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Quantification of PFAS in rice and maize: Validation of a UHPLC-HRMS/MS isotopic dilution approach in support to food safety

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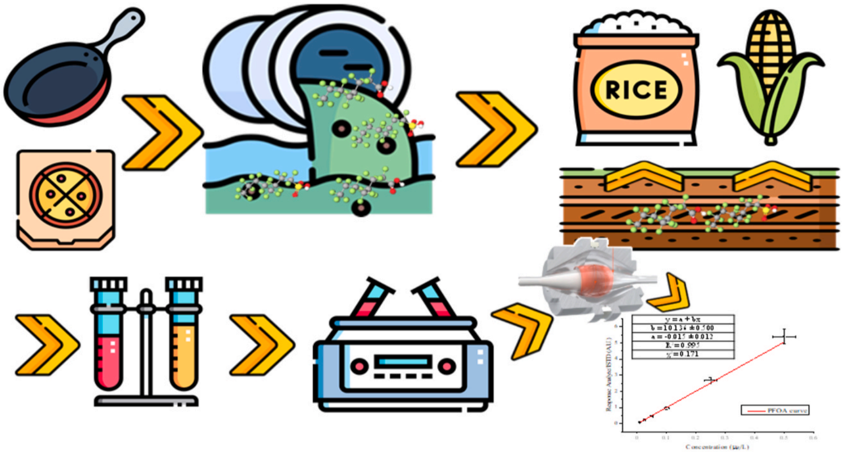
HIGHLIGHTS

- Analytical quantification of PAFS in food matrices.
- Metrological approach for uncertainty budget evaluation.
- UHPLC-HRMS/MS isotopic dilution approach for PFAS analysis.
- Support the revision of current guidelines and legislation.
- Support food safety of rice and maize in Europe.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, an analytical method for the quantification of per and poly fluoroalkyl substances (PFAS) in rice and maize has been developed and then validated with a metrological approach. PFAS are a group of human-made chemicals used in a variety of industries and consumer products for their water- and grease-resistant properties. Studies have shown that PFAS can contaminate soil and water, and there is concern about their bioaccumulation in edible plants, fruits, and cereals. The presence of PFAS has been identified in rice and other food products, including maize, as indicated by studies and scientific literature. This is particularly alarming since some PFAS have been associated with adverse health effects and rice and maize account for over 20% of the annual food intake worldwide. Despite this evidence, the regulation currently in place is not covering cereal matrices and limits of quantification for matrices encompassed by the current legislation are defined for a small group of PFAS. In this study an UHPLC-HRMS/MS based method was validated, obtaining a LOQ (Limit Of Quantification) ranging between 2 ng/kg and 32 ng/kg and robustness in line with EU guidelines and

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recommendation for PFAS in food. Additionally, a metrological approach was employed to estimate the uncertainty budget, utilizing modeling and experimental methods, and comparing the outcomes, aiming to characterize with high accuracy PFAS in rice and maize and support control bodies to assess contamination in suspected areas. A comparison of uncertainty of different approaches was conducted after applying the method to 30 real samples.

Thousands of synthetic compounds, known as per- and polyfluoroalkyl substances (PFAS), are extensively utilized in food packaging and various materials due to their fat, fire, and water resistance properties. PFAS are produced at industrial level primarily in the form of polymers (Perfluoroalkyl-Chemicals-Pfas) and they are characterized by the presence of one of the strongest bonds in nature, the carbon-fluorine bond. In Fig. S1, in the Supplementary Information (SI), the molecular structure of two representative monomers, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), is depicted, representing carboxylic acids and sulfonic acids, respectively, which are the two categories studied in this work.

PFAS, grouped in the "forever chemicals" due to their enduring persistence, can disrupt into monomers, exhibiting heightened mobility and significant stability (Ohagan, 2008). Since they are present in many production chains, industrial wastes containing excess PFAS are quite often released into the environment, thus contaminating groundwater and air (Schiavone and Portesi, 2023). This is particularly alarming since they are well-documented for carcinogenic and reprotoxic effects on humans, for their role in vaccine resistance, and for their impact on the immune system (McCarthy et al., 2021; Goodrum et al., 2021; Roth et al., 2020; Hekster et al., 2003). While studies on the toxicological effects of these compounds span decades, only recently researches have confirmed their adverse effects on humans, prompting exponential concern within the scientific community and capturing global attention. In particular, both EU and US authorities have responded by implementing strategies to control or prevent PFAS contamination, culminating in the proposals to eliminate these substances from the production chain, as required by law (Echa-Publishes-Pfas-Restriction-Proposal; EC, 2010).

Legislation moved also forward regarding the methods of sampling and analysis for controlling PFAS according to Commission Implementing Regulation (EU) 2022/1428 of August 24, 2022 (The Europen and Commission, 2022).

At the EU level, the European Food Safety Authority (EFSA) took one of the initial actions in 2019 by recommending a Tolerable Weekly Intake (TWI) for key PFAS, including PFOA, PFOS, perfluorohexanesulfonate (PFHxS), and perfluorononanoic acid (PFNA). Subsequently, the European Union has issued guidelines and regulations to address PFAS contamination in food and feed. The most recent update on maximum PFAS levels in EU food regulations is outlined in Commission Regulation (2023)/915 of April 25, 2023 (FAO. Commission Regulation (EU) 2023/915 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006, 2023). Even though PFAS have been found in a wide range of foods, including staples like rice and maize, analytical techniques and standardized methods that can meet the Regulation's recommended targeted limits are lacking.

Furthermore, EU regulation 2023/915 currently regulates only four PFAS and covers a limited range of matrices such as meat, fish, and eggs.

In table 4.2, p119 [11], the maximum levels (ML) for PFAS in different matrices set by EU regulation 2023/915 for PFAS are summarized, where the maximum level applies to the wet weight.

Also the Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022), that sets the limits of quantification (LOQ) in $\mu g/kg$ w.w. for the four individual PFAS (PFOS, PFOA, PFNA, PFHxS), encompasses few matrices (table 4[12]), not including rice and maize.

On the other hand, both rice and maize are highly consumed foods and constitute the basis of the diet for many people. In particular, rice, being a globally consumed staple, especially in Asian countries with extensive industries, represents a matrix of relevant concern for contamination (Ghisi et al., 2019), also considering that the daily exposure to PFAS through rice consumption has already been demonstrated (Yamazaki et al., 2023). In Europe, Italy is the country that produces the most rice, and Piedmont is the most rapidly expanding region on the continent in this regard, producing 1.6 million tons annually and accounting for 52% of Italy's total production.

From an analytical point of view, the current method for the quantification of PFAS mainly relies on Liquid Chromatography-Mass Spectrometry (LC-MS), which is widely used for PFAS analysis due to its sensitivity and selectivity. Tandem mass spectrometry (MS/MS) enhances selectivity and sensitivity by allowing the detection of specific PFAS ions. However, considering the LOQ recommended by recent guidelines (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022), significant improvements are required, spanning from sample preparation to mass spectrometry parameters. Additionally, consistent approaches are needed for the validation of the optimized analytical procedure and for the estimation of the related measurement uncertainties, a parameter of paramount importance for evaluating method performances.

This work addresses the relevant needs related to the development of analytical tools for assessing the level of PFAS in matrices that are not yet covered by the current legislation, encompassing PFAS molecules beside the 4 regulated ones. To this end, a sensitive and reproducible method based on Isotope Dilution Mass Spectrometry combined with a metrological approach for the quantification of 21 PFAS molecules (thus well beyond the 4 regulated ones) was developed and validated.

By a metrological approach, it is possible to assess the metrological traceability to the International System of Units "SI" and the uncertainty budget associated with the measurements. Regarding the traceability, it is worth noting that isotope dilution mass spectrometry (IDMS) is well recognized as one of a few analytical methods which have the potential for being used as primary methods, i.e. a method that ultimately allows the traceability to the SI units (Richter, 1997). In this work, the use of suitable commercial certified reference materials (CRMs) in the IDMS analysis has allowed us to check the quality of the measurements, to validate the analytical methods and support the evaluation of the proper way to assess uncertainty budget.

For estimating the uncertainty budget associated with the measurements, two approaches (bottom-up and intralaboratory) were performed and compared to assess the more reliable one.

The method validated allowed to quantify PFAS in rice and maize samples collected by Italian control bodies (Northwest region), in order to monitor samples coming from areas suspected to be contaminated. Furthermore, through application in various matrices, the method has demonstrated excellent elasticity. This underscores the potential to address the current analytical gap in tracing PFAS, encompassing not only the four regulated substances but also including PFAS in matrices that are not yet covered by legislation.

The goals of the study are to develop and validate an analytical method by using a metrological approach, able to: i. quantify in an accurate and reliable manner a large group PFAS, well beyond the 4 regulate ones; ii. to assess the analytical performances of the method in food matrices that are not yet covered by the existing legislation i.e. rice and maize, that are at the basis of the diet for a large part of the population worldwide, therefore constituting a possible relevant source of PFAS intake.

The analytical challenges faced in this work are not trivial, since the optimization of the method for the analysis of PFAS is strongly matrix dependent, and maize and rice require sample preparation procedures that are quite different than those required for matrices already included i the legislation (e.g. egg, fish, meat). Besides already regulated PFAS, there is a need for analytical tools for the detection and measurement of emerging and novel perfluoroalkyl substances. This work is aiming to address this need, with a focus on short chain PFAS that are expected to be found in vegetables and cereals. In the long term, the outcomes. i.e. the assessment of LOD and LOQs for specific PFAS/matrices combinations, with corresponding uncertainties, are expected to support the regulatory framework that, for PFAS, is constantly under development, both on the EU and global level. At a higher level, this work will support the protection of a safe and sustainable food system, with a focus on food matrices that are particularly relevant for developing countries.

1. Materials and analytical methods

1.1. Sample information

In the framework of the collaboration with the national authorities for the screening and monitoring of food and feed in the Northwest of Italy a lot of rice samples were collected in 2022–2023 for screening of different contaminants. The method was developed for maize as well, for investigating its possible contamination. thirty rice samples and two maize samples provided by the control bodies were analyzed in this study.

1.2. Chemicals

Methanol, Acetonitrile, Formic acid, Ammonium acetate and water all LC/MS grade purchased by Carlo Erba (Cornaredo, Milan, Italy), Copure© QuEChERS Extraction Pouch from Biocomma (Shenzhen, Guangdong, China), Cleanert MAS-Q dSPE from Agela (Tianjin, China). Two CRMs as a mixture of 21 PFASs (Table in SI) from C_4 to C_{18} both carboxylic and sulfonic acids, a mixture of 13 PFASs (Table in SI) labeled with ^{13}C purchased from Wellington Laboratories (Wellington Laboratories Inc., Ontario, Canada). The analytes chosen in the mixtures include the few PFASs already legislated and officially monitored (EC, 2010). In Fig. 1 the analytical workflow of the developed method for the quantification of 21 PFASs in rice and maize.

1.3. Sample extraction

For the extraction of PFAS, 5 *g* of the samples (powdered by means of Buchi ceramic miller) were weighed in an analytical balance and then put in a 50 mL conical tube. 10 mL of CH_3CN and 10 mL of H_2O were added to the sample and, subsequently, all acidified with 150 μL of formic acid. After that, a QuEChERS (1 *g* NaCl + 4 *g* $MgSO_4$) pouch was added to help extraction of analytes into the organic phase and to absorb the water. The mix was vortexed for 1 min and centrifuged at 4000 rpm, 4 °*C* for 5 min. Subsequently, 5 mL of the supernatant were collected and transferred in a 15 mL conical tube with 150 mg of primary secondary amine (PSA), 150 mg of C_{18} and 900 mg $MgSO_4$ performing a dispersive solid phase extraction (dSPE). Then the mix was vortexed and centrifuged with the same conditions. The supernatant was collected and 5 μL were injected into the UHPLC-HRMS/MS system (Yun et al., 2023; Organtini et al., 2023; Genualdi et al., 2021). The sample preparation process is summarized in Fig. S2 (SI).

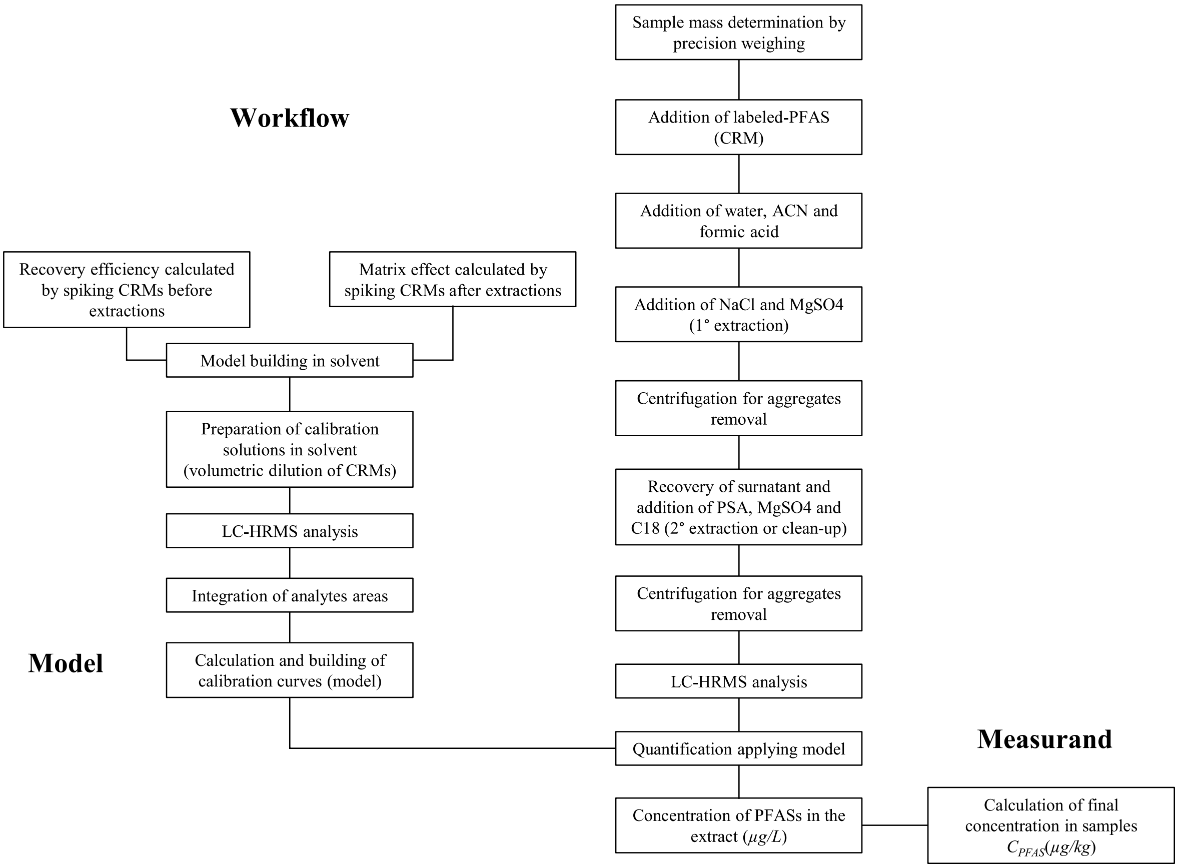

Fig. 1. Workflow of the method developed for the quantification of 21 PFASs in rice and maize. From the analytical model built to obtain a reliable measurement of the measurand.

The background contamination was evaluated by means of a procedural blank (5 *g* of Mass Spectrometry grade water treated as sample), procedural blank spiked post extraction, and negative rice samples experiments. The results of these tests showed that there are only two analytes that cause a background contamination, PFOA at 10 ppt and PFBA in the order of 1 ppt, both lower than LOQs.

For the intralaboratory validation, a sample was spiked with 100 μL of the 10 ng/mL solution of analytes and the internal standards (ISTD) resulting in a final concentration of 0.2 μg/kg.

1.4. UHPLC-HRMS method

The analysis of target PFASs was performed using an UHPLC (Vanquish Flex, Thermo Fisher Scientific, USA) coupled with a HRMS (Q-Exactive Plus, Thermo Fisher Scientific, USA) equipped with an Heated Electrospray Ionization (HESI) source using the Parallel Reaction Monitoring (PRM) approach already tested for targeted analysis (Roberts et al., 2023). The retention time windows for each analyte and transitions are detailed in the supplementary information (SI) together with instrumental parameters.

To obtain the mathematical model for the quantification of the analytes, the isotopic dilution approach was applied, so each PFAS area was divided by the area of the labeled compound more physically and chemically similar, obtaining a ratio. The labeled internal standards chosen for each analyte are reported in the SI (Table SI6). This approach was chosen in order to obtain the most reproducible and correct quantification and to overcome the different matrix effects. To this end, the proper choice of the correct fragment ion is also crucial. In the SI, the chromatograms of each analyte PRM trace for the standard solutions at 0.1 μg/L is reported.

2. Results and discussion

2.1. Method validation

As a first step, the **linearity** of the method was evaluated with CRMs in solvent. To build the calibration curves the software *CCC* (Lecuna et al., 2020) was used. This software uses as inputs the different concentrations of the solutions and the response of the MS measurement, calculated as the ratio between the area of the analyte and the area of the internal standard (ISTD), accompanied by their respective uncertainties. The uncertainties of the concentrations and the ratios are, respectively, the propagation of errors for each dilution step and the standard deviation of the response between the different replicates. Then, the calibration curves are calculated by the Weighted Total Least Squares (WTLS) algorithm. The OriginLab™ v2022 was used to investigate the R^2 values applying the linear regression. To assess **repeatability** and **reproducibility** of the method (Rolle et al., 2017), the standard solutions were prepared by two different operators in different days at 6 different concentrations of the CRM containing the 21 PFAS: 0.010 μg/L, 0.025 μg/L, 0.050 μg/L, 0.100 μg/L, 0.250 μg/L and 0.500 μg/L (equivalent to 0.005 μg/kg, 0.013 μg/kg, 0.025 μg/kg, 0.050 μg/kg, 0.125 μg/kg and 0.250 μg/kg in matrix). Six different curves were prepared and for each curve a triplicate analysis was performed. Then, a calibration curve was built applying a linear fit to the mean of 3 instrumental replicates for each of the 6 mixtures. Eventually, the concentrations of the analytes were calculated by interpolating data into the calibration curves. Fig. 2 shows the calibration curve for PFOA.

The calibration curves obtained in different days for all compounds showed linearity (R^2) above 0.99 and chi-square χ^2 below 1 for almost all analytes (with few exceptions below 2) in this way linearity and errors of the model were positively established. To confirm the linearity in real samples, the calibration curves were built in rice and maize matrices as well, by spiking the same levels of CRMs in the final extract of negative samples. The results showed linearity values greater than 0.99 for almost all the compounds in rice and maize, the only exception being the PFBA (0.98 in rice and 0.97 in maize), probably due to the less response in the matrix of labeled PFBA that affected the linearity and to the presence of interferences near the chromatographic peak.

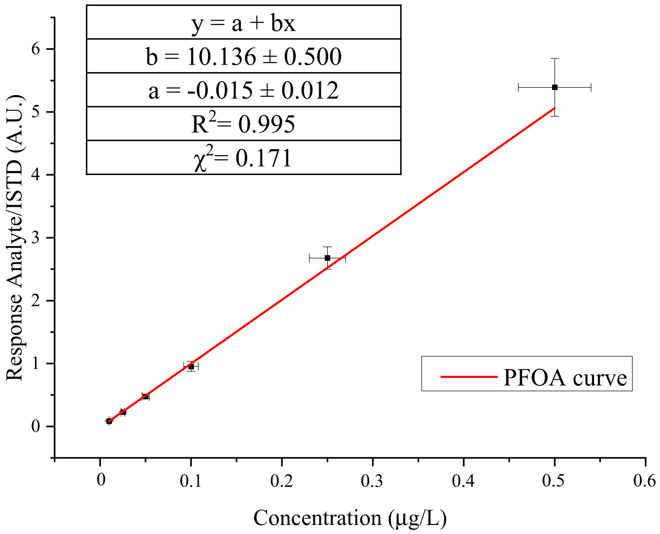

Fig. 2. Calibration curve of PFOA where the response is the ratio of itself and labeled PFOA in the range from 0.010 μg/L to 0.500 μg/L, with equation, linearity and chi-square values.

To evaluate the **precision** and **repeatability** of the method, the percentage of the relative standard deviation (RSD%) calculated at 0.005 μg/kg for six real replicates in solution was also calculated and is shown in Table 1.

The nature and chemistry of some molecules is quite different from the others, such as in the case of the shorter and more polar PFBA. These differences led to less reproducible results in terms of ionization and response into the mass spectrometer, especially at low levels. Also the longer chain molecules, i. e. from C_{11} compounds PFUdA for carboxylic acids and L-PFDoS for sulfonic acids, have a great RSD% compared to the other analytes.

To assess the **sensitivity** of the method, Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated from the calibration curves in solution for each analyte, following the EURACHEM guidelines (Rasul et al., 2018). The selected approach, differing from the EURL-POPs one, takes into account a different calculated value for each PFAS analyzed. LOQs varied from 0.002 μg/kg for PFOS to 0.032 μg/kg for PFODA as shown in Table 2. Compounds like PFOA (0.018 μg/kg) with different responses ionization and fragmentation were found in between.

According to the latest European guidelines and recommendations on food and feed analysis (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022) the method allows to reach a good level of quantification for all 21 analytes in rice and maize.

Table 1
RSD% calculated for all the 21 compounds at 0.005 μg/kg, lower level of the calibration curve in solution. The values ranged from 1.56 % to 32.95%.

Analyte	RSD%	Analyte	RSD%
L-PFBA	32.95	PFDA	7.93
PFPeA	5.15	L-PFNS	8.41
PFBS	2.07	PFUdA	13.52
PFHxA	6.10	L-PFDS	4.74
L-PFPeS	5.25	PFDoA	8.58
PFHpA	1.56	PFTrA	16.13
L-PFHxS	7.23	L-PFDoS	18.47
PFOA	5.74	PFTeDA	11.31
L-PFHpS	2.24	PFHxDA	9.99
PFNA	8.44	PFODA	8.01
L-PFOS	8.07		

Table 2
LOQ, in *µg/kg*, calculated for all the 21 compounds in the intralaboratory study. The values ranged from 0.002 to 0.032 µg/kg.

Compound	LOQ *[µg/kg]*	Compound	LOQ *[µg/kg]*
PFBA	0.031	PFDA	0.019
PFPeA	0.018	L-PFNS	0.005
L-PFBS	0.015	PFUdA	0.016
PFHxA	0.022	L-PFDS	0.004
L-PFPeS	0.020	PFDoA	0.008
PFHpA	0.014	PFTrA	0.015
L-PFHxS	0.009	L-PFDoS	0.003
PFOA	0.018	PFTeDA	0.026
L-PFHpS	0.003	PFHxDA	0.024
PFNA	0.007	PFODA	0.032
L-PFOS	0.002		

It has to be taken into account that rice is a matrix not yet encompassed by the current legislation, however the calculated LOQs for the 4 regulated PFASs are quite in line with recommended (recLOQs) in the EURL POPs guidelines. It is particularly interesting to note that the limits of quantification for PFOS, PFOA, PFNA are still slightly above the recLOQ (≤0.010 µg/kg for the first two, and ≤0.005 µg/kg for the latter) in vegetables (that is the most similar to rice among all matrices considered in the document)), while the quantification limit of PFHxS is already well below the recLOQ (0.015 µg/kg). Furthermore, in the latest legislation (FAO. Commission Regulation (EU) 2023/915 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006, 2023), where more stringent limits are defined, rice is also not included. Therefore, no official LOQs are currently established for this matrix.

For evaluating to which extent the experimental results are representation of the actual concentration of the analytes in the real samples, the **Matrix Effect** (ME) was calculated by spiking CRMs after the extraction of negative rice and maize samples (according to EURL POPs guidelines, ISO/IEC Guide 58:1993)). As shown in Fig. 3, ME was between −20% and +20% in rice and between −15% and +38% for maize.

In comparison with other data in literature (Li et al., 2009; Meng et al., 2022), the ME can be considered negligible in the majority of the cases for both matrices. However, some exceptions were found, such as for PFBS in maize samples where an interference was found in the transition 298.941 *m/z* -> 79.975 *m/z*. This problem was overcome by finding a new transition 298.941 *m/z* -> 98.976 *m/z* which was selected to obtain a good quantification in the matrix. This can be explained by the natural abundance of SO_3^- groups (79.975 *m/z*), while it is rarer the same group added with a CF_2 molecule (98.976 *m/z*).

The **absolute recovery** (i.e. the ratio between the amount of analyte measured in matrix and the amount measured in solvent) was evaluated by spiking four samples (injected 3 times each) and the solvent with the CRMs, at concentration of 0.2 µg/kg.

Absolute recoveries (Fig. 4) resulted in values higher than 80% for all the compounds analyzed. However, some compounds deviated from the average (that was around 98%) due to their chemical properties and solubility in acetonitrile used for the extraction. An example is the PFBA, the shortest and more polar compound, which results in the worst absolute recovery. This problem can be solved by using the **apparent recovery** approach as stated in the EURL POPs guidelines (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022; Burns et al., 2003). The apparent recovery is obtained using the isotope dilution approach and it allows to correct the absolute recovery for extraction/clean-up losses and matrix-effects (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022). For example, the apparent recovery of PFBA and PFODA (less polar) were calculated to be 81.9%, and 99.2% respectively, while the absolute recoveries were 128% (PFBA) and 114% (PFODA).

These values are in line with the values indicated in the guidelines (European Union Reference Laboratory for halogenated POPs and in Feed and Food, 2022), where the acceptable values range from 80% to 120% for compliance testing and 65–135% for monitoring purposes).

As a fundamental step of the metrological validation of a method, the **uncertainty value** must be provided, for estimating the reliability of the quantification and for calculating the error associated with the measurement itself (Meyer, 2007). Eurolab and Eurachem established analytical validation guidelines in different technical reports (Rasul et al., 2018; European Federation of National Associations of Measurement Testing and Analytical Laboratories, 2006). Here, the two approaches (bottom-up approach and intralaboratory approach) were tested and the results were compared, in order to provide insight on what can be used as a reference method.

For the **bottom-up approach** all sources of error were taken into account so the uncertainties of the values on the two dimensions of the calibration curve (x axis and y axis - see Fig. 4) were calculated. The uncertainties on concentrations for the dilutions of standard solutions (x-axis) were obtained by applying the propagation of the uncertainties

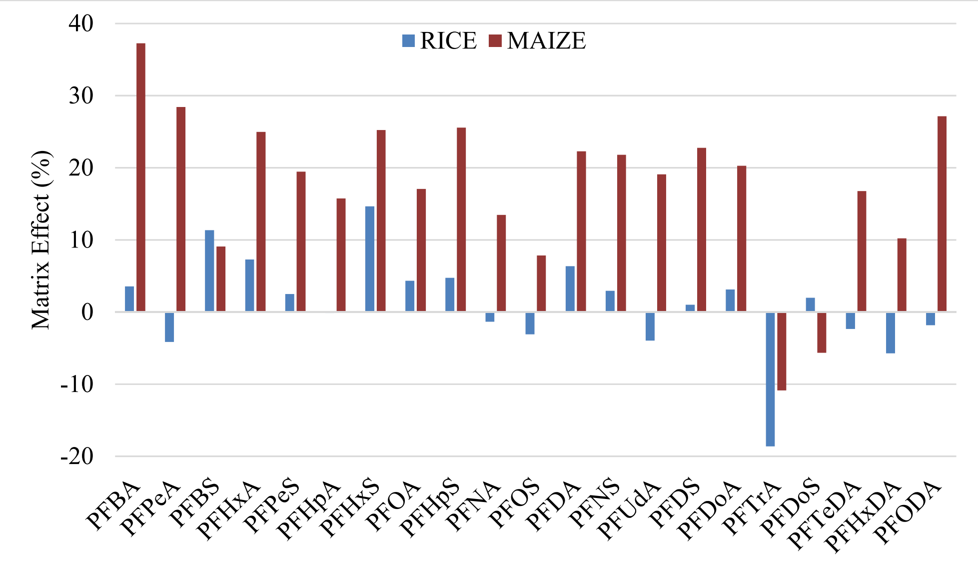

Fig. 3. Chart with the calculated ME in percentage for all the analytes in rice and maize matrices. The ME ranged from −20% and +20% in rice and between −15% and +38% for maize.

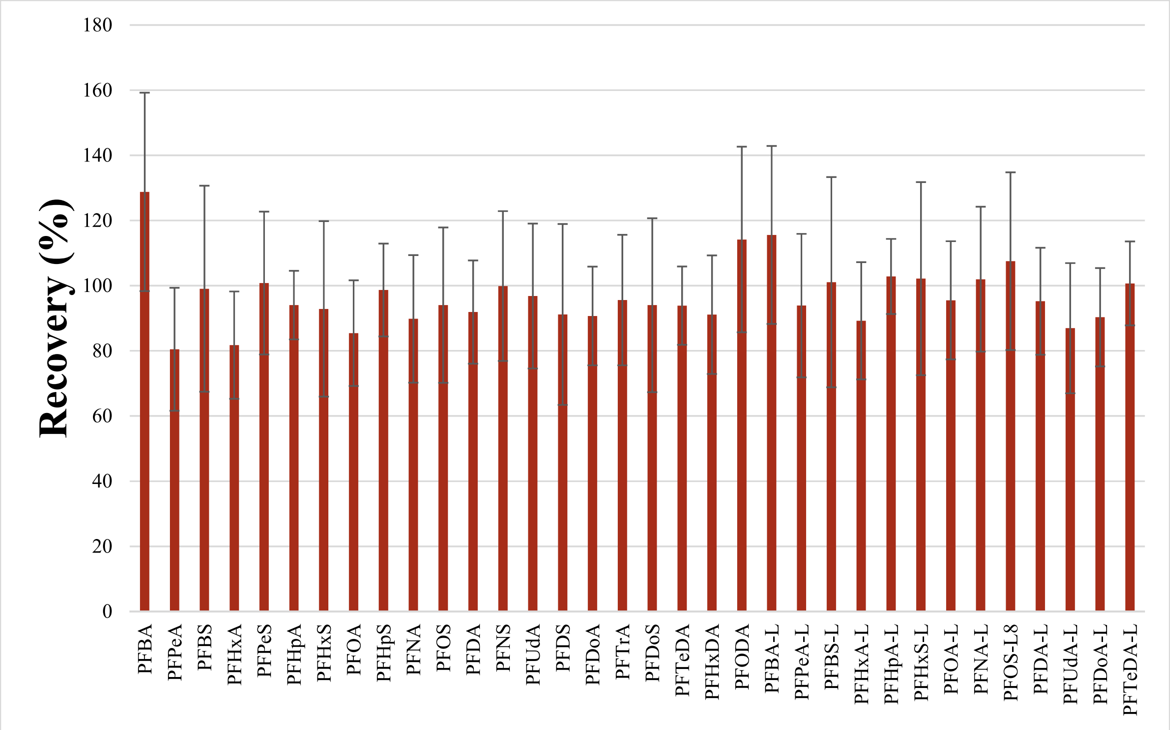

Fig. 4. Chart with the calculated recovery at 0.2 μg/kg in percentage for all the analytes and internal standard in rice matrices. The recovery ranged from 80% to 128%.

related to each specific pipette used, taking also into account the already declared uncertainties of the CRMs on the available certificate of analysis. For the calculation of the y-axis error, the propagation of the calculated standard deviations for the three replicates of each analyte and each labeled compound was performed, summing the squared errors of the two relative uncertainties because of the ratio calculated to obtain the response analyte/ISTD. An overview of the whole budget of uncertainties is depicted by the Ishikawa diagram built, starting from the model (Equation (1)), in Fig. 5.where C_{ext} is the concentration of PFASs in the extract ($\mu g/L$), f_d is the dilution factor and C_{PFAS} is the final concentration of PFASs in samples C_{PFAS} ($\mu g/kg$).

Once the equation of the model is clear, as well as for the contributors to the budget of uncertainty, the following formula (Equation (2)) is applied to calculate the uncertainty:

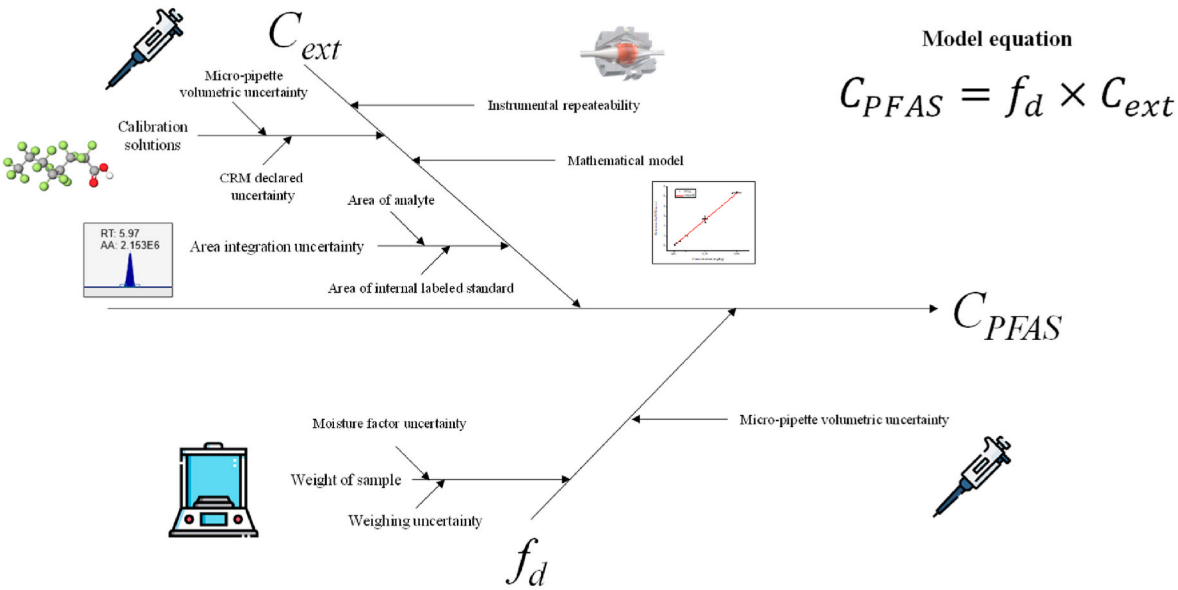

Fig. 5. Ishikawa diagram for bottom-up budget uncertainty evaluation in which all the contributors to the various calculated values of uncertainty are reported. *This figure has been designed using images from Flaticon.com (Icons from figures and graphical).

$$C_{PFAS} = f_d \times C_{ext} \tag{1}$$

$$u(y) = \sqrt{\sum u_i^2(y)} = \sqrt{u_{CRM}^2 + u_{weight}^2 + u_{MW}^2 + u_{dil}^2 + u_{int}^2} \tag{2}$$

where u_{CRM} is the contribution for CRMs, u_{weight} for weighing operations, u_{MW} for the molecular weight, u_{dil} for the dilution operations and u_{int} for the integration steps.

The uncertainty evaluation, carried out by the **intralaboratory approach**, was performed according to guidelines (Meyer, 2007), and the formula used is showed below (Equation (3)):

$$u(y) = \sqrt{s_{Rw}^2 + \Delta_{ref}^2 + u_{ref}^2 + \frac{SD^2_{(spiked\ samples)}}{n}} \tag{3}$$

where s_{Rw} is the overall run to run variation, Δ_{ref} is the difference between the reference and the calculated concentration, u_{ref} is the uncertainty of the reference material and SD (Ohagan, 2008)$_{(spiked\ samples)}/n$ is the standard deviation divided by $n = 4$, where n is the number of replicates.

The results of the two approaches (Fig. 6), reported in comparison with the corresponding relative standard deviation RDS%, demonstrates that the bottom-up approach is not able to provide realistic estimation of uncertainties for the different analytes. In fact, bottom up values are quite similar for all the analytes, while the RDS% are noticeably different from one analyte to another.

For example, the RSD% for PFDS is around 2.5%, while for PFBS one is 11%, but from the modeling approach the final uncertainty seems to be higher from PFDS side.

It appears that the intralaboratory approach gives more reliable values, better reflecting the complexity of the matrix and of the method, and the different chemical properties of the analytes. As a general consideration, the modeling approach seems to provide an underestimation of the percent relative uncertainty, while the single intralaboratory approach permits to correctly evaluate the uncertainty taking into account the experimental contribution.

2.2. Monitoring of real samples: rice and maize

The analysis of the samples results in one rice quantified in PFBA and ten rice with some PFAS only detected. In the maize samples there were no findings. The PFAS detected in the food samples are reported in the chart below; all the rice samples not shown in Fig. 7 have no PFAS detected.

According to literature the uptake of PFAS in fruit and vegetables is confirmed to be preferred by short and ultrashort PFAS due to the polarity properties.

3. Conclusions

The method validated in this work has provided a robust and precise approach to assess the level of contamination of PFAS in rice and maize. The uncertainty budget was well established and its calculation was defined aiming to harmonize the scientific community to quantify these contaminants in food and feed, providing inputs to control bodies and legislation. In comparison to other methodologies, this method is able to reach analytical performances with a few extraction steps and this is a significant outcome. The same approach is under validation on other matrices, and applied on triple quadrupole mass spectrometers and the preliminary results are quite satisfactory and in line with the new legislative requirements.

The results shown here are in line with some of the latest scientific papers published on the same matrices (Hwang et al., 2024; Genualdi S et al., 2024).

Also, it is the first time the meteorological approach is used for the method validation of PFAS in this type of matrices and, in general, there are few works in literature focused on the metrological aspects of measuring PFAS in food. Here, the comparison of different approaches for estimating the uncertainty budget of the method has provided significant insight on what is the best way for evaluating the precision and the accuracy of the methods. This tool is of paramount importance for industrial and control laboratories that have to check and confirm the compliance of the product to legislative requirements. In addition, the same analytical approach is well fitted, beside quantifying target analytes, for screening purposes. The results obtained by full-scan measurements of the same matrices are under evaluation. The advantage of having optimized and validated a method using an HRMS relies on the fact that target and screening methods can be performed at the same time, therefore providing an exhaustive set of information in one single

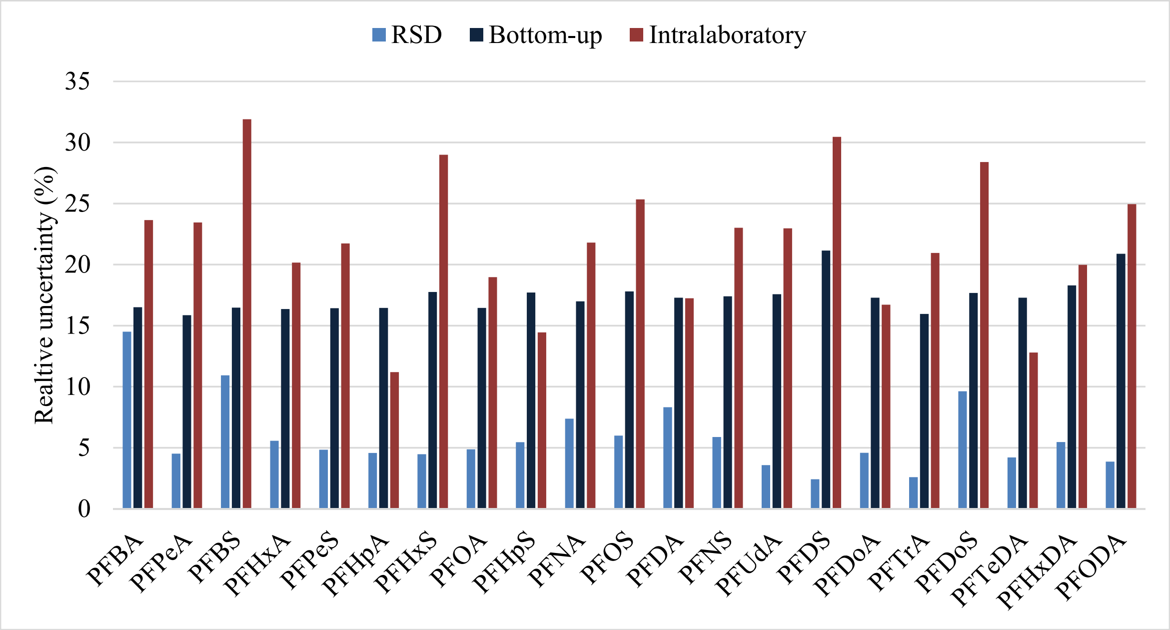

Fig. 6. Chart with the comparison between the bottom-up, intralaboratory uncertainties and RSD% for all the 21 compounds analyzed in the study. The bottom-up approach is too steady for all compounds compared to the differences in relative standard deviation for the different analytes. The intralaboratory relative uncertainty ranged from 12% to 32%.

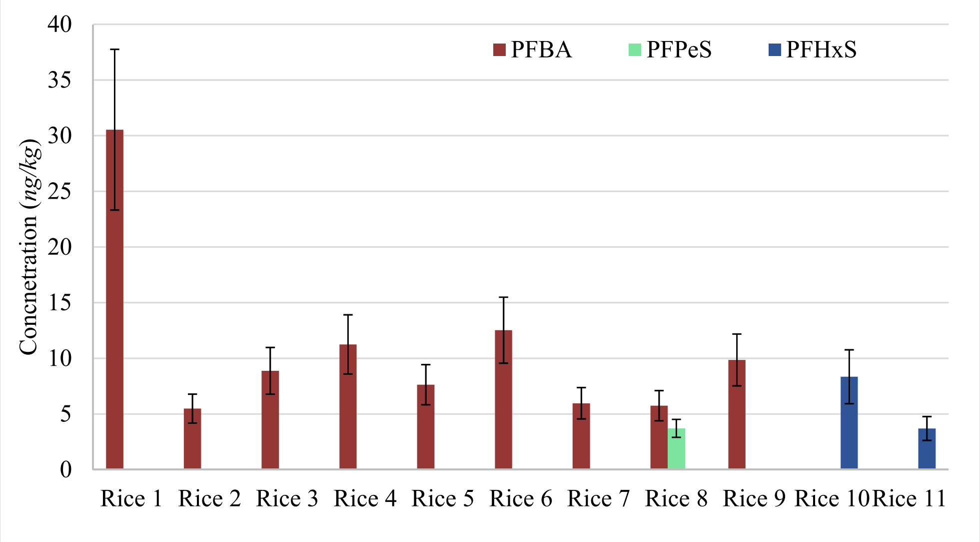

Fig. 7. Quantification of PFAS in Rice samples expressed in *ng/kg*. PFBA mainly detected and few cases of PFPeS and PFHxS found, but all findings were around or below LOQs.

measurement batch, able to assess the safety of the products.

In relation to the matrices targeted in this work, the screening of 30 rice samples and 2 maize samples from the north-west of Italy demonstrates that in this area in general no relevant levels of contamination are present. However, in rice sample “1”, 0.030 μg/kg of PFBA has been detected, which exceeds the levels of other PFAS compounds and therefore warrants additional attention.

In general, the results obtained from this study can support the revision of current guidelines and legislation. Providing more information about food contamination caused by the presence of PFAS, particularly short-chained ones, in vegetable matrices from samples collected by Italian control bodies will support EFSA’s goal of seeking data and assisting authorities in updating analytical guidelines. Moreover, once the legislation is updated and officially published regarding new matrices and emerging PFAS, accurate quantification of PFAS will be of paramount importance. This study aims to assess the uncertainty budget calculation, which will be necessary to evaluate positive and negative findings in real samples.

This includes food matrices such as rice and maize, which form the basis of the diet for a large portion of the population but are not yet addressed by existing regulations. This updating process will be facilitated by the coordinated efforts of reference laboratories, research facilities, and metrological institutes. At the EU level, these efforts are backed by the European Metrology Network on Safe and Sustainable Food.

CRediT authorship contribution statement

Schiavone Consolato: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Romaniello Francesco:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Rossi Andrea Mario:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Avolio Rosa:** Writing – review & editing, Resources. **Abete Maria Cesarina:** Writing – review & editing, Resources. **Portesi Chiara:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142690.

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