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LC-MS Solutions for

# PFAS in Food

## Accepted AOAC First Action Method to SMPR 2023.003

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# Introduction

Per- and polyfluoroalkyl substances (PFAS) are broad group of thousands of manufactured chemicals characterized by fluorinated organic chains. This includes perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), the two most commonly found and historically most widely used PFAS compounds. Widely known as "forever chemicals", PFAS are persistent pollutants taken up by plants, they can bioaccumulate in animals, impact our soils, waterways and farms, and have records of adverse health effects.

PFAS have been used in manufacturing of consumer goods like carpet, clothing and shoes, cookware, and packaging for decades. Their effectiveness as both an oil and water repellent, a lubricant, as well as a fire retardant, made these substances popular for use in furniture production, linings for take-out containers, firefighting, medicine and countless other applications. Many industries have moved away from using PFAS, but due to that historic use, and since they are extremely difficult to destroy, PFAS are prolific in our environment as residue, leachate, or in legacy products.

With years of data on exposure, scientists have determined adverse health effects stemming from contact with some of the most prolific of these chemicals, and further study is underway. The historical and widespread use has resulted in finding PFAS in the environment and in our food supply. Even after phasing these out from new products, old materials still end up in landfill, and in some industries, they're still used where we don't have a suitable replacement. Widespread contamination has led to a necessity of testing, and many groups globally have been working on solving this problem.

Many LC-MS/MS methods have been developed for the targeted analysis of PFAS in environmental samples. Most notably EPA Methods 537, 537.1, 533, 1633. These methods demonstrate testing in the environmental space has been going on for nearly two decades, as EPA 537 V1.1 was finalized in 2009 with years of work prior to putting it together. ASTM International, a group working towards the creation of voluntary consensus standards, also developed methods: D7979, D8421, D7968, and D8535.[1-7] These methods, similar to those developed by the EPA, are focused on soils, biosolids, but none were designed for testing food products. In the vacuum of food testing methods, EPA 1633's inclusion of tissue samples led to the testing of farm animals using this method, but ultimately, it was not designed for testing human food products.

Methods developed by food protection agencies, notably C-01.02 from the FDA and USDA's CLG - PFAS 2.03[8-9] have been single laboratory validated for PFAS in food. The FDA has recently published an update C-010.03[10], a single laboratory validated method for 30 PFAS in 9 matrixes, expanding capabilities from the C-01.02.

AOAC International, an industry-led standardization body for food safety had called for methods to test 30 PFAS compounds from 11 food categories to address the lack of standardized food centric methods. The Standard Method Performance Requirements document SMPR 2023.003 detailed the necessary specifications. We present Shimadzu's response, an accepted First Action method to AOAC's OMA: a single LC-MS/MS method to address the needs of routine testing labs.

## Table 1: PFAS Target Analytes, Abbreviations, and CAS Numbers

| Name | Abbreviation | CAS No. |
|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 |
| Perfluorooctanoic acid | PFOA | 335-67-1 |
| Perfluorononanoic acid | PFNA | 375-95-1 |
| Perfluorodecanoic acid | PFDA | 335-76-2 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 |
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 |

The list of PFAS in AOAC's SMPR is covered largely by the same standards used in EPA method 1633, with the addition of PFUnDS, PFTrDS, and 10:2 FTS.

# Instrument Setup

Two Shimadzu LCMS-8060NX triple quadrupole LC/MS/MS instruments with heated electrospray ionization (ESI) in negative mode, using Lab Solutions Software, were optimized and coordinated in two different locations. These were used for the collection of all data to rapidly meet the requirements set in SMPR 2023.003 in a collaborative effort. Methods submitted to AOAC undergo a review and approved methods are published in the Official Methods of Analysis or OMA. First they are published as First Action where they have done a single lab validation. After a First Action acceptance, an inter-laboratory study is needed for submission for approval to a Final Action method. Setting up our initial instrumentation parameters in two locations and duplicating our settings sets a foundation for future validation work.

Mass spectrometry source and ionization conditions were first optimized by introducing the mixed PFAS standard into the Shimadzu 8060NX MS detector. In these experiments, the following were tested: collision induced dissociation (CID) gas pressure, desolvation line (DL) temperature, drying gas flow rate, heating block temperature, heating gas flow rate, interface temperature, interface voltage, and nebulizer gas flow rate. Small changes in these parameters can affect the sensitivity of the detector and the most optimal conditions can differ compound-to-compound. Determining the best setup for a given method provides the most sensitive results from the detector.

The results were assessed to maximize the response of the various PFAS, with particular focus given to the EU regulated PFAS: PFOA, PFHxS, PFNA, and PFOS.

These optimization experiments were conducted on both instruments at the two selected Shimadzu research centers in Columbia, Maryland, USA and in Tokyo, Japan. Thirty-eight different variables were tested in each location on all 30 target PFAS analytes for a total of 1984 computations. At the Maryland location, the heating gas flow rate was also tested against the response of PFOA, PFHxS, PFNA, and PFOS as these targets required the most sensitive responses per the SMPR. Confirmation of these optimized conditions in two location allowed for duplicate systems to be run concurrently and share the research workload. LabSolutions CS software allows for real time sharing of data across the globe to facilitate rapid decision making and collaboration across teams.

LCMS-8060NX Triple Quadrupole Mass Spectrometer

## Table 2: MRM Transitions, CE and Quantitation Isotopes

| Analyte | Quantitation Ion | Qualifier Ion | Quantitation Ion CE | Qualifier Ion CE | Quantitation labeled Isotope |
|---|---|---|---|---|---|
| PFBA | 212.9 > 168.8 | | 10 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | 8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | 9 | 22 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | 9 | 16 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | 11 | 17 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | 10 | 16 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | 10 | 18 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | 13 | 17 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | 19 | 26 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | 13 | 25 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | 12 | 28 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | 33 | 26 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | 39 | 30 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | 44 | 33 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | 49 | 39 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | 54 | 39 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | 54 | 43 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | 45 | 45 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | 52 | 52 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | 54 | 48 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | 54 | 53 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | 31 | 25 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | 25 | 25 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | 27 | 28 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | 7 | 27 | $^{13}$C3-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | 12 | 29 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | 31 | 31 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | 36 | 36 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | 26 | 46 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | 27 | 45 | $^{13}C_7$-PFUnA |

## Table 3: Isotopically Labeled Internal Standards

| Analyte | Quantitation Ion | Qualifier Ion | Quantitation Ion CE | Qualifier Ion CE |
|---|---|---|---|---|
| $^{13}C_4$-PFBA-ES | 217 > 172 | | 10 | |
| $^{13}C_5$-PFPeA-ES | 268 > 222.9 | | 8 | |
| $^{13}C_5$-PFHxA-ES | 318 > 272.9 | | 9 | |
| $^{13}C_4$-PFHpA-ES | 367 > 321.9 | | 9 | |
| $^{13}C_8$-PFOA-ES | 421 > 375.9 | | 9 | |
| $^{13}C_9$-PFNA-ES | 472 > 426.9 | | 10 | |
| $^{13}C_6$-PFDA-ES | 519 > 473.8 | | 11 | |
| $^{13}C_7$-PFUnA_ES | 570 > 524.8 | | 11 | |
| $^{13}C_2$-PFDoA_ES | 615 > 569.8 | | 11 | |
| $^{13}C_2$-PFTeDA_ES[a] | 715 > 669.7 | | 13 | |
| $^{13}C_3$-PFBS-ES | 302 > 79.8 | | 32 | |
| $^{13}C_3$-PFHxS-ES | 402 > 79.8 | | 42 | |
| $^{13}C_8$-PFOS_ES | 507 > 79.8 | | 55 | |
| $^{13}C_8$-PFOSA_ES | 506 > 77.8 | | 33 | |
| $^{13}C_3$-HFPO-DA-ES | 287 > 168.8 | | 7 | |
| $^{13}C_2$-4:2 FTS-ES | 329 > 80.9 | 329 > 308.8 | 26 | 18 |
| $^{13}C_2$-6:2 FTS-ES[a] | 429 > 80.9 | 429 > 408.8 | 35 | 23 |
| $^{13}C_2$-8:2 FTS-ES[a] | 529 > 79.8 | 529 > 508.8 | 44 | 24 |

[a] not used in SLV, but optional. $^{13}C_2$-isotopes tend to suffer more interference particularly for higher mass compounds

# PFAS Target Limits and Matrix Categories Set by SMPR 2023.003

**Table 4**: PFAS Target Limits Set by SMPR 2023.003

| SMPR Category | Category 1 (ug/kg) | Category 2 (ug/kg) | Category 3 (ug/kg) |
|---|---|---|---|
| egg | ≤0.3 | ≤3 | ≤3 |
| seafood | ≤0.3 | ≤3 | ≤3 |
| meat & fish | ≤0.1 | ≤1 | ≤1 |
| offal | ≤0.4 | ≤4 | ≤4 |
| produce | ≤0.01 | ≤1 | ≤0.1 |
| coffee | ≤0.3 | ≤3 | ≤3 |
| milk | ≤0.01 | ≤1 | ≤0.1 |
| protein powder | ≤0.08 | ≤1 | ≤0.8 |
| fish oil | ≤0.5 | ≤5 | ≤5 |
| baby food | ≤0.01 | ≤1 | ≤0.1 |
| pet food | ≤0.5 | ≤5 | ≤5 |

With AOAC being an international organization, existing EU regulations were taken into consideration. Four of the target PFAS are already regulated in certain foods in Europe: PFOS, PFOA, PFNA, and PFHxS. These are considered in category 1 of table 4. These are some of the most studied PFAS compounds with respect to exposure and testing, so they require lower limits of quantitation and stricter tolerances. They are regulated in the first 4 matrices of the table: eggs, seafood, meat, fish, and offal. Category 2 includes only PFBA and PFPeA, these are PFAS with only one specific MS/MS transition and have the highest limits of all the targeted compounds. Category 3 contains the remaining 24 PFAS targets which include perfluoroalkyl acids and their precursors. For the study, we used representative examples from each matrix category listed in the SMPR; the products validated for the study per category are shown in table 5.

**Table 5**: Matrix categories from the SMPR and corresponding products validated in this study

| SMPR Matrix Category | Product validated in study |
|---|---|
| Produce | Carrots |
| Coffee | Organic brewed coffee |
| Milk (liquid) | Whole Milk (3.7% fat) |
| Dairy powders and plant-based protein powder | Organic plant-based protein powder |
| Eggs | Whole eggs |
| Seafood | Frozen shrimp |
| Fish meat and meat of terrestrial mammals | Raw tuna filet |
| Edible offal of terrestrial animals | Beef kidney |
| Fish oil | Fish oil capsules |
| Foods for infants and young children | Strained pumpkin and sweet potato |
| Pet food and animal feed | Bagged dry dog food |

# Liquid Chromatography Optimization

In order to ensure the best possible outcomes, the chromatographic conditions, including flow rate, gradient, injection volume were evaluated to find an optimal setup. The TQ 8060NX triple quadrupole LCMS was optimized across over 1900 parameters on two different units duplicated in two locations to ensure replication of the setup. The columns were optimized, as were the gradient conditions and flow rates. The final conditions chosen and used in the study are shown in Table 6. These conditions maximized separation of the PFAS analytes and their sensitivity while minimizing noise and systemic contamination.

**Table 6**: Chromatographic instrument settings

| Instrument Parameter | Value |
|---|---|
| Mobile Phase A | 2mM Ammonium Acetate in 5 / 95 = MeCN / Water |
| Mobile Phase B | MeCN |
| Analytical Column | Shimpack Scepter C18-120, 3um, 2.1 x 100 mm |
| Delay Column | Shimpack Scepter C18-120, 3um, 2.1 x 50 mm |
| Oven Temperature | 40 °C |

| Time, min | Flow rate mL/min | Mobile Phase Composition | |
|---|---|---|---|
| | | A % | B % |
| 0 | 0.3 | 80 | 20 |
| 10 | 0.3 | 0 | 100 |
| 10.01 - 12 | 0.6 | 0 | 100 |
| 12.01 - 15 | 0.3 | 80 | 20 |

# Chromatography Optimization and Interferences

An example chromatogram is shown from a sample of carrots which were used to demonstrate the extraction of PFAS from produce. All 30 target PFAS and the respective isotopically labelled internal standards were separated in a nine-minute chromatographic run. A Shimpack Scepter C18-120 delay column was used to isolate possible background contaminant PFAS compounds and avoid coelution with target analytes.

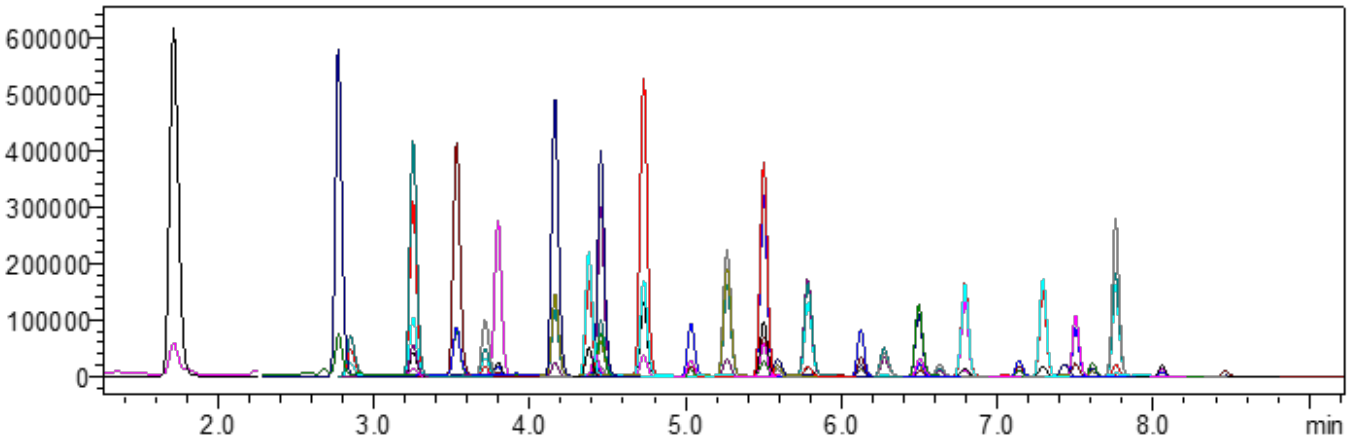



**Figure 2**: Chromatogram of 0.1 ng/g PFAS in a carrot, representing extraction from produce, with separation of all peaks in nine minutes

Nexera HPLC, most used on LCMS-8060NX

Part of optimization for chromatography involved the identification and separation of PFOA from potential cholic acid interferences, per the requirements set forth by AOAC INTERNATIONAL. Three were noted in SMPR 2023.003, Taurodeoxycholic acid (TCDA), Taurochenodeoxycholic acid (TCDCA) and Tauroursodeoxycholic acid (TUDCA).

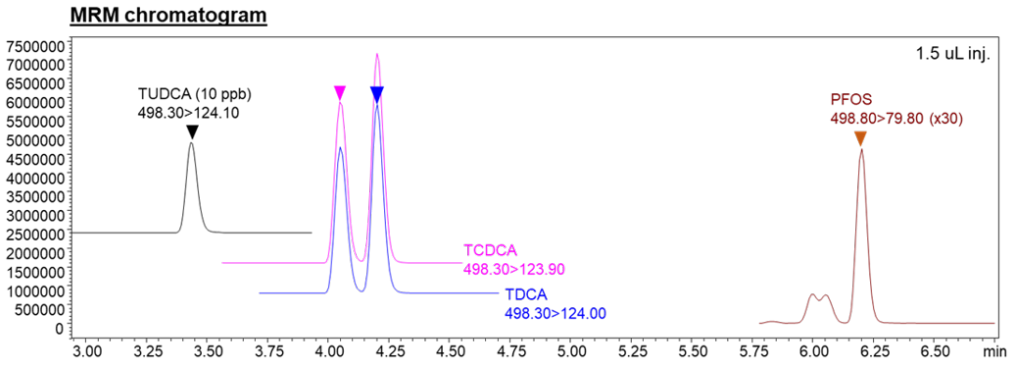



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

# Sample Preparation and Analysis

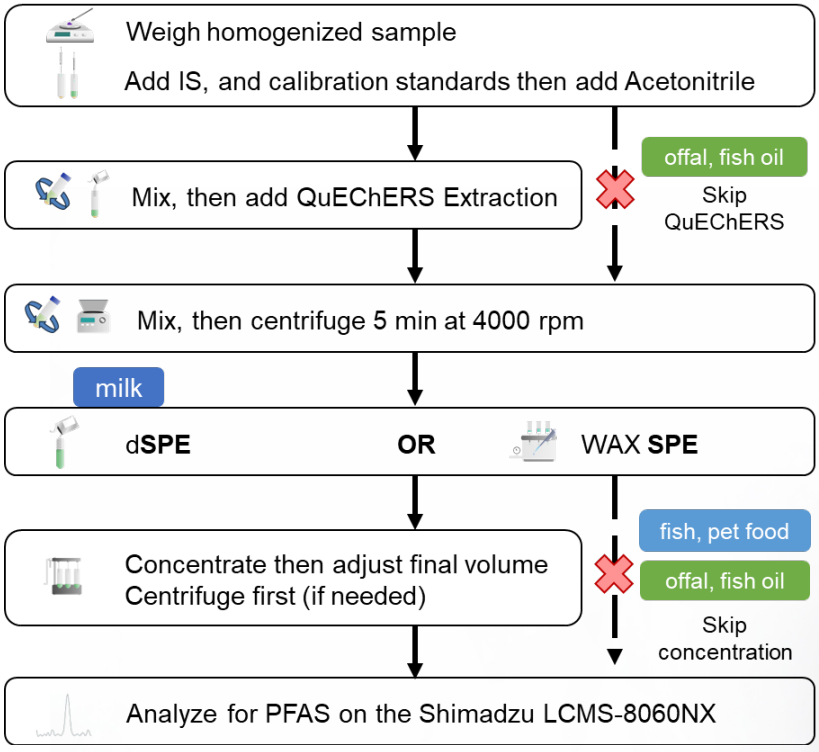
Consistent sample preparation is key to obtaining the necessary repeatability to meet SMPR 2023.003. A general method was therefore developed for sample preparation involving cleanup with QuEChERS and SPE, then preconcentrating the samples before running the analysis on the Shimadzu LCMS-8060NX. Each matrix type was run using the same instrument method.

Knowing the challenges faced in routine labs, the goal in creating this method was to use the same LCMS-8060NX method, keep the workflow of sample preparation the same but simplify it wherever possible. We chose to omit steps on matrices where we found they were unnecessary, saving time, reagents, and consumable costs. For some matrices some components of the sample preparation did not improve results, so we investigated omitting these steps. We evaluated recovery and repeatability without the addition of the QuEChERS reagents and process, and without concentration after SPE extraction. In the case of milk, dispersive SPE proved more efficient than cartridge SPE.

We found that for offal and fish oil, a QuEChERS treatment was unnecessary, with better or equal recovery and repeatability when compared to the general method versus omitting this step. Similarly, concentration post-SPE cleanup was unnecessary for fish tissue, offal, fish oil, and animal feed or pet food.

The general workflow shown in chart 1 walks through the general procedure of: homogenization, QuEChERS where needed, followed by SPE for all samples, then concentration (again only where needed) before injection to an LC-MS/MS system. Were we could skip a step, we highlighted to do so, so we could streamline the procedure to save testing labs time and money.

**Chart 1**: General workflow of PFAS sampling




# PFAS Confirmation Using QTOF LC/MS

The presence of PFAS compounds that do not have a confirmation ion, such as PFBA and PFPeA, must be confirmed if detected in a sample at or above the LOQ. Confirmation may be by using another column of a different stationary phase, or by using a High Resolution Mass Spectrometer (HRMS), such as the Shimadzu LCMS-9050 Quadrupole Time-of-Flight (QTOF) Liquid Chromatograph Mass Spectrometer. HRMS determines the atomic masses of organic molecules with a high degree of accuracy.

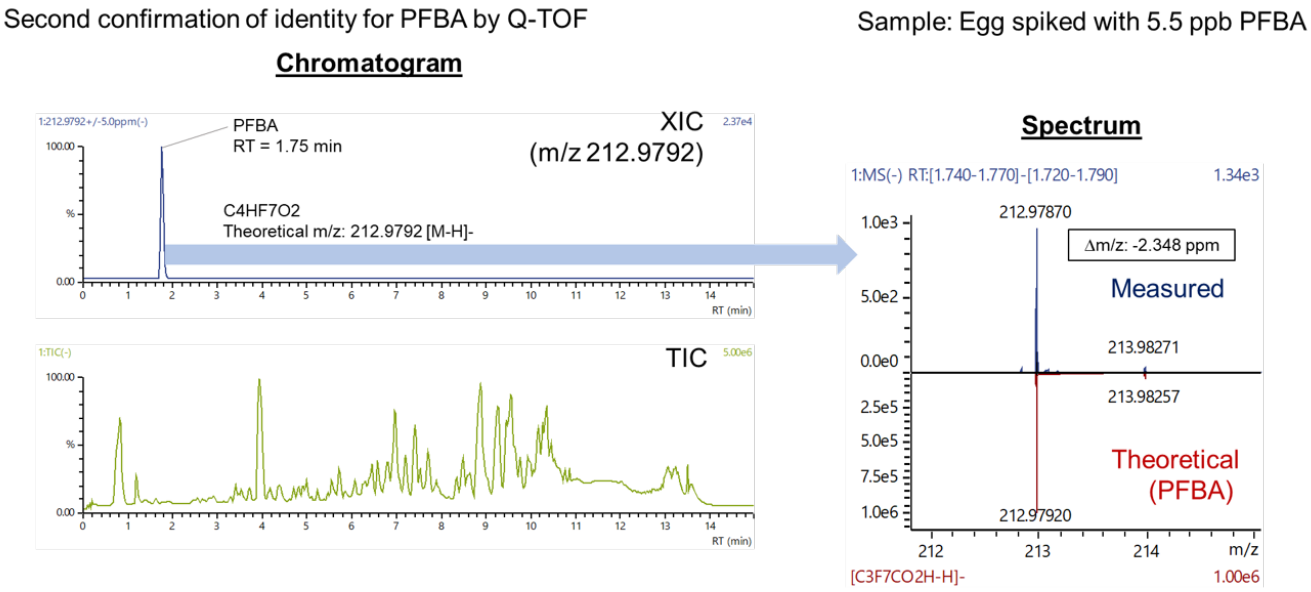



**Figure 4**: Confirmation by LC-QTOF for analytes without reference ion, example in egg for PFBA showing both the chromatogram as TIC and extracted ion chromatogram, followed by the mass spectrum compared to the theoretical reference.

Analysis using the LCMS-9050 begins by injecting the extracted sample into the LC operating with the same analytical conditions as used on the LC-MS/MS. Once the analytes elute from the column, they are ionized. The ions travel along the spectrometer's length, separating by their relative charge and mass and a ratio of charge-to-mass is recorded to five decimal places. This accurate mass from the unconfirmed target analyte in the sample can be compared to theoretical values for the mass of the target analyte or to the retention time and accurate mass of a calibration standard injected under the same conditions. Figure 4 shows and extracted ion chromatogram for the exact mass of PFBA (212.9792 m/z) in an egg matrix.

LCMS-9050 Quadrupole Time-of-Flight
Liquid Chromatograph Mass Spectrometer

# Analysis Results

We used the SMPR as guide for the required LOQ and tested in 3 concentration points: one below the estimated LOQ, and two between 2 – 100 times the estimated LOQ.

Figure 5 shows the recoveries and RSD results for the fish matrix samples, it is used as an example as fish is one of the matrices regulated in the EU. The RSD limit for fish for the 4 EU-regulated compounds was 20%, the scale on the right for RSD does not exceed 20% demonstrating results were well within the required limit. The recovery was required between 80-120%, and on the left scale for recovery rate, this was met for 5, 1 and 0.1 ug/kg level spiked samples.

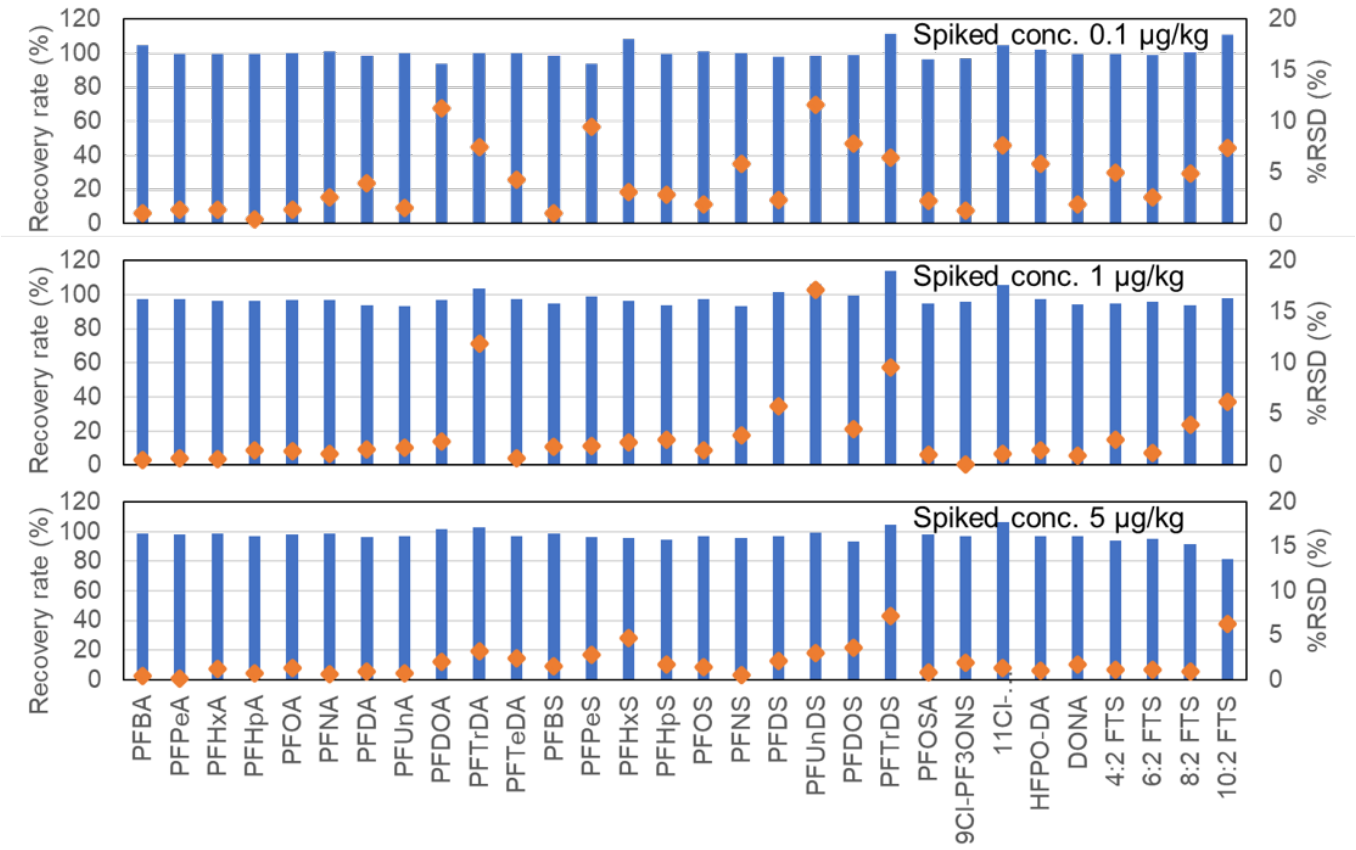



**Figure 5**: Recoveries and RSD results for the fish matrix samples

The following page organizes the 30 PFAS included in the AOAC SMPR into groupings based on existing regulations in Europe for category 1, and differing requirements for PFBA and PFPeA than the remaining PFAS targets. EU-regulated matrices for PFAS include eggs, seafood, fish meat and meat of terrestrial animals, and offal. These food groups have stricter limits for PFOS, PFOA, PFNA and PFHxS ,and make up the first quadrant of the table. Category 2 includes only PFBA and PFPeA, these are PFAS with only one specific MS/MS transition and have the highest limits of all the targeted compounds. Category 3 contains the remaining 24 PFAS targets which include perfluoroalkyl acids and their precursors.

Limits for each target in each category are followed by the limits of quantitation achieved. Each limit in category 1 was met, in some cases over an order of magnitude lower than the requested value, even as they were some of the lowest concentrations due to existing EU regulations. All limits in category 2 and 3 were met at the SMPR level or we could quantitate lower.

**Table 7**: Obtained LOQs and SMPR target limits for all matrices Values shown in ug/kg

| PFAS | Egg | Seafood | Fish | Offal | Produce | Coffee | Milk | Protein powder | Fish oil | Baby food | Pet food |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Limit (ppb) in SMPR per matrix for all Category A | ≤0.3 | ≤0.3 | ≤0.1 | ≤0.4 | ≤0.01 | ≤0.3 | ≤0.01 | ≤0.08 | ≤0.5 | ≤0.01 | ≤0.5 |
| PFOA | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFOS | 0.055 | 0.055 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFNA | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| PFHxS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| Limit (ppb) in SMPR per matrix for all Category B | ≤3 | ≤3 | ≤1 | ≤4 | ≤1 | ≤3 | ≤1 | ≤1 | ≤5 | ≤1 | ≤5 |
| PFBA | 0.055 | 0.055 | 0.1 | 0.2 | 0.055 | 0.055 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| PFPeA | 0.055 | 0.006 | 0.1 | 0.2 | 0.055 | 0.006 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| Limit (ppb) in SMPR per matrix for all Category C | ≤3 | ≤3 | ≤1 | ≤4 | ≤0.1 | ≤3 | ≤0.1 | ≤0.8 | ≤5 | ≤0.1 | ≤5 |
| PFHxA | 0.055 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| PFHpA | 0.006 | 0.055 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFDA | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| PFUnA | 0.006 | 0.006 | 0.1 | 0.2 | 0.055 | 0.006 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| PFDoA | 0.006 | 0.055 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFTrDA | 0.006 | 0.055 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| PFTeDA | 0.055 | 0.055 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| PFBS | 0.006 | 0.055 | 0.1 | 0.2 | 0.055 | 0.006 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFPeS | 0.006 | 0.055 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFHpS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| PFNS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFDS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFUnDS | 0.055 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.1 | 0.5 |
| PFDoS | 0.055 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| PFTrDS | 0.055 | 0.055 | 0.1 | 0.2 | 0.055 | 0.55 | 0.01 | 0.55 | 0.25 | 0.1 | 0.5 |
| PFOSA | 0.55 | 0.055 | 0.1 | 0.2 | 0.006 | 0.55 | 0.01 | 0.55 | 0.25 | 0.1 | 0.5 |
| 9Cl-PF3ONS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| 11Cl-PF3OUdS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| HFPO-DA | 0.006 | 0.055 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.55 | 0.25 | 0.01 | 0.5 |
| DONA | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.006 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| 4:2 FTS | 0.55 | 0.055 | 0.1 | 0.2 | 0.055 | 0.055 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| 6:2 FTS | 0.006 | 0.55 | 0.1 | 0.2 | 0.055 | 0.006 | 0.01 | 0.055 | 0.25 | 0.01 | 0.5 |
| 8:2 FTS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.55 | 0.01 | 0.006 | 0.25 | 0.01 | 0.5 |
| 10:2 FTS | 0.006 | 0.006 | 0.1 | 0.2 | 0.006 | 0.055 | 0.01 | 0.055 | 0.5 | 0.01 | 0.5 |

# Results and Discussion

The same procedure from the test samples was used to process the calibration standards. A linear model provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Branched and linear isomers of PFHxS and PFOS were integrated together. Blank matrixes were analyzed in triplicate, depending on the matrix. Four different concentrations from below the LOQ required by the SMPR, and ranging up to 500 times the estimated LOQ, were also analyzed in triplicate to assess recovery and repeatability throughout the calibration and across matrices. Recovery and repeatability at the LOQ in egg matrix for each analyte in category A are given in table 8 to highlight existing EU regulations. Remaining data is appended.

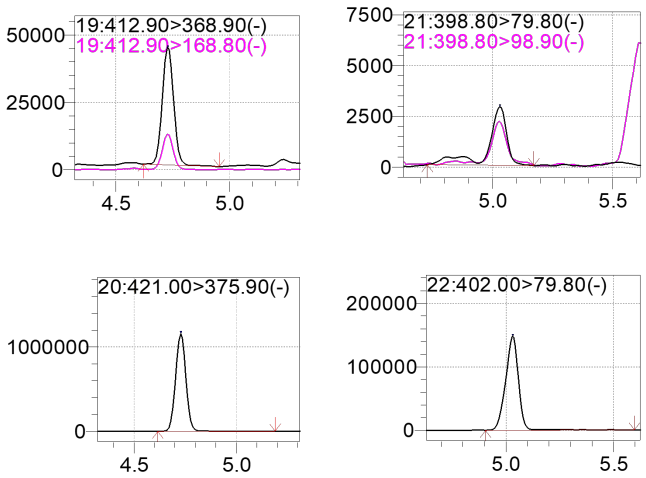



**Figure 6**: LOQ peaks for PFHxS, and PFOA and their corresponding internal standards

**Table 8**: Recovery and repeatability for select PFAS at each concentration level in eggs.

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| **PFOA** | Blank | 0 | | | |
| | 0.006 | 0.005 | 5.5 | 6.19 | 88.9 |
| | 0.055 | 0.054 | 2.41 | 2.46 | 97.9 |
| | 0.55 | 0.557 | 1.11 | 1.1 | 101.3 |
| | 5.5 | 6.338 | 2.33 | 2.02 | 115.2 |
| **PFNA** | Blank | 0 | | | |
| | 0.006 | 0.005 | 1.66 | 1.67 | 99.1 |
| | 0.055 | 0.056 | 2.05 | 2.01 | 102 |
| | 0.55 | 0.592 | 0.59 | 0.54 | 107.6 |
| | 5.5 | 6.122 | 8.8 | 7.91 | 111.3 |
| **PFOS** | Blank | 0.002 | | | |
| | 0.006 | 0.006 | 5.17 | 5.28 | 98.1 |
| | 0.055 | 0.051 | 2.3 | 2.49 | 92.4 |
| | 0.55 | 0.52 | 0.29 | 0.31 | 94.6 |
| | 5.5 | 5.563 | 1.78 | 1.76 | 101.2 |
| **PFHxS** | Blank | -0.001 | | | |
| | 0.006 | 0.006 | 21.39 | 19.16 | 111.7 |
| | 0.055 | 0.051 | 1.16 | 1.24 | 93.7 |
| | 0.55 | 0.533 | 1.27 | 1.32 | 96.9 |
| | 5.5 | 5.779 | 1.15 | 1.09 | 105.1 |

The LOQs for all matrices and compounds were determined according to the SMPR, with retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30% all taken into consideration. Figure 6 includes examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards. The LOQ for each analyte was determined based on the specifications listed in the SMPR. The appended application notes detail the process and provide data for each matrix category.

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Foods for Infants and Young Children (Baby Food) by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in baby food meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in pumpkin and sweet potato puree baby food using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.1 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.1 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.1 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.01 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.01 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.01 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.01 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.01 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.01 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.1 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.1 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.01 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.01 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.01 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.01 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.01 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.01 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.01 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.1 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.01 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.1 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.1 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.01 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.01 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.01 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.01 |

## ■ Sample Preparation and Analysis Conditions

Samples were prepared by spiking blended baby food samples in triplicate at four different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using baby food test portions spiked with concentrations of 0.005, 0.01, 0.05, 0.10, 0.50 and 1.0 ng/g. Quantitation was carried out on additional baby food samples spiked in triplicate at 0.01, 0.10, 0.50 and 1.0 ng/g. Since standards were extracted in a baby food matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

Multiple jars of baby food were purchased, combined, and blended. Ten-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were shaken for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute, allowed to cool to room temperature and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, the extract was concentrated to less than 1 mL, then adjusted to a final 1 mL in a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

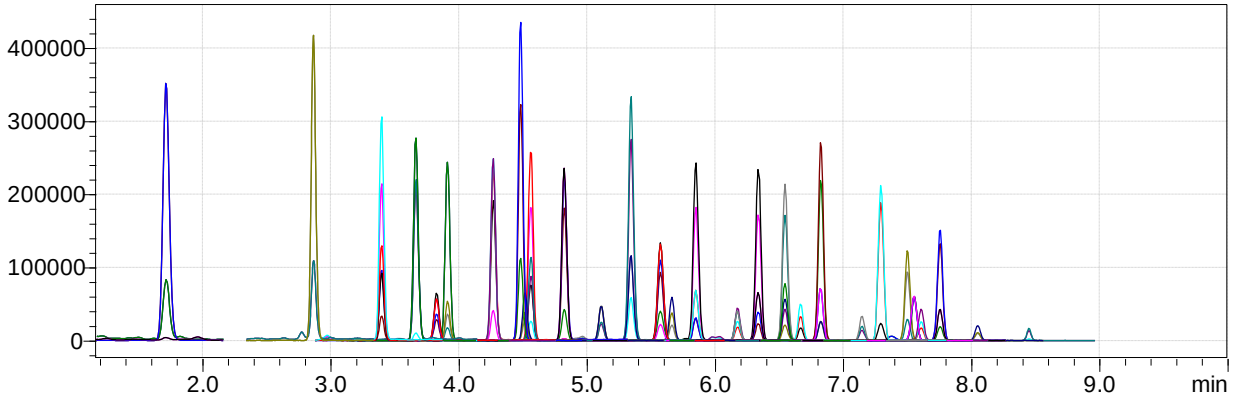



**Figure 2**: Chromatogram of 0.1 ng/g PFAS in a baby food matrix with separation of all peaks in nine minutes

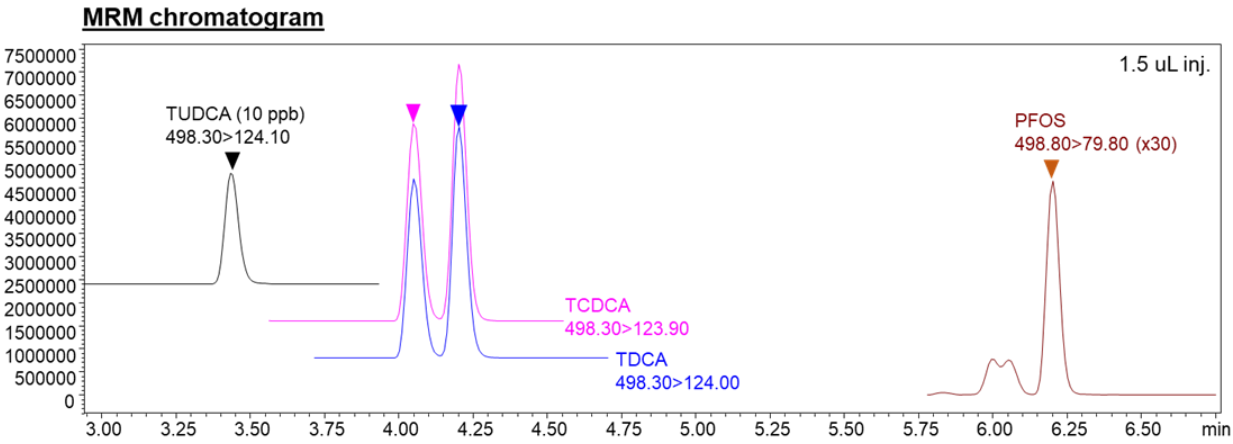



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

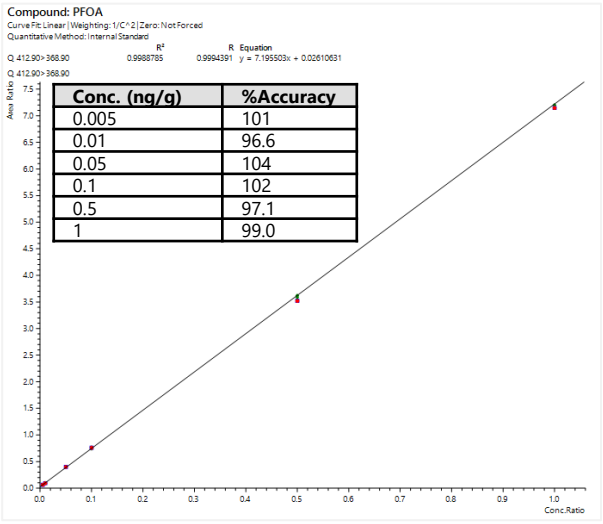
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

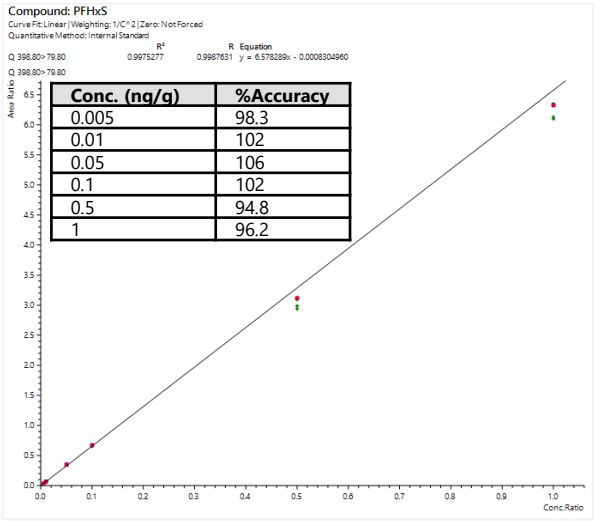
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



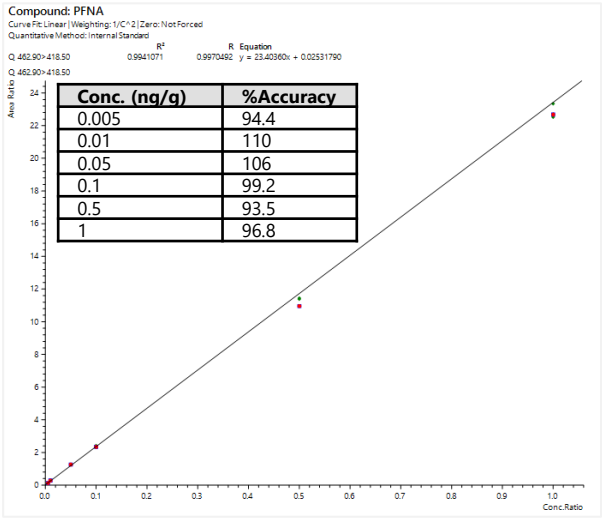

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 101 |
| 0.01 | 96.6 |
| 0.05 | 104 |
| 0.1 | 102 |
| 0.5 | 97.1 |
| 1 | 99.0 |

**Figure 4**: PFHxS Calibration Curve



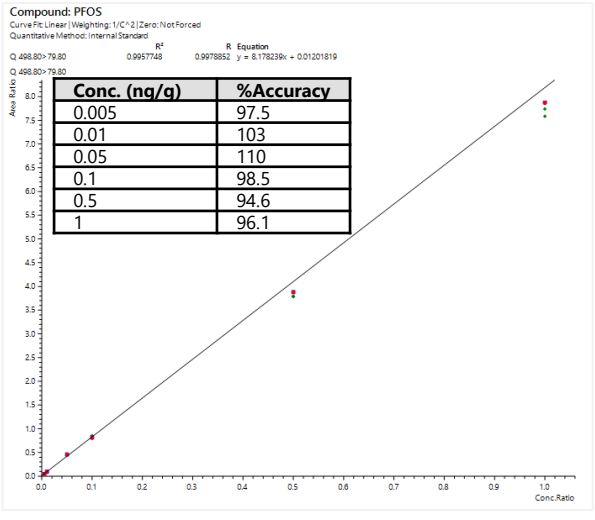

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 98.3 |
| 0.01 | 102 |
| 0.05 | 106 |
| 0.1 | 102 |
| 0.5 | 94.8 |
| 1 | 96.2 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 94.4 |
| 0.01 | 110 |
| 0.05 | 106 |
| 0.1 | 99.2 |
| 0.5 | 93.5 |
| 1 | 96.8 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 97.5 |
| 0.01 | 103 |
| 0.05 | 110 |
| 0.1 | 98.5 |
| 0.5 | 94.6 |
| 1 | 96.1 |

Blank matrixes and at least three different concentrations ranging from the SMPR required LOQ to 100 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery (%) |
|---|---|---|---|---|---|
| PFBA | Blank | 0.000 | | | |
| | 0.1 | 0.095 | 3.90 | 4.10 | 95.1 |
| | 0.5 | 0.506 | 0.38 | 0.37 | 101.2 |
| | 1 | 1.006 | 0.46 | 0.46 | 100.6 |
| PFPeA | Blank | 0.008 | | | |
| | 0.01 | 0.011 | 6.52 | 5.76 | 113.3 |
| | 0.1 | 0.101 | 0.91 | 0.90 | 100.9 |
| | 0.5 | 0.481 | 0.53 | 0.55 | 96.1 |
| | 1 | 0.976 | 0.40 | 0.41 | 97.6 |
| PFHxA | Blank | ND | | | |
| | 0.01 | 0.010 | 2.82 | 2.89 | 97.5 |
| | 0.1 | 0.099 | 1.85 | 1.86 | 99.4 |
| | 0.5 | 0.475 | 1.26 | 1.32 | 95.0 |
| | 1 | 0.977 | 1.37 | 1.41 | 97.7 |
| PFHpA | Blank | ND | | | |
| | 0.01 | 0.010 | 3.20 | 3.33 | 96.2 |
| | 0.1 | 0.104 | 2.60 | 2.49 | 104.1 |
| | 0.5 | 0.488 | 1.90 | 1.95 | 97.6 |
| | 1 | 0.990 | 0.35 | 0.35 | 99.0 |
| PFOA | Blank | ND | | | |
| | 0.01 | 0.010 | 3.04 | 3.07 | 99.3 |
| | 0.1 | 0.102 | 1.95 | 1.92 | 101.7 |
| | 0.5 | 0.495 | 0.95 | 0.96 | 99.0 |
| | 1 | 0.994 | 0.38 | 0.38 | 99.4 |
| PFNA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 5.44 | 5.13 | 106.2 |
| | 0.1 | 0.100 | 2.35 | 2.36 | 99.6 |
| | 0.5 | 0.480 | 2.24 | 2.33 | 96.1 |
| | 1 | 0.975 | 1.83 | 1.88 | 97.5 |
| PFDA | Blank | ND | | | |
| | 0.01 | 0.010 | 5.19 | 5.04 | 103.0 |
| | 0.1 | 0.102 | 1.17 | 1.15 | 102.0 |
| | 0.5 | 0.490 | 2.71 | 2.76 | 98.0 |
| | 1 | 1.000 | 0.52 | 0.52 | 100.0 |
| PFUnA | Blank | ND | | | |
| | 0.01 | 0.010 | 8.96 | 8.91 | 100.6 |
| | 0.1 | 0.104 | 0.97 | 0.94 | 103.6 |
| | 0.5 | 0.495 | 1.99 | 2.01 | 98.9 |
| | 1 | 0.995 | 3.20 | 3.22 | 99.5 |
| PFDoA | Blank | ND | | | |
| | 0.01 | 0.012 | 10.48 | 9.10 | 115.2 |
| | 0.1 | 0.103 | 6.81 | 6.63 | 102.8 |
| | 0.5 | 0.467 | 1.61 | 1.72 | 93.4 |
| | 1 | 0.957 | 3.90 | 4.08 | 95.7 |
| PFTrDA | Blank | ND | | | |
| | 0.1 | 0.103 | 1.04 | 1.01 | 103.0 |
| | 0.5 | 0.504 | 1.34 | 1.33 | 100.8 |
| | 1 | 1.024 | 3.89 | 3.80 | 102.4 |
| PFTeDA | Blank | ND | | | |
| | 0.1 | 0.117 | 5.89 | 5.03 | 117.3 |
| | 0.5 | 0.557 | 3.35 | 3.01 | 111.3 |
| | 1 | 1.139 | 3.42 | 3.00 | 113.9 |
| PFBS | Blank | ND | | | |
| | 0.01 | 0.010 | 15.82 | 15.43 | 102.5 |
| | 0.1 | 0.107 | 3.90 | 3.64 | 107.2 |
| | 0.5 | 0.503 | 2.70 | 2.68 | 100.7 |
| | 1 | 1.023 | 3.93 | 3.84 | 102.3 |
| PFPeS | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 5.20 | 4.62 | 112.6 |
| | 0.1 | 0.104 | 2.75 | 2.63 | 104.4 |
| | 0.5 | 0.483 | 3.68 | 3.80 | 96.7 |
| | 1 | 0.969 | 1.64 | 1.70 | 96.9 |

| | | | | | |
|---|---|---|---|---|---|
| PFHxS | Blank | 0.001 | | | |
| | 0.01 | 0.011 | 7.99 | 7.45 | 107.4 |
| | 0.1 | 0.101 | 2.16 | 2.15 | 100.5 |
| | 0.5 | 0.457 | 2.40 | 2.62 | 91.5 |
| | 1 | 0.941 | 2.06 | 2.19 | 94.1 |
| PFHpS | Blank | ND | | | |
| | 0.01 | 0.009 | 2.86 | 3.10 | 92.2 |
| | 0.1 | 0.098 | 2.71 | 2.75 | 98.6 |
| | 0.5 | 0.461 | 4.58 | 4.97 | 92.1 |
| | 1 | 0.949 | 3.25 | 3.42 | 94.9 |
| PFOS | Blank | ND | | | |
| | 0.01 | 0.009 | 5.72 | 6.56 | 87.2 |
| | 0.1 | 0.099 | 3.74 | 3.78 | 99.1 |
| | 0.5 | 0.462 | 0.06 | 0.06 | 92.3 |
| | 1 | 0.943 | 1.66 | 1.76 | 94.3 |
| PFNS | Blank | ND | | | |
| | 0.01 | 0.010 | 4.62 | 4.59 | 100.8 |
| | 0.1 | 0.103 | 2.44 | 2.37 | 103.0 |
| | 0.5 | 0.495 | 1.33 | 1.34 | 99.1 |
| | 1 | 0.991 | 2.71 | 2.74 | 99.1 |
| PFDS | Blank | ND | | | |
| | 0.01 | 0.008 | 1.62 | 2.04 | 79.3 |
| | 0.1 | 0.106 | 6.18 | 5.83 | 105.9 |
| | 0.5 | 0.508 | 1.40 | 1.38 | 101.6 |
| | 1 | 1.020 | 3.31 | 3.24 | 102.0 |
| PFUnDS | Blank | ND | | | |
| | 0.1 | 0.098 | 3.71 | 3.76 | 98.5 |
| | 0.5 | 0.498 | 0.55 | 0.55 | 99.6 |
| | 1 | 1.005 | 1.80 | 1.79 | 100.5 |
| PFDoS | Blank | ND | | | |
| | 0.01 | 0.008 | 10.92 | 13.51 | 80.8 |
| | 0.1 | 0.097 | 2.81 | 2.91 | 96.7 |
| | 0.5 | 0.407 | 0.85 | 1.05 | 81.4 |
| | 1 | 0.808 | 1.21 | 1.50 | 80.8 |
| PFTrDS | Blank | ND | | | |
| | 0.1 | 0.100 | 2.46 | 2.45 | 100.4 |
| | 0.5 | 0.504 | 5.22 | 5.17 | 100.8 |
| | 1 | 1.028 | 4.62 | 4.50 | 102.8 |
| PFOSA | Blank | ND | | | |
| | 0.01 | 0.010 | 10.68 | 10.71 | 99.7 |
| | 0.1 | 0.101 | 4.27 | 4.24 | 100.9 |
| | 0.5 | 0.478 | 1.86 | 1.94 | 95.6 |
| | 1 | 0.961 | 0.25 | 0.26 | 96.1 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 7.75 | 7.30 | 106.2 |
| | 0.1 | 0.104 | 3.58 | 3.44 | 104.0 |
| | 0.5 | 0.485 | 2.06 | 2.13 | 96.9 |
| | 1 | 0.987 | 2.03 | 2.06 | 98.7 |
| 11Cl-PF3OUdS | Blank | ND | | | |
| | 0.01 | 0.010 | 0.69 | 0.66 | 104.4 |
| | 0.1 | 0.099 | 3.76 | 3.79 | 99.2 |
| | 0.5 | 0.478 | 2.25 | 2.35 | 95.7 |
| | 1 | 0.941 | 4.44 | 4.72 | 94.1 |
| HFPO-DA | Blank | ND | | | |
| | 0.01 | 0.011 | 18.97 | 17.88 | 106.1 |
| | 0.1 | 0.108 | 1.46 | 1.35 | 107.7 |
| | 0.5 | 0.503 | 0.93 | 0.92 | 100.6 |
| | 1 | 1.009 | 1.21 | 1.19 | 100.9 |
| DONA | Blank | ND | | | |
| | 0.01 | 0.011 | 3.65 | 3.49 | 104.5 |
| | 0.1 | 0.103 | 2.15 | 2.09 | 103.0 |
| | 0.5 | 0.486 | 1.91 | 1.96 | 97.3 |
| | 1 | 0.995 | 0.40 | 0.41 | 99.5 |

| | | | | | |
|---|---|---|---|---|---|
| 4:2 FTS | Blank | 0.000 | | | |
| | 0.01 | 0.012 | 11.06 | 9.41 | 117.5 |
| | 0.1 | 0.105 | 3.82 | 3.64 | 104.9 |
| | 0.5 | 0.492 | 2.05 | 2.09 | 98.2 |
| | 1 | 0.999 | 0.47 | 0.47 | 99.9 |
| 6:2 FTS | Blank | ND | | | |
| | 0.01 | 0.011 | 7.86 | 7.17 | 109.6 |
| | 0.1 | 0.104 | 4.90 | 4.72 | 103.9 |
| | 0.5 | 0.492 | 1.23 | 1.25 | 98.5 |
| | 1 | 0.981 | 2.00 | 2.04 | 98.1 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 1.06 | 0.94 | 112.5 |
| | 0.1 | 0.101 | 4.33 | 4.29 | 100.9 |
| | 0.5 | 0.451 | 1.90 | 2.11 | 90.2 |
| | 1 | 0.895 | 3.04 | 3.40 | 89.5 |
| 10:2 FTS | Blank | ND | | | |
| | 0.01 | 0.012 | 10.15 | 8.88 | 114.3 |
| | 0.1 | 0.104 | 3.84 | 3.69 | 104.0 |
| | 0.5 | 0.470 | 2.59 | 2.76 | 94.0 |
| | 1 | 0.911 | 2.92 | 3.20 | 91.1 |

ND = average results less than zero

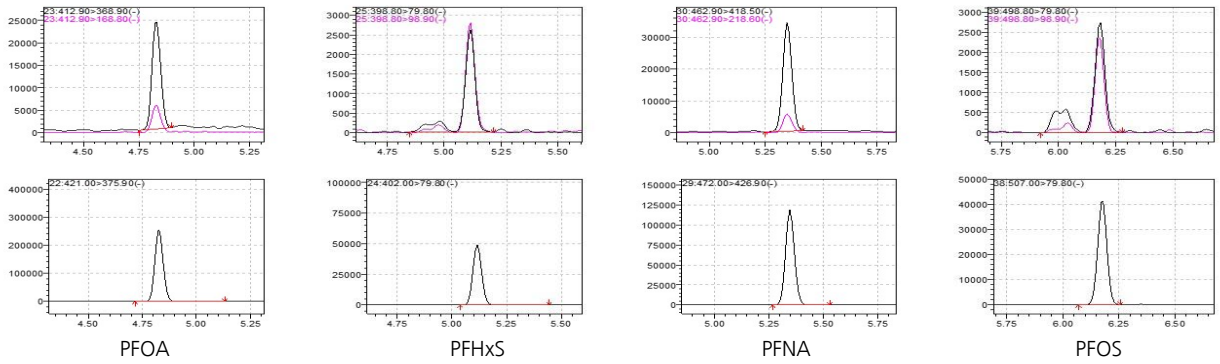



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a baby food matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Egg by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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[1] Shimadzu Scientific Instruments, Inc.

## User Benefits

- Validated method for 30 PFAS in Egg meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in eggs using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.055 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.055 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.055 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.0055 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.0055 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.0055 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.0055 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.0055 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.0055 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.0055 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.055 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.0055 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.0055 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.0055 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.0055 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.055 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.0055 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.0055 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.055 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.055 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.055 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.55 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.0055 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.0055 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.0055 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.55 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.0055 |

## ■ Sample Preparation and Analysis Conditions

Large brown eggs were purchased locally. Samples were prepared cracking and removing the whole egg from the shell and grinding with dry ice for 30 seconds at 4000 rpm. The ground material was placed in a beaker. Test portions were spiked in triplicate at four different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram test portions spiked with concentrations of 0.001, 0.01, 0.1, 1.0 and 10.0 ng/g. Quantitation was carried out on additional whole egg samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in an egg matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, the extract was concentrated to dryness and dissolved in 0.4 mL of a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

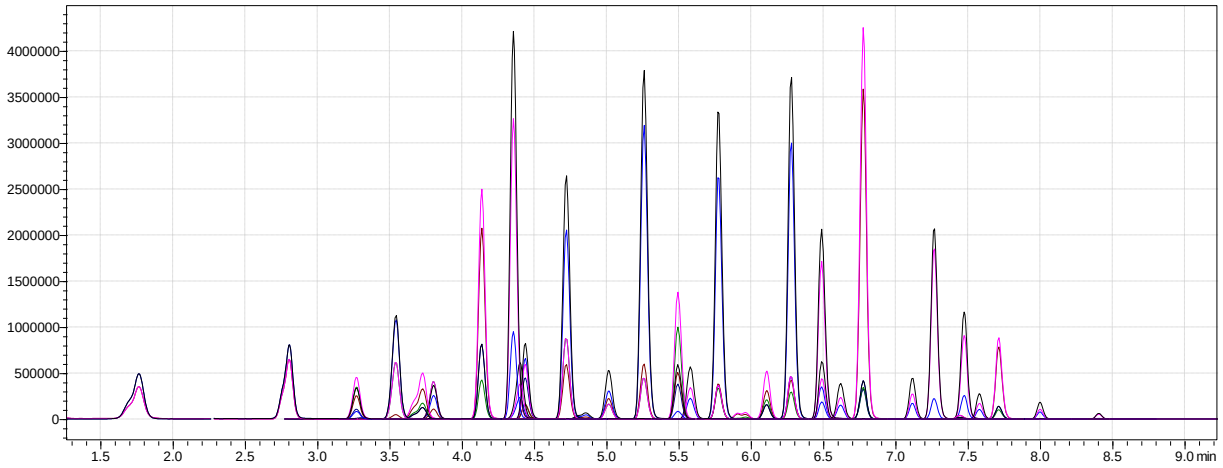



**Figure 2**: Chromatogram of 0.55 ng/g PFAS in an egg matrix with separation of all peaks in nine minutes

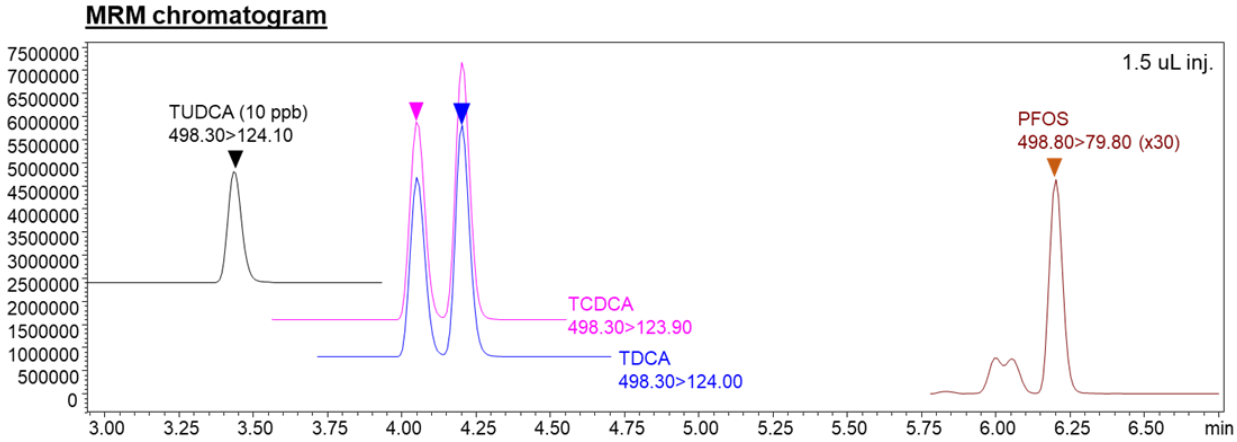



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

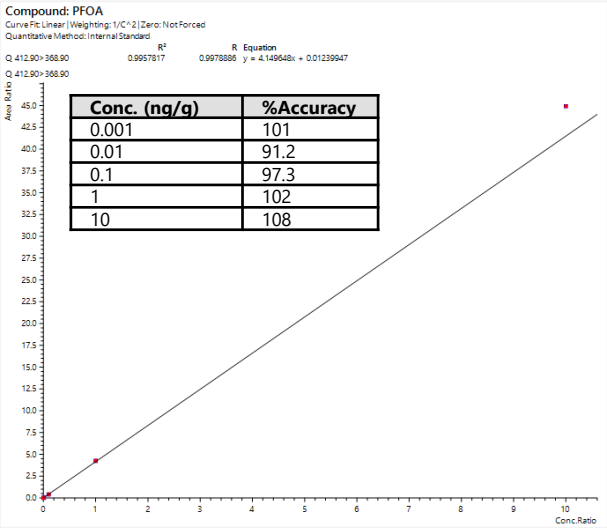
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

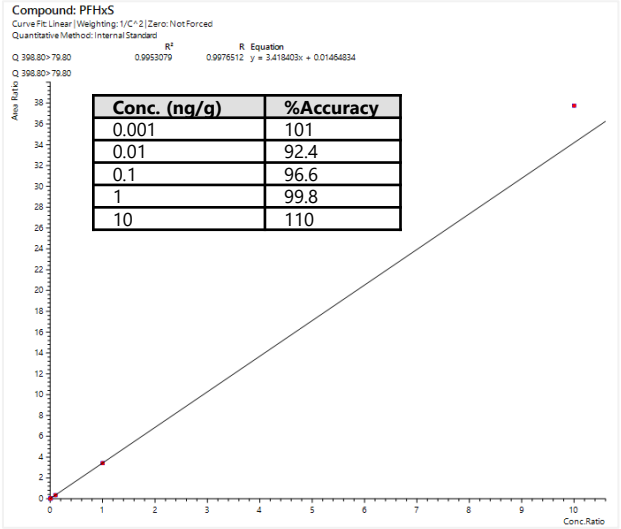
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



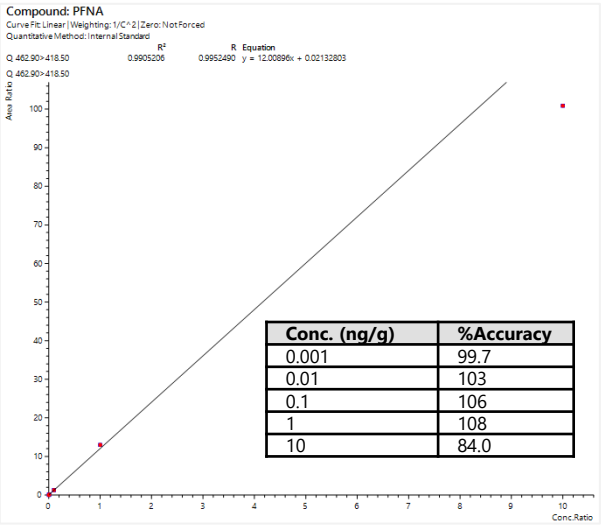

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 91.2 |
| 0.1 | 97.3 |
| 1 | 102 |
| 10 | 108 |

**Figure 4**: PFHxS Calibration Curve



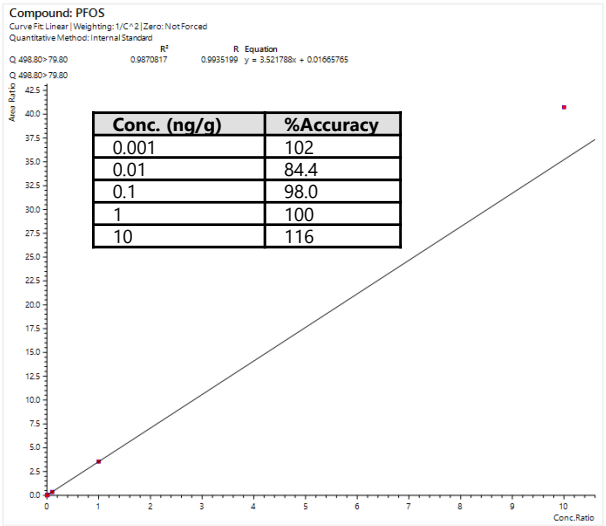

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 92.4 |
| 0.1 | 96.6 |
| 1 | 99.8 |
| 10 | 110 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.7 |
| 0.01 | 103 |
| 0.1 | 106 |
| 1 | 108 |
| 10 | 84.0 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 102 |
| 0.01 | 84.4 |
| 0.1 | 98.0 |
| 1 | 100 |
| 10 | 116 |

Blank matrixes and four different concentrations ranging from below the SMPR required LOQ to approximately 20 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | 0.002 | | | |
| | 0.055 | 0.051 | 1.38 | 1.48 | 93.3 |
| | 0.55 | 0.528 | 1.30 | 1.36 | 95.9 |
| | 5.5 | 5.664 | 0.26 | 0.26 | 103.0 |
| PFPeA | Blank | 0.004 | | | |
| | 0.0055 | 0.006 | 10.48 | 9.67 | 108.4 |
| | 0.055 | 0.050 | 4.96 | 5.42 | 91.5 |
| | 0.55 | 0.486 | 0.50 | 0.57 | 88.3 |
| | 5.5 | 5.364 | 0.87 | 0.90 | 97.5 |
| PFHxA | Blank | 0.004 | | | |
| | 0.0055 | 0.005 | 4.52 | 4.82 | 93.8 |
| | 0.055 | 0.055 | 0.62 | 0.62 | 100.3 |
| | 0.55 | 0.550 | 1.37 | 1.36 | 100.0 |
| | 5.5 | 6.107 | 1.10 | 0.99 | 111.1 |
| PFHpA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 4.39 | 4.62 | 94.9 |
| | 0.055 | 0.053 | 1.04 | 1.09 | 95.6 |
| | 0.55 | 0.542 | 0.49 | 0.50 | 98.5 |
| | 5.5 | 6.271 | 0.92 | 0.80 | 114.0 |
| PFOA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 5.50 | 6.19 | 88.9 |
| | 0.055 | 0.054 | 2.41 | 2.46 | 97.9 |
| | 0.55 | 0.557 | 1.11 | 1.10 | 101.3 |
| | 5.5 | 6.338 | 2.33 | 2.02 | 115.2 |
| PFNA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 1.66 | 1.67 | 99.1 |
| | 0.055 | 0.056 | 2.05 | 2.01 | 102.0 |
| | 0.55 | 0.592 | 0.59 | 0.54 | 107.6 |
| | 5.5 | 6.122 | 8.80 | 7.91 | 111.3 |
| PFDA | Blank | ND | | | |
| | 0.0055 | 0.005 | 3.66 | 3.91 | 93.7 |
| | 0.055 | 0.055 | 0.98 | 0.99 | 99.7 |
| | 0.55 | 0.569 | 2.10 | 2.03 | 103.5 |
| | 5.5 | 6.085 | 7.05 | 6.37 | 110.7 |
| PFUnA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 3.20 | 3.35 | 95.5 |
| | 0.055 | 0.057 | 2.15 | 2.10 | 102.8 |
| | 0.55 | 0.587 | 0.85 | 0.80 | 106.8 |
| | 5.5 | 6.281 | 8.37 | 7.33 | 114.2 |
| PFDoA | Blank | ND | | | |
| | 0.0055 | 0.004 | 12.06 | 16.81 | 71.7 |
| | 0.055 | 0.047 | 6.41 | 7.53 | 85.1 |
| | 0.55 | 0.504 | 1.88 | 2.05 | 91.6 |
| | 5.5 | 5.892 | 1.60 | 1.49 | 107.1 |
| PFTrDA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 9.21 | 10.21 | 90.2 |
| | 0.055 | 0.047 | 3.12 | 3.63 | 85.8 |
| | 0.55 | 0.515 | 0.81 | 0.86 | 93.6 |
| | 5.5 | 6.927 | 3.26 | 2.59 | 125.9 |
| PFTeDA | Blank | 0.002 | | | |
| | 0.0055 | 0.006 | 24.12 | 21.48 | 112.3 |
| | 0.055 | 0.040 | 0.61 | 0.83 | 73.5 |
| | 0.55 | 0.508 | 0.91 | 0.98 | 92.4 |
| | 5.5 | 7.220 | 3.10 | 2.36 | 131.3 |
| PFBS | Blank | ND | | | |
| | 0.0055 | 0.004 | 7.72 | 10.25 | 75.4 |
| | 0.055 | 0.051 | 0.61 | 0.66 | 93.1 |
| | 0.55 | 0.534 | 2.17 | 2.23 | 97.1 |
| | 5.5 | 5.763 | 1.50 | 1.43 | 104.8 |
| PFPeS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 17.67 | 18.11 | 97.6 |
| | 0.055 | 0.051 | 4.60 | 4.99 | 92.2 |
| | 0.55 | 0.516 | 0.67 | 0.71 | 93.9 |
| | 5.5 | 5.494 | 1.49 | 1.49 | 99.9 |

| | | | | | |
|---|---|---|---|---|---|
| PFHxS | Blank | ND | | | |
| | 0.0055 | 0.006 | 21.39 | 19.16 | 111.7 |
| | 0.055 | 0.051 | 1.16 | 1.24 | 93.7 |
| | 0.55 | 0.533 | 1.27 | 1.32 | 96.9 |
| | 5.5 | 5.779 | 1.15 | 1.09 | 105.1 |
| PFHpS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 2.93 | 2.98 | 98.3 |
| | 0.055 | 0.057 | 3.84 | 3.73 | 103.0 |
| | 0.55 | 0.515 | 1.29 | 1.38 | 93.6 |
| | 5.5 | 5.058 | 3.91 | 4.25 | 92.0 |
| PFOS | Blank | 0.002 | | | |
| | 0.0055 | 0.006 | 5.17 | 5.28 | 98.1 |
| | 0.055 | 0.051 | 2.30 | 2.49 | 92.4 |
| | 0.55 | 0.520 | 0.29 | 0.31 | 94.6 |
| | 5.5 | 5.563 | 1.78 | 1.76 | 101.2 |
| PFNS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 10.26 | 11.84 | 86.7 |
| | 0.055 | 0.048 | 1.71 | 1.94 | 88.1 |
| | 0.55 | 0.523 | 2.52 | 2.65 | 95.1 |
| | 5.5 | 6.736 | 3.30 | 2.69 | 122.5 |
| PFDS | Blank | ND | | | |
| | 0.0055 | 0.005 | 2.25 | 2.47 | 91.3 |
| | 0.055 | 0.050 | 2.42 | 2.67 | 90.5 |
| | 0.55 | 0.503 | 1.82 | 1.99 | 91.5 |
| | 5.5 | 5.541 | 3.26 | 3.23 | 100.8 |
| PFUnDS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 4.47 | 5.03 | 88.9 |
| | 0.055 | 0.049 | 3.86 | 4.36 | 88.7 |
| | 0.55 | 0.536 | 2.55 | 2.62 | 97.4 |
| | 5.5 | 6.074 | 2.62 | 2.37 | 110.5 |
| PFDoS | Blank | ND | | | |
| | 0.0055 | 0.004 | 7.73 | 11.46 | 67.5 |
| | 0.055 | 0.048 | 1.70 | 1.94 | 87.7 |
| | 0.55 | 0.548 | 1.64 | 1.65 | 99.7 |
| | 5.5 | 5.444 | 3.69 | 3.72 | 99.0 |
| PFTrDS | Blank | ND | | | |
| | 0.055 | 0.045 | 1.86 | 2.25 | 82.5 |
| | 0.55 | 0.514 | 1.37 | 1.46 | 93.4 |
| | 5.5 | 6.457 | 3.92 | 3.34 | 117.4 |
| PFOSA | Blank | ND | | | |
| | 0.0055 | 0.005 | 10.12 | 12.42 | 81.5 |
| | 0.055 | 0.054 | 2.96 | 3.00 | 98.7 |
| | 0.55 | 0.539 | 1.73 | 1.77 | 98.1 |
| | 5.5 | 5.676 | 2.21 | 2.14 | 103.2 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 6.82 | 6.82 | 100.0 |
| | 0.055 | 0.048 | 1.26 | 1.45 | 86.6 |
| | 0.55 | 0.507 | 1.59 | 1.73 | 92.2 |
| | 5.5 | 7.191 | 2.75 | 2.10 | 130.7 |
| 11Cl-PF3OUdS | Blank | 0.002 | | | |
| | 0.0055 | 0.006 | 4.92 | 4.23 | 116.2 |
| | 0.055 | 0.045 | 0.96 | 1.18 | 81.4 |
| | 0.55 | 0.494 | 2.01 | 2.23 | 89.9 |
| | 5.5 | 7.332 | 3.60 | 2.70 | 133.3 |
| HFPO-DA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 10.01 | 10.88 | 92.0 |
| | 0.055 | 0.052 | 0.50 | 0.53 | 94.7 |
| | 0.55 | 0.533 | 1.25 | 1.29 | 96.9 |
| | 5.5 | 5.695 | 1.62 | 1.56 | 103.5 |
| DONA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 3.26 | 3.24 | 100.6 |
| | 0.055 | 0.057 | 0.53 | 0.51 | 104.2 |
| | 0.55 | 0.583 | 0.31 | 0.29 | 106.1 |
| | 5.5 | 6.148 | 9.36 | 8.38 | 111.8 |
| 4:2 FTS | Blank | 0.017 | | | |
| | 0.0055 | 0.005 | 3.67 | 4.43 | 82.7 |
| | 0.055 | 0.052 | 3.28 | 3.44 | 95.5 |
| | 0.55 | 0.548 | 2.03 | 2.04 | 99.6 |
| | 5.5 | 5.733 | 2.18 | 2.09 | 104.2 |

| | | | | | |
|---|---|---|---|---|---|
| 6:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 14.57 | 15.09 | 96.6 |
| | 0.055 | 0.053 | 2.80 | 2.88 | 97.2 |
| | 0.55 | 0.538 | 1.99 | 2.03 | 97.9 |
| | 5.5 | 5.480 | 1.60 | 1.61 | 99.7 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.004 | 2.76 | 3.47 | 79.5 |
| | 0.055 | 0.056 | 3.32 | 3.29 | 101.2 |
| | 0.55 | 0.613 | 2.81 | 2.52 | 111.6 |
| | 5.5 | 4.938 | 3.12 | 3.47 | 89.8 |
| 10:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 10.90 | 13.79 | 79.1 |
| | 0.055 | 0.052 | 1.12 | 1.17 | 95.5 |
| | 0.55 | 0.596 | 2.71 | 2.50 | 108.3 |
| | 5.5 | 4.553 | 4.08 | 4.93 | 82.8 |

ND = average results less than zero

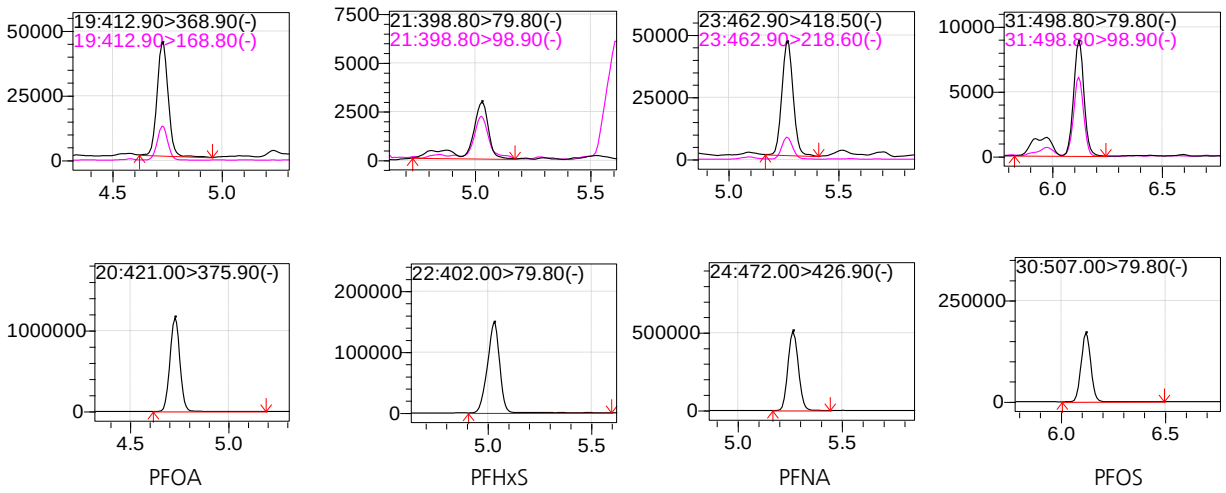



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in an egg matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Fish by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in Fish meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in fish using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at three concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.1 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.1 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.1 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.1 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.1 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.1 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.1 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.1 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.1 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.1 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.1 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.1 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.1 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.1 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.1 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.1 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.1 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.1 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.1 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.1 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.1 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.1 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.1 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.1 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.1 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.1 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.1 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.1 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.1 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.1 |

## ■ Sample Preparation and Analysis Conditions

Samples were prepared by dicing the edible portion of tuna filets and grinding with dry ice. The ground material was placed in a freezer overnight. Test portions were spiked in triplicate at three different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram test portions spiked with concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 ng/g. Quantitation was carried out on additional tuna samples spiked in triplicate at 0.1, 1.0, and 5.0 ng/g. Since standards were extracted in a tuna matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were shaken by hand for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. A 0.5 mL aliquot was transferred to a LC vial and acidified with formic acid.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

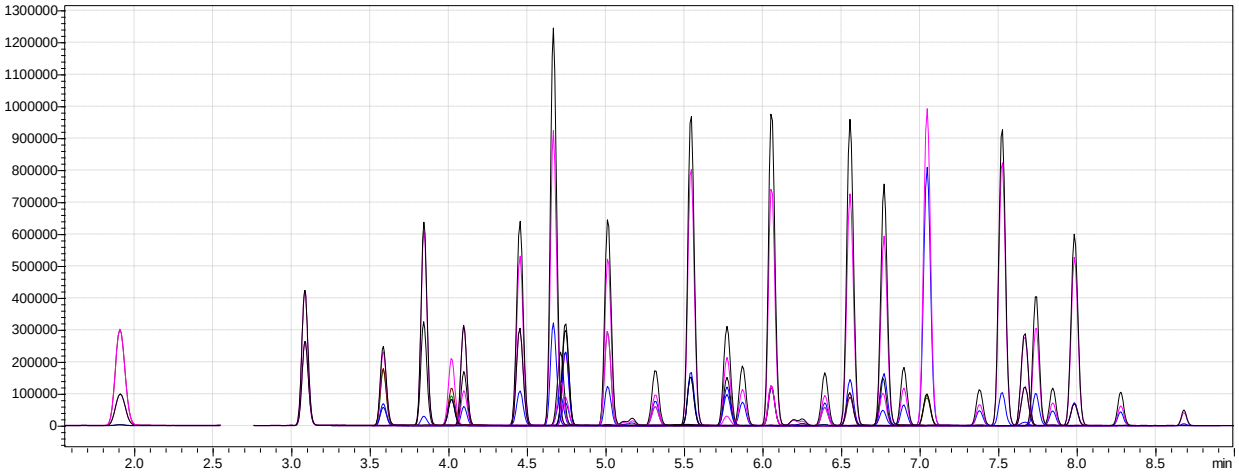



**Figure 2**: Chromatogram of 1.0 ng/g PFAS in a fish matrix with separation of all peaks in nine minutes

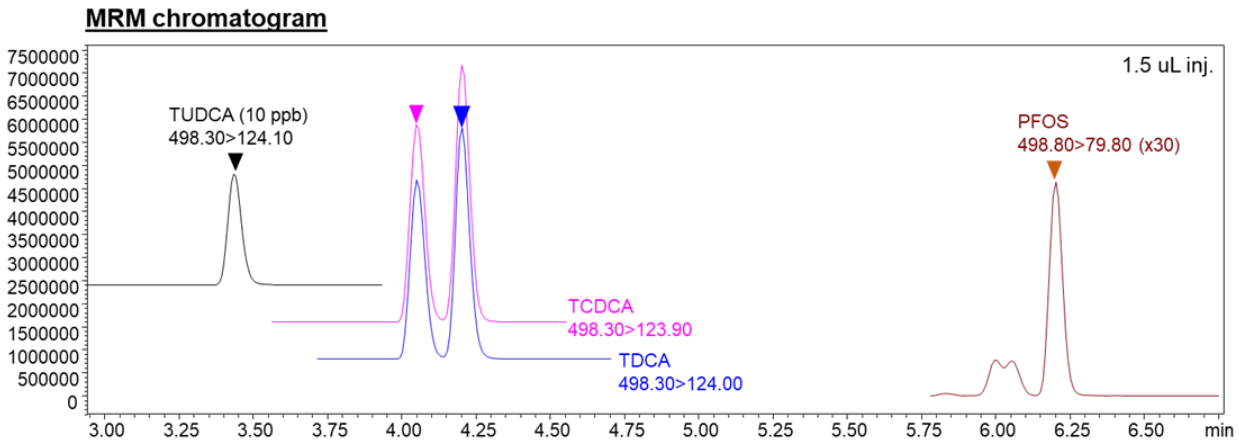



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

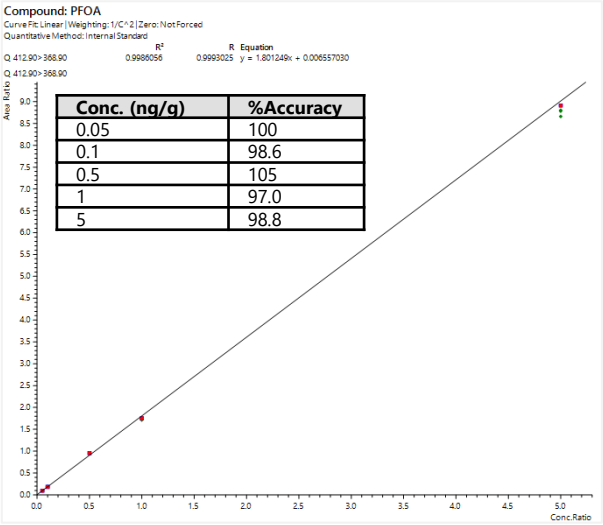
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

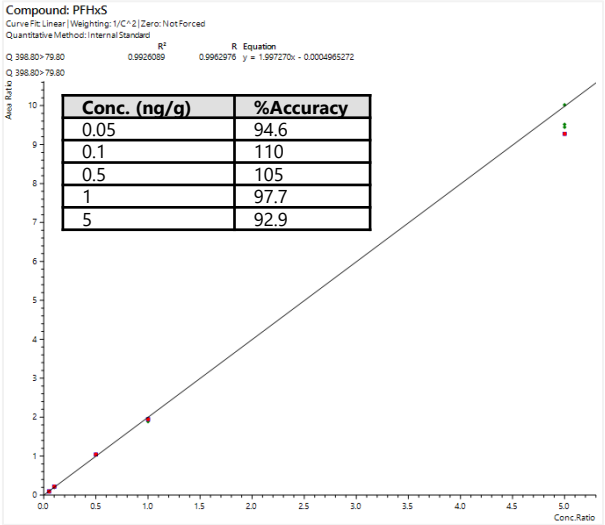
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



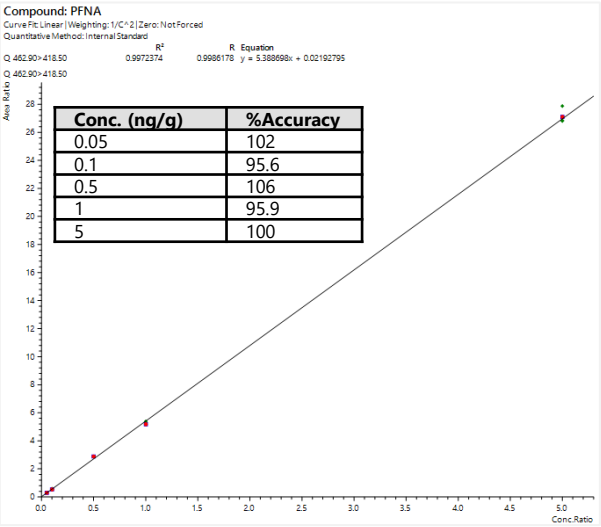

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.05 | 100 |
| 0.1 | 98.6 |
| 0.5 | 105 |
| 1 | 97.0 |
| 5 | 98.8 |

**Figure 4**: PFHxS Calibration Curve



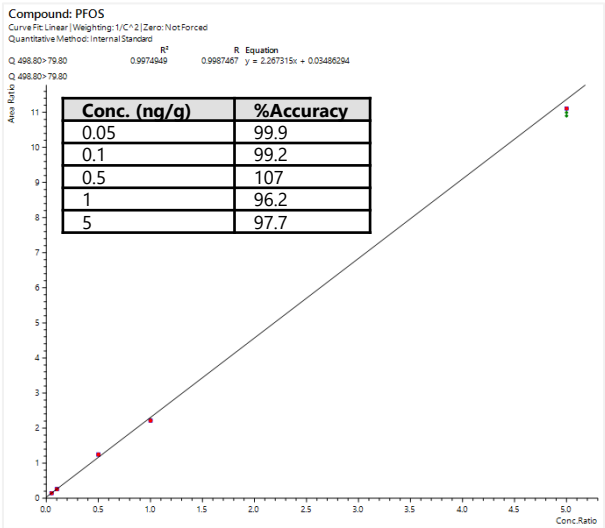

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.05 | 94.6 |
| 0.1 | 110 |
| 0.5 | 105 |
| 1 | 97.7 |
| 5 | 92.9 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.05 | 102 |
| 0.1 | 95.6 |
| 0.5 | 106 |
| 1 | 95.9 |
| 5 | 100 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.05 | 99.9 |
| 0.1 | 99.2 |
| 0.5 | 107 |
| 1 | 96.2 |
| 5 | 97.7 |

Blank matrixes and three different concentrations ranging from the SMPR required LOQ to 50 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | 0.004 | | | |
| | 0.1 | 0.103 | 2.51 | 2.43 | 103.4 |
| | 1 | 0.970 | 0.21 | 0.21 | 97.0 |
| | 5 | 4.830 | 0.79 | 0.82 | 96.6 |
| PFPeA | Blank | ND | | | |
| | 0.1 | 0.096 | 2.11 | 2.20 | 96.2 |
| | 1 | 0.976 | 0.46 | 0.47 | 97.6 |
| | 5 | 4.920 | 0.61 | 0.62 | 98.4 |
| PFHxA | Blank | 0.002 | | | |
| | 0.1 | 0.099 | 1.64 | 1.66 | 98.8 |
| | 1 | 0.957 | 0.55 | 0.58 | 95.7 |
| | 5 | 4.883 | 1.72 | 1.77 | 97.6 |
| PFHpA | Blank | ND | | | |
| | 0.1 | 0.100 | 0.44 | 0.44 | 100.1 |
| | 1 | 0.980 | 1.72 | 1.76 | 98.0 |
| | 5 | 4.998 | 1.42 | 1.42 | 100.0 |
| PFOA | Blank | ND | | | |
| | 0.1 | 0.100 | 1.50 | 1.51 | 99.6 |
| | 1 | 0.960 | 1.43 | 1.49 | 96.0 |
| | 5 | 4.852 | 0.81 | 0.84 | 97.0 |
| PFNA | Blank | ND | | | |
| | 0.1 | 0.101 | 2.74 | 2.72 | 100.9 |
| | 1 | 0.984 | 1.11 | 1.12 | 98.4 |
| | 5 | 5.039 | 2.25 | 2.24 | 100.8 |
| PFDA | Blank | 0.000 | | | |
| | 0.1 | 0.098 | 3.36 | 3.42 | 98.2 |
| | 1 | 0.936 | 1.39 | 1.48 | 93.6 |
| | 5 | 4.800 | 0.57 | 0.59 | 96.0 |
| PFUnA | Blank | 0.002 | | | |
| | 0.1 | 0.100 | 1.65 | 1.65 | 99.8 |
| | 1 | 0.932 | 1.44 | 1.55 | 93.2 |
| | 5 | 4.896 | 1.63 | 1.66 | 97.9 |
| PFDoA | Blank | ND | | | |
| | 0.1 | 0.093 | 10.48 | 11.29 | 92.9 |
| | 1 | 0.939 | 2.17 | 2.31 | 93.9 |
| | 5 | 4.833 | 1.42 | 1.47 | 96.6 |
| PFTrDA | Blank | 0.004 | | | |
| | 0.1 | 0.107 | 8.56 | 8.05 | 106.4 |
| | 1 | 0.996 | 3.93 | 3.95 | 99.6 |
| | 5 | 4.889 | 4.60 | 4.71 | 97.8 |
| PFTeDA | Blank | 0.009 | | | |
| | 0.1 | 0.114 | 8.25 | 7.23 | 114.1 |
| | 1 | 1.029 | 12.47 | 12.12 | 102.9 |
| | 5 | 4.860 | 5.60 | 5.76 | 97.2 |
| PFBS | Blank | ND | | | |
| | 0.1 | 0.100 | 2.77 | 2.78 | 99.7 |
| | 1 | 0.945 | 1.76 | 1.86 | 94.5 |
| | 5 | 4.836 | 1.26 | 1.30 | 96.7 |
| PFPeS | Blank | ND | | | |
| | 0.1 | 0.092 | 9.10 | 9.85 | 92.4 |
| | 1 | 0.990 | 2.20 | 2.22 | 99.0 |
| | 5 | 4.857 | 1.96 | 2.01 | 97.2 |
| PFHxS | Blank | 0.002 | | | |
| | 0.1 | 0.105 | 6.51 | 6.21 | 104.9 |
| | 1 | 0.968 | 2.02 | 2.09 | 96.8 |
| | 5 | 4.838 | 3.11 | 3.21 | 96.7 |
| PFHpS | Blank | 0.001 | | | |
| | 0.1 | 0.099 | 3.85 | 3.88 | 99.1 |
| | 1 | 0.925 | 2.29 | 2.47 | 92.5 |
| | 5 | 4.613 | 0.35 | 0.38 | 92.2 |

| | | | | | |
|---|---|---|---|---|---|
| PFOS | Blank | 0.003 | | | |
| | 0.1 | 0.102 | 1.89 | 1.85 | 102.2 |
| | 1 | 0.968 | 0.60 | 0.62 | 96.8 |
| | 5 | 4.834 | 0.90 | 0.93 | 96.7 |
| PFNS | Blank | ND | | | |
| | 0.1 | 0.101 | 6.29 | 6.26 | 100.4 |
| | 1 | 0.929 | 2.48 | 2.68 | 92.9 |
| | 5 | 4.840 | 1.61 | 1.66 | 96.8 |
| PFDS | Blank | ND | | | |
| | 0.1 | 0.097 | 4.10 | 4.24 | 96.7 |
| | 1 | 0.982 | 2.63 | 2.68 | 98.2 |
| | 5 | 4.877 | 3.65 | 3.74 | 97.6 |
| PFUnDS | Blank | 0.000 | | | |
| | 0.1 | 0.106 | 13.25 | 12.58 | 105.3 |
| | 1 | 1.054 | 7.89 | 7.49 | 105.4 |
| | 5 | 4.759 | 6.56 | 6.90 | 95.2 |
| PFDoS | Blank | 0.005 | | | |
| | 0.1 | 0.114 | 16.66 | 14.59 | 114.1 |
| | 1 | 1.084 | 20.30 | 18.72 | 108.4 |
| | 5 | 4.694 | 2.70 | 2.88 | 93.9 |
| PFTrDS | Blank | 0.008 | | | |
| | 0.1 | 0.119 | 14.78 | 12.41 | 119.1 |
| | 1 | 1.113 | 24.60 | 22.11 | 111.3 |
| | 5 | 4.911 | 7.02 | 7.15 | 98.2 |
| PFOSA | Blank | 0.000 | | | |
| | 0.1 | 0.096 | 2.16 | 2.25 | 96.0 |
| | 1 | 0.945 | 0.97 | 1.03 | 94.5 |
| | 5 | 4.898 | 0.67 | 0.68 | 98.0 |
| 9Cl-PF3ONS | Blank | ND | | | |
| | 0.1 | 0.097 | 1.14 | 1.17 | 97.2 |
| | 1 | 0.965 | 0.44 | 0.45 | 96.5 |
| | 5 | 4.901 | 1.82 | 1.86 | 98.0 |
| 11Cl-PF3OUdS | Blank | ND | | | |
| | 0.1 | 0.097 | 6.63 | 6.82 | 97.3 |
| | 1 | 0.943 | 3.67 | 3.89 | 94.3 |
| | 5 | 4.796 | 3.06 | 3.20 | 95.9 |
| HFPO-DA | Blank | 0.004 | | | |
| | 0.1 | 0.102 | 6.03 | 5.91 | 101.9 |
| | 1 | 0.964 | 1.36 | 1.41 | 96.4 |
| | 5 | 4.778 | 0.21 | 0.22 | 95.5 |
| DONA | Blank | 0.001 | | | |
| | 0.1 | 0.100 | 1.42 | 1.43 | 99.6 |
| | 1 | 0.956 | 0.90 | 0.94 | 95.6 |
| | 5 | 4.974 | 1.10 | 1.11 | 99.5 |
| 4:2 FTS | Blank | 0.000 | | | |
| | 0.1 | 0.099 | 3.73 | 3.77 | 98.9 |
| | 1 | 0.955 | 1.62 | 1.69 | 95.5 |
| | 5 | 4.718 | 1.86 | 1.97 | 94.4 |
| 6:2 FTS | Blank | 0.001 | | | |
| | 0.1 | 0.098 | 2.91 | 2.98 | 97.7 |
| | 1 | 0.944 | 1.03 | 1.09 | 94.4 |
| | 5 | 4.664 | 1.00 | 1.07 | 93.3 |
| 8:2 FTS | Blank | 0.010 | | | |
| | 0.1 | 0.107 | 4.31 | 4.02 | 107.2 |
| | 1 | 1.096 | 9.63 | 8.79 | 109.6 |
| | 5 | 5.041 | 19.12 | 18.97 | 100.8 |
| 10:2 FTS | Blank | 0.008 | | | |
| | 0.1 | 0.110 | 5.70 | 5.21 | 109.4 |
| | 1 | 1.029 | 7.31 | 7.10 | 102.9 |
| | 5 | 4.028 | 8.96 | 11.12 | 80.6 |

ND = average results less than zero

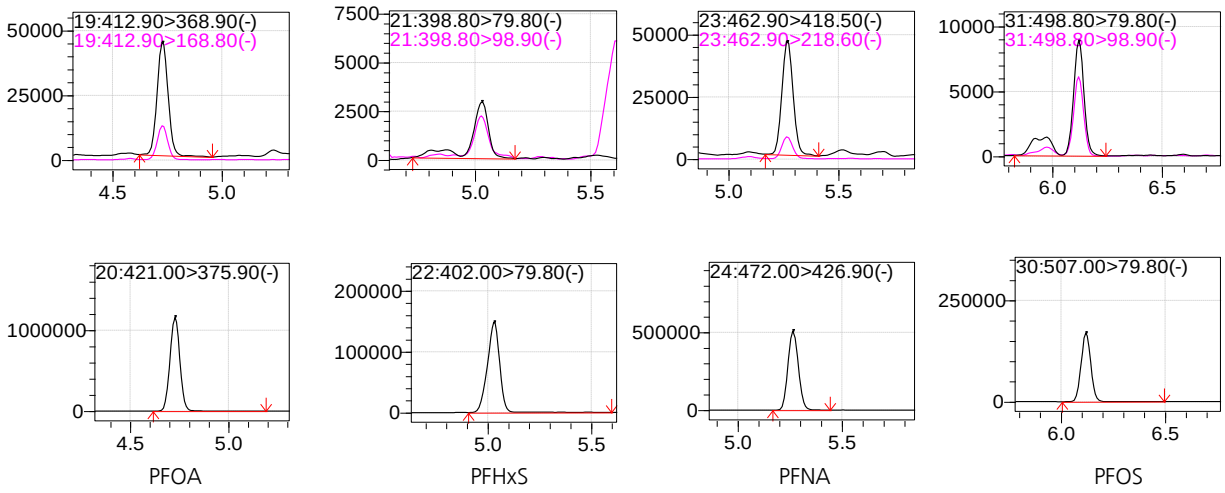



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a fish matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Protein Powder by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in Protein Powder meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in protein powder using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.055 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.055 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.055 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.055 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.055 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.0055 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.0055 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.0055 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.055 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.055 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.055 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.055 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.055 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.0055 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.0055 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.055 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.055 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.055 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.055 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.055 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.55 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.55 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.0055 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.0055 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.55 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.055 |

## ■ Sample Preparation and Analysis Conditions

Organic, plant-based protein powder was purchased locally. Test portions were weighed directly from the store packaging and spiked in triplicate at four different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram protein powder test portions spiked with concentrations of 0.001, 0.01, 0.1, 1.0 and 10.0 ng/g. Quantitation was carried out on additional protein powder samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in a protein powder matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, 10 mL of water and 10 mL of acetonitrile was added. The samples were shaken for 1 minute and a QuEChERS packet was added. The sample was shaken again for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 13 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, the extract was concentrated to dryness and dissolved in 0.4 mL of a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

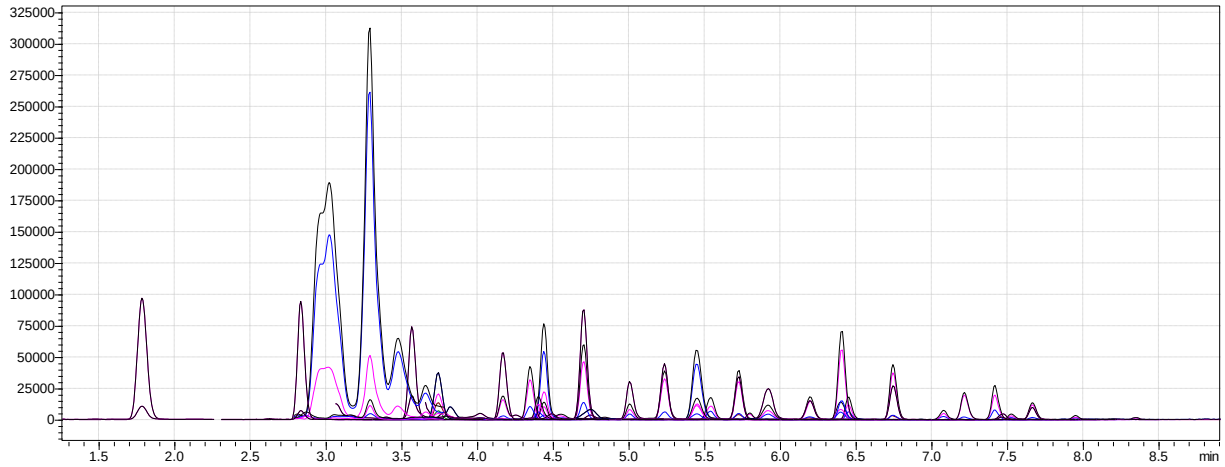



**Figure 2**: Chromatogram of 0.055 ng/g PFAS in a protein powder matrix with separation of all peaks in nine minutes. The chromatogram shows other co-eluting peaks between 2.7 and 3.6 minutes, but they did not interfere.

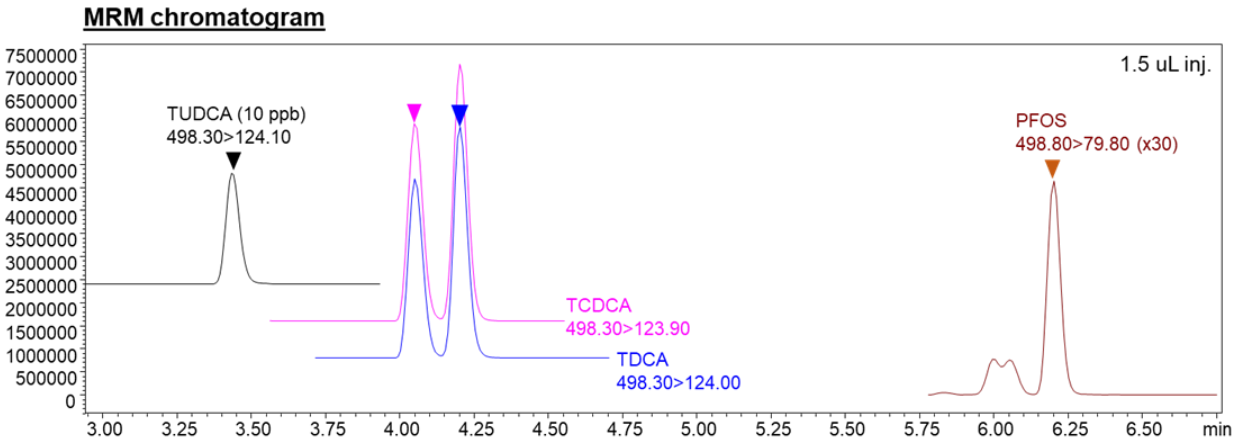



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

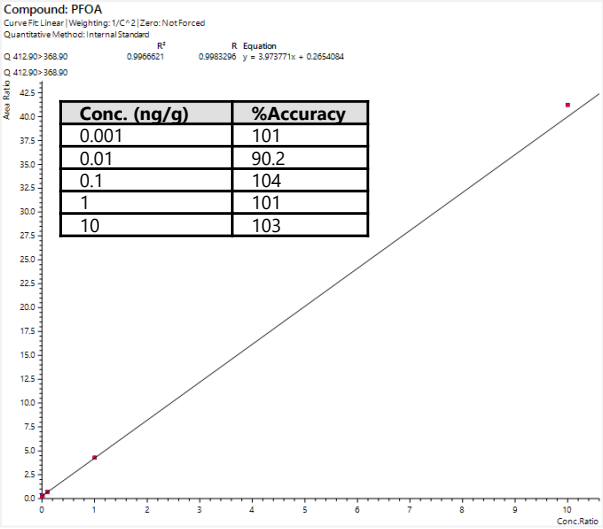
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

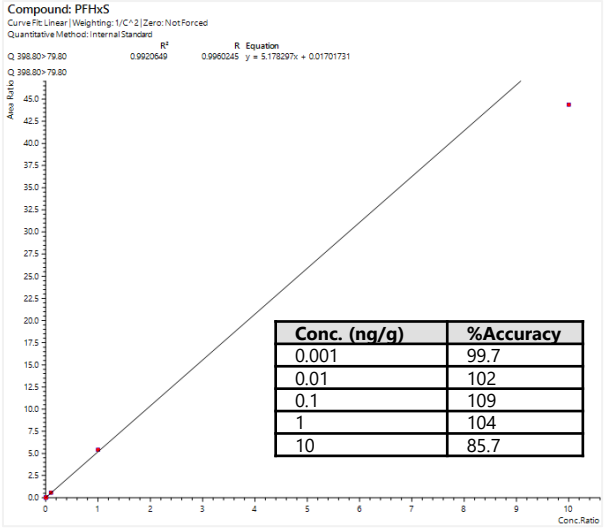
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



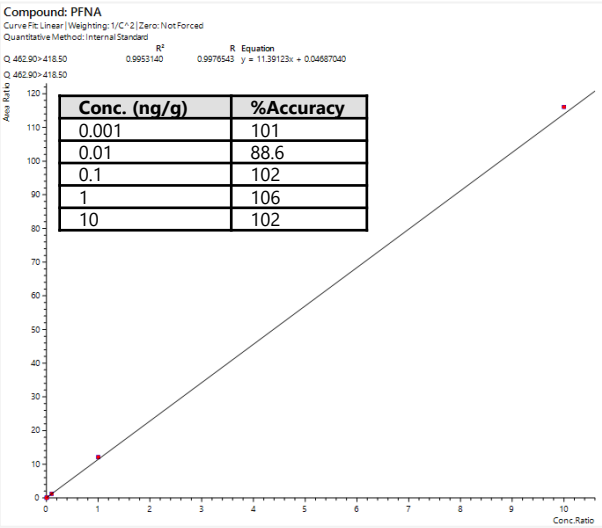

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 90.2 |
| 0.1 | 104 |
| 1 | 101 |
| 10 | 103 |

**Figure 4**: PFHxS Calibration Curve



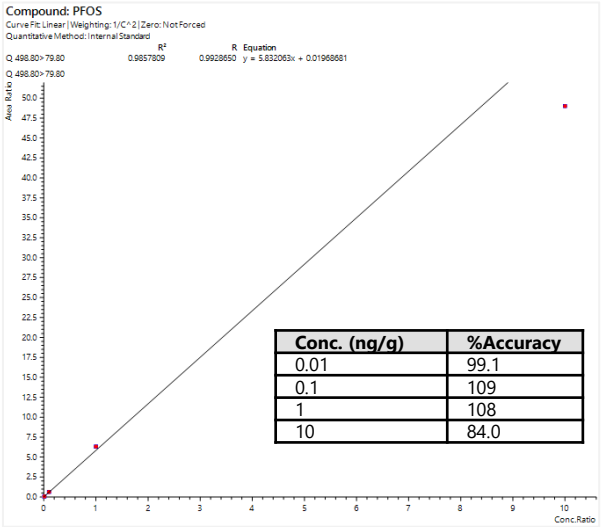

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.7 |
| 0.01 | 102 |
| 0.1 | 109 |
| 1 | 104 |
| 10 | 85.7 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 88.6 |
| 0.1 | 102 |
| 1 | 106 |
| 10 | 102 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.01 | 99.1 |
| 0.1 | 109 |
| 1 | 108 |
| 10 | 84.0 |

Blank matrixes and at least three different concentrations ranging from below the SMPR required LOQ to approximately 70 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | ND | | | |
| | 0.055 | 0.071 | 6.83 | 5.31 | 128.7 |
| | 0.55 | 0.599 | 2.41 | 2.22 | 108.9 |
| | 5.5 | 6.077 | 0.70 | 0.64 | 110.5 |
| PFPeA | Blank | ND | | | |
| | 0.055 | 0.061 | 3.70 | 3.36 | 110.2 |
| | 0.55 | 0.598 | 0.29 | 0.27 | 108.6 |
| | 5.5 | 6.275 | 2.88 | 2.53 | 114.1 |
| PFHxA | Blank | ND | | | |
| | 0.055 | 0.049 | 2.50 | 2.81 | 89.1 |
| | 0.55 | 0.546 | 2.08 | 2.10 | 99.2 |
| | 5.5 | 5.758 | 1.32 | 1.26 | 104.7 |
| PFHpA | Blank | 0.005 | | | |
| | 0.055 | 0.057 | 1.46 | 1.41 | 103.3 |
| | 0.55 | 0.541 | 4.25 | 4.32 | 98.4 |
| | 5.5 | 5.339 | 2.40 | 2.47 | 97.1 |
| PFOA | Blank | 0.000 | | | |
| | 0.055 | 0.060 | 4.83 | 4.40 | 109.7 |
| | 0.55 | 0.614 | 2.34 | 2.10 | 111.6 |
| | 5.5 | 5.962 | 2.80 | 2.58 | 108.4 |
| PFNA | Blank | ND | | | |
| | 0.0055 | 0.006 | 10.70 | 10.53 | 101.7 |
| | 0.055 | 0.060 | 0.67 | 0.61 | 109.2 |
| | 0.55 | 0.604 | 2.45 | 2.23 | 109.8 |
| | 5.5 | 6.158 | 2.84 | 2.54 | 111.9 |
| PFDA | Blank | ND | | | |
| | 0.0055 | 0.005 | 4.86 | 5.09 | 95.6 |
| | 0.055 | 0.058 | 1.52 | 1.45 | 104.8 |
| | 0.55 | 0.589 | 3.72 | 3.48 | 107.1 |
| | 5.5 | 5.996 | 3.88 | 3.55 | 109.0 |
| PFUnA | Blank | ND | | | |
| | 0.0055 | 0.007 | 21.51 | 18.99 | 113.3 |
| | 0.055 | 0.059 | 6.09 | 5.73 | 106.3 |
| | 0.55 | 0.595 | 2.56 | 2.36 | 108.2 |
| | 5.5 | 5.655 | 4.91 | 4.78 | 102.8 |
| PFDoA | Blank | ND | | | |
| | 0.055 | 0.060 | 10.10 | 9.34 | 108.2 |
| | 0.55 | 0.597 | 6.61 | 6.09 | 108.6 |
| | 5.5 | 5.748 | 7.59 | 7.26 | 104.5 |
| PFTrDA | Blank | ND | | | |
| | 0.0055 | 0.004 | 12.72 | 17.77 | 71.6 |
| | 0.055 | 0.052 | 2.20 | 2.30 | 95.5 |
| | 0.55 | 0.561 | 2.15 | 2.10 | 102.0 |
| | 5.5 | 5.583 | 3.40 | 3.35 | 101.5 |
| PFTeDA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 4.51 | 4.94 | 91.2 |
| | 0.055 | 0.049 | 5.97 | 6.69 | 89.3 |
| | 0.55 | 0.568 | 1.33 | 1.29 | 103.3 |
| | 5.5 | 5.432 | 4.41 | 4.46 | 98.8 |
| PFBS | Blank | ND | | | |
| | 0.055 | 0.062 | 11.70 | 10.33 | 113.2 |
| | 0.55 | 0.620 | 7.16 | 6.35 | 112.7 |
| | 5.5 | 5.952 | 6.67 | 6.16 | 108.2 |
| PFPeS | Blank | 0.001 | | | |
| | 0.055 | 0.063 | 3.61 | 3.14 | 114.8 |
| | 0.55 | 0.602 | 6.16 | 5.62 | 109.5 |
| | 5.5 | 6.168 | 0.86 | 0.77 | 112.2 |
| PFHxS | Blank | ND | | | |
| | 0.0055 | 0.006 | 6.85 | 6.02 | 113.8 |
| | 0.055 | 0.059 | 3.86 | 3.60 | 107.1 |
| | 0.55 | 0.587 | 1.17 | 1.10 | 106.7 |
| | 5.5 | 5.486 | 2.16 | 2.17 | 99.7 |
| PFHpS | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 15.90 | 14.87 | 106.9 |
| | 0.055 | 0.058 | 2.05 | 1.94 | 105.6 |
| | 0.55 | 0.563 | 3.63 | 3.55 | 102.3 |
| | 5.5 | 6.008 | 7.58 | 6.94 | 109.2 |

| | | | | | |
|---|---|---|---|---|---|
| PFOS | Blank | ND | | | |
| | 0.055 | 0.057 | 17.30 | 16.79 | 103.0 |
| | 0.55 | 0.603 | 4.60 | 4.20 | 109.6 |
| | 5.5 | 5.325 | 2.41 | 2.49 | 96.8 |
| PFNS | Blank | ND | | | |
| | 0.055 | 0.054 | 4.15 | 4.24 | 98.0 |
| | 0.55 | 0.610 | 8.84 | 7.97 | 110.9 |
| | 5.5 | 6.174 | 5.02 | 4.48 | 112.3 |
| PFDS | Blank | 0.003 | | | |
| | 0.055 | 0.056 | 18.37 | 18.08 | 101.6 |
| | 0.55 | 0.532 | 3.72 | 3.85 | 96.6 |
| | 5.5 | 5.808 | 6.93 | 6.56 | 105.6 |
| PFUnDS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 10.13 | 12.93 | 78.4 |
| | 0.055 | 0.056 | 3.07 | 3.03 | 101.4 |
| | 0.55 | 0.561 | 3.32 | 3.25 | 101.9 |
| | 5.5 | 4.688 | 3.45 | 4.04 | 85.3 |
| PFDoS | Blank | 0.003 | | | |
| | 0.0055 | 0.006 | 7.39 | 6.50 | 113.7 |
| | 0.055 | 0.058 | 4.98 | 4.75 | 104.8 |
| | 0.55 | 0.523 | 3.60 | 3.79 | 95.0 |
| | 5.5 | 4.703 | 1.74 | 2.04 | 85.5 |
| PFTrDS | Blank | ND | | | |
| | 0.55 | 0.695 | 2.08 | 1.65 | 126.4 |
| | 5.5 | 6.531 | 6.31 | 5.32 | 118.8 |
| PFOSA | Blank | ND | | | |
| | 0.055 | 0.053 | 7.62 | 7.91 | 96.3 |
| | 0.55 | 0.614 | 3.36 | 3.01 | 111.7 |
| | 5.5 | 5.397 | 2.50 | 2.55 | 98.1 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 3.21 | 2.76 | 116.4 |
| | 0.055 | 0.063 | 2.40 | 2.10 | 114.3 |
| | 0.55 | 0.607 | 3.84 | 3.48 | 110.3 |
| | 5.5 | 5.687 | 1.52 | 1.47 | 103.4 |
| 11Cl-PF3OUdS | Blank | ND | | | |
| | 0.0055 | 0.004 | 9.37 | 12.62 | 74.2 |
| | 0.055 | 0.060 | 7.15 | 6.56 | 109.0 |
| | 0.55 | 0.557 | 0.90 | 0.89 | 101.3 |
| | 5.5 | 5.777 | 3.99 | 3.80 | 105.0 |
| HFPO-DA | Blank | 0.030 | | | |
| | 0.55 | 0.663 | 8.31 | 6.89 | 120.5 |
| | 5.5 | 6.199 | 8.09 | 7.18 | 112.7 |
| DONA | Blank | ND | | | |
| | 0.0055 | 0.005 | 5.20 | 5.91 | 88.0 |
| | 0.055 | 0.060 | 3.83 | 3.54 | 108.2 |
| | 0.55 | 0.546 | 4.27 | 4.30 | 99.3 |
| | 5.5 | 5.671 | 1.33 | 1.29 | 103.1 |
| 4:2 FTS | Blank | 0.012 | | | |
| | 0.055 | 0.054 | 5.74 | 5.85 | 98.0 |
| | 0.55 | 0.589 | 2.02 | 1.89 | 107.1 |
| | 5.5 | 5.963 | 5.17 | 4.77 | 108.4 |
| 6:2 FTS | Blank | 0.004 | | | |
| | 0.055 | 0.071 | 15.56 | 12.10 | 128.6 |
| | 0.55 | 0.582 | 4.10 | 3.87 | 105.9 |
| | 5.5 | 5.729 | 4.83 | 4.64 | 104.1 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 7.43 | 7.54 | 98.6 |
| | 0.055 | 0.055 | 7.09 | 7.10 | 99.8 |
| | 0.55 | 0.553 | 5.34 | 5.32 | 100.5 |
| | 5.5 | 5.004 | 4.06 | 4.46 | 91.0 |
| 10:2 FTS | Blank | ND | | | |
| | 0.055 | 0.055 | 10.56 | 10.61 | 99.5 |
| | 0.55 | 0.673 | 8.99 | 7.35 | 122.3 |
| | 5.5 | 5.601 | 11.11 | 10.91 | 101.8 |

ND = average results less than zero

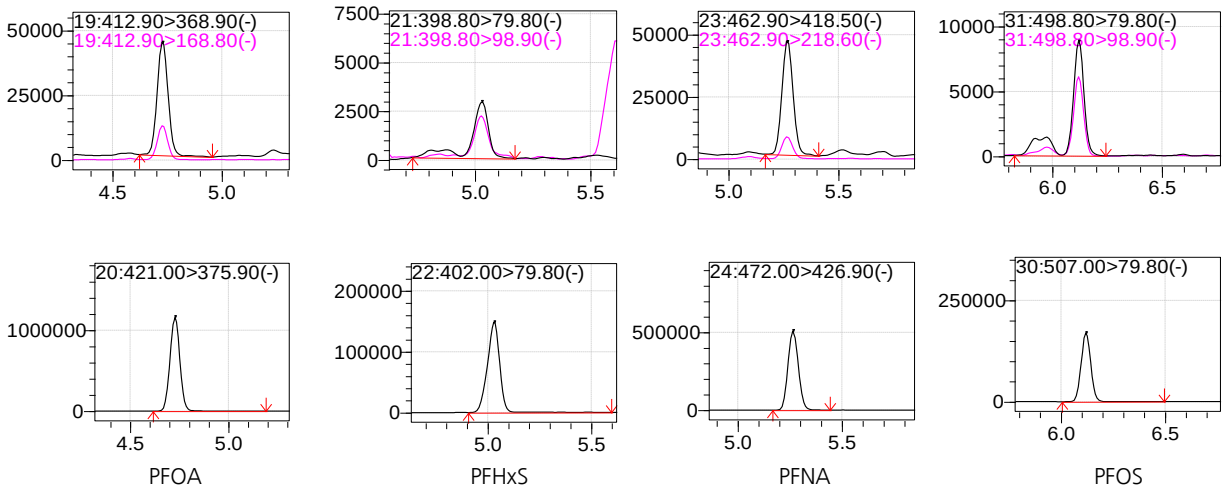



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a protein powder matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Pet Food by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

William Lipps[1], Toshiya Matsubara[1], Dominika Gruszecka[1], Nozomi Maeshima[2], Yui Higashi[2], Manami Kobayashi[2]

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## User Benefits

- Validated method for 30 PFAS in Pet Food meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, Low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in pet food using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at three concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.5 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.5 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.5 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.5 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.5 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.5 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.5 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.5 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.5 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.5 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.5 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.5 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.5 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.5 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.5 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.5 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.5 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.5 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.5 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.5 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.5 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.5 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.5 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.5 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.5 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.5 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.5 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.5 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.5 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.5 |

## ■ Sample Preparation and Analysis Conditions

Dog food was purchased locally. Sub samples were removed from the bag and crushed with dry ice. The ground material placed into containers and stored in a freezer overnight to allow the dry ice to sublimate away completely. Test portions were spiked in triplicate at three different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 2- gram test portions spiked with concentrations of 0.25, 0.5, 2.5, 5, 12.5, and 25 ng/g. Quantitation was carried out on additional dog food samples spiked in triplicate at 0.5, 5.0, and 25 ng/g. Since standards were extracted in a pet food matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

2-gram portions were weighed, spiked with target analytes and internal standards, 10 mL of water and 10 mL of acetonitrile was added. The samples were shaken by hand for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. A 0.5 mL aliquot of the eluted solution was transferred to a vial and acidified with formic acid.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

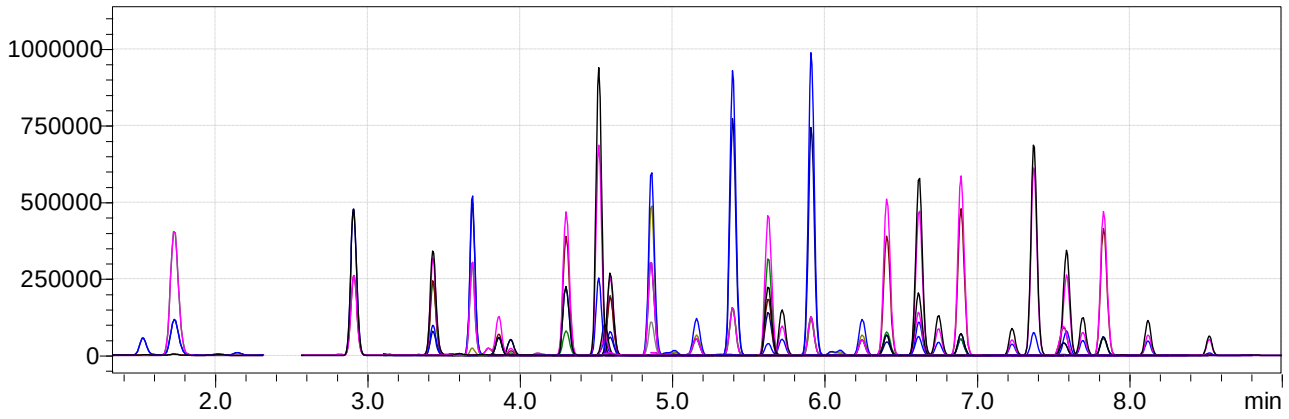



**Figure 2**: Chromatogram of 5 ng/g PFAS in a pet food matrix with separation of all peaks in nine minutes

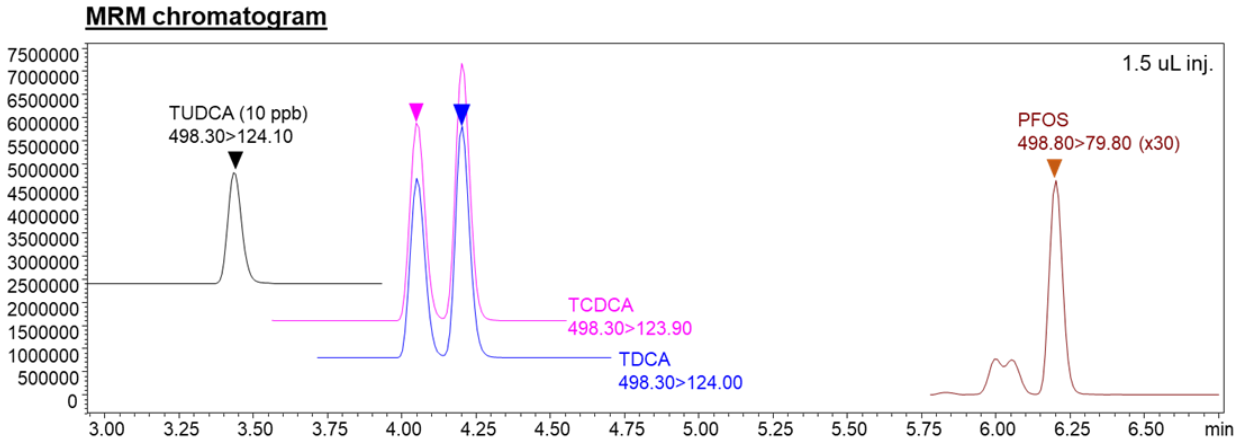



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

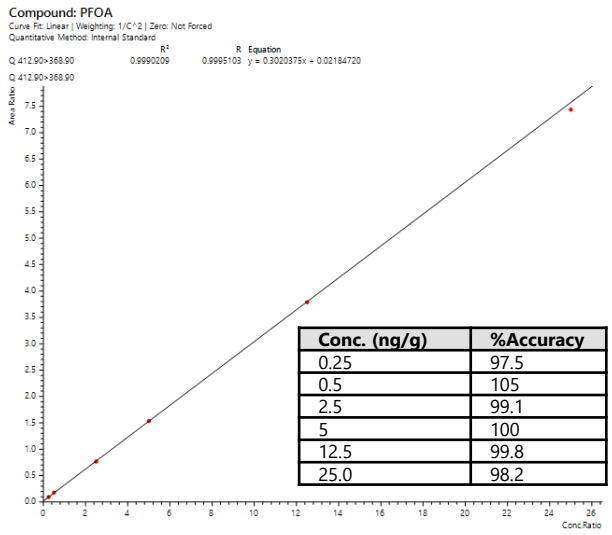
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

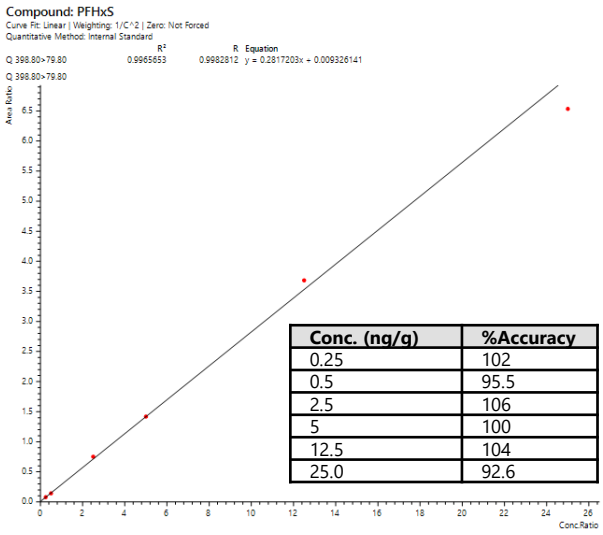
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



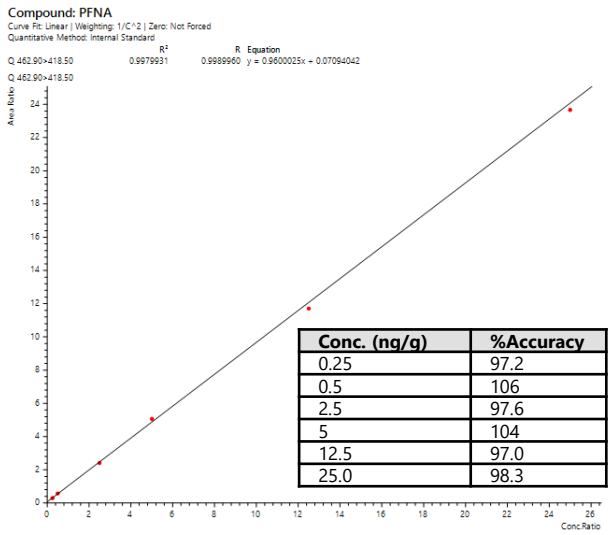

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.25 | 97.5 |
| 0.5 | 105 |
| 2.5 | 99.1 |
| 5 | 100 |
| 12.5 | 99.8 |
| 25.0 | 98.2 |

**Figure 4**: PFHxS Calibration Curve



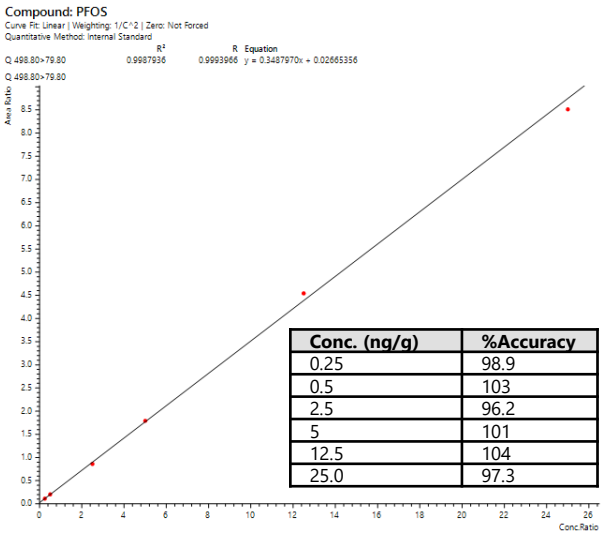

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.25 | 102 |
| 0.5 | 95.5 |
| 2.5 | 106 |
| 5 | 100 |
| 12.5 | 104 |
| 25.0 | 92.6 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.25 | 97.2 |
| 0.5 | 106 |
| 2.5 | 97.6 |
| 5 | 104 |
| 12.5 | 97.0 |
| 25.0 | 98.3 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.25 | 98.9 |
| 0.5 | 103 |
| 2.5 | 96.2 |
| 5 | 101 |
| 12.5 | 104 |
| 25.0 | 97.3 |

Blank matrixes and three different concentrations ranging from the SMPR required LOQ to 50 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | ND | | | |
| | 0.5 | 0.541 | 13.70 | 12.66 | 108.2 |
| | 5 | 4.956 | 1.69 | 1.71 | 99.1 |
| | 25 | 22.753 | 2.35 | 2.58 | 91.0 |
| PFPeA | Blank | ND | | | |
| | 0.5 | 0.514 | 0.65 | 0.63 | 102.8 |
| | 5 | 5.071 | 0.59 | 0.58 | 101.4 |
| | 25 | 23.462 | 0.29 | 0.31 | 93.9 |
| PFHxA | Blank | ND | | | |
| | 0.5 | 0.502 | 2.66 | 2.65 | 100.3 |
| | 5 | 5.088 | 0.25 | 0.25 | 101.8 |
| | 25 | 24.247 | 1.07 | 1.10 | 97.0 |
| PFHpA | Blank | ND | | | |
| | 0.5 | 0.559 | 3.74 | 3.35 | 111.8 |
| | 5 | 5.073 | 0.85 | 0.84 | 101.4 |
| | 25 | 23.973 | 0.36 | 0.38 | 95.9 |
| PFOA | Blank | ND | | | |
| | 0.5 | 0.525 | 2.82 | 2.68 | 105.0 |
| | 5 | 5.147 | 0.81 | 0.79 | 102.9 |
| | 25 | 24.564 | 0.42 | 0.42 | 98.3 |
| PFNA | Blank | ND | | | |
| | 0.5 | 0.513 | 0.76 | 0.74 | 102.8 |
| | 5 | 5.101 | 0.95 | 0.93 | 102.0 |
| | 25 | 24.739 | 0.64 | 0.65 | 99.0 |
| PFDA | Blank | ND | | | |
| | 0.5 | 0.525 | 1.19 | 1.14 | 105.1 |
| | 5 | 5.197 | 1.80 | 1.74 | 103.9 |
| | 25 | 24.522 | 0.76 | 0.78 | 98.1 |
| PFUnA | Blank | ND | | | |
| | 0.5 | 0.541 | 1.25 | 1.16 | 108.3 |
| | 5 | 5.141 | 2.02 | 1.97 | 102.8 |
| | 25 | 23.863 | 0.76 | 0.79 | 95.4 |
| PFDoA | Blank | ND | | | |
| | 0.5 | 0.482 | 4.82 | 5.00 | 96.4 |
| | 5 | 4.919 | 3.08 | 3.13 | 98.4 |
| | 25 | 23.291 | 1.75 | 1.88 | 93.2 |
| PFTrDA | Blank | ND | | | |
| | 0.5 | 0.490 | 6.38 | 6.51 | 97.9 |
| | 5 | 4.921 | 5.49 | 5.58 | 98.4 |
| | 25 | 24.337 | 0.87 | 0.90 | 97.4 |
| PFTeDA | Blank | ND | | | |
| | 0.5 | 0.508 | 1.01 | 0.99 | 101.7 |
| | 5 | 4.858 | 2.87 | 2.96 | 97.2 |
| | 25 | 24.158 | 1.93 | 2.00 | 96.6 |
| PFBS | Blank | ND | | | |
| | 0.5 | 0.533 | 11.52 | 10.80 | 106.7 |
| | 5 | 5.091 | 1.32 | 1.30 | 101.8 |
| | 25 | 22.671 | 0.89 | 0.98 | 90.7 |
| PFPeS | Blank | ND | | | |
| | 0.5 | 0.428 | 13.61 | 15.89 | 85.6 |
| | 5 | 4.429 | 0.35 | 0.39 | 88.6 |
| | 25 | 19.906 | 5.80 | 7.28 | 79.6 |
| PFHxS | Blank | ND | | | |
| | 0.5 | 0.539 | 2.23 | 2.07 | 107.7 |
| | 5 | 4.900 | 2.96 | 3.02 | 98.0 |
| | 25 | 22.645 | 2.36 | 2.60 | 90.6 |
| PFHpS | Blank | ND | | | |
| | 0.5 | 0.494 | 2.61 | 2.64 | 98.7 |
| | 5 | 5.026 | 0.82 | 0.81 | 100.5 |
| | 25 | 24.918 | 1.45 | 1.46 | 99.7 |

| | | | | | |
|---|---|---|---|---|---|
| PFOS | Blank | ND | | | |
| | 0.5 | 0.475 | 6.50 | 6.84 | 95.0 |
| | 5 | 4.930 | 3.29 | 3.34 | 98.6 |
| | 25 | 22.823 | 1.41 | 1.55 | 91.3 |
| PFNS | Blank | ND | | | |
| | 0.5 | 0.512 | 7.18 | 7.01 | 102.3 |
| | 5 | 4.926 | 2.71 | 2.75 | 98.5 |
| | 25 | 22.611 | 0.29 | 0.32 | 90.4 |
| PFDS | Blank | ND | | | |
| | 0.5 | 0.512 | 9.79 | 9.57 | 102.3 |
| | 5 | 4.921 | 1.68 | 1.71 | 98.4 |
| | 25 | 23.794 | 2.55 | 2.68 | 95.2 |
| PFUnDS | Blank | ND | | | |
| | 0.5 | 0.531 | 3.02 | 2.84 | 106.1 |
| | 5 | 5.145 | 1.75 | 1.70 | 102.9 |
| | 25 | 24.117 | 1.78 | 1.84 | 96.5 |
| PFDoS | Blank | ND | | | |
| | 0.5 | 0.502 | 5.59 | 5.57 | 100.5 |
| | 5 | 5.103 | 2.54 | 2.49 | 102.1 |
| | 25 | 23.501 | 3.05 | 3.25 | 94.0 |
| PFTrDS | Blank | ND | | | |
| | 0.5 | 0.511 | 5.99 | 5.87 | 102.1 |
| | 5 | 4.752 | 5.22 | 5.49 | 95.0 |
| | 25 | 23.836 | 1.86 | 1.95 | 95.3 |
| PFOSA | Blank | ND | | | |
| | 0.5 | 0.523 | 0.95 | 0.91 | 104.7 |
| | 5 | 5.139 | 0.62 | 0.61 | 102.8 |
| | 25 | 24.134 | 0.55 | 0.57 | 96.5 |
| 9Cl-PF3ONS | Blank | ND | | | |
| | 0.5 | 0.494 | 2.51 | 2.54 | 98.9 |
| | 5 | 5.095 | 1.51 | 1.49 | 101.9 |
| | 25 | 22.288 | 0.81 | 0.91 | 89.1 |
| 11Cl-PF3OUdS | Blank | ND | | | |
| | 0.5 | 0.501 | 1.86 | 1.86 | 100.1 |
| | 5 | 4.919 | 3.34 | 3.39 | 98.4 |
| | 25 | 23.572 | 1.67 | 1.78 | 94.3 |
| HFPO-DA | Blank | ND | | | |
| | 0.5 | 0.360 | 1.91 | 2.66 | 72.0 |
| | 5 | 5.044 | 1.04 | 1.03 | 100.9 |
| | 25 | 24.698 | 2.86 | 2.89 | 98.8 |
| DONA | Blank | ND | | | |
| | 0.5 | 0.493 | 6.51 | 6.59 | 98.7 |
| | 5 | 4.744 | 2.14 | 2.25 | 94.9 |
| | 25 | 21.780 | 7.33 | 8.41 | 87.1 |
| 4:2 FTS | Blank | ND | | | |
| | 0.5 | 0.582 | 4.99 | 4.29 | 116.4 |
| | 5 | 5.084 | 1.85 | 1.82 | 101.6 |
| | 25 | 23.506 | 0.26 | 0.28 | 94.0 |
| 6:2 FTS | Blank | ND | | | |
| | 0.5 | 0.545 | 3.78 | 3.47 | 109.0 |
| | 5 | 5.080 | 2.71 | 2.66 | 101.6 |
| | 25 | 24.619 | 6.15 | 6.24 | 98.5 |
| 8:2 FTS | Blank | ND | | | |
| | 0.5 | 0.543 | 0.85 | 0.79 | 108.6 |
| | 5 | 5.626 | 3.15 | 2.80 | 112.5 |
| | 25 | 23.754 | 3.35 | 3.52 | 95.0 |
| 10:2 FTS | Blank | ND | | | |
| | 0.5 | 0.502 | 10.12 | 10.08 | 100.4 |
| | 5 | 5.372 | 0.85 | 0.79 | 107.4 |
| | 25 | 22.176 | 3.46 | 3.90 | 88.7 |

ND = average results less than zero

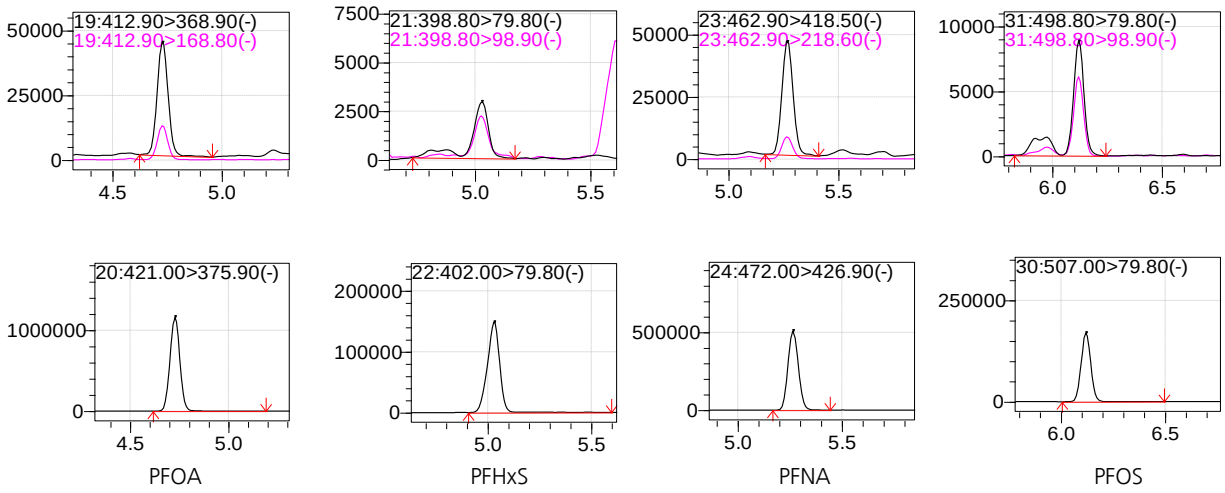



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a pet food matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Produce by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in produce meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, Low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in carrots using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.055 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.055 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.0055 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.0055 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.0055 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.0055 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.0055 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.055 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.0055 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.0055 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.0055 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.055 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.0055 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.0055 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.0055 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.0055 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.0055 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.0055 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.0055 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.0055 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.055 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.0055 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.0055 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.0055 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.0055 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.0055 |

## ■ Sample Preparation and Analysis Conditions

Samples were prepared by dicing the tuber portion of carrots and grinding with dry ice. The ground material was placed in a freezer overnight. Test portions were spiked in triplicate at four different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram carrot test portions spiked with concentrations of 0.001, 0.01, 0.1, 1.0 and 10.0 ng/g. Quantitation was carried out on additional carrot samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in a carrot matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, the extract was concentrated to dryness and dissolved in 0.4 mL of a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

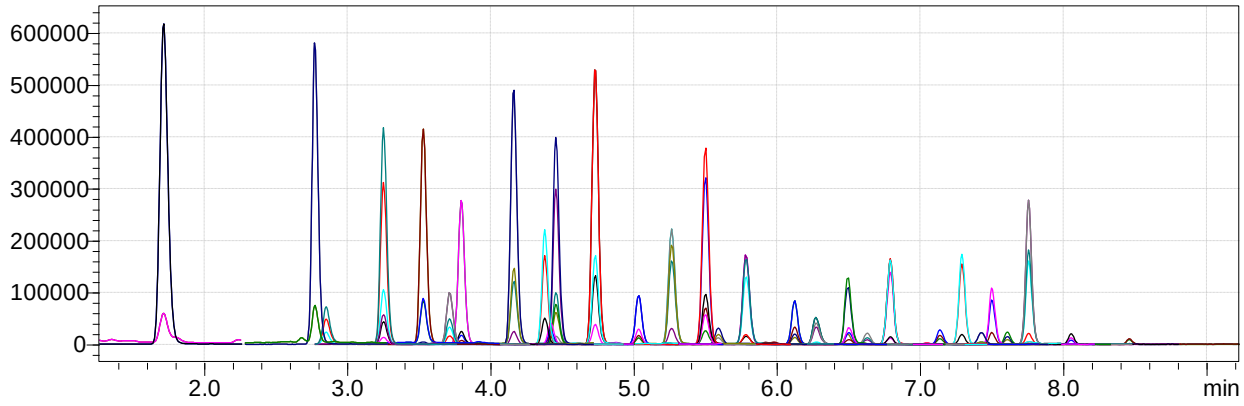



**Figure 2**: Chromatogram of 0.055 ng/g PFAS in a carrot matrix with separation of all peaks in nine minutes

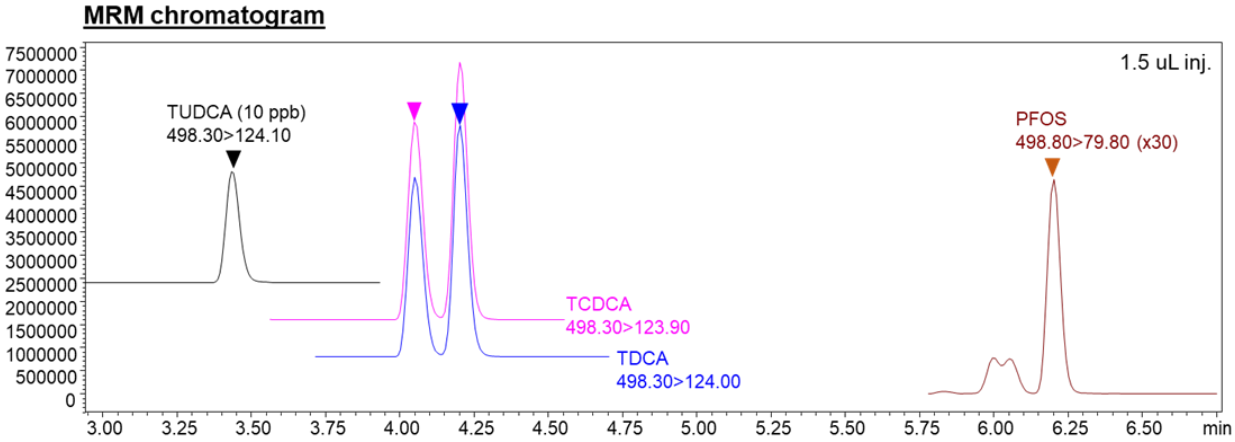



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

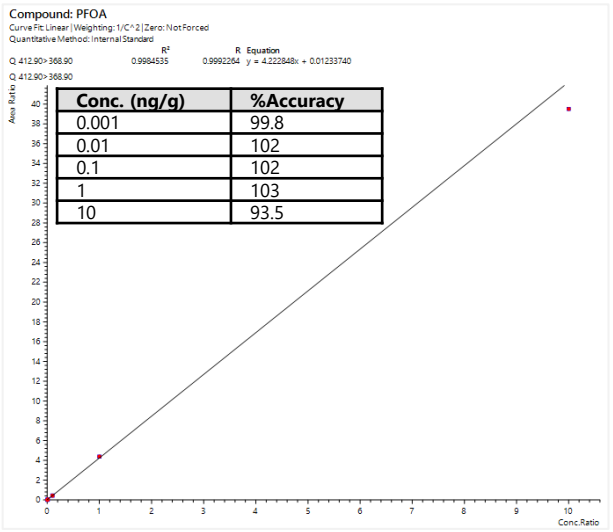
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

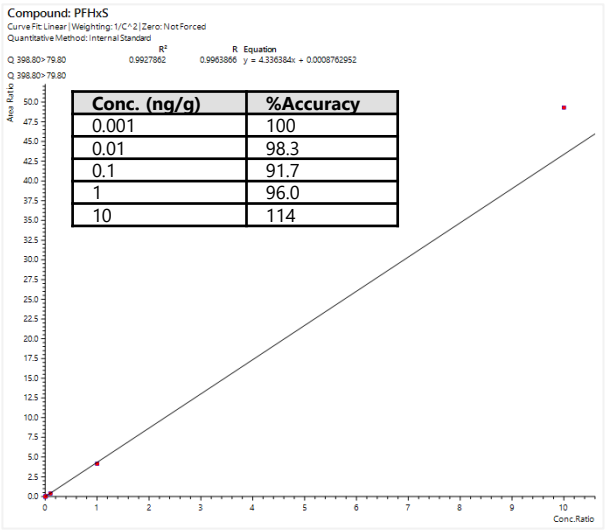
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



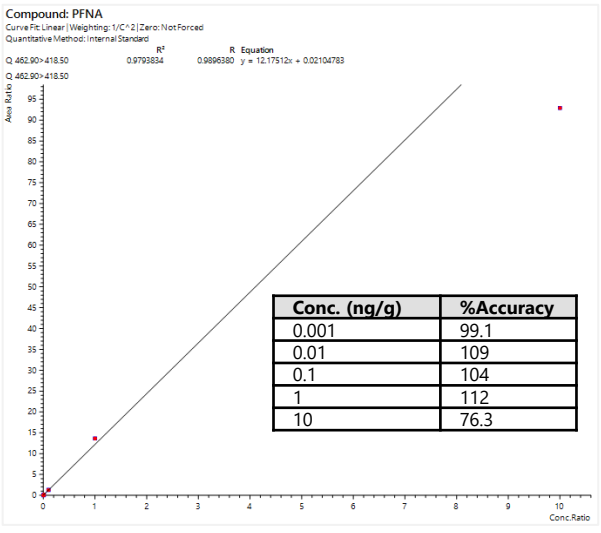

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.8 |
| 0.01 | 102 |
| 0.1 | 102 |
| 1 | 103 |
| 10 | 93.5 |

**Figure 4**: PFHxS Calibration Curve



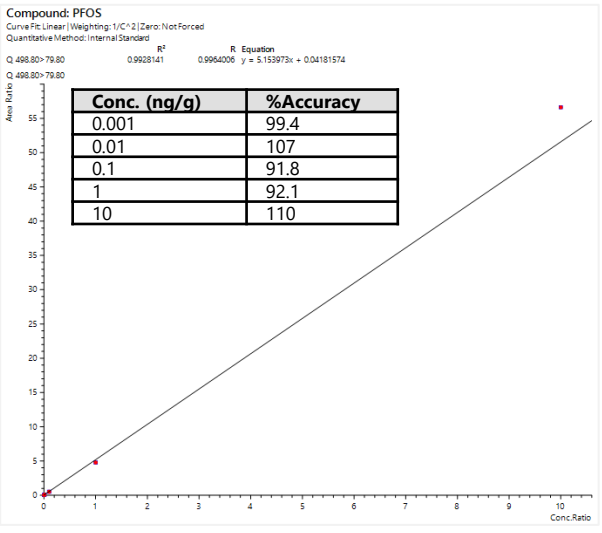

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 100 |
| 0.01 | 98.3 |
| 0.1 | 91.7 |
| 1 | 96.0 |
| 10 | 114 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.1 |
| 0.01 | 109 |
| 0.1 | 104 |
| 1 | 112 |
| 10 | 76.3 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.4 |
| 0.01 | 107 |
| 0.1 | 91.8 |
| 1 | 92.1 |
| 10 | 110 |

Blank matrixes and at least three different concentrations ranging from below the SMPR required LOQ to approximately 500 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | 0.000 | | | |
| | 0.055 | 0.047 | 1.74 | 2.03 | 85.5 |
| | 0.55 | 0.504 | 0.40 | 0.44 | 91.7 |
| | 5.5 | 5.485 | 0.32 | 0.32 | 99.7 |
| PFPeA | Blank | ND | | | |
| | 0.0055 | 0.005 | 9.98 | 11.62 | 85.9 |
| | 0.055 | 0.051 | 0.40 | 0.44 | 92.4 |
| | 0.55 | 0.519 | 1.91 | 2.02 | 94.3 |
| | 5.5 | 5.895 | 0.52 | 0.48 | 107.2 |
| PFHxA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 9.51 | 11.41 | 83.4 |
| | 0.055 | 0.057 | 3.03 | 2.94 | 103.0 |
| | 0.55 | 0.533 | 1.36 | 1.40 | 96.9 |
| | 5.5 | 6.089 | 0.92 | 0.83 | 110.7 |
| PFHpA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 2.80 | 3.20 | 87.5 |
| | 0.055 | 0.054 | 1.46 | 1.50 | 97.1 |
| | 0.55 | 0.558 | 0.87 | 0.86 | 101.4 |
| | 5.5 | 6.131 | 1.21 | 1.09 | 111.5 |
| PFOA | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 3.55 | 3.30 | 107.3 |
| | 0.055 | 0.055 | 1.16 | 1.16 | 99.8 |
| | 0.55 | 0.560 | 0.65 | 0.64 | 101.7 |
| | 5.5 | 5.954 | 0.45 | 0.42 | 108.2 |
| PFNA | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 1.45 | 1.38 | 104.8 |
| | 0.055 | 0.057 | 0.67 | 0.64 | 103.6 |
| | 0.55 | 0.592 | 2.73 | 2.54 | 107.7 |
| | 5.5 | 5.168 | 0.60 | 0.64 | 94.0 |
| PFDA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 14.07 | 15.03 | 93.6 |
| | 0.055 | 0.058 | 1.85 | 1.77 | 104.7 |
| | 0.55 | 0.606 | 0.85 | 0.78 | 110.1 |
| | 5.5 | 6.447 | 1.36 | 1.16 | 117.2 |
| PFUnA | Blank | 0.000 | | | |
| | 0.0055 | 0.004 | 4.53 | 5.65 | 80.3 |
| | 0.055 | 0.052 | 1.79 | 1.89 | 94.8 |
| | 0.55 | 0.548 | 2.22 | 2.23 | 99.6 |
| | 5.5 | 6.023 | 0.49 | 0.45 | 109.5 |
| PFDoA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 6.79 | 7.44 | 91.2 |
| | 0.055 | 0.044 | 4.99 | 6.22 | 80.2 |
| | 0.55 | 0.483 | 16.54 | 18.85 | 87.8 |
| | 5.5 | 4.796 | 1.74 | 2.00 | 87.2 |
| PFTrDA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 7.05 | 8.34 | 84.6 |
| | 0.055 | 0.041 | 1.95 | 2.58 | 75.4 |
| | 0.55 | 0.477 | 11.69 | 13.46 | 86.9 |
| | 5.5 | 4.357 | 2.07 | 2.61 | 79.2 |
| PFTeDA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 5.88 | 6.00 | 97.9 |
| | 0.055 | 0.043 | 1.99 | 2.51 | 79.3 |
| | 0.55 | 0.484 | 10.52 | 11.96 | 88.0 |
| | 5.5 | 4.333 | 1.80 | 2.29 | 78.8 |
| PFBS | Blank | 0.003 | | | |
| | 0.055 | 0.050 | 2.00 | 2.21 | 90.6 |
| | 0.55 | 0.520 | 0.56 | 0.59 | 94.5 |
| | 5.5 | 5.669 | 0.06 | 0.06 | 103.1 |
| PFPeS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 1.13 | 1.16 | 97.5 |
| | 0.055 | 0.054 | 2.88 | 2.95 | 97.7 |
| | 0.55 | 0.504 | 1.66 | 1.82 | 91.6 |
| | 5.5 | 5.637 | 1.20 | 1.17 | 102.5 |
| PFHxS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 6.82 | 7.21 | 94.5 |
| | 0.055 | 0.050 | 1.82 | 1.98 | 91.8 |
| | 0.55 | 0.514 | 1.02 | 1.09 | 93.5 |
| | 5.5 | 5.182 | 10.95 | 11.62 | 94.2 |

| | | | | | |
|---|---|---|---|---|---|
| PFHpS | Blank | 0.002 | | | |
| | 0.0055 | 0.007 | 10.64 | 8.67 | 122.8 |
| | 0.055 | 0.060 | 3.50 | 3.22 | 108.8 |
| | 0.55 | 0.545 | 6.17 | 6.22 | 99.1 |
| | 5.5 | 6.013 | 0.38 | 0.35 | 109.3 |
| PFOS | Blank | ND | | | |
| | 0.0055 | 0.006 | 3.72 | 3.77 | 98.9 |
| | 0.055 | 0.050 | 2.49 | 2.75 | 90.4 |
| | 0.55 | 0.511 | 1.08 | 1.16 | 93.0 |
| | 5.5 | 5.627 | 0.15 | 0.15 | 102.3 |
| PFNS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 18.78 | 20.65 | 90.9 |
| | 0.055 | 0.048 | 1.61 | 1.86 | 86.5 |
| | 0.55 | 0.488 | 12.40 | 13.98 | 88.7 |
| | 5.5 | 4.823 | 2.00 | 2.28 | 87.7 |
| PFDS | Blank | 0.000 | | | |
| | 0.0055 | 0.004 | 5.30 | 6.84 | 77.5 |
| | 0.055 | 0.046 | 3.56 | 4.24 | 84.1 |
| | 0.55 | 0.501 | 10.91 | 11.96 | 91.2 |
| | 5.5 | 5.495 | 3.06 | 3.06 | 99.9 |
| PFUnDS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 0.86 | 0.91 | 95.0 |
| | 0.055 | 0.055 | 2.30 | 2.31 | 99.7 |
| | 0.55 | 0.594 | 1.35 | 1.25 | 107.9 |
| | 5.5 | 6.127 | 0.92 | 0.82 | 111.4 |
| PFDoS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 18.94 | 16.15 | 117.3 |
| | 0.055 | 0.064 | 1.96 | 1.68 | 117.0 |
| | 0.55 | 0.638 | 13.06 | 11.26 | 116.0 |
| | 5.5 | 6.836 | 1.31 | 1.05 | 124.3 |
| PFTrDS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 1.42 | 1.45 | 97.9 |
| | 0.055 | 0.051 | 0.89 | 0.95 | 93.2 |
| | 0.55 | 0.529 | 7.67 | 7.97 | 96.2 |
| | 5.5 | 5.722 | 4.76 | 4.58 | 104.0 |
| PFOSA | Blank | ND | | | |
| | 0.0055 | 0.005 | 4.88 | 5.54 | 88.1 |
| | 0.055 | 0.053 | 1.66 | 1.71 | 96.6 |
| | 0.55 | 0.528 | 1.40 | 1.46 | 96.0 |
| | 5.5 | 5.828 | 2.20 | 2.07 | 106.0 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 3.20 | 2.93 | 109.1 |
| | 0.055 | 0.059 | 1.42 | 1.33 | 106.2 |
| | 0.55 | 0.604 | 2.48 | 2.26 | 109.8 |
| | 5.5 | 6.603 | 0.15 | 0.13 | 120.0 |
| 11Cl-PF3OUdS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 0.95 | 0.97 | 97.4 |
| | 0.055 | 0.050 | 1.57 | 1.73 | 91.0 |
| | 0.55 | 0.530 | 11.47 | 11.91 | 96.4 |
| | 5.5 | 6.030 | 2.40 | 2.19 | 109.6 |
| HFPO-DA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 4.01 | 4.32 | 92.9 |
| | 0.055 | 0.052 | 1.37 | 1.43 | 95.4 |
| | 0.55 | 0.538 | 0.79 | 0.81 | 97.8 |
| | 5.5 | 5.755 | 0.75 | 0.72 | 104.6 |
| DONA | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 5.41 | 5.26 | 102.8 |
| | 0.055 | 0.056 | 0.30 | 0.30 | 101.5 |
| | 0.55 | 0.590 | 0.87 | 0.81 | 107.3 |
| | 5.5 | 5.373 | 0.51 | 0.53 | 97.7 |
| 4:2 FTS | Blank | ND | | | |
| | 0.0055 | 0.006 | 11.31 | 11.28 | 100.2 |
| | 0.055 | 0.049 | 0.96 | 1.08 | 88.8 |
| | 0.55 | 0.544 | 0.21 | 0.21 | 98.9 |
| | 5.5 | 5.793 | 0.25 | 0.24 | 105.3 |

| | | | | | |
|---|---|---|---|---|---|
| 6:2 FTS | Blank | 0.002 | | | |
| | 0.055 | 0.051 | 0.85 | 0.92 | 92.4 |
| | 0.55 | 0.493 | 0.67 | 0.74 | 89.7 |
| | 5.5 | 5.107 | 1.99 | 2.14 | 92.8 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 3.26 | 2.78 | 117.2 |
| | 0.055 | 0.057 | 3.83 | 3.68 | 104.0 |
| | 0.55 | 0.649 | 7.42 | 6.29 | 118.1 |
| | 5.5 | 6.487 | 2.75 | 2.33 | 117.9 |
| 10:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 3.15 | 3.39 | 93.0 |
| | 0.055 | 0.050 | 0.86 | 0.95 | 90.4 |
| | 0.55 | 0.512 | 11.03 | 11.85 | 93.1 |
| | 5.5 | 4.647 | 2.25 | 2.66 | 84.5 |

ND = average results less than zero

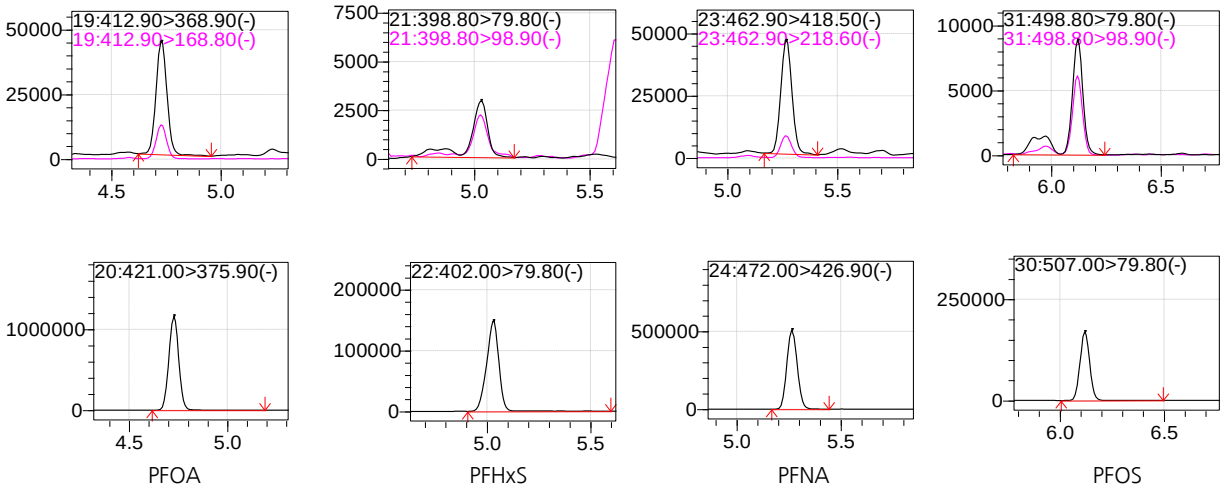



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a carrot matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Offal by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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[1] Shimadzu Scientific Instruments, Inc.

## User Benefits

- Validated method for 30 PFAS in Offal meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in beef kidney using Acetonitrile and Solid Phase Extraction (SPE) followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at five concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.2 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.2 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.2 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.2 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.2 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.2 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.2 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.2 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.2 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.2 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.2 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.2 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.2 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.2 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.2 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.2 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.2 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.2 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.2 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.2 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.2 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.2 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.2 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.2 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.2 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.2 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.2 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.2 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.2 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.2 |

## ■ Sample Preparation and Analysis Conditions

Samples were prepared by cutting locally purchased beef kidney into slices, freezing and grinding with dry ice. The ground material was placed in a freezer overnight. Test portions were spiked in triplicate at five different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10-gram kidney test portions spiked with concentrations of 0.1, 0.5, 1.5, 5.0 and 15 ng/g. Quantitation was carried out on additional kidney samples spiked in triplicate at 0.2, 0.4, 1, 4, and 10 ng/g. Since standards were extracted in a kidney matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram test portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with a basic methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

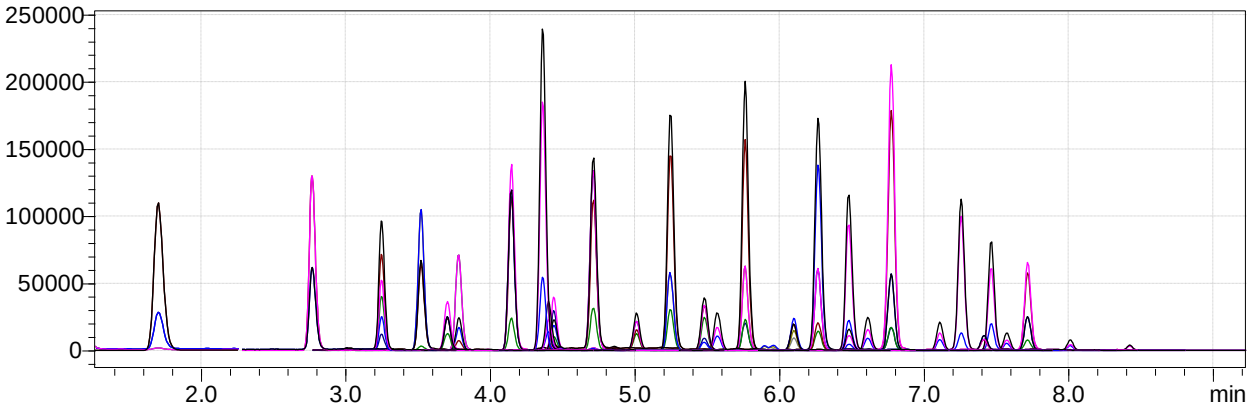



**Figure 2**: Chromatogram of 0.4 ng/g PFAS in a beef kidney matrix with separation of all peaks in nine minutes

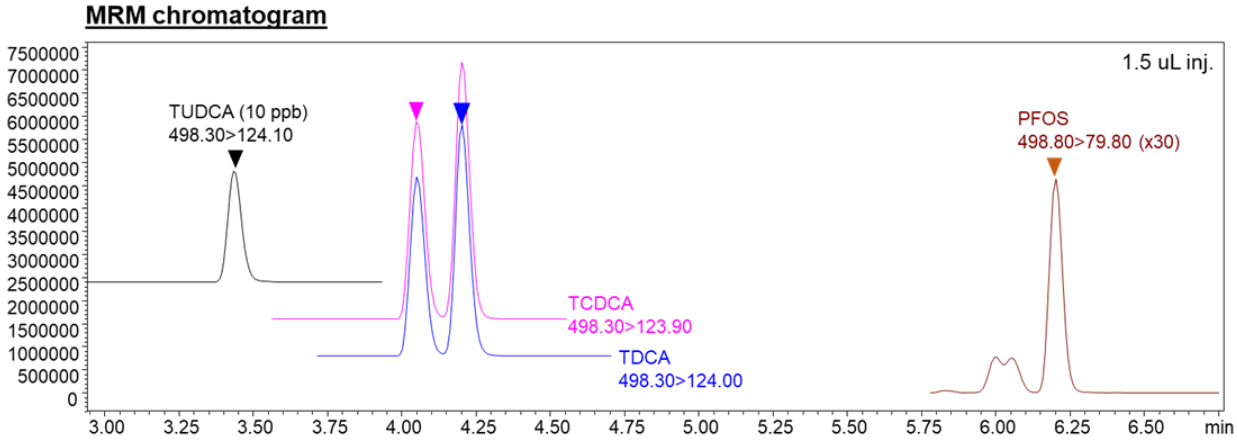



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

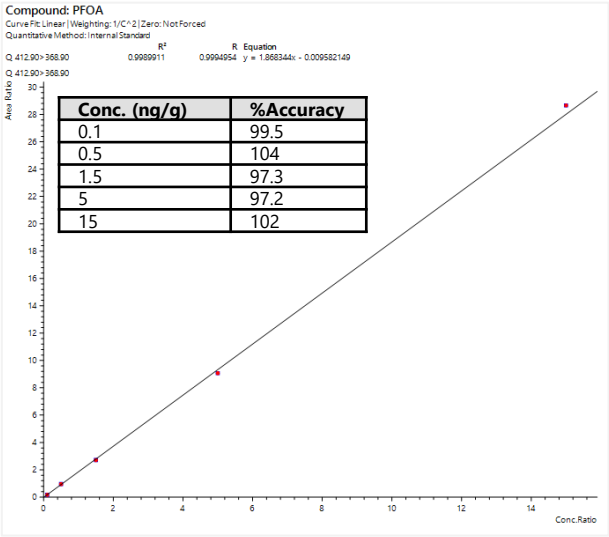
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

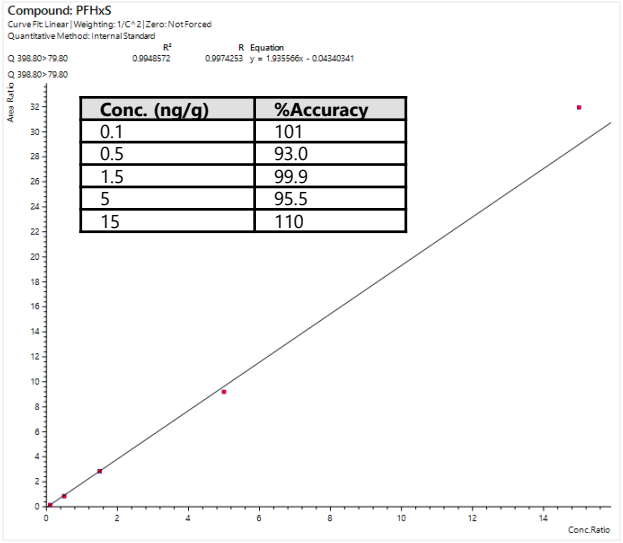
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



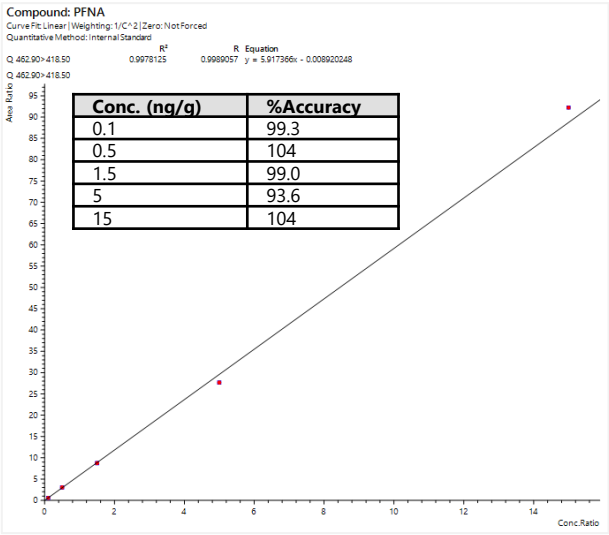

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 99.5 |
| 0.5 | 104 |
| 1.5 | 97.3 |
| 5 | 97.2 |
| 15 | 102 |

**Figure 4**: PFHxS Calibration Curve



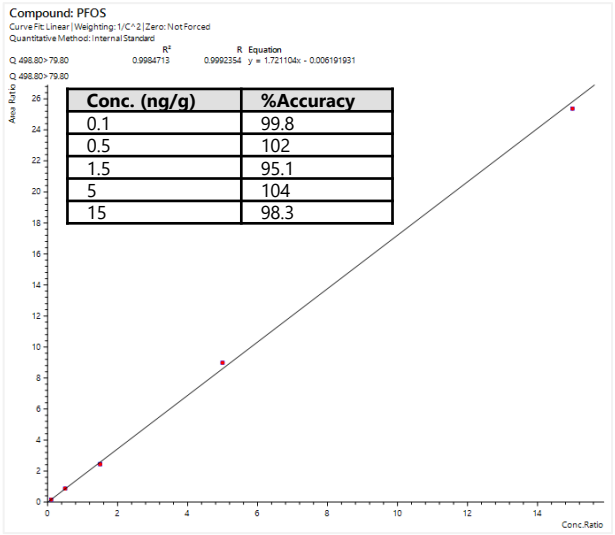

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 101 |
| 0.5 | 93.0 |
| 1.5 | 99.9 |
| 5 | 95.5 |
| 15 | 110 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 99.3 |
| 0.5 | 104 |
| 1.5 | 99.0 |
| 5 | 93.6 |
| 15 | 104 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 99.8 |
| 0.5 | 102 |
| 1.5 | 95.1 |
| 5 | 104 |
| 15 | 98.3 |

Blank matrixes and five different concentrations ranging from below the SMPR required LOQ to 25 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | 0.002 | | | |
| | 0.2 | 0.212 | 3.23 | 3.04 | 106.3 |
| | 0.4 | 0.442 | 1.78 | 1.61 | 110.5 |
| | 1.0 | 1.131 | 5.12 | 4.52 | 113.1 |
| | 4.0 | 4.102 | 2.55 | 2.49 | 102.5 |
| | 10 | 10.121 | 3.84 | 3.80 | 101.2 |
| PFPeA | Blank | 0.010 | | | |
| | 0.2 | 0.213 | 3.58 | 3.35 | 106.7 |
| | 0.4 | 0.434 | 1.53 | 1.41 | 108.5 |
| | 1.0 | 1.068 | 4.36 | 4.08 | 106.8 |
| | 4.0 | 3.908 | 2.66 | 2.72 | 97.7 |
| | 10 | 9.583 | 2.29 | 2.38 | 95.9 |
| PFHxA | Blank | 0.003 | | | |
| | 0.2 | 0.216 | 1.08 | 1.00 | 107.9 |
| | 0.4 | 0.454 | 4.87 | 4.29 | 113.5 |
| | 1.0 | 1.195 | 6.03 | 5.04 | 119.5 |
| | 4.0 | 4.441 | 3.84 | 3.46 | 111.0 |
| | 10 | 10.797 | 4.18 | 3.87 | 108.0 |
| PFHpA | Blank | 0.007 | | | |
| | 0.2 | 0.208 | 4.47 | 4.30 | 103.8 |
| | 0.4 | 0.412 | 0.46 | 0.45 | 102.9 |
| | 1.0 | 1.014 | 5.75 | 5.67 | 101.4 |
| | 4.0 | 3.690 | 1.90 | 2.06 | 92.3 |
| | 10 | 9.120 | 3.12 | 3.42 | 91.2 |
| PFOA | Blank | 0.012 | | | |
| | 0.2 | 0.221 | 3.89 | 3.52 | 110.7 |
| | 0.4 | 0.453 | 2.65 | 2.34 | 113.3 |
| | 1.0 | 1.174 | 6.21 | 5.29 | 117.4 |
| | 4.0 | 4.331 | 2.69 | 2.48 | 108.3 |
| | 10 | 10.503 | 3.84 | 3.65 | 105.0 |
| PFNA | Blank | 0.007 | | | |
| | 0.2 | 0.205 | 3.75 | 3.67 | 102.1 |
| | 0.4 | 0.435 | 2.77 | 2.55 | 108.9 |
| | 1.0 | 1.139 | 7.18 | 6.30 | 113.9 |
| | 4.0 | 4.204 | 4.53 | 4.31 | 105.1 |
| | 10 | 10.421 | 2.91 | 2.79 | 104.2 |
| PFDA | Blank | 0.020 | | | |
| | 0.2 | 0.211 | 3.42 | 3.25 | 105.3 |
| | 0.4 | 0.433 | 0.55 | 0.51 | 108.1 |
| | 1.0 | 1.053 | 8.41 | 7.98 | 105.3 |
| | 4.0 | 3.936 | 2.52 | 2.56 | 98.4 |
| | 10 | 9.547 | 3.61 | 3.78 | 95.5 |
| PFUnA | Blank | 0.011 | | | |
| | 0.2 | 0.213 | 1.89 | 1.77 | 106.4 |
| | 0.4 | 0.433 | 4.47 | 4.12 | 108.3 |
| | 1.0 | 1.094 | 4.58 | 4.18 | 109.4 |
| | 4.0 | 4.070 | 2.89 | 2.84 | 101.7 |
| | 10 | 9.848 | 4.64 | 4.71 | 98.5 |
| PFDoA | Blank | 0.016 | | | |
| | 0.2 | 0.226 | 3.26 | 2.88 | 113.2 |
| | 0.4 | 0.462 | 3.03 | 2.62 | 115.5 |
| | 1.0 | 1.124 | 2.03 | 1.80 | 112.4 |
| | 4.0 | 4.365 | 2.26 | 2.07 | 109.1 |
| | 10 | 10.375 | 3.51 | 3.38 | 103.7 |
| PFTrDA | Blank | 0.001 | | | |
| | 0.2 | 0.214 | 2.86 | 2.66 | 107.3 |
| | 0.4 | 0.433 | 3.99 | 3.69 | 108.2 |
| | 1.0 | 0.973 | 7.74 | 7.95 | 97.3 |
| | 4.0 | 3.927 | 1.39 | 1.41 | 98.2 |
| | 10 | 9.297 | 2.55 | 2.74 | 92.9 |
| PFTeDA | Blank | 0.008 | | | |
| | 0.2 | 0.205 | 2.66 | 2.60 | 102.5 |
| | 0.4 | 0.427 | 0.99 | 0.92 | 106.8 |
| | 1.0 | 0.990 | 4.18 | 4.22 | 99.0 |
| | 4.0 | 3.880 | 0.98 | 1.02 | 97.0 |
| | 10 | 9.041 | 0.56 | 0.62 | 90.4 |
| PFBS | Blank | 0.011 | | | |
| | 0.2 | 0.194 | 11.88 | 12.27 | 96.8 |
| | 0.4 | 0.415 | 9.99 | 9.64 | 103.6 |
| | 1.0 | 1.035 | 4.94 | 4.78 | 103.5 |
| | 4.0 | 3.824 | 0.17 | 0.18 | 95.6 |
| | 10 | 9.594 | 7.39 | 7.71 | 95.9 |

| | | | | | |
|---|---|---|---|---|---|
| PFPeS | Blank | 0.025 | | | |
| | 0.2 | 0.225 | 6.70 | 5.95 | 112.8 |
| | 0.4 | 0.455 | 2.64 | 2.31 | 113.9 |
| | 1.0 | 1.042 | 3.84 | 3.69 | 104.2 |
| | 4.0 | 4.009 | 0.78 | 0.78 | 100.2 |
| | 10 | 9.704 | 3.97 | 4.09 | 97.0 |
| PFHxS | Blank | 0.026 | | | |
| | 0.2 | 0.203 | 6.37 | 6.29 | 101.3 |
| | 0.4 | 0.383 | 1.65 | 1.72 | 95.7 |
| | 1.0 | 0.962 | 5.37 | 5.58 | 96.2 |
| | 4.0 | 3.953 | 4.07 | 4.12 | 98.8 |
| | 10 | 9.357 | 3.12 | 3.34 | 93.6 |
| PFHpS | Blank | 0.025 | | | |
| | 0.2 | 0.216 | 6.23 | 5.77 | 107.9 |
| | 0.4 | 0.456 | 5.34 | 4.68 | 114.0 |
| | 1.0 | 1.110 | 7.64 | 6.88 | 111.0 |
| | 4.0 | 4.110 | 2.21 | 2.15 | 102.8 |
| | 10 | 9.886 | 4.35 | 4.40 | 98.9 |
| PFOS | Blank | 0.027 | | | |
| | 0.2 | 0.212 | 1.90 | 1.80 | 105.9 |
| | 0.4 | 0.429 | 1.97 | 1.84 | 107.1 |
| | 1.0 | 1.092 | 4.66 | 4.27 | 109.2 |
| | 4.0 | 4.071 | 5.24 | 5.15 | 101.8 |
| | 10 | 10.156 | 7.04 | 6.94 | 101.6 |
| PFNS | Blank | 0.015 | | | |
| | 0.2 | 0.223 | 2.50 | 2.24 | 111.4 |
| | 0.4 | 0.437 | 4.84 | 4.43 | 109.4 |
| | 1.0 | 1.142 | 7.23 | 6.33 | 114.2 |
| | 4.0 | 4.119 | 1.61 | 1.56 | 103.0 |
| | 10 | 9.991 | 5.09 | 5.09 | 99.9 |
| PFDS | Blank | ND | | | |
| | 0.2 | 0.214 | 6.66 | 6.23 | 106.8 |
| | 0.4 | 0.444 | 7.66 | 6.91 | 110.9 |
| | 1.0 | 1.122 | 11.22 | 10.00 | 112.2 |
| | 4.0 | 4.189 | 0.80 | 0.77 | 104.7 |
| | 10 | 9.983 | 4.23 | 4.24 | 99.8 |
| PFUnDS | Blank | 0.030 | | | |
| | 0.2 | 0.239 | 9.94 | 8.31 | 119.6 |
| | 0.4 | 0.438 | 3.92 | 3.59 | 109.5 |
| | 1.0 | 1.005 | 5.98 | 5.95 | 100.5 |
| | 4.0 | 4.038 | 3.74 | 3.71 | 101.0 |
| | 10 | 9.503 | 5.35 | 5.63 | 95.1 |
| PFDoS | Blank | 0.016 | | | |
| | 0.2 | 0.232 | 4.32 | 3.73 | 115.9 |
| | 0.4 | 0.459 | 2.71 | 2.36 | 114.7 |
| | 1.0 | 1.081 | 0.83 | 0.77 | 108.1 |
| | 4.0 | 4.281 | 3.52 | 3.29 | 107.0 |
| | 10 | 9.839 | 0.96 | 0.98 | 98.4 |
| PFTrDS | Blank | 0.009 | | | |
| | 0.2 | 0.222 | 5.77 | 5.20 | 111.0 |
| | 0.4 | 0.475 | 12.42 | 10.46 | 118.7 |
| | 1.0 | 1.088 | 2.72 | 2.50 | 108.8 |
| | 4.0 | 4.264 | 4.76 | 4.47 | 106.6 |
| | 10 | 9.680 | 2.69 | 2.78 | 96.8 |
| PFOSA | Blank | ND | | | |
| | 0.2 | 0.232 | 24.78 | 21.34 | 116.1 |
| | 0.4 | 0.419 | 13.60 | 12.98 | 104.7 |
| | 1.0 | 1.110 | 6.80 | 6.12 | 111.0 |
| | 4.0 | 4.157 | 5.28 | 5.09 | 103.9 |
| | 10 | 10.422 | 6.27 | 6.02 | 104.2 |
| 9Cl-PF3ONS | Blank | 0.016 | | | |
| | 0.2 | 0.202 | 2.37 | 2.34 | 101.1 |
| | 0.4 | 0.428 | 5.16 | 4.83 | 106.9 |
| | 1.0 | 1.143 | 4.80 | 4.20 | 114.3 |
| | 4.0 | 4.194 | 2.87 | 2.74 | 104.9 |
| | 10 | 10.461 | 6.84 | 6.54 | 104.6 |
| 11Cl-PF3OUdS | Blank | 0.008 | | | |
| | 0.2 | 0.210 | 2.53 | 2.41 | 104.9 |
| | 0.4 | 0.458 | 8.34 | 7.29 | 114.5 |
| | 1.0 | 1.123 | 7.60 | 6.77 | 112.3 |
| | 4.0 | 4.352 | 2.84 | 2.61 | 108.8 |
| | 10 | 10.038 | 3.95 | 3.94 | 100.4 |

| | | | | | |
|---|---|---|---|---|---|
| HFPO-DA | Blank | 0.016 | | | |
| | 0.2 | 0.207 | 3.86 | 3.72 | 103.6 |
| | 0.4 | 0.418 | 4.55 | 4.36 | 104.5 |
| | 1.0 | 1.084 | 6.85 | 6.32 | 108.4 |
| | 4.0 | 3.938 | 4.03 | 4.09 | 98.4 |
| | 10 | 9.601 | 3.20 | 3.34 | 96.0 |
| DONA | Blank | 0.011 | | | |
| | 0.2 | 0.211 | 1.14 | 1.08 | 105.2 |
| | 0.4 | 0.428 | 0.55 | 0.51 | 107.0 |
| | 1.0 | 1.042 | 6.69 | 6.43 | 104.2 |
| | 4.0 | 3.827 | 1.91 | 2.00 | 95.7 |
| | 10 | 9.590 | 2.75 | 2.86 | 95.9 |
| 4:2 FTS | Blank | 0.016 | | | |
| | 0.2 | 0.213 | 5.91 | 5.54 | 106.7 |
| | 0.4 | 0.422 | 6.26 | 5.92 | 105.6 |
| | 1.0 | 1.056 | 6.91 | 6.54 | 105.6 |
| | 4.0 | 3.881 | 1.99 | 2.05 | 97.0 |
| | 10 | 9.579 | 3.63 | 3.79 | 95.8 |
| 6:2 FTS | Blank | 0.028 | | | |
| | 0.2 | 0.218 | 15.62 | 14.33 | 109.1 |
| | 0.4 | 0.431 | 2.45 | 2.27 | 107.7 |
| | 1.0 | 1.149 | 5.08 | 4.42 | 114.9 |
| | 4.0 | 4.304 | 3.14 | 2.92 | 107.6 |
| | 10 | 10.209 | 6.68 | 6.55 | 102.1 |
| 8:2 FTS | Blank | 0.022 | | | |
| | 0.2 | 0.164 | 2.83 | 3.46 | 81.8 |
| | 0.4 | 0.340 | 4.56 | 5.36 | 85.0 |
| | 1.0 | 0.952 | 1.18 | 1.24 | 95.2 |
| | 4.0 | 3.974 | 9.63 | 9.70 | 99.3 |
| | 10 | 9.327 | 3.07 | 3.29 | 93.3 |
| 10:2 FTS | Blank | 0.016 | | | |
| | 0.2 | 0.217 | 9.19 | 8.47 | 108.5 |
| | 0.4 | 0.489 | 14.16 | 11.58 | 122.4 |
| | 1.0 | 1.251 | 8.26 | 6.61 | 125.1 |
| | 4.0 | 4.956 | 4.67 | 3.77 | 123.9 |
| | 10 | 11.777 | 6.18 | 5.25 | 117.8 |

ND = average results less than zero

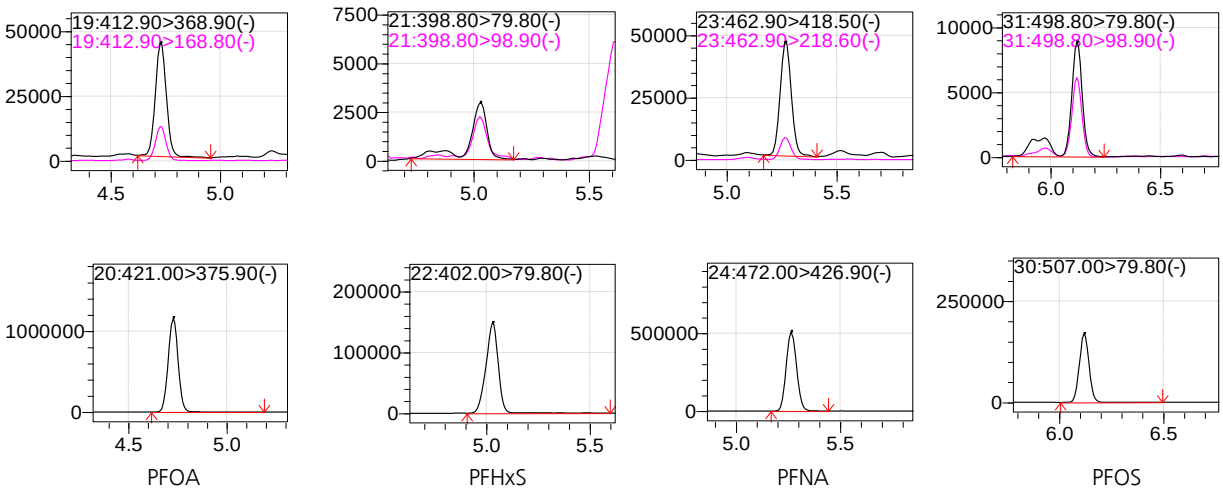



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in an offal matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Milk by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in Milk meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in milk using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at three concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.01 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.01 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.01 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.01 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.01 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.01 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.01 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.01 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.01 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.01 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.01 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.01 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.01 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.01 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.01 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.01 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.01 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.01 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.01 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.01 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.01 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.01 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.01 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.01 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.01 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.01 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.01 |

## ■ Sample Preparation and Analysis Conditions

Whole milk (3.7 % fat) was purchased and sampled directly from the carton. Test portions were spiked in triplicate at three different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram test portions spiked with 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 ng/g of each target analyte. Quantitation was carried out on additional whole milk samples spiked in triplicate at 0.01, 0.10 and 1.0 ng/g. Since standards were extracted in milk matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 150 µL of formic acid and 10 mL of acetonitrile was added. The samples were shaken by hand for 10 seconds and a QuEChERS packet was added. The sample was shaken again for 5 minutes and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube to which additional QuEChERS reagent was added. The sample was shaken for 10 seconds by hand, for 5 minutes on a shaker, and then centrifuged for 5 minutes at 4000 rpm. An aliquot was removed to another tube and concentrated to less than 1 ml under nitrogen, reconstituted to 1 ml in a methanol-water mixture, transferred to a 1.5 mL tube and centrifuged for 10 minutes at 15,000 rpm. The supernatant was transferred to an LC vial for analysis.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

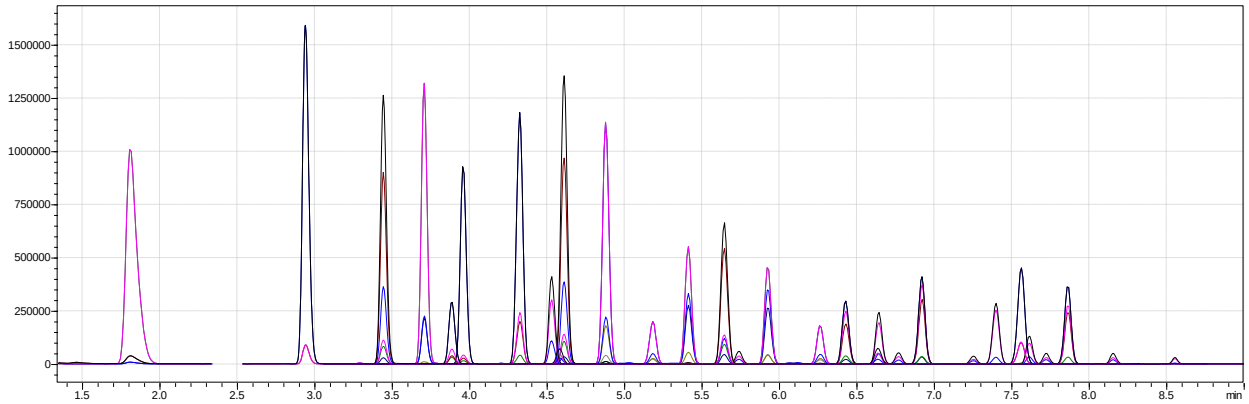



**Figure 2**: Chromatogram of 0.1 ng/g PFAS in a milk matrix with separation of all peaks in nine minutes

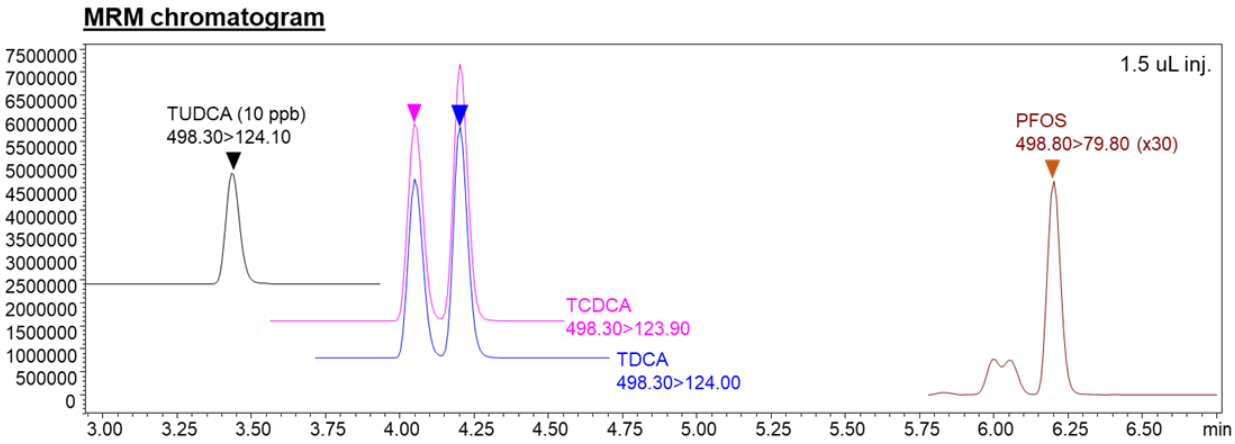



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

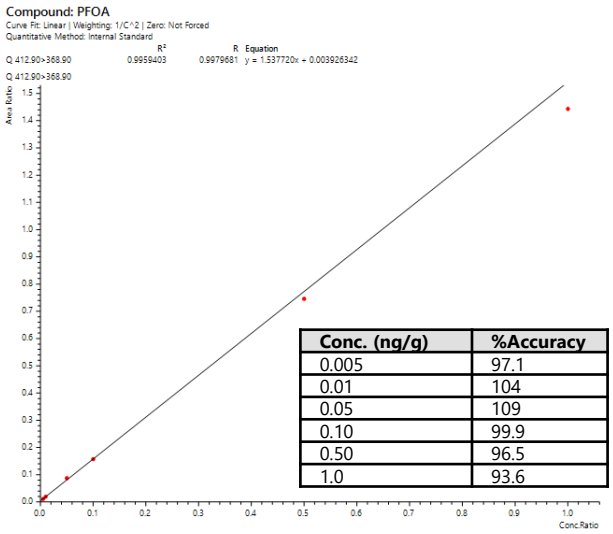
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

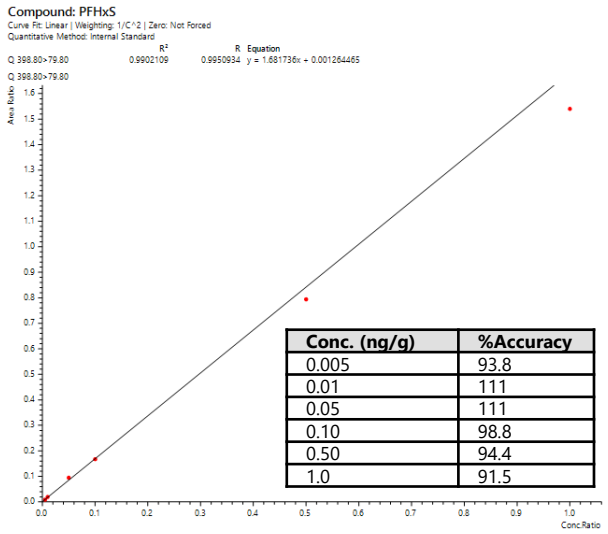
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



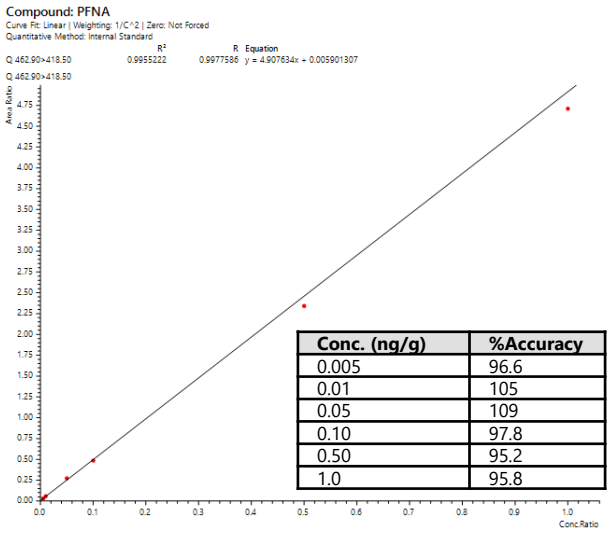

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 97.1 |
| 0.01 | 104 |
| 0.05 | 109 |
| 0.10 | 99.9 |
| 0.50 | 96.5 |
| 1.0 | 93.6 |

**Figure 4**: PFHxS Calibration Curve



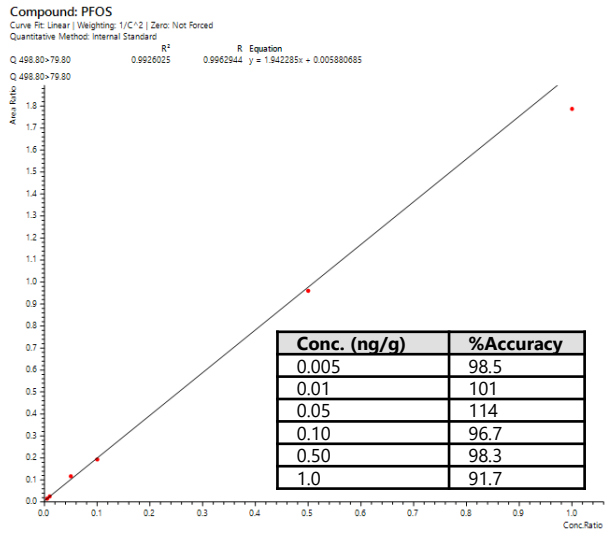

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 93.8 |
| 0.01 | 111 |
| 0.05 | 111 |
| 0.10 | 98.8 |
| 0.50 | 94.4 |
| 1.0 | 91.5 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 96.6 |
| 0.01 | 105 |
| 0.05 | 109 |
| 0.10 | 97.8 |
| 0.50 | 95.2 |
| 1.0 | 95.8 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.005 | 98.5 |
| 0.01 | 101 |
| 0.05 | 114 |
| 0.10 | 96.7 |
| 0.50 | 98.3 |
| 1.0 | 91.7 |

Blank matrixes and three different concentrations ranging from the SMPR required LOQ to 100 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | 0.000 | | | |
| | 0.01 | 0.012 | 8.20 | 7.02 | 116.8 |
| | 0.1 | 0.103 | 2.10 | 2.02 | 103.5 |
| | 1 | 0.973 | 0.57 | 0.58 | 97.3 |
| PFPeA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 2.63 | 2.45 | 107.1 |
| | 0.1 | 0.099 | 0.66 | 0.66 | 99.3 |
| | 1 | 0.947 | 0.38 | 0.40 | 94.7 |
| PFHxA | Blank | ND | | | |
| | 0.01 | 0.010 | 1.74 | 1.67 | 104.4 |
| | 0.1 | 0.096 | 0.93 | 0.97 | 96.1 |
| | 1 | 0.947 | 0.10 | 0.11 | 94.7 |
| PFHpA | Blank | 0.001 | | | |
| | 0.01 | 0.011 | 7.89 | 6.79 | 116.2 |
| | 0.1 | 0.099 | 0.45 | 0.45 | 99.4 |
| | 1 | 0.968 | 1.50 | 1.55 | 96.8 |
| PFOA | Blank | ND | | | |
| | 0.01 | 0.010 | 11.84 | 11.35 | 104.3 |
| | 0.1 | 0.098 | 2.89 | 2.93 | 98.5 |
| | 1 | 0.977 | 1.00 | 1.02 | 97.7 |
| PFNA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 0.96 | 0.86 | 112.2 |
| | 0.1 | 0.100 | 0.92 | 0.93 | 99.5 |
| | 1 | 0.976 | 0.61 | 0.62 | 97.6 |
| PFDA | Blank | 0.001 | | | |
| | 0.01 | 0.011 | 2.91 | 2.59 | 112.7 |
| | 0.1 | 0.104 | 0.42 | 0.40 | 104.2 |
| | 1 | 0.994 | 2.71 | 2.73 | 99.4 |
| PFUnA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 1.20 | 1.08 | 111.5 |
| | 0.1 | 0.101 | 0.84 | 0.83 | 100.9 |
| | 1 | 0.980 | 0.72 | 0.74 | 98.0 |
| PFDoA | Blank | 0.000 | | | |
| | 0.01 | 0.012 | 13.65 | 11.65 | 117.1 |
| | 0.1 | 0.101 | 1.54 | 1.52 | 101.3 |
| | 1 | 0.951 | 1.47 | 1.55 | 95.1 |
| PFTrDA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 2.40 | 2.24 | 106.9 |
| | 0.1 | 0.101 | 1.60 | 1.59 | 100.9 |
| | 1 | 0.994 | 0.68 | 0.68 | 99.4 |
| PFTeDA | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 4.14 | 3.80 | 108.8 |
| | 0.1 | 0.098 | 0.67 | 0.68 | 98.2 |
| | 1 | 0.974 | 3.40 | 3.49 | 97.4 |
| PFBS | Blank | ND | | | |
| | 0.01 | 0.012 | 8.49 | 7.19 | 118.1 |
| | 0.1 | 0.102 | 1.65 | 1.62 | 101.6 |
| | 1 | 0.958 | 0.25 | 0.26 | 95.8 |
| PFPeS | Blank | ND | | | |
| | 0.01 | 0.010 | 11.26 | 11.41 | 98.7 |
| | 0.1 | 0.102 | 1.05 | 1.03 | 101.9 |
| | 1 | 0.953 | 1.62 | 1.70 | 95.3 |
| PFHxS | Blank | 0.000 | | | |
| | 0.01 | 0.010 | 10.50 | 10.76 | 97.6 |
| | 0.1 | 0.099 | 3.88 | 3.89 | 99.6 |
| | 1 | 0.956 | 1.36 | 1.42 | 95.6 |
| PFHpS | Blank | ND | | | |
| | 0.01 | 0.010 | 7.26 | 6.85 | 106.0 |
| | 0.1 | 0.105 | 2.27 | 2.17 | 104.4 |
| | 1 | 0.983 | 2.10 | 2.14 | 98.3 |

| | | | | | |
|---|---|---|---|---|---|
| PFOS | Blank | ND | | | |
| | 0.01 | 0.010 | 6.44 | 6.54 | 98.4 |
| | 0.1 | 0.099 | 2.57 | 2.59 | 99.2 |
| | 1 | 0.961 | 1.32 | 1.37 | 96.1 |
| PFNS | Blank | ND | | | |
| | 0.01 | 0.010 | 10.24 | 10.20 | 100.4 |
| | 0.1 | 0.102 | 2.86 | 2.81 | 101.8 |
| | 1 | 0.992 | 2.58 | 2.60 | 99.2 |
| PFDS | Blank | ND | | | |
| | 0.01 | 0.009 | 3.74 | 3.95 | 94.8 |
| | 0.1 | 0.103 | 6.13 | 5.95 | 103.0 |
| | 1 | 1.015 | 1.22 | 1.20 | 101.5 |
| PFUnDS | Blank | ND | | | |
| | 0.01 | 0.011 | 2.19 | 2.01 | 109.3 |
| | 0.1 | 0.105 | 4.78 | 4.56 | 105.0 |
| | 1 | 0.988 | 3.49 | 3.54 | 98.8 |
| PFDoS | Blank | ND | | | |
| | 0.01 | 0.010 | 8.88 | 9.15 | 97.0 |
| | 0.1 | 0.101 | 2.24 | 2.22 | 100.6 |
| | 1 | 0.963 | 2.27 | 2.36 | 96.3 |
| PFTrDS | Blank | ND | | | |
| | 0.01 | 0.010 | 5.12 | 4.83 | 106.0 |
| | 0.1 | 0.099 | 3.30 | 3.32 | 99.4 |
| | 1 | 0.990 | 2.08 | 2.10 | 99.0 |
| PFOSA | Blank | ND | | | |
| | 0.01 | 0.011 | 3.59 | 3.36 | 106.8 |
| | 0.1 | 0.099 | 1.83 | 1.85 | 99.0 |
| | 1 | 0.950 | 0.55 | 0.58 | 95.0 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.01 | 0.010 | 4.42 | 4.31 | 102.5 |
| | 0.1 | 0.103 | 0.96 | 0.94 | 102.5 |
| | 1 | 0.971 | 3.15 | 3.25 | 97.1 |
| 11Cl-PF3OUdS | Blank | ND | | | |
| | 0.01 | 0.009 | 5.32 | 5.61 | 94.8 |
| | 0.1 | 0.101 | 1.10 | 1.09 | 100.8 |
| | 1 | 0.992 | 1.22 | 1.23 | 99.2 |
| HFPO-DA | Blank | ND | | | |
| | 0.01 | 0.010 | 7.16 | 7.19 | 99.6 |
| | 0.1 | 0.099 | 2.17 | 2.18 | 99.3 |
| | 1 | 0.932 | 0.80 | 0.86 | 93.2 |
| DONA | Blank | 0.000 | | | |
| | 0.01 | 0.010 | 0.80 | 0.77 | 103.9 |
| | 0.1 | 0.099 | 0.42 | 0.42 | 98.9 |
| | 1 | 0.961 | 2.12 | 2.21 | 96.1 |
| 4:2 FTS | Blank | ND | | | |
| | 0.01 | 0.011 | 4.97 | 4.63 | 107.5 |
| | 0.1 | 0.102 | 2.75 | 2.69 | 102.1 |
| | 1 | 0.989 | 1.28 | 1.29 | 98.9 |
| 6:2 FTS | Blank | 0.001 | | | |
| | 0.01 | 0.012 | 11.91 | 10.39 | 114.6 |
| | 0.1 | 0.106 | 5.06 | 4.80 | 105.5 |
| | 1 | 0.984 | 1.40 | 1.43 | 98.4 |
| 8:2 FTS | Blank | 0.001 | | | |
| | 0.01 | 0.012 | 8.01 | 6.86 | 116.7 |
| | 0.1 | 0.097 | 1.53 | 1.57 | 97.1 |
| | 1 | 0.888 | 0.23 | 0.26 | 88.8 |
| 10:2 FTS | Blank | 0.000 | | | |
| | 0.01 | 0.011 | 10.94 | 10.09 | 108.4 |
| | 0.1 | 0.095 | 3.27 | 3.43 | 95.4 |
| | 1 | 0.862 | 1.22 | 1.42 | 86.2 |

ND = average results less than zero

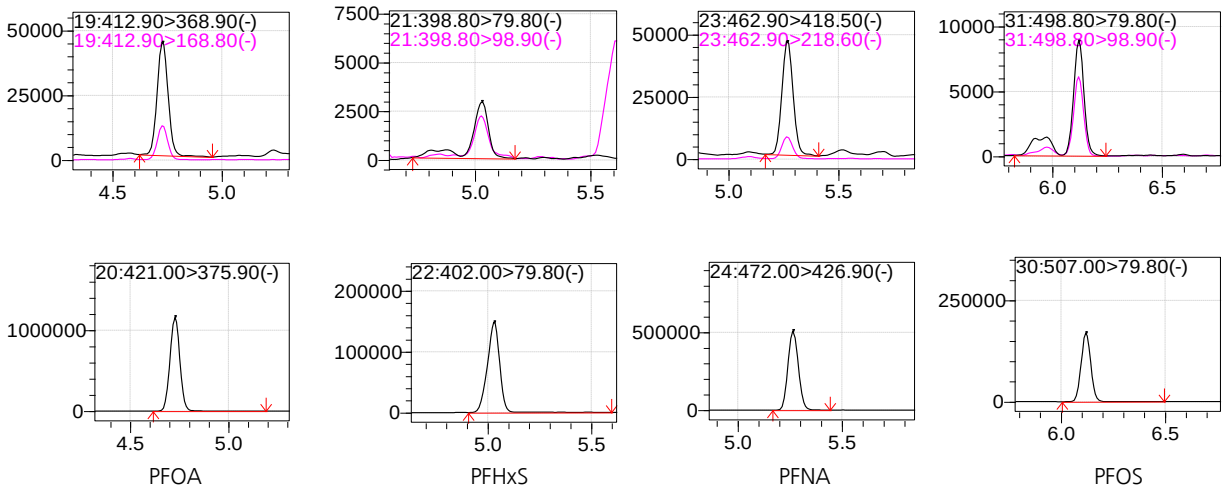



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a high fat milk matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Fish Oil by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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[1] Shimadzu Scientific Instruments, Inc.

## User Benefits

- Validated method for 30 PFAS in fish oil meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in fish oil followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at five concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.25 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.25 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.25 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.25 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.25 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.25 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.25 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.25 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.25 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.25 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.25 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.25 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.25 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.25 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.25 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.25 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.25 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.25 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.25 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.25 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.25 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.25 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.25 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.25 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.25 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.25 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.25 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.25 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.25 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.5 |

## ■ Sample Preparation and Analysis Conditions

Fish oil soft gels were purchased locally. Samples were prepared by cutting the tip of the capsule with scissors and squeezing the oil out into a 50 ml tube. Test portions were spiked in triplicate at five different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram fish oil test portions spiked with concentrations of 0.1, 0.5, 1.5, 5.0 and 15.0 ng/g. Quantitation was carried out on additional fish oil samples spiked in triplicate at 0.25, 0.5, 1.0, 5.0, and 10 ng/g. Since standards were extracted in a fish oil matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

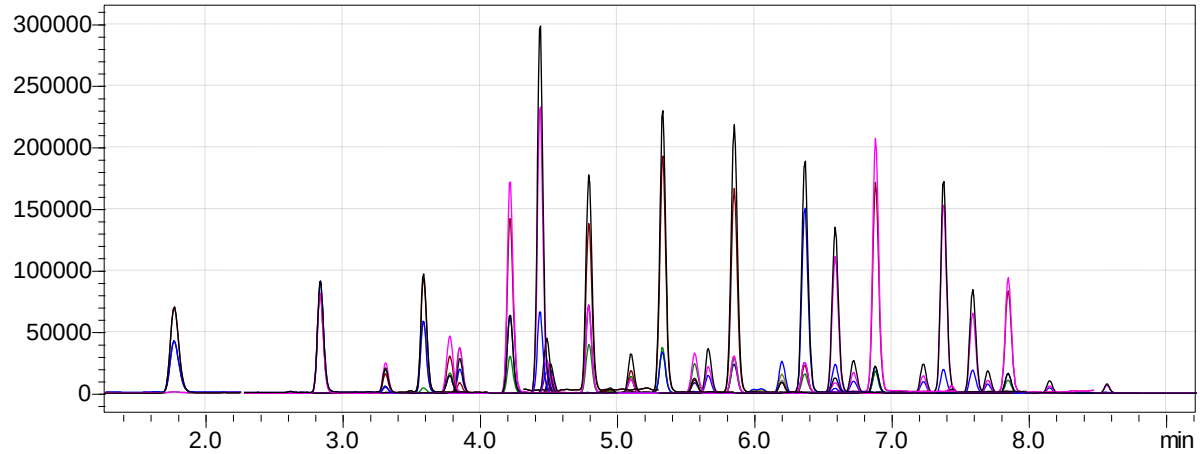



**Figure 2**: Chromatogram of 0.5 ng/g PFAS in a fish oil matrix with separation of all peaks in nine minutes

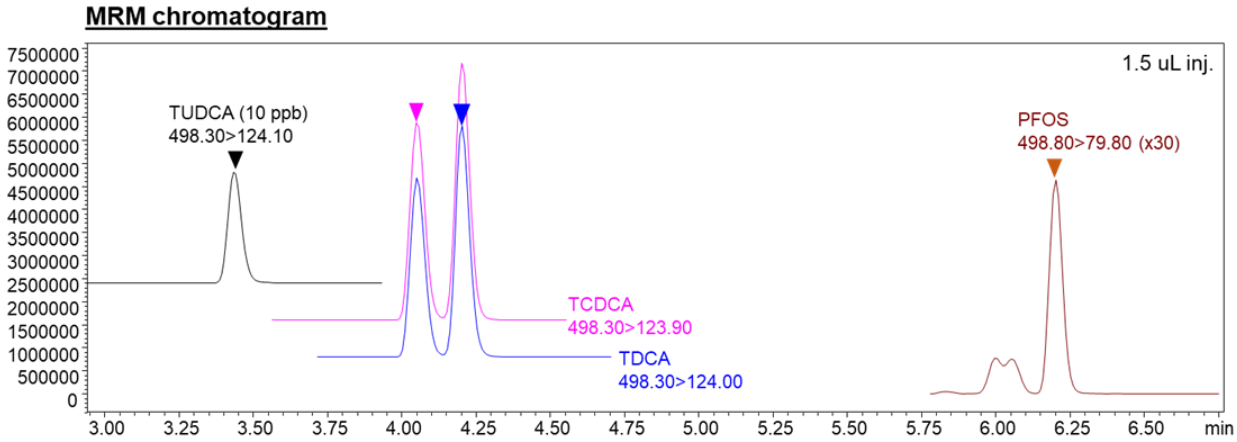



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

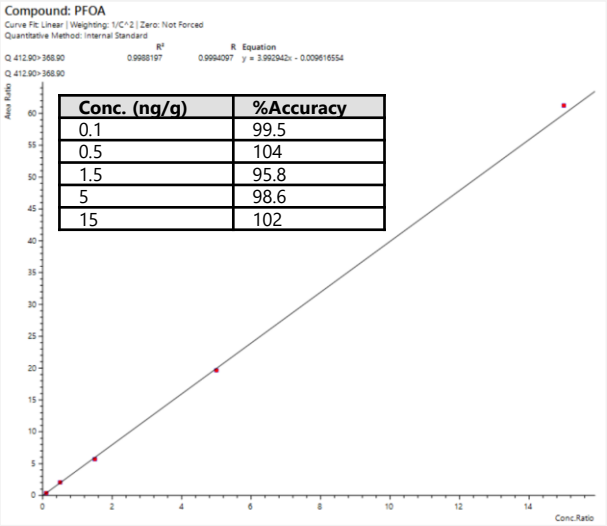
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

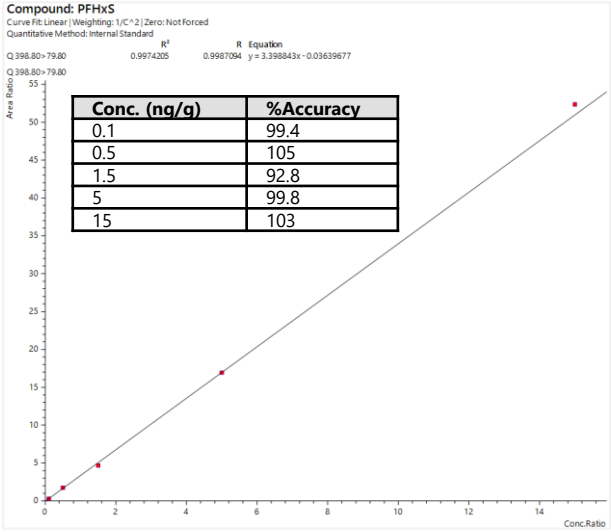
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



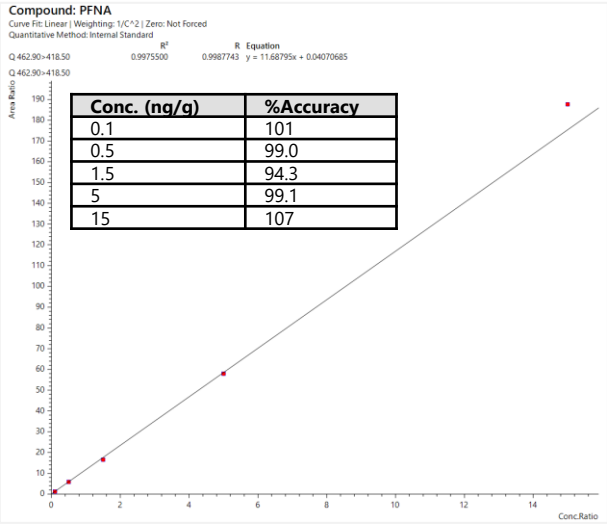

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 99.5 |
| 0.5 | 104 |
| 1.5 | 95.8 |
| 5 | 98.6 |
| 15 | 102 |

**Figure 4**: PFHxS Calibration Curve



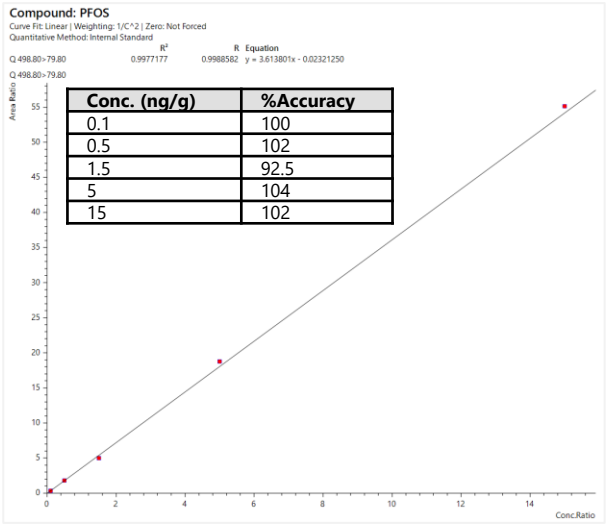

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 99.4 |
| 0.5 | 105 |
| 1.5 | 92.8 |
| 5 | 99.8 |
| 15 | 103 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 101 |
| 0.5 | 99.0 |
| 1.5 | 94.3 |
| 5 | 99.1 |
| 15 | 107 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.1 | 100 |
| 0.5 | 102 |
| 1.5 | 92.5 |
| 5 | 104 |
| 15 | 102 |

Blank matrixes and five different concentrations ranging from below the SMPR required LOQ to 20 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | ND | | | |
| | 0.25 | 0.254 | 6.17 | 6.07 | 101.7 |
| | 0.5 | 0.434 | 7.21 | 8.31 | 86.8 |
| | 1.0 | 1.017 | 3.90 | 3.83 | 101.7 |
| | 5.0 | 5.338 | 7.41 | 6.94 | 106.8 |
| | 10 | 10.414 | 3.17 | 3.04 | 104.1 |
| PFPeA | Blank | ND | | | |
| | 0.25 | 0.249 | 2.87 | 2.88 | 99.7 |
| | 0.5 | 0.447 | 5.37 | 6.00 | 89.5 |
| | 1.0 | 1.025 | 2.20 | 2.15 | 102.5 |
| | 5.0 | 5.247 | 8.12 | 7.74 | 104.9 |
| | 10 | 10.238 | 2.98 | 2.91 | 102.4 |
| PFHxA | Blank | 0.008 | | | |
| | 0.25 | 0.264 | 4.76 | 4.50 | 105.6 |
| | 0.5 | 0.483 | 4.25 | 4.40 | 96.6 |
| | 1.0 | 1.103 | 1.17 | 1.06 | 110.3 |
| | 5.0 | 5.537 | 6.70 | 6.05 | 110.7 |
| | 10 | 10.836 | 5.22 | 4.81 | 108.4 |
| PFHpA | Blank | ND | | | |
| | 0.25 | 0.266 | 3.07 | 2.87 | 106.7 |
| | 0.5 | 0.482 | 4.35 | 4.51 | 96.4 |
| | 1.0 | 1.089 | 2.63 | 2.42 | 108.9 |
| | 5.0 | 5.556 | 8.68 | 7.81 | 111.1 |
| | 10 | 10.898 | 4.01 | 3.68 | 109.0 |
| PFOA | Blank | 0.009 | | | |
| | 0.25 | 0.270 | 3.15 | 2.92 | 108.0 |
| | 0.5 | 0.470 | 2.78 | 2.96 | 94.0 |
| | 1.0 | 1.022 | 1.14 | 1.11 | 102.2 |
| | 5.0 | 5.292 | 5.17 | 4.88 | 105.8 |
| | 10 | 10.508 | 1.59 | 1.51 | 105.0 |
| PFNA | Blank | ND | | | |
| | 0.25 | 0.261 | 1.91 | 1.83 | 104.3 |
| | 0.5 | 0.473 | 2.11 | 2.23 | 94.7 |
| | 1.0 | 1.054 | 2.91 | 2.76 | 105.4 |
| | 5.0 | 5.453 | 3.67 | 3.36 | 109.0 |
| | 10 | 10.879 | 1.39 | 1.27 | 108.8 |
| PFDA | Blank | 0.006 | | | |
| | 0.25 | 0.265 | 6.96 | 6.57 | 105.9 |
| | 0.5 | 0.491 | 3.98 | 4.05 | 98.2 |
| | 1.0 | 1.054 | 0.81 | 0.77 | 105.4 |
| | 5.0 | 5.568 | 3.46 | 3.11 | 111.4 |
| | 10 | 10.804 | 1.37 | 1.26 | 108.1 |
| PFUnA | Blank | 0.010 | | | |
| | 0.25 | 0.259 | 6.56 | 6.34 | 103.4 |
| | 0.5 | 0.529 | 4.65 | 4.39 | 105.9 |
| | 1.0 | 1.040 | 3.08 | 2.96 | 104.0 |
| | 5.0 | 5.453 | 4.99 | 4.58 | 109.0 |
| | 10 | 11.149 | 1.21 | 1.08 | 111.5 |
| PFDoA | Blank | 0.011 | | | |
| | 0.25 | 0.252 | 6.11 | 6.06 | 100.8 |
| | 0.5 | 0.552 | 2.93 | 2.65 | 110.5 |
| | 1.0 | 1.076 | 7.91 | 7.35 | 107.6 |
| | 5.0 | 5.845 | 4.88 | 4.17 | 116.9 |
| | 10 | 11.709 | 1.20 | 1.02 | 117.1 |
| PFTrDA | Blank | 0.019 | | | |
| | 0.25 | 0.235 | 18.57 | 19.79 | 93.8 |
| | 0.5 | 0.544 | 9.18 | 8.43 | 108.9 |
| | 1.0 | 1.064 | 1.30 | 1.22 | 106.4 |
| | 5.0 | 5.459 | 4.67 | 4.27 | 109.2 |
| | 10 | 10.772 | 1.80 | 1.67 | 107.8 |
| PFTeDA | Blank | 0.028 | | | |
| | 0.25 | 0.202 | 19.65 | 24.26 | 81.0 |
| | 0.5 | 0.472 | 2.76 | 2.93 | 94.3 |
| | 1.0 | 0.994 | 7.50 | 7.54 | 99.4 |
| | 5.0 | 5.695 | 7.96 | 6.99 | 113.9 |
| | 10 | 11.144 | 2.97 | 2.67 | 111.4 |
| PFBS | Blank | ND | | | |
| | 0.25 | 0.240 | 3.96 | 4.13 | 96.0 |
| | 0.5 | 0.467 | 6.35 | 6.79 | 93.5 |
| | 1.0 | 0.920 | 0.46 | 0.50 | 92.0 |
| | 5.0 | 4.573 | 4.12 | 4.51 | 91.4 |
| | 10 | 9.796 | 2.10 | 2.15 | 98.0 |

| | | | | | |
|---|---|---|---|---|---|
| PFPeS | Blank | ND | | | |
| | 0.25 | 0.276 | 7.43 | 6.72 | 110.5 |
| | 0.5 | 0.471 | 3.21 | 3.41 | 94.1 |
| | 1.0 | 1.079 | 4.42 | 4.10 | 107.9 |
| | 5.0 | 5.282 | 2.68 | 2.53 | 105.6 |
| | 10 | 11.338 | 4.10 | 3.62 | 113.4 |
| PFHxS | Blank | 0.013 | | | |
| | 0.25 | 0.262 | 4.08 | 3.89 | 104.9 |
| | 0.5 | 0.482 | 1.97 | 2.04 | 96.4 |
| | 1.0 | 0.941 | 1.99 | 2.12 | 94.1 |
| | 5.0 | 4.740 | 4.58 | 4.83 | 94.8 |
| | 10 | 9.739 | 0.49 | 0.51 | 97.4 |
| PFHpS | Blank | ND | | | |
| | 0.25 | 0.266 | 3.21 | 3.02 | 106.3 |
| | 0.5 | 0.464 | 5.18 | 5.59 | 92.8 |
| | 1.0 | 1.066 | 2.45 | 2.30 | 106.6 |
| | 5.0 | 5.037 | 0.35 | 0.35 | 100.7 |
| | 10 | 11.747 | 3.06 | 2.61 | 117.5 |
| PFOS | Blank | 0.008 | | | |
| | 0.25 | 0.242 | 5.29 | 5.46 | 96.8 |
| | 0.5 | 0.464 | 3.63 | 3.90 | 92.9 |
| | 1.0 | 0.922 | 3.99 | 4.33 | 92.2 |
| | 5.0 | 4.614 | 4.50 | 4.88 | 92.3 |
| | 10 | 9.836 | 2.56 | 2.60 | 98.3 |
| PFNS | Blank | ND | | | |
| | 0.25 | 0.256 | 5.54 | 5.41 | 102.4 |
| | 0.5 | 0.488 | 4.03 | 4.14 | 97.5 |
| | 1.0 | 1.011 | 3.78 | 3.74 | 101.1 |
| | 5.0 | 4.918 | 0.85 | 0.86 | 98.4 |
| | 10 | 11.269 | 1.63 | 1.44 | 112.7 |
| PFDS | Blank | 0.004 | | | |
| | 0.25 | 0.261 | 2.69 | 2.58 | 104.2 |
| | 0.5 | 0.480 | 7.88 | 8.21 | 96.0 |
| | 1.0 | 0.986 | 3.78 | 3.84 | 98.6 |
| | 5.0 | 4.884 | 2.71 | 2.77 | 97.7 |
| | 10 | 11.074 | 2.04 | 1.84 | 110.8 |
| PFUnDS | Blank | 0.038 | | | |
| | 0.25 | 0.229 | 11.65 | 12.74 | 91.4 |
| | 0.5 | 0.471 | 3.69 | 3.92 | 94.2 |
| | 1.0 | 0.936 | 5.08 | 5.43 | 93.6 |
| | 5.0 | 4.868 | 5.07 | 5.21 | 97.3 |
| | 10 | 9.633 | 2.03 | 2.10 | 96.3 |
| PFDoS | Blank | 0.014 | | | |
| | 0.25 | 0.240 | 17.49 | 18.23 | 95.9 |
| | 0.5 | 0.463 | 3.94 | 4.26 | 92.5 |
| | 1.0 | 1.008 | 8.40 | 8.34 | 100.8 |
| | 5.0 | 5.083 | 3.24 | 3.19 | 101.7 |
| | 10 | 10.273 | 3.84 | 3.74 | 102.7 |
| PFTrDS | Blank | 0.013 | | | |
| | 0.25 | 0.217 | 10.40 | 11.99 | 86.8 |
| | 0.5 | 0.449 | 2.72 | 3.03 | 89.9 |
| | 1.0 | 1.017 | 8.78 | 8.64 | 101.7 |
| | 5.0 | 5.471 | 5.86 | 5.36 | 109.4 |
| | 10 | 11.963 | 3.60 | 3.01 | 119.6 |
| PFOSA | Blank | ND | | | |
| | 0.25 | 0.254 | 5.16 | 5.08 | 101.7 |
| | 0.5 | 0.497 | 7.54 | 7.59 | 99.4 |
| | 1.0 | 0.998 | 6.46 | 6.48 | 99.8 |
| | 5.0 | 5.054 | 3.82 | 3.78 | 101.1 |
| | 10 | 10.768 | 3.10 | 2.88 | 107.7 |
| 9Cl-PF3ONS | Blank | 0.007 | | | |
| | 0.25 | 0.261 | 4.09 | 3.91 | 104.5 |
| | 0.5 | 0.500 | 1.76 | 1.76 | 100.1 |
| | 1.0 | 0.976 | 3.38 | 3.46 | 97.6 |
| | 5.0 | 4.989 | 5.16 | 5.17 | 99.8 |
| | 10 | 10.804 | 1.33 | 1.23 | 108.0 |
| 11Cl-PF3OUdS | Blank | 0.004 | | | |
| | 0.25 | 0.276 | 0.75 | 0.68 | 110.5 |
| | 0.5 | 0.496 | 3.36 | 3.39 | 99.3 |
| | 1.0 | 1.036 | 9.19 | 8.87 | 103.6 |
| | 5.0 | 5.483 | 4.11 | 3.75 | 109.7 |
| | 10 | 12.079 | 4.51 | 3.73 | 120.8 |

| | | | | | |
|---|---|---|---|---|---|
| HFPO-DA | Blank | 0.010 | | | |
| | 0.25 | 0.251 | 4.55 | 4.53 | 100.4 |
| | 0.5 | 0.469 | 7.34 | 7.81 | 93.9 |
| | 1.0 | 0.876 | 3.07 | 3.50 | 87.6 |
| | 5.0 | 4.538 | 4.21 | 4.63 | 90.8 |
| | 10 | 9.461 | 3.98 | 4.21 | 94.6 |
| DONA | Blank | ND | | | |
| | 0.25 | 0.244 | 3.92 | 4.01 | 97.7 |
| | 0.5 | 0.466 | 4.58 | 4.92 | 93.1 |
| | 1.0 | 1.026 | 1.17 | 1.14 | 102.6 |
| | 5.0 | 5.390 | 9.33 | 8.65 | 107.8 |
| | 10 | 10.210 | 3.70 | 3.62 | 102.1 |
| 4:2 FTS | Blank | ND | | | |
| | 0.25 | 0.250 | 10.10 | 10.10 | 99.9 |
| | 0.5 | 0.480 | 2.93 | 3.05 | 96.0 |
| | 1.0 | 1.107 | 7.81 | 7.05 | 110.7 |
| | 5.0 | 5.443 | 9.61 | 8.83 | 108.9 |
| | 10 | 10.788 | 7.37 | 6.83 | 107.9 |
| 6:2 FTS | Blank | ND | | | |
| | 0.25 | 0.267 | 12.51 | 11.70 | 106.9 |
| | 0.5 | 0.511 | 13.05 | 12.78 | 102.1 |
| | 1.0 | 1.079 | 9.92 | 9.19 | 107.9 |
| | 5.0 | 5.342 | 8.95 | 8.38 | 106.8 |
| | 10 | 10.826 | 9.54 | 8.81 | 108.3 |
| 8:2 FTS | Blank | 0.009 | | | |
| | 0.25 | 0.225 | 9.23 | 10.25 | 90.1 |
| | 0.5 | 0.483 | 0.38 | 0.39 | 96.6 |
| | 1.0 | 1.016 | 0.85 | 0.84 | 101.6 |
| | 5.0 | 5.945 | 10.53 | 8.86 | 118.9 |
| | 10 | 10.140 | 3.49 | 3.44 | 101.4 |
| 10:2 FTS | Blank | 0.003 | | | |
| | 0.5 | 0.478 | 10.34 | 10.82 | 95.6 |
| | 1.0 | 1.060 | 8.61 | 8.13 | 106.0 |
| | 5.0 | 5.939 | 9.65 | 8.13 | 118.8 |
| | 10 | 11.502 | 2.70 | 2.35 | 115.0 |

ND = average results less than zero

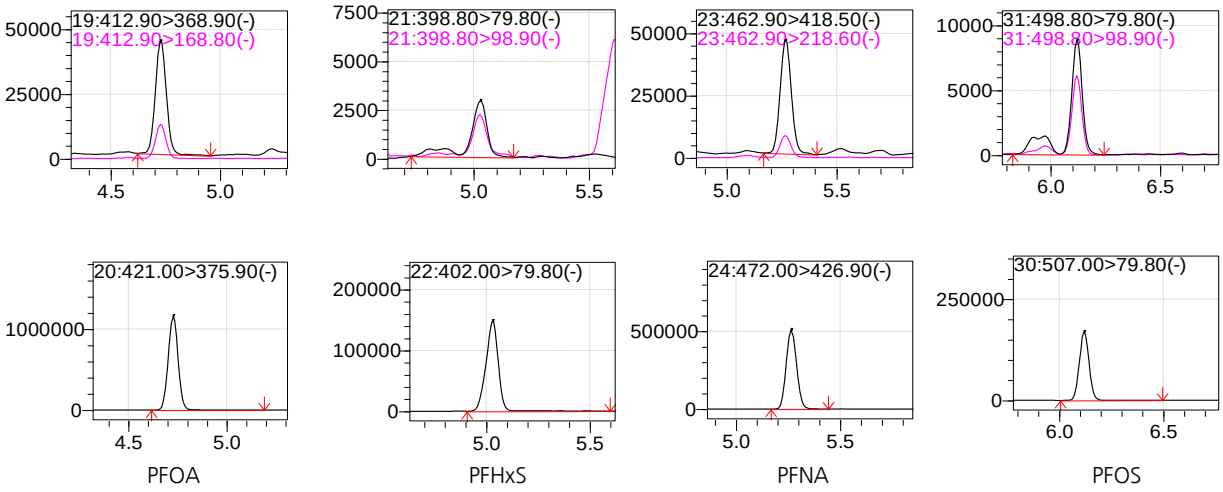



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a fish oil matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Coffee by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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## User Benefits

- Validated method for 30 PFAS in Coffee meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, with SPE cleanup
- Simple and rapid extraction

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in brewed coffee followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.055 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.0055 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.055 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.0055 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.0055 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.0055 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.055 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.0055 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.055 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.055 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.055 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.0055 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.0055 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.0055 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.055 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.055 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.055 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.055 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.055 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.055 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.55 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.55 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.0055 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.055 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.055 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.55 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.055 |

## ■ Sample Preparation and Analysis Conditions

Organic brewed coffee was purchased locally. Samples were prepared by shaking the bottle and removing aliquots directly from the original container. Test portions were spiked in triplicate at four different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10- gram test portions spiked with concentrations of 0.001, 0.01, 0.1, 1.0 and 10.0 ng/g. Quantitation was carried out on additional samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in a brewed coffee matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, the extract was concentrated to dryness and dissolved in 0.4 mL of a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

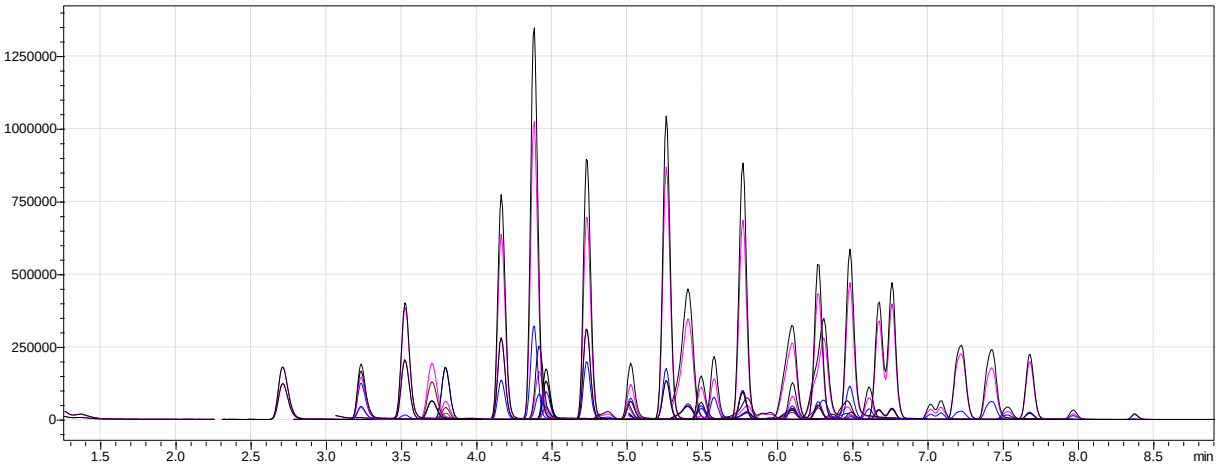



**Figure 2**: Chromatogram of 0.55 ng/g PFAS in a coffee matrix with separation of all peaks in nine minutes

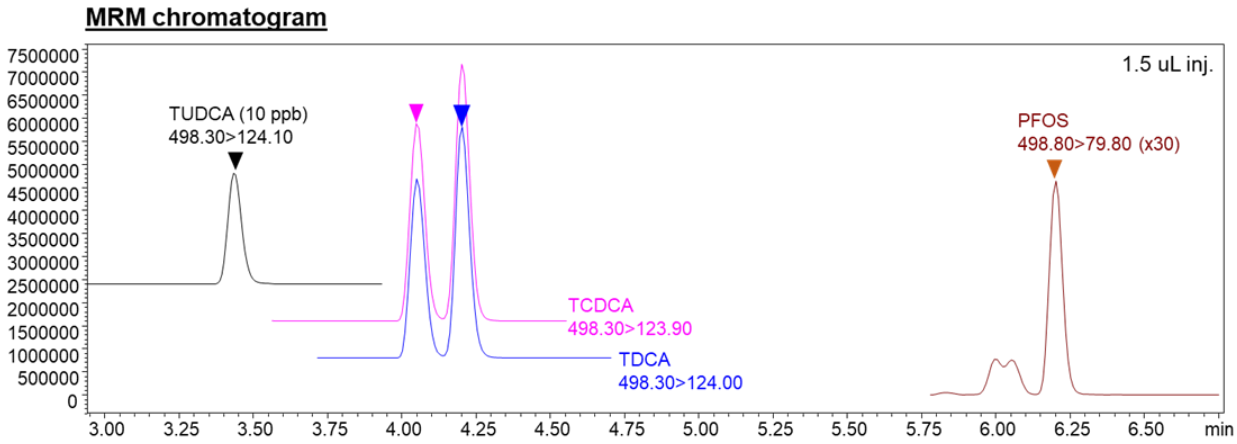



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

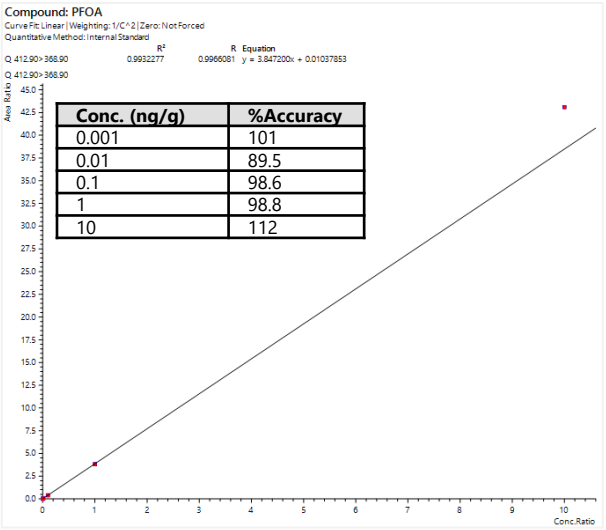
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_3$-PFBS |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_2$-PFDoA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

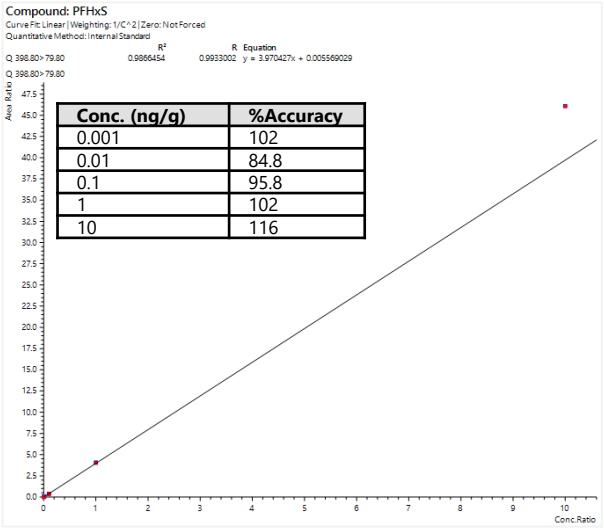
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

**Figure 3**: PFOA Calibration Curve



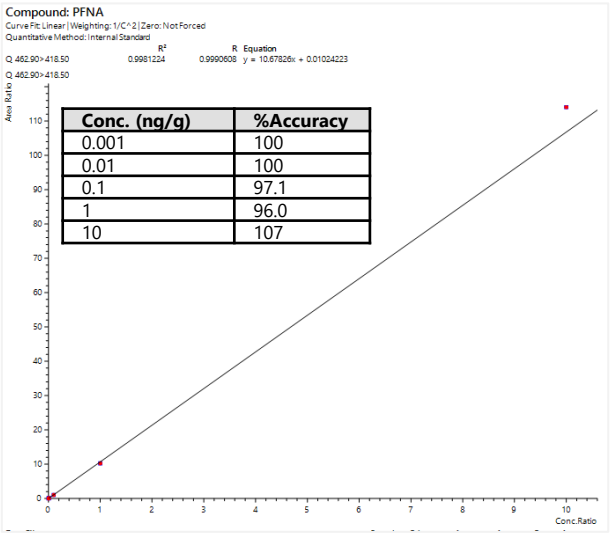

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 89.5 |
| 0.1 | 98.6 |
| 1 | 98.8 |
| 10 | 112 |

**Figure 4**: PFHxS Calibration Curve



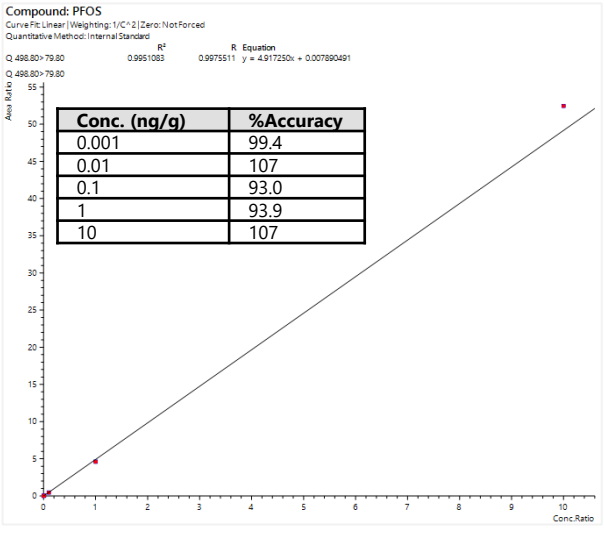

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 102 |
| 0.01 | 84.8 |
| 0.1 | 95.8 |
| 1 | 102 |
| 10 | 116 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 100 |
| 0.01 | 100 |
| 0.1 | 97.1 |
| 1 | 96.0 |
| 10 | 107 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.4 |
| 0.01 | 107 |
| 0.1 | 93.0 |
| 1 | 93.9 |
| 10 | 107 |

Blank matrixes and at four different concentrations ranging from below the SMPR required LOQ to approximately 20 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery% |
|---|---|---|---|---|---|
| PFBA | Blank | ND | | | |
| | 0.055 | 0.049 | 4.19 | 4.75 | 88.1 |
| | 0.55 | 0.526 | 2.00 | 2.09 | 95.8 |
| | 5.5 | 5.553 | 0.31 | 0.30 | 101.0 |
| PFPeA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 8.23 | 8.41 | 97.9 |
| | 0.055 | 0.051 | 3.42 | 3.67 | 93.1 |
| | 0.55 | 0.504 | 1.34 | 1.46 | 91.7 |
| | 5.5 | 5.274 | 1.07 | 1.12 | 95.9 |
| PFHxA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 17.58 | 18.38 | 95.7 |
| | 0.055 | 0.054 | 1.08 | 1.11 | 97.2 |
| | 0.55 | 0.534 | 3.22 | 3.32 | 97.2 |
| | 5.5 | 5.744 | 1.98 | 1.89 | 104.4 |
| PFHpA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 16.77 | 17.85 | 94.0 |
| | 0.055 | 0.053 | 1.46 | 1.53 | 96.0 |
| | 0.55 | 0.530 | 2.95 | 3.07 | 96.3 |
| | 5.5 | 5.549 | 2.10 | 2.08 | 100.9 |
| PFOA | Blank | ND | | | |
| | 0.0055 | 0.005 | 2.36 | 2.62 | 89.9 |
| | 0.055 | 0.053 | 0.55 | 0.58 | 95.7 |
| | 0.55 | 0.555 | 0.67 | 0.66 | 100.9 |
| | 5.5 | 5.798 | 2.46 | 2.33 | 105.4 |
| PFNA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 2.91 | 3.48 | 83.4 |
| | 0.055 | 0.052 | 4.07 | 4.26 | 95.4 |
| | 0.55 | 0.523 | 4.73 | 4.97 | 95.2 |
| | 5.5 | 5.581 | 3.29 | 3.24 | 101.5 |
| PFDA | Blank | 0.000 | | | |
| | 0.0055 | 0.004 | 14.51 | 17.41 | 83.4 |
| | 0.055 | 0.051 | 3.33 | 3.56 | 93.6 |
| | 0.55 | 0.521 | 2.63 | 2.78 | 94.7 |
| | 5.5 | 5.445 | 2.10 | 2.12 | 99.0 |
| PFUnA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 10.28 | 11.12 | 92.4 |
| | 0.055 | 0.052 | 3.02 | 3.22 | 93.8 |
| | 0.55 | 0.520 | 0.78 | 0.82 | 94.6 |
| | 5.5 | 5.420 | 4.17 | 4.23 | 98.5 |
| PFDoA | Blank | 0.001 | | | |
| | 0.055 | 0.056 | 11.37 | 11.21 | 101.4 |
| | 0.55 | 0.504 | 1.40 | 1.53 | 91.7 |
| | 5.5 | 5.587 | 3.54 | 3.48 | 101.6 |
| PFTrDA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 13.20 | 14.63 | 90.2 |
| | 0.055 | 0.043 | 3.55 | 4.53 | 78.2 |
| | 0.55 | 0.450 | 1.87 | 2.29 | 81.9 |
| | 5.5 | 5.272 | 3.37 | 3.51 | 95.9 |
| PFTeDA | Blank | ND | | | |
| | 0.055 | 0.037 | 2.40 | 3.59 | 66.9 |
| | 0.55 | 0.440 | 2.75 | 3.43 | 80.1 |
| | 5.5 | 5.363 | 4.01 | 4.12 | 97.5 |
| PFBS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 10.02 | 10.89 | 92.0 |
| | 0.055 | 0.051 | 1.92 | 2.07 | 92.7 |
| | 0.55 | 0.515 | 1.65 | 1.76 | 93.5 |
| | 5.5 | 5.359 | 0.78 | 0.80 | 97.4 |
| PFPeS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 9.29 | 9.22 | 100.7 |
| | 0.055 | 0.060 | 2.99 | 2.75 | 108.9 |
| | 0.55 | 0.547 | 1.80 | 1.81 | 99.5 |
| | 5.5 | 5.546 | 2.99 | 2.96 | 100.9 |
| PFHxS | Blank | ND | | | |
| | 0.0055 | 0.006 | 12.64 | 12.35 | 102.3 |
| | 0.055 | 0.054 | 1.14 | 1.16 | 97.8 |
| | 0.55 | 0.538 | 1.75 | 1.79 | 97.8 |
| | 5.5 | 5.752 | 1.80 | 1.72 | 104.6 |
| PFHpS | Blank | 0.001 | | | |
| | 0.055 | 0.052 | 6.88 | 7.25 | 94.9 |
| | 0.55 | 0.484 | 1.62 | 1.84 | 88.1 |
| | 5.5 | 4.930 | 1.72 | 1.91 | 89.6 |

| | | | | | |
|---|---|---|---|---|---|
| PFOS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 14.22 | 15.79 | 90.1 |
| | 0.055 | 0.048 | 1.11 | 1.26 | 88.1 |
| | 0.55 | 0.493 | 2.30 | 2.57 | 89.7 |
| | 5.5 | 5.395 | 7.45 | 7.59 | 98.1 |
| PFNS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 18.55 | 20.76 | 89.3 |
| | 0.055 | 0.056 | 1.30 | 1.28 | 101.6 |
| | 0.55 | 0.573 | 4.06 | 3.89 | 104.2 |
| | 5.5 | 5.419 | 4.92 | 4.99 | 98.5 |
| PFDS | Blank | 0.007 | | | |
| | 0.055 | 0.051 | 8.80 | 9.47 | 93.0 |
| | 0.55 | 0.497 | 2.55 | 2.83 | 90.3 |
| | 5.5 | 5.096 | 4.15 | 4.48 | 92.7 |
| PFUnDS | Blank | 0.005 | | | |
| | 0.055 | 0.047 | 10.89 | 12.89 | 84.5 |
| | 0.55 | 0.401 | 4.99 | 6.85 | 72.9 |
| | 5.5 | 5.537 | 9.92 | 9.85 | 100.7 |
| PFDoS | Blank | 0.003 | | | |
| | 0.055 | 0.047 | 13.08 | 15.26 | 85.7 |
| | 0.55 | 0.478 | 5.59 | 6.44 | 86.8 |
| | 5.5 | 4.933 | 1.55 | 1.73 | 89.7 |
| PFTrDS | Blank | 0.001 | | | |
| | 0.055 | 0.038 | 6.92 | 10.10 | 68.5 |
| | 0.55 | 0.437 | 3.70 | 4.65 | 79.5 |
| | 5.5 | 5.021 | 3.90 | 4.27 | 91.3 |
| PFOSA | Blank | ND | | | |
| | 0.055 | 0.046 | 12.00 | 14.31 | 83.9 |
| | 0.55 | 0.605 | 7.45 | 6.77 | 110.0 |
| | 5.5 | 4.924 | 3.98 | 4.45 | 89.5 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 13.50 | 13.00 | 103.8 |
| | 0.055 | 0.054 | 0.32 | 0.33 | 98.0 |
| | 0.55 | 0.538 | 1.85 | 1.89 | 97.8 |
| | 5.5 | 5.888 | 7.98 | 7.45 | 107.0 |
| 11Cl-PF3OUdS | Blank | 0.003 | | | |
| | 0.0055 | 0.007 | 13.02 | 11.27 | 115.5 |
| | 0.055 | 0.047 | 9.48 | 11.20 | 84.6 |
| | 0.55 | 0.451 | 1.20 | 1.46 | 82.0 |
| | 5.5 | 4.916 | 3.70 | 4.14 | 89.4 |
| HFPO-DA | Blank | ND | | | |
| | 0.055 | 0.046 | 2.80 | 3.33 | 84.3 |
| | 0.55 | 0.550 | 3.03 | 3.03 | 99.9 |
| | 5.5 | 5.770 | 2.77 | 2.64 | 104.9 |
| DONA | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 3.27 | 3.18 | 102.8 |
| | 0.055 | 0.053 | 3.57 | 3.69 | 96.7 |
| | 0.55 | 0.547 | 2.87 | 2.89 | 99.4 |
| | 5.5 | 5.721 | 1.55 | 1.49 | 104.0 |
| 4:2 FTS | Blank | 0.005 | | | |
| | 0.055 | 0.051 | 6.44 | 6.97 | 92.5 |
| | 0.55 | 0.557 | 1.34 | 1.33 | 101.2 |
| | 5.5 | 5.534 | 1.07 | 1.06 | 100.6 |
| 6:2 FTS | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 6.71 | 7.33 | 91.5 |
| | 0.055 | 0.056 | 16.30 | 16.11 | 101.2 |
| | 0.55 | 0.559 | 10.64 | 10.46 | 101.7 |
| | 5.5 | 5.406 | 4.45 | 4.53 | 98.3 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 19.62 | 20.35 | 96.4 |
| | 0.055 | 0.054 | 8.94 | 9.00 | 99.3 |
| | 0.55 | 0.609 | 2.99 | 2.69 | 110.9 |
| | 5.5 | 7.143 | 6.46 | 4.97 | 129.9 |
| 10:2 FTS | Blank | 0.001 | | | |
| | 0.055 | 0.043 | 10.17 | 12.82 | 79.4 |
| | 0.55 | 0.443 | 3.81 | 4.73 | 80.4 |
| | 5.5 | 5.042 | 6.27 | 6.84 | 91.7 |

ND = average results less than zero

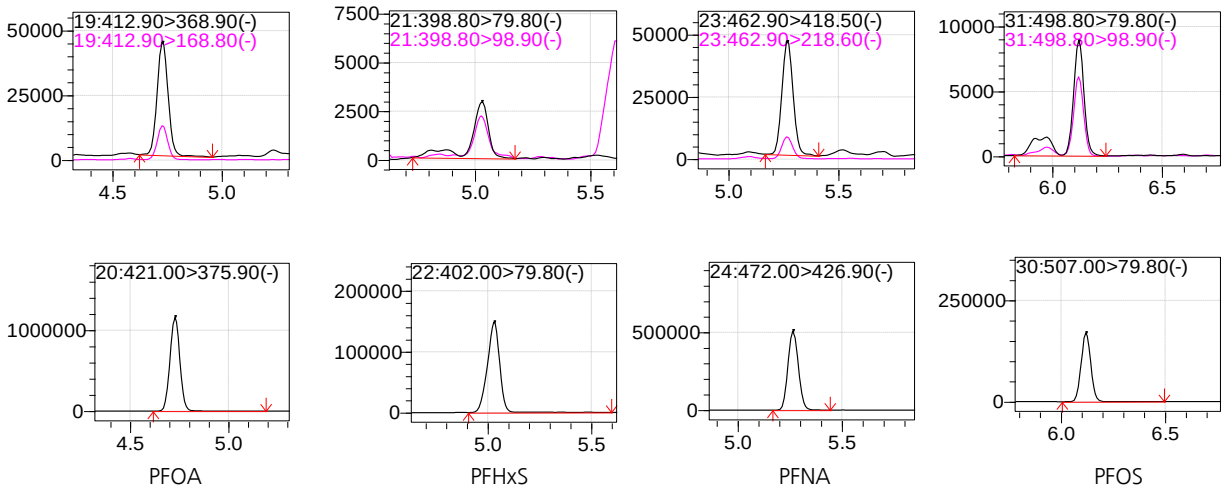



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a coffee matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## ■ Reference

1) AOAC SMPR 2023.003

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

# Determination of 30 PFAS in Seafood by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

William Lipps[1], Toshiya Matsubara[1], Dominika Gruszecka[1]
[1] Shimadzu Scientific Instruments, Inc.

## User Benefits

- Validated method for 30 PFAS in seafood (shrimp) meeting all criteria of AOAC SMPR 2023.003
- High precision, excellent recovery, low Limit of Quantification (LOQ)
- Simple and rapid extraction using QuEChERS

## ■ Introduction

Per-and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in shrimp using the QuEChERS technique followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at three concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.

**Figure 1**: Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1**: PFAS Analytes, Acronyms, CAS No. and Method LOQ

| Analyte name | Acronym | CAS No. | LOQ (ppb) |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | 375-22-4 | 0.055 |
| Perfluoropentanoic acid | PFPeA | 2706-90-3 | 0.0055 |
| Perfluorohexanoic acid | PFHxA | 307-24-4 | 0.0055 |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | 0.055 |
| Perfluorooctanoic acid | PFOA | 335-67-1 | 0.0055 |
| Perfluorononanoic acid | PFNA | 375-95-1 | 0.0055 |
| Perfluorodecanoic acid | PFDA | 335-76-2 | 0.0055 |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | 0.0055 |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | 0.055 |

| | | | |
|---|---|---|---|
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | 0.055 |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | 0.055 |
| Perfluorobutanesulfonic acid | PFBS | 375-73-5 | 0.055 |
| Perfluoropentansulfonic acid | PFPeS | 2706-91-4 | 0.055 |
| Perfluorohexanesulfonic acid | PFHxS | 355-46-4 | 0.0055 |
| Perfluoroheptanesulfonic acid | PFHpS | 375-92-8 | 0.0055 |
| Perfluorooctanesulfonic acid | PFOS | 1763-23-1 | 0.055 |
| Perfluorononanesulfonic acid | PFNS | 68259-12-1 | 0.0055 |
| Perfluorodecanesulfonic acid | PFDS | 335-77-3 | 0.0055 |
| Perfluoroundecanesulfonic acid | PFUnDS | 749786-16-1 | 0.0055 |
| Perfluorododecanesulfonic acid | PFDoS | 79780-39-5 | 0.0055 |
| Perfluorotridecanesulfonic acid | PFTrDS | 791563-89-8 | 0.055 |
| Perfluorooctanesulfonamide | PFOSA | 754-91-6 | 0.055 |
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | 0.0055 |
| 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | 0.0055 |
| Hexafluoropropylene oxide dimer acid | HFPO-DA | 13252-13-6 | 0.055 |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA | 919005-14-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid | 4:2 FTS | 757124-72-4 | 0.055 |
| 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid | 6:2 FTS | 27619-97-2 | 0.55 |
| 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid | 8:2 FTS | 39108-34-4 | 0.0055 |
| 1H,1H, 2H, 2H-Perfluorododecane sulfonic acid | 10:2 FTS | 120226-60-0 | 0.0055 |

## ■ Sample Preparation and Analysis Conditions

Samples were prepared by spiking prepared shrimp samples in triplicate at three different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using shrimp test portions spiked with concentrations of 0.001, 0.01, 0.10, 1.0, and 10.0 ng/g. Quantitation was carried out on additional shrimp samples spiked in triplicate at 0.0055, 0.055, 0.55, and 5.5 ng/g. Since standards were extracted in a shrimp matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

Frozen shrimp were thawed at room temperature and then crushed in a grinder with dry ice for 30 seconds at 4000 rpm. The ground sample was stored in a freezer overnight to remove all the dry ice. Ten-gram portions were weighed, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and a QuEChERS packet was added. The sample was shaken for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. For greater sensitivity, 2 mL of the extract was concentrated in 400 µL of a methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

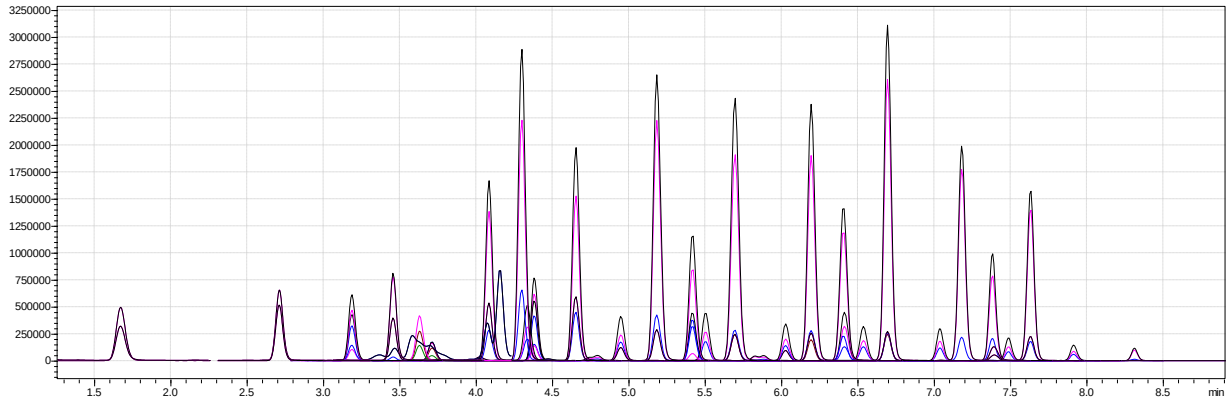


**Figure 2**: Chromatogram of 0.55 ng/g PFAS in shrimp matrix with separation of all peaks in nine minutes

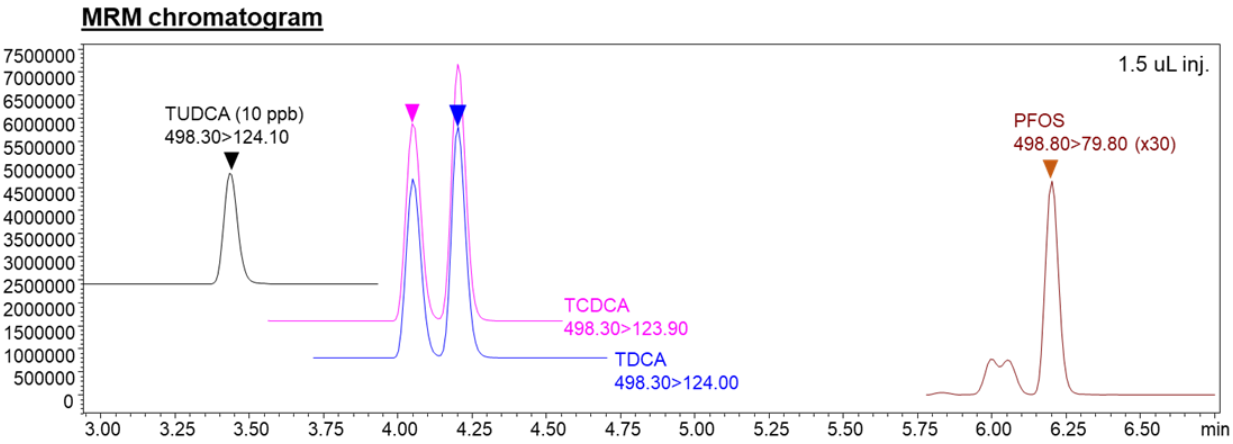



**Figure 3**: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

**Table 2**: MRM Transitions and Internal Standard Associations

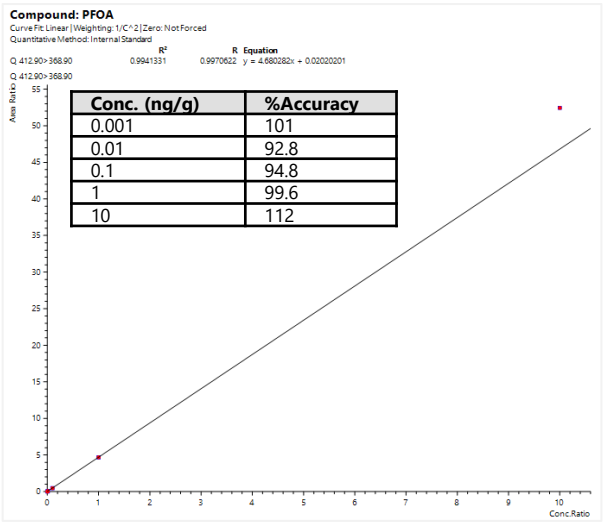
| Analyte | Quantitation Ion | Qualifier Ion | Internal Standard |
|---|---|---|---|
| PFBA | 212.9 > 168.8 | | $^{13}C_4$-PFBA |
| PFPeA | 262.9 > 218.8 | | $^{13}C_5$-PFPeA |
| PFHxA | 313 > 268.8 | 313 > 118.9 | $^{13}C_5$-PFHxA |
| PFHpA | 362.9 > 318.9 | 362.9 > 168.8 | $^{13}C_4$-PFHpA |
| PFOA | 412.9 > 368.9 | 412.9 > 168.8 | $^{13}C_8$-PFOA |
| PFNA | 462.9 > 418.5 | 462.9 > 218.6 | $^{13}C_9$-PFNA |
| PFDA | 512.9 > 468.95 | 512.9 > 268.55 | $^{13}C_6$-PFDA |
| PFUnA | 562.9 > 518.95 | 562.9 > 269 | $^{13}C_7$-PFUnA |
| PFDoA | 612.9 > 268.6 | 612.9 > 168.6 | $^{13}C_7$-PFUnA |
| PFTrDA | 663 > 618.6 | 663 > 168.5 | $^{13}C_7$-PFUnA |
| PFTeDA | 713 > 669 | 713 > 168.5 | $^{13}C_7$-PFUnA |
| PFBS | 298.8 > 79.8 | 298.8 > 98.9 | $^{13}C_5$-PFHxA |
| PFPeS | 348.8 > 79.8 | 348.8 > 98.9 | $^{13}C_4$-PFHpA |
| PFHxS | 398.8 > 79.8 | 398.8 > 98.9 | $^{13}C_3$-PFHxS |
| PFHpS | 448.8 > 79.8 | 448.8 > 99 | $^{13}C_6$-PFDA |
| PFOS | 498.8 > 79.8 | 498.8 > 98.9 | $^{13}C_8$-PFOS |
| PFNS | 548.9 > 79.8 | 548.8 > 99 | $^{13}C_7$-PFUnA |
| PFDS | 598.8 > 99 | 598.8 > 79.8 | $^{13}C_7$-PFUnA |
| PFUnDS | 649 > 99 | 649 > 80 | $^{13}C_8$-PFOS |
| PFDoS | 699 > 80 | 699 > 99 | $^{13}C_2$-PFDoA |
| PFTrDS | 749 > 80 | 749 > 279.6 | $^{13}C_7$-PFUnA |
| PFOSA | 498 > 78 | 498 > 477.95 | $^{13}C_8$-PFOSA |
| 9Cl-PF3ONS | 530.8 > 350.8 | 532.9 > 352.95 | $^{13}C_8$-PFOS |
| 11Cl-PF3OUdS | 631 > 451 | 632.9 > 452.95 | $^{13}C_7$-PFUnA |
| HFPO-DA | 284.8 > 168.8 | 284.8 > 118.9 | $^{13}C_3$-HFPO-DA |
| DONA | 376.9 > 250.8 | 376.9 > 84.9 | $^{13}C_4$-PFHpA |
| 4:2 FTS | 326.9 > 80.9 | 326.9 > 306.8 | $^{13}C_2$-4:2 FTS |
| 6:2 FTS | 426.9 > 80.8 | 426.9 > 406.9 | $^{13}C_2$-6:2FTS |
| 8:2 FTS | 526.9 > 506.45 | 526.9 > 80.55 | $^{13}C_3$-PFHxS |
| 10:2 FTS | 626.9 > 606.7 | 626.9 > 80.9 | $^{13}C_7$-PFUnA |

## ■ Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were ±25% of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHXS and PFOS were integrated together.

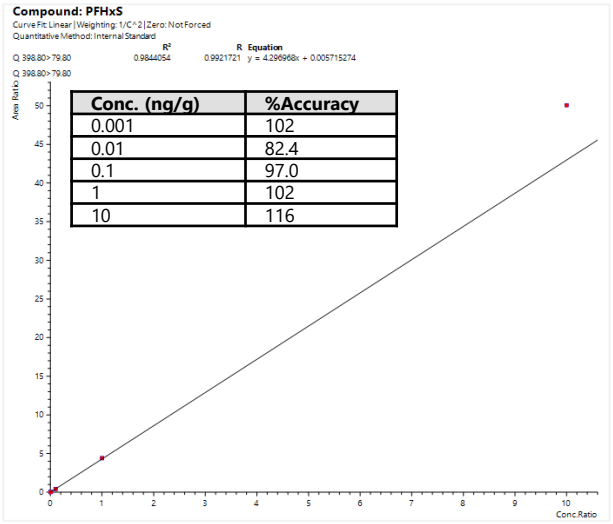
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3. During the single laboratory validation, the shrimp matrix contained an unknown interference coeluting with the $^{13}C_3$-PFBS isotope so $^{13}C_5$-PFHxA was used instead.

**Figure 3**: PFOA Calibration Curve



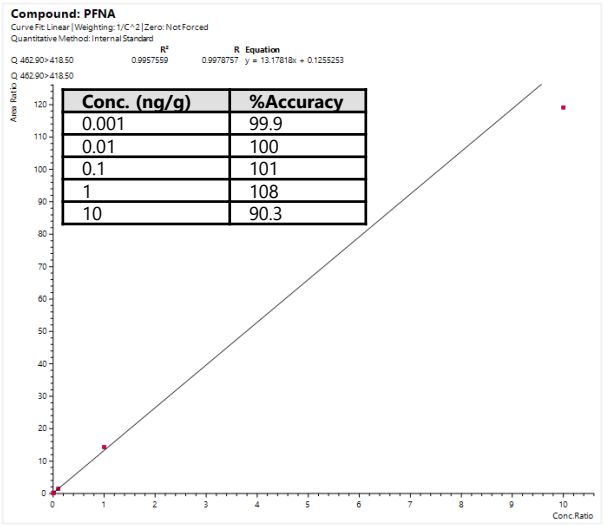

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 101 |
| 0.01 | 92.8 |
| 0.1 | 94.8 |
| 1 | 99.6 |
| 10 | 112 |

**Figure 4**: PFHxS Calibration Curve



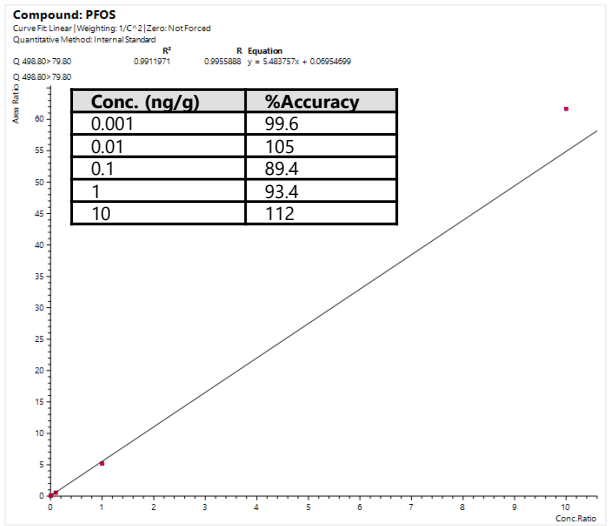

| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 102 |
| 0.01 | 82.4 |
| 0.1 | 97.0 |
| 1 | 102 |
| 10 | 116 |

**Figure 5**: PFNA Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.9 |
| 0.01 | 100 |
| 0.1 | 101 |
| 1 | 108 |
| 10 | 90.3 |

**Figure 6**: PFOS Calibration Curve




| Conc. (ng/g) | %Accuracy |
|---|---|
| 0.001 | 99.6 |
| 0.01 | 105 |
| 0.1 | 89.4 |
| 1 | 93.4 |
| 10 | 112 |

Blank matrixes and at least three different concentrations ranging from below the SMPR required LOQ to approximately 20 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrices and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of ±30%. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a S/N > 10. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3**: Recovery and repeatability for each analyte at each spike concentration

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | Standard Deviation | %RSD | Average Recovery (%) |
|---|---|---|---|---|---|
| PFBA | Blank | 0.007 | | | |
| | 0.055 | 0.056 | 0.98 | 0.98 | 100.3 |
| | 0.55 | 0.518 | 0.32 | 0.34 | 94.1 |
| | 5.5 | 5.478 | 0.35 | 0.35 | 99.6 |
| PFPeA | Blank | ND | | | |
| | 0.0055 | 0.005 | 3.52 | 3.81 | 92.6 |
| | 0.055 | 0.054 | 2.17 | 2.23 | 97.6 |
| | 0.55 | 0.517 | 0.67 | 0.71 | 94.1 |
| | 5.5 | 5.751 | 0.26 | 0.25 | 104.6 |
| PFHxA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 9.08 | 9.50 | 95.6 |
| | 0.055 | 0.057 | 1.48 | 1.43 | 103.7 |
| | 0.55 | 0.538 | 0.66 | 0.67 | 97.8 |
| | 5.5 | 5.871 | 0.15 | 0.14 | 106.8 |
| PFHpA | Blank | 0.001 | | | |
| | 0.0055 | 0.005 | 3.90 | 4.06 | 96.1 |
| | 0.055 | 0.051 | 0.75 | 0.82 | 92.0 |
| | 0.55 | 0.518 | 0.26 | 0.28 | 94.2 |
| | 5.5 | 6.161 | 1.25 | 1.12 | 112.0 |
| PFOA | Blank | ND | | | |
| | 0.0055 | 0.006 | 6.16 | 5.89 | 104.6 |
| | 0.055 | 0.053 | 0.67 | 0.69 | 95.8 |
| | 0.55 | 0.543 | 1.59 | 1.61 | 98.8 |
| | 5.5 | 6.303 | 0.67 | 0.58 | 114.6 |
| PFNA | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 7.32 | 6.77 | 108.1 |
| | 0.055 | 0.056 | 0.68 | 0.67 | 102.1 |
| | 0.55 | 0.583 | 0.55 | 0.52 | 106.0 |
| | 5.5 | 5.340 | 2.53 | 2.61 | 97.1 |
| PFDA | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 4.45 | 4.38 | 101.6 |
| | 0.055 | 0.056 | 0.72 | 0.71 | 101.9 |
| | 0.55 | 0.572 | 0.86 | 0.83 | 104.0 |
| | 5.5 | 5.827 | 2.00 | 1.89 | 105.9 |
| PFUnA | Blank | ND | | | |
| | 0.0055 | 0.007 | 6.07 | 4.92 | 123.3 |
| | 0.055 | 0.056 | 2.29 | 2.27 | 100.9 |
| | 0.55 | 0.579 | 1.03 | 0.98 | 105.2 |
| | 5.5 | 5.789 | 1.91 | 1.82 | 105.3 |
| PFDoA | Blank | 0.001 | | | |
| | 0.055 | 0.054 | 6.52 | 6.67 | 97.7 |
| | 0.55 | 0.528 | 3.51 | 3.66 | 96.0 |
| | 5.5 | 5.863 | 3.10 | 2.91 | 106.6 |
| PFTrDA | Blank | ND | | | |
| | 0.055 | 0.063 | 2.31 | 2.01 | 115.0 |
| | 0.55 | 0.586 | 3.89 | 3.65 | 106.6 |
| | 5.5 | 6.215 | 2.56 | 2.26 | 113.0 |
| PFTeDA | Blank | 0.001 | | | |
| | 0.055 | 0.055 | 5.22 | 5.23 | 99.7 |
| | 0.55 | 0.555 | 4.27 | 4.23 | 100.8 |
| | 5.5 | 6.313 | 3.03 | 2.64 | 114.8 |
| PFBS | Blank | 0.001 | | | |
| | 0.055 | 0.051 | 5.55 | 5.93 | 93.6 |
| | 0.55 | 0.527 | 2.83 | 2.95 | 95.8 |
| | 5.5 | 5.940 | 2.31 | 2.14 | 108.0 |
| PFPeS | Blank | 0.002 | | | |
| | 0.0055 | 0.004 | 15.88 | 20.67 | 76.8 |
| | 0.055 | 0.055 | 3.92 | 3.89 | 100.7 |
| | 0.55 | 0.598 | 3.29 | 3.02 | 108.8 |
| | 5.5 | 5.713 | 0.71 | 0.68 | 103.9 |

| | | | | | |
|---|---|---|---|---|---|
| PFHxS | Blank | ND | | | |
| | 0.0055 | 0.004 | 14.59 | 17.62 | 82.8 |
| | 0.055 | 0.052 | 2.32 | 2.45 | 94.9 |
| | 0.55 | 0.535 | 1.06 | 1.09 | 97.2 |
| | 5.5 | 6.070 | 0.25 | 0.23 | 110.4 |
| PFHpS | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 20.96 | 18.27 | 114.8 |
| | 0.055 | 0.057 | 9.51 | 9.14 | 104.0 |
| | 0.55 | 0.590 | 8.60 | 8.02 | 107.2 |
| | 5.5 | 6.223 | 4.30 | 3.80 | 113.1 |
| PFOS | Blank | 0.001 | | | |
| | 0.055 | 0.053 | 5.12 | 5.28 | 96.9 |
| | 0.55 | 0.517 | 1.77 | 1.88 | 94.0 |
| | 5.5 | 5.700 | 1.03 | 0.99 | 103.6 |
| PFNS | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 5.17 | 4.42 | 117.0 |
| | 0.055 | 0.052 | 3.68 | 3.86 | 95.1 |
| | 0.55 | 0.526 | 3.20 | 3.35 | 95.6 |
| | 5.5 | 6.117 | 4.19 | 3.76 | 111.2 |
| PFDS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 9.46 | 8.11 | 116.6 |
| | 0.055 | 0.056 | 0.87 | 0.85 | 102.6 |
| | 0.55 | 0.569 | 4.82 | 4.67 | 103.4 |
| | 5.5 | 6.148 | 4.56 | 4.08 | 111.8 |
| PFUnDS | Blank | ND | | | |
| | 0.0055 | 0.004 | 15.95 | 19.84 | 80.4 |
| | 0.055 | 0.056 | 5.75 | 5.66 | 101.5 |
| | 0.55 | 0.594 | 0.61 | 0.56 | 107.9 |
| | 5.5 | 5.614 | 0.85 | 0.84 | 102.1 |
| PFDoS | Blank | 0.000 | | | |
| | 0.0055 | 0.007 | 14.15 | 11.90 | 118.9 |
| | 0.055 | 0.056 | 5.90 | 5.80 | 101.7 |
| | 0.55 | 0.607 | 4.68 | 4.24 | 110.3 |
| | 5.5 | 5.949 | 2.90 | 2.68 | 108.2 |
| PFTrDS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 8.10 | 7.94 | 102.0 |
| | 0.055 | 0.057 | 4.16 | 4.03 | 103.2 |
| | 0.55 | 0.581 | 5.25 | 4.97 | 105.7 |
| | 5.5 | 6.228 | 3.96 | 3.50 | 113.2 |
| PFOSA | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 9.45 | 8.91 | 106.1 |
| | 0.055 | 0.055 | 3.80 | 3.81 | 99.8 |
| | 0.55 | 0.556 | 0.78 | 0.77 | 101.1 |
| | 5.5 | 5.973 | 1.75 | 1.61 | 108.6 |
| 9Cl-PF3ONS | Blank | 0.000 | | | |
| | 0.0055 | 0.004 | 7.22 | 8.57 | 84.3 |
| | 0.055 | 0.054 | 1.65 | 1.67 | 98.6 |
| | 0.55 | 0.570 | 0.78 | 0.75 | 103.6 |
| | 5.5 | 5.896 | 2.62 | 2.44 | 107.2 |
| 11Cl-PF3OUdS | Blank | 0.001 | | | |
| | 0.0055 | 0.006 | 5.66 | 5.06 | 112.0 |
| | 0.055 | 0.054 | 1.81 | 1.85 | 97.8 |
| | 0.55 | 0.546 | 3.03 | 3.06 | 99.2 |
| | 5.5 | 6.022 | 3.61 | 3.30 | 109.5 |
| HFPO-DA | Blank | 0.002 | | | |
| | 0.0055 | 0.005 | 6.55 | 6.59 | 99.4 |
| | 0.055 | 0.053 | 2.17 | 2.24 | 96.9 |
| | 0.55 | 0.517 | 3.93 | 4.18 | 94.0 |
| | 5.5 | 5.653 | 0.95 | 0.93 | 102.8 |

| | | | | | |
|---|---|---|---|---|---|
| DONA | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 2.04 | 2.07 | 98.7 |
| | 0.055 | 0.060 | 3.96 | 3.59 | 110.1 |
| | 0.55 | 0.619 | 2.37 | 2.10 | 112.6 |
| | 5.5 | 5.714 | 1.95 | 1.87 | 103.9 |
| 4:2 FTS | Blank | ND | | | |
| | 0.055 | 0.052 | 1.92 | 2.05 | 93.6 |
| | 0.55 | 0.535 | 0.35 | 0.36 | 97.2 |
| | 5.5 | 5.663 | 0.80 | 0.78 | 103.0 |
| 6:2 FTS | Blank | 0.018 | | | |
| | 0.055 | 0.048 | 4.12 | 4.66 | 88.4 |
| | 0.55 | 0.495 | 1.60 | 1.78 | 90.0 |
| | 5.5 | 5.290 | 1.26 | 1.31 | 96.2 |
| 8:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.005 | 5.61 | 6.54 | 85.8 |
| | 0.055 | 0.060 | 1.83 | 1.67 | 110.1 |
| | 0.55 | 0.609 | 3.59 | 3.25 | 110.6 |
| | 5.5 | 5.790 | 0.83 | 0.79 | 105.3 |
| 10:2 FTS | Blank | 0.000 | | | |
| | 0.0055 | 0.006 | 13.17 | 12.02 | 109.5 |
| | 0.055 | 0.065 | 2.75 | 2.34 | 117.8 |
| | 0.55 | 0.641 | 2.69 | 2.31 | 116.6 |
| | 5.5 | 6.102 | 4.75 | 4.28 | 110.9 |

ND = average results less than zero

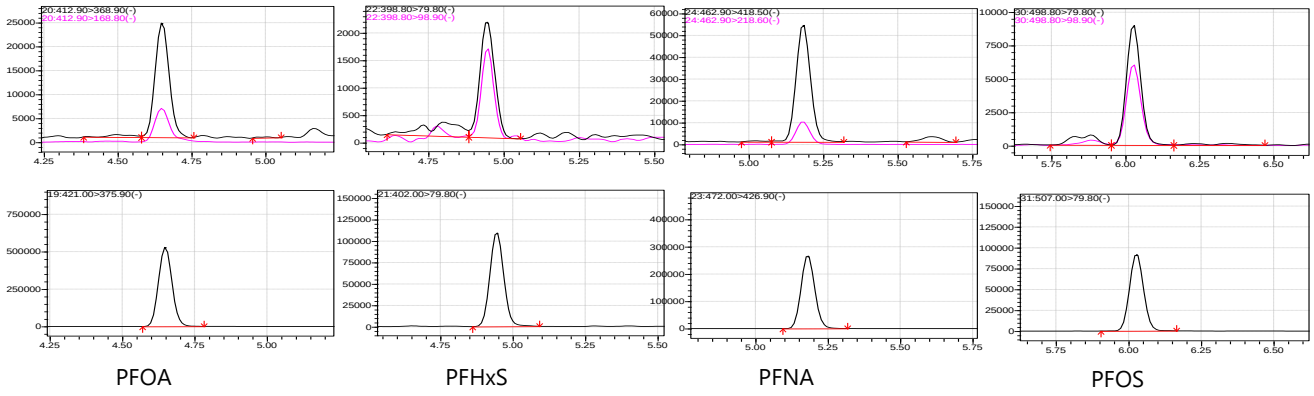



**Figure 7**: LOQ Peaks with Internal Standards

## ■ Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a shrimp matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

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ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

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