Holding Steady: Lessons from the PFAS Front Line

Food testing scientists share the lessons, technologies, and support that helped them achieve reliable PFAS results, and prepare for what comes next

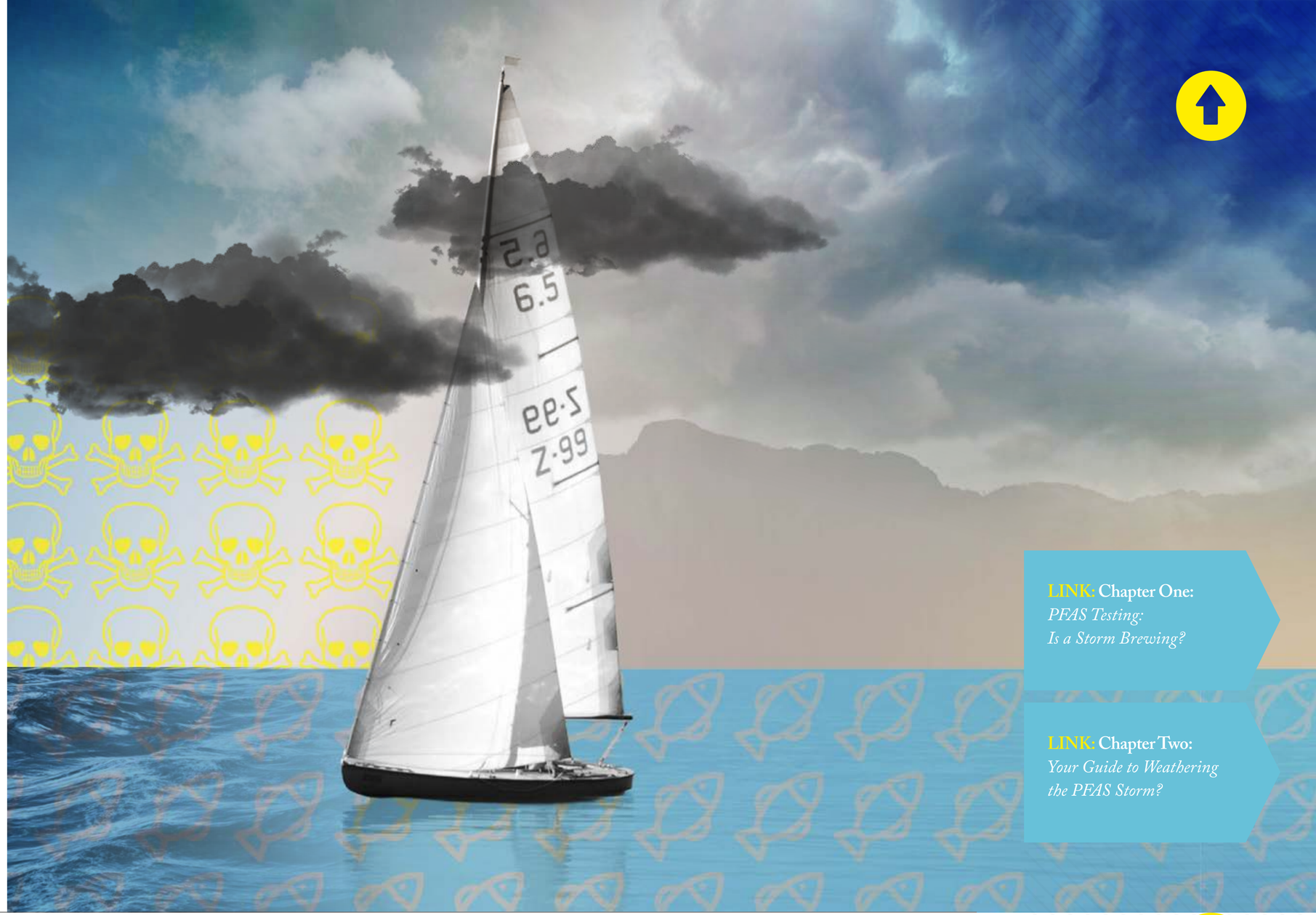


the Analytical Scientist x Agilent Technologies

PFAS testing in food remains shrouded in uncertainty as food manufacturers and testing labs attempt to navigate evolving regulations, the prospect of increased testing requirements, and the absence of the standardized methods available for environmental analysis. With no EPA method specifically designed for food, laboratories often need to develop and validate their own workflows – sometimes for individual matrices. As we discussed in the first chapter of our PFAS Testing eBook series, "Is a Storm Brewing?," the stakes are considerable, with PFAS-related consumer lawsuits on the rise.

Fortunately, practical solutions are available, as we examined in chapter two, "Your Guide to Weathering the PFAS Storm," which explored how laboratories can establish reliable, contamination-resistant PFAS testing workflows. Translating that guidance into everyday practice, however, is rarely straightforward. That's why, in this third chapter, we hear directly from scientists who have established PFAS testing programs and confronted the challenges firsthand.

Drawing on their experience using Agilent systems to analyze PFAS in food, our expert panel discusses initial surprises, setbacks, and lessons learned on their way to achieving reliable results. They also share advice for laboratories getting started and consider what still needs to change as target lists expand and regulatory expectations evolve.



LINK: Chapter One:
*PFAS Testing:
Is a Storm Brewing?*

LINK: Chapter Two:
*Your Guide to Weathering
the PFAS Storm?*

Meet the Experts



Guillermo Sánchez González, Chemical LC Coordinator, Analytica Alimentaria GmbH

Guillermo Sánchez González is a chemical engineer with more than 12 years of experience in liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (LC-HRMS). He joined Analytica Alimentaria in 2014 and now serves as Chemical LC Coordinator. Analytica Alimentaria is an independent laboratory with sites in Berlin, Germany, and Almería, Spain, analyzing more than 60,000 samples each year. Its work focuses primarily on detecting pesticides and contaminants in fruits, vegetables, and other foodstuffs. In his current role, González helps develop and implement LC methods to address emerging food safety challenges. This includes the laboratory's PFAS workflow for fruits and vegetables, developed in response to new European guidance and regulatory limits. His focus is on achieving the sensitivity, contamination control, and quality assurance required to produce reliable results at very low concentrations.



Natalia Soares Quinete, Associate Professor, Department of Chemistry and Biochemistry, Institute of Environment, Florida International University

Natalia Soares Quinete has a background in chemical engineering and analytical chemistry and leads the Emerging Contaminants of Concern Research Lab at Florida International University (FIU). Her research focuses on developing mass spectrometry methods for targeted and non-targeted analysis and applying them to emerging contaminants in environmental, biological, and food samples. She began studying PFAS during her PhD in 2007 and expanded this work from surface water to seafood after establishing her lab at FIU in 2020, investigating bioaccumulation and potential dietary exposure. Her food research has focused primarily on fish, shellfish, and other marine organisms. Alongside targeted analysis of regulated PFAS, her team uses high-resolution mass spectrometry for suspect and non-target screening to identify a broader range of contaminants.

Devon Firesinger, Senior Biological Scientist, University of South Florida

Devon Firesinger has a background spanning chemistry, marine science, and geochemistry, with a particular focus on anthropogenic environmental contaminants. At the University of South Florida College of Marine Science, Firesinger supports fieldwork, sample preparation, and laboratory instrumentation, including the use of triple quadrupole LC-MS to quantify PFAS in environmental and tissue samples. This PFAS research grew from broader efforts to understand emerging contaminants in Tampa Bay. Firesinger helps collect, process, archive, and analyze fish tissues and sediments, with a particular interest in popular recreational fish species, as well as oysters and barnacles. This work helps assess patterns of contamination and their potential implications for ecological and human health.



Lorna De Leoz, Global Food Segment Director, Agilent Technologies

Lorna De Leoz works with key opinion leaders, food testing laboratories, regulatory and standards groups, and applied scientists to understand the real-world challenges of PFAS analysis in food. Her previous experience as a bench scientist gives her a practical appreciation of method development, troubleshooting, and reliable data generation. In her current role, she helps translate customer insights and emerging regulatory trends into practical workflows that support confident results in complex food matrices.



Taking PFAS Seriously

By tracing our panelists' routes into PFAS research, we reveal how established analytical expertise is being adapted to meet the field's unusual technical and regulatory demands – from preventing background contamination and working with complex matrices to achieving ultralow detection limits and navigating an evolving regulatory landscape.

When and why did your organizations begin focusing on PFAS testing?

Natalia Soares Quinete: I started studying PFAS during my PhD in 2007, when they were still commonly referred to as PFCs. After joining FIU in spring 2020 and establishing my lab, I saw a major knowledge gap around PFAS environmental monitoring, distribution, and source characterization. Very little work was being done in Florida, particularly South Florida.

We began by trying to understand the distribution of PFAS in surface water, which naturally expanded into seafood. We wanted to determine whether PFAS in the water were accumulating in marine organisms, so we analyzed tuna, lobster, oysters, and other seafood. We also wanted to understand the potential importance of seafood as a dietary exposure pathway for humans.

Our extensive work with water, biota, and biosolids, along with our experience in LC-MS/MS, PFAS method development, and quantification, gave us a very good starting point. We were certainly not beginning from scratch. However, even our work with seafood proved much more complex than analyzing environmental samples. High lipid and fat content introduces challenges that you do not encounter to the same extent with water.

Guillermo Sánchez González: The key development for us was the publication of a guidance document by the European Reference Laboratory in May 2022, which recommended specific limits for PFAS that should not be exceeded in fruit and vegetables. The European Commission also introduced limits for four key PFAS compounds in foods of animal origin. From that point onward, my team and I began researching the subject and found that the migration of these chemicals into food was common. We therefore began developing an analytical method.

Devon Firesinger: I was not involved in the college's initial move into PFAS analysis. A previous postdoctoral researcher worked alongside application chemists from Agilent to develop an approach based on EPA Method 1633. They focused particularly on refining the EMR cartridge methodology for fish tissues.

We were already analyzing pesticides and other contaminants of emerging concern, so it made sense to add PFAS to our existing sampling efforts. We were well prepared from a sampling perspective, but updating the instrument, establishing all the correct transitions, and optimizing the system was a major undertaking.

What challenges are laboratories most likely to underestimate when establishing a reliable PFAS testing workflow?

Quinete: The greatest challenge for most people is probably



contamination. Obtaining a clean blank can be difficult. Most current methodologies involve many steps, and every step introduces another opportunity for contamination.

With food, you need to first homogenize the sample, then do an extraction and clean-up, each step creates the potential for contamination. You also need a delay column to separate PFAS originating from the solvents or system from PFAS in the sample.

Even seemingly minor choices can create problems. Researchers have reported PFAS leaching from certain brands of pipette tips, despite the tips being made from polypropylene and therefore not being expected to contain PFAS.

We have also found that some batches of solvent can contain high PFAS levels. Even when you buy from the same supplier, an individual bottle or lot can present a problem. Laboratories working with PFAS should always test their solvents before using them. We learned this the hard way: after encountering substantial contamination, we checked every reagent and eventually traced it back to the solvent.

Another challenge is the specificity of method validation. In our experience with seafood, you cannot necessarily develop one validated protocol for every type of seafood. A method developed for fish may need to be validated for a particular type of fish, and its performance can vary between species because the matrices differ. That was something we definitely underestimated.

Firesinger: When you consider how many PFAS compounds are out there, becoming an expert in measuring everything is a daunting prospect. Some compromise is inevitable; we obviously cannot measure 10,000 compounds. But my biggest learning curve was simply getting sufficient time on the instrument and running pilot samples through the workflow.

Sánchez: In my case, it was probably the extremely low limits that must be achieved when analyzing fruit and vegetables. Because PFAS are present in such a wide range of materials, preventing external contamination is crucial, particularly when working at the very low concentrations required in this area.

"Obtaining a clean blank can be difficult. Most current methodologies involve many steps, and every step introduces another opportunity for contamination."



Lessons from the Front Line

Sensitivity may be essential in PFAS analysis, but it is only one part of an effective testing solution. Here, our contributors discuss the importance of robust performance across complex matrices, effective sample cleanup and contamination control – and explain how Agilent technology and expertise have helped them meet these demands.

How did you determine which PFAS testing solution would best meet your laboratory's needs?

Quinete: We needed a robust system capable of analyzing environmental, biological, and food samples at low parts-per-trillion concentrations while maintaining reliable performance across complex matrices. We considered instruments from several vendors before selecting the Agilent 6470 triple quadrupole LC/MS, which offered the performance we needed without requiring extensive cleaning, maintenance, or troubleshooting.

We recently began participating in an interlaboratory study and initially wondered whether our system would provide sufficient sensitivity. A few minor adjustments were required, but we are now achieving very high sensitivity for all the compounds. I am very happy with its performance.

We had also begun implementing EMR-Lipid cartridges before the interlaboratory study. Although these were not the newer PFAS-specific cartridges, we tested them on seafood and saw a considerable improvement in cleanup compared with our previous approach.

Previously, we used graphitized carbon together with magnesium silicate. That approach provided some cleanup, but it did not remove as much fat. When we froze the samples, we would still see a precipitating forming. So far, the Agilent system has met all our requirements.

Agilent has also been very helpful whenever we have needed assistance. Customer service has been excellent, and we have had access to several application notes that have supported our work in different ways.

Sánchez: In our analysis of fruit and vegetables, we monitor the four main PFAS regulated in Europe, along with four additional compounds that we consider relevant to vegetable samples. For several years, we have also analyzed difluoroacetic acid (DFA) – an ultrashort-chain PFAS and the principal metabolite of the pesticide flupyradifurone – using a separate method. More recently, we developed and validated a high-resolution method specifically for TFA in vegetables.

TFA produces only one useful fragment, which makes confident identification more difficult. Using the Agilent QTOF system gives us the high mass accuracy needed to report positive results with greater confidence.

Agilent also supported the implementation of our broader PFAS method by providing its available application notes and an HPLC conversion kit containing fluorine-free components, which helped us minimize contamination originating from the instrument.

Firesinger: EPA Method 1633 provided a broadly accepted framework for bringing some consistency to what had previously been a "Wild West" of laboratory approaches. Because Method 1633 specifies weak anion exchange (WAX) cartridges, we tried those. However, we did not achieve the same recoveries as I had with the EMR cartridges. When Agilent introduced the newer cartridges, I found them much better. The pass-through method is simpler than conditioning, loading, washing, and eluting from a conventional solid-phase extraction cartridge.

When I compared batches of the same samples using the two approaches, the difference in the time required – including for evaporation – was striking. That was one aspect of PFAS analysis that surprised me. I was accustomed to processing many samples, but that is much harder with PFAS workflows.

Which elements of the Agilent PFAS workflow have made the greatest practical difference in your laboratory, and what has been the overall impact of implementing the workflow?

Sánchez: One of the most important considerations is having access to the latest technology required for PFAS analysis – we have implemented our PFAS method on an Agilent 6495C system and have recently acquired a 6495D, which offers improved sensitivity, which could be very useful for PFAS analysis. But if I had to highlight one key element from Agilent, I would choose the software; MassHunter makes the work much easier.

Firesinger: We installed the PFAS-free conversion kit and made the other necessary adaptations. In addition to the substantial time savings I mentioned, we also try to make our work as environmentally sustainable as possible. Some analytical processes use large quantities of solvent and plastic, so any opportunity to reduce that consumption is valuable. Agilent quantified those savings in a recent

application note, and they were considerable.

Quinete: Although we have not yet fully implemented the PFAS workflow designed by Agilent, the sample preparation method appears very simple. From watching the demonstration video, I do not think it would require extensive training. It is a straightforward protocol that any graduate student or other researcher could follow in the laboratory.

I am particularly excited about the Captiva EMR PFAS Food cartridge. It looks very promising for cleaning up challenging matrices such as meat, milk, and eggs. We look forward to testing it and seeing how it could improve our workflow.

Lorna De Leoz: At a recent conference, I saw a presentation from a leading food testing laboratory that had implemented the complete Agilent workflow, from the EMR cartridges and hybrid multisampler to the 6495D. In addition to the matrices covered in our application notes, that laboratory validated the workflow for infant formula and dietary supplements. The presenter particularly highlighted the hybrid multisampler, which allowed the laboratory to inject acetonitrile-rich extracts without drying them down while still obtaining good peak shapes. Seeing laboratories implement the complete workflow successfully and validate it for their own matrices demonstrates its broader practical impact.

Where has Agilent support proved most valuable?

Firesinger: I did not have as much time working alongside the previous postdoctoral researcher on the LC-MS system as I would have liked, so I had to teach myself quite a lot. I reached out to Limian Zhao and Emily Parry at Agilent, and they were crucial in helping me understand the PFAS workflow (check out Chapter Two, "Your Guide to Weathering the PFAS Storm," which features both Limian and Emily).

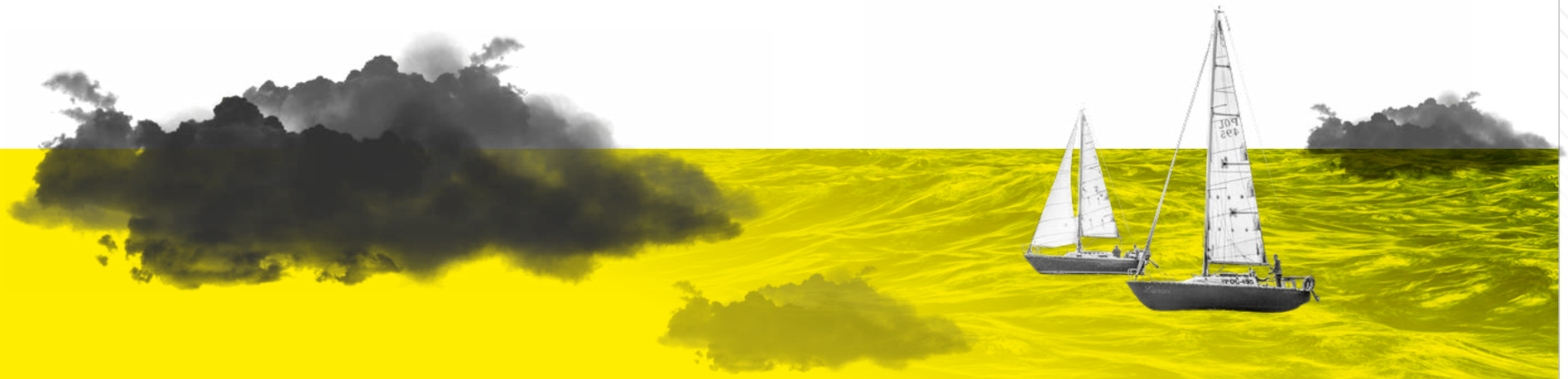
I had already extracted many samples for the previous postdoc, but I needed support with routine LC maintenance and best practices for dealing with instrument downtime. Emily spent considerable time helping me establish a quantitative method. She also helped me configure the extracted and non-extracted internal standards in an older version of the quantitative analysis software, which was essential to getting the method working. Her assistance was crucial in getting the quantitative method working, and the workflow is now much more streamlined.

I would also highlight the work of the field engineers. Problems outside your control inevitably occur, including power surges, instrument shutdowns, turbo-pump failures, and quadrupole-driver faults. I am not an expert in diagnosing those issues, so having field engineers who work tirelessly to support us at critical times is extremely valuable.

Quinete: The Agilent team has been very supportive throughout the interlaboratory study. We have weekly meetings where we provide feedback, and the team offers guidance on setting up the methodology and troubleshooting any problems.

My group has worked with PFAS for a long time, so we already had our own methodology. Moving away from your established method and implementing someone else's is surprisingly difficult. Initially, we were not seeing peaks with the new method, even though they appeared when we ran our existing method. Agilent helped us investigate those problems and suggested adjustments and alternative approaches to try. The team has been very helpful with every challenge we have encountered during method development.





Lessons from Difficult PFAS Samples

Quinete: We have encountered significant challenges with seafood and other biological samples. We have analyzed different types of tissue, including high-lipid tissues such as kidney and liver, and those can be disastrous.

We were assessing the distribution of PFAS across tissues from larger marine animals, including dolphins and manatees. These are very challenging matrices because of their high lipid and protein content. During method development and validation, one of my postdoctoral researchers ran around 50 calibration samples, quality controls, and other samples.

By the following day, the instrument cone was a complete mess, and its performance had declined substantially. We had to stop the run and clean the source and capillary. The matrix cleanup had clearly been insufficient; we could see how dirty the system had become and how its performance deteriorated during the run.

At the time, we did not know about the Agilent EMR cartridges. We were using dispersive solid-phase extraction, but we found that diluting the samples before reinjection improved matters. A dedicated cartridge would probably have been a better solution, and that is something we would now implement.

These extracts can also be intensely colored. Lipids are therefore not the only concern – the strong pigmentation may require additional cleanup steps.

Firesinger: Fish muscle tissue has not generally been problematic, although bile salts are a known concern. You need to ensure that they elute well away from the PFOS peak.

In oyster tissue, we observed a substantial peak eluting close to a different PFAS compound. It had a very similar mass and initially appeared to indicate a high concentration of that PFAS in the oysters. However, it eluted at a different retention time, and we identified it as a possible unsaturated fatty acid. It was simply a matrix component with very similar characteristics to the PFAS compound.

Quinete: What has been described by Devon illustrates an important point for anyone working with biological or food samples. If you observe an unexpectedly high concentration, you need to consider whether it could be an interference.

Many food analysis methods now monitor additional transitions associated with fatty acids to ensure that they do not coincide with the PFAS peaks. This is particularly important for some short-chain PFAS, although a fatty acid has also been reported with the same transition as PFOS. Laboratories need to watch carefully for these "ghost peaks."

De Leoz: We have seen this in eggs and other animal-derived foods, where naturally occurring bile acids can interfere with PFOS measurements because they overlap with PFOS transitions. They can be separated chromatographically, but mass spectrometry alone is not always sufficient – particularly when using a triple quadrupole instrument for targeted analysis.

Quinete: We use a pentafluorophenyl, or PFP, column, which provides better separation for halogenated compounds compared to the more commonly used C18 column.

We also monitor relevant fatty acid transitions and have not observed them eluting at the same time as our PFAS compounds. However, when we first began this work, someone asked how we knew that a particular peak was genuinely the PFAS rather than a fatty acid. We therefore ran additional tests and incorporated the other transitions into our method to confirm peak identity.

Best Practices and Future Priorities

Reliable PFAS testing starts with the fundamentals: fit-for-purpose instrumentation, rigorous contamination control, matrix-specific validation, and quality checks at every stage. But laboratories must also prepare for a moving target, with broader compound lists, limited reference materials, fragmented regulations, and growing demand for standardized, automated, and more comprehensive methods.

What practical advice would you give a laboratory establishing its first PFAS testing workflow?

Quinete: Choosing instrumentation is difficult when the budget is limited. You might purchase a triple quadrupole LC-MS system capable of reaching extremely low concentrations or choose a Q-TOF, which may not achieve the same sensitivity, but allows you to perform a broader range of analyses and provides greater mass accuracy. That additional accuracy can give you more confidence that you are genuinely detecting a PFAS rather than a fatty acid or another interference.

For most laboratories, the hardest part is getting started. Before trying to reach very low concentrations, you need a strong foundation for controlling contamination. Without that, you will never obtain reliable results. You need clean blanks and a robust set of quality controls from the beginning.

Laboratories should invest time in analyzing solvent, procedural, and equipment blanks to identify potential contamination sources. PFAS analysis is rigorous. We begin with a calibration curve and regularly analyze continuing calibration verification standards, as well as an initial calibration verification standard. Where available, we also use certified reference materials.

Food analysis introduces further requirements. Laboratories need to account for matrix effects and validate the method for each type of food matrix being analyzed.

Sánchez: I would add four quality control recommendations. First, include a calibration curve in every batch that covers the required quantification limit for each compound, together with an appropriate blank. Second, inject a quality control sample at the end of the sequence to confirm that all samples have been quantified correctly. Third, correct each result using the corresponding isotope-labeled internal standard. Finally, whenever you obtain a positive result, repeat the entire analytical process to confirm it.

De Leoz: I agree with the importance of running blanks consistently and questioning everything. Every material, reagent, consumable, and even the water can be a potential source of contamination. Test and validate everything before incorporating it into the method.

Laboratories should also use a method appropriate to the matrix. Food



testing does not currently have the same prescriptive methods that apply to some environmental analyses, such as EPA Methods 537 and 1633. The workflow should therefore be designed and validated specifically for food.

Firesinger: Sample preparation for PFAS analysis is slow, so my main advice is to choose equipment that provides sufficient throughput. The aim is to keep the LC-MS system supplied with samples rather than allowing sample preparation to become a bottleneck.

What needs to change to support the future of PFAS testing?

Firesinger: One important gap is the availability of certified reference materials containing a sufficiently broad range of PFAS. When I began validating the method, it was difficult to find a fish material with known concentrations. The available materials generally have certified values for only a few compounds, with reference values for several others.

I also use a less expensive in-house reference material. By analyzing it consistently, we can establish an expected value and track performance over time. However, a certified value is still preferable, despite the cost.

Quinete: I completely agree. We also need isotope-labeled internal standards for more of the PFAS we are trying to measure. The current food analysis list includes around 30 compounds, and exact internal standards are still unavailable for some of them.

There is also a clear need for standardized methodology, which is one reason the Agilent-led interlaboratory study is important. Many laboratories currently follow EPA Method 1633, even though it is not ideal for food, while laboratories with more food analysis experience may use different protocols.

Even if laboratories follow different protocols, we still need agreement on questions such as the acceptable surrogate-recovery range for each compound. Method 1633 was established through work involving multiple laboratories, allowing its performance and variability to be characterized. We need the same kind of interlaboratory evidence for food analysis.

More automated protocols would also help reduce the potential for human error during sample preparation.

Finally, non-target analysis needs to become a more established part of the toolbox. When we measure only 30, 40, 50, or even 100 PFAS, we are missing much of the picture because thousands of other compounds may be present. Some laboratories, including ours, are conducting non-target analysis to an extent, but it is still far from routine.

Sánchez: I agree with Natalia. More than 12,000 compounds fall within the PFAS category, and it is impossible to monitor all of them individually.

One emerging approach is to monitor the terminal products into which PFAS precursors ultimately degrade, including difluoroacetic acid and trifluoroacetic acid. In my view, this is a sensible strategy because these compounds can indicate the use or presence of their precursors. I am not suggesting that long-chain PFAS no longer need to be analyzed. However, measuring ultrashort-chain PFAS such as DFA and TFA may provide a more complete picture of exposure.

De Leoz: Another important gap is toxicological data. Not all PFAS are the same, and some are more toxic, persistent and bioaccumulative than others. We need a clearer understanding of which PFAS compounds present the greatest health concern so that monitoring, risk

"We need isotope-labeled internal standards for more of the PFAS we are trying to measure. The current food analysis list includes around 30 compounds, and exact internal standards are still unavailable for some of them."

assessment, and regulatory efforts can be prioritized effectively.

Regulatory requirements also remain fragmented. The European Union regulates four PFAS compounds, including their sum, in certain foods. Other regions and countries are still evolving their approaches, and regulatory scrutiny continues to increase. In the United States, for example, the US FDA has used Import Alert 99-48 to detain imported foods due to PFAS contamination concerns. The AOAC standard and US FDA's method include 30 PFAS analytes, while the USDA method includes 16 PFAS compounds.

Greater international harmonization, grounded in scientific evidence and toxicological data, is still needed for a clearer direction.

What are the key lessons laboratories should take from this discussion?

De Leoz: What stands out from this discussion is how many different factors can affect the reliability of PFAS analysis. Clean sample preparation, contamination control, matrix-specific validation, quality control, data analysis, and robust instrumentation all contribute to the final result. We have also heard clearly where laboratories still need more support – from standardized methods, reference materials, and isotope-labeled internal standards to greater automation, stronger interlaboratory evidence, and clearer toxicological data. As target lists continue to grow, laboratories will need workflows that can evolve with them, alongside new ways of identifying PFAS beyond established target lists. Continued collaboration between laboratories, regulators, researchers, and technology providers will be essential. The experiences shared here show how much progress has already been made – and where the community needs to focus next.



Technologies on the Front Line

The Agilent PFAS testing workflow, now AOAC Official Method 2025.11 (First Action), is designed to address the unique challenges of PFAS analysis in food. That workflow brings together food-specific sample preparation, PFAS-ready LC components, and sensitive LC-MS/MS detection.

Captiva EMR PFAS Food cartridges use a pass-through clean-up approach that retains matrix interferences while allowing PFAS analytes to pass into the final extract. The approach reduces steps such as conditioning and washing compared to conventional solid phase extraction workflows. By minimizing sample handling, laboratories can simplify PFAS sample preparation while reducing solvent consumption and potential opportunities for contamination or analyte loss. In addition, because the pass-through approach removes matrix components rather than trapping and recovering analytes, it can provide a more scalable approach as PFAS target lists continue to evolve.

The InfinityLab Hybrid Multisampler helps laboratories handle the acetonitrile-rich extracts produced by QuEChERS sample preparation. Its Feed Injection capability introduces small quantities of extract in a series of pulses, allowing strong-solvent samples to be injected without first being diluted or dried down. This can also improve the peak shape of early-eluting PFAS.

Elsewhere in the workflow, **PFAS-ready LC components** help control background contamination. The Agilent conversion kit replaces fluoropolymer-containing parts, while a delay column separates PFAS originating from the mobile phase or LC system from compounds present in the sample.

The Agilent 6495D Triple Quadrupole LC/MS is designed for the large multianalyte methods increasingly required for PFAS analysis in food. By maintaining sensitivity and precision at low dwell times, it supports the analysis of expanded PFAS panels without compromising data quality. The system is also engineered for mechanical robustness, with stable performance over more than 10,000 injections, supporting reproducible results in routine food testing laboratories.

Combined with the **MassHunter software**, these technologies provide an integrated route from sample preparation to final results.

LINK: PFAS Testing in Food, Beverages & Food Packaging