AOAC SMPR® 2023.003

Standard Method Performance Requirements **(SMPRs®) for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed**

Intended Use: Compliance Monitoring by Trained Technicians

Purpose:

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*SM and AOAC *Performance Tested Methods*SM programs. AOAC SMPRs also may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Quantitative analysis of selected PFAS in produce, beverages, dairy products, eggs, seafood, meat products, and feed (*see* Tables 1–3). Preference will be given to methods applicable to all analyte/matrix combinations listed in Table 4 and as many other analyte/matrix category combinations as possible.

2 Analytical Technique

Mass spectrometry-based methods

3 Definitions

Limit of quantitation (LOQ).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Matrix blank.—Sample with PFAS <30% of LOQ that is brought through entire measurement procedure and analyzed in the same manner as a test sample.

Procedural blank.—Sample that does not contain the matrix that is brought through entire measurement procedure and analyzed in the same manner as a test sample.

Recovery.—Ratio of the calculated concentration versus the expected concentration, expressed as a percentage.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator (in the same laboratory) and repeated in the same day. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation ($\%RSD_r$).

Reproducibility.—Variation arising when identical test materials are analyzed in different laboratories by different operators on different instruments. Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation ($\%RSD_R$).

4 Method Performance Requirements

See Tables 4-7.

5 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include at least procedural blanks and matrix spikes. Procedural blank levels should be ≤30% of the levels in samples analyzed in each batch. In the case of unavoidable background contamination from solvent bottles, etc., subtraction of procedural blank concentrations may be performed. Measures should be taken to reduce background contamination during each stage of sampling and analysis. Materials used should be free of PFAS and contact with fluoropolymer materials should be avoided.

Selectivity of the method should be evaluated to demonstrate that known cholic acid interferences including TDCA, TCDCA, and TUDCA (*see* Appendix A) do not co-elute with the PFOS *m/z* 499→80 MS/MS transition when using low-resolution mass spectrometry. Baseline separation of these compounds must be achieved between the cholic acids and all PFOS isomers represented by the branched standard, or it should be demonstrated that these interferences are removed prior to the chromatographic separation (during extraction and/or cleanup steps). The MS/MS transition *m/z* 499→124 is present in all three cholic acids and should be monitored to confirm separation or removal of cholic acids from samples. This evaluation is not necessary when using high-resolution mass spectrometry.

For PFAS with only one specific MS/MS transition (e.g., PFBA and PFPeA), a second confirmation of identity (e.g., high-resolution mass spectrometry) is needed if reporting results from the analysis of food samples.

A branched PFOS standard should be included in the analysis for retention time confirmation of isomers. The PFOS standards used for ∑PFOS analysis should be specified.

6 Reference Material(s)

U.S. National Institute of Standards and Technology (NIST) has multiple reference materials (RMs) in production, and interested parties should check https://shop.nist.gov to keep up to date on any newly available RMs.

Australian National Measurement Institute: https://www.industry.gov.au/national-measurement-institute/chemical-and-biological-measurement-services/proficiency-testing-services

FAPAS: www.fapas.com

7 Validation Guidance

Method Validation

Validation must be conducted at the target LOQ and at least two additional concentration levels within 2–100x of the target LOQ. In each case, a suitable matrix blank should be spiked at least in triplicate.

LOQ is the lowest concentration of mass of the analyte in the test material that has been validated with acceptable performance (recovery and repeatability) by applying the complete analytical method and identification criteria (1). The following positive identification criteria are to be met simultaneously:

(*1*) Retention time (each analyte and internal standard) should match with the average of the calibration

points in the same sequence with a tolerance of 1%.

(*2*) Include all visible qualifier transition signals with an S/N ratio ≥3:1 (method must include specific technique used to calculate S/N).

(*3*) Ion ratio(s) of the diagnostic ions shall correspond to those in the calibration points of the same sequence with ±30% relative tolerance.

A matrix blank is considered suitable if it contains no more than 30% of the target LOQ level for the given analyte. For method validation, method developers should select at least one representative matrix from each matrix category listed in Table 3. Preference will be given to methods applicable to all analyte/matrix combinations listed in Table 4 and as many other analyte/matrix category combinations as possible.

If a suitable matrix blank cannot be found for matrices that contain higher levels of incurred PFAS (>30% of the target LOQ for that analyte), spiking experiments should be conducted for the affected analytes at two concentration levels in the range of 2–50x the analyte in the evaluated matrix and then the incurred PFAS concentration is subtracted from spiked samples for recovery calculations.

In the case where there is unavoidable background contamination of PFAS in the matrix blanks where incurred residues are present, LOQ can be calculated for those specific analytes by processing seven "blank" samples through all steps of the method by use of the following equation, where S_s is equal to the sample standard deviation of the replicate "blank" samples:

$$LOQ = 10*S_s$$

Recovery is the fraction or percentage of analyte concentration that is measured when the test sample is analyzed using the entire method. For isotope dilution quantification, response of the target compound is normalized to the response of its isotopically labeled analog or the response of the isotopically labeled analog of another compound with chemical and retention time similarities.

8 Maximum Time-to-Result

None.

9 References

(1) EURL for halogenated POPs in feed and food (2022) *Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed*, version 1.2 of 11 May 2022. https://eurl-pops.eu/core-workinggroups#_pfas

(2) Commission Regulation (EU) 2022/2388 amending Regulation (EC) No. 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs (December 7, 2022) *Off. J. Eur. Union* **L316**/38

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Table 1. Target analytes			
No.	Name	Abbreviation	CAS No.
1	Perfluorobutanoic acid	PFBA	375-22-4
2	Perfluoropentanoic acid	PFPeA	2706-90-3
3	Perfluorohexanoic acid	PFHxA	307-24-4
4	Perfluoroheptanoic acid	PFHpA	375-85-9
5	Perfluorooctanoic acid	PFOA	335-67-1
6	Perfluorononanoic acid	PFNA	375-95-1
7	Perfluorodecanoic acid	PFDA	335-76-2
8	Perfluoroundecanoic acid	PFUnA	2058-94-8
9	Perfluorododecanoic acid	PFDoA	307-55-1
10	Perfluorotridecanoic acid	PFTrDA	72629-94-8
11	Perfluorotetradecanoic acid	PFTeDA	376-06-7
12	Perfluorobutanesulfonic acid	PFBS	375-73-5
13	Perfluoropentansulfonic acid	PFPeS	2706-91-4
14	Perfluorohexanesulfonic acid	PFHxS	355-46-4
15	Perfluoroheptanesulfonic acid	PFHpS	375-92-8
16	Perfluorooctanesulfonic acid	PFOS	1763-23-1
17	Perfluorononanesulfonic acid	PFNS	68259-12-1
18	Perfluorodecanesulfonic acid	PFDS	335-77-3
19	Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1
20	Perfluorododecanesulfonic acid	PFDoS	79780-39-5
21	Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8
22	Perfluorooctanesulfonamide	PFOSA	754-91-6
23	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
24	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
25	Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
26	4,8-Dioxa-3*H*-perfluorononanoic acid	DONA	919005-14-4
27	1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2 FTS	757124-72-4
28	1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2
29	1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2 FTS	39108-34-4
30	1*H*,1*H*, 2*H*, 2*H*-Perfluorododecane sulfonic acid	10:2 FTS	120226-60-0

Table 2. Additional analytes to consider			
No.	Name	Abbreviation	CAS No.
1	6:2 Fluorotelomer phosphate monoester	6:2PAP	57678-01-0
2	8:2 Fluorotelomer phosphate monoester	8:2PAP	57678-03-2
3	6:2 Fluorotelomer phosphate diester	6:2diPAP	57677-95-9
4	8:2 Fluorotelomer phosphate diester	8:2diPAP	114519-85-6
5	Capstone product A: 1-Propanaminium, *N,N*-dimethyl-*N*-oxide-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide	Capstone A	80475-32-7
6	Capstone product B: 1-Propanaminium, *N*-(carboxymethyl)-*N,N*-dimethyl-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide	Capstone B	34455-29-3
7	2-Perfluorobutyl ethanol (4:2)	4:2 FTOH	2043-47-2
8	2-Perfluorohexyl ethanol (6:2)	6:2 FTOH	647-42-7
9	2-Perfluorooctyl ethanol (8:2)	8: 2 FTOH	678-39-7
10	2-Perfluorodecyl ethanol (10:2)	10:2 FTOH	865-86-1

Table 3. Target matrices	
Matrix category	Typical representative examples
Produce	Fruits, vegetables, tubers, fungi, fruit/vegetable juice
Coffee	Beans, grounds, instant
Milk (liquid)	Milk
Dairy powders and plant-based protein powders	Powdered milk, adult milk-based powders (ex: protein powder, animal- and plant based)
Eggs	Eggs, egg whites
Seafood (crustaceans and mollusks)	Oysters, shrimp, clams
Fish meat and meat of terrestrial animals (raw, cooked, processed)	Fish fillet, meat (ex: beef, chicken, pork)
Edible offal of terrestrial animals	Edible offal
Fish oil	Fish oil
Foods for infants and young children (baby food)	Fruit- and vegetable-based baby foods, infant formula
Pet food and animal feed	Pet food, animal feed (ex: grain, silage, corn, hay, finished feed product)

Table 4. Target limits of quantification (LOQ) for PFOS, PFOA, PFNA, and PFHxS in EU-regulated matrices[a]				
	LOQ, µg/kg[b]			
Matrix category	PFOS	PFOA	PFNA	PFHxS
Eggs	≤0.3	≤0.3	≤0.3	≤0.3
Seafood (crustaceans and mollusks)	≤0.3	≤0.3	≤0.3	≤0.3
Fish meat and meat of terrestrial animals	≤0.1	≤0.1	≤0.1	≤0.1
Edible offal of terrestrial animals	≤0.4	≤0.4	≤0.4	≤0.4
[a] Matrices with maximum levels established for PFOS, PFOA, PFNA, and PFHxS (individually and as a sum) by Commission Regulation (EU) 2022/2388 (2).				
[b] Target LOQs expressed on w/w basis in samples as received for testing. Values may be revised in the future based on new toxicological studies and hazard assessments.				

Table 5. Target limits of quantification (LOQ) for PFOS, PFOA, PFNA, and PFHxS in other matrices				
	LOQ, µg/kg[a]			
Matrix category	PFOS	PFOA	PFNA	PFHxS
Produce	≤0.01	≤0.01	≤0.01	≤0.01
Coffee	≤0.3	≤0.3	≤0.3	≤0.3
Milk (liquid)	≤0.01	≤0.01	≤0.01	≤0.01
Dairy powders and plant-based protein powders	≤0.08	≤0.08	≤0.08	≤0.08
Fish oil	≤0.5	≤0.5	≤0.5	≤0.5
Food for infants and young children (baby food)	≤0.01	≤0.01	≤0.01	≤0.01
Feed	≤0.5	≤0.5	≤0.5	≤0.5
[a] Target LOQs expressed on w/w basis in samples as received for testing. Values may be revised in the future based on new toxicological studies and hazard assessments.				

Table 6. Target limits of quantification (LOQ) for other PFAS		
Matrix category	LOQ, µg/kg[a,b]	
	PFBA and PFPeA	Other PFAS
Eggs	≤3	≤3
Seafood (crustaceans and mollusks)	≤3	≤3
Fish meat and meat of terrestrial animals	≤1	≤1
Edible offal of terrestrial animals	≤4	≤4
Produce	≤1	≤0.1
Coffee	≤3	≤3
Milk (liquid)	≤1	≤0.1
Dairy powders	≤1	≤0.8
Fish oil	≤5	≤5
Food for infants and young children (baby food)	≤1	≤0.1
Feed	≤5	≤5
[a] Target LOQs expressed on w/w basis in samples as received for testing. Values may be revised in the future based on new toxicological studies and hazard assessments.		
[b] Target LOQs calculated by multiplying LOQs from Tables 4 and 5 by a factor of 10. Minimum LOQ for PFBA and PFPeA was set to 1 µg/kg.		

Table 7. Recovery, repeatability, and reproducibility		
Parameter	PFOS, PFOA, PFHxS, and PFNA in regulated matrices (*see* Table 4)	PFOS, PFOA, PFHxS, and PFNA in other matrices and all other analytes[a]
Recovery, %	80–120	65–135
Repeatability (RSD_r), %	≤20	≤25
Reproducibility (RSD_R), %	≤40	≤40
[a] For analytes without commercially available matching isotopically labeled standards, recoveries within 40-140% and RSD_r ≤30% could be acceptable.		

Appendix A. Known cholic acid interferences		
Common name	Abbreviation	CAS No.
Taurodeoxycholic acid	TDCA	516-50-7
Taurochenodeoxycholic acid	TCDCA	516-35-8
Tauroursodeoxycholic acid	TUDCA	14605-22-2