

# Analysis of PFAS in Milk by LC-MS/MS

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#### **Abstract**

The ubiquitous use of per- and polyfluoroalkyl substances (PFAS) in everyday products has resulted in the accumulation of these compounds in the environment. Due to the prevalence of these compounds in environmental sources, such as water and soil, they are finding avenues into food commodities meant for human consumption. Dairy milk is one such commodity that can be impacted by environmental contaminants, such as PFAS, so it is important to implement extensive, robust, and accurate testing. In this work, a sensitive and reliable method was developed for the analysis of PFAS in milk by LC-MS/MS at levels as low as  $0.01~\mu g/kg$ .

## Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured organic compounds that are used in a wide array of applications and products. The carbon-fluorine bond is very stable resulting in compounds that do not readily degrade in the environment. As such, these compounds have been detected in drinking water, soil, wastewater, food commodities, and biological matrices. There are several potential adverse health effects associated with exposure to PFAS. These include increased cholesterol levels, decreased birth weight, lower antibody response to vaccines, kidney and testicular cancer, pregnancy-induced hypertension (preeclampsia), and changes in liver enzymes [1]. Since food sources have been identified as one of the avenues of exposure to PFAS, it is important to have robust, reliable, and sensitive methods to monitor these compounds in foods intended for human consumption.

## **Related Products**

- Force C18 3 μm, 50 mm x 2.1 mm (cat.# 9634352)
- Force C18 EXP guard cartridge, 5 mm x 2.1 mm (963450252)
- PFAS delay column (cat. # 27854)
- PFAS 28 calibration standard (cat.# 30734)
- 50 mL centrifuge tube (cat.# 25846)
- Q-sep QuEChERS extraction salt packet (cat.# 25847)
- Q-sep QuEChERS dSPE 15 mL centrifuge tube (cat.# 26126)
- Polypropylene vial (cat.# 23243)
- Polyethylene screw cap (cat.# 23244)

More than six billion people around the world consume dairy milk and milk products [2] so it is imperative to monitor PFAS contamination in these products. The European Food Safety Authority (EFSA) has assessed four PFAS that make up half of the lower bound exposure: perfluorooctane sulfonic acid (PFOS); perfluorooctanoic acid (PFOA); perfluorohexane sulfonic acid (PFHxS); and perfluorononaoic acid (PFNA). Toddlers and children were found to have two-fold higher exposure [3], and this could potentially be from their consumption of milk and other related food items. The EFSA recommends limiting exposure to these four PFAS to no more than 4.4 nanograms per kilogram of body weight per week [3]. In the United States, Maine Department of Agriculture, Conservation, and Forestry (DACF) established an action threshold for PFOS in milk at 210 parts per trillion [4].

In this work, a method was developed for the analysis of PFAS in milk by LC-MS/MS, including the four PFAS assessed by the EFSA and 24 other compounds included in the European Union Reference Laboratory for Halogenated POPs (persistent organic pollutants) in Feed and Food guidance document. This guidance document outlines required limits of quantitation for the four PFAS analytes identified by EFSA in milk to be as low as  $\leq 0.01 \,\mu\text{g/kg}$  [5]. This target LOQ was used in these experiments and applied to all 28 PFAS. One potential complication of this analysis is the presence of coextractables, such as bile acids. Bile acids tauroursodeoxycholic acid (TUDCA); taurochenodeoxycholic acid (TCDCA); and taurodeoxycholic acid (TDCA) can be present in high concentrations in samples of biological origin and share the same mass transition as PFOS. To accurately quantify PFOS, the bile acids need to either be removed from the sample during sample preparation or separated from PFOS chromatographically. In this application, a workflow was developed for the analysis of 28 PFAS in milk. To prepare samples, a QuEChERS approach coupled with dSPE was implemented. This workflow returned exceptional results for analysis of the four PFAS assessed by the EFSA and showed excellent method accuracy and precision for most target compounds.



## **Experimental**

## Chromatographic Method

LC-MS/MS analysis of PFAS in milk was performed on a Waters ACQUITY Premier system coupled with a Waters XEVO TQ Absolute triple quadrupole mass spectrometer. Note that a PFAS delay column was installed between the mixer and injector to prevent any potential PFAS contamination upstream of the injector from coeluting with PFAS in the samples. The method conditions are as follows:

## Columns:

• Analytical column: Force C18, 50 mm x 2.1 mm, 3 μm (cat.# 9634352) • Guard column: Force C18 EXP, 5 mm x 2.1 mm (cat.# 963450252)

• PFAS delay column, 50 mm x 2.1 mm, 5 μm (cat.# 27854)

Injection volume:

Mobile phase A: Water, 5 mM ammonium acetate Mobile phase B: Methanol:acetonitrile (50:50)

Flow rate: 0.6 mL/min Temperature:

50 °C

Gradient: Time (min) %B 0.00

5 3.00 40 70 9.00 9.50 95 100 10.50 100 11.00 5 11.01 13.00 5

Ion mode: Negative ESI Mode: Scheduled MRM

## Sample and Standard Preparation

All products and solvents were tested for PFAS contamination prior to use. Milk samples were obtained locally and included reduced-fat milk (Sample 1); fat-free milk (Sample 2); and low-fat milk (Sample 3). Ten gram samples of milk were weighed into 50 mL centrifuge tubes (cat.# 25846) and fortified at 0.01 or 0.05 µg/kg. Isotopically labeled internal standards were spiked into the sample at 0.1 µg/kg and briefly vortexed. Acetonitrile (10 mL), formic acid (150 µL), and a Q-sep QuEChERS extraction salt packet (cat.# 25847) were added to the sample and vortexed for approximately 30 seconds. The samples were transferred to a shaker table and shaken for 10 minutes. The samples were then centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a Q-sep QuEChERS dSPE 15 mL centrifuge tube containing 900 mg MgSO4, 300 mg PSA, 150 mg GCB (cat.# 26126) and vortexed for approximately 30 seconds followed by centrifugation for 5 minutes at 4200 rpm. The supernatant was aliquoted (6 mL) into clean 15 mL centrifuge tubes and dried down at 35 °C for 90 minutes using a blowdown evaporator. The samples were reconstituted in methanol:water (60:40) (400 μL), vortexed for approximately 30 seconds, and centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a polypropylene vial (cat.# 23243) and capped with a polyethylene screw cap (cat.# 23244) and 5 µL was injected for analysis.

The working calibration standards were prepared using Restek's PFAS 28 calibration standard (cat.# 30734), and the standards for other PFAS were obtained separately. The standards were aliquoted into polypropylene LC vials and diluted in 60:40 methanol:water to a concentration range of 0.03-50 ng/mL. Each calibration curve included seven or eight concentration levels depending on analyte sensitivity. Internal standards were spiked in the calibration standards at 1.5 ng/mL. Solvent calibration using isotope dilution was performed for quantitation. The mass transitions and the isotopically labeled internal standards that were used for each analyte are shown in Table I.



**Table I:** Precursor lons, Product lons, and Isotopically Labeled Internal Standards Used for the Analysis of PFAS in Milk by LC-MS/MS

| Compound     | Precursor 1 | Product 1 | Precursor 2 | Product 2 | Internal Standard |
|--------------|-------------|-----------|-------------|-----------|-------------------|
| PFBA         | 212.94      | 168.89    | NA          | NA        | M4PFBA            |
| PFPeA        | 262.93      | 218.89    | NA          | NA        | M5PFPeA           |
| PFHxA        | 313.10      | 119.03    | 313.10      | 268.88    | M5PFHxA           |
| PFHpA        | 363.16      | 169.06    | 363.16      | 319.09    | M4PFHpA           |
| PFOA         | 413.10      | 168.90    | 413.10      | 368.96    | M8PFOA            |
| PFNA         | 463.10      | 219.02    | 463.10      | 419.01    | M9PFNA            |
| PFDA         | 513.17      | 219.06    | 513.17      | 469.16    | M6PFDA            |
| PFUnDA       | 562.78      | 268.82    | 562.78      | 518.80    | M7-PFUnDA         |
| PFDoA        | 612.52      | 318.90    | 612.52      | 568.80    | M2-PFDoA          |
| PFTrDA       | 662.78      | 168.87    | 662.78      | 618.74    | M2-PFTeDA         |
| PFTeDA       | 712.78      | 168.87    | 712.78      | 668.74    | M2-PFTeDA         |
| PFBS         | 298.97      | 79.97     | 298.97      | 98.89     | M3PFBS            |
| PFPeS        | 349.10      | 79.98     | 349.10      | 98.98     | M5PFHxA           |
| PFHxS        | 398.80      | 79.82     | 398.80      | 98.82     | M3PFHxS           |
| PFHpS        | 448.78      | 79.82     | 448.78      | 98.82     | M9PFNA            |
| PFOS         | 498.78      | 79.89     | 498.78      | 98.82     | M8PFOS            |
| PFNS         | 548.65      | 79.89     | 548.65      | 98.82     | M7-PFUnDA         |
| PFDS         | 598.78      | 98.82     | 598.79      | 79.89     | M2-PFDoA          |
| PFUnDS       | 648.65      | 79.88     | 648.65      | 98.82     | M2-PFDoA          |
| PFTrDS       | 748.59      | 79.88     | 748.90      | 98.88     | M2-PFTeDA         |
| PFDoS        | 698.71      | 79.89     | 698.71      | 98.88     | M2-PFTeDA         |
| FOSA         | 497.75      | 77.87     | 497.75      | 477.76    | M7-PFUnDA         |
| ADONA        | 376.90      | 84.97     | 376.90      | 250.93    | M4PFHpA           |
| HFPO-Da      | 284.87      | 168.89    | 284.87      | 184.83    | M5PFHxA           |
| 9Cl-PF3ONS   | 530.78      | 350.85    | 532.84      | 352.78    | M7-PFUnDA         |
| 11Cl-PF3OUdS | 630.71      | 450.81    | 632.71      | 452.81    | M2-PFDoA          |
| Capstone A   | 526.90      | 180.96    | 526.90      | 506.91    | M9PFNA            |
| Capstone B   | 568.81      | 548.85    | 568.81      | 548.90    | M9PFNA            |

## **Results & Discussion**

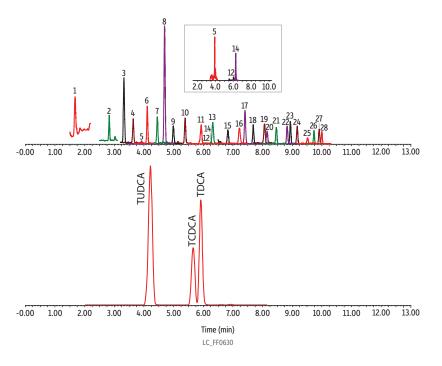
# Chromatographic Performance

Bile acids are often a concern when analyzing tissue samples for PFAS. Taurodeoxycholic acid (TDCA) is an endogenous bile acid that is formed in the liver and can be detected at high concentrations in biological samples. This compound, along with tauroursodeoxycholic acid (TUDCA) and taurochenodeoxycholic acid (TCDCA), all share the same mass transition (499>80) as PFOS and can also potentially interfere with FOSA. As a result, bile acids either need to be removed from the sample or be chromatographically resolved from the impacted compounds. It can be challenging to selectively remove bile acids from the sample without also removing analytes of interest; therefore, the chromatographic approach was taken here for the analysis of PFAS in milk samples.

Methanol and acetonitrile were both tested as organic mobile phases. Using a scouting gradient, methanol was tested first, and the results indicated that the use of this organic mobile phase would require significantly more method development to be able to potentially resolve the interferences. When using acetonitrile, all three bile acids were easily resolved from PFOS using the scouting conditions, but sensitivity for some of the PFAS was reduced compared to when using methanol. Therefore, a 50:50 mixture of the two organic modifiers was used, and it provided good sensitivity and resolution of the bile acids from PFOS. The final LC-MS/MS method for the analysis of PFAS in milk used a 50:50 mixture of methanol and acetonitrile and can be seen in Figure 1.



Figure 1: Top: Chromatogram of 28 PFAS and Internal Standards (The PFAS that can potentially be affected by bile acid coelution are PFOS and FOSA.) Bottom: Chromatogram of Three Bile Acids Analyzed Using the Same Chromatographic Conditions



| Peaks   | Precursor<br>Ion 1 | Product<br>Ion | Column<br>Dimensions:             | Force C18 (cat.# 9634352)<br>50 mm x 2.1 mm ID  |
|---|--------------------|----------------|-----------------------------------|---|
| Perfluorobutanoic acid (PFBA)                                     | 212.94             | 168.89         | Particle Size:                    | 3 μm  |
| 2. Perfluoropentanoic acid (PFPeA)                                | 262.93             | 218.89         | Pore Size:                        | 100 Å   |
| 3. Perfluorobutane sulfonic acid (PFBS)                           | 298.96             | 79.97          | Guard Column:                     | Force EXP C18 guard cartridge 5 mm, 2.1 mm ID (cat.# 963450252)   |
| 4. Perfluorohexanoic acid (PFHxA)                                 | 313.09             | 119.03         | Temp.:                            | 50 ℃  |
| 5. Hexafluoropropylene oxide dimer acid (HFPO-DA)                 | 284.86             | 168.88         | Standard/Sample                   | PFAS 28 calibration standard (cat.# 30734)  |
| 6. Perfluoropentane sulfonic acid (PFPeS)                         | 349.10             | 79.98          |                                   | Other standards obtained externally.  |
| 7. Perfluoroheptanoic acid (PFHpA)                                | 363.15             | 169.06         | Diluent:                          | Methanol:water (60:40)  |
| 8. Ammonium 4,8-dioxa-3 <i>H</i> -perfluorononanoate (ADONA)      | 376.90             | 84.97          | Conc.:                            | Analytes spiked at 0.01 μg/kg in milk   |
| 9. Perfluorohexane sulfonic acid (PFHxS)                          | 398.80             | 79.82          | Inj. Vol.:<br><b>Mobile Phase</b> | 5 μL  |
| 10. Perfluorooctanoic acid (PFOA)                                 | 413.09             | 168.90         | A:                                | Water, 5 mM ammonium acetate  |
| 11. Perfluoroheptane sulfonic acid (PFHpS)                        | 448.78             | 79.82          | А.<br>В:                          | Methanol:acetonitrile (50:50)   |
| 12. 1-Propanaminium, N-(carboxymethyl)-N,N-dimethyl-3-            |                    | 15102          | D.                                | Time (min) Flow (mL/min) %A %B  |
| [[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, |                    |                |                                   | 0.00 0.6 95 5   |
| hydroxide (Capstone B)  | 568.81             | 548.85         |                                   | 3.00 0.6 60 40  |
| 13. Perfluorononanoic acid (PFNA)                                 | 463.09             | 219.01         |                                   | 9.00 0.6 30 70  |
| 14. 1-Propanaminium, N,N-dimethyl-N-oxide-3-                      | 100.00             | 223.01         |                                   | 9.50 0.6 5 95   |
| [[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, |                    |                |                                   | 10.50 0.6 0 100   |
| hydroxide (Capstone A)  | 526.90             | 180.96         |                                   | 11.00 0.6 0 100   |
| 15. Perfluorooctane sulfonic acid (PFOS)                          | 498.78             | 79.89          |                                   | 11.01 0.6 95 5  |
| 16. Perfluorodecanoic acid (PFDA)                                 | 513.16             | 219.06         |                                   | 13.00 0.6 95 5  |
| 17. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)   | 530.77             | 350.85         | Man Dunanan                       | 200 have  |
| 18. Perfluorononane sulfonic acid (PFNS)                          | 548.65             | 79.89          | Max Pressure:<br>Detector         | <b>360 bar</b> Waters Xevo TO Absolute  |
| 19. Perfluoroundecanoic acid (PFUnA)                              | 562.78             | 268.82         | Ion Mode:                         | ESI-  |
| 20. Perfluorooctane sulfonic acid (FOSA)                          | 497.75             | 77.87          | Mode:                             | MRM   |
| 21. Perfluorodecane sulfonic acid (PFDS)                          | 598.78             | 98.82          | Instrument                        | Waters ACQUITY Premier  |
| 22. Perfluorododecanoic acid (PFDoA)                              | 612.52             | 318.90         | Sample Preparation                | Ten grams of milk were weighed into a 50 mL centrifuge tube (cat.# 25846)   |
| 23. 11-Chloroeicosafluoro-3-oxanonane-1-sulfonate (11Cl-PF3OUdS)  | 630.71             | 450.81         |                                   | and fortified at 0.01 µg/kg. Isotopically labeled internal standards (50 µL)  |
| 24. Perfluoroundecane sulfonic acid (PFUnDS)                      | 648.65             | 79.88          |                                   | were spiked into the sample and briefly vortexed. Acetonitrile (10 mL); formic  |
| 25. Perfluorotridecanoic acid (PFTrDA)                            | 662.78             | 168.87         |                                   | acid (150 µL); and a Q-sep QuEChERS extraction salt packet (cat.# 25847)  |
| 26. Perfluorododecane sulfonic acid (PFDoS)                       | 698.71             | 79.89          |                                   | were added to the sample and vortexed for ~30 seconds. The samples were   |
| 27. Perfluorotetradecanoic acid (PFTeDA)                          | 712.78             | 168.87         |                                   | transferred to a shaker table and shaken for 10 minutes. The samples were then  |
| 28. Perfluorotridecane sulfonic acid (PFTrDS)                     | 748.59             | 79.88          |                                   | centrifuged for 5 minutes at 4200 rpm. The supernatant was aliquoted into a   |
| , ,   |                    |                |                                   | Q-sep QuEChERS dSPE 15 mL centrifuge tube containing 900 mg MgSO4; 300  |
|   |                    |                |                                   | mg PSA; and 150 mg GCB (cat.# 26126) and then vortexed for ~30 seconds  |
|   |                    |                |                                   | followed by centrifuging for 5 minutes at 4200 rpm. The supernatant was aliquoted (6 mL) into a clean 15 mL centrifuge tube and dried down at 35 °C |
|   |                    |                |                                   | for 90 minutes using a Biotage TurboVap. The samples were reconstituted in  |
|   |                    |                |                                   | methanol:water 60:40 (400 µL); vortexed for ~30 seconds; and centrifuged for  |
|   |                    |                |                                   | 5 minutes at 4200 rpm. The supernatant was aliquoted into a polypropylene vial  |
|   |                    |                |                                   | (cat.# 23243) and capped with a polyethylene screw cap (cat.# 23244) and 5 μL   |
|   |                    |                |                                   | was injected for analysis.  |
|   |                    |                | Notes                             | A PFAS delay column (cat.# 27854) was installed before the injector.  |
|   |                    |                |                                   |   |

## Linearity, Accuracy, & Precision

For this LC-MS/MS method for the analysis of PFAS in milk, calibration was performed across a range of 0.03/0.1-50 ng/mL at seven or eight different concentrations. To assess linearity, 1/x weighting was used. Good linearity was demonstrated by  $R^2$  values  $\geq 0.991$  for all compounds (Table III).

Three different milk samples were obtained and fortified at 1X target LOQ and 5X target LOQ levels ( $0.01~\mu g/kg$  and  $0.05~\mu g/kg$ , respectively). Blank samples were prepared in duplicate and analyzed in triplicate. Milk samples were prepared in triplicate and analyzed in triplicate. Analytes detected in the blank samples are identified by an asterisk in Tables II and III, and incurred levels were subtracted from the fortified samples. Excellent recoveries were obtained at the LOQ spiking level for all four of the PFAS assessed by the EFSA. These recoveries were 101-112% at  $0.01~\mu g/kg$ , and excellent precision was demonstrated by %RSDs of 3.81-16.6% (Table II).

Table II: Percent Recoveries and %RSD at 0.01 µg/kg for the Four PFAS Assessed by EFSA

|       | Average Percent Recovery (%RSD) n=9 Sample 1 Sample 2 Sample 3 |            |            |  |  |  |
|-------|--|------------|------------|--|--|--|
|       |  |            |            |  |  |  |
| PFOA* | 108 (5.09)   | 102 (6.21) | 105 (7.74) |  |  |  |
| PFNA  | 112 (7.97)   | 106 (8.19) | 101 (16.6) |  |  |  |
| PFHxS | 112 (5.93)   | 106 (7.25) | 106 (12.8) |  |  |  |
| PFOS* | 109 (7.25)   | 109 (3.81) | 102 (6.72) |  |  |  |

<sup>\*</sup>Incurred levels were present in the blank samples for these analytes and were subtracted from the fortified samples.

Most of the 28 PFAS monitored in these experiments had acceptable recoveries within the 80–120% range. Some long-chain PFAS showed low percent recoveries, namely PFTrDA, PFTeDA, PFUnDS, PFTrDS, PFDoS, FOSA, and Capstone B, which contain carbon chains of eight to thirteen carbons. Low recoveries were not observed in Sample 2, the fat-free milk, indicating that the presence of milk fat in the other samples could potentially be hindering the recoveries for these compounds. A summary of the linear dynamic range, R² values, percent recoveries, and percent RSD values can be seen in Table III for all 28 PFAS.



Table III: Linearity, Accuracy, and Precision in Milk

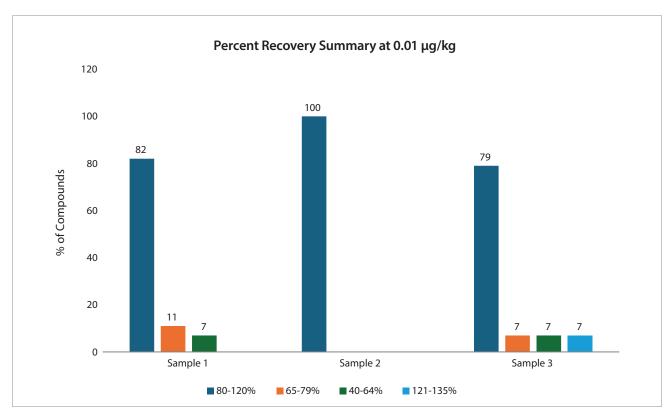
|              | Linear Range (ng/mL) |       |                | Percent Recovery (Percent RSD) n=9 |             |             |             |             |             |
|--------------|----------------------|-------|----------------|------------------------------------|-------------|-------------|-------------|-------------|-------------|
| Compound     |                      | 111.1 | D2             | Sam                                | ple 1       | Sam         | ple 2       | Sam         | ple 3       |
|              | Low                  | High  | R <sup>2</sup> | 0.01 μg/kg                         | 0.05 μg/kg  | 0.01 µg/kg  | 0.05 μg/kg  | 0.01 µg/kg  | 0.05 μg/kg  |
| PFBA*        | 0.1                  | 50    | 0.996          | 90.1 (4.81)                        | 105 (7.06)  | 103 (8.61)  | 116 (2.91)  | 115 (11.5)  | 116 (4.95)  |
| PFPeA        | 0.1                  | 50    | 0.996          | 105 (7.89)                         | 110 (5.45)  | 102 (6.72)  | 111 (2.55)  | 107 (10.8)  | 114 (2.80)  |
| PFHxA        | 0.1                  | 50    | 0.996          | 114 (10.6)                         | 113 (5.13)  | 104 (8.11)  | 112 (5.49)  | 118 (11.3)  | 113 (6.11)  |
| PFHpA        | 0.03                 | 50    | 0.997          | 117 (5.09)                         | 112 (7.31)  | 108 (7.14)  | 115 (2.69)  | 111 (16.2)  | 118 (4.21)  |
| PFOA*        | 0.1                  | 50    | 0.995          | 108 (5.09)                         | 109 (4.62)  | 102 (6.21)  | 113 (2.56)  | 105 (7.74)  | 113 (3.28)  |
| PFNA         | 0.03                 | 50    | 0.996          | 112 (7.97)                         | 109 (2.63)  | 106 (8.19)  | 110 (3.81)  | 101 (16.6)  | 101 (10.4)  |
| PFDA         | 0.03                 | 50    | 0.997          | 111 (9.02)                         | 113 (5.55)  | 107 (6.08)  | 112 (4.13)  | 112 (7.38)  | 115 (3.26)  |
| PFUnDA       | 0.03                 | 50    | 0.992          | 104 (7.52)                         | 109 (11.6)  | 103 (10.9)  | 107 (7.57)  | 109 (8.90)  | 110 (4.54)  |
| PFDoA        | 0.03                 | 50    | 0.997          | 110 (7.57)                         | 109 (4.34)  | 102 (6.72)  | 112 (2.76)  | 108 (9.77)  | 114 (2.45)  |
| PFTrDA       | 0.03                 | 50    | 0.999          | 75.9 (12.5)                        | 65.8 (7.68) | 86.7 (8.95) | 73.3 (8.64) | 71.8 (11.0) | 68.8 (12.2) |
| PFTeDA       | 0.03                 | 50    | 0.997          | 83.2 (14.5)                        | 83.8 (12.4) | 103 (6.91)  | 109 (8.95)  | 74.1 (17.2) | 78.4 (11.3) |
| PFBS*        | 0.1                  | 50    | 0.995          | 106 (11.9)                         | 109 (11.4)  | 110 (11.2)  | 112 (5.21)  | 103 (7.18)  | 109 (5.32)  |
| PFPeS        | 0.1                  | 50    | 0.996          | 113 (6.32)                         | 119 (5.78)  | 105 (6.19)  | 113 (4.87)  | 114 (8.03)  | 115 (5.08)  |
| PFHxS        | 0.03                 | 50    | 0.996          | 112 (5.93)                         | 114 (5.29)  | 106 (7.25)  | 111 (3.59)  | 106 (12.8)  | 113 (3.92)  |
| PFHpS        | 0.03                 | 50    | 0.995          | 117 (5.14)                         | 121 (9.15)  | 112 (5.57)  | 116 (3.81)  | 121 (5.04)  | 119 (5.32)  |
| PFOS*        | 0.1                  | 50    | 0.995          | 109 (7.25)                         | 110 (7.21)  | 109 (3.81)  | 113(4.03)   | 102 (6.72)  | 107 (4.62)  |
| PFNS         | 0.03                 | 50    | 0.999          | 114 (17.1)                         | 88.5 (18.1) | 110 (11.5)  | 90.1 (15.4) | 103 (15.5)  | 84.3 (19.9) |
| PFDS         | 0.03                 | 50    | 0.997          | 92.6 (11.7)                        | 95.8 (6.01) | 101 (7.31)  | 100 (9.33)  | 99.2 (12.3) | 102 (11.8)  |
| PFUnDS       | 0.03                 | 50    | 0.997          | 74.2 (9.48)                        | 95.5 (9.11) | 100 (9.94)  | 113 (9.25)  | 81.4 (20.7) | 104 (12.7)  |
| PFTrDS       | 0.03                 | 50    | 0.994          | 67.9 (15.6)                        | 46.2 (9.53) | 88.1 (14.1) | 95.5 (10.6) | 43.2 (15.1) | 55.5 (6.04) |
| PFDoS        | 0.03                 | 50    | 0.997          | 55.6 (9.8)                         | 59.6 (8.22) | 96.3 (10.0) | 103 (9.33)  | 62.1 (11.5) | 73.9 (7.29) |
| FOSA         | 0.1                  | 50    | 0.992          | 60.8 (12.6)                        | 59.4 (10.2) | 92.6 (8.16) | 95.5 (9.41) | 87.1 (15.3) | 84.0 (12.5) |
| ADONA        | 0.03                 | 50    | 0.996          | 111 (7.17)                         | 109 (7.91)  | 93.8 (11.3) | 98.9 (4.52) | 93.8 (9.54) | 104 (6.00)  |
| HFPO-Da      | 0.1                  | 50    | 0.996          | 106 (13.7)                         | 110 (7.69)  | 111 (15.4)  | 114 (12.7)  | 125 (9.00)  | 124 (4.31)  |
| 9Cl-PF3ONS   | 0.03                 | 50    | 0.992          | 94.5 (3.37)                        | 99.3 (8.09) | 96.6 (10.3) | 98.9 (7.64) | 102 (12.8)  | 106 (6.08)  |
| 11Cl-PF3OUdS | 0.03                 | 50    | 0.997          | 93.5 (6.74)                        | 97.8 (6.78) | 103 (12.2)  | 114 (10.0)  | 97.7 (11.2) | 102 (13.1)  |
| Capstone A   | 0.1                  | 50    | 0.991          | 120 (12.1)                         | 126 (9.27)  | 102 (16.5)  | 108 (7.34)  | 119 (6.44)  | 114 (5.06)  |
| Capstone B   | 0.1                  | 50    | 0.992          | 92.1 (11.6)                        | 68.3 (10.9) | 85.1 (15.2) | 71.7 (8.24) | 84.6 (15.3) | 71.4 (10.6) |

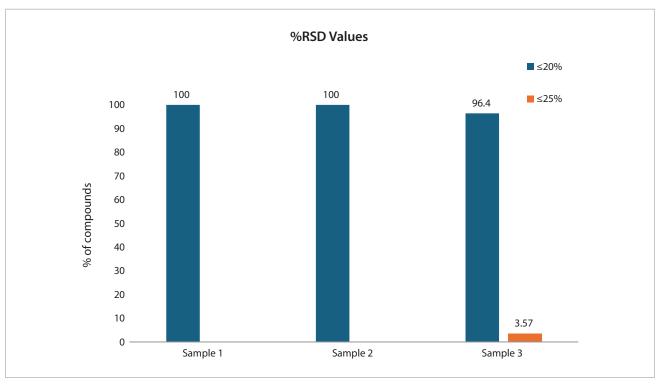
 $<sup>{}^*</sup> Incurred\ levels\ were\ present\ in\ the\ blank\ samples\ for\ these\ analytes\ and\ were\ subtracted\ from\ the\ fortified\ samples.$ 

Figure 2 outlines the results for the analysis of all 28 PFAS in milk samples using this LC-MS/MS method. At 0.01  $\mu$ g/kg, 82% of analytes meet the 80–120% recovery range for Sample 1; 100% did for Sample 2; and 79% did for Sample 3. All but one analyte, PFUnDS, returned a %RSD value of  $\leq$ 20%.



**Figure 2:** Percentage of Analytes in Each Sample Meeting the Acceptable Accuracy and Precision Criteria at 0.01  $\mu$ g/kg for the Analysis of PFAS in Milk







### Conclusion

This workflow provides straightforward and reliable methodology for the analysis of 28 PFAS in milk by LC-MS/MS. The method effectively separates target analytes from bile acid interferences that share the same mass transition. With a fast, simple sample preparation using QuEChERS extraction and dSPE cleanup, this workflow is ideal for high-throughput PFAS testing labs. The results showed exceptional results for the four PFAS assessed by the EFSA, and acceptable results for the majority of the other analytes. The recoveries of a few of the long-chain PFAS returned low values, which may be related to the presence of fat in milk samples. Although the recoveries for these compounds were low, the precision of the method was still within the acceptable range. This method can easily be implemented in labs to confidently monitor PFAS contamination in dairy milk.

#### References

- 1. Agency for Toxic Substances and Disease Registry. PFAS information for clinicians. Fact sheet. January 2024. https://www.atsdr.cdc.gov/pfas/docs/PFAS-info-for-clinicians-508.pdf
- 2. Food and Agricultural Organization of the United Nations. Gateway to diary production and products. 2024. https://www.fao.org/dairy-production-products/en
- 3. D. Schrenk, et al., Risk to human health related to the presence of perfluoroalkyl substances in food, EFSA J 18, (9) 2020. https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6223
- 4. Maine Department of Agriculture, Conservation, & Forestry. PFAS response. 202. https://www.maine.gov/dacf/ag/pfas/pfas-response.shtml
- 5. European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food. Guidance document on analytical parameters for the determination of per- and polyfluoroalkyl substances (PFAS) in food and feed. Version 1.2. May 2022. https://eurl-pops.eu/news/guidance-document-pfas/guidance-document-pfas



## Force C18 LC Column

- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- High carbon load (20%) offers high hydrophobic retention.

| Catalog No. | Product Name                              | Units |
|-------------|---|-------|
| 9634352     | Force C18 LC Column, 3 µm, 50 mm x 2.1 mm | ea.   |





## Force C18 Guard Cartridge

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.
- • For use with 3 or 5  $\mu m$  Force LC columns. For 1.8  $\mu m$  Force columns, use a 0.2  $\mu m$  UltraShield filter.

| Catalog No. | Product Name                              | Units |
|-------------|---|-------|
| 963450252   | Force C18 Guard Cartridge, 5 x 2.1 mm EXP | 3-pk. |



# **PFAS Delay Column**

- Traps system-related PFAS, preventing interference and ensuring accurate trace-level analysis of PFAS in samples.
- Universal compatibility: works with
  - any HPLC or UHPLC up to 15,000 psi (1034 bar);
  - both FPP and SPP analytical columns; and
  - all stationary phases.
- Highly retentive of system-related PFAS; no breakthrough even with extended equilibration times.
- Easy installation with standard fittings.

| Catalog No. | Product Name                                     | Units |
|-------------|--|-------|
| 27854       | PFAS Delay Column, 5 µm, 50 x 2.1 mm HPLC Column | ea.   |



## **PFAS 28 Calibration Standard**

#### Contains:

11-chloroeicosafluoro-3-oxaundecane-1sulfonic acid (11Cl-PF3OUdS) (763051-92-9)

1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS) (39108-34-4)

1H,1H,2H,2H-Perfluorodexane sulfonic acid (4:2 FTS) (75105-34-4)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (4:2 FTS) (27619-97-2)
4,8-dioxa-3H-perfluorononanoic acid (ADONA) (919005-14-4)

9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS) (756426-58-1)

2-(Heptafluoropropoxy)2,3,3,3tetrafluoropropionic acid (HFPO-DA) (13252-13-6) N-ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)\* (2991-50-6)

N-methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)\* (2355-31-9)

Perfluoro-1-decanesulfonic acid (PFDS) (335-77-3)

Perfluoro-1-nonanesulfonic acid (PFNS) (68259-12-1)

Perfluoro-1-octanesulfonamide (FOSA) (754-91-6)

Perfluoro-1-pentanesulfonic acid (PFPeS) (2706-91-4)

Perfluorobutanesulfonic acid (PFBS) (375-73-5)

Perfluorobutanoic acid (PFBA) (375-22-4)

Perfluorodecanoic acid (PFDA) (335-76-2)

Perfluorododecanoic acid (PFDOA) (307-55-1)

Perfluoroheptanesulfonic acid (PFHpS) (375-92-8)

Perfluoroheptanoic acid ((PFHpA) (375-85-9)

Perfluorohexanesulfonic acid (PFHxS)\* (355-46-4)

Perfluorohexanoic acid ((PFHxA) (307-24-4)

Perfluorononanoic acid (PFNA) (375-95-1)

Heptadecafluorooctanesulfonic acid (PFOS)\* (1763-23-1)

Perfluorooctanoic acid (PFOA)\* (335-67-1)

Perfluoropentanoic acid (PFPeA) (2706-90-3)

Perfluorotetradecanoic acid (PFTeDA) (376-06-7)

Perfluorotridecanoic acid (PFTrDA) (72629-94-8)

Perfluoroundecanoic acid (PFUnA) (2058-94-8)

\*Technical grade compound containing both branched and linear isomers; see certificate for details.

| Catalog No. | Concentration | Solvent                               | Volume     | Units |
|-------------|---------------|---------------------------------------|------------|-------|
| 30734       | 1μg/mL        | Methanol (1 mM KOH)/2-Propanol (98:2) | 1 mL/ampul | ea.   |







# **Q-sep QuEChERS Extraction Salt Packets**

- Free-flowing salts transfer easily and completely.
- Easy-open packets eliminate the need for a second empty tube for salt transfer.
- Convenient slim packets fit perfectly into tubes to prevent spills.
- Ready-to-use tubes, no glassware required.
- Pre-weighed, ultra-pure extraction salts.
- Ideal for original unbuffered, AOAC (2007.01), and European (EN 15662) QuEChERS methods.

| Catalog No. | Product Name  | Units  |
|-------------|---|--------|
| 25847       | Q-sep QuEChERS Extraction Salt Packets Only (Original), 4 g MgSO4, 1 g NaCl | 50-pk. |



# **Q-sep QuEChERS dSPE**

- Packaged in foil subpacks of 10 for enhanced protection and storage stability.
- Ready-to-use tubes, no glassware required.
- Pre-weighed, ultra-pure sorbents.
- Support original unbuffered, AOAC (2007.01), European (EN 15662), and minimultiresidue QuEChERS methods.

| Catalog No. | Product Name  | Units  |
|-------------|---|--------|
| 26126       | Q-sep QuEChERS dSPE 15 mL Centrifuge Tube, Contains 900 mg MgSO4,<br>300 mg PSA, 150 mg GCB | 50-pk. |





# **Empty Centrifuge Tubes**

| Catalog No. | Product Name                                       | Units  |
|-------------|--|--------|
| 25846       | Empty Centrifuge Tubes, 50 mL, Polypropylene w/Cap | 50-pk. |



# **Limited-Volume Screw-Thread Polypropylene Vials**

| Catalog No. | Product Name  | Units   |
|-------------|---|---------|
| 23243       | Limited-Volume Screw-Thread Polypropylene Vials, 9 mm, 700 μL, 12 x 32 mm | 100-pk. |



# Solid-Top Polyethylene Caps, Screw-Thread

| Catalog No. | Product Name  | Units   |
|-------------|---|---------|
| 23244       | 2.0 mL, 9 mm Solid-Top Polyethylene Caps, Screw-Thread, | 100-pk. |
|             | 10 mil thick membrane. Clear                            | •       |



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