Application note | 004043

Food and beverage

Screening and quantitation of perfluoroalkyl and polyfluoroalkyl substances (PFAS) residues in foods using LC-MS/MS

Authors

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Keywords

Chromeleon software, perfluoroalkyl and polyfluoroalkyl substances (PFAS), fish, egg, LC-MS/MS, TSQ Quantis Plus MS

Goal

The objective of this work is to demonstrate the capability of LC-MS/MS, using a Thermo Scientific™ TSQ Quantis™ Plus Mass Spectrometer, for trace-level quantitation of PFAS residues in fresh fish tissues and eggs. The method performance was assessed as per the SANTE 2021 V2 guideline to ensure compliance with the EU maximum limit requirements (MRL).

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of compounds with variable carbon chains and fluorine. The carboxylic and sulfonic acid functional groups exhibit hydrophilic and hydrophobic properties. PFAS compounds exhibit high thermal stability, low reactivity, and strong carbon-fluorine bonding. They have been widely used in applications such as construction, electronic manufacturing, fire-fighting foams, cookware, packaging, textiles, and pharmaceuticals.[1] These chemicals are non-biodegradable, so they remain permanent, bio-accumulating substances.[2]

Exposure to these chemicals is a significant risk for human beings due to their effect on the reproductive and immune systems. Human exposure to PFAS can occur by inhalation of dust and airborne volatiles or by ingestion from food packaging. However, the most prevalent route is the consumption of contaminated food or drinking water. PFAS can enter the animal food chain through feed, water, and soil ingestion by foraging farm animals, thereby resulting in the contamination of products such as milk, eggs, and meat. Food can also get contaminated through the presence of PFAS in food packaging or processing equipment.[3]

The European Food Safety Authority (EFSA) CONTAM panel assessed the risk associated with the consumption of PFAS-contaminated food, looking at four substances—PFOA, PFOS, PFNA, and PFHxS. Fish (tissue) was identified as the principal contributor to the exposure, followed by fruit and fruit products, and eggs and egg products.[4] The European Union Reference Laboratory (EURL) published the "Guidance Document on Analytical Parameters for the Determination of Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed" to harmonize the analytical performances.[5] The Guidance Document principles were then adopted by EU Regulation 2022/1428. Finally, the limits were set in foods by Commission Regulation (EU) 2022/2388 of 7th December 2022, entering into force on 1st January 2023, and amending Regulation 1881/2006 (repealed and substituted by Commission Regulation (EU) 2023/915 of 25 April 2023).[6,7] The recommended limit of quantitation (LOQ) for PFOS, PFOA, PFNA, and PFHxS in fish meat and meat is 0.1 µg/kg for monitoring purposes as per the EC 2022/1431 document.[8]

This work reports a modified version of the QuEChERS method. A sensitive, selective, and robust analytical workflow was developed for the determination of PFAS compounds in fish tissue and egg using a TSQ Quantis Plus MS, which met low levels (µg/kg) of detection, thereby fulfilling the LOQ requirement. Finally, the developed method was applied to PFAS monitoring of real food samples according to recommendation 2022/1431.

Experimental

Chemicals and apparatus

- Fisher Chemical™ Acetonitrile, Optima™ LC/MS grade (Cat. No. A955-4)
- Fisher Chemical™ Methanol, Optima™ LC/MS grade (Cat. No. A456-4)
- Fisher Chemical™ Water, Optima™ LC/MS grade (Cat. No. W64)
- Fisher Chemical™ Ammonium acetate (Cat. No. A637-500)
- Analytical balance (ACZET, CY2202, San Diego, CA) and precision balance (ACZET, CY205C, San Diego, CA)
- Thermo Scientific™ Vortex mixer (Cat. No. 88880017TS)
- Thermo Scientific™ Sorvall™ ST8 Ventilated Benchtop Centrifuge, Refrigerated (Cat. No. 75007203)
- Thermo Scientific™ Finnpipettes: 20–200 µL (Cat. No. 4641080N), 100–1,000 µL (Cat. No. 4641100N), 1–10 mL (Cat. No. 4642110)
- Thermo Scientific™ QuEChERS EN 15662 Method Extraction Kit, pouch of 4 g magnesium sulfate (anhydrous), 1 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate (Cat. No. S1-10-EN-POT)
- Thermo Scientific™ Anhydrous magnesium sulfate (Cat. No. 80020-415-500), PSA (Cat. No. 80020-429-100), C18 (Cat. No. 80020-430-100), and bulk graphitized carbon black (GCB) (Cat. No. 80020-431-50)
- Thermo Scientific™ PFAS Upgrade Kit (Vanquish Flex) (Cat. No. 80100-62144)
- Thermo Scientific™ Viper™ Capillary, 0.18 × 350 mm, MP35N (Cat. No. 6042.2337)
- Thermo Scientific™ Vanquish™ Pump Mixer, 35 µL set, VF-P1 (Cat. No. 6044.3870)
- Thermo Scientific™ nanoViper™ Capillary, 75 µm × 750 mm (Cat. No. 6041.5780)

Standards

A mix standard of PFAS (100 µg/mL) in methanol:water (ISO 17034 certified) was purchased from LGC Standards (Germany). The standard stock solution contained perfluorobutanoic acid (PFBA), nonafluoro-1-butanesulfonate (PFBS), 4:2 fluorotelomer sulfonate (4:2FTS), perfluorohexanoic acid (PFHxA), perfluoropentanesulfonic acid (PFPS), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS), 6:2 fluorotelomer sulfonate (6:2FTS), perfluorooctanoic acid (PFOA), perfluoroheptanesulfonic acid (PFHpS), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), 8:2 fluorotelomer sulfonate (8:2FTS), perfluorononae sulfonate (PFNS), perfluorodecanoic acid (PFDA), *N*-methylperfluorooctane-sulfonamidoacetic acid (NMeFOSAA), pentafluorodecanesulfonate (PFDS), *N*-ethylperfluorooctane-sulfonamidoacetic acid (NEtFOSAA), perfluoroundecanoic acid (PFUnDA), perfluorooctanesulfonimide (PFOSA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), and perfluoropentanoic acid (PFPeA). This solution was further diluted in methanol appropriately and utilized for the recovery study and calibration standards preparation. The calibration curve was prepared in the range of 0.05–5 ng/mL (50–5,000 pg/mL).

Sample preparation

Fresh eggs (white and yolk) were mixed thoroughly to get a uniform homogeneous test portion. The fish sample was collected from the local market and kept in a freezer until analysis. After reaching room temperature, the fish sample was cut into small pieces. Fish pieces (approx. 200 g) were homogenized using a heavy-duty mixer and grinder, and 3 g (±0.1 g) of homogenized sample was weighed in a 50 mL polypropylene (PP) tube separately. The PFAS standard mix was spiked before the extraction, vortexed for 1 min, and kept for 20 min. Water (15 mL) and acetonitrile (15 mL) were added to the sample, which was vortexed for 1 min and kept in an ultrasound bath for 15 min. The EN QuEChERS salt was added; the sample was vortexed and then centrifuged at 10°C at 2,147 *g* for 5 min. The supernatant (8 mL) was transferred into another 15 mL PP tube containing 400 mg PSA, 400 mg C18, 10 mg GCB, and 1,000 mg $MgSO_4$. The tube was vortexed for 1 min at 2,500 rpm and centrifuged at 2,147 *g* for 10 min. The cleaned extract (6 mL) was evaporated to dryness under a gentle stream of nitrogen gas at 40°C. The final extract was reconstituted in 0.3 mL with diluent methanol:water (50:50, v/v), centrifuged at 11,180 *g*, and injected into the LC-MS/MS.

Analysis

The LC-MS/MS analysis was performed using a Thermo Scientific™ Vanquish™ Flex UHPLC coupled to the TSQ Quantis Plus triple quadrupole mass spectrometer, including a heated electrospray ionization (HESI) ion source. The best ionization was achieved in the negative polarity (HESI-). The chromatographic separation was performed on a Thermo Scientific™ Accucore™ C18 Column (100 × 2.1 mm, 2.6 µm) with a Thermo Scientific™ Hypersil GOLD™ Column as a PFAS delay column (50 × 2.1 mm, 1.9 µm). The LC mobile phase consisted of 2 mM ammonium acetate in water (A) and 2 mM ammonium acetate in methanol (B). The detailed UHPLC and mass spectrometer parameters are reported in Table 1. A short length delay column with similar characteristics was utilized to reduce the isobaric contamination. This delay column was connected between the mobile phase mixer and the injector port. The delay column tends to have good retention and separates the PFAS-interfering signals coming from either the chromatographic system or solvents. The chromatographic separation was achieved for the branched and linear isomers. The total PFOS was obtained by the sum of linear and branched PFOS, and the branched PFOS isomers were quantified against the linear PFOS standard in the hypothesis of an equimolar response.[5]

Data analysis

The data acquisition was performed by using the instrument conditions in Table 1 with the selected reaction monitoring (SRM) and processing carried out by using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.3.2.

Table 1. LC-MS/MS instrument conditions.

UHPLC instrumentation	Vanquish Flex UHPLC (Thermo Scientific™ Vanquish™ Quaternary Pump F, Cat. No. VF-P20-A)			
LC analytical column	Accucore C18 column, 100 × 2.1 mm, 2.6 µm (Cat. No. 17326-102130)			
LC trap column	Hypersil GOLD C18 column, 50 × 4.6 mm, 1.9 µm (Cat. No. 25002-054630)			
Sample compartment temp.	10°C (Still air) (Thermo Scientific™ Vanquish™ Split Sampler FT, Cat. No. VF-A10-A)			
Column oven temp.	40°C (Thermo Scientific™ Vanquish™ Column Compartment, Cat. No. VH-C10-A)			
Injection volume	20 µL			
Needle wash	50:50:50 (acetonitrile:MeOH:water, v/v)			
Mobile phase	A phase: 2 mM ammonium acetate in water B phase: 2 mM ammonium acetate in methanol			
Inline filter	35 µL, VF-P1 (Cat. No. 6044.3870)			
Total run time	20.0 min			
LC gradient program	Time (min)	Flow rate (mL/min)	%B	Curve
	0.0	0.300	10	5
	0.5	0.300	10	5
	2.0	0.300	60	5
	10.0	0.300	98	5
	13.0	0.300	98	5
	13.5	0.300	10	5
	20.0	0.300	10	5
Mass spectrometer instrumentation	**TSQ Quantis Plus triple quadrupole mass spectrometer**			
Acquisition method type	tSRM mode			
Ion source type	H-ESI			
Spray voltage	Static, Negative 1,500 V			
Sheath gas	50 Arb			
Aux gas	10 Arb			
Sweep gas	0 Arb			
Ion transfer tube temp.	225°C			
Vaporizer temp.	300°C			

The compound-specific SRM transitions and retention times are shown in Table 2. In the data acquisition, two or more transitions were selected for all target analytes except PFBA and PFPeA in time-based SRM mode. For all target analytes, the most intense product ion was selected as the quantitative ion, and other ions were selected as qualifiers. PFBA and PFPeA have only one transition (i.e., quantitative). In the case of a few analytes, a pseudo-ion was selected for confirmation/quantitation purposes. The user-defined parameters, including identity and confirmation, retention time (±0.1 min), linearity (>0.99 with residuals ±20%), recovery (70%–120%), and precision (<20%), were set in the data processing method as per the SANTE guideline as well as the Commission Implementing Regulation (EU) 2022/1428.[5,6,7,9]

Table 2. List of PFAS compounds with their SRM transitions, CE, RT, and R^2.

Sr. no.	Compound	RT (min)	Parent (*m/z*)	Quant (*m/z*)	CE (V)	Confirm. (*m/z*)	CE (V)	R^2
1	Perfluorobutanoic acid (PFBA)	2.85	212.9	168.9	7.7	212.9	5	0.9939
2	Perfluoropentanoic acid (PFPeA)	4.80	263	218.9	5	263	5	0.9947
3	Nonafluoro-1-butanesulfonate (PFBS)	4.98	299	80	25	99	25	0.9966
4	4:2 Fluorotelomer sulfonate (4:2FTS)	5.32	327	81	28	307	20	0.9934
5	Perfluorohexanoic acid (PFHxA)	5.35	313	269	12	119	10	0.9862
6	Perfluoropentanesulfonic acid (PFPeS)	5.40	349	80	25	99	25	0.9894
7	Perfluoroheptanoic acid (PFHpA)	5.85	362.9	169	15	319.1	10	0.9985
8	Perfluorohexanesulfonic acid (PFHxS)	5.88	398.9	79.9	36	98.9	25	0.9981
9	6:2 Fluorotelomer sulfonate (6:2FTS)	6.38	427	407	30	80	21	0.9958
10	Perfluorooctanoic acid (PFOA)	6.41	412.9	369	10	169	25	0.9943
11	Perfluoroheptanesulfonic acid (PFHpS)	6.43	448.9	80.1	37	99	35	0.9919
12	Perfluorononanoic acid (PFNA)	7.02	462.9	419	10	169	25	0.9937
13	Perfluorooctanesulfonic acid (PFOS)	7.05	498.9	80	46	99	40	0.9934
14	8:2 Fluorotelomer sulfonate (8:2FTS)	7.63	527	81	30	506.9, 527	24, 5	0.9881
15	Perfluorononane sulfonate (PFNS)	7.65	549	549	30	99, 80	5, 30	0.9917
16	Perfluorodecanoic acid (PFDA)	7.65	512.9	269	15	469.1, 512.9	10, 5	0.9938
17	*N*-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	7.96	570	419	18	483, 570	16, 5	0.9912
18	Pentafluorodecanesulfonate (PFDS)	8.28	599	80	40	99	35	0.9971
19	*N*-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	8.25	584	419	20	483, 526, 584	18, 15, 5	0.9847
20	Perfluoroundecanoic acid (PFUnDA)	8.30	562.9	269	15	518.9	10	0.9873
21	Perfluorooctanesulfonimide (PFOSA)	8.75	498	78	25	169	15	0.9961
22	Perfluorododecanoic acid (PFDoA)	8.85	612.9	169	25	569, 612.9	12, 5	0.9949
23	Perfluorotridecanoic acid (PFTrDA)	9.45	663	169	20	618	12	0.9916
24	Perfluorotetradecanoic acid (PFTeDA)	9.92	713	169	20	669	12	0.9877

Laboratory environment

A few researchers reported that PFAS are observed in the environment, as fluorine-based polymers, and PTFE-based materials.[10] A variety of PTFE materials were used in most of the laboratories. The usage of PTFE and fluorine-based polymeric material was avoided where possible during PFAS analysis. As a preventive measure and to avoid interferences, all the required glassware and plasticware were washed with LC-MS grade methanol before use. There was no usage of PTFE and fluorine-based polymeric material during PFAS analysis. The UHPLC solvent tubing was changed from PTFE to PEEK, and an additional delay column was used between the mobile phase mixer and the sample loop. Further, the PFAS were monitored regularly through the procedural blanks.

Method validation

As per the guidance document, the analytical parameters—linearity, trueness, precision, method robustness, and the limit of quantitation—were considered for the quantitation of PFAS in food. The linearity was plotted in the range of 0.05–5 ng/mL for all target analytes. The instrument response for each analyte was assessed with respect to the concentration of the analyte. The quantification of most analytes was achieved using a matrix-based (procedural standards) linearity by following linear curve fitting. Method accuracy and precision were evaluated through spiking an uncontaminated matrix, which is a representative edible portion. Two different spiking levels (0.10 and 0.20 µg/kg) were studied with six replicates at each level in fish, whereas 0.1 and 0.3 µg/kg were spiked in egg along with their unspiked matrix as well as procedural solvent blank. The method recoveries were assessed by measuring absolute concentration using a procedural matrix-based calibration curve. The limit of quantitation (LOQ) was set as the lowest successfully validated analyte level, for which the identification criteria, trueness, and precision were met.[11]

Results and discussion

Sample preparation

Both selected matrices contain high fat and protein content, along with vitamins and minerals. Such matrices are challenging and may lead to lower extractability of PFAS compounds. Stecconi et al. have reported an acetonitrile-based extraction with QuEChERS salts followed by SPE clean-up in the fish matrix.[3]

The matrices were extracted by following the EN-QuEChERS method with minor modifications involving a reduction in sample size, water addition, and an increase in volume of extraction solvent. The use of citrate buffer enabled the ionization of the fatty matrix interferences and reduced the matrix background. The optimized clean-up provided excellent chromatographic peaks by reducing the matrix background noise, and there is no adverse impact of the clean-up material on the target PFAS analytes. The quantitation of PFAS was quite challenging due to its trace-level sensitivity requirement. To mitigate the requirement, the cleaned extract was enriched through evaporation and reconstituted. This approach enabled the reduction of the sample dilution and achieved the desired limits of quantitation. This method provided a highly selective analytical approach that did not show any interference signal at the retention time of the target analyte.

LC-MS/MS analysis

The laboratory environment needs to remain clean and free from PFAS contamination. The PFAS kit contains a solvent PEEK tubing, which avoids incurred contamination from PTFE tubings. PFAS provided better ionization efficiency in negative polarity as $[M-H]^-$ (deprotonation). The spray voltage of 1,500 V and collision-induced dissociation gas pressure (2 mTorr) offered the best sensitivity. The sheath and auxiliary gas were set to normal based on the flow rate. The optimized spray voltage and the CID gas flow enhanced the sensitivity by 1.5–2X for PFAS compounds as compared to the default value of 1.5 mTorr. The Accucore C18 column and optimized mobile phase with gradient program offered the best symmetrical peak shape for all the target compounds with excellent resolution (Figure 1).

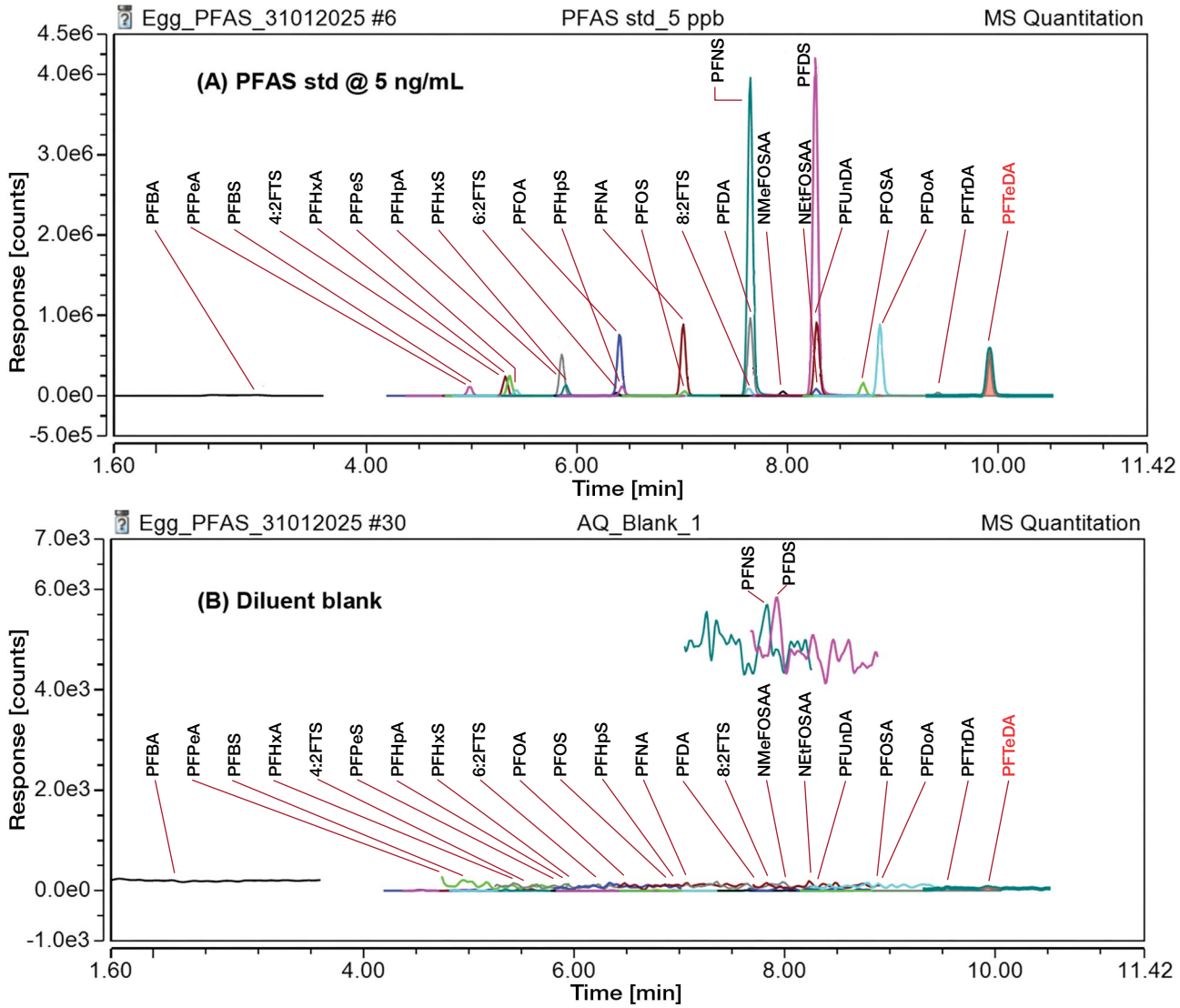

Figure 1. Total ion chromatogram observed for PFAS neat standard at 5 ng/mL (A) and diluent blank (B).

The interference signals were observed for PFBA, PFHxA, and PFHxS in the fish and egg matrix at different retention times, more than 1 min of target RT, and chromatographically well separated from each other. The matrix interference for PFOS was observed in the egg matrix for 498.93→80 and 498.93→499 transitions at closer retention and in-between branched and linear isomers, whereas the 498.93→99 transition was absent. This interference was confirmed through the standard addition techniques, and the PFOS (linear) retention was observed at the tailing part of the interference signal. To avoid any ambiguity, a 498.93→99.0 transition was used for the accurate and precise quantitation purpose in the egg matrix (Figure 2).

The sample dilution with acetonitrile:water (1:1) disturbs the peak shape through band broadening and peak distortion/splitting for early eluting signals, such as PFBA and PFPeA. To avoid that issue, the organic solvent strength was changed to methanol:water (1:1), which resulted in the best peak shape with separation of branched and linear isomers (Figure 3). Also, a strong solvent loop helped to increase the injection volume, certainly without disturbance in peak shapes.

All target analytes had two transitions, except PFBA and PFPeA which carry a single transition due to poor fragmentation and response of the confirming ion. User-defined parameters for data processing were set in the data processing method. Based on these pre-defined parameters, the data was processed automatically. For identification, a minimum of two transitions was considered—quantitation and confirmation. In Figures 4 and 5, an identification of PFOA in egg and fish was demonstrated at 0.1 ng/g with two transitions, 413→369 (Quant) and 413→169 (Qual), with the ion ratio in comparison with a standard, which is within ±30% as per the SANTE guideline.[9] Further, the quantification of both branched and linear isomers is demonstrated in Figure 6.

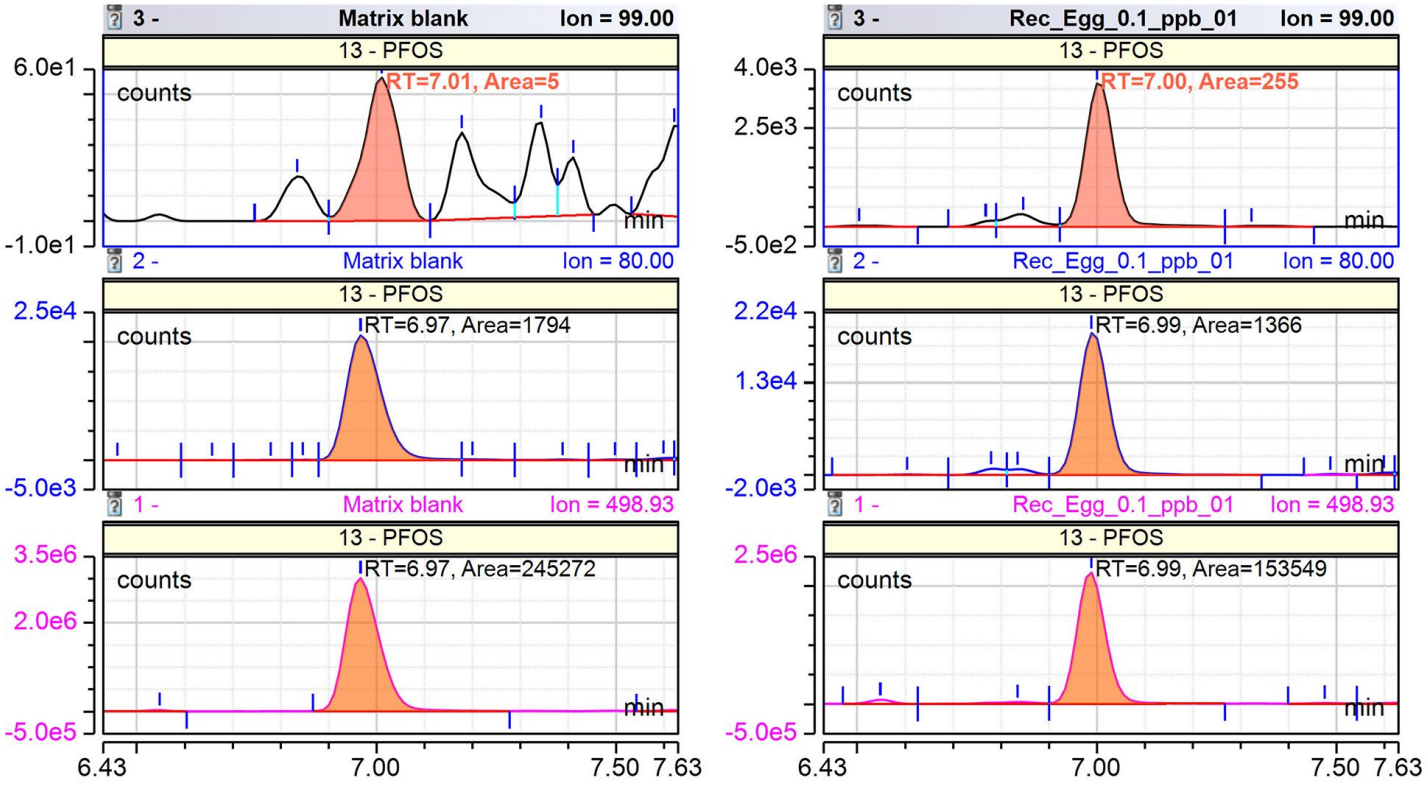

Figure 2. Differentiation of the isobaric signal from PFOS in the egg matrix through the standard addition technique at 0.1 µg/kg.

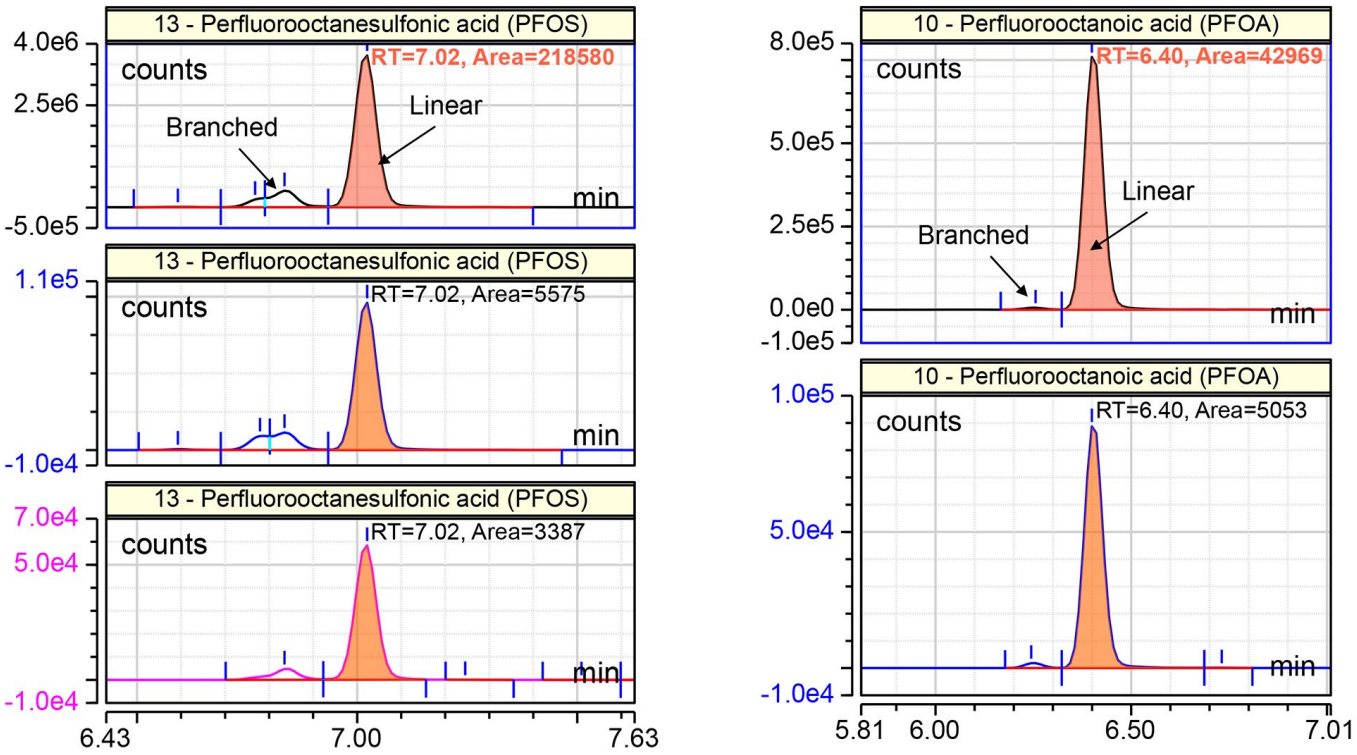

Figure 3. Chromatographic separation between branched and linear isomers of PFOS and PFOA at 5 ng/mL.

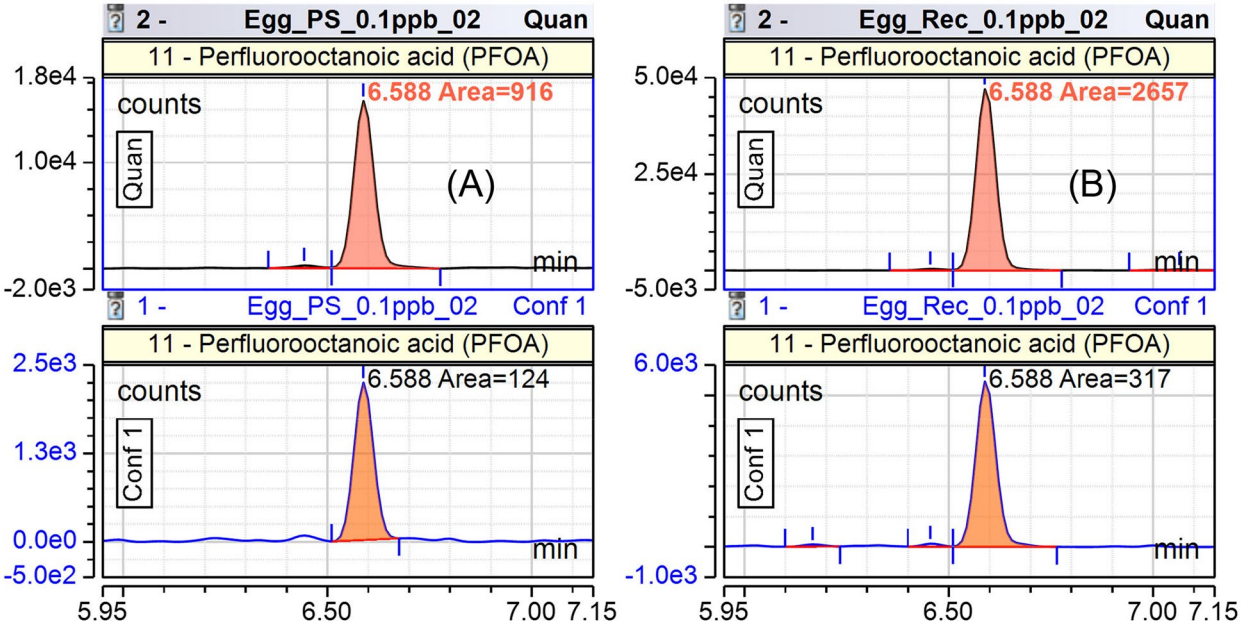

Figure 4. Extracted ion chromatogram for PFOA at 0.1 ng/g matrix standard (A), and 0.1 ng/g spiked egg sample (B).

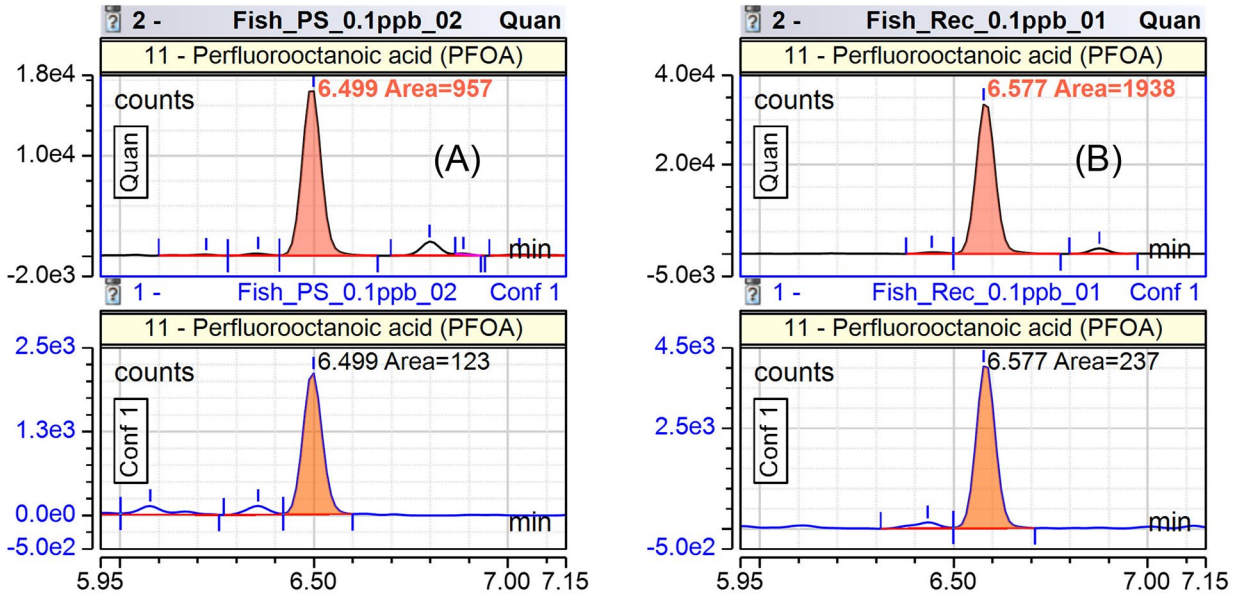

Figure 5. Extracted ion chromatogram for PFOA at 0.1 ng/g matrix standard (A), and 0.1 ng/g spiked fish sample (B).

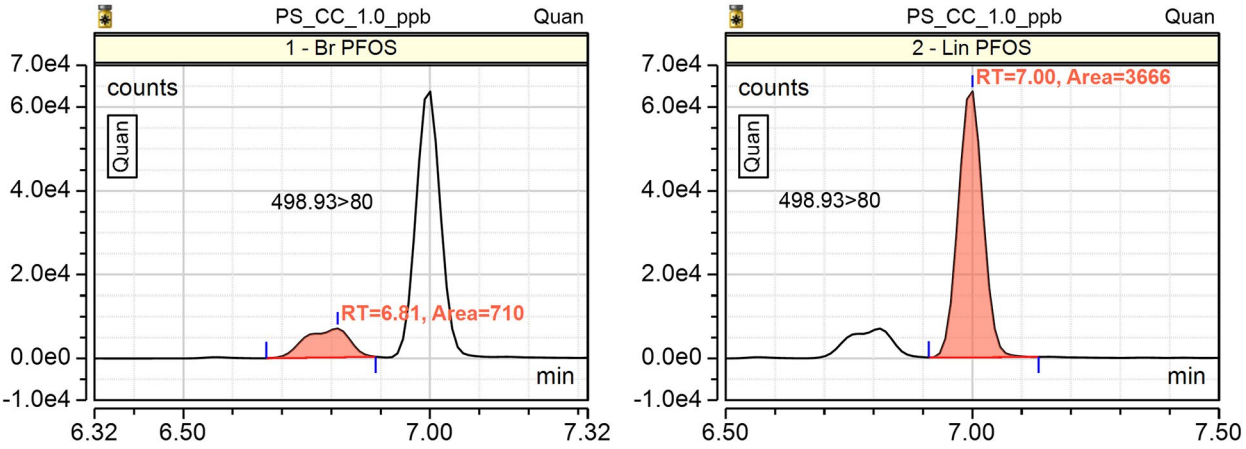

Figure 6. Distribution of branched and linear isomer for PFOS at 1.0 µg/kg.

Calculations for PFOS [branched (Br.) and linear (Lin.) isomers]

Area for Br. PFOS = 710

Area for Lin. PFOS = 3,666

Total area for PFOS = 4,376 with a concentration of 1 µg/kg

$$\text{Calculated conc. for Br. PFOS} = \frac{\text{Area of Br. PFOS}}{\text{Area of Total PFOS}} \times \text{conc. of PFOS (µg/kg)} = 0.162\ \text{µg/kg}$$

$$\text{Calculated conc. for Lin. PFOS} = \frac{\text{Area of Lin. PFOS}}{\text{Area of Total PFOS}} \times \text{conc. of PFOS (µg/kg)} = 0.837\ \text{µg/kg}$$

Calculated conc. for total PFOS (µg/kg) = Cal. conc. for Br. PFOS + Cal. conc. for Lin. PFOS = 0.999 µg/kg

Method performance

The linearity was plotted in the range of 0.05–5 µg/kg for all analytes in egg and fish. The selected linearity range offered an excellent correlation coefficient (≥0.99) with ±20% residuals for all target analytes in the fish tissue and egg. The matrix-based (procedural) calibration standards were compared with the neat standards, and there is matrix enhancement observed for a few analytes. To harmonize the results, a matrix-based linearity was plotted to quantify the absolute concentration of PFAS in two matrices. The lowest calibration level showed good sensitivity with ≥10 signal-to-noise ratio with two transition confirmations for all target analytes except PFBA and PFPeA. The sample preparation protocol involved a diluted extract, which was enriched through pre-concentration and injected into the LC-MS/MS. This approach offered optimum dilution and clean extraction to achieve the desired sensitivity requirement with EU regulatory limits and meet the SANTE guideline criteria.

The limit of quantitation (LOQ) values were 0.1 ng/g with acceptable recoveries (70%–120%) and precision (<20%) observed in both matrices. The recovery experiment was carried out at higher levels (0.1 and 0.2 ng/g in fish, and 0.1 and 0.3 ng/g in egg) to demonstrate the performance of the method. Most of the analytes showed average recoveries in the range of 70%–120% with <20% RSD, as shown in Table 3, except a few, which were within the acceptance criteria of the SANTE guideline.[9] A few analytes—PFHxS, PFTeDA, PFPeA, and PFPS—showed recoveries <70% as well as >120%, but the precision was excellent, and hence considered for the final method. The optimized method was tested for repeatability of results (0.2 µg/kg) obtained throughout the batch of multiple injections (n=22) by considering a commercial food testing lab schedule. The repeatability of a representative analyte (PFOS, PFNA, PFOA, and PFHxS) was within 10% (acceptable limit), as shown in Figure 7.

Table 3. Recoveries and precision for all target PFAS compounds.

		Egg				Fish tissue			
		0.1 µg/kg		0.3 µg/kg		0.1 µg/kg		0.2 µg/kg	
Sr. no.	Name of compound	% Rec	%RSD	% Rec	%RSD	% Rec	%RSD	% Rec	%RSD
1	PFBA	112.1	18.8	73.5	11.3	100.8	13.5	97.3	12.4
2	PFPeA	59.4	15.9	76.7	15.9	93.1	19.6	89.7	12.5
3	PFBSA	95.0	9.5	69.8	13.7	87.0	6.5	95.2	7.5
4	4:2FTS	89.6	9.9	75.3	17.4	103.8	17.4	87.7	12.3
5	PFHxA	104.9	10.0	73.1	17.0	74.1	17.5	84.5	10.1
6	PFPS	91.8	9.3	64.2	17.1	90.8	13.7	97.1	13.3
7	PFHpA	95.2	9.2	76.0	12.9	101.2	16.5	88.9	15.2
8	PFHxS	95.0	7.5	67.3	13.2	86.5	12.9	89.9	7.7
9	6:2FTS	89.0	9.3	75.2	15.1	104.6	17.3	77.7	15.8
10	PFOA	94.5	8.5	71.5	12.8	97.7	9.3	91.8	3.3
11	PFHpS	95.6	7.8	65.0	14.6	105.8	7.5	86.9	3.8
12	PFNA	98.1	8.4	71.1	8.8	99.1	10.3	87.4	5.2
13	PFOS	94.4	9.0	66.7	6.9	99.8	18.9	97.3	14.1
14	PFDA	77.0	8.0	86.9	10.5	90.3	15.2	89.3	7.3
15	8:2FTS	88.5	10.3	85.1	8.5	81.9	17.1	69.1	12.3
16	PFNS	86.6	8.5	81.8	10.0	106.2	8.4	90.4	12.9
17	NMeFOSAA	77.0	11.3	89.2	14.8	112.2	13.5	93.0	17.3
18	PFDS	92.0	8.9	92.5	13.1	90.9	12.9	94.0	17.7
19	NEtFOSAA	68.7	19.8	114.4	14.4	114.7	16.2	74.3	11.8
20	PFUNDA	86.5	7.4	85.6	8.7	95.7	12.7	89.5	17.9
21	PFOSA	89.0	12.2	102.5	20.1	96.5	12.0	87.2	14.0
22	PFDoA	79.7	10.1	107.6	11.9	96.3	16.1	83.0	10.3
23	PFTrDA	92.4	13.4	115.8	11.4	76.9	16.1	75.1	10.4
24	PFTeDA	70.7	17.2	162.5	11.3	103.1	15.2	86.6	19.0

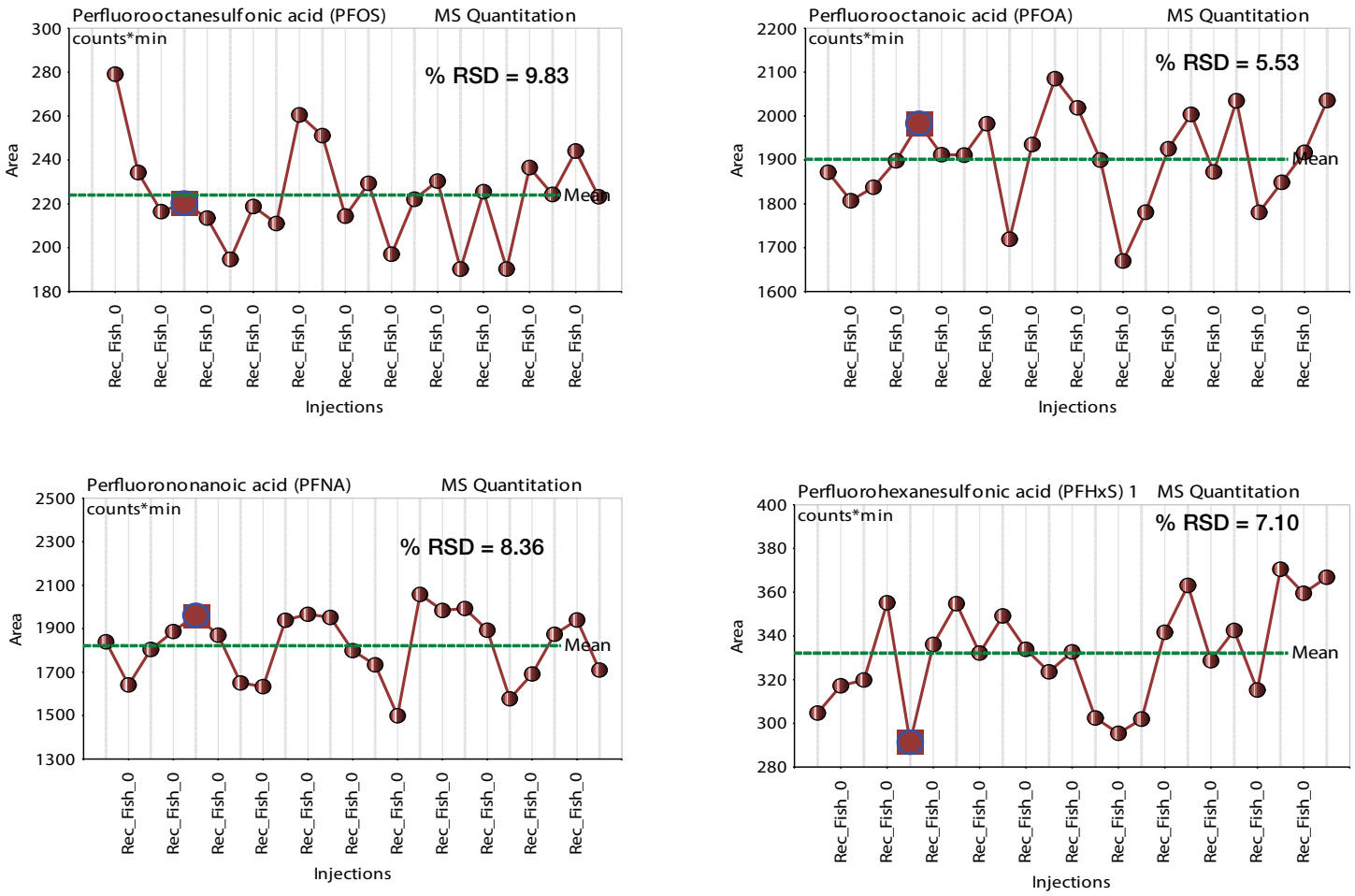

Figure 7. Repeatability of representative analytes (PFOS, PFOA, PFNA, and PFHxS) shown at 0.2 ng/g level in fish tissue.

Conclusions

This work offers a complete workflow for the trace-level detection and quantification of PFAS concentration in fish and egg by using a Thermo Scientific™ LC-HESI-MS/MS (TSQ Quantis Plus MS). A generic approach for sample preparation, the EN-QuEChERS method, is followed by sample extraction and clean-up and then enrichment, achieving the desired sensitivity (0.1 ng/g), which is below the EU requirements, and acceptable performance (recoveries and precision). This approach enables compliance with the EU MRLs and method performance as per the EU requirements. The sample preparation and analytical conditions offer a total workflow without any interference or carryover issues during analysis. Also, this approach helps to keep the LC-MS/MS clean and operate without a breakdown, which can assist commercial labs in further increasing their high throughput.

References

1. Stramenga, A.; Tavoloni, T.; Stecconi, T.; Galarini, R.; Giannotti, M.; Siracusa, M.; Ciriaci, M; Bacchiocchi, S.; Piersanti, A. Perfluoroalkyl and polyfluoroalkyl substances (PFASs): An optimized LC-MS/MS procedure for feed analysis. *J. Chromatogr. B* **2021,** *1186,* 123009. https://doi.org/10.1016/j.jchromb.2021.123009
2. Panieri, E.; Baralic, K.; Djukic-Cosic, D.; Buha Djordjevic, A.; Saso, L. PFAS molecules: a major concern for human health and the environment. *Toxics* **2022,** *10*(2), 44. https://doi.org/10.3390/toxics10020044
3. Stecconi, T.; Tavoloni, T.; Stramenga, A.; Bacchiocchi, S.; Barola, C.; Dubbini, A.; Galarini, R.; Moretti, S.; Sagratini, G.; Piersanti, A. A LC-MS/MS procedure for the analysis of 19 perfluoroalkyl substances in food fulfilling recent EU regulations requests. *Talanta* **2024,** *266*, Part 2, 125054. https://doi.org/10.1016/j.talanta.2023.125054
4. EFSA, Risk to human health related to the presence of perfluoroalkyl substances in food, *EFSA J.* **2020,** *18,* e06223. https://doi.org/10.2903/j.efsa.2020.6223
5. Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed. https://eurl-pops.eu/user/pages/05.news/17.Guidance-Document-PFAS/Guidance%20Document%20PFAS%20V2.0%20(incl.%20Annex%20V2.0).pdf?g-64cde584
6. Commission Implementing Regulation (EU) 2022/1428 of 24 August 2022 Laying Down Methods of Sampling and Analysis for the Control of Perfluoroalkyl Substances in Certain Foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1428
7. European Commission, Commission Regulation (EU) 2022/2388 of 7 December 2022 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Perfluoroalkyl Substances in Certain Foodstuffs (Text with EEA Relevance). http://data.europa.eu/eli/reg/2022/2388/oj/eng2022
8. COMMISSION RECOMMENDATION (EU) 2022/1431 on the Monitoring of Perfluoroalkyl Substances in Food. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022H1431
9. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. https://food.ec.europa.eu/system/files/2023-11/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf
10. Chiesa, L.M.; Nobile, M.; Malandra, R.; Pessina, D.; Panseri, S.; Labella, G.F.; Arioli, F. Food safety traits of mussels and clams: distribution of PCBs, PBDEs, OCPs, PAHs and PFASs in sample from different areas using HRMS-Orbitrap® and modified QuEChERS extraction followed by GC-MS/MS. *Food Additives & Contaminants: Part A* **2018,** *35*(5), 959–971. https://doi.org/10.1080/19440049.2018.1434900
11. Robouch, P.; Stroka, J.; Haedrich, J.; Schaechtele, A.; Wenzl, T. Guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food, Publications Office, 2016, https://data.europa.eu/doi/10.2787/8931

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