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An interlaboratory validation study for the assessment of low limits of quantification for PFAS in foods of plant origin

Consolato Schiavone [a], Francesco Romaniello [a,*], Christina Riemenschneider [b], Alexander Schächtele [b], Andrea Mario Rossi [a], Chiara Portesi [a]

[a] *Quantum Metrology and Nano Technologies Division, Istituto Nazionale di Ricerca Metrologica (INRiM), Strada delle Cacce, 91, 10135, Turin, Italy*

[b] *European Union Reference Laboratory (EURL) for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food, 79114, Freiburg, Germany*

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) extensively contaminate the environment and can enter the food chain. Fruits and vegetables represent a significant, yet under-regulated, human exposure pathway due to the uptake of PFAS by plants from contaminated soil/water. PFAS exposure is linked to adverse health effects. EFSA has established a strict group tolerable weekly intake of 4.4 $ng/kg_{bw}/week$ for PFOA, PFOS, PFHxS, and PFNA, leading the EU to introduce specific regulations. However, EU maximum levels in plant-derived foods are lacking because the achievability of limits of quantification (LOQ) of 0.001 μg/kg poses significant analytical challenges. This study presents the results of an extensive interlaboratory validation study among European laboratories using various triple quadrupoles and high-resolution mass spectrometers. The aim was to assess method robustness and achievability of analytical LOQs required by EU guidance with a focus on the EFSA-4 PFAS. The interlaboratory validation study involved 240 food extracts prepared in two different laboratories by two operators each. The quantification was performed across four laboratories and seven analytical setups. The validated method showed robustness, with an apparent recovery between 65% and 135%, uncertainty and RSD below 25%, and consistency with current EU guidelines. The application of internal ^{13}C-labeled standards and the use of very sensitive mass spectrometers proved to be a critical factor in achieving low LOQs.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS), often called "forever chemicals" (O'Hagan, 2008) due to their exceptional stability, are a large group of synthetic compounds with over 7 million molecules identified by the Organisation for Economic Co-operation and Development (OECD) in PubChem (Schymanski et al., 2023). Their unique chemical structure—featuring a polar head and a fluorinated carbon chain—gives them remarkable properties and makes them invaluable in countless industrial and consumer products (Glüge et al., 2020).

However, this widespread utility, combined with their chemical stability, has led to pervasive environmental contamination (Abunada et al., 2020; Evich et al., 2022), since PFAS can readily enter our ecosystems through industrial emissions and waste, polluting soil, air, and water (Brusseau et al., 2020; Crone et al., 2019; Li & Kannan, 2024, Schiavone & Portesi, 2023). Consequently, these substances, including more mobile monomers like perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), can contaminate the food chain, posing significant health risks (Cao & Ng, 2021; Fenton et al., 2021; Hekster et al., 2003, International Agency for Research on Cancer, 2025).

While the presence of PFAS in animal products (Mertens et al., 2025; Pickard et al., 2022) and drinking water (Teymoorian et al., 2023) is increasingly recognized, with maximum levels already set by EU legislation (Commission Regulation (EU) 2023/915), there's a critical lack of data. From the EU side, PFAS in foods of plant origin are legislated only with target limits of quantification (LOQs) (Commission, Recommendation (EU) 2022/1431, Piva et al., 2023; Sznajder-Katarzyńska et al., 2018). This is a significant concern because plants can absorb PFAS from contaminated soil and irrigation water (Costello & Lee, 2024; Felizeter et al., 2021; Gu et al., 2023; Scearce et al., 2025), leading to accumulation in edible tissues, which form a substantial part of the human diet.

Recognizing these risks, the European Union has progressively set a tolerable weekly intake (TWI) for four key PFAS (PFOA, PFOS, PFHxS,

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* Corresponding author.
E-mail address: f.romaniello@inrim.it (F. Romaniello).

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and PFNA) (European Food Safety Authority, 2020) and has implemented regulations (Commission Regulation (EU) 2022/2388, Commission Regulation (EU) 2023/915, Commission, Recommendation (EU) 2022/1431). Despite these efforts, a crucial gap remains: the absence of comprehensive maximum levels for PFAS in many plant-based foods. Effective compliance with any current or future maximum levels, especially in the lower nanogram-per-kilogram (*ng/kg*) range, requires robust and harmonized analytical methods capable of achieving the low LOQs recommended by EU monitoring guidelines. The approach for determination of LOQs needs to follow the principles as described in the latest guidance document published by the European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food (EURL for halogenated POPs in Feed and Food, 2024).

Extending prior work in the literature, this study establishes and validates comprehensive procedures covering all critical analytical stages, from sample preparation to instrumental quantification. A systematic evaluation was conducted of the state-of-the-art strategies for PFAS analysis applied to a large and representative panel of plant matrices and analytes.

Selected matrices included both fruits and vegetables, and particular attention was devoted to minimize contamination and matrix effects in accordance with current guidance (EURL for halogenated POPs in Feed and Food, 2024; Iannone et al., 2024; Meng et al., 2022).

In addition, a broad range of PFAS was encompassed, classified into three main groups based on their functional groups: PerFluoroalkyl Carboxylic Acids “PFCA” (e.g., PFOA, PFNA), PerFluoroalkyl Sulfonic Acids “PFSA” (e.g., PFOS, PFHxS), and novel PFAS, which include distinct subclasses such as fluorotelomer sulfonates (e.g., 6:2 FTS, 8:2 FTS), ether-based PFAS (e.g., GenX, DONA), and sulfonamides (e.g., FOSA), representing precursor substances and newer alternatives to legacy PFAS (Liu et al., 2024; Pancras et al., 2024).

Solid-phase extraction, QuEChERS, and ion-pair extraction approaches were comparatively assessed to identify a rapid, robust, and cost-effective procedure suitable for trace-level determination and compliant with EURL PFAS recommendations (EURL for halogenated POPs in Feed and Food, 2024). In parallel, an interlaboratory validation study, as previously done for other matrices (Hofman et al., 2025; Nilsson et al., 2021; Whitaker et al., 2021), was conducted across four European laboratories, involving multiple operators, diverse LC–MS platforms (triple quadrupole and high-resolution systems), and representative fruit and vegetable matrices, to evaluate method robustness, transferability, and applicability across varied plant-based food compositions.

This study aimed to assess the robustness and achievability of very low limits of quantification (LOQs) for PFAS in foods of plant origin, in accordance with stringent and harmonized European guidelines (EURL for halogenated POPs in Feed and Food, 2024, Commission Regulation (EU) 2022/2388, Commission Regulation (EU) 2023/915, Commission, Recommendation (EU) 2022/1431). To this end, a comprehensive interlaboratory validation was conducted to evaluate a harmonized analytical workflow across multiple laboratories, operators, matrices, and LC–MS platforms, with particular emphasis on the EFSA-regulated PFAS (PFOA, PFOS, PFHxS, and PFNA). By examining the consistency of results across laboratories and instrumental configurations, the study addresses the uncertainty budget inherent to interlaboratory evaluations and assesses the robustness and practical achievability of PFAS determination at challenging low LOQs, extending approaches previously applied at the intralaboratory level (EUROLAB, 2007; Schiavone et al., 2024).

2. Material and methods

2.1. *Interlaboratory procedure*

Two European laboratories, the National Metrology Institute of Italy (INRIM) and the EURL-POPs (Freiburg, Germany), organised this interlaboratory validation study. Both laboratories used the same experimental and validation protocol, i.e., sample material, sample preparation method, evaluation protocol, and criteria, to guarantee comparability of the results. However, the panel of analytes slightly differed between the two laboratories aiming to investigate variables like different standard panels provided by different producers (see Table S1).

2.2. *Chemicals and reference standards*

All used chemicals were of analytical grade (>98% purity), and the water used for all preparations and mobile phases was MilliQ filtered by the LC Pak filter Merck (Darmstadt, Germany). Native reference standards and isotope-labeled internal standards were obtained as individual solutions or mixtures in methanol or isopropanol. Details are provided in Appendix A (Chapters: Chemicals and reference standards – INRIM and Chemicals and reference standards – EURL-POPs).

2.3. *Experimental and validation protocol*

2.3.1. *Sample material*

In this interlaboratory validation study, a matrix group validation for PFAS in fruits and vegetables according to **chapter 2.2.2** of the EURL PFAS Guidance Document (EURL for halogenated POPs in Feed and Food, 2024) was performed. Three different types of fruits and vegetables, respectively, were selected. The matrices chosen were apple (APP), banana (BAN), grape (GRA), lettuce (LET), potato (POT), and tomato (TOM). In this way, not only some of the most popular fruits and vegetables in Europe (Eurostat, 2024) but also food of plant origin with high water and acid content (European Commission, SANTE/2017/10632, Rev. 5, 2023) were covered. All samples were purchased from a local supermarket in Germany and mixed and homogenised at the EURL POPs. An aliquot of 300 *g* of each matrix was shipped cooled to INRIM.

2.3.2. *Sample preparation*

Sample preparation was performed by INRIM and EURL POPs. All fruits and vegetables were fortified with PFAS by two technicians per laboratory and in duplicate each. Samples were fortified (with PFAS listed in Table S1) prior to extraction at four different levels, i.e., 0.001 μg/kg, 0.010 μg/kg, 0.050 μg/kg, and 0.100 μg/kg fresh weight (f.w.). Afterwards, the samples were vortexed in order to get equilibration of the analytes of interest and extracted. Different matrices were extracted on different days. Moreover, a procedural blank (sample that does not contain the matrix but analysed in the same manner as the spiked samples) and a matrix blank (non-fortified matrix analysed in the same manner as the spiked samples) were prepared for each sequence. In total, six procedural blanks, six matrix blanks, and twelve samples fortified at 0.001 μg/kg, 0.010 μg/kg, 0.050 μg/kg, and 0.100 μg/kg fresh weight (f.w.) were prepared per operator.

2.3.3. *Sample extraction*

Sample extraction was applied by EURL POPs and INRIM and followed an ion pair extraction method and principles reported in Annex V2.0 of the EURL PFAS Guidance Document (EURL for halogenated POPs in Feed and Food, 2024). In brief, 100 μL isotope-labeled internal standard solution and 10 mL water were added to a 2 *g* aliquot of fresh sample material and shaken by hand. The polypropylene centrifuge tubes used for all the extraction steps (both 50 mL and 15 mL, produced by APTACA Spa, Asti, Italy). This was followed by the addition of 2 mL of 0.5 *M* tetrabutylammonium hydrogen sulphate solution at a pH of 10 adjusted by means of sodium hydroxide. The pH was measured by means of a SevenMulti (Mettler Toledo, GmbH, Schwerzenbach, Switzerland) pH meter. Afterwards, 4 mL of 0.25 *M* sodium carbonate solution and 20 mL methyl tert-butyl ether were added, shaken for 25 min at room temperature, and centrifuged for 10 min at 4000 rpm. A 10 mL aliquot of the supernatant was evaporated to dryness at 40 °*C* under N_2 stream, and the residue was reconstituted with 300 μL MeOH/1% formic acid in water (2:1, *v:v*) in order to maximize sensitivity and solubility of PFAS. To ensure a complete dissolution an ultrasonic bath for 15 min was used. Finally, the extract was filtered and centrifuged for 5 min at 10000 rpm using a 0.45 μm cellulose acetate centrifuge filter (Spin-X®, purchased from Sigma-Aldrich). Three polypropylene (PP) vials (both vials and caps were purchased from Phenomenex, Torrance, California, USA) were filled with almost 100 μL of final extract each and kept stored frozen at −20 °C. Extracts were analysed by four different laboratories on five instruments, as depicted in Fig. 1.

2.3.4. Instrumental analysis

The strategy (according to Fig. 1) was to analyse PFAS by including high-end and middle-class mass spectrometers as well as techniques operating in high and low resolution, aiming to represent a realistic perspective of the analytical capabilities of laboratories across Europe.

The Waters Corporation and Sciex DemoLabs (all details provided in Table 1) were selected to include experimental data obtained from different instruments, further complementing the analyses performed by EURL POPs and INRIM.

Based on their sensitivity in PFAS analysis, mass spectrometers were classified as high-end and the latest release triple quadrupoles (in group "A", divided into A1, A1#, and A2), middle-class aged instrumentation triple quadrupoles (in group "B"), and high-resolution (in group “C”) systems (see Table 1 for the instrumentation list and from Table S2 to Table S21 for the LC-MS conditions).

To evaluate as many variables as possible, not only differences in mass spectrometers but also variations in chromatographic conditions (e.g., column manufacturers, selectivities, dimensions, injection volumes, and mobile phases) were taken into account (from Table S2 to Table S21).

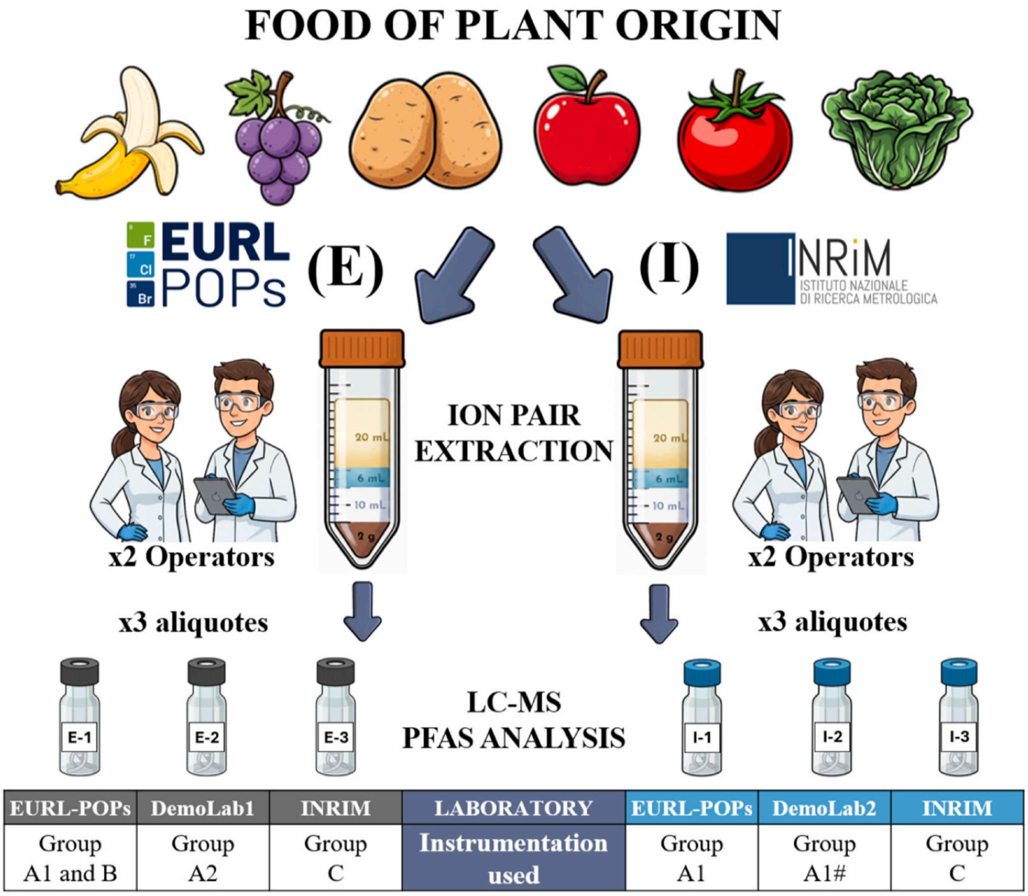
Table 1

Classification of mass spectrometer employed by each participating institute.

Institute	Mass Spectrometer	Release year	Classification
EURL POPs (European Union Reference Laboratory for Halogenated POPs in Feed and Food), Freiburg, Germany	Triple quadrupole 6490	2012	Group B
	Triple quadrupole Xevo™ TQ Absolute	2022	Group A
INRIM (inside Metrological Infrastructure for Food Safety “**IMPreSA**” laboratory), Turin, Italy	Orbitrap QExactive+	2016	Group C
Waters Corporation DemoLab, Milan, Italy	Triple quadrupole Xevo™ TQ Absolute	2022	Group A
Sciex DemoLab, Darmstadt, Germany	SCIEX 7500+ system	2024	Group A

While A1 and A1# are identical triple quadrupoles located in different laboratories, A2 is a triple quadrupole from a different manufacturer featuring a distinct physical design for both the ionization source and the fragmentation chamber.

The workflow of the validation study is described in **Fig. 1**.



EURL-POPs	DemoLab1	INRIM	LABORATORY	EURL-POPs	DemoLab2	INRIM
Group A1 and B	Group A2	Group C	Instrumentation used	Group A1	Group A1#	Group C

Fig. 1. Workflow of the interlaboratory validation study. From matrix selection to PFAS quantification.

Triple quadrupole mass spectrometers (groups A and B) operated in Multiple Reaction Monitoring (MRM) mode provide enhanced sensitivity and robustness, resulting in lower and more reproducible limits of quantification for targeted PFAS at ultra-trace concentration levels.

On the contrary, high-resolution mass spectrometer (group "C"), while offering superior mass accuracy and signal-to-noise ratio, often exhibits lower signal intensity for certain analytes—particularly those characterized by reduced ionization efficiencies or increased susceptibility to matrix interferences (de Hoffmann & Stroobant, 2007)—even when operated in Parallel Reaction Monitoring (PRM) mode as done for this study. Therefore, a direct comparison between high-resolution and triple quadrupole platforms is essential to achieve a comprehensive and realistic evaluation of LOQ performance, balancing analytical sensitivity, selectivity, and retrospective screening capabilities in ultra-trace PFAS analysis.

Moreover, this extensive approach was chosen to ensure the robustness and reproducibility of analytical results for PFAS in food of plant origin.

The details of the analytical methods, including chromatographic and mass spectrometry conditions, are described in Appendix A (Chapter: LC-MS parameters and conditions). For peak identification, the criteria according to chapter 2.4.3 of the EURL PFAS Guidance Document (EURL for halogenated POPs in Feed and Food, 2024) were applied.

The workflow of the validation study is described in Fig. 1.

The sample preparation protocol is summarized in Table S22.

2.3.5. Data evaluation and validation criteria

INRIM and EURL POPs data evaluation was carried out by the respective laboratories responsible for extract preparation. Raw data generated by Waters Corporation and Sciex DemoLab were sent to INRIM and EURL POPs, respectively, for further evaluation. An overview of the experimental design is given in Table S22. Further information is given in Appendix A (Chapter: Validation protocol, parameter, and criteria). Verification of validation parameters, i.e., trueness, precision, and LOQ, was performed by INRIM and EURL POPs in line with the EURL PFAS Guidance Document of 2024 and Commission implementing Regulation (EU) 2022/1428, i.e., the LOQ is the lowest validated level for which the criteria (apparent recovery between 65% and 135%, relative standard deviation ≤ 25%, and data inclusion thresholds with a compliance ≥75%) for identification, trueness, and precision are met. The complete data obtained for each analyte, matrix, and instrumentation are shown in Tables S23, S24, S25, and S26.

2.3.6. Statistical analysis

In addition to the validation criteria, a statistical evaluation was performed to determine whether analytical performance differed significantly as a function of instrumental group, matrix, spiking level, and extract-producing laboratory. Statistical analyses were restricted to the validated working range of (0.010 - 0.100) μg/kg, because the 0.001 μg/kg level contained the highest proportion of unstable and missing results and was therefore considered unsuitable for balanced factorial comparison. Apparent recovery (AR), absolute trueness error (|AR-100|), and relative standard deviation (RSD) were used as response variables. Because |AR-100| and RSD showed asymmetry, ANOVA models were fitted on log-transformed variables [log(|AR-100|) and log (RSD)], whereas AR was additionally analysed on the raw scale. Outliers were removed conservatively only when AR exceeded 500% or RSD exceeded 500%. For each extract set, factorial ANOVA was used with instrumental group, matrix, and spiking level as fixed factors. Paired Student's t-tests were then applied for targeted comparisons between detection systems, spiking levels, selected matrix pairs, and between EURL POPs and INRIM extracts on matched analyte-matrix-spike combinations. Statistical significance was set at $\alpha = 0.05$. ANOVA tables, paired comparisons, and the list of excluded outliers are reported in Tables S27, S28, S29, and S30.

3. Results of interlaboratory study

3.1. Procedural blank evaluation

Due to the ubiquitous distribution of PFAS, contamination of samples during sample preparation is an important topic to be addressed. In order to improve the robustness and sensitivity of the method, various sample preparation techniques (i.e., solid-liquid extraction combined with solid-phase extraction, the modified QuEChERS method, and ion-pair extraction without any further clean-up; all these extraction and clean-up approaches were in accordance with Annex V2.0 of the EURL PFAS Guidance Document (EURL for halogenated POPs in Feed and Food, 2024)) were tested, and the level of analytes, listed in Table S1, in procedural blanks was compared (data not presented). Ion pair extraction consistently yielded cleaner procedural blanks, which we attribute to its simplified nature involving fewer steps and thus mitigating potential contamination. This approach was similar to other studies (Roberts et al., 2023) that used only NaCl as salts and did not require any cleanup steps, unlike conventional QuEChERS extraction, where more types of salts and a cleanup step are required. Furthermore, ion pair extraction without any further clean-up offers benefits in terms of reduced sample preparation time and cost efficiency, as it relies exclusively on solvent usage, thereby avoiding expensive cartridges (like those used in SPE), which could otherwise contribute to higher blank contamination (as for powders in QuEChERS). PFAS found with the ion pair approach at a concentration of at least 0.001 μg/kg in the procedural blanks are shown in Table 2, where the concentrations (in *μg/kg*) are reported as the mean of 5 replicates, analysed by EURL POPs with the A1 instrument.

Using the two other approaches, the following additional analytes were detected in a range between 0.001 μg/kg and 0.080 μg/kg: PFHxA, PFHpA, PFNA, PFUnDA, PFDoDA, PFBS, PFHxS, PFOS, 8:2 FTS, and FOSA.

3.2. Results

In order to visualize the sensitivity of the sample preparation method in combination with the respective LC-MS instruments (Table 3, where "Values detected" represent the minimum number of values required within a given data evaluation approach to avoid excluding outliers when more than 20% of the total possible values are affected), the results are presented as heat maps (see Figs. 2–4) in the following sections.

The maps show a comparative assessment of the LOQ achieved (when the parameters previously described in section 2.3.5 are contemporarily met) for various PFAS in fruit and vegetable matrices across multiple analytical setups. Among the list of analytes, the ones in red are lacking an isotope-labeled internal standard. Each instrument exhibits different performances, favoring variations in LOQ throughout various analytes and matrices. The color gradient ranging from dark green to orange explains these differences, with lower LOQs indicating higher sensitivity and better detection capability; on the contrary, higher LOQs suggest analytical challenges, i.e., poor ionization efficiency or significant matrix suppression.

The heat map utilizes a color-coded scale to indicate the estimated LOQ of the analytes, where:

Table 2
Average concentration of PFAS (in *μg/kg*) detected in 5 procedural blanks for the ion pair approach tested.

	Analyte	Concentration (*μg/kg*)
Carboxylic acids	PFBA	0.019 ± 0.005
	PFPeA	0.009 ± 0.002
	PFOA	0.002 ± 0.002
Precursors	6:2 FTS	0.003 ± 0.003

Table 3
Overview of approaches applied for the assessment of the interlaboratory validation study dataset, divided by the laboratory performing the extraction, matrices, classification of instrumentation, minimum and maximum number of detected values to estimate the LOQ, number of evaluated analytes (PFAS), and description of evaluation.

Data evaluation approach #	Extracts	Matrices	LC-MS group					Values detected (min)	PFAS	Description	Cross ref. to results
			A1	A1#	A2	B	C				
1	EURL POPs	all	x		x	x	x	24 (18)	30	Comparison middle class vs. high-end instrumentation	Fig. 2
2	INRIM	all	x	x			x	24 (18)	23		
3	EURL POPs	fruits vegetables	x		x		x	12 (10) 12 (10)	30 30	Comparison of all instrumentation type for fruits and vegetables	Fig. 3
4	INRIM	fruits	x					12 (10)	EFSA 4	Comparison between same type of instrument but different laboratories	Fig. 4
		vegetables						12 (10)	EFSA 4		
	INRIM	fruits		x				12 (10)	EFSA 4		
		vegetables						12 (10)	EFSA 4		
5	EURL POPs	fruits	x					12 (10)	EFSA 4	Comparison between same instrument but extracts of different laboratories	
		vegetables						12 (10)	EFSA 4		
	INRIM	fruits	x					12 (10)	EFSA 4		
		vegetables						12 (10)	EFSA 4		

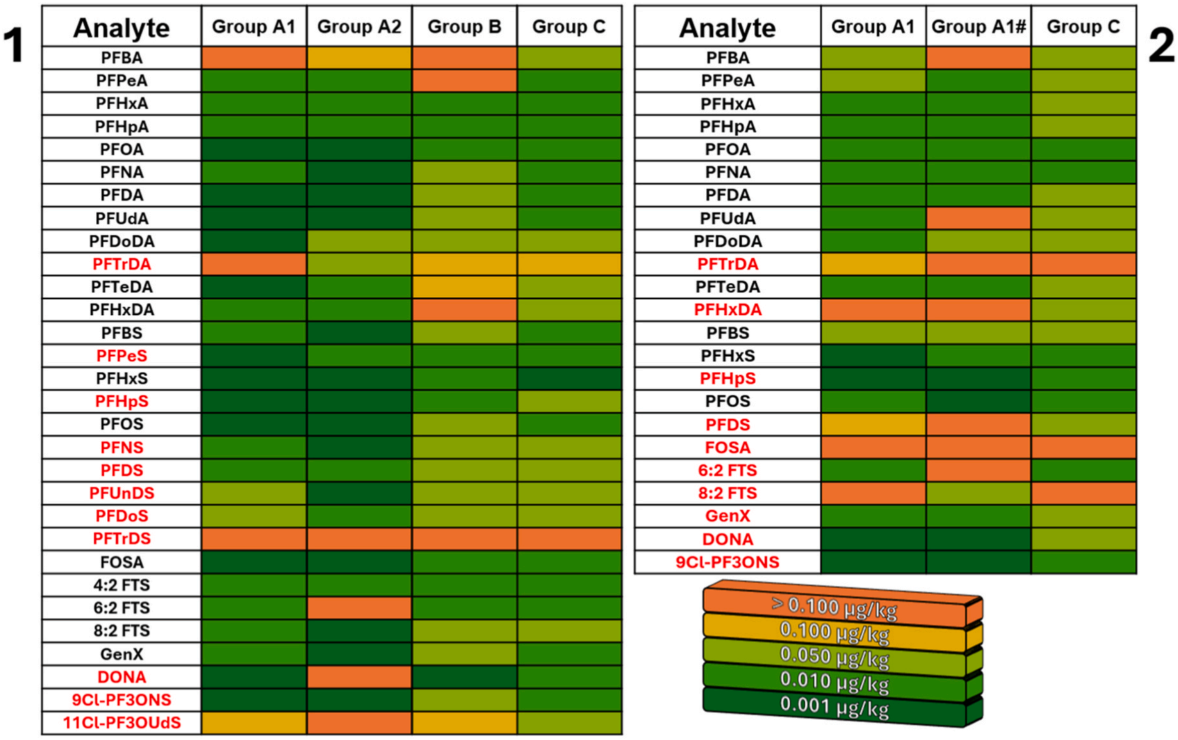


Fig. 2. Overview of LOQs obtained from EURL POPs (1) and INRIM extracts (2) for all matrices by different LC-MS systems. Grouping of LC-MS systems is described in section 2.3.4 and Table 1. Analytes in red are lacking an isotope-labeled internal standard. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

- **Dark green** represents the lowest validated concentration of **0.001** µg/kg;
- **Green** corresponds to **0.010** µg/kg;
- **Light green** stands for **0.050** µg/kg;
- **Yellow** indicates **0.100** µg/kg;
- **Orange** for concentrations **exceeding 0.100** µg/kg.

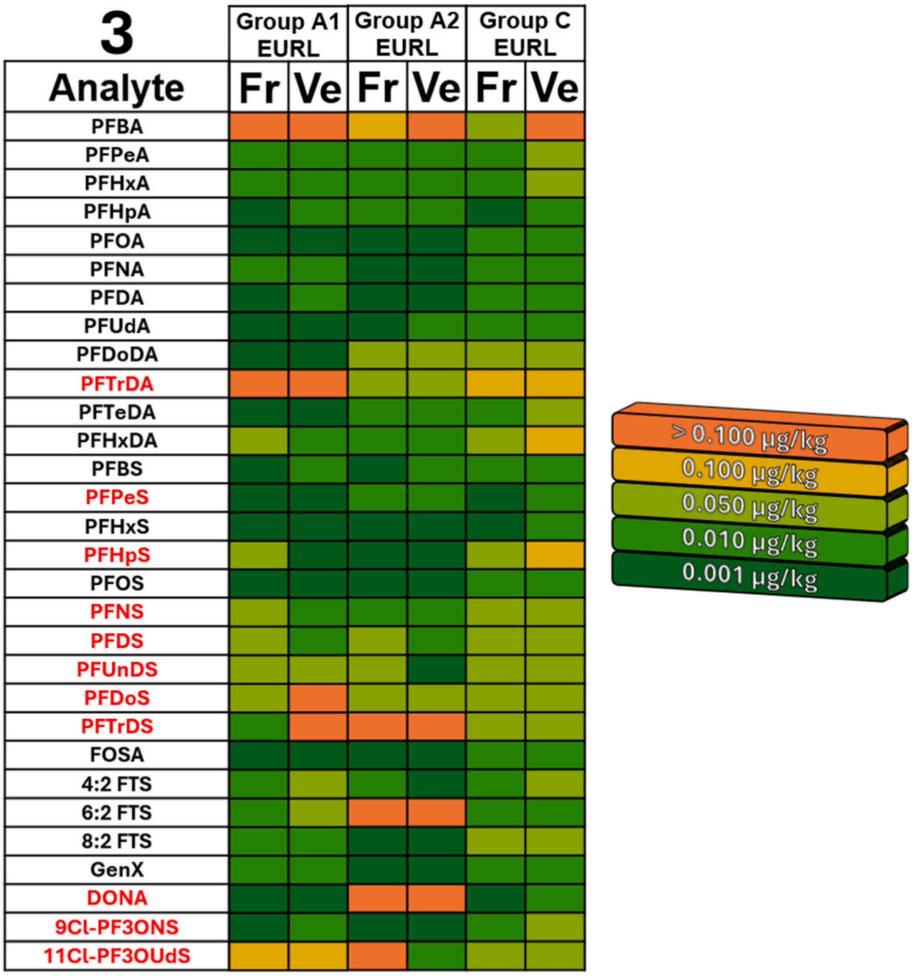


Fig. 3. Comparison of LOQs of 30 PFAS between fruit (Fr) and vegetable (Ve) extracts within the two group "A" instrumentations fabricated from different producers and the group "C" mass spectrometer (approach 3 of Table 3).

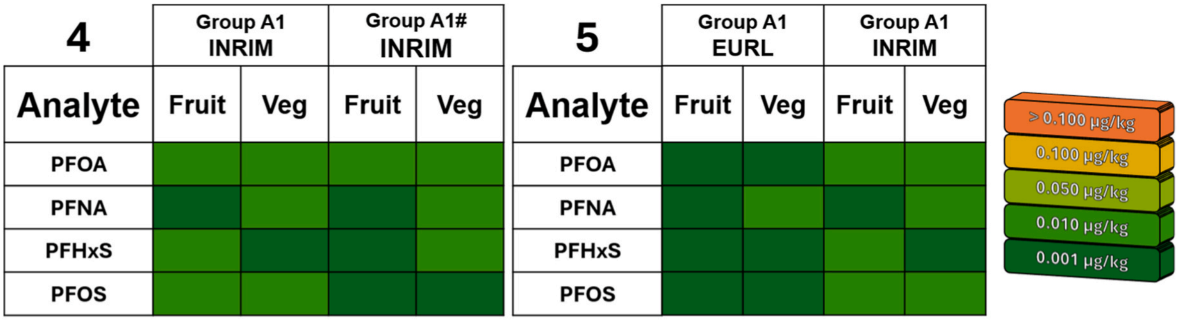


Fig. 4. Comparison of EFSA-regulated PFAS LOQ between the same instrument but different laboratories on INRIM extracts (left - approach 4 of Table 3); comparison of EFSA-regulated PFAS LOQ between different laboratories but the same instrument on EURL-POPs and INRIM extracts (right - approach 5 of Table 3).

3.2.1. Performance overview between different instruments on the different extracts

In order to estimate whether an LOQ of 0.001 µg/kg can be achieved for PFAS, different LC-MS technologies were tested and compared. Both EURL POPs and INRIM extracts were analysed with high-end instruments from the same manufacturer in different laboratories ("A1 and A1#") and with a high-resolution instrument ("C") in the same laboratory (approaches 1 and 2 in Table 3).

For complementary information, EURL POP extracts were analysed with a second high-end mass spectrometer from a different manufacturer ("A2") and a middle-class instrument ("B") (approach 1 in Table 3).

In Fig. 2 the LOQs obtained for the different instruments using EURL POPs (1) and INRIM (2) extracts are visualized.

In general, the lowest LOQs of 0.001 µg/kg could be achieved using high-end mass spectrometers (see A1, A2, and A1# in Fig. 2). The high-end instruments tested in this study are two triple quadrupoles (TQ) a Xevo™ TQ Absolute from Waters Corporation and a Triple Quadrupole/Linear Ion Trap hybride instrument (QTRAP) SCIEX 7500+ system. Both types of instruments are known for their improved sensitivity for detection of PFAS in water samples (Nieland et al., 2021; Organtini & Adams, 2022).

This overall visualization was supported by the statistical analysis. In the EURL POPs dataset, the instrumental group had a significant effect on both trueness error and precision (factorial ANOVA on $\log|AR\text{-}100|$ and logRSD, both $p < 0.005$; Table S27). Pairwise comparisons further showed that Group B and Group A1 triple quadrupoles did not differ significantly in trueness error or precision by taking into account all spiking levels tested ($p = 0.183$ and $p = 0.943$, respectively), while Group B performance was strongly affected by LOQ sensitivity for the 0.001 μg/kg level (Table S28).

From Fig. 2 clearly emerges the need for an isotope-labeled internal standard in PFAS quantification, because 84% of orange-colored cells represent PFAS quantified without the ^{13}C homologous. This is strengthened by the FOSA example, where $^{13}C_8$-FOSA was employed (EURLPOPs (1)) or not (INRIM (2)). The resulting LOQ of FOSA for EURL POPs extracts (1) ranged from 0.001 μg/kg *to* 0.010 μg/kg. However, no LOQ could be derived for FOSA from INRIM extracts (2). The lack of an isotope labeled standard led to a high relative standard deviation (>25%) and thus, none of the spiking levels of FOSA fulfilled the validation criteria. The high fluctuation of data does not depend on the instrumentation group but only on the lack of the internal standard (ISTD).

Digging into the results, it is possible to estimate an LOQ for the perfluoro carboxylic and sulfonic acids (PFCA and PFSA) from C5 to C12 (excluding EFSA-4 PFAS, which are discussed in Section 3.2.3). Based on the distribution of validated LOQs, almost half of the PFAS (49.5%) are validated at 0.010 μg/kg; moreover, 29% of PFAS resulted in a validated limit of quantification of 0.050 μg/kg, and 20.6% at 0.001 μg/kg, indicating that more than 99% of validated LOQs were ≤0.050 μg/kg. These results are consistent with previously published studies. Although most PFAS have been validated at an LOQ of 0.010 μg/kg, only the EFSA-4 method has achieved an LOQ of 0.001 μg/kg (Aβhoff et al., 2024). Other studies have reported similarly low LOQs; however, they encountered limitations for specific PFAS, such as PFPeA, for which relatively high LOQs were observed in fruits and vegetables (0.100 μg/kg and 0.025 μg/kg, respectively) (Kause et al., 2024).

The effect of spiking level was also statistically significant in this study. In the EURL POPs extracts, the 0.010 μg/kg level showed significantly poorer trueness and precision than both 0.050 μg/kg and 0.100 μg/kg (paired *t*-test, $p < 0.005$ for all), confirming that the lowest routinely workable level within the validated range is also the most statistically susceptible to fluctuation (Table S29). The same pattern was observed for INRIM extracts, where 0.010 μg/kg was again significantly poorer than 0.050 μg/kg for both trueness and precision (both $p < 0.005$) (Table S29).

In general, other PFAS (differing from PFCA and PFSA) demonstrated good analytical performance, achieving validated LOQs in the range of 0.001 μg/kg and 0.100 μg/kg (with few exceptions due to the lack of appropriate internal standards), with 62% of compounds showing LOQ ≤0.010 μg/kg.

3.2.2. Comparison of LOQ for 30 PFAS in fruits and vegetables with different group instrumentation

A comparison of LOQs for 30 PFAS obtained from fruit and vegetable extracts prepared by EURL POPs and analysed with the two group “A” LC-MS/MS systems and the group “C” mass spectrometer, all from different producers, is shown in Fig. 3.

For instance, PFBA analysed with the group “C” mass spectrometer, due to the nature of the technique already described in section 2.3.4, could overcome interferences thanks to the PRM high-resolution selectivity. This result exemplifies the lower limits of quantification obtained in fruit matrices relative to vegetables.

The persistently high LOQ observed for PFBA with triple quadrupoles and for vegetables in high resolution (Fig. 2), relative to other PFCAs (C5–C12), presents a critical analytical challenge. This LOQ does not reflect poor sensitivity for this analyte; rather, it is due to interferences that likely originate from overall background contamination which comprehend endogenous matrix content, reagents, and laboratory environment.

Moreover, the source of contamination could also be exogenous, and it is notoriously difficult to control and can arise from multiple sources within the laboratory environment (e.g., ventilation systems) (Morales-McDevitt et al., 2021; Yao et al., 2018) as well as from the analytical workflow itself. High-purity solvents (e.g., methanol, water), essential for extraction and mobile phases, are a common vector, with contamination levels often varying significantly between different analytical solvent batches or suppliers. Furthermore, laboratory personnel are a potential source; contamination can be introduced from clothing or through the use of personal care products and cosmetics, many of which contain or are packaged with fluorinated compounds.

The instrumentation geometry of the mass spectrometer/ion source increases this variability chance. Different mass spectrometer manufacturers employ distinct ion source geometries and ion-guiding technologies. This varied design can significantly change the instrument's susceptibility to background chemical noise. Moreover, the specific design of the fragmentation and collision cells can influence ion fragmentation pathways and the potential for in-source or in-cell degradation of precursor contaminants, which could erroneously contribute to the target analyte signal (Smith, K. M., & Rainville, P. D., 2020).

Collectively, these factors lead to elevated LOQs—especially for short-chain PFAS such as PFBA—with published studies (Zhang et al., 2020) on the matrices examined here reporting LOQs ≥0.220 μg/kg. By contrast, within this interlaboratory validation study, an LOQ of ≤0.050 μg/kg could be achieved for most of the PFAS in fruits and vegetables (Fig. 4), representing a substantial improvement. Once again, the procedural blank evaluation performed at the very beginning of the study helped to obtain low LOQ levels.

The EFSA-4 PFAS demonstrated excellent sensitivity, achieving an LOQ of 0.001 μg/kg in fruits and vegetables for PFOA, PFOS, and PFHxS by means of both A1 and A2 instruments. A focus on EFSA-4 PFAS with high-end instrumentation will be better discussed in section 3.2.3.

As discussed in section 2.3.4, for PFAS with carbon chain lengths greater than five, the HRMS platform (group C) showed higher LOQs than group A, attributable to lower absolute signal intensity in PRM mode and reduced ionization efficiency with increased sensitivity to matrix effects for longer-chain compounds, whereas MRM acquisition on triple quadrupole instruments provides higher sensitivity for targeted ultra-trace analysis.

Statistically, it is evident that matrix effects were significant and varied within instrumental platforms. In the EURL POPs extracts, matrix had a significant main effect on trueness error and precision (both $p < 0.0001$), and selected paired comparisons showed that, for example, banana performed worse than grape both in trueness error and precision ($p < 0.05$ for both), while potato was similar to tomato, showing good trueness error ($p = 0.997$) and precision ($p = 0.728$). A similar pattern was observed in INRIM, where banana again showed worse trueness and precision than grape (both $p < 0.0002$) (Tables S27 and S30). These results support the hypothesis that LOQ differences are not instrument-driven only but also matrix and laboratory dependent.

3.2.3. High-end instrumentation performance (group A1) on LOQs of EFSA-4 PFAS in fruits and vegetables

The European Union has established target LOQs for PFAS in fruits, vegetables, and starchy roots and tubers (Commission, Recommendation (EU) 2022/1431). Regarding carboxylic acids, the targeted LOQ is ≤ 0.001 μg/kg for PFOA and PFNA, while it is ≤ 0.002 μg/kg and 0.004 μg/kg for PFOS and PFHxS sulfonic acid, respectively. Furthermore, if the indicative levels of PFOS (≥0.010 μg/kg), PFOA (≥0.010 μg/kg), PFNA (≥0.005 μg/kg), and PFHxS (≥0.015 μg/kg) are exceeded in the samples, the causes of the PFAS contamination must be investigated in accordance with the Commission Recommendation.

In this regard, a focus on the EFSA-regulated PFAS was done in Fig. 4 (showing results for **approaches 4** and **5**; see Table 3), which depicts, respectively, the comparison of the same type of instrument used in different laboratories to analyse the same extracts and, on the other hand, the same instrument in the same laboratory employed to quantify PFAS in extracts from two laboratories.

The cross-laboratory comparison further showed that the same platform did not perform identically in all settings. Within the INRIM extract set, Group A1 and Group A1# did not differ significantly in trueness error (paired *t*-test, $p = 0.959$), but Group A1 showed significantly lower RSD than Group A1# ($p = 0.0241$), indicating that laboratory-dependent implementation still affected repeatability even when instrument class was the same (Table S28).

The focus on the EFSA-regulated PFAS allows to evaluate how the proposed and validated analytical approach is aligned to legislative requirements and official monitoring purposes. In this study, an LOQ of 0.001 μg/kg was achieved for fruits by group "A" instrumentation on EURL-POPs extracts (Fig. 4). In general, sulfonates, such as PFOS and PFHxS, performed the best, resulting most of the time in an LOQ of 0.001 μg/kg. In literature, it is already demonstrated (Aβhoff et al., 2024; Kause et al., 2024) that LOQs obtained in fruit matrices are typically worse than those in vegetables for some PFAS (i.e., PFPeA, PFHxA. PFHpA). Even using high-end instrumentation (i.e., the latest TQ released on the market), PFOA could have an LOQ above 0.025 μg/kg for some laboratories (Kause et al., 2024) and 0.001 μg/kg for others (Aβhoff et al., 2024). These results demonstrate that it is possible to reach 0.001 μg/kg as an LOQ for fruits and vegetables, but it remains up to a lot of variables, being factors of manner, along with only the instrumentation used, the laboratory involved, and the operator's, from sample preparation to the analysis.

4. Conclusions

This interlaboratory validation study demonstrates that ultra-trace determination of PFAS in foods of plant origin is achievable with a robustness and transferability that aligns with the needs of European monitoring and, ultimately, future risk-management measures. A total of 240 fortified food extracts, 40 for each of six representative fruit and vegetable matrices (apple, banana, grape, lettuce, potato, and tomato), were generated and evaluated across four laboratories using seven analytical setups. When assessed against predefined performance requirements for apparent recovery (65–135%), precision (RSD ≤25%), and data inclusion (≥75% compliance), the results show that reliable quantification at very low ng/kg levels is not an "exceptional" outcome, but a realistic objective when the entire measurement chain is controlled. Across the first two evaluation approaches (i.e., PFAS considered over all matrices and instruments), 87% of the target PFAS were successfully validated with LOQs ≤0.100 μg/kg, and more than 99% of these achieved LOQs ≤0.050 μg/kg, indicating strong analytical consistency among laboratories.

The interlaboratory validation study clarifies that, once modern LC–MS platforms are in place, the practical achievability of low LOQs is driven less by nominal instrument sensitivity than by metrological control of the workflow: rigorous contamination prevention and monitoring (procedural and matrix blanks), management of matrix-dependent ionization effects, and, critically, the use of homologous ^{13}C-labeled internal standards to consolidate trueness and precision at the lowest fortification levels. In line with this, higher LOQs were primarily associated with analytes lacking dedicated isotopic labeled analogues (e.g., FOSA), rather than reflecting an intrinsic limitation of the instrumental platforms.

The findings also support a pragmatic strategy for official control and routine surveillance. High-end triple quadrupole instruments operated in MRM mode (Group A) provided the most consistent route to the lowest LOQs for targeted monitoring, reaching 0.001 μg/kg for the EFSA-4 PFAS in fruits and vegetables under best-performing conditions. At the same time, high-resolution PRM, while not generally optimal for maximizing signal intensity for longer-chain PFAS, proved valuable when selectivity is decisive (i.e., its ability to mitigate PFBA interferences). The persistent difficulty for PFBA further underlines a key conclusion that sensitivity alone cannot resolve: for certain short-chain PFAS, LOQs can remain limited by ubiquitous background contamination originating from laboratory environments, solvents, and ventilation systems. Nevertheless, even for PFBA, validated LOQs as low as 0.050 μg/kg were achieved, representing a clear improvement over literature-reported values and reinforcing that systematic contamination control can considerably shift practical performance.

These descriptive findings were supported by statistical analysis: factorial ANOVA demonstrated significant effects of instrumental group, matrix, and spiking level on validation performance. Thus, the study shows that achieving ultra-trace PFAS LOQs in foods of plant origin depends not only on instrumental sensitivity but also on the combined control of extraction, matrix effects, and laboratory implementation.

Taken together, this work not only confirms the method's reliability for harmonized occurrence data generation across Europe, but also identifies where further gains are most likely, particularly through improving its feasibility for routine implementation, and adoption of homologous labeled standards (including those that have only recently become commercially available), targeted follow-up evaluation of PFAS previously lacking such analogues, and continued optimization of extraction and chromatographic conditions to broaden applicability across additional foods, instruments, and emerging PFAS not yet fully encompassed by the validated scope.

CRediT authorship contribution statement

Consolato Schiavone: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Francesco Romaniello:** Writing – review & editing, Validation, Investigation. **Christina Riemenschneider:** Writing – review & editing, Methodology, Investigation, Data curation. **Alexander Schächtele:** Writing – review & editing, Methodology, Data curation. **Andrea Mario Rossi:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Chiara Portesi:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Chiara Portesi reports financial support was provided by EURAMET European Metrology Programme for Innovation and Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2026.112315.

Data availability

Data will be made available on request.

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