Application Note

Food

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 food stuffs.[1] 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 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 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.

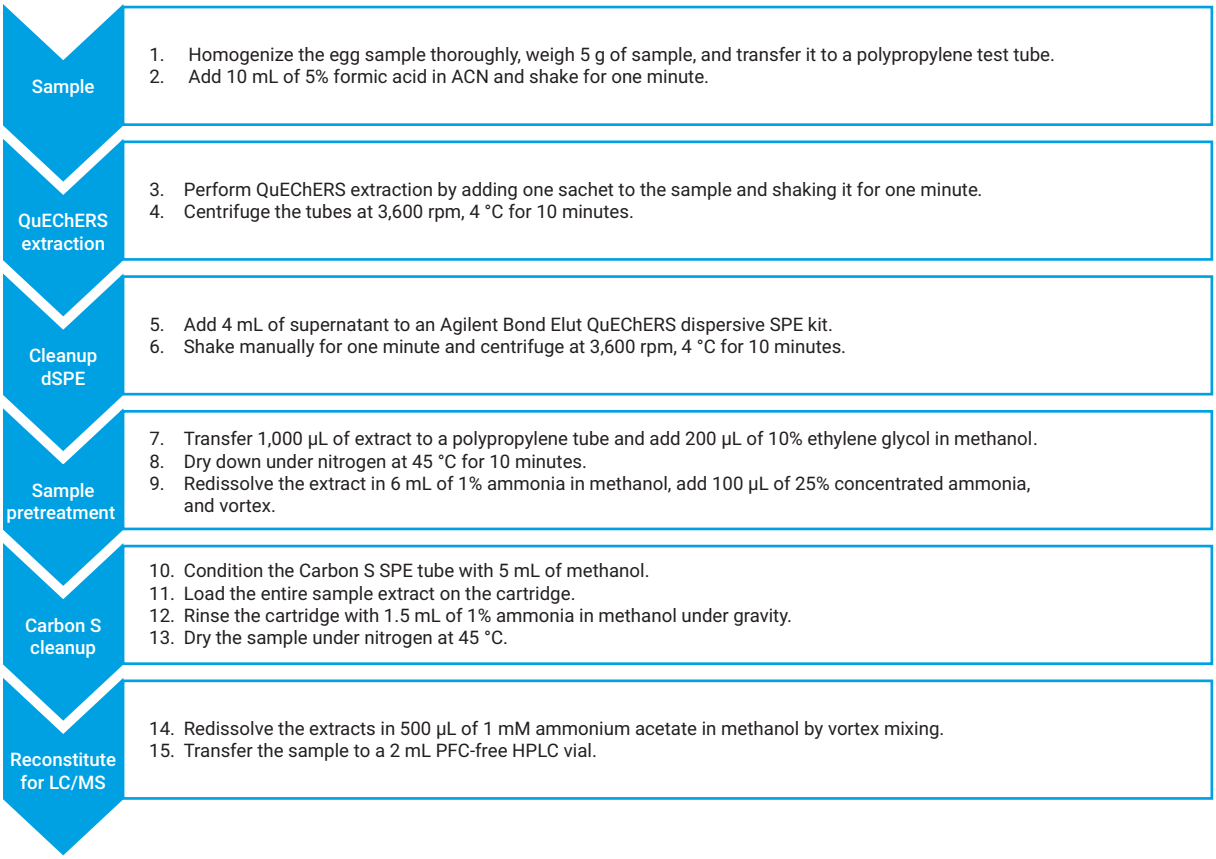

Figure 1. Sample preparation workflow diagram.

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.

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.

Table 1. Compounds, retention times, and concentration ranges.

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	$^{13}C_6$-PFDA	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

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).

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.

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 1: Time (s) 7, Solvent ACN, Seat backflush and needle wash Step 2: Time (s) 7, Solvent MeOH, Seat backflush and needle wash Step 3: Time (s) 7, Solvent 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

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

Results and discussion

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 R^2 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. 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.

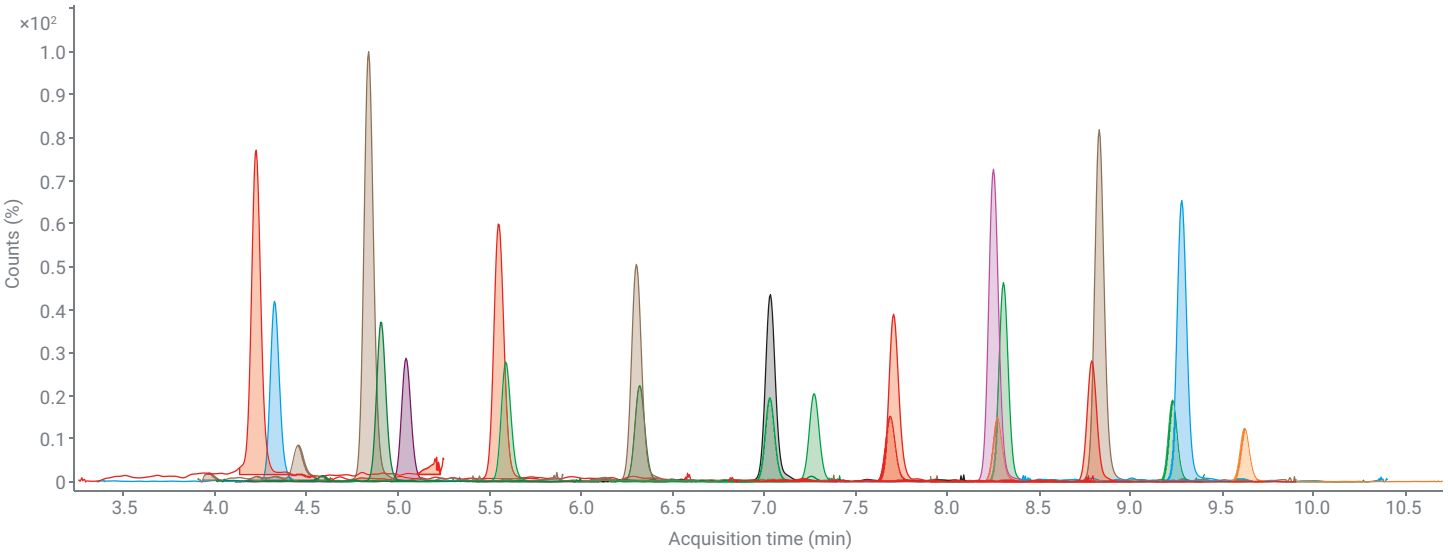

Figure 2. Extracted MRM chromatogram of blank egg samples spiked with 21 PFAS targets at 0.4 to 1 µg/kg (calibration standard 3). Internal standards are not shown.

Table 4. Average recovery and precision results (N = 5).

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

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.

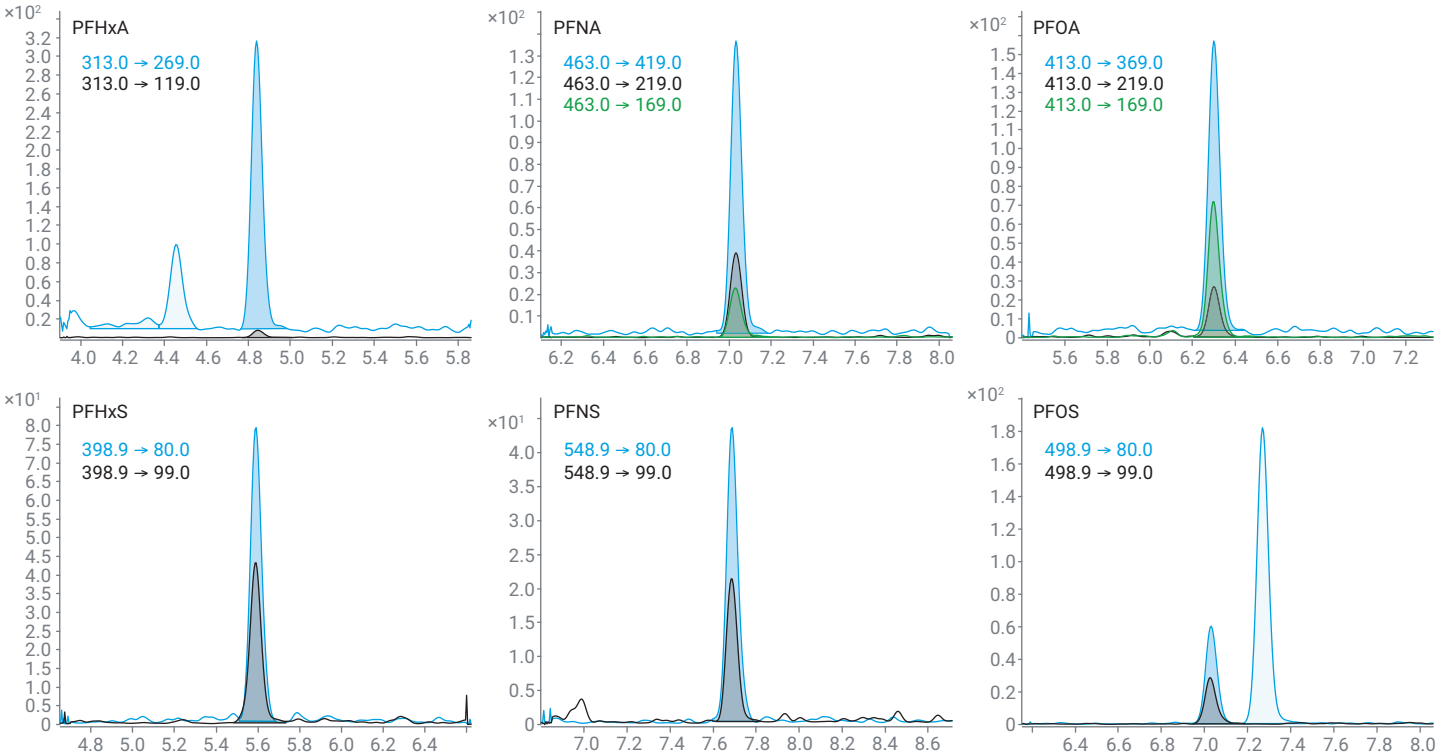

Figure 3A. Chromatograms of PFHxA, PFNA, PFOA, PFHxS, PFNS, and PFOS at the CAL 1 level (0.14 or 0.15 µg/kg). Both the quantifier and the qualifier transitions are shown.

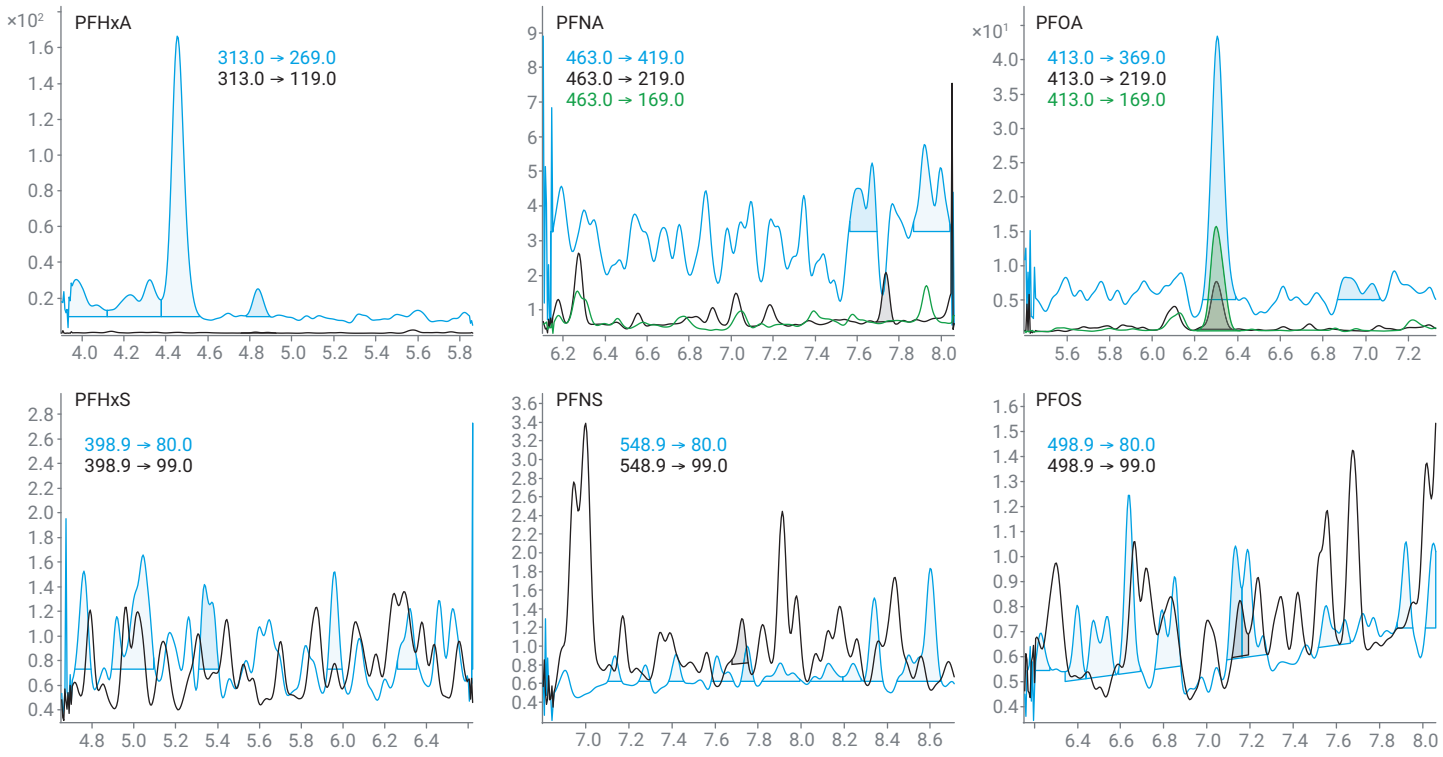

Figure 3B. Chromatograms of PFHxA, PFNA, PFOA, PFHxS, PFNS, and PFOS of the method blank. Both the quantifier and the qualifier transition are shown.

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]

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. This method is now routinely in use for the determination of PFAS in eggs.

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.
3. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed; SANTE 11312/2021.

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