer 1 mL of Acetonitrile and add to dSPE Kit Tube (150 mg MgSO₄ and 50 mg PSA)
- Vortex for 30 seconds and centrifuge
- Remove 1 mL clean Acetonitrile and analyze, or proceed to Step 2 SPE Protocol

Step 2. Solid Phase Extraction (SPE) Protocol

Pre-treatment:

Transfer 500 µL of clean acetonitrile from QuEChERS Protocol and dilute to ~15 mL with water

Solid Phase Extraction

Cartridge: Strata[®]-X-AW 200 mg, 3 mL
Part No.: 8B-S038-FBJ
Condition: Add 3 aliquots of 2 mL 0.3 % NH₄OH/ACN
Equilibrate: 3 mL Water
Load: ~15 mL of diluted QuEChERS Extract
Wash: 5 mL Water
Elute: 4 mL 0.3 % NH₄OH/ACN
Dry: Evaporate to near dryness and reconstitute to 500 µL

Transfer to autosampler vial for LC-MS/MS analysis

LC-MS/MS Conditions

Column: Luna Omega 1.6 µm PS C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4752-AN
Mobile Phase: A: 5 mM Ammonium Acetate in Water
 B: Acetonitrile
Gradient:

Time (min)	% B
0	40
0.5	40
3	90
3.1	100
4	100

Flow Rate: 0.55 mL/min
Injection: 20 µL
Temperature: 40 °C
UHPLC System: Agilent[®] 1290
Detection: Agilent 6460 QQQ
Analytes:

1. PFBA	9. PFHpS	17. Et-FOSE
2. PFPeA	10. PFOS	18. Et-FOSA
3. PFBS	11. PFNA	19. PFDS
4. PFHxA	12. FOSA	20. PFUdA
5. PFHpA	13. Me-FOSE	21. PFDoA
6. PFHxS	14. 8:2 FTS	22. PFTrDA
7. 6:2 FTS	15. Me-FOSA	23. PFTeDA
8. PFOA	16. PFDA	



Results and Discussion

Optimized LC-MS/MS conditions were explored, where ammonium fluoride is often a nice negative ion-mode enhancing modifier. Ammonium acetate, acetic acid, and ammonium formate were also screened (**Figure 1**), with ammonium formate yielding the best ionization efficiency and sensitivity for these particular compounds, and branched isomer resolution was maintained (**Figure 2**).

The system was calibrated from 0.05 ppb – 1000 ppb (**Figure 3**), with a 0.05 ppb LLOQ (**Figure 4**) and 0.5 ppb mid level (**Figure 5**).

Butter, cheese, egg, milk, and fish samples were screened using various QuEChERS-like extractions, including C18 dSPE, C18 dSPE with a hexane, PSA d-SPE with a hexane, C18+GCB dSPE, C18+GCB dSPE with hexane, and PSA dSPE (**Figure 6**). The PSA dSPE with the traditional water+acetonitrile extraction solvents gave the best recoveries for all of the samples with a 1 ng/g sensitivity in matrix (**Figures 7-11**), and then further optional clean-up using a Strata[®]-X-AW (mixed-mode reversed phase – weak anion-exchange) solid phase extraction yielded sensitivity in matrix down to 0.1 ng/g (**Figure 12**).

Conclusion

Even with decreased use of PFAS on commercial products, PFAS contamination will persist throughout the environment due to their bio-accumulative properties. Thus, demand for PFAS analysis is not limited only to environmental samples, but also at low levels in food matrices for human consumption. Presented is a fast and sensitive LC-MS/MS method developed to meet PFAS analysis needs in diverse food samples, including dairy and fish, down to low ppb levels. The use of the QuEChERS technique along with further clean up using solid phase extraction is an effective extraction and clean up procedure to ensure the optimum effectiveness of LC-MS/MS analysis.

Acknowledgement

We would like to provide special thanks to Weck Laboratories for contributing this application.



Figure 1:
Mobile phase buffer sensitivity screens

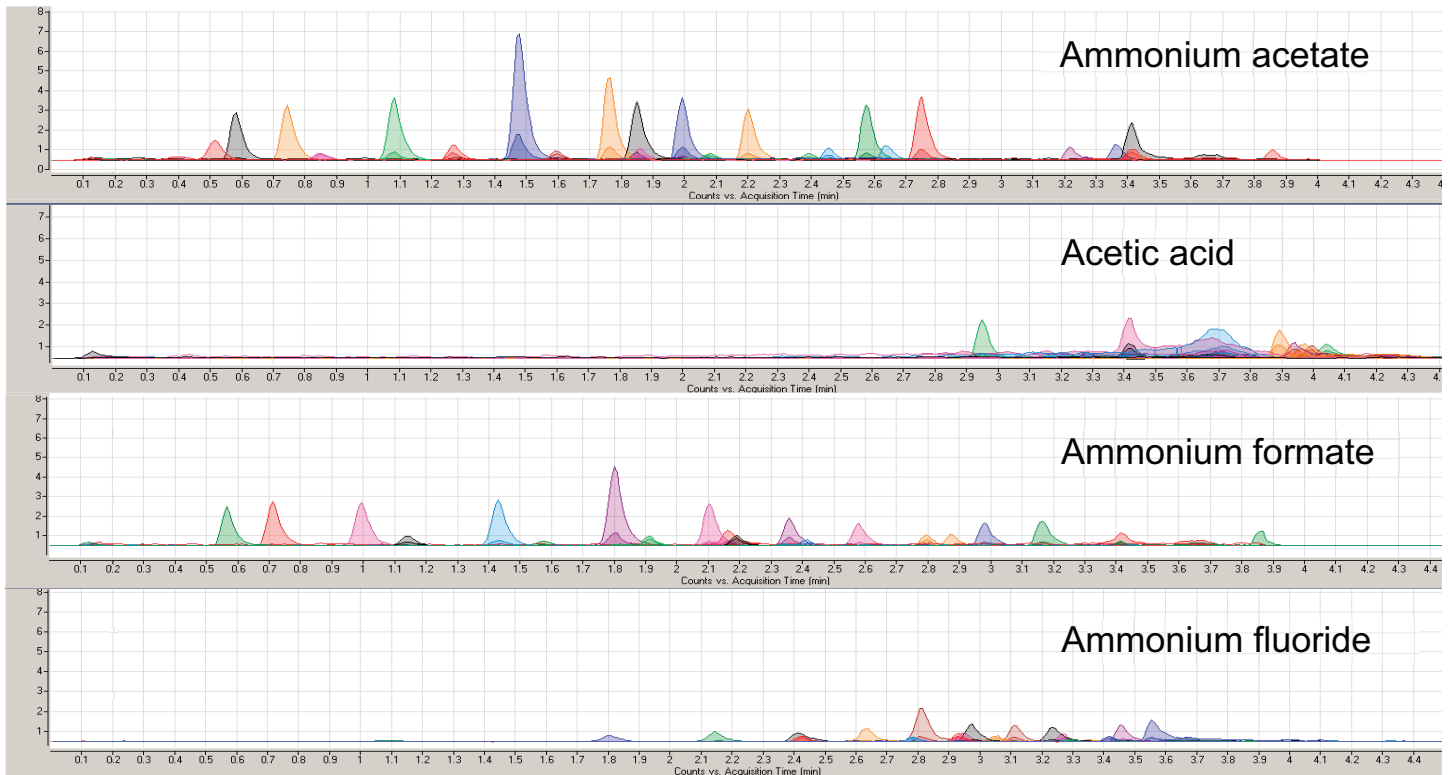


Figure 2:
Branched isomer resolution using Ammonium Formate Buffer

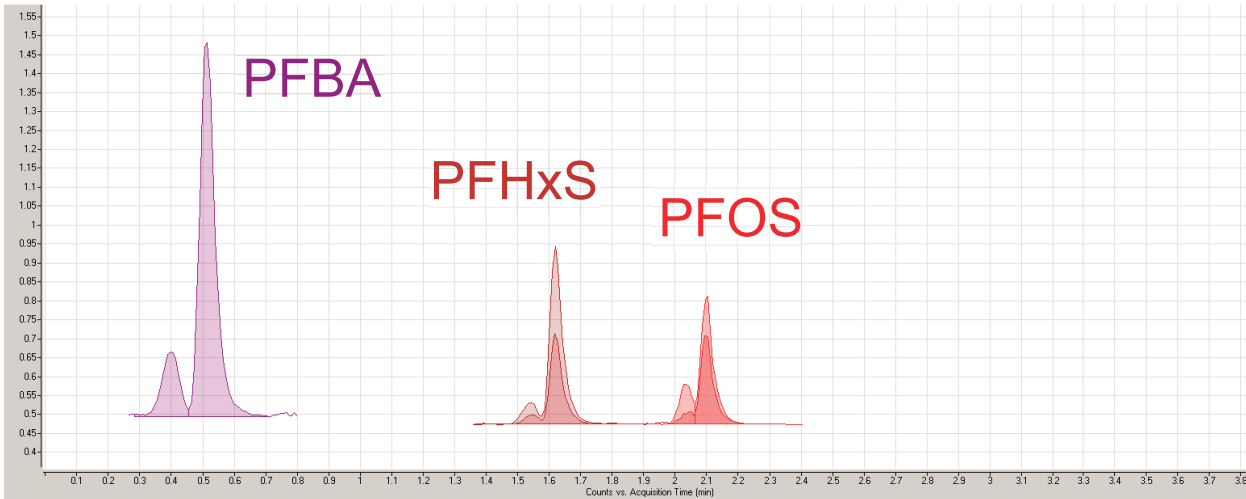


Figure 3:
Dynamic Range (0.05-1000 ppb)

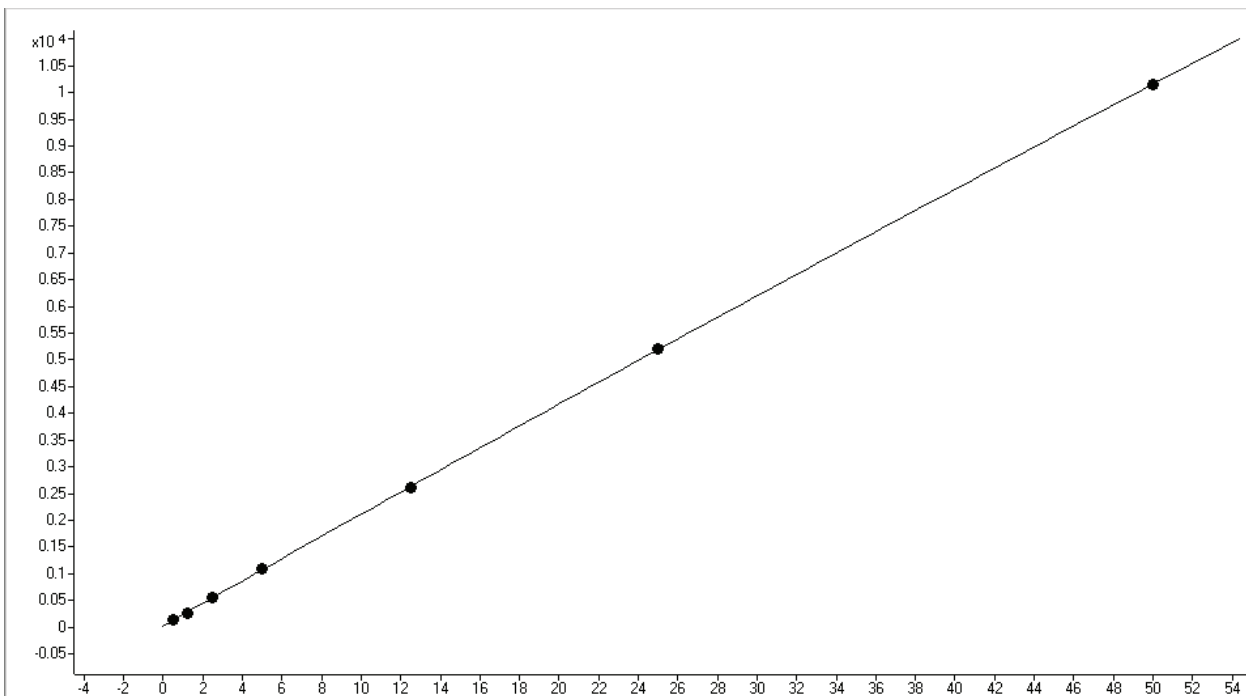
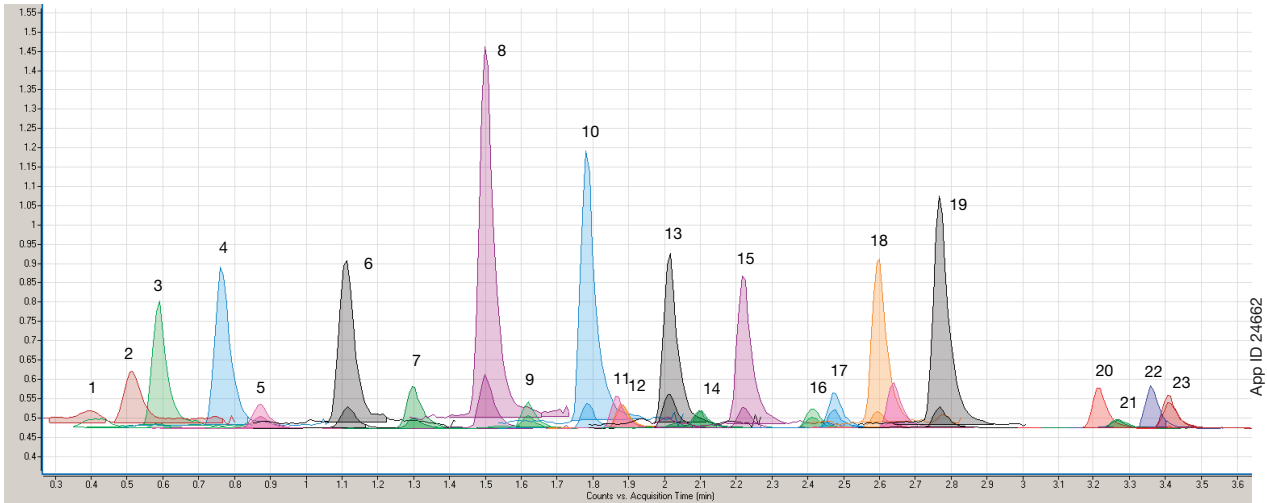
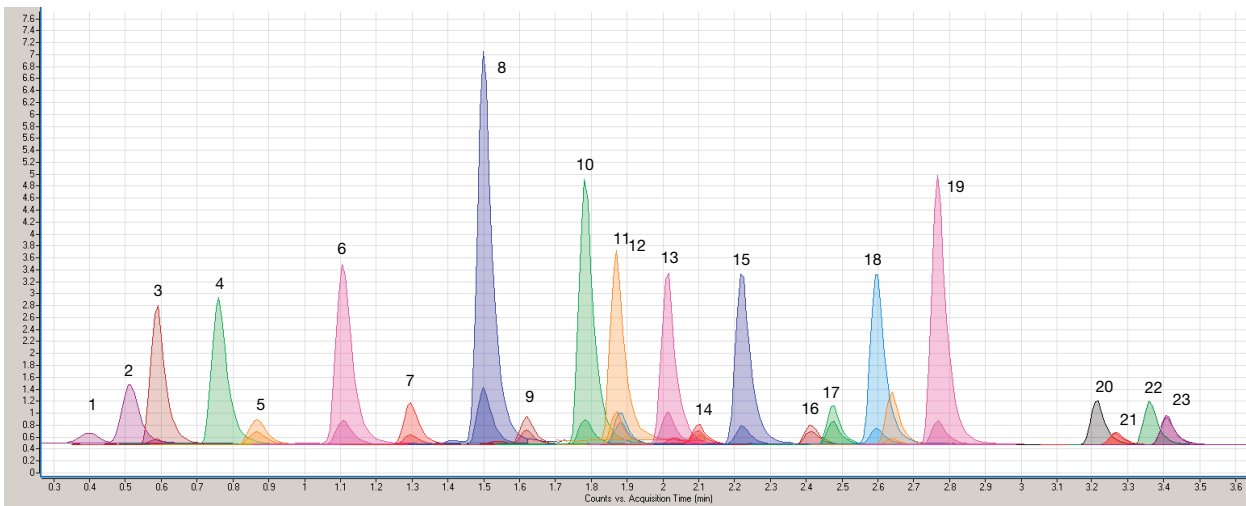


Figure 4:
Chromatogram of a 0.05 ppb LLOQ



App ID 24662

Figure 5:
Chromatogram of 0.5 ppb mid-level standard



App ID 24665

Figure 6:
Water + Acetonitrile with PSA dSPE
From left to right: Blank, Butter, Cheese, Egg, Milk, and Fish

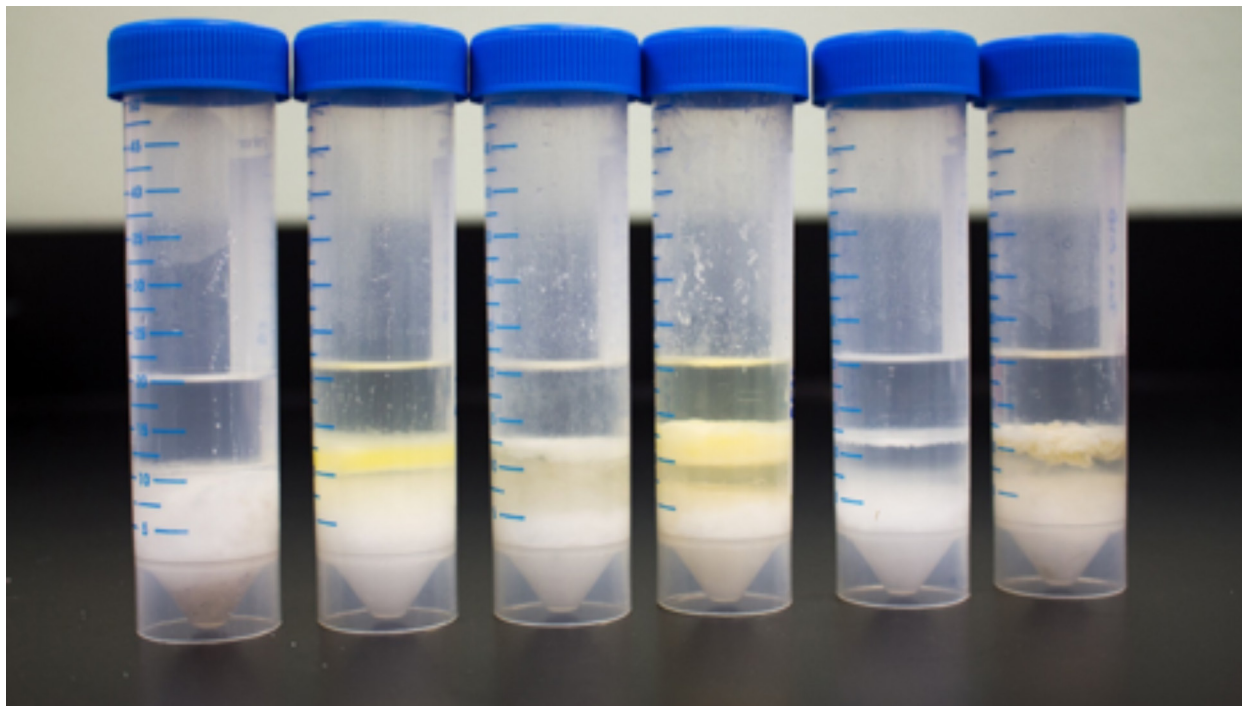


Figure 7:
QuEChERS Recoveries 1 ng/g (n=4)

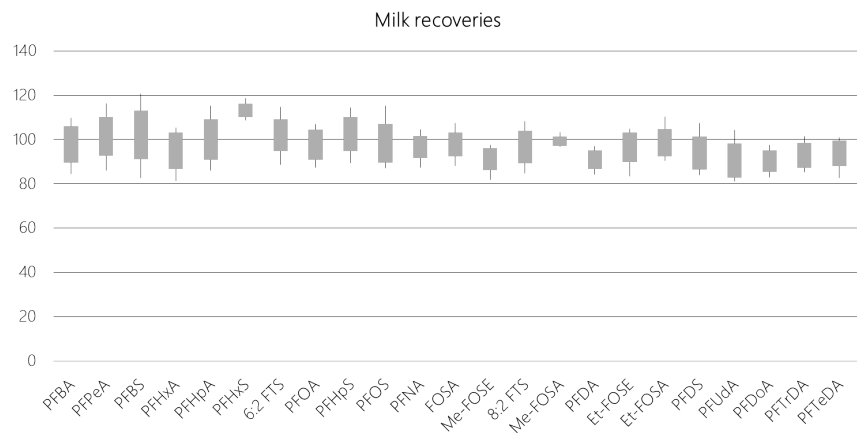


Figure 8:
QuEChERS Recoveries 1 ng/g (n=4)

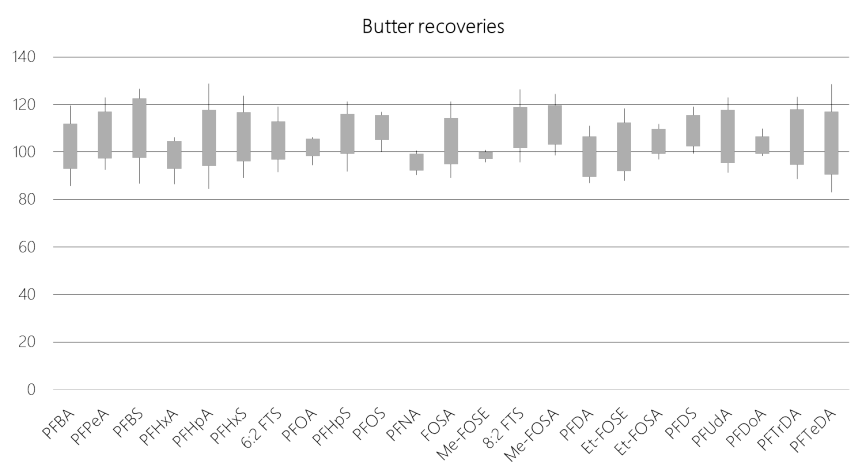


Figure 9:
QuEChERS Recoveries 1ng /g (n=4)

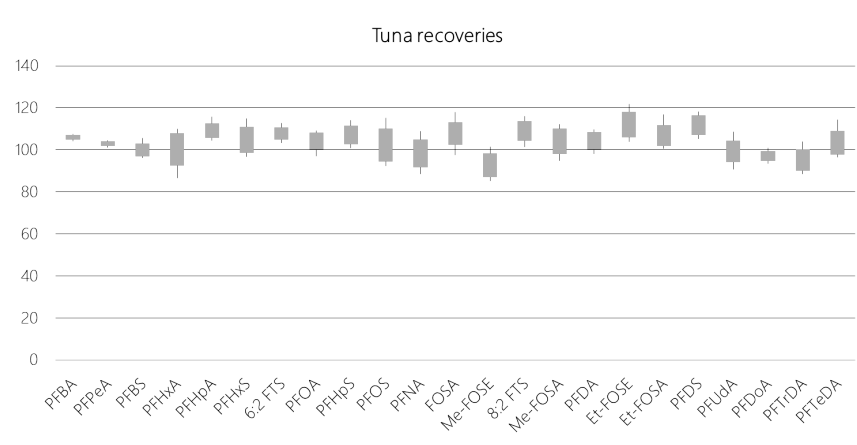


Figure 10:
QuEChERS Recoveries 1 ng/g (n=4)

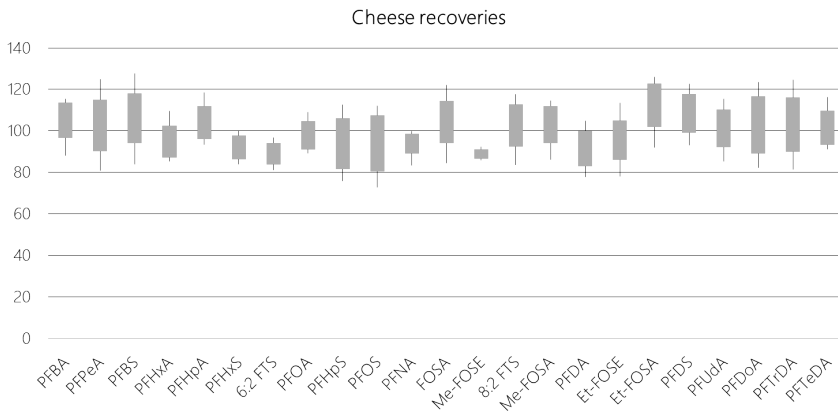


Figure 11:
QuEChERS Recoveries 1 ng/g (n=4)

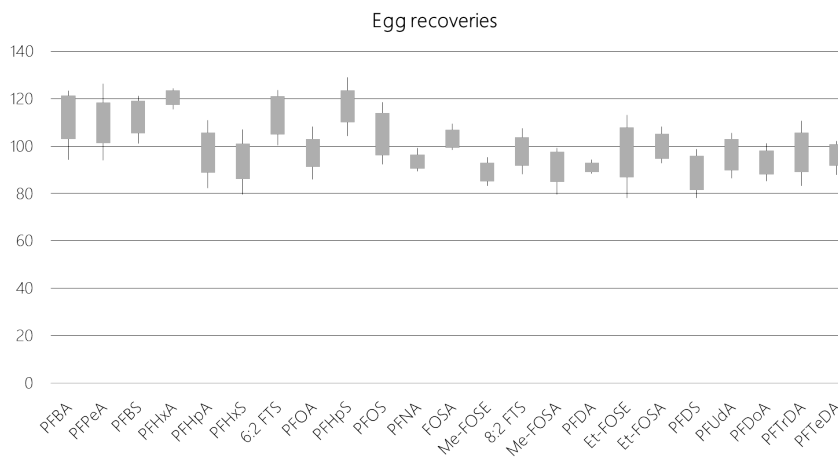
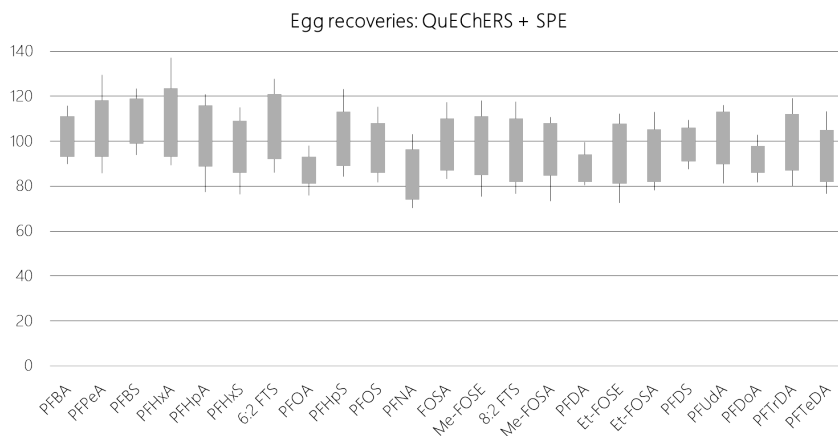


Figure 12:
QuEChERS + SPE Recoveries 0.1 ng/g (n=4)



APPLICATION

Luna® Omega Ordering Information

1.6 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-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJO-9508
					for ID: 2.1 mm ID

⁺SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

5 µm Minibore Columns (mm)		SecurityGuard Cartridges (mm)			
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0* (10/pk)
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJO-7605
					for ID: 2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)		SecurityGuard Cartridges (mm)		
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* (10/pk)
PS C18	00B-4753-YO	00D-4753-YO	00F-4753-YO	AJO-7605
				for ID: 2.0 - 3.0 mm

5 µm Analytical Columns (mm)		SecurityGuard Cartridges (mm)			
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* (10/pk)
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJO-7606
					for ID: 3.1-8.0 mm

⁺SecurityGuard Analytical cartridges require holder, Part No.: KJO-4282

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Strata®-X-AW Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S038-EBJ	3 mL (50/box)
	100 mg	8B-S038-ECH	6 mL (30/box)
	200 mg	8B-S038-FBJ	3 mL (50/box)
	200 mg	8B-S038-FCH	6 mL (30/box)
	500 mg	8B-S038-HBJ	3 mL (50/box)
	500 mg	8B-S038-HCH	6 mL (30/box)

roQ™ Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Kits		
6.0 g MgSO ₄ , 1.5 g NaOAc	50/pk	KSO-8911*
EN 15662 Method Extraction Kits		
4.0 g MgSO ₄ , 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KSO-8909*
Original Non-buffered Method Extraction Kits		
4.0 g MgSO ₄ , 1.0 g NaCl	50/pk	KSO-8910
6.0 g MgSO ₄ , 1.5 g NaCl	50/pk	KSO-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KSO-8911-NC and KSO-8909-NC

roQ dSPE Kits

dSPE kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg MgSO ₄ , 25 mg PSA, 25 mg C18E	100/pk	KSO-8913
150 mg MgSO ₄ , 25 mg PSA, 2.5 mg GCB	100/pk	KSO-8914
150 mg, MgSO ₄ , 25 mg PSA, 7.5 mg GCB	100/pk	KSO-8915
150 mg MgSO ₄ , 25 mg PSA	100/pk	KSO-8916
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18E, 50 mg GCB	100/pk	KSO-8917
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18E	100/pk	KSO-8918
150 mg MgSO ₄ , 50 mg PSA, 50 mg GCB	100/pk	KSO-8919
150 mg MgSO ₄ , 50 mg PSA	100/pk	KSO-8920
15 mL dSPE Kits		
900 mg MgSO ₄ , 150 mg PSA, 150 mg C18E	50/pk	KSO-8921
900 mg MgSO ₄ , 150 mg PSA, 15 mg GCB	50/pk	KSO-8922
900 mg MgSO ₄ , 150 mg PSA, 45 mg GCB	50/pk	KSO-8923
900 mg MgSO ₄ , 150 mg PSA	50/pk	KSO-8924
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18E, 400 mg GCB	50/pk	KSO-8925
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18E	50/pk	KSO-8926
1200 mg MgSO ₄ , 400 mg PSA, 400 mg GCB	50/pk	KSO-8927
1200 mg MgSO ₄ , 400 mg PSA	50/pk	KSO-8928



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