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Application Note

Evaluation of the Performance of a UPLC-MS/MS Method for the Determination of PFAS in Drinking Water, for Checking Compliance with the EU Drinking Waters Directive, Using an Interlaboratory Study

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Waters previously developed a method for the determination of per-and polyfluoroalkyl substances (PFAS) in drinking water based on direct injection liquid chromatography with tandem mass spectrometry (LC-MS/MS) that was suitable for checking compliance with the revised EU Drinking Water Directive. This application brief shows the successful evaluation of the performance of this method by interlaboratory study using ACQUITY UPLC™ Systems fitted with the PFAS Analysis Kit and Isolator Columns and Xevo™ TQ-XS Tandem Quadrupole Mass Spectrometers with the UniSpray™ ion source. A reference material containing twenty PFAS was sent to seven Waters™ laboratories, along with standard solutions containing native PFAS compounds and isotopically labelled analogues. The reference material was diluted upon receipt to create a test sample with the

PFAS compounds at 0.01 µg/L. No assessment of the results for perfluorobutanoic acid (PFBA) was possible due to contamination experienced by most laboratories. For the other PFAS, the trueness of the method was determined to be within the range of 96 to 110%. Close agreement was observed with the repeatability within each laboratory and the reproducibility between laboratories both being <20% RSD.

Benefits

- The method was successfully implemented, in seven different Waters laboratories, with varying levels of experience of PFAS analysis
- The performance of the method, as demonstrated by this interlaboratory study, provides users with confidence in the ease of implementation and its suitability for testing drinking water for PFAS for regulatory compliance
- Implementation is supported utilizing our outcome-based support model to ensure customer success

Introduction

PFAS, the so-called “forever chemicals”, have been detected in the environment of many countries. For decades, evidence has been growing that links their widespread occurrence in the environment with adverse effects on human health and ecology.[1] PFAS have been used in many industrial applications and are found in consumer goods such as clothing, food packaging, cookware, cosmetics, and carpet as well as in fire-fighting foam. The use of PFAS in so many products, and the fact they do not biodegrade, has resulted in the contamination of drinking water supplies on a global scale, from multiple sources. There are not yet any federally enforceable standards for PFAS in drinking water in the United States but the US Environmental Protection Agency (EPA) is committed to developing a PFAS National Primary Drinking Water Regulation for publication by late 2022.[2] Instead of a standard, in 2016, the EPA established a non-enforceable health advisory level for PFOA and PFOS in drinking water.[3] In the absence of a federal drinking water standard, numerous US states have enacted limits for PFAS in drinking water, surface water, and groundwater based on different health effects.[4] In Europe, levels of PFAS in drinking water are now regulated.[5] The objective of the Drinking Water Directive concerning the quality of water intended for human consumption, is to protect human health from adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean. The revised Drinking Water

Directive, which entered into force January 2020, includes a limit of 0.1 µg/L for a sum of twenty individual PFAS.

We have previously reported the development of a direct injection method for PFAS suitable for checking compliance with the 2020 EU Drinking Water Directive. A limit of quantitation (LOQ) of 0.001 µg/L was achieved for each individual PFAS to be confident of implementing the 0.1 µg/L sum of the twenty PFAS limit, without the need for any pre-concentration steps. The method used an ACQUITY Premier BEH™ Shield RP18 Column on an ACQUITY UPLC I-Class PLUS System with Xevo TQ-XS Tandem Quadrupole Mass Spectrometer and the unique UniSpray ion source.[6] Although PFAS respond well using electrospray ionization in negative ion mode, UniSpray was shown to exhibit consistent gains in sensitivity for all twenty PFAS. In addition, the novel MaxPeak™ High Performance Surfaces (HPS) technology of the ACQUITY Premier UPLC Column provided an increase in sensitivity for the longer-chain PFAS. Here, we report the results of an interlaboratory study to assess the ease of implementation of the method and to further evaluate the performance of the method.

Results and Discussion

Laboratories were supplied with:

- Analytical protocol, including a list of the analytes of interest and internal standards (see Table 1), the instrument configuration, the method, and parameters to be used, guidance documents and the analytical run sequence
- Bespoke water reference material and native PFAS and isotopically labelled PFAS stock solutions, supplied by ERA™ and Wellington Laboratories.

The compounds listed in the European regulation comprise two series, one with perfluoro carboxylic acid (PFCA) and one with perfluoro sulphonic acids (PFSA), both in the range 4 to 13 carbons, making up 20 in total (Table 1). Although most of these PFAS are well known, and are included in US EPA methods, there are three that are relatively “new” in this context, the C11, C12, and C13 PFSA (PFUnDS, PFDoDS, and PFTrS).

Name	Abbreviation	Internal standard
Perfluorobutanoic acid	PFBA	$^{13}C_4$-PFBA
Perfluoropentanoic acid	PFPeA	$^{13}C_5$-PFPeA
Perfluorohexanoic acid	PFHxA	$^{13}C_5$-PFHxA
Perfluoroheptanoic acid	PFHpA	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	PFOA	$^{13}C_8$-PFOA
Perfluorononanoic acid	PFNA	$^{13}C_9$-PFNA
Perfluorodecanoic acid	PFDA	$^{13}C_6$-PFDA
Perfluoroundecanoic acid	PFUnDA	$^{13}C_7$-PFUnDA
Perfluorododecanoic acid	PFDoDA	$^{13}C_2$-PFDoDA
Perfluorotridecanoic acid	PFTrDA	–
Perfluorobutyl sulfonate	PFBS	$^{13}C_3$-PFBS
Perfluoropentane sulfonate	PFPS	–
Perfluorohexyl sulfonate	PFHxS	$^{13}C_3$-PFHxS
Perfluoroheptane sulfonate	PFHpS	–
Perfluorooctyl sulfonate	PFOS	$^{13}C_8$-PFOS
Perfluorononane sulfonate	PFNS	–
Perfluorodecane sulfonate	PFDS	–
Perfluoroundecane sulfonate	PFUnDS	–
Perfluorododecane sulfonate	PFDoDS	–
Perfluorotridecane sulfonate	PFTrDS	–

Table 1. List of PFAS included in this study and the stable isotope analogues used as internal standards.

The original method was developed on an ACQUITY UPLC System fitted with FL-based Sample Manager (SM) so was modified by each laboratory for use on their FTN-based systems. Participants were instructed to install the PFAS Analysis Kit and Isolator Column, as per the guide, prior to use.[7] This establishes a complete flow path for analyzing PFAS-containing samples while minimizing interference from background contamination. It

involves removing and replacing components such as some tubing assemblies that contain known PFAS and separating any unavoidable interference from sources such as mobile phase by delaying their signal from the sample using an isolator column.

Participants from seven laboratories were asked to determine the concentration of the analytes from replicate injections (n=6) of a water test sample containing twenty PFAS compounds at 0.01 µg/L, using suitable calibration graphs prepared from the analysis of standards in LC-MS grade water, with stable isotope analogues as internal standards where available, as instructed in the protocol. All the participants were busy facilities with varying experience of PFAS analysis. As laboratories only had limited lime to set up the method on their systems, this study provided valuable feedback on the ease of implementation of this method and any critical issues encountered. No assessment of the results for PFBA was possible due to the relative magnitude of contamination experienced by some of the laboratories. This reiterates the need for careful consideration to be given to sources of contamination during the planning of any investigation into PFAS levels in water. In cases where suitable mitigation measures were taken (e.g., using positive displacement pipettes, rinsing any glassware before use, using bottled water for mobile phase, and flushing all UPLC lines thoroughly), laboratories reported satisfactory performance for PFBA (n=3; trueness 98%, RSD_r=4%, RSD_{RL}=5.8%).

Each of the laboratories successfully implemented the chromatographic method to provide sufficient retention of the most polar PFAS and exhibited Gaussian chromatographic peak shape and stable retention times throughout. The method was shown to be highly sensitive as can be seen from the chromatograms from the analysis of the standard at 0.001 µg/L (Figure 1).

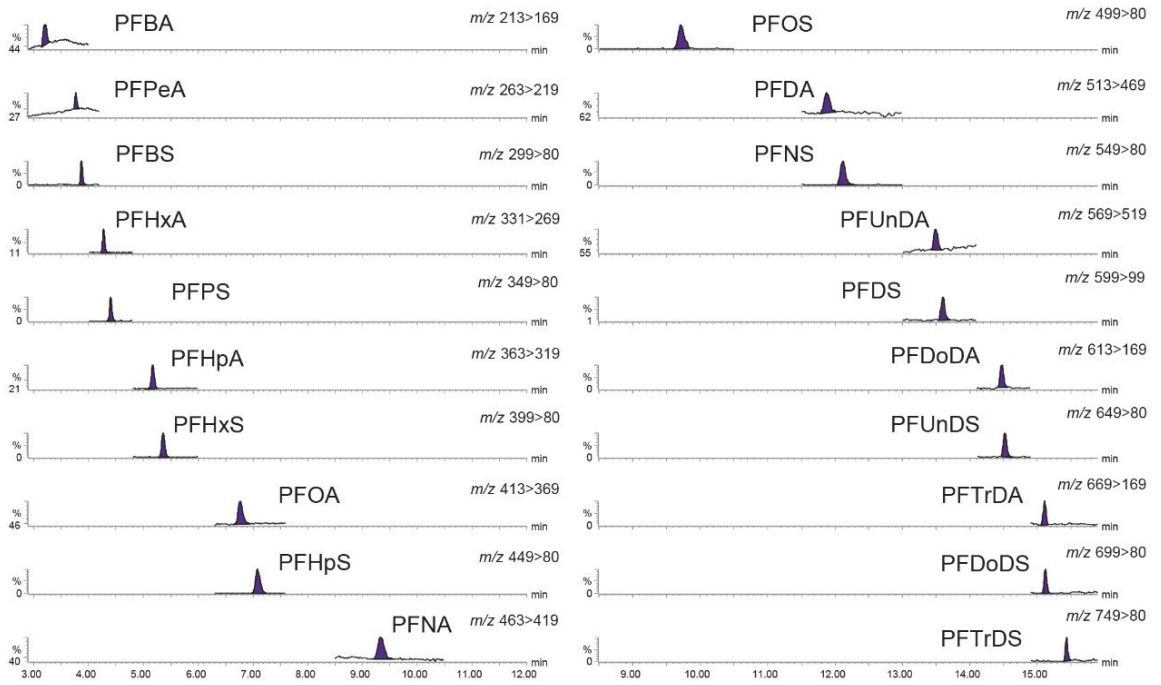

Figure 1. Typical chromatograms showing PFAS from analysis of the standard at 0.001 µg/L.

Calibration graphs exhibited excellent linearity of response, typically using a linear fit with a 1/x weighting, r^2 >0.99 and residuals <20% (Figure 2).

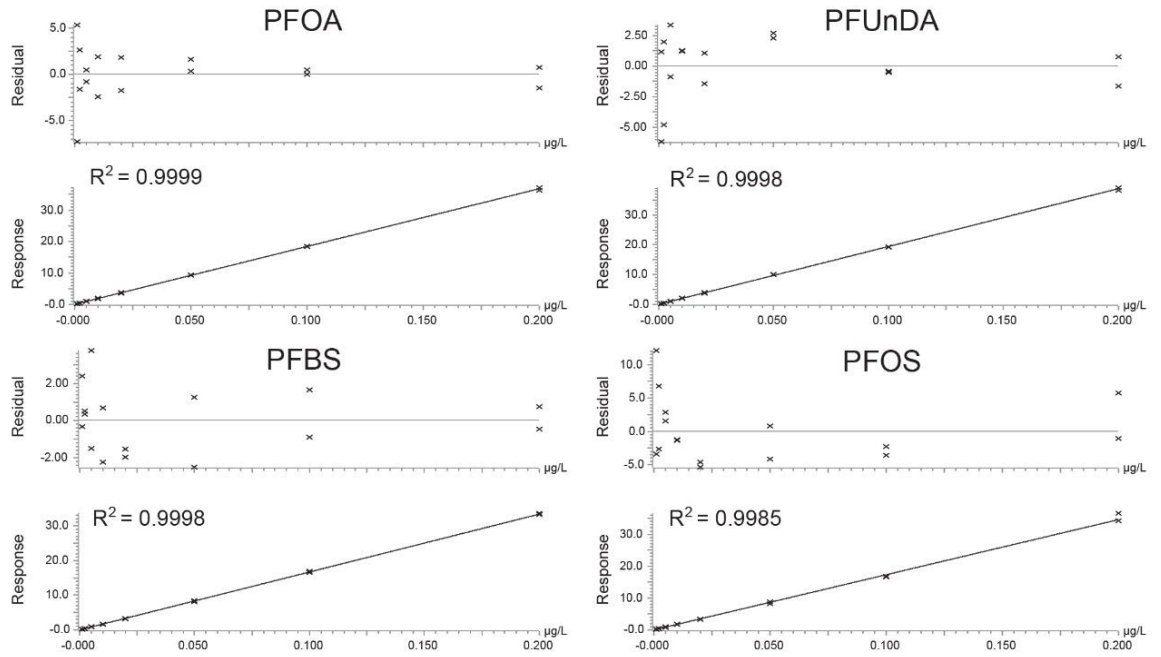

Figure 2. Typical calibration graphs for a selection of PFAS over the range 0.001–0.20 µg/L in LC-MS grade water.

The laboratories demonstrated good accuracy for the quantification of all the PFAS in the water test sample (Table 2) except for PFBA. Trueness was shown to be between 96 and 110%, repeatability within each laboratory (RSD_r) between 10 and 16% and values for reproducibility between laboratories (RSD_{RL}) were between 10 and 19%. Results from the validation are shown in Table 2 and summarized Figures 3 and 4. In each case, ion ratios and retention times from the analysis of the water test sample agreed well with commonly used criteria.[8]

	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	
Repeatability (% RSD_r)	11%	10%	12%	11%	11%	10%	10%	11%	12%	
Reproducibility (% RSD_{RL})	15%	15%	17%	19%	13%	14%	12%	19%	10%	
Trueness (%)	104%	104%	103%	105%	102%	103%	102%	110%	101%	

	PFBS	PFPS	PFHxS	PFHpS	PFOS	PFNS	PFDS	PFUnDS	PFDoDS	PFTrDS
Repeatability (% RSD_r)	10%	10%	11%	10%	11%	10%	11%	11%	14%	16%
Reproducibility (% RSD_{RL})	12%	14%	14%	15%	14%	13%	12%	10%	13%	19%
Trueness (%)	103%	99%	103%	100%	104%	100%	101%	101%	99%	96%

Table 2. Summary of results from the interlaboratory study.

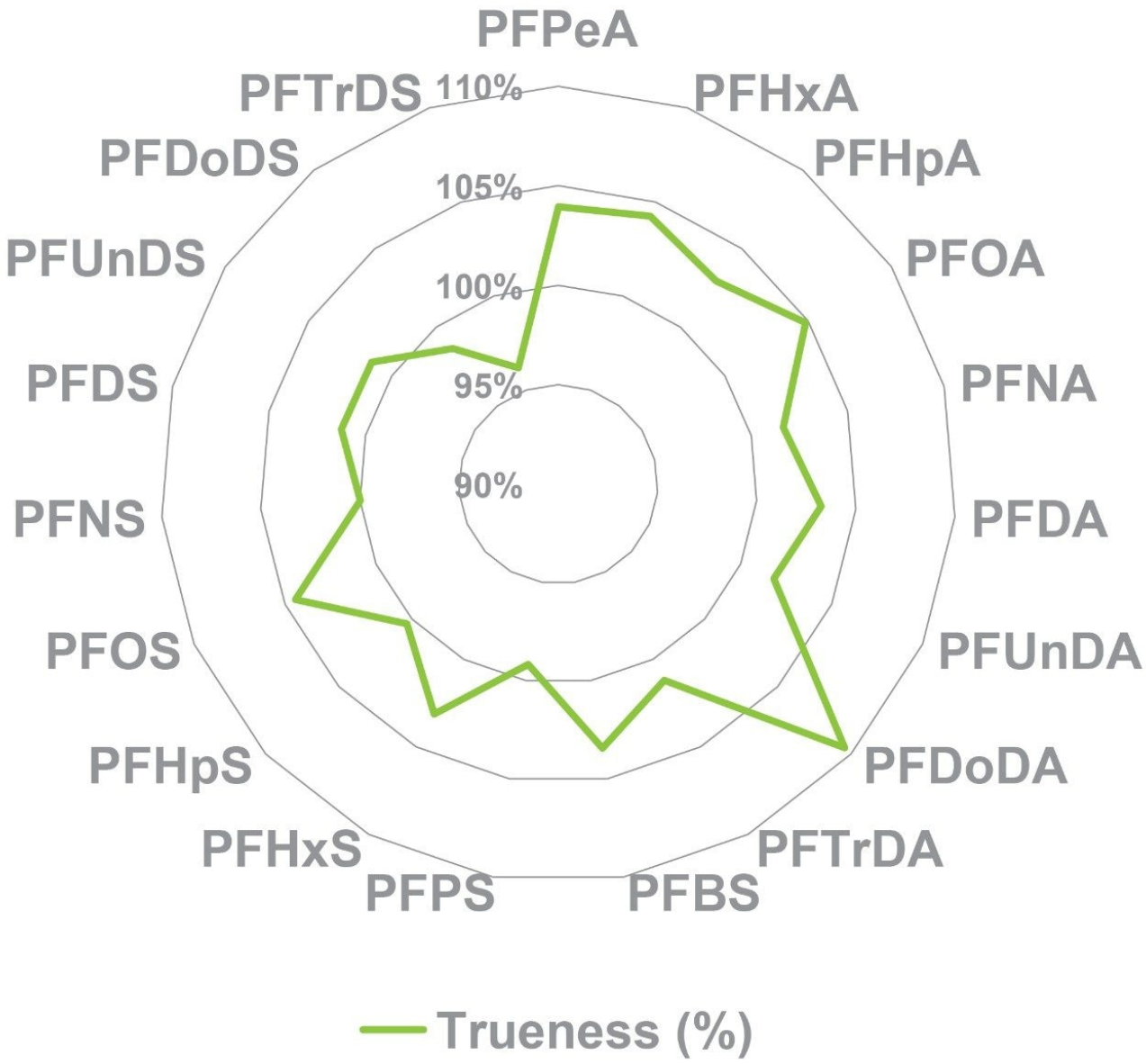

Figure 3. Summary of the trueness values for PFAS from the analysis of the water test sample at 0.01 µg/L.

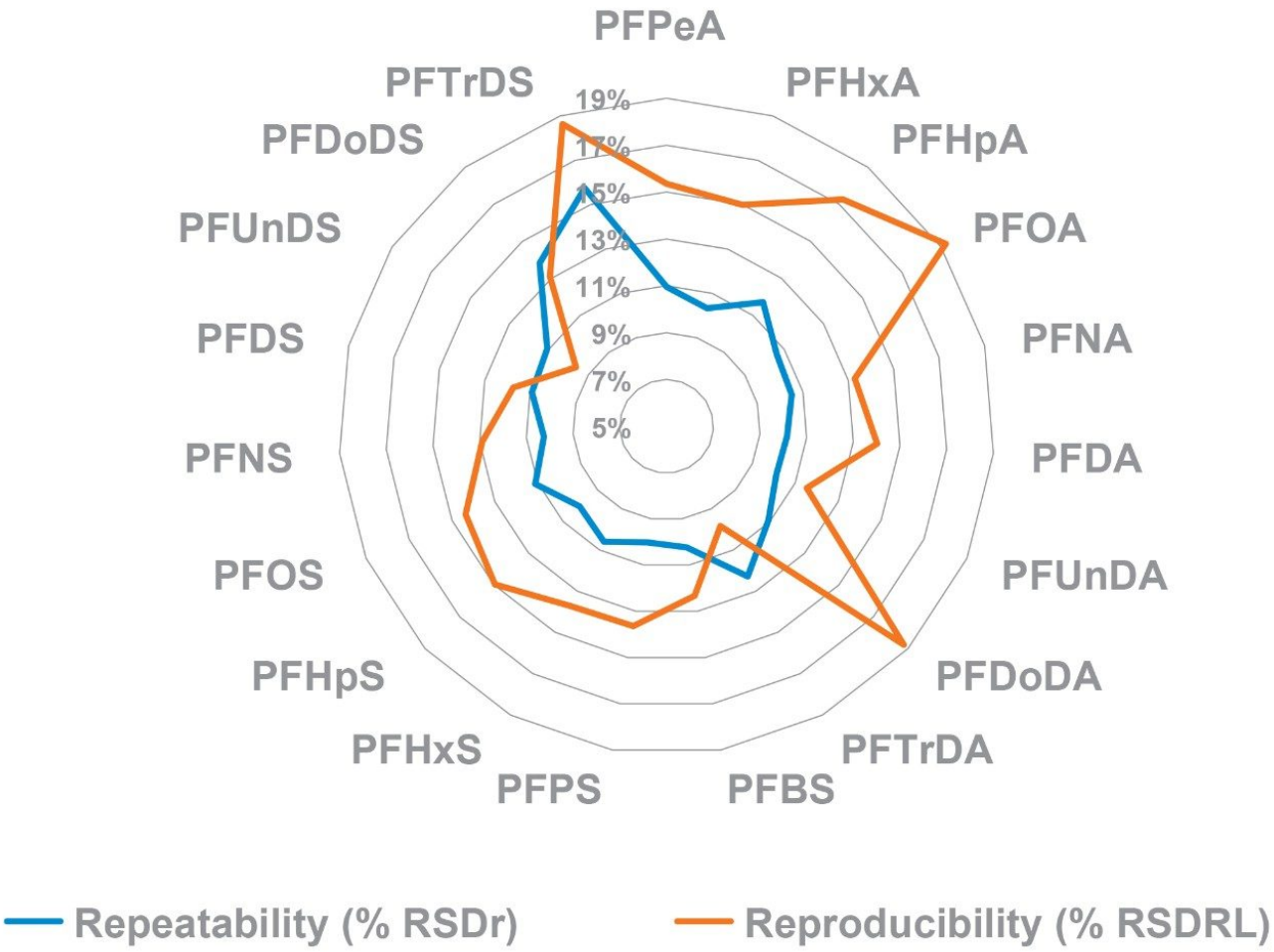

Figure 4. Summary of the values for repeatability within each laboratory (RSD_r) and for reproducibility between laboratories (RSD_{RL}) for PFAS from the analysis of the water test sample at 0.01 µg/L.

Conclusion

The performance of the method for the determination of twenty PFAS in water was investigated using an interlaboratory study. Each laboratory successfully implemented the method, including installation of the ACQUITY UPLC PFAS Kit, using the start-up guide, and demonstrated stable chromatography and satisfactory sensitivity. Participants demonstrated good accuracy for the quantification of the PFAS except PFBA in a water

test sample. Trueness was shown to be between 96 and 100%, and values for repeatability within each laboratory and reproducibility between laboratories were all <20%. All but three laboratories experienced issues relating to contamination with PFBA. This study confirms that this direct injection UPLC-MS/MS method can be implemented in multiple laboratories and is suitable for checking drinking water supplies for compliance with various regulatory and advisory limits globally.

References

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