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Analyzing PFAS in Fruits and Vegetables

Overview of Current Analytical Approaches

Quantitation of PFAS in Chicken Eggs

Analytical Solutions for PFAS in Food

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Extraction and Matrix Clean-up Method for Analyzing Novel Per- and Polyfluoroalkyl Ether Acids and Other PFAS in Fruits and Vegetables

An efficient approach for evaluating levels of 45 PFAS in produce

Introduction

Per- and polyfluoroalkyl ether acids (PFEAs) are a subclass of per- and polyfluoroalkyl substances (PFAS) that are detected with increasing frequency in environmental matrices. Diet can be an important route of PFEA exposure, but the presence of PFEAs in food is poorly understood. Furthermore, extraction methods for food samples exist for traditionally studied PFAS, however their suitability for PFEAs and other novel PFAS remains unknown. Analytical complications arise because many PFEAs are unstable in aprotic polar solvents and the majority of them lack isotopically labeled internal standards. To address these challenges, a recent study developed an extraction and matrix cleanup method to quantify

45 PFAS, including 13 PFEAs, three perfluoroalkane sulfonamides, and six fluorotelomer carboxylic acids, in ten types of fruits and vegetables.[1] The method was then applied to the analysis of blueberries from gardens that were potentially exposed to PFAS by a nearby industrial site.

Method Development

The method focused on a total of 45 PFAS, including 11 perfluoroalkyl carboxylic acids (PFCAs, C4-C14), seven perfluoroalkyl sulfonic acids (PFSAs, C4-C10), ten per- and polyfluoroalkyl ether carboxylic acids (PFECAs), three per- and polyfluoroalkyl ether sulfonic acids (PFESAs), six fluorotelomer carboxylic acids (FTCAs), three fluorotelomer sulfonic acids (FTSs), three perfluoroalkane sulfonamides (FASAs), and two perfluoroalkane sulfonamido acetic acids (FASAAs). The fruits and vegetables used for method development were obtained from Whole Foods market. Six of the produce samples had high water content (blueberry, blackberry, grape, peach, squash, and tomato), two were high in starch (corn, potato), one contained high levels of fat (pecan), and one was high in fiber (okra).

WORKFLOW

In the first steps of the extraction and matrix cleanup protocol, frozen samples were homogenized with a stainless-steel tissue homogenizer before being spiked with internal standard (IS). Vortexing, sonicating, and centrifuging were then performed in triplicate in basic methanol. The three supernatants resulting from this step were then combined into a single vial and frozen overnight to precipitate out the starch. The extract was then diluted with water to achieve a solution with 8% methanol content.

A solid phase extraction (SPE) step using automated SPE clean up with WAX cartridge to remove color and other chemical interferences. The solvent was then evaporated under nitrogen and reconstituted in 5 M ammonium acetate in methanol/water (10:90 by volume) to correspond to the mobile phase in the liquid chromatograph (LC). 200 µL was injected into an Agilent 1290 Infinity HPLC with a Zorbax Eclipse Plus C18 column that was interfaced with an Agilent 6495 triple quadrupole mass spectrometer. Mobile phase A was 5 mM ammonium acetate in water and B was 5 mM ammonium acetate in methanol/water (5:95). A 28-minute gradient was run. Each sample was injected twice using two different sheath gas temperatures because some of the PFECAs disintegrated in the source at high temperatures, while other analytes such as the PFSAs responded better with higher temperatures. The low temperature method used a 250°C sheath gas whereas the high temperature method used 400°C.

INTERNAL STANDARDS

The method was validated by performing spike-recovery experiments at spike

levels of 1 ng/g in all ten matrices and 0.1 ng/g in two matrices. For PFAS without a corresponding isotopically labeled internal standard, using an IS with a similar chromatographic retention time generated the most accurate recoveries compared to structurally similar standards.

Spike recoveries at the 1 ng/g level obtained excellent recoveries (75-125%) for the compounds that had mass labeled analogs while those without isotopically labeled standards yielded a wider range of recoveries (50-150%). Overall, depending upon the matrix, recoveries of 38–44 PFAS (including 10–13 PFEAs) fell within 50–150% for samples spiked at 1 ng/g.

Recovery trends at the spike level of 0.1 ng/g were similar to the higher level, in that analytes with labeled standards had more accurate recoveries. The lower-level recoveries ranged 50–150% for 40 and 38 PFAS in blueberries and corn, respectively, indicating the method performed reasonably well even at low levels. Given their somewhat broad range of recoveries, results for compounds that lack mass labeled analogs as internal standards should be considered semi-quantitative.

METHOD QUANTIFICATION LIMITS

There are several approaches for determining method quantification limits (MQLs). These methodologies include an instrumental limit, a method blank-based limit, a matrix blank-based limit, and a matrix spike-based limit. The recent study combined the method blank-based limit with a correction factor for matrix effects. Although the most accurate approach is the matrix spiked-based method, that technique requires determination of a calibration curve in each individual matrix. The goal of the study, however, was to rapidly assess numerous different fruit and vegetable matrices which would make the matrix spiked-based method too labor intensive.

In the strategy employed in the study, the calibration curve was determined by preparing it in the solvent which was extracted through all the same steps as the sample. Procedural solvent blanks were used as well. For quality control, matrix spikes were included. In addition, the responses of the internal standards in the matrix were measured in order to obtain a matrix correction factor that allowed the MQL to be adjusted. The matrix factor was determined by comparing the response in a known matrix-spike sample with that in pure solvents. The MQL of a particular matrix was subsequently calculated by taking

the product of the matrix factor multiplied by the MQL for the solvent. Method quantification limits of PFAS in pure solvents were determined as the lowest calibration level with an accuracy between 70% and 130%. By using this approach, a balance of accuracy and time was achieved.

The MQLs of 45 PFAS, including 13 PFEAs, in the ten matrices had values ranging from 0.025 to 0.25 ng/g. For the more starchy and fatty matrices—potatoes and pecans—a larger sample mass was extracted which led to higher MQLs that were nonetheless very good.

Application to Home Grown Produce

Fluoroethers were emitted into the air and surface water through wastewater discharges by a fluorochemical manufacturer in North Carolina. These emissions led to contamination of public drinking water as well as private well water. Residents had concerns about the safety of the fruits and vegetables growing in their gardens and questioned whether the produce was impacted by fluoroether uptake from contaminated soil or irrigation water. To address community concerns, the new PFAS method was applied to eight blueberry samples that were obtained from homes in the vicinity of the fluorochemical plant from 2015 to 2019. Samples were collected from five different locations, designated A through E. Six PFEAs were detected at elevated levels with their sums ranging from 0.6 to 14.4 ng/g. The three most prevalent were PMPA, PFO2HxA, and PFMOAA, which were present at levels up to 8.5, 3.9, and 2.1 ng/g, respectively.

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There was further interest in determining whether the PFAS were contained primarily within the blueberries or on the external surface and if they could be removed by washing with either water or methanol. Unfortunately, measurements revealed that the PFAS levels did not change substantially after washing with either solvent, suggesting that >90% of the PFAS were contained within the bulk of the fruit. This demonstrated that washing the blueberries would not be effective for reducing human exposure.

To gauge what the detected levels meant in terms of human exposure, the high concentration of PMPA found in the blueberries was compared to measured levels in local drinking water. Nearby wells have measured 1000 ng/L of PMPA and higher; 1000 ng/L was used for the comparison. An eight-ounce glass of water containing 1000 ng/L PMPA equates to the consumption of 237 ng PMPA. Similarly, one ounce of contaminated blueberries with 8.51 ng/g PMPA equals an intake of 241 ng PMPA. Therefore, the two exposure routes are equivalent in terms of the mass of PMPA that is ingested. This suggests that dietary uptake could be an important additional exposure route to that of drinking water in this community.

Conclusion

A method was developed for the quantification of 45 PFAS, including 13 fluoroethers, in fruits and vegetables. The method was validated in ten different fruit and vegetable matrices. For PFAS for which an isotopically labeled internal standards were available, excellent spike recoveries were obtained in the range of 75-125%. For PFAS that lacked isotopically labeled internal standards, retention time based internal standards were utilized, the majority of which yielded acceptable recoveries of 50-150%.

The new method was then applied to the analysis of blueberries from a PFAS-impacted community in North Carolina. Elevated levels of six fluoroethers were detected in those blueberries. The investigation also found that washing the blueberries was inefficient in terms of reducing human exposure, as the contaminants were present in the bulk of the berries. A quick calculation revealed that ingestion of the blueberries delivered an equivalent PMPA exposure to that of drinking the community's contaminated water. Therefore, dietary uptake is another significant PFAS exposure route to be considered in that region. This application demonstrated the utility of the novel method for sensitively quantifying 45 PFAS in many fruits and vegetables and its potential value to human health.

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Overview of Current Analytical Approaches

for PFAS Screening in Food Items

Prevailing methodologies, recent advances, and case study

By LCGC Staff

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that have been under scrutiny for their widespread use and ubiquitous nature in the environment, including food. Potential sources of PFAS contamination in food include contact with food packaging materials, irrigation of farm products with polluted water, and the use of tainted soils and fertilizers for crops. To date, several studies have reported the presence of PFAS in meat, seafoods, vegetables, and fast foods, which raises considerable concern due to their persistence, bioaccumulation, and toxicity (PBT).

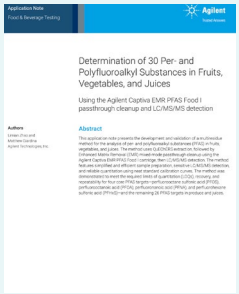
Several instrumental methodologies and separation techniques have been employed for the detection and quantification of PFAS, such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), Orbitrap MS, and ion mobility mass spectrometry (IMMS). Common separation techniques adopted for the extraction of PFAS in food items offer both advantages and limitations. Although knowledge gaps and research needs still exist, analytical approaches for the identification and quantification of PFAS in food items have benefited from recent advances.[1]

PFAS OVERVIEW

PFAS are a group of synthetic chemicals that have been used in a vast array of industrial and consumer products. Applications include water resistant clothing, non-stick cookware, food packaging, personal care products, and firefighting foams. Due to their persistent nature, they are frequently detected in a variety of environmental matrices such as air, water, and soil, as well as food. Their strong carbon-fluorine bonds give PFAS very high resistance to degradation such that these compounds are commonly referred to as forever chemicals.

A myriad of compounds fall under the category of PFAS but the most studied substances are perfluoroalkyl acids (PFAAs), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). For food safety, perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkanesulfonic acids (PFSAs), fluorotelomers with hydroxyl end groups, and perfluorinated sulfonamides are commonly monitored and reported.

The pervasive sources of PFAS[2] in daily life and their interconnections make human exposure nearly impossible to avoid. Industrial emissions, sewer discharge, military airbase runoff, landfill leaching, and consumer products are potential routes of contact. Exposure is also greatly influenced by diet, as food packaging, water, and both plant and animal food stuffs can contain PFAS. The health risks from PFAS consumption are many, including cancers, low birth weights, developmental delays, thyroid disease, infertility, and immunosuppression, as well as a host of other health issues.[3] Moreover, some PFAS can remain in the body for many years, even decades. Due to the persistence and adverse health effects of PFAS, numerous analytical methodologies have been developed in order to understand and monitor these hazardous materials.


Application Note
Food & Beverage Testing
Agilent
Determination of 30 Per- and Polyfluoroalkyl Substances in Fruits, Vegetables, and Juices
Using the Agilent Captiva EMR-PFAS Food I passthrough cleanup and LC/MS/MS detection
Authors
Abstract

APPLICATION NOTE

Determination of 30 Per- and Polyfluoroalkyl Substances in Fruits, Vegetables, and Juices

Sample Preparation for PFAS Analysis: Extraction

The literature contains a variety of techniques for the extraction of PFAS in food items of plant and animal origin. Most of them involve Ion-pair extraction (IPE), Solid-Liquid extraction (SLE), Solid Phase extraction (SPE), or QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe).

IPE was the earliest extraction method, which involves using a counter ion of

opposite charge to distribute the analyte of interest into the organic phase. Tetra-n-butylammonium (TBA) was reportedly used as the ion pairing agent with anionic PFAS. This reduced the polarity of PFAS and transfered it into the organic phase. A limitation of the technique is the co-extraction of matrix with the organic solvent which can potentially affect the sensitivity of PFAS analysis. Alkaline-based IPE is preferred compared to acid digestion because it is less prone to oxidation of the PFAS precursors.

There are benefits and limitations that should be considered when choosing an extraction method.

SLE removes soluble components from solid matrices using a suitable solvent such as acetonitrile (ACN) or methanol. Commonly used for soil and sediment samples, SPE is faster and less laborious compared to other traditional methods. It is used for direct extraction of liquid samples such as milk and fish due to the advantages of extraction and clean up in one step. A high throughput on-line SPE method has been developed for PFAS extraction. Although manual SPE is less expensive, more sample volume is needed. On-line SPE works with less sample and minimal sample preparation, but a potential limitation is the clogging of the SPE column due to particulate matter.

QuEChERS is one of the most recently used approaches for routine extraction of PFAS in food items due to the low cost of materials and ability to extract a wide variety of organic pollutants. Initially designed for multi-residue analysis of pesticides in vegetables and fruits, it is now being extended to PFAS extraction in food because of its broad application range. Studies have shown good recoveries for many PFAS compounds. QuEChERS uses $MgSO_4$, NaCl, primary and secondary amine (PSA), C_{18} and graphitized carbon black (GCB), which are applied based on the type of food samples being processed.

There are benefits and limitations that should be considered when choosing an extraction method. For example, IPE is basically used for extracting an ionic PFAS by making use of an ion pairing agent. One of its limitations is that there is poor recovery of some PFAS as well as matrix interference. SLE uses a small amount of sample for the extraction but consumes a large volume of solvent. In addition, this method cannot extract a wide range of PFAS, for example, nonpolar long chain and hydrophilic short chain PFSA (C_4-C_8) and PFCA (C_4-C_{14}).
SPE has high selectivity for PFAS compounds, and it has advantages for both

extraction and clean up. However, some commercial sorbents cannot extract short chain PFCAs, and some sorbents can be very expensive to buy. QuEChERS is suitable for both ionic and non-ionic PFAS. It is also a cheaper method compared to SPE. However, there could be loss of PFAS when coupled to SPE clean up. It has also been reported that there could be background contamination with this method.

Sample Preparation for PFAS Analysis: Clean Up

A clean up step is necessary in order to remove endogenous materials from the PFAS extract and facilitate interference-free analysis. Clean up techniques are categorized as retentive and non-retentive. In retentive mode, different forms of SPE are used, such as WAX or hydrophilic lipophilic balance (HLB) sorbents. HLBs have poor recovery for short chain PFAS whereas WAX cartridges were reported to yield better recoveries. The mechanism of the WAX cartridge operation involves maintaining it at pH = 4 so that it is positively charged, including during the washing step. Anionic PFAS are adsorbed onto the cartridge through electrostatic interactions and then eluted via an increase in pH.

APPLICATION NOTE

Simplifying Sample Preparation for PFAS in Food and Feed Analysis

Non-retentive methods, comprising dispersive SPE (dSPE) with graphitized carbon can be combined with other techniques for clean up. They are suitable for all PFAS except perfluorinated compounds with aromatic properties. The methods are effective for complete elimination of matrix interference although fatty food items may not be effectively removed due to the presence of gelatinous precipitate if only graphitized carbon SPE is used. Multiple clean up steps can be applied to complement graphitized carbon SPE.

Magnetic Solid Phase extraction (MSPE) was initially developed for traditional contaminants but is now being applied to the extraction of PFAS in food items. Essentially, the technique works by introducing magnetic adsorbent to sample solutions in which the PFAS compounds interact with the adsorbent through electrostatic interactions, hydrophilic interactions, or hydrogen bonding. The analytes are then collected using an external magnetic field. No centrifugation or filtration is required, and only small sample and solvent volumes are needed. Huang and colleagues reported short extraction time and good recoveries of PFAS, as well as good limits of detection (LODs) and limits of quantitation (LOQs) in fish muscle samples using MSPE.[4] This suggests that it could be a promising

technique for the extraction of PFAS and should be explored for other food classes.

Molecularly imprinted polymers (MIPs) are characterized by high selectivity and affinity for targeted molecules. They are synthesized by co-polymerizing one or more functional monomers and a cross linker around a template which is the target of interest. The template is then removed from the complex to form a memory site. This site selectively recognizes and binds to the targeted analyte in the matrix solution. Zou et al synthesized and applied a hydrophilic MIP functionalized with phenolic resin in dispersive mode for the selective extraction of long chain PFAS in pork samples. They reported low LODs and excellent recoveries with short extraction time for the analytes.[5] MIPs could be used to complement cartridges with poor recovery of PFAS with carbon chain lengths less than 8.

Targeted analysis is the gold standard for compound determination and quantification.

Analysis of PFAS

Once the PFAS have been extracted from foods and cleaned up, advanced analytical instrumentation is required for their analysis. Factors to be considered for the testing of PFAS via LC-MS/MS includes the type of mobile phase, the LC column, and the ion source. Incorrect selection of any of these vital components can ultimately affect the chemical space of PFAS being screened and detected. Most ionization sources for PFAS analysis use negative electrospray ionization (ESI). The sources are coupled to a tandem quadrupole (TQ) to enable the acquisition of mass spectrometry data based on the precursor and fragment ions which are then used for detection and quantification of PFAS analytes. ESI is often used for PFAS research because of its increased sensitivity, wide dynamic range, and better linearity compared to atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI).

Reversed phase LC columns are typically utilized for separation of the analytes. For example, C_{18} columns with smaller particle size and internal diameter reduce separation time and increase the resolution of PFAS peaks. For the mobile phase, ammonium formate/acetate (5 mM) and methanol are often used. Organic modifiers such as tetrabutylammoniumhydrogen sulfate (TBAS) may be added if short chain PFAS elute too early.

Targeted analysis is the gold standard for compound determination and

quantification. For PFAS, scientists have been monitoring thousands of compounds but there are many thousands more that are known to exist yet have no available analytical standards or even scientific knowledge on their fate and transformation. The EPA Master List contains over 10,000 PFAS which shows that there is still much to be understood.

Non-targeted analysis has recently emerged to provide a more specific assessment of PFAS in environmental and biological samples, helping with the identification of known compounds that are now routinely monitored by targeted measurements. Non-targeted analyses play a critical role in uncovering novel PFAS and their transformation products.

Non-targeted analysis has recently emerged to provide a more specific assessment of PFAS in environmental and biological samples...

Due to its high resolving power (RP) and selectivity, high resolution mass spectrometry (HRMS) has become a powerful tool for known target and exploratory analysis of novel PFAS in various environmental consumer products and biological matrices without the need for reference standards. The more commonly used instrumentation includes High Resolution Mass Spectrometry instruments, and Ion Mobility MS. HRMS instruments are very sensitive and allow the collection of data using either data independent acquisition (DIA) or data dependent acquisition (DDA).

In DIA, all the selected ions in the mass/charge range are fragmented and ionized. This ensures comprehensive detection of all peaks and acquisition of MS/MS without using specific filters or thresholds. In DDA mode, only the most intense precursor ions are then selected for MS/MS. In this way, the DDA generates a very simple data set that can be analyzed with more straightforward approaches.

While negative ESI is the most common ionization mode, cationic and zwitterionic compounds require positive ion mode. Different approaches are highlighted in the literature for the identification of PFAS, however, both non-targeted analysis (NTA) and suspect screening analysis (SSA) generally follow a similar workflow.

The first step is sample preparation, during which an attempt is made to be as non-selective as possible so that a wide range of PFAS may be extracted from the food. LC-HRMS is then employed, and the data is processed. Data processing for NTA normally includes steps such as peak detection, mass filtering, molecular

formula assignment, and a database search for a possible structure match. There are commercially available software packages for PFAS as well as open-source software. Statistical analysis may also be used for further elucidation of the data.

Preliminary data revealed that of the 30 PFAS screened, 29 were present in the seafood samples.

Ion mobility mass spectrometry has also been increasingly used for PFAS analysis as it provides additional information besides retention time and accurate mass by measuring an ion's collision cross section (CCS). This offers benefits in terms of the separation of isomers as well as co-eluting compounds with branched and long chain PFAS. Interestingly, a correlation has been observed between CCS and m/z for different PFAS classes.[6] IMMS has been successfully applied to the characterization of emerging PFAS species and isomer content in environmental samples. Although there is limited information on the application for resolving isomeric PFAS compounds in food items, the technique offers promise in this field.

Case Study: PFAS in Tuna and Lobster

A recent study investigated the presence of PFAS in blackfin tuna and lobsters. Thirty samples of each marine animal were collected in coastal Biscayne Bay, Florida from May 2021 to March 2023 during their peak seasons. These recreational fish are commonly consumed in the region due to their nutritional benefits and rich protein content.

The sample preparation protocol involved spiking 1 g tissue with an internal standard and using 5 mL 0.01 M KOH:MeOH solvent. After homogenization and double centrifugation, the supernatant was concentrated and then cleaned up via dSPE (50 mg GCB + 100 mg Florisil®). The clean supernatant layer was then passed through a 0.2 µm filter before being transferred to an appropriate vial for analysis by LC-MS/MS.

Preliminary data revealed that of the 30 PFAS screened, 29 were present in the seafood samples. In tuna, perfluorooctanesulfonic acid (PFOS), perfluorotetradecanoic (PFTD) acid, and perfluorotridecanoic acid (PFTrDA) were the most abundant. N-methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA) had the highest concentration in the lobster samples which has been attributed to the fact that long chain PFAS tend to absorb to particulate matter which makes them more available to benthic organisms.
The PFAS in the seafoods were then further characterized using LC-Trapped Ion

Mobility Spectrometry (LC-TIMS).[7] In the preliminary data, PFAS were detected and assigned trend lines based on their mobility. A total of 118 features were found in the tuna samples and 137 features in lobsters.

More cyclic perfluorinated compounds were uniquely present in the fish and lobster. In addition, two PFAS were found in all of the samples: the first is a fluorotelomer carboxylic acid used as a fire retardant, while the other is a precursor to legacy PFAS. These results suggest that leveraging ion mobility separation capabilities can help detect more chemical features that are not captured by targeted analysis and potentially other high resolution mass spectrometric methods.

Conclusion

The pervasiveness of PFAS impels their extensive study and close monitoring in the interest of human health. Scientists around the globe have been working to further the identification and understanding of these hazardous substances using advanced analytical instrumentation and methodologies. For extraction prior to analysis, common techniques include IPE, SLE, SPE, and QuEChERS. SPE stationary phases should be selected based on the chemistry of interaction and preferential selectivity to some PFAS classes. Highly selective sorbents for PFAS extraction, such as MIPs and MSPE, are promising techniques to compensate for the deficiencies of commercially available SPEs.

Negative ion ESI LC-MS/MS is encouraged for PFAS targeted analysis; however, this method is prone to matrix interference. The use of HRMS for non-targeted analysis can help reduce interferences and aid the elucidation of novel PFAS in complex food items. Ion mobility spectrometry is growing exponentially in its application to food science. This technology's separation capabilities should be explored for screening more emerging perfluorinated compounds in food. As analytical advances continue, their contribution to PFAS analysis will deepen insight into food contamination.

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Quantitation of Per- and Polyfluoroalkyl Substances (PFAS) in Chicken Eggs for Human Consumption

Using Agilent Bond Elut Carbon S solid phase extraction cartridges and an Agilent 6475 triple quadrupole LC/MS system

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ABSTRACT

The European Commission regulation on maximum levels for certain contaminations in food describes maximum tolerated levels of four per- and polyfluoroalkyl substance (PFAS) compounds in various food matrices such as meat and fish products, as well as eggs. Additionally, there is a European Commission recommendation to monitor additional PFAS components. Other regions around the world are working on similar regulations. This application note presents the development and validation of a multicomponent method for the analysis of 21 PFAS compounds in chicken eggs. The method incorporates a sample extraction using QuEChERS cleanup, followed by solid-phase extraction using Agilent Bond Elut Carbon S cartridges. Quantitative analysis was performed by LC/MS/MS using the Agilent 1290 Infinity II LC system coupled to the Agilent 6475A triple quadrupole LC/MS. The method was validated according to SANTE guidance 11312/2021, monitoring analyte extraction recoveries, linearity, sensitivity (method detection limits), and reproducibility.

Introduction

PFAS are a group of more than 8,000 synthetic organofluorine chemicals that were first developed in the 1940s. The chemical characteristics that have led to their extensive use as surfactants and coatings in a wide range of commercial applications include resistance to heat, water, oil, grease, and stains. Commercial applications of PFAS include cosmetics, food packaging, nonstick cookware, firefighting foams, electronic devices, aircraft, vehicles, and various textiles (such as carpets, leather products, furniture, clothing, surgical gowns, and more). The chemical structure of PFAS molecules includes a chain of strong carbon-fluorine bonds, making them resistant to environmental degradation. As such, these chemicals tend to be pervasive, persistent, and environmentally stable. The main exposure routes to PFAS for humans include contaminated water and food.

In April 2023, the European Commission published the regulation (2023/915) on maximum levels of certain contaminants in foodstuffs. This regulation describes the maximum levels in fish, meat, and egg products for four PFAS components. The maximum tolerated levels are for PFOS, PFOA, PFNA, and PFHxS, at levels of 1.0, 0.30, 0.70, and 0.30 µg/kg, respectively. Additionally, the sum of the four components has a maximum level of 1.7 µg/kg in eggs. Other than this regulation, there is also an EU Commission Recommendation (2022/1431) in place on the monitoring of PFAS substances in food.[2] This recommendation mentions that member states should monitor, if possible, the presence of compounds that are similar to PFOS, PFOA, PFNA, and PFHxS, and suggests 18 different components in this regard. Additionally, the measurement of other PFAS components should also be taken into consideration. The limits of quantification in eggs should be at or below 0.30 µg/kg for PFOS, PFOA, PFNA, and PFHxS. For other PFAS components, no requested limit of quantification is mentioned.

Analysis of eggs can be challenging due to the presence of matrix interferences such as cholesterol, lipids, bile acids, and proteins. This application note describes efficient sample cleanup using QuEChERS in combination with Agilent Bond Elut Carbon S solid phase extraction cartridges followed by LC/MS/MS analysis.

Experimental

SAMPLE COLLECTION

All eggs used for this method were intended for human consumption.

CHEMICALS AND REAGENTS

For this study, LC/MS-grade acetonitrile and methanol were acquired from Actu-All Chemicals BV. The water that was used was ultrapurified (Milli-Q). Ammonium acetate, formic acid, and ethylene glycol were purchased from Sigma-Aldrich. Ammonia was obtained from Themo Scientific.

STANDARDS AND SOLUTIONS

PFAS standards were obtained from Wellington Laboratories. The PFAS components used in this study, including their internal standards, are listed in **Table 1**.

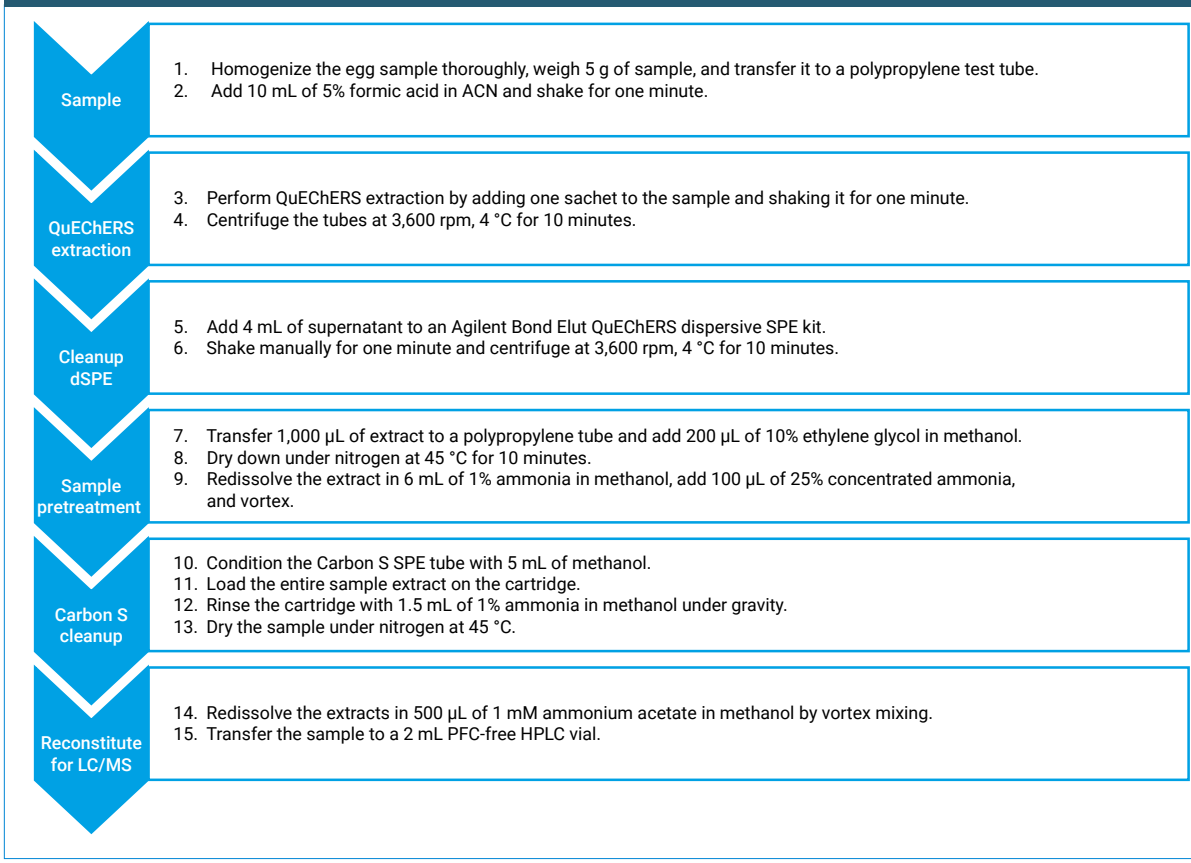
SAMPLE EXTRACTION

As shown in **Figure 1**, egg samples were homogenized manually, and five grams were transferred to a polypropylene test tube. Internal standards were added to each sample, calibrant, or QC sample. For each sample, 10 mL of 5% formic acid in ACN was added, and the tubes were shaken for one minute. One sachet of Agilent Bond Elut QuEChERS extraction kit, AOAC method (part number 5982-5755) was added to each sample tube and shaken for one minute. The tubes

TABLE 1: Method performance data (continued on next page).

Compound	Retention Time (min)	Internal Standard	Calibration Curve (µg/kg)	QC Low Concentration (µg/kg)	QC High Concentration (µg/kg)
PFPeA	4.22	$^{13}C_5$-PFPeA	0.15 to 2.0	0.3	1.5
PFBS	4.32	$^{13}C_3$-PFBS	0.13 to 1.8	0.27	1.33
PFHxA	4.84	$^{13}C_5$-PFHxA	0.15 to 2.0	0.3	1.5
PFPeS	4.90	$^{13}C_5$-PFPeA	0.15 to 2.0	0.3	1.5
HFPO-DA	5.04	$^{13}C_5$-PFHxA	0.3 to 4.0	0.6	3
PFHpA	5.55	$^{13}C_4$-PFHpA	0.15 to 2.0	0.3	1.5
PFHxS	5.59	$^{13}C_3$-PFHxS	0.14 to 1.9	0.29	1.43
PFOA	6.90	$^{13}C_8$-PFOA	0.15 to 2.0	0.3	1.5
PFHpS	6.32	$^{13}C_4$-PFHpA	0.14 to 1.91	0.29	1.43
PFOS	7.03	$^{13}C_8$-PFOS	0.14 to 1.92	0.29	1.44
PFNA	7.04	$^{13}C_9$-PFNA	0.15 to 2.0	0.3	1.5
PFNS	7.69	$^{13}C_9$-PFNA	0.15 to 2.0	0.3	1.5
PFDA	7.71	$^{13}C_6$-PFNA	0.15 to 2.0	0.3	1.5
PFOSA	8.25	$^{13}C_2$-PFDoDA	0.3 to 4.0	0.6	3
PFDS	8.27	$^{13}C_2$-PFDoDA	0.14 to 1.93	0.29	1.45
PFUnDA	8.31	$^{13}C_7$-PFUnDA	0.15 to 2.0	0.3	1.5
PFUdS	8.79	$^{13}C_7$-PFUnDA	0.3 to 4.0	0.6	3
PFDoDA	8.83	$^{13}C_2$-PFDoDA	0.15 to 2.0	0.3	1.5
PFDoS	9.24	$^{13}C_2$-PFTDA	0.3 to 4.0	0.6	3
PFTrDA	9.29	$^{13}C_2$-PFTDA	0.15 to 2.0	0.3	1.5
PFTrDS	9.64	$^{13}C_2$-PFTDA	0.3 to 4.0	0.6	3

FIGURE 1: Sample preparation workflow diagram.



were then centrifuged at 3,600 rpm at 4 °C for 15 minutes. Following this, 4 mL of the supernatant was transferred to an Agilent Bond Elut QuEChERS Fruits and Vegetables with Fats and Waxes, dispersive SPE kit (part number 5982-5156). The tubes were sealed and hand shaken vigorously for one minute. The samples were centrifuged at 3,600 rpm, 4 °C for 10 minutes. Following this, 1,000 µL of the sample was transferred to a 17 × 100 mm polypropylene test tube. 200 µL of 10% ethylene glycol in methanol was added to the tube. The sample was then dried under nitrogen at 45 °C for 10 minutes. The extract was redissolved in 6 mL of 1% ammonia in methanol, followed by the addition of 100 µL of 25% concentrated ammonia. The tubes were vortexed and ready for the cleanup step with Agilent Bond Elut Carbon S SPE 250 mg/6 cc cartridge (part number 5610-2082). The Carbon S SPE columns were first conditioned with 5 mL methanol. The entire extract was loaded onto the cartridge, followed by a rinse with 1.5 mL of 1% ammonia in methanol under gravity. Extracts were dried under nitrogen at 45 °C. The dried extracts were redissolved in 500 µL 1 mM ammonium acetate in methanol by vortex mixing. The extracts were transferred to 2 mL PFC-free HPLC vials with caps (part numbers 5191-8150 and 5191-8151) and ready for LC/MS/MS analysis.

CALIBRATION STANDARDS, EXTRACT SPIKES, AND QC SAMPLES

For the preparation of calibration standards, matrix blanks, and QC samples, 5 grams of blank egg matrix were transferred to a plastic tube. For calibration standards and QC samples, both PFAS standards were added as internal standards before sample preparation. Standards and QCs then underwent the same sample preparation as the samples. For the extract spike sample used for matrix effect determination during validation, the blank egg matrix underwent sample preparation. The PFAS standards and internal standards were spiked after the Carbon S SPE step.

TABLE 2: LC conditions.

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 1290 ZORBAX Eclipse plus C18 guard column, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Delay Column	Agilent InfinityLab PFC delay column, 4.6 × 30 mm (p/n 5062-8100)
Column Temperature	50 °C
Injection Volume	5 µL
Needle Wash	**Step** / **Time (s)** / **Solvent** 1 / 7 / ACN / Seat backflush and needle wash 2 / 7 / MeOH / Seat backflush and needle wash 3 / 7 / Water / Seat backflush and needle wash
Flow Rate	0.4 mL/min
Mobile Phase	A) 5 mM ammonium acetate in water B) 5 mM ammonium acetate in methanol
Gradient	**Time (min)** / **%A** / **%B** 0 / 90 / 10 0.5 / 90 / 10 2.5 / 45 / 55 9 / 10 / 90 9.5 / 0 / 100 11.5 / 0 / 100 11.6 / 90 / 10 14 / 90 / 10

To test for PFAS background during extraction and LC/MS analysis, a method blank was created. To prepare this sample, a tube was taken without egg sample, which underwent all steps as described in the extraction.

The internal standard concentration in each sample was 1 µg/kg. Calibration curves consisted of six levels, and varied per PFAS component from either 0.13 to 1.8 µg/kg for some PFAS components, up to 0.3 to 4 µg/kg for other PFAS components. QC samples in four replicates were used both in low-level (0.3 or 0.6 µg/kg depending on PFAS component) and high-level (ranging from 1.3 to 3 µg/kg depending on PFAS component). The exact calibration ranges are shown in **Table 1**.

INSTRUMENTATION

Sample analysis was performed using a 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II high-speed pump (G7120A), an Agilent 1290 Infinity II multisampler equipped with multiwash option (G7167B), and an Agilent 1290 Infinity II multicolumn thermostat (G7167B). The LC system was modified for PFAS analysis using the Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006). The LC system was coupled to an Agilent 6475A triple quadrupole LC/MS equipped with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter Workstation software (version 12.0) was used for data acquisition and analysis. The optimized MRM settings for the different PFAS components were taken from the PFAS dMRM database (G1736AA).

TABLE 3: MS conditions.

Parameter	Value
MS	Agilent 6475A triple quadrupole LC/MS with Agilent Jet Stream ESI source
Scan Type	Dynamic MRM (dMRM)
Cycle Time	300 ms
Total MRMs	69 MRMs and one dummy positive transition
Source Parameters	
Polarity	Negative
Gas Flow	250 °C, 11 L/min
Sheath Gas	375 °C, 11 L/min
Nebulizer Gas	25 psi
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

The LC and MS method parameters are listed in **Tables 2 and 3**, respectively. The positive dummy transition was added at the end of each injection to prevent instrument charging due to measuring in only negative ionization mode.

Results and discussion

The method was validated according to SANTE 11312/2021 guidance. All analytes had consistent retention times with RSDs over the entire run of less than 0.05%. All analytes also had excellent calibration curve R2 values of greater than 0.993 for a six-point curve using linear fit with no weighting and ignore origin, except for PFHpS and PFPeS, where a weighting of 1/X was applied.

Calibration performance

The method was validated according to SANTE 11312/2021 guidance.[3] All analytes had consistent retention times with RSDs over the entire run of less than 0.05%. All analytes also had excellent calibration curve R2 values of greater than 0.993 for a six-point curve using linear fit with no weighting and ignore origin, except for

FIGURE 2: Extracted MRM chromatogram of blank egg samples spiked with 21 PFAS targets at 0.4 to 1 µg/kg (calibration standard 3). Internal standards are not shown.

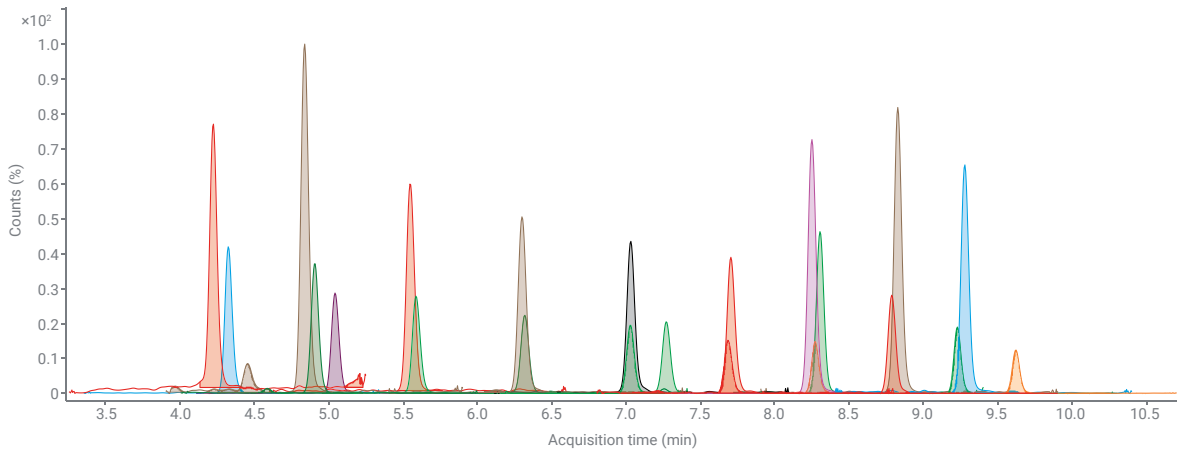
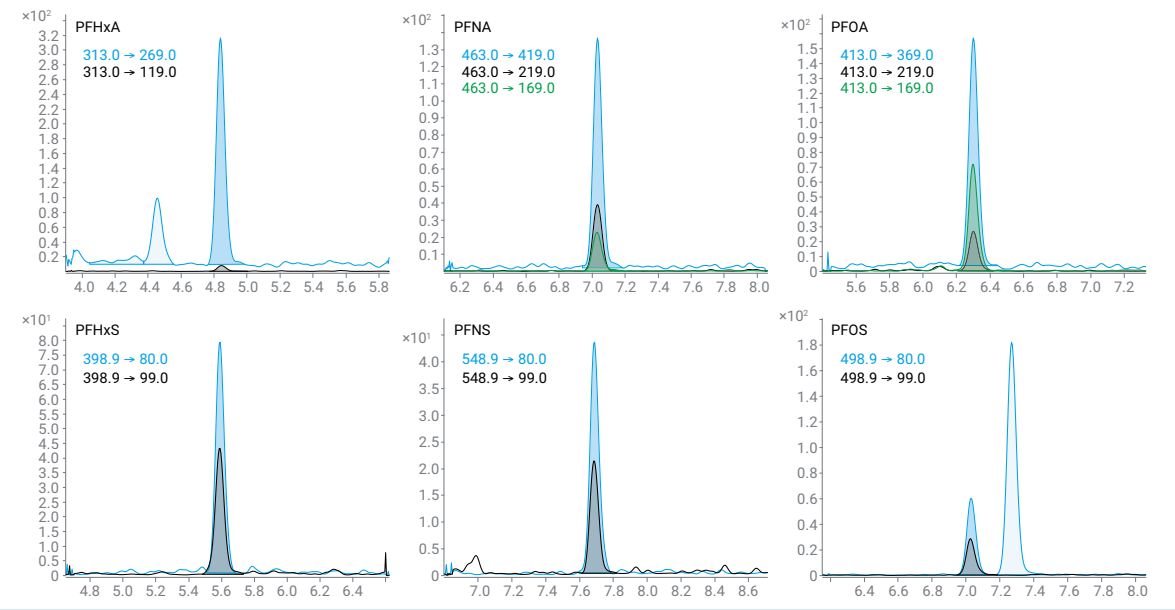


FIGURE 3A: Chromatograms of PFHxA, PFNA, PFOA, PFHxS, PFNS, and PFOS at the CAL 1 level (0.14 or 0.15 µg/kg). Both the quantifier and the qualifier transitions are shown.



PFHpS and PFPeS, where a weighting of 1/X was applied. The MRM chromatogram shown in **Figure 2** demonstrates good separation and detection of the target PFAS.

RECOVERY, PRECISION, AND MATRIX EFFECT

For determination of recovery and precision, the QC low and QC high samples were prepared as five individual preparations per level and injected on the LC/MS. Additionally, for determination of the matrix effect, a system suitability sample (PFAS components in mobile phase) was compared to the matrix blank sample with postspiking at the corresponding concentration.

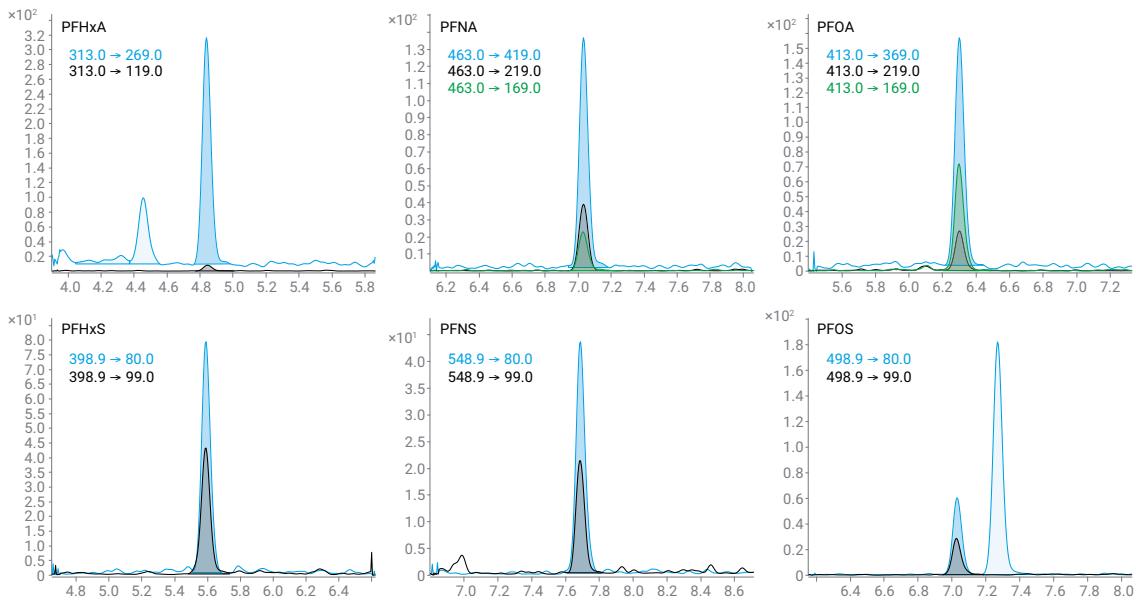
As shown in **Table 4**, all PFAS components demonstrated excellent recoveries between 93.5 to 109.0%, which is well within the acceptable limit of 70 to 120%. The precision values were all better than 10.3%, with the exception of PFOSA, which went up to 17.5%. The higher RSD value for PFOSA is attributed to the unavailable corresponding stable label internal standard for PFOSA, and therefore another internal standard was used. However, all values are well within the SANTE acceptable value of ≤ 20%.

The matrix effect study demonstrated that there is hardly any matrix effect. For all components, the peak responses were approximately the same area for the extract spike samples compared to the system suitability sample.

TABLE 4: Method performance data (continued on next page).

Compound	QC Low		QC High	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)
HFPO-DA	97.0	5.1	99.8	1.6
PFBS	93.5	2.2	102.2	0.5
PFDA	102.1	1.4	96.0	1.0
PFDoDA	98.9	4.8	101.2	1.1
PFDoS	102.9	4.4	100.6	3.9
PFDS	107.4	9.0	101.3	4.1
PFHpA	98.3	4.0	103.7	2.9
PFHpS	95.5	10.3	104.0	2.4
PFHxA	99.8	2.0	100.1	2.2
PFHxS	105.4	4.1	98.1	1.0
PFNA	102.7	4.2	101.1	2.9
PFNS	102.6	7.8	99.8	4.0
PFOA	98.9	4.0	100.0	2.1
PFOS	100.9	5.2	102.4	4.7
PFOSA	109.0	17.5	99.5	16.9
PFPeA	99.4	5.6	103.1	0.8
PFPeS	97.3	5.3	104.5	2.6
PFTrDA	103.5	5.8	101.0	3.1
PFTrDS	99.0	6.0	98.9	3.2
PFUdS	96.0	9.7	98.8	1.9
PFUnDA	102.6	5.1	100.9	2.4

FIGURE 3A: Chromatograms of PFHxA, PFNA, PFOA, PFHxS, PFNS, and PFOS at the CAL 1 level (0.14 or 0.15 µg/kg). Both the quantifier and the qualifier transitions are shown.



METHOD DETECTION LIMITS

For the sensitivity of the method, the lowest calibration standard was used. The deviation from the back-calculated concentration was calculated and the chromatograms were monitored. The deviations from the back-calculated concentration (percent error) for all PFAS components at the CAL 1 level was 15.2% or better, which is well within the acceptable limit of ≤ ± 20%.

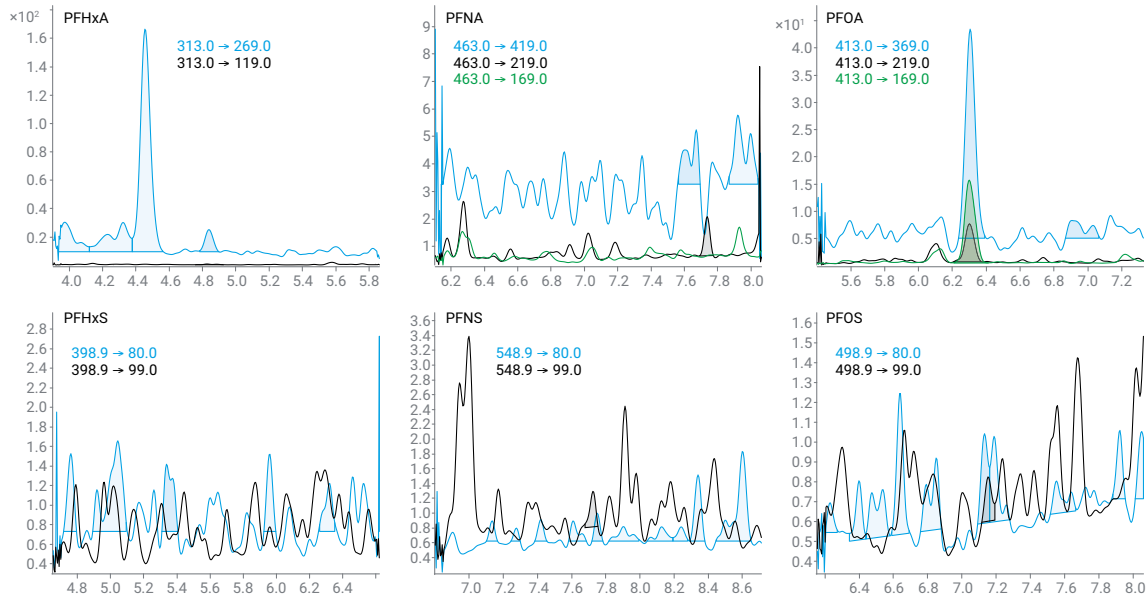
Figure 3A shows chromatograms of PFOS, PFOA, PFNA, and PFHxS (the four PFAS components in the EU regulation) in addition to two other groups, PFNS and PFHxA, at the lowest calibration level. To demonstrate the selectivity of the method, the chromatograms of the method blank are shown in Figure 3B. A small amount of PFOA is visible in the method blank, but for all other PFAS components, the method blank did not show any peaks.

The samples used for the SANTE validation were cross-checked on two different LC/MS/MS systems in two different laboratories.

Results in eggs for human consumption

This method is now in use for routine quantification of eggs coming from various sources. In the first run following validation, 30 different egg batches were tested. Only one batch was positive for PFOS, at a concentration of 0.147 µg/kg, which is just above the lowest calibration point at 0.144 µg/kg for this component. This is well below the allowed 1.0 µg/kg as indicated by the European Commission regulation.[1]

FIGURE 3B: Chromatograms of PFHxA, PFNA, PFOA, PFHxS, PFNS, and PFOS of the method blank. Both the quantifier and the qualifier transition are shown.



Conclusion

An efficient method for the quantification of PFAS in eggs has been demonstrated. The combination of QuEChERS sample preparation with Agilent Bond Elut Carbon S solid phase extraction cartridges leads to excellent recoveries and reproducibility. Additionally, the midrange Agilent 6475A triple quadrupole LC/MS system was sufficient to fulfill the European Commission regulation and recommendation, even in a challenging matrix such as eggs. The 21 selected PFAS components fulfilled the validation according to SANTE guidelines.

REFERENCES

1. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels of certain contaminants in food repealing Regulation (EC) No 1881/2006.
2. Commission Recommendation (EU) 2022/1431 of 24 August 2022 on the monitoring of perfluoroalkyl substances in food.
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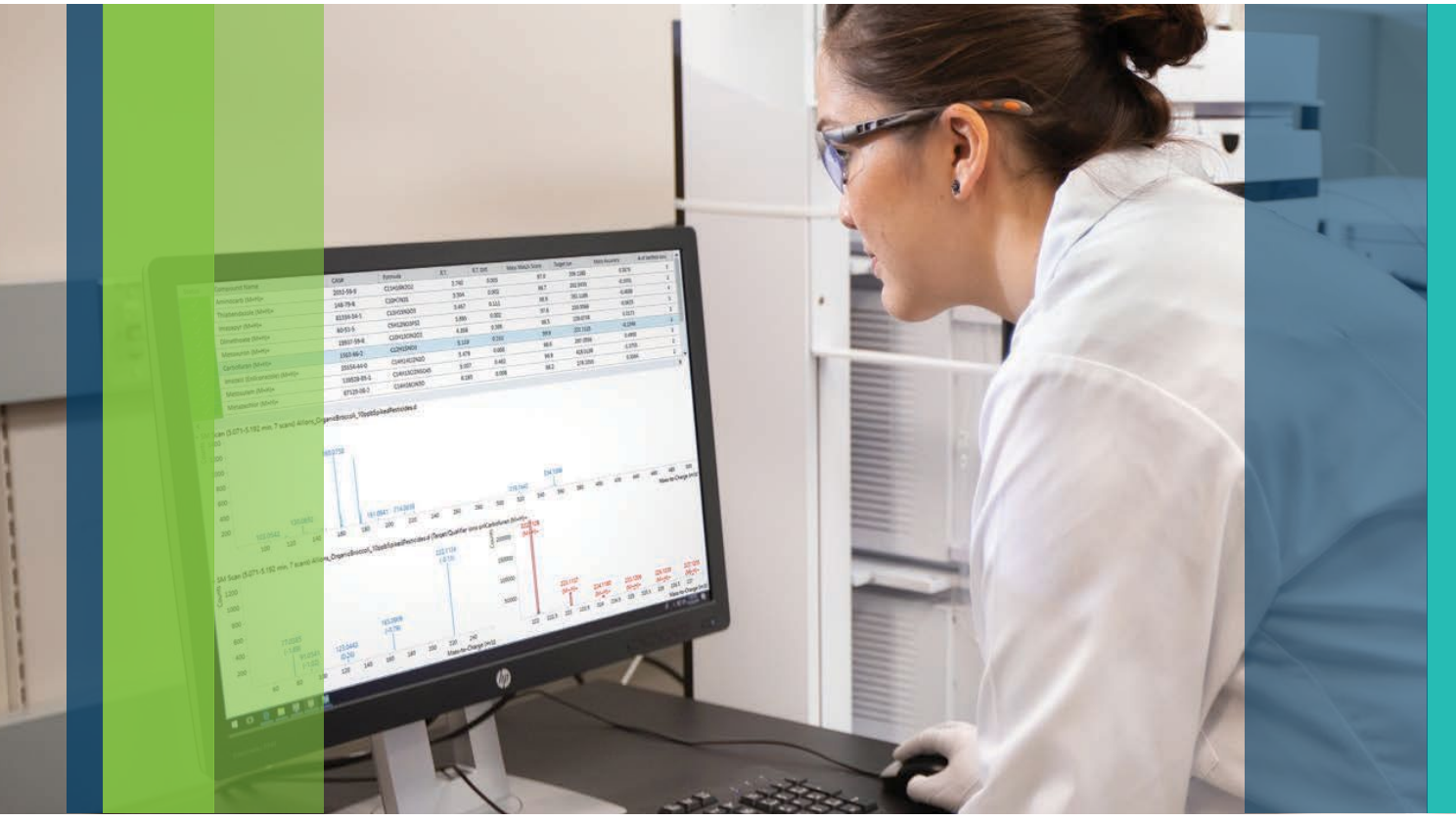

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