Guidance for Per- and Polyfluoroalkyl Substances: Analytical

Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants composed of thousands of human-made, fluorinated organic chemicals. The actual number of compounds is continuously changing, as some PFAS are no longer produced in the United States due to regulatory and voluntary actions, while new ones are created as alternatives. Phased-out PFAS still exist in the environment, human bodies, and some products due to their extreme environmental persistence, presence in waste streams, and ongoing global production.

The Minnesota Pollution Control Agency (MPCA) requires lab accreditation through the Minnesota Environmental Lab Accreditation Program (MNELAP) for all PFAS analytical data submitted to the MPCA. The MPCA and MNELAP work together to ensure analytical methods are available for accreditation as they develop and undergo validation to satisfy program requirements. Laboratories must incorporate the data quality objectives in this document if a PFAS method is followed. Laboratories and methods accredited by MNELAP can be found on their website Search for Accredited Laboratories. The performance-based criteria included in this guidance document outlines specific quality processes for sample preparation, instrument calibration, and analysis when working with PFAS.

Purpose and objectives

This guidance is intended for those producing, reporting, or reviewing PFAS data used to support MPCA program work. The purpose of the guidance is to provide data quality objectives and consistency with data quality. Visit https://www.pca.state.mn.us/about-mpca/science-and-data to access current versions of PFAS guidance including Guidance for per-and polyfluoroalkyl substances (PFAS): Sampling (p-eao2-27).

Isotope Dilution Analysis (IDA)

Isotope dilution technique involves quantitation of a compound of interest using a labeled isotope of that very compound. A variety of isotopically labeled analogs are added to each sample prior to extraction, or prior to analysis when extraction isn't required. The isotopically labeled analogs, sometimes referred to as surrogates or extracted internal standard analytes, function as both a surrogate standard (calculation of the recovery of the standard) and internal standard (used in the calculation of the target compounds). Include isotope analog recovery for each sample and analyte in the data report. Analog recoveries need to be within 100 $\pm$ 30% for regulatory work and 100 $\pm$ 50% for screening work, the MPCA expects data to be qualified if outside these limits. Analogs are added to samples prior to preparation and/or analysis depending on the sample matrix; for example:

- Aqueous samples: added to samples prior to extraction.
- Solid samples and biota: added after homogenization and subsampling, prior to addition of water or extraction solvent.
- Serial dilution: Aqueous film forming foam (AFFF) and other foams: added after final dilution.

Instrument and analyte identification

The analytical technique of choice for PFAS is liquid chromatography - mass spectrometry - mass spectrometry (LC/MS/MS). Quantify analytes by comparing the product ion of one precursor ion and retention time in samples to calibration standards. Additional product ions and their ion ratios can be used to distinguish analytes from matrix interference. It is recommended that branched standards are used when available, PFBA and PFPeA are exceptions. Use the ion transition recommendations below when monitoring for two or more ion transitions from parent to characteristic product ions. Ion transition ratio criteria should be determined based on information obtained from standards and used to detect potential bias in sample results.

Ion Transitions:

- PFOA: 413 → 369, 413 → 319, 413 → 269
- PFOS: 499 → 80, 499 → 99, 499 → 130
- PFHxS: 399 → 80, 399 → 99, 399 → 130
- PFBS: 299 → 80, 299 → 99, 299 → 130
- 4:2FTS: 327 → 307
- 6:2FTS: 427→ 407
- 8:2FTS: 527 → 507
- N-EtFOSAA:584 → 419
- NMeFOSAA:570 → 419

Quantitate samples by integrating the total response, accounting for peaks that are identified as linear and branched isomers. Sum the different transitions. Documentation of the primary and confirmation transitions is required. If these transitions are not used, the reason must be technically justified and documented.

Interferences

Within a review process, laboratories must have a documented process to review and limit cross-contamination. PFAS can be found in laboratory items such as polytetrafluoroethylene products (PTFE), solvent lines, aluminum foil, and methanol. These items could lead to method interferences, isotope dilution recovery issues and elevated baselines in chromatograms. Laboratory equipment and supplies that contact samples should be analyzed and contain less than 1/2 the MPCA defined method reporting limit for each PFAS method analyte and isotope performance standards.

Standards

Certified analytical standards are required, when available. Products vary in purity and isomer profiles that can compromise accuracy, precision, and reproducibility of data. Linear and branched isomers are not available for all analytes. Standards must be stored in glass or polypropylene ampules following manufacturer's directions, insuring proper storage and shelf life. Investigate stability of prepared analytical standards as some PFAS analytes form methyl esters over time in methanolic solutions.

Perfluoroalkyl carboxylic acids (PFCAs) including perfluorooctanoic acid (PFOA) have been widely recognized as persistent environmental contaminants. The accurate quantification of PFCAs is dependent on the stability in calibration solutions, as they are important criteria of quantification.

Stability studies indicate that no methyl esters (perfluorooctanoate MePFOA, and methyl formate) were detected in methanol solutions immediately after preparation. MePFOA was detected in calibration solutions stored around 4 months and increased with increase in methyl formate. PFCAs including PFOA should be used immediately after preparation when methanol is used as a solvent. Standard integrity can be increased by the addition of 1% sodium hydroxide to prevent the formation of the methyl esters.

Calibration

Mass calibration is done once or twice a year or as described by manufacturer. Analytical calibration curves should be run at the beginning of each day. The calibration curve must contain at least six consecutive points, but preferably 8-10, non-zero calibration standards containing a consistent amount of stable isotope internal standards. Select the simplest curve fit possible. A linear curve fit is not likely due to the nature of PFAS. The lowest calibration point must be at or below the method reporting limit. Run appropriate blanks with the calibration curve. A calibration verification (ICV) from a source separate from the calibration standard must be analyzed after each calibration curve and before sample analyses can begin. Calibration curves should be evaluated against its regression analysis and standards equal to or less than the MPCA defined method reporting limit should be within ± 40% of the true value. All other calibration points should be within ± 30% of the true value.

Continuing calibration verifications (CCV) must be run prior to sample analysis, after every 10 field samples, and after the analytical sequence. The CCV acceptance criteria must be within 30% of true value. A standard at the MPCA defined method reporting limit must be analyzed prior to each analytical batch to document the instrument's ability to accurately quantitate down to the method reporting limit concentration. The acceptance criteria for the method reporting limit verification is 100 $\pm$ 40% of true known value. If these criteria are not met, the method reporting limit has been set too low and must be confirmed again at a higher concentration.

Calibration criteria for methods using isotope dilution must calibrate with the isotopically labeled analogs of the analytes. Laboratories must include the isotope analog recoveries for each sample and analyte in data reports, including the calibration curve data.

Instrument blanks

The ubiquitous nature of PFAS makes it critical to analyze instrument blanks to determine if the instrument is potentially affected by PFAS concentrations. Instrument blanks must be analyzed after highest calibration standard and daily prior to sample analysis. The concentration of each analyte must be $\leq$ 1/2 the method reporting limit.

Quality control samples

Recommended QA samples for scanning PFAS analysis:

- Method blanks- one per batch of field samples, not to exceed 20 field samples. Same media as associated field samples and undergoes same sample prep. Each analyte must be $\leq$ the method reporting limit.
- Instrument blank- minimum of 1 prior to start of daily analysis and after samples exceeding quantitation range. Must contain internal standards.
- Sample duplicate (DUP) - minimum 1 per batch of 20 field samples or fewer.
- Lab control spike (LCS) – One per analytical batch. LCS must contain all project specific PFAS analytes in same media as associated samples. The recovery acceptance for each target analyte is 100 $\pm$ 50% and the relative percent difference (RPD) of the recoveries $\leq$ 50%.
- Matrix spike and Matrix spike duplicate (MS/MSD) - one pair prepared with each analytical batch. The recovery acceptance for each analyte is 100 $\pm$ 50% and the RPD is ≤ 50%.

Recommended QA samples for regulatory PFAS analysis:

- Method blanks- two per batch of field samples, not to exceed 20 field samples. Same media as associated field samples and undergoes same sample prep. Each analyte must be $\leq$ 1/2 the method reporting limit.
- Instrument blank- minimum of 1 prior to start of daily analysis and after samples exceeding quantitation range. Must contain internal standards.
- Sample duplicate (DUP) - minimum 1 per batch of 20 field samples or fewer.

- Lab control spike (LCS) – Run in triplicate, at 3 levels per analytical batch (low, medium, high) if samples concentration within corresponding batch is unknown. LCS must contain all project specific PFAS analytes in same media as associated samples. The recovery acceptance for each target analyte is 100 ± 30% and the percent relative standard deviation (RSD) of the recoveries ≤ 30%. If samples are prescreened, or have historical levels, then an LCS concentration at a level relative to the samples is run in triplicate.
- Matrix spike and Matrix spike duplicate (MS/MSD) - one pair prepared with each analytical batch. The recovery acceptance for each analyte is 100 ± 30% and the RPD is ≤ 30%.

Representative sample

The following is recommended to ensure a representative sample/subsample is used for analysis:

- Use the entire sample for solid phase extraction (SPE) of aqueous samples. Samples that are unlikely to pass entirely through the SPE due to high TSS or other matrix interferences, spike with an isotopically labeled solution prior to centrifuging, decant the liquid portion. Resuspend the solids with a solvent, centrifuge, and add the decanted solvent to the sample for analysis.
- Sample filtration is not recommended with high particulate samples because retention of PFAS onto SPE filters is likely. If a sample is filtered a qualifier must be applied to the samples in the final report.
- Samples can be centrifuged to reduce sample particulate. This is not recommended unless target analyte absorption has been investigated.
- High PFAS concentrations can overload SPE cartridge capacity. Serial dilutions are recommended for known high concentration samples, such as AFFF.
- Homogenize soil samples prior to subsampling. SPE is not ideal for soil extraction.
- Cleanup procedures must be done on associated batch QC samples (method blank, lab control samples) if matrix interferences occur. PFAS loss may occur when extracts are evaporated to dryness or at temperatures greater than 30°C.

Dilutions

When isotope dilution samples require a dilution, the volume of the diluent contains the same concentration of labeled isotope compounds as what was originally spiked into the sample. The isotope recovery results from the initial analysis should not be used to adjust the data from the secondary dilution analysis.

When non-isotope dilution analyses require a dilution, quantitate target compounds and surrogates relative to internal standards. Note results are from a dilution on the final report. These results are not recovery-corrected.

Total Suspended Solids (TSS) results applying a dilution-like approach to amount of sample run through the SPE must be documented in the final lab report and Electronic Data Deliverable (EDD).

For EPA Draft Method 1633, the dilution(s) must be documented clearly within the final report. For instance, if a dilution is made prior to extraction and a dilution was made to the extracted aliquot (up to a 10x), the final report must show the correct dilution factor for accurate result reporting and transparency.

Method detection limits and method reporting limits

Method reporting limits are based upon performance-based method criteria and performance base instrument criteria and how they behave in each individual laboratory. Each lab has their own equipment and levels of background contamination. The MPCA requires data reporting to the reporting limit, also referred to as the minimum level of quantitation (LOQ) or minimum level (ML). Laboratories must establish MDL's for each target analyte according to the procedure at 40 CFR Part 136, Appendix B. The table below includes compounds and their corresponding reporting limit goals the MPCA would like to work towards however, they may not be achievable for all compounds or by all laboratories.

Compound	CAS number	Aqueous RL goals (ng/L)	Solid RL goals (ng/g) Dry weight	*Biota RL goals (ng/g) Wet weight
Carboxylic Acids (C_4-C_{12} common acids)				
Perfluorobutanoic acid (PFBA)	375-22-4	4	50	50
Perfluoropentanoic acid (PFPeA)	2706-90-3	4	50	50
Perfluorohexanoic acid (PFHxA)	307-24-4	4	50	50
Perfluoroheptanoic acid (PFHpA)	375-85-9	4	50	50
Perfluorooctanoic acid (PFOA)	335-67-1	4	50	50
Perfluorononanoic acid (PFNA)	375-95-1	4	50	50
Perfluorodecanoic acid (PFDA)	335-76-2	4	50	50
Perfluoroundecanoic acid (PFUnA)	2058-94-8	4	100	100
Perfluorododecanoic acid (PFDoA)	307-55-1	4	50	50
Carboxylic Acids (C_{13}-C_{18} less common acids)				
Perfluorotridecanoic Acid (PFTrA)	72629-94-8	4	50	50
Perfluorotetradecanoic acid (PFTeA)	376-06-7	4	50	50
Sulfonates (C_4-C_{12} common sulfonates)				
Perfluorobutanesulfonic acid (PFBS)	375-73-5	4	50	50
Perfluoropentanesulfonic acid (PFPeS)	2706-91-4	4	50	50
Perfluorohexanesulfonic acid (PFHxS)	355-46-4	4	50	50
Perfluoroheptanesulfonic Acid (PFHpS)	375-92-8	4	50	50
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	4	50	50
Perfluorononanesulfonic acid (PFNS)	474511-07-4	4	50	50
Perfluorodecanesulfonic acid (PFDS)	335-77-3	4	50	50
Perfluorododecanesulfonic acid (PFDoS)	79780-39-5	4	50	50
Amides				
Perfluorooctane Sulfonamide (FOSA)	754-91-6	4	50	50
N-methyl perfluorooctane sulfonamidoacetic acid (NMeFOSAA)	2355-31-9	4	50	50
N-ethyl perfluorooctane sulfonamidoacetic acid (NEtFOSAA)	2991-50-6	4	50	50
Telomer Sulfonic Acids				

Compound	CAS number	Aqueous RL goals (ng/L)	Solid RL goals (ng/g) Dry weight	*Biota RL goals (ng/g) Wet weight
4:2 Fluorotelomer sulfonic acid (4:2 FTSA)	757124-72-4	4	100	100
6:2 Fluorotelomer sulfonic acid (6:2 FTSA)	27619-97-2	4	100	100
8:2 Fluorotelomer sulfonic acid (8:2 FTSA)	39108-34-4	4	100	100
N-Methyl perfluorooctane sulfonamidoethanol (N-MeFOSE)	24448-09-7	4	50	50
N-Ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE)	1691-99-2	4	50	50
N-Methyl perfluorooctane sulfonamide (MeFOSA)	31506-32-8	4	50	50
N-Ethyl perfluorooctane sulfonamide (EtFOSA)	4151-50-2	4	50	50
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11CL-PF3OUdS)	763051-92-9	4	50	50
3H-Perfluoro-3-[(3-methoxy-propoxy) propanoic acid] (ADONA)	919005-14-4	4	50	50
Hexafluoropropylene oxide dimer acid (HFPO-DA)	13252-13-6	4	50	50
9-Chlorohexadecafluoro-3-oxane-1-sulfonic acid (9Cl-PF3ONS)	756426-58-1	4	50	50

*Biota reporting limits will depend on the biota sampled. Biota can cause matrix enhancement and greatly increase the method reporting limits.

Frequently Asked Questions (FAQs)

What is considered a modified method?

A modified method is when a method that is not followed exactly as written and/or utilized for a matrix that is not specified in the method.

Can lab accreditation add more methods?

Yes, lab accreditation and MPCA work very closely with labs and EPA to be sure accreditation can offer what is needed. PFAS methods and analytes are constantly progressing.

Can alternate labeled isotopes be utilized?

Yes, labeled isotopes can have variable commercial availability and many PFAS methods are performance based. If you have reproducibility studies to demonstrate your ability to quantify accurately from a specific labeled isotope and it is documented, you can utilize alternate labeled isotopes.

What if we are unable to meet this guidance acceptance criteria?

If any guidance criteria is not able to be met, qualify the data on the final lab report and electronic data deliverable

Why are the batch QC accuracy and precision limits set at the levels chosen? For each matrix?

As an agency we are encouraging good science. We understand that those limits may not be achievable now, with current technologies, for every compound. However, we are encouraging laboratory improvements as well as consistent data defensibility.

Can PFAS data be reported to the method detection limit (MDL)?

The MPCA requires data reporting to the MPCA defined reporting limit, also referred to as the minimum level of quantitation (LOQ) or minimum level (ML). Laboratories must establish MDL's for each target analyte according to the procedure at 40 CFR Part 136, Appendix B.

Why are multiple levels of CCV and or LCS encouraged?

LCS should be run at a level relative to the field sample batch levels. The guidance allows for a single level to be utilized if sample concentrations are known. Running an LCS at levels unrelative to the sample defeats the purpose of the QC sample.

Why run LCS in triplicate at 3 different levels?

The relative standard deviation (RSD) measures the precision of the average of your results. The three different levels accommodate unknown sample concentrations and ensures the QC sample is relative to the data within the corresponding batch.

References

Code of Federal Regulations Title 40 part 136, Appendix B