# Determination of 30 PFAS in Fish and Pet Food by LC-MS/MS

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## 1. Introduction

PFAS can enter the food through a contaminated environment and bioaccumulate in tissues. Contamination can also occur during food processing or with exposure to packaging. As PFAS have been linked to serious health effects, accurate testing methods are needed, especially for foods at risk of contamination like fish and pet food. In this work, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in fish and pet food using QuEChERS. Instrument sensitivity was sufficient to meet LOQ requirements without sample preconcentration . Preparation was followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to the LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

In this study, we spiked samples at three concentrations in triplicate. Standards were matrix-matched and extracted, spikes were quantified using isotope dilution. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR; including the stricter requirements for EU regulated compounds in fish tissue.

## 2. Methods

Samples were prepared by dicing the edible portion of locally purchased tuna filets and grinding with dry ice. Sub-samples of locally purchased dog food were removed from the packaging and crushed with dry ice for 30 seconds at 4,000 rpm. For both types of samples, the ground material was placed into containers and stored in a freezer overnight. Test portions were spiked in triplicate at three different concentrations with 30 native PFAS and 16 isotopically labeled internal standards.

For fish, 10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile were added. For pet food, only 2-gram portions were needed, spiked with target analytes and internal standards, then 10 mL of water and 10 mL of acetonitrile were added. The samples were shaken and a QuEChERS procedure was followed. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol. 0.5 mL were transferred to a vial and acidified with formic acid.

Adequate separation of all compounds was achieved in nine minutes, including separation of PFOA from potential cholic acid interferences (Figure 2). For this study, we 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 S/N ratio of PFOA, PFHxS, PFNA, and PFOS which are regulated PFAS in the EU.

**Fig. 1** Nexera™ and LCMS™-8060NX

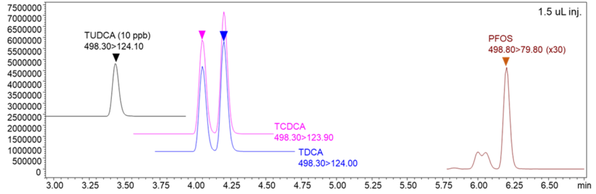


**Fig. 2** Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

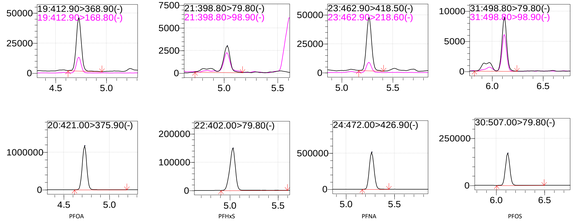


**Fig. 3** LOQ peaks with internal standards in fish for PFOA, PFHxS, PFNA, and PFOS, meeting the requirements for EU regulations of fish tissue

## 3. Results

Calibration standards were processed the same as samples. 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 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 the lowest concentration are given in Table 1 for fish and Table 2 for pet food.

The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023_003.

The LOQs for all matrixes and compounds were 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 S/N > 10, peaks with IS for fish tissue are shown in Figure 3. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ.

**Table. 1** Recovery and repeatability for each analyte at the lowest spike concentration in fish tissue

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | %RSD (n=3) | Average Recovery% |
|---|---|---|---|---|
| PFBA | 0.1 | 0.103 | 2.43 | 103.4 |
| PFPeA | 0.1 | 0.096 | 2.2 | 96.2 |
| PFHxA | 0.1 | 0.099 | 1.66 | 98.8 |
| PFHpA | 0.1 | 0.1 | 0.44 | 100.1 |
| **PFOA** | **0.1** | **0.1** | **1.51** | **99.6** |
| **PFNA** | **0.1** | **0.101** | **2.72** | **100.9** |
| PFDA | 0.1 | 0.098 | 3.42 | 98.2 |
| PFUnA | 0.1 | 0.1 | 1.65 | 99.8 |
| PFDoA | 0.1 | 0.093 | 11.29 | 92.9 |
| PFTrDA | 0.1 | 0.107 | 8.05 | 106.4 |
| PFTeDA | 0.1 | 0.114 | 7.23 | 114.1 |
| PFBS | 0.1 | 0.1 | 2.78 | 99.7 |
| PFPeS | 0.1 | 0.092 | 9.85 | 92.4 |
| **PFHxS** | **0.1** | **0.105** | **6.21** | **104.9** |
| PFHpS | 0.1 | 0.099 | 3.88 | 99.1 |
| **PFOS** | **0.1** | **0.102** | **1.85** | **102.2** |
| PFNS | 0.1 | 0.101 | 6.26 | 100.4 |
| PFDS | 0.1 | 0.097 | 4.24 | 96.7 |
| PFUnDS | 0.1 | 0.106 | 12.58 | 105.3 |
| PFDoS | 0.1 | 0.114 | 14.59 | 114.1 |
| PFTrDS | 0.1 | 0.119 | 12.41 | 119.1 |
| PFOSA | 0.1 | 0.096 | 2.25 | 96 |
| 9Cl-PF3ONS | 0.1 | 0.097 | 1.17 | 97.2 |
| 11Cl-PF3OUdS | 0.1 | 0.097 | 6.82 | 97.3 |
| HFPO-DA | 0.1 | 0.102 | 5.91 | 101.9 |
| DONA | 0.1 | 0.1 | 1.43 | 99.6 |
| 4:2 FTS | 0.1 | 0.099 | 3.77 | 98.9 |
| 6:2 FTS | 0.1 | 0.098 | 2.98 | 97.7 |
| 8:2 FTS | 0.1 | 0.107 | 4.02 | 107.2 |
| 10:2 FTS | 0.1 | 0.11 | 5.21 | 109.4 |

Bolded are the 4 PFAS regulated in the EU in fish tissue; the stricter requirements of the SMPR for this matrix are met using this method.

**Table. 2** Recovery and repeatability for each analyte at the lowest spike concentration in pet food

| Analyte | Spike conc. (ppb) | Average conc. (ppb) | %RSD (n=3) | Average Recovery% |
|---|---|---|---|---|
| PFBA | 0.5 | 0.541 | 12.66 | 108.2 |
| PFPeA | 0.5 | 0.514 | 0.63 | 102.8 |
| PFHxA | 0.5 | 0.502 | 2.65 | 100.3 |
| PFHpA | 0.5 | 0.559 | 3.35 | 111.8 |
| PFOA | 0.5 | 0.525 | 2.68 | 105 |
| PFNA | 0.5 | 0.513 | 0.74 | 102.8 |
| PFDA | 0.5 | 0.525 | 1.14 | 105.1 |
| PFUnA | 0.5 | 0.541 | 1.16 | 108.3 |
| PFDoA | 0.5 | 0.482 | 5 | 96.4 |
| PFTrDA | 0.5 | 0.49 | 6.51 | 97.9 |
| PFTeDA | 0.5 | 0.508 | 0.99 | 101.7 |
| PFBS | 0.5 | 0.533 | 10.8 | 106.7 |
| PFPeS | 0.5 | 0.428 | 15.89 | 85.6 |
| PFHxS | 0.5 | 0.539 | 2.07 | 107.7 |
| PFHpS | 0.5 | 0.494 | 2.64 | 98.7 |
| PFOS | 0.5 | 0.475 | 6.84 | 95 |
| PFNS | 0.5 | 0.512 | 7.01 | 102.3 |
| PFDS | 0.5 | 0.512 | 9.57 | 102.3 |
| PFUnDS | 0.5 | 0.531 | 2.84 | 106.1 |
| PFDoS | 0.5 | 0.502 | 5.57 | 100.5 |
| PFTrDS | 0.5 | 0.511 | 5.87 | 102.1 |
| PFOSA | 0.5 | 0.523 | 0.91 | 104.7 |
| 9Cl-PF3ONS | 0.5 | 0.494 | 2.54 | 98.9 |
| 11Cl-PF3OUdS | 0.5 | 0.501 | 1.86 | 100.1 |
| HFPO-DA | 0.5 | 0.36 | 2.66 | 72 |
| DONA | 0.5 | 0.493 | 6.59 | 98.7 |
| 4:2 FTS | 0.5 | 0.582 | 4.29 | 116.4 |
| 6:2 FTS | 0.5 | 0.545 | 3.47 | 109 |
| 8:2 FTS | 0.5 | 0.543 | 0.79 | 108.6 |
| 10:2 FTS | 0.5 | 0.502 | 10.08 | 100.4 |

Pet food is not additionally regulated.

## 4. Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph measured 30 PFAS compounds in fish and pet food meeting criteria set by AOAC SMPR 2023.003, including stricter requirements for 4 PFAS that are regulated in the EU for fish. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between isomers, and a separation between PFOS and potentially interfering cholic acids in only nine minutes.