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Nota de aplicación

Analysis of 28 EU Regulated and Recommended PFAS in Food via LC-MS/MS – Part 1: Vegetable, Fruit, and Baby Food

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Abstract

This study introduces an optimized LC-MS/MS methodology for the comprehensive analysis of Per- and Polyfluoroalkyl Substances (PFAS) in vegetable, fruit, and baby food. The method demonstrates an exceptionally low limit of quantification, reaching 0.0005 µg/kg for some compounds in matrix, while accurately detecting and quantifying the PFAS compounds listed in the Commission Recommendation (EU) 2022/1431. The combination of enhanced sensitivity of the Xevo™ TQ Absolute MS System, with the increased clean-up efficacy of a prototype bilayer dual-phase GCB/WAX SPE cartridge gave excellent recoveries, between 87 and 116% for the mandatory PFAS, and between 65 and 131% for the majority of the recommended compounds along with repeatability (RSD_r) ≤10%.

Benefits

- Sensitive quantitative method for the analysis of mandatory, recommended, and considered PFAS in the EU Commission recommendation allowed for all PFAS to be incorporated into a single method meeting requirements
- Extremely low limits of quantification (down to 0.0005 µg/kg) in vegetable, fruit, and baby food allowing to

surpass the criteria set by the EURL POPs guidelines

- Excellent method recoveries and repeatability, which complies with the acceptance criteria in the guidelines
- Good recovery for FOSA using a new bilayer dual-phase SPE cartridge containing GCB and WAX for sample clean-up, allowing to effectively clean-up neutral PFAS
- Enhanced time effectiveness compared to prior UPLC™ methods, resulting in a 50% reduction in sample analysis time, while ensuring baseline separation between linear and branched PFAS compounds and potential interferences

Introduction

Per- and Polyfluoroalkyl Substances (PFAS) constitute a class of synthetic compounds widely used in various industrial and commercial applications, known for their persistence and potential adverse health effects. Regulatory bodies have increasingly prioritized stringent monitoring and control of PFAS levels in food due to their presence in agricultural environments and food chains, posing potential health risks to consumers.

In the European Union (EU), Commission Regulation (EU) 2022/2388, amending Regulation (EC) No 1881/2006, applying from 1 January 2023, sets individual maximum levels for PFOS, PFOA, PFNA, and PFHxS, together with a maximum level for the sum of those PFAS, in foods of animal origin.[1] Commission Recommendation (EU) 2022/1431, in force from September 2022, recommends Member States should test for the presence of the same four PFAS during the years 2022, 2023, 2024, and 2025 in a wider range of foodstuffs than covered in 2022/2388, and also suggests the monitoring for a larger list of PFAS in various foodstuffs.[2]

Commission Implementing Regulation (EU) 2022/1428 (CIR), in force from September 2022, provides methods of sampling and analysis for the control of PFAS in certain foodstuffs, it also provides acceptance criteria for validation of methods and information on reporting and interpretation of results.[3] Furthermore, in 2022, the EURL POPs released a guidance document for PFAS methods, which provides information on expected method performance and limits of quantification.[4,5] AOAC Standard Method Performance Requirements (SMPRs®) describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single laboratory validation, or a multi-site collaborative study. AOAC SMPRs can be used as acceptance criteria for verification in scenarios beyond Commission Implementing Regulation (EU) 2022/1428. AOAC SMPR 2023.003 for PFAS in a variety of foodstuffs has been published.[6]

Part 1 of this study focuses on the development of a sensitive LC-MS/MS method tailored for the detection and quantification of ultra-trace levels of PFAS in vegetable, fruit, and baby food to address the need for reliable analytical methods and to meet the criteria set in the recent guidelines. Part 2 will focus on the determination of PFAS in animal products.

Experimental

Standards and Solutions

All standards were purchased from Wellington Laboratories. The following standards were used to prepare stock solutions:

Name of commercial standard	Concentration of individual PFAS in MeOH (µg/mL)	Purpose
PFAC30PAR	1	Natives standard mix
L-PFUdS	50	Native standards
L-PFDoS	50	Native standards
L-PFTrDS	50	Native standards
6:2FTAB (Capstone B)	50	Native standards
DPOSA (Capstone A)	10	Native standards
M3-HFPODA	50	Extraction internal standard mix
MPFAC-24ES	1	Extraction internal standard mix
MPFAC-C-IS	2	Injection internal standard mix

A native PFAS mix stock solution (500 ng/mL of each analyte) was prepared in methanol and was used for serial dilutions. An extraction internal standard (EIS) mix solution (1 ng/mL of each labelled analyte) was prepared in methanol and was used to spike food samples prior to extraction. A mix of EIS and injection internal standards (IIS) was prepared in water:methanol 1:1 (all proportions in this work were v:v). The EIS + IIS mix included 0.2 ng/mL of each labelled analyte; this solution served as diluent for the calibration curve. Finally, an IIS mix solution (20 ng/mL of each labelled analyte) was prepared in water:methanol 1:1 and was used to spike each LC vial after extraction and clean-up.

A solvent calibration curve in the range of 0.00125–5 ng/mL (equivalent to 0.00025–1 µg/kg in actual sample)

was prepared and used for sample analysis.

Sample Preparation

Prior to any operation in the laboratory, good practices for preventing or minimising PFAS contamination from the environment and reagents was followed (details described in the White Paper 720007905 < https://www.waters.com/waters/library.htm?cid=511436&lid=135116171&lcid=135116170>).[7]

Test samples consisted of vegetable (tomato), fruit (apple), and baby food (an organic smooth puree made of fruit and yogurt) which were purchased at a local grocery store. The edible portions of tomato and apple were cut into thin slices, and homogenized using a kitchen blender. Samples were stored in a freezer (-20 °C) and thawed in a refrigerator (4 °C) overnight prior to extraction.

2.5 grams of sample were placed into a 15 mL centrifuge Falcon tube, spiked with 100 µL of EIS (at a concentration of 0.04 µg/kg), and vortexed for a few seconds.

The samples were extracted with 6 mL of 0.3% ammonium hydroxide methanolic solution and vortex mixing the tubes for 10 min. After centrifugation at 5000 g, the supernatant was quantitatively transferred into an empty clean tube. The extraction step was repeated, and approximately 12 mL of extract were combined and concentrated to 0.5 mL under a gentle nitrogen stream at 50 ºC. Each sample was reconstituted up to 8 mL using reagent water. The pH of each sample was checked with test strips, and corrected by adding a few drops of a 50% formic acid solution (if needed) to ensure the pH of the reconstituted extract was below 6 units.

The extract was loaded onto a prototype bilayer dual-phase SPE cartridge from Waters™ containing weak anion exchange (WAX) and graphitized carbon black (GCB). The cartridge used for clean-up of food samples has the GCB layer on top of the WAX layer. The cartridge was previously conditioned with 15 mL of 1% methanolic ammonium hydroxide, and with 5 mL of 0.3 M formic acid in water. After loading the sample, the cartridge was washed with 10 mL of reagent water and 5 mL of 1:1 0.1 M formic acid:methanol. After drying the cartridge for 10 seconds, the analytes were eluted with 5 mL of 1% methanolic ammonium hydroxide, collecting the eluate in a clean Falcon tube.

The eluate was concentrated under a gentle nitrogen stream at 50 ºC until about one drop, and then reconstituted with 0.5 mL of water:methanol 1:1. The reconstitution was performed in two steps. First 250 µL of methanol were added and the tube was vortexed. Then 250 µL of water was added and the tube was vortexed again. This approach is meant to increase the solubility of less polar PFAS.

The reconstituted cleaned-up samples (0.5 mL) were transferred into polypropylene LC vials (p/n: 186005219 < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186005219-polypropylene-12-x-32-mm-screw-neck-vial-700--l-volume-100-pk.html>) with polyethylene cap (p/n: 186000305 < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186000305-blue-12-x-32-mm-screw-neck-cap-and-preslit-ptfe-silicone-septum-.html>). 5 µL of IIS solution was added to each vial (0.2 ng/mL of each labelled IIS resulted in vial), which were then vortexed and placed in the autosampler for injection. The method is illustrated in Figure 1, and resulted in a concentration factor of five.

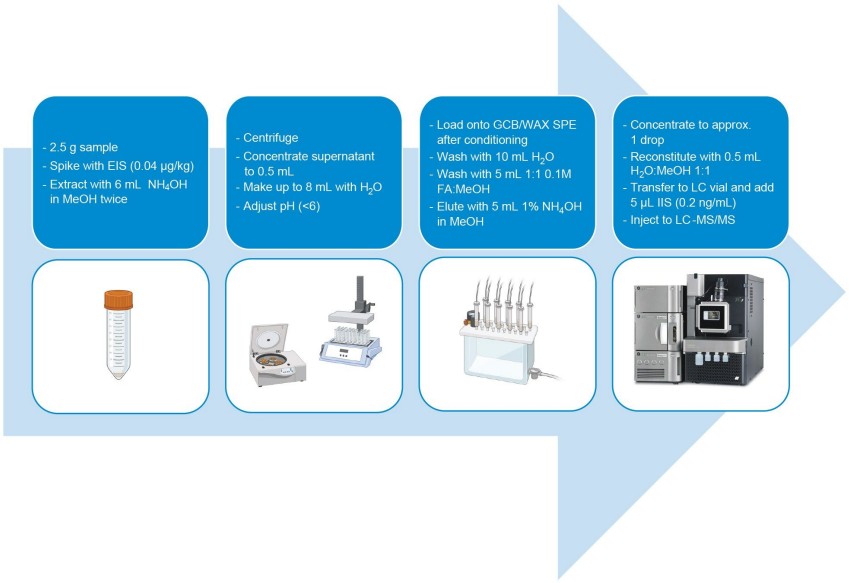

Figure 1. Scheme of the extraction and clean-up protocol for vegetable, fruit, and baby food samples.

LC Conditions

LC system:	ACQUITY™ PREMIER UPLC with PFAS Analysis Kit
Vials:	Polypropylene autosampler vial (p/n: 186005219) with polyethylene cap (p/n: 186000305)

Analytical column:	ACQUITY Premier UPLC BEH™ C_{18}, 2.1 x 50 mm, 1.7 µm (p/n: 186009452)
Isolator column:	Atlantis™ Premier BEH C_{18} AX 2.1 x 50 mm, 5.0 µm (p/n: 186009407)
Column temperature:	35 °C
Sample temperature:	10 °C
Injection volume:	5 µL
Flow rate:	0.3 mL/min
Mobile phase A:	2 mM ammonium acetate in water
Mobile phase B:	2 mM ammonium acetate in methanol/acetonitrile 1/1 (v/v)

Gradient Table

Time (min)	%A	%B	Curve
0.0	95	5	0
0.5	75	25	6
3.0	50	50	6
6.5	15	85	6
7.0	5	95	6
8.5	5	95	6
9.0	95	5	6
11.0	95	5	6

MS Conditions

MS system:	Xevo TQ Absolute
Ionization mode:	Electrospray negative
Source temperature:	100 °C
Capillary voltage:	0.5 kV
Desolvation temperature:	350 °C
Desolvation flow rate:	900 L/hr
Cone flow rate:	150 L/hr
MRM method:	See Appendix for Full MRM Method details

Data Management

Software:	waters_connect™ for quantitation

Method Performance Assessment

The performance of the method was evaluated using the criteria set in the EURL POPs PFAS guidance document and AOAC SMPR.[4,6] For the intra-lab method performance, trueness was assessed by spiking samples of tomato, apple, and baby food with a mixture of 28 native PFAS at three concentration levels, in triplicate. Solvent blanks, procedural blanks, matrix blanks, and fortified samples were then extracted and analysed as described in the previous section. Repeatability of the method was assessed by relative standard deviation under repeatability conditions intra-day (RSD_r). Apparent recoveries and RSD_r were calculated for each level:

Level 1: corresponding to the method-lower limit of quantification (m-LLOQ)

Level 2: corresponding to 10x m-LLOQ

Level 3: corresponding to the method-upper limit of quantification (m-ULOQ)

The analytical sequence of each batch was composed of two different sets for solvent calibrants (≥6 points per each curve, excluding blank) bracketing incurred, and fortified samples.

Results and Discussion

The method includes 28 PFAS that are listed in the Commission Recommendation (EU) 2022/1431, including mandatory, recommended and PFAS “for consideration” (see Table 1 for the list of PFAS).

Analyte	RT (min)	i-LOD (ng/mL)	i-LOQ (ng/mL)
PFBA	2.23	0.00075	0.0025
PFPeA	3.04	0.00075	0.0025
PFBS	3.63	0.000375	0.00125
PFHxA	3.86	0.000375	0.00125
GenX	4.10	0.00075	0.0025
PFPeS	4.29	0.000375	0.00125
PFHpA	4.54	0.00075	0.0025
ADONA	4.70	0.000375	0.00125
PFHxS	4.92	0.000375	0.00125
PFOA	5.14	0.00075	0.0025
PFHpS	5.48	0.000375	0.00125
Capstone B	5.61	0.0075	0.025
PFNA	5.70	0.00075	0.0025
Capstone A	5.70	0.00375	0.0125
PFOS	5.98	0.000375	0.00125
PFDA	6.18	0.0015	0.005
9Cl-PF3ONS	6.25	0.000375	0.00125
PFNS	6.45	0.000375	0.00125
PFUnDA	6.60	0.00075	0.0025
PFDS	6.78	0.000375	0.00125
FOSA	6.78	0.000375	0.00125
PFDoDA	6.98	0.00075	0.0025
11Cl-PF3OUdS	7.05	0.000375	0.00125
PFUnDS	7.12	0.00075	0.0025
PFTrDA	7.30	0.0015	0.005
PFDoDS	7.42	0.000375	0.00125
PFTeDA	7.61	0.0015	0.005
PFTrDS	7.67	0.0075	0.025

Table 1. Retention time (RT), instrument-limit of detection (i-LOD) and instrument-limit of quantification (i-LOQ) of 28 PFAS compounds listed in the Commission Recommendation (EU) 2022/1431 using the Xevo-TQ Absolute Mass Spectrometer.

The EIS were spiked prior to sample extraction and used to correct the native compounds for extraction recovery and matrix effects. The IIS were added to the sample after clean-up and used to correct the extraction standards for reconstitution variations, matrix effects, and potential injection variations. With the presence of EIS and IIS, matrix-matching was not necessary for routine sample analysis. This approach has been discussed in a previous study.[8]

Chromatography

Baseline separation of linear and branched-PFAS was achieved on a 50 mm BEH C_{18} column after scaling down a previously developed LC method from 22 to 11 minutes of run time. A combination of methanol and acetonitrile in the organic mobile phase was used to obtain baseline separation of PFAS from potential interferences commonly found in certain types of foods (***e.g.***, separating cholic acids from PFOS). Figure 2 shows the chromatographic trace of PFHxS and PFOS, where it can be noted that linear and branched PFAS can be quantified either individually or as a sum given to the excellent chromatographic separation. More information regarding LC optimization can be found in our previous work App Note 720008108.[9]

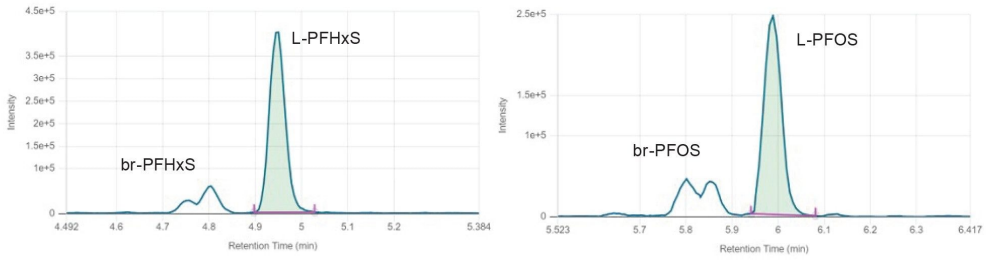

Figure 2. Chromatographic traces of a tomato sample spiked with 0.01 µg/kg of PFHxS (on the left) and PFOS (on the right). L=linear, br=branched.

The ratio of the chromatographic retention time (RT) of the analyte to that of the IS (***i.e.*** relative RT of the analyte) corresponded to that of the calibration standard with a deviation ≤1%.

The first eluting compound (PFBA, RT=2.23 min) presents a RT >6 times the column void time (t_0=0.37), thus enough retention was obtained for all PFAS included in this study.

Linearity and Limits of Quantification

The response of solvent standards, which bracketed the incurred and fortified samples, were plotted to construct the calibration curve using 1/x weighting factor. The linear range of the method differed across different analytes and matrices. Coefficients of determination of the calibration curves (R^2) were >0.99, and residuals were within ±20% in the majority of the cases. Ion ratios from sample extracts were within ±30% (relative) of average of calibration standards from same sequence.

The instrument limit of detection (i-LOD)[10] was calculated on solvent standards (signal-to-noise ratio ≥3 at the LOD level) and were as low as 0.0004 ng/mL for certain individual PFAS (equivalent to 2 fg on column).

The method lower and upper limits of quantification (m-LLOQ and m-ULOQ) were adopted as the lowest and highest level of the calibration range, respectively, provided that m-LLOQ presented a signal-to-noise ratio ≥10 (noise processing=peak-to-peak). m-LLOQ were as low as 0.0005 µg/kg in the tested samples. i-LOD, i-LOQ and retention times are reported in Table 1, while m-LLOQ, method linear range and linearity for each food commodity can be found in Tables 2, 3, and 4.

Notably, for the four mandatory PFAS (PFOS, PFOA, PFNA, and PFHxS) the method allows to accurately quantify as low as 0.0005 µg/kg in all food commodities tested. Figure 3 presents the calibration curves and residuals plot, whilst Figure 4 shows an example chromatogram at the m-LLOQ level.

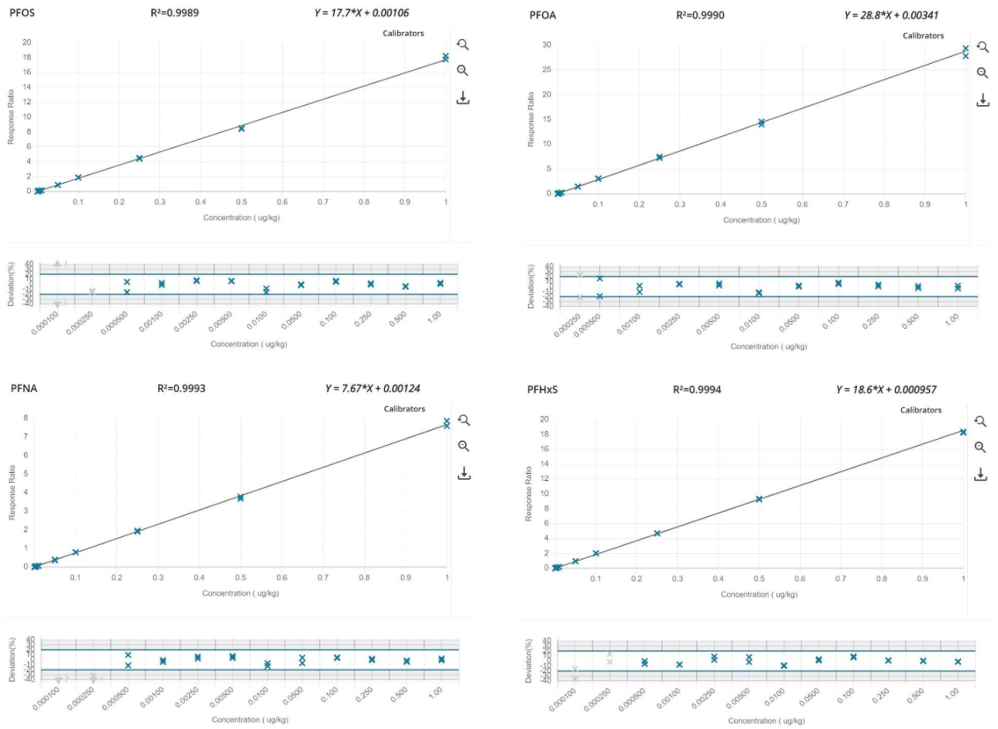

Figure 3. Calibration curves and residuals plots of the EU mandatory PFAS (PFOS, PFOA, PFNA, and PFHxS).

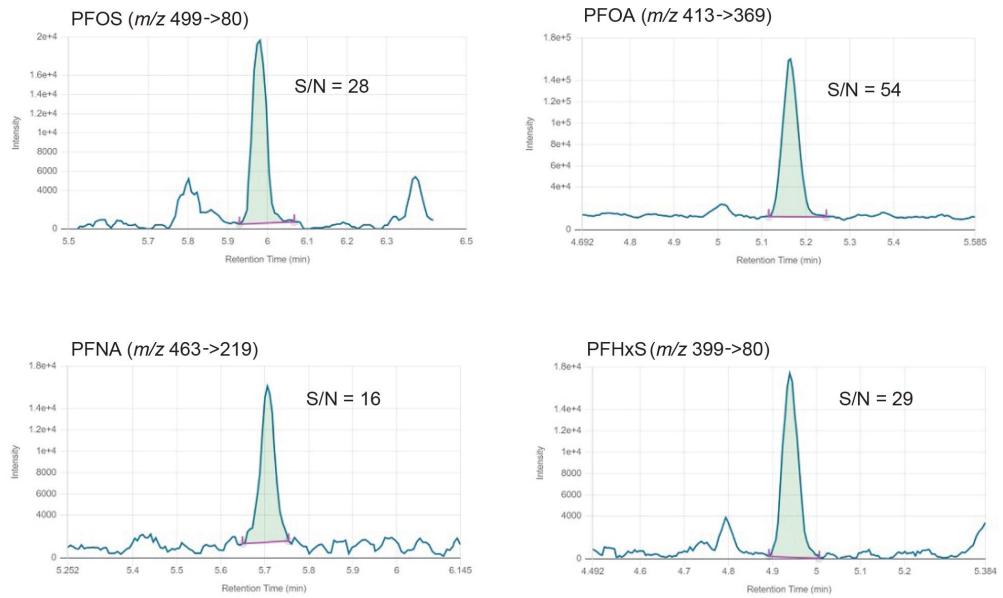

Figure 4. Chromatographic traces of 0.0005 µg/kg of EU mandatory PFAS in an apple sample. S/N: signal-to-noise ratio (noise processing=peak-to-peak).

Trueness and Repeatability

Trueness, determined as apparent recovery, was assessed by recovery experiments which involved spiking a blank sample at three concentration levels. When considering the entire panel of native PFAS together, mean percentage recovery across all fortification levels was 101±14% for tomato (min=65%, max=131%), 98±13% for apple (min=65%, max=131%), and 99±13% for baby food (min=66%, max=127%). For the four mandatory compounds (PFOS, PFOA, PFNA, and PFHxS) apparent recoveries were between 84 and 116%. For the remaining PFAS recommended and considered in Commission Recommendation (EU) 2022/1431 for monitoring purposes, apparent recoveries were within 65 and 131%. Both sets of results meet the acceptance criteria for trueness described in the CIR, EURL POPS guidelines, and AOAC SMPR. Repeatability (RSD_r) ranged between 0.4 and 10% across all matrices and at all fortification levels, within the limits described for RSD_r in the AOAC SMPR*. The only exceptions were PFDS, 9Cl-PF3ONS, and 11Cl-PF3OUdS, whose recoveries in apple and baby food were within the criteria, but in tomato recoveries were 53, 52, and 58%, respectively. Nevertheless, since RSD_r were well below 10%, for these three compounds, it could be possible to apply a recovery correction factor, as precision is not compromised. Recoveries and RSD_r are reported in Tables 2, 3, and 4, and plotted in Figures 5, 6, and 7.

Analyte	m-LOD (µg/kg)	m-LLOQ (µg/kg)	Method linear range (µg/kg)	Rec% (RSD,%) at LLOQ	Rec% (RSD,%) at 10*LLOQ	Rec% (RSD,%) at ULOQ
PFOS	0.00015	0.0005	0.0005–1	104 (5)	102 (5)	113 (2)
PFOA	0.00015	0.0005	0.0005–1	87 (2)	99 (1)	110 (1)
PFNA	0.00015	0.0005	0.0005–1	84 (4)	102 (4)	112 (0.4)
PFHxS	0.00015	0.0005	0.0005–1	94 (6)	106 (3)	114 (1)
PFBA	N/A	N/A	0.1–1	104 (1)	113 (0.2)	110 (1)
PFPeA	0.00075	0.0025	0.0025–1	119 (2)	106 (1)	112 (1)
PFHxA	0.00015	0.0005	0.0005–1	106 (1)	94 (2)	107 (1)
PFHpA	0.0003	0.001	0.001–1	109 (5)	102 (2)	115 (0.4)
PFDA	0.0015	0.005	0.005–1	117 (2)	106 (1)	115 (0.4)
PFUnDA	0.00075	0.0025	0.0025–1	97 (10)	104 (5)	110 (2)
PFDoDA	0.0015	0.005	0.005–1	114 (2)	103 (4)	108 (3)
PFTrDA	0.0015	0.005	0.005–1	87 (3)	96 (7)	81 (9)
PFTeDA	0.0015	0.005	0.005–1	96 (7)	90 (4)	93 (7)
PFBS	0.0003	0.001	0.001–1	108 (4)	101 (2)	114 (2)
PFPeS	0.0003	0.001	0.001–1	131 (4)	124 (1)	129 (2)
PFHpS	0.0003	0.001	0.001–1	124 (1)	114 (1)	127 (2)
PFNS	0.0003	0.001	0.001–1	72 (5)	77 (3)	65 (3)
PFDS	0.00075	0.0025	0.0025–1	47 (10)	73 (1)	40 (4)
PFUnDS	0.00075	0.0025	0.0025–1	82 (6)	119 (3)	112 (4)
PFDoDS	0.0015	0.005	0.005–1	87 (7)	77 (8)	70 (8)
PFTrDS	0.0015	0.005	0.005–1	86 (9)	77 (4)	85 (6)
FOSA	0.00075	0.0025	0.0025–1	85 (4)	102 (3)	112 (2)
9Cl-PF3ONS	0.00075	0.0025	0.0025–1	55 (8)	50 (1)	50 (0.4)
11Cl-PF3OUdS	0.0003	0.001	0.001–1	50 (0.3)	74 (6)	49 (4)
GenX	0.0003	0.001	0.001–1	94 (8)	99 (3)	108 (2)
ADONA	0.00015	0.0005	0.0005–1	95 (2)	102 (1)	99 (1)
Capstone A	N/A	N/A	N/A	N/A	N/A	N/A
Capstone B	N/A	N/A	N/A	N/A	N/A	N/A

Table 2. Method performance parameters in tomato: method limit of detection (m-LOD) and method lower limit of quantification (m-LLOQ), linear range, percentage recovery at three levels, and respective percentage relative standard deviation under repeatability conditions (RSD_r%, n=3). ULOQ=method upper limit of quantification (equivalent to the highest level of the calibration curve).

Analyte	m-LOD (µg/kg)	m-LLOQ (µg/kg)	Method linear range (µg/kg)	Rec% (RSD,%) at LLOQ	Rec% (RSD,%) at 10*LLOQ	Rec% (RSD,%) at ULOQ
PFOS	0.00015	0.0005	0.0005–1	91 (6)	104 (4)	115 (1)
PFOA	0.00015	0.0005	0.0005–1	91 (5)	97 (2)	113 (2)
PFNA	0.00015	0.0005	0.0005–1	87 (4)	94 (1)	112 (1)
PFHxS	0.00015	0.0005	0.0005–1	98 (2)	101 (4)	116 (1)
PFBA	N/A	N/A	N/A	N/A	N/A	N/A
PFPeA	0.00075	0.0025	0.0025–1	90 (3)	95 (1)	114 (1)
PFHxA	0.0003	0.001	0.001–1	95 (5)	101 (0.5)	109 (1)
PFHpA	0.0003	0.001	0.001–1	102 (1)	92 (2)	112 (1)
PFDA	0.0003	0.001	0.001–1	89 (7)	97 (3)	110 (1)
PFUnDA	0.0003	0.001	0.001–1	113 (7)	96 (2)	108 (3)
PFDoDA	0.0015	0.005	0.005–1	100 (4)	113 (6)	113 (3)
PFTrDA	0.0015	0.005	0.005–1	112 (4)	106 (7)	117 (2)
PFTeDA	0.0015	0.005	0.005–1	79 (7)	65 (5)	71 (1)
PFBS	0.0003	0.001	0.001–1	99 (7)	95 (0.4)	115 (1)
PFPeS	0.0003	0.001	0.001–1	106 (3)	112 (4)	131 (1)
PFHpS	0.0003	0.001	0.001–1	100 (3)	111 (5)	125 (0.2)
PFNS	0.0003	0.001	0.001–1	91 (10)	76 (2)	80 (1)
PFDS	0.0003	0.001	0.001–1	95 (1)	69 (1)	72 (2)
PFUnDS	0.0003	0.001	0.001–1	83 (7)	71 (5)	91 (1)
PFDoDS	0.0015	0.005	0.005–1	84 (9)	67(10)	67 (3)
PFTrDS	0.003	0.01	0.01–1	102 (8)	101 (3)	100 (3)
FOSA	0.00015	0.0005	0.0005–1	93 (6)	99 (4)	113 (2)
9Cl-PF3ONS	0.0003	0.001	0.001–1	98 (6)	94 (3)	110 (1)
11Cl-PF3OUdS	0.0003	0.001	0.001–1	102 (6)	84 (2)	87 (1)
GenX	0.00015	0.0005	0.0005–1	99 (10)	109 (4)	113 (3)
ADONA	0.00015	0.0005	0.0005–1	89 (7)	107 (1)	112 (3)
Capstone A	N/A	N/A	N/A	N/A	N/A	N/A
Capstone B	N/A	N/A	N/A	N/A	N/A	N/A

Table 3. Method performance parameters in apple: method limit of detection (m-LOD) and method lower limit of quantification (m-LLOQ), linear range, percentage recovery at three levels and respective percentage relative standard deviation under repeatability conditions (RSD_r%, n=3). ULOQ=method upper limit of quantification (equivalent to the highest level of the calibration curve).

Analyte	m-LOD (µg/kg)	m-LLOQ (µg/kg)	Method linear range (µg/kg)	Rec% (RSD,%) at LLOQ	Rec% (RSD,%) at 10*LLOQ	Rec% (RSD,%) at ULOQ
PFOS	0.00015	0.0005	0.0005–1	112 (6)	101 (3)	114 (2)
PFOA	0.00015	0.0005	0.0005–1	113 (6)	107 (1)	109 (0.4)
PFNA	0.00015	0.0005	0.0005–1	87 (8)	103 (1)	105 (0.4)
PFHxS	0.00015	0.0005	0.0005–1	111 (9)	110 (4)	113 (1)
PFBA	N/A	N/A	N/A	N/A	N/A	N/A
PFPeA	0.0015	0.005	0.005–1	95 (9)	96 (3)	111 (1)
PFHxA	0.0003	0.001	0.001–1	109 (5)	88 (2)	100 (1)
PFHpA	0.0003	0.001	0.001–1	102 (1)	98 (3)	113 (1)
PFDA	0.00075	0.0025	0.0025–1	102 (6)	98 (5)	105 (2)
PFUnDA	0.0003	0.001	0.001–1	108 (10)	97 (3)	104 (1)
PFDoDA	0.0015	0.005	0.005–1	108 (8)	98 (2)	109 (1)
PFTrDA	0.0015	0.005	0.005–1	99 (5)	81 (1)	98 (9)
PFTeDA	0.00075	0.0025	0.0025–1	97 (1)	69 (3)	73 (4)
PFBS	0.0003	0.001	0.001–1	113 (4)	101 (1)	110 (2)
PFPeS	0.0003	0.001	0.001–1	81 (9)	67 (4)	87 (6)
PFHpS	0.0003	0.001	0.001–1	96 (6)	81 (4)	101 (10)
PFNS	0.0003	0.001	0.001–1	104 (1)	104 (8)	127 (4)
PFDS	0.0003	0.001	0.001–1	81 (9)	113 (8)	107 (8)
PFUnDS	0.0003	0.001	0.001–1	86 (7)	75 (1)	79 (0.3)
PFDoDS	0.0015	0.005	0.005–1	77 (3)	66 (6)	77 (5)
PFTrDS	0.0015	0.005	0.005–1	87 (9)	89 (8)	77 (9)
FOSA	0.00015	0.0005	0.0005–1	81 (7)	103 (5)	112 (2)
9Cl-PF3ONS	0.00015	0.0005	0.0005–1	109 (10)	118 (1)	117 (1)
11Cl-PF3OUdS	0.00015	0.0005	0.0005–1	113 (5)	115 (8)	104 (3)
GenX	0.0003	0.001	0.001–1	103 (5)	107 (2)	103 (1)
ADONA	0.00015	0.0005	0.0005–1	112 (7)	109 (3)	102 (1)
Capstone A	N/A	N/A	N/A	N/A	N/A	N/A
Capstone B	N/A	N/A	N/A	N/A	N/A	N/A

Table 4. Method performance parameters in baby food: method limit of detection (m-LOD) and method lower limit of quantification (m-LLOQ), linear range, percentage recovery at three levels and respective percentage relative standard deviation under repeatability conditions (RSD_r%, n=3). ULOQ=method upper limit of quantification (equivalent to the highest level of the calibration curve).

(*) No assessment of intermediate precision (RSDR) was made during this study.

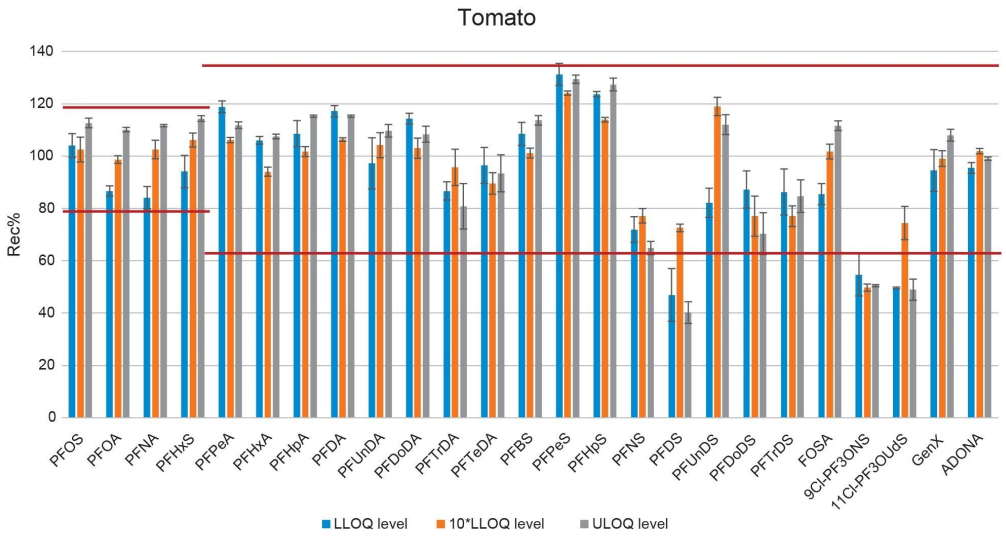

Figure 5. Bar-plot representing the recovery of PFAS in tomato at three fortification levels. Red lines represent the thresholds set by the EURL POPs guidelines.

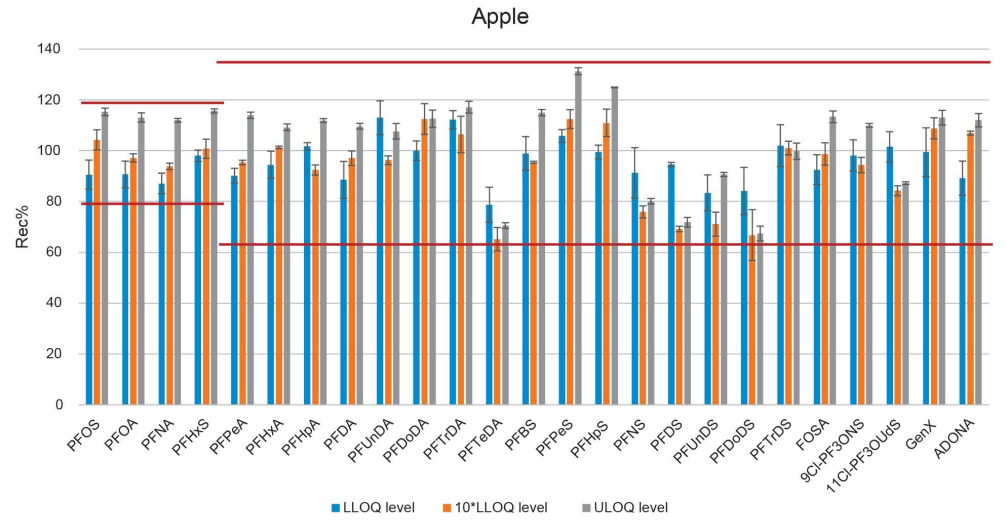

Figure 6. Bar-plot representing the recovery of PFAS in apple at three fortification levels. Red lines represent the thresholds set by the EURL POPs guidelines.

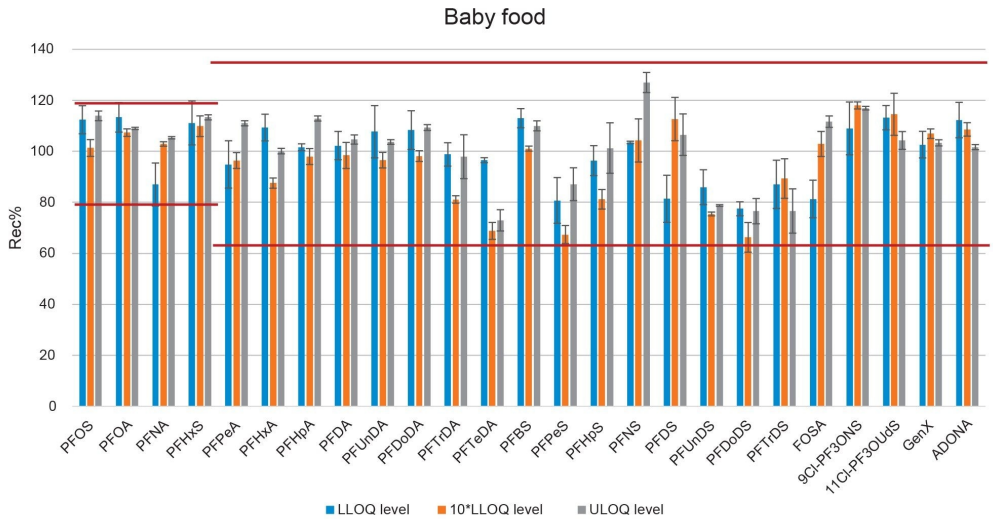

Figure 7. Bar-plot representing the recovery of PFAS in baby food at three fortification levels. Red lines represent the thresholds set by the EURL POPs guidelines.

During this study, a remarkably high contamination of PFBA was encountered not only in matrix samples, but also in solvents and reagents such as formic acid and methanol. PFBA was found in procedural blanks and in both tomato and apple at 0.2 µg/kg. More worryingly, PFBA was also found in baby food at an estimated concentration of 17.5 µg/kg (extrapolated from the calibration curve). PFBA contamination varied from batch to batch, but was always present in all types and brands of methanol that were tested, making it impossible to obtain accurate recovery data for this compound in the present work.

Many PFAS compounds were also found in matrix blanks at levels close to the m-LLOQ. In such cases, blank subtraction was performed in the calculation of recovery.

Capstone A and B were investigated within the method development phase, and appeared to yield to very low process recoveries (below 10%). To better understand the causes of this, an experiment was conducted in which a solvent standard at 0.5 ng/mL was loaded onto the GCB/WAX cartridge after conditioning, and each fraction of the SPE process was collected and analysed after dilution with water or methanol to obtain a final composition of 1:1 water:methanol in the vial. As it can be seen in Figure 8, about 31 and 37% of the analyte was lost in the second washing step for Capstone A and B, respectively. Some researchers[11] also found that these zwitterionic compounds were not efficiently retained on a GCB/WAX SPE cartridge, and suggested to separate capstones from the other PFAS, and to analyse capstones via an extraction-dilution-injection approach. Another factor

contributing to these low recoveries could be the impact of potential ion suppression phenomena in ESI affecting these compounds. Furthermore, as no labelled internal standards were commercially available for capstone A and capstone B, and due to their peculiar chemical structure, to achieve a more accurate quantification a standard addition approach could be carried out.

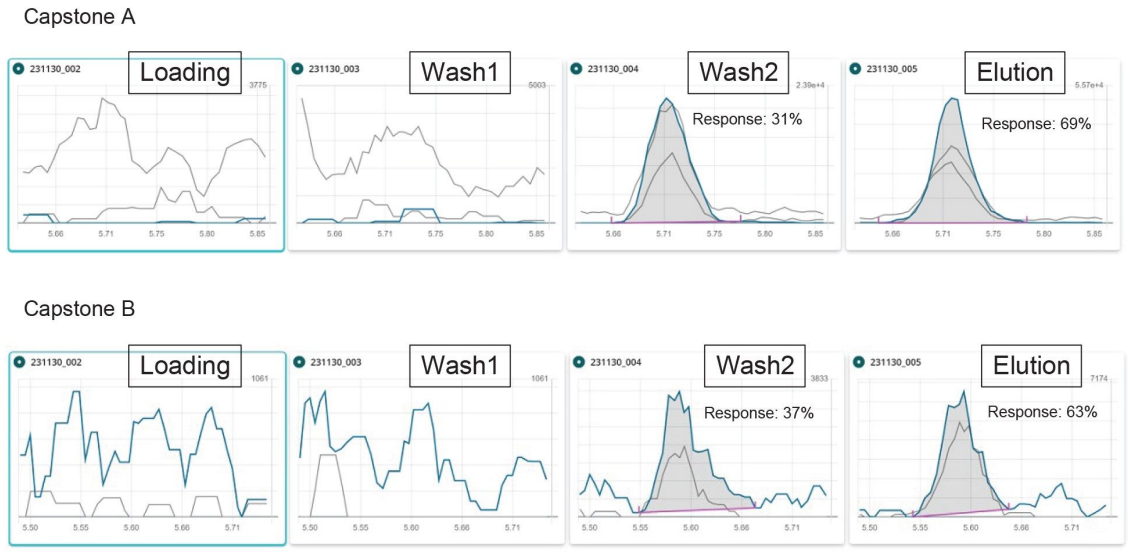

Figure 8. Chromatograms of capstone A (top) and capstone B (bottom), for each fraction of the SPE protocol. Wash1=wash step with water, wash2=wash step with 1:1 0.1 M formic acid:methanol. Blue trace: quantifier transition, grey traces: qualifier transitions.

Notably, the recoveries for FOSA were in the range 85–112%, indicating that the sample preparation and clean-up approach herein presented can be suitable for the determination of neutral PFAS, in addition to the ionic PFAS. A similar experiment to the one described for capstones has been carried out for FOSA, showing almost full recovery of this compound in the eluate fraction (see Figure 9).

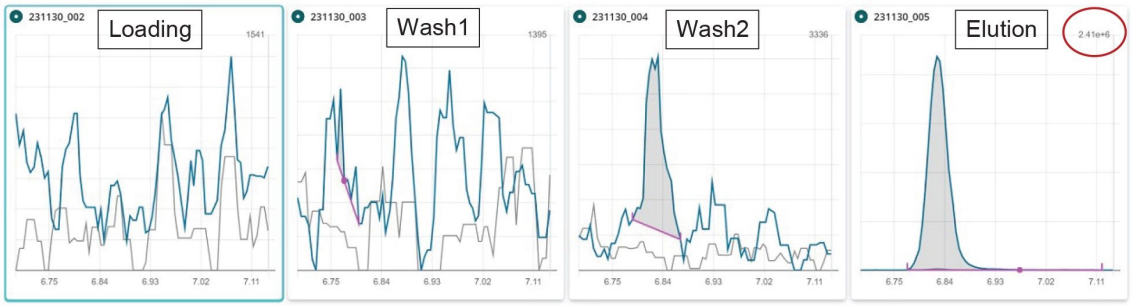

Figure 9. Chromatograms of FOSA for each fraction of the SPE protocol. Wash1=wash step with water, wash2=wash step with 1:1 0.1 M formic acid:methanol. Blue trace: quantifier transition, grey traces: qualifier transitions.

Conclusion

This study presents an optimized LC-MS/MS method for the comprehensive analysis of PFAS in vegetable, fruit, and baby food. The method demonstrated an exceptionally low limit of quantification (down to 0.0005 µg/kg for some compounds) accurately detecting and quantifying the PFAS compounds listed in the Commission Recommendation (EU) 2022/1431.

The increased cleanup efficacy of the new GCB/WAX SPE cartridge, in combination with the enhanced sensitivity of the Xevo TQ Absolute MS System, produced excellent recoveries, between 87 and 116% for the mandatory PFAS, and between 65 and 131% for the majority of the recommended and considered compounds, with RSD_r ≤10%.

The method also demonstrated excellent recoveries for FOSA, a neutral PFAS which was usually very challenging to retain onto weak anionic exchange cartridges.

Limitations were observed in the analysis of capstone A and B, due to their complex zwitterionic structure, and for PFBA, due to contamination in solvents and reagents. Therefore, more work should be done to investigate on best approaches to reduce PFBA contamination, as well as to obtain higher recoveries for capstones.

The second part of the present study will concern a workflow for the determination of PFAS in animal products.

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Appendix

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	CV (V)	CE (eV)	Internal standard	Type of internal standard
PFBA	212.9	169	10	10	^{13}C-PFBA	
		19		14		
PFPeA	262.9	219	10	10	$^{13}C_5$-PFPeA	
		19		14		
PFBS	298.9	80	15	30	$^{13}C_8$-PFOS	
		99		28		
PFHxA	312.9	269	5	10	$^{13}C_5$-PFHxA	
		119		22		
GenX (HFPO-DA)	285	169	10	8	$^{13}C_3$-GenX	
		119		30		
PFPeS	348.9	80	30	32	$^{13}C_8$-PFOS	
		99		30		
PFHpA	362.9	319	15	10	$^{13}C_4$-PFHpA	
		169		18		
ADONA	376.9	251	10	12	$^{13}C_3$-GenX	
		85		28		
PFHxS	398.9	80.1	10	36	$^{13}C_8$-PFOS	
		99.1		34		
PFOA	412.9	369	10	10	$^{13}C_8$-PFOA	
		169		18		
PFHpS	448.9	80	15	40	$^{13}C_8$-PFOS	
		99		36		
Capstone B	568.9	223.1	20	15	$^{13}C_8$-PFOS	
		549.1		13		
PFNA	462.9	219	10	18	$^{13}C_9$-PFNA	
		418.9		12		
Capstone A	527	507	20	12	$^{13}C_8$-PFOS	
		181		15		
PFOS	498.9	80	30	44	$^{13}C_8$-PFOS	
		99		38		
PFDA	512.9	219	15	18	$^{13}C_6$-PFDA	
		468.9		12		
9Cl-PF3ONS	530.9	351	15	26	$^{13}C_8$-PFOS	
		83		26		
PFNS	548.9	80.1	20	46	$^{13}C_8$-PFOS	
		99.1		42		
PFUnDA	562.9	269	25	20	$^{13}C_7$-PFUnDA	
		518.9		12		
PFDS	598.9	80.1	25	50	$^{13}C_8$-PFOS	
		99.1		46		
FOSA	497.9	77.9	40	30	$^{13}C_8$-FOSA	
		83		30		
PFDoDA	612.9	169	30	28	^{13}C-PFDoDA	
		568.9		14		
11Cl-PF3OUdS	630.9	450.9	30	30	$^{13}C_8$-PFOS	
		82.9		30		

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	CV (V)	CE (eV)	Internal standard	Type of internal standard
PFUnDS	649.1	80	40	52	^{13}C-PFDoDA	
		99		48		
PFTrDA	662.9	169	5	30	$^{13}C_2$-PFTeDA	
		618.9		10		
PFDoDS	699.1	80	40	56	$^{13}C_2$-PFTeDA	
		99		52		
PFTeDA	712.9	668.9	14	14	$^{13}C_2$-PFTeDA	
		168.9		30		
PFTrDS	749.1	80	40	64	$^{13}C_2$-PFTeDA	
		99		54		
$^{13}C_3$-PFBA	217	172	10	10	$^{13}C_3$-PFBA	Extraction (MPFAC-24ES + M3-HFPODA)
$^{13}C_5$-PFPeA	267.9	223	10	10	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES + M3-HFPODA)
$^{13}C_5$-PFHxA	317.9	272.9	10	6	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES + M3-HFPODA)
		119.9		18		
$^{13}C_4$-PFHpA	366.9	321.9	15	10	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES+ M3-HFPODA)
		169		15		
$^{13}C_8$-PFOA	420.9	375.9	5	8	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES+ M3-HFPODA)
		172		16		
$^{13}C_9$-PFNA	471.9	426.9	10	10	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES + M3-HFPODA)
		223		18		
$^{13}C_6$-PFDA	518.9	223	20	15	^{13}C-PFDA	Extraction (MPFAC-24ES + M3-HFPODA)
		473.9		7		
$^{13}C_7$-PFUnDA	569.9	524.9	9	8	^{13}C-PFDA	Extraction (MPFAC-24ES + M3-HFPODA)
		274		14		
^{13}C-PFDoDA	614.9	569.9	20	10	^{13}C-PFDA	Extraction (MPFAC-24ES + M3-HFPODA)
		169		22		
$^{13}C_2$-PFTeDA	715	168.9	18	25	^{13}C-PFDA	Extraction (MPFAC-24ES + M3-HFPODA)
		219		25		
$^{13}C_3$-PFBS	301.9	80	10	30	^{13}C-PFOS	Extraction (MPFAC-24ES + M3-HFPODA)
		99		25		
$^{13}C_3$-PFHxS	401.9	80.1	10	38	^{13}C-PFOS	Extraction (MPFAC-24ES + M3-HFPODA)
		99.1		30		
$^{13}C_8$-PFOS	506.9	80.1	15	40	^{13}C-PFOS	Extraction (MPFAC-24ES + M3-HFPODA)
		99.1		40		
$^{13}C_8$-FOSA	505.9	78.1	20	27	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES + M3-HFPODA)
$^{13}C_3$-GenX	287	169	10	12	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES + M3-HFPODA)
		119		12		
$^{13}C_3$-PFBA	216	172	10	10		Injection (MPFAC-C-IS)
$^{13}C_2$-PFOA	415	370	10	10	-	Injection (MPFAC-C-IS)
		169		15		
^{13}C-PFOS	503	80.1	5	40	-	Injection (MPFAC-C-IS)
		99.1		40		
^{13}C-PFDA	515	219	20	15	-	Injection (MPFAC-C-IS)
		470		10		

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