Agilent InfinityLab

Workflow Ordering Guide

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Edible Fish Tissue

PFAS are a group of more than 5,000 synthetic organofluorine chemicals used in industry and consumer products since the 1940s. Commercial applications of PFAS include cosmetics, food packaging, nonstick cookware, firefighting foams, electronic devices, aircraft, vehicles, and various textiles. Their chain of strong carbon-fluorine bonds makes them resistant to environmental degradation, and thus pervasive, persistent, and bio-accumulative over long-term exposure. Contaminated water and food, including seafood, are considered the main exposure routes for humans to PFAS.

The European Commission has established maximum levels for PFAS allowed in certain foods[1], laid out in Regulation (EC) 2022/2388,[2] that went into effect as of January 2023.

The regulation specifically focuses on limits for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS), which are types of PFAS that are or have been widely used for commercial and industrial purposes. The regulation includes information on the maximum levels allowed for each of these chemicals in fish muscle meat. Fish meat intended for the production of foods for infants and young children must not exceed 2 mg/kg, while fish meat intended for adults must not exceed 8 mg/kg.

While the FDA has not established a safe level of PFAS in fish, several states have issued fish consumption advisories based on PFOS levels in freshwater fish.[3] The levels, however, vary from state to state.

Analysis of fish tissue extracts can be challenging due to the presence of matrix interferences such as fats and lipids. The Captiva EMR–Lipid pass-through cleanup technique efficiently removes major lipid classes without analyte loss. Removal of lipid interferences assures minimal matrix ionization effects and improves method reliability and ruggedness. Agilent scientists and collaborators have developed a rugged extraction method for the determination of 25 PFAS in a large-scale study comprising 25 fish species using Captiva EMR-Lipid (Figure 1) followed by LC/MS/MS analysis (Tables 1 & 2).[4]

Achieve uncompromising accuracy with Agilent PFAS analysis solutions that include:

1. Our InfinityLab PFC-free HPLC conversion kit* includes everything you need to ensure your 1290 Infinity II instruments and 1290 Infinity II high-speed pump are free of PFAS contaminants:
   - Tubing
   - Inline filter
   - Bottle head assembly
   - Delay column with InfinityLab Quick Connect LC fitting
2. Sample preparation solutions, including Bond Elut PFAS WAX solid phase extraction (SPE) cartridges and Carbon S products
3. PFAS chemical standards
4. Analytical HPLC columns
5. PFAS-free HPLC supplies and solvents

** Although the kit is customer-installable, Agilent offers supplemental installation by a service professional. To add this service to your order, ask for part number H5949A.*

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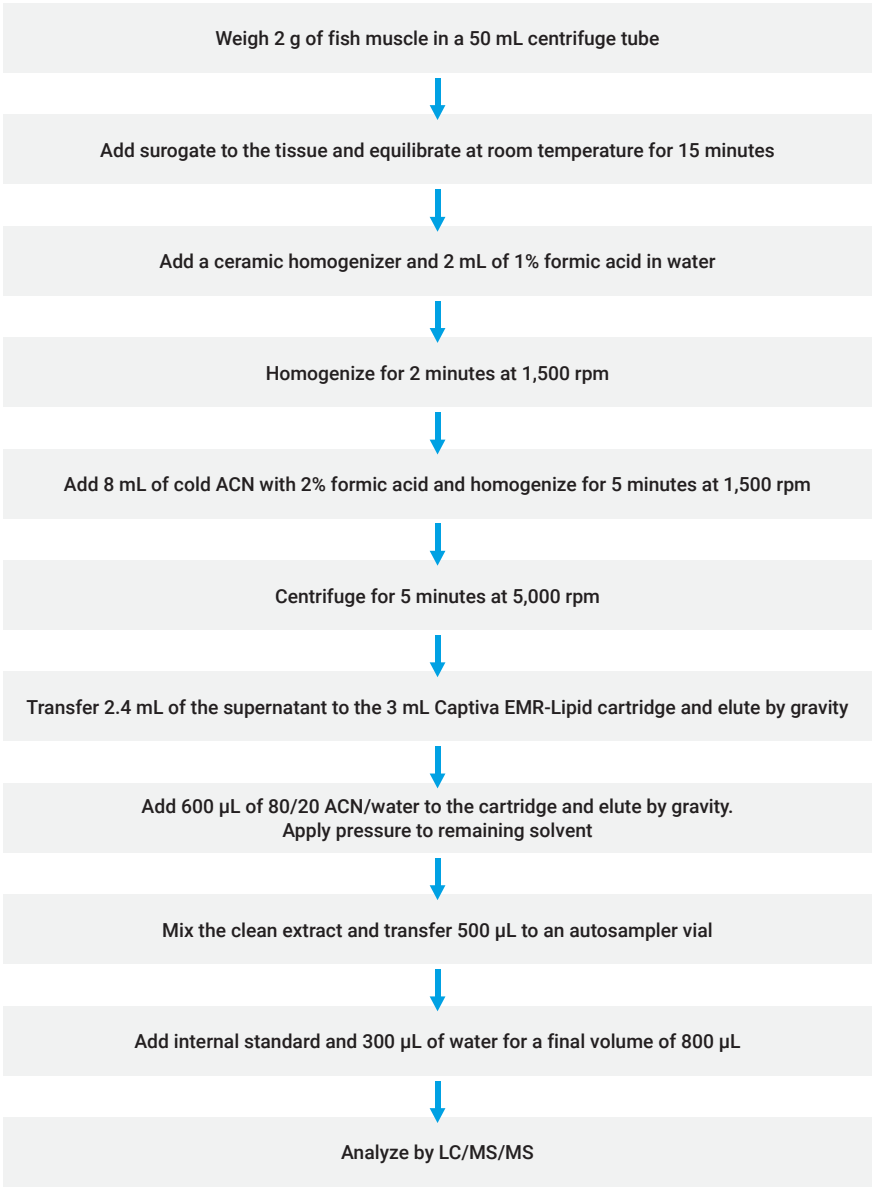

Figure 1. Sample preparation workflow diagram[4]

Table 1. LC conditions.[4]

Parameter	Value
LC	Agilent 1290 Infinity II LC
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) with Agilent InfinityLab Quick Change inline filter, 0.2 µm (p/n 5067-1603)
Delay Column	Agilent ZORBAX Eclipse Plus 95 Å C18 column, 4.6 × 50 mm, 3.5 µm (p/n 959943-902)
Column Temperature	50 °C
Injection Volume	20 µL
Mobile Phase	**A)** 20 mM ammonium acetate in 95/5 water/ACN **B)** 10 mM ammonium acetate in 95/5 ACN/water
Gradient	**Time (min) / % A / % B / Flow (mL/min)** 0 / 100 / 0 / 0.300 6 / 70 / 30 / 0.300 9 / 50 / 50 / 0.300 16 / 15 / 85 / 0.300 17 / 0 / 100 / 0.300 20 / 0 / 100 / 0.300 21 / 100 / 0 / 0.300 31 / 100 / 0 / 0.300

Table 2. MS conditions.[4]

Parameter	Value
MS	Agilent 6470B triple quadrupole LC/MS with Agilent Jet Stream ESI source
Source Parameters	
Polarity	Negative
Drying Gas	230 °C, 4 L/min
Sheath Gas	250 °C, 12 L/min
Nebulizer Gas	15 psi
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

Best Practices for PFAS Analysis Using LC/MS/MS

Polytetrafluoroethylene (PTFE), used in all major UHPLC systems to ensure chemical inertness, can be a major source of contamination during analysis, resulting in high levels of PFAS background that exceed regulatory quantification and detection limits.

Common sources of PFAS contamination & background interference

- HPLC pump head and seals
- Solvent lines and tubing
- Solvents
- Filters
- Vials and caps (PTFE-containing septa)
- Fluoropolymer tubing in sample preparation devices
- Bottles and bottle caps
- Labware

Steps to reduce PFAS background from the system[6]

1. Use the PFC-free LC (5004-0006) conversion kit to replace PFC materials from the system. The PFC-free conversion kit includes:

    a. InfinityLab PFC delay column which delays potential PFC impurities from the mobile phases and across the analytical run. Install the delay column after the UHPLC pump but before the injector/autosampler and should not be used as a separation column.

    b. PFC-free bottle head assembly (5004-0004), including: PP solvent line; stainless steel solvent inlet filter (01018-60025), PEEK ferrule 1/8" with stainless steel lock ring (0100-1919), PFC-free InfinityLab Stay Safe cap with PP cap insert and PP tubing fitting

    c. Pump head adapter assembly, does not contain filter (G1312-60001)

    d. InfinityLab Quick Change inline filter (5067-1602) with PEEK/stainless steel filter discs (5067-1613). Use of the inline filter upstream of the delay column removes particulates and percipitates, preserving delay column life.

    e. PFC-free multi wash tubing kit (5004-0003) including: PEEK tubing (0890-1761) cut to length, PEEK ferrules 1/16" with stainless steel lock ring (0100-1690)

    f. PFC-free vials and caps, tested for PFAS applications: 2 mL polypropylene vials (5191-8150) and compatible screw caps with bilayer polypropylene/silicone septa (5191-8151), allowing multiple injections from individual vials due to resealing capability. Standard vial caps containing PTFE membrane septa would add PFAS background.
2. The degasser and solvent selection valve should be bypassed as part of the installation. Agilent suggests vacuum degassing or helium sparkling to degas the mobile phases, if necessary.
3. Since PFAS can stick to the needle and needle seat after high concentration injections, make sure to use the multi-wash program with at least 12-20 second wash cycles of the needle and needle seat. If necessary, use multiple wash solvents. To check for high PFAS contamination in the needle or seat, perform an injection in bypass mode. If contamination disappears, replace the needle and needle seat.
4. Ensure the solvents being used are free of PFAS by using cellulose filter membranes when filtering solvents.

Easy selection and ordering information

To order items from the table below, click on "View MyList" and add them to your Favorite Products. Your favorite products list will be saved for future orders. If it is your first time using Favorite Products, you will need to provide your email for account verification. Existing Agilent account holders can log in, while new users will need to register. This feature is available only in e-commerce enabled regions. You can also order the items in the table through your regular sales and distributor channels.

Analysis of Per and Polyfluoroalkyl substances in edible fish tissue using Captiva EMR-Lipid and LC/MS/MS[4]. View MyList.

Product Category	Description	Part Number
Sample preparation	Captiva EMR-Lipid cartridge	5190-1003
	Ceramic homogenizers, 50 mL tubes, 100/pk	5982-9313
	Positive Pressure Manifold 48 (PPM-48)	5191-4101
	50 mL Centrifuge tubes and caps, 25/pk	5610-2049
	15 mL Centrifuge tubes and caps, 50/pk	5610-2039
Analytical column	ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 100 mm, 1.8 µm, 1200 bar pressure limit, 1/pk	959758-902
Guard column	ZORBAX RRHD Eclipse Plus C18 2.1 × 5 mm, 1.8 µm guard, 3/pk	821725-901
PFC-free LC kit w/delay column	InfinityLab PFC-free HPLC Conversion Kit	5004-0006
Delay column	InfinityLab PFC Delay Column, 4.6 x 30 mm, 1200 bar (replacement)	5062-8100
Other HPLC supplies	InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC	5067-5957
	Stainless steel solvent inlet filter, 12-14 µm (Solvent inlet filter for seal wash)	01018-60025
	InfinityLab Quick Change inline filter assembly, for UHPLC*	5067-1603
	InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC	5067-6166
	Quick Turn capillary 0.12 x 280 mm, for connection from column to detector	5500-1191
	InfinityLab Quick Turn LC fitting (fitting for p/n 5067-5957)	5067-5966
	InfinityLab solvent bottle, clear, 1000 mL, with cap	9301-6528
	InfinityLab solvent bottle, clear, 125 mL, with cap	9301-6527
	InfinityLab solvent bottle, clear, 500 mL, with cap	9301-6523
	InfinityLab Stay Safe starter kit including Stay Safe cap (GL45 thread, 4 ports) and 6 L waste can	5043-1221
	InfinityLab Stay safe charcoal filter with time strip, for 6 L waste can	5043-1193
Vials and caps	2 mL polypropylene screw style vials, certified PFC-free, 100/pk	5191-8150
	9 mm screw style cap with polypropylene/silicone septa, certified PFC-free, 100/pk	5191-8151
Solvent filtration supplies**	IInfinityLab solvent filtration assembly (includes glass funnel 250 mL, membrane holder glass base, glass flask 1 L, and aluminum clamp)	5191-6776
	Regenerated Cellulose Filter membrane 47 mm, 0.20 µm, 100/pk	5191-4340
Solvents	InfinityLab Ultrapure LC/MS Acetonitrile, 1 L (for needle wash)	5191-4496
	LC/MS grade MeOH, 1 L (for organic mobile phase, SPE reagent, diluent, and needle wash)	5190-6896
	InfinityLab Ultrapure LC/MS Water, 1 L	5191-4498
Standards***	PFC Calibration Standard (For use with system performance evaluation, troubleshooting, and ongoing system evaluation)	ITA-70

* Recommended between guard cartridge and analytical column.

** If using solvents other than those listed in this table, use the InfinityLab Solvent Filtration assembly to filter prior to analysis.

*** More information at: www.agilent.com/chem/standards

References

1. Food Safety Magazine. 2022. EU Sets Limits for PFAS in Certain Foods.
2. Publications Office (europa.eu). 2022. Regulation (EU) 2022/2388, Official Journal of the European Union.
3. ACS Publications. 2023. Toxic PFAS found in freshwater fish. Environmental Science & Technology, 101(3).
4. Pulster E. L., & Giardina M. 2022. Analysis of Per and Polyfluoroalkyl substances in edible fish tissue using Captiva EMR-Lipid and LC/MS/MS, *Agilent Technologies application note*, publication number 5994-5227EN.
5. Frontiers. 2022. Assessing per- and polyfluoroalkyl substances (PFAS) in sediments and fishes in a large, urbanized estuary and the potential human health implications. Frontiers in Marine Science, volume 9, article 1046667.
6. Kamuf M. *et al.* 2021. Reduce PFAS Background with Agilent PFC-free HPLC conversion kit. An ideal solution for trace level PFAS analysis with LC/MS/MS, *Agilent Technologies application note*, publication number 5994-2291EN.

Agilent CrossLab services

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