

Perfluoroalkyl Substances (PFAS) in Water by LC/MS/MS - PBM

Parameter Perfluoroalkyl Substances (Perfluorobutane Sulfonate (PFBS), Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA)) in Waters

Analytical Method Solid Phase Extraction (SPE), LC/MS/MS

Introduction This method is applicable to the quantitative determination of perfluorinated alkyl substances in waters.

Method Summary Water samples are fortified with isotope dilution standards and passed through an SPE cartridge to extract the target analytes. Target compounds are eluted from the solid phase with methanol and the extract is evaporated to dryness. Internal standard is added and the volume is adjusted to a known volume with methanol:water. Analysis for PFBS, PFOS and PFOA is by isotope dilution liquid chromatography tandem mass spectrometry (LC/MS/MS).

This method may be applied to other perfluorinated alkyl acids in waters provided the performance requirements and data quality objectives are met.

This method is performance-based. Laboratories may adopt alternative options to improve performance or efficiency provided all stated performance requirements and prescribed (mandatory) elements are met.

MDL(s) and EMS Analyte Codes	<u>Analyte</u>	<u>CAS No.</u>	<u>Approx. MDL (µg/L)</u>	<u>EMS Analyte Code</u>
	Perfluorobutane Sulfonate (PFBS)	375-73-5	0.01 – 0.10	Defined on request
	Perfluorooctane Sulfonate (PFOS)	1763-23-1	0.01 – 0.10	Defined on request
	Perfluorooctanoic Acid (PFOA)	335-67-1	0.01 – 0.10	Defined on request

EMS Method Code(s) ***Refer to [EMS Parameter Dictionary](#) on the ministry website for all current EMS codes.

Matrix Freshwater, Seawater, Groundwater, Wastewater.

- Interferences and Precautions**
- a) All reagents and solvents should be pesticide residue purity or higher to minimize interference or contamination problems. Avoid the use of all sources of Teflon®/PTFE including PFC-containing caps.
 - b) Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably depending on the nature of the water. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source, or low recoveries on the SPE sorbent. Total organic carbon (TOC) is a good indicator of humic content of the sample.
 - c) Contaminants have been found in reagents, glassware, tubing, glass disposable pipettes, filters, degassers, aluminum foil, PTFE products and other apparatus that release perfluorinated compounds. These materials and supplies must be demonstrated to be free from interferences by analysis of laboratory reagent blanks under the same conditions as for tested samples. If found, take measures to remove the contamination or qualify test results; background subtraction of blank contamination is not allowed.
 - d) Relatively large quantities of preservatives may be added to sample bottles. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks particularly when new lots of reagents are acquired.
 - e) SPE cartridges can be a source of interferences. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that

contamination does not preclude analyte identification and quantitation.

- f) The Liquid Chromatography system used should consist, as much as practical, of sample solution or eluent-contacting components free of PTFE and PFC target analytes.
- g) Degassers are important to continuous LC operation and most commonly are made of fluorinated polymers. To enable use, an isolator column should be placed after the degasser and prior to the sample injection valve to separate the PFCs in the sample from the PFCs in the LC system.

Sample Handling and Preservation

Container: HDPE or Polypropylene (PP). HDPE is recommended where extended lists of PFAA substances will be tested (some sorption of higher chain PFAAs to PP has been reported). Avoid PTFE.

Preservation: Chlorinated waters must be preserved with sodium thiosulfate (~80 mg/L of sodium thiosulfate is recommended, which can neutralize up to ~15 mg/L free chlorine), or with Tris base (Trizma®) at 5 g/L. Preservation is not required for non-chlorinated samples.

Storage: Store samples at $\leq 10^{\circ}\text{C}$ during shipment to the laboratory and at $\leq 6^{\circ}\text{C}$ at the laboratory. Avoid freezing to prevent sample breakage. Storage of extracts and standards at room temperature is preferred to prevent sorption onto container surfaces when refrigerated. However, standards and extracts may be stored refrigerated if stabilized at room temperature prior to use (Ref: EPA 537).

Stability

Holding Time:

Samples: Extract samples within 28 days of collection (Ref: ASTM D7979-15).

Extracts: Sample extracts may be held for up to 40 days before instrumental analysis.

Procedure

Calibration Standard Stock:

If possible, purchase all calibration standards as technical grade standards or neat materials. Standards or neat materials that contain only the linear isomer can be substituted only if technical grade (linear and branched isomers) standards or suitable neat technical materials cannot be purchased. PFOS calibration standards must be purchased as technical grade (containing branched and linear isomers).

Extraction:

Detailed sample extraction procedures are not provided in this method. Consult EPA Method 537 or ASTM Method D7979-15 for more detailed guidance (see references). A summary of the extraction method is as follows:

- a) Transfer a representative aliquot of sample (including any particulate matter) to a suitable pre-calibrated container. An indirect measurement may be made by marking the level of the sample on the bottle or by weighing the sample and bottle. Because some PFAAs adsorb to surfaces, do not transfer the sample to a graduated cylinder for volume measurement.

Note: Where samples contain more than ~1% solids, the solids must be separated and extracted separately with extracts or test results combined to represent total sample concentrations.

- b) Add isotopically-labelled isotope dilution (ID) internal standards to all samples and quality control samples prior to extraction.
- c) Pass the samples through a conditioned SPE column and extract with a methanol solution.
- d) Concentrate the extract to dryness under a gentle stream of nitrogen in a water bath to remove all the water/methanol mix.
- e) Reconstitute to 1 mL with methanol:water solution.
- f) Transfer an aliquot to a polypropylene autosampler vial and analyze by LC/MS/MS.

Instrumental Analysis:

Detailed instrumental analysis procedures are not provided in this method. Consult EPA Method 537 or ASTM Method D7979-15 for more detailed guidance (see references).

Extracts must be analyzed by LC/MS/MS. A C₁₈ column or any column that provides adequate resolution, peak shape, capacity, accuracy and precision is used.

Use a minimum five-point initial calibration over the desired working range to meet the performance requirements outlined in US EPA Method 537.

If required, dilutions may be conducted on high level samples using the standard isotope dilution corrections if the diluted ID standards can still be accurately measured. Alternatively, a smaller portion of sample may be extracted and analyzed or higher amounts of the ID standards may be added.

Most PFAAs are produced by two different processes. One process gives rise to linear PFAAs only while the other process produces both linear and branched isomers. Thus, both branched and linear PFAAs can potentially be found in the environment. For the compounds that give rise to more than one peak (particularly PFOS), all the chromatographic peaks observed in the standard must be integrated and the areas totaled. Chromatographic peaks in a sample must be integrated in the same way as the calibration standard.

As noted above, PFOS (and most likely PFBS) has linear and branched isomers. In an attempt to reduce PFOS bias, it is required that the transition m/z 499 → m/z 80 be used as the quantitation transition. Some MS/MS instruments, such as conventional ion traps, may not be able to scan a product ion with such a wide mass difference from the precursor ion; therefore, they may not be used for this method if PFOS analysis is to be conducted.

Any analytical method options selected for this analysis must meet or exceed the performance requirements specified below.

Accuracy and Precision requirements are distinct from daily QC requirements and apply to measures of long-term method performance (averages and standard deviations). Achievement of these requirements is to be demonstrated during initial and ongoing method revalidation studies. For Initial Validations, averages of at least 8 Laboratory Control Samples must be assessed. Ongoing Revalidations (performance reviews) should assess QC data encompassing longer periods (e.g. 6 months to 1 year). A minimum frequency of 2 years is recommended for Ongoing Revalidations.

Accuracy Requirement: Laboratories must demonstrate method accuracy (measured as average recovery) through repeat analysis of Laboratory Control Samples at concentrations above ten times the MDL. Recovery must be between 80-120% of the true value.

Precision Requirement: Laboratories must demonstrate method precision through repeat analysis of Laboratory Control Samples at concentrations above ten times the MDL. Precision, measured as percent relative standard deviation (%RSD), must be ≤15% for all analytes.

Sensitivity Requirement: Where possible, the method should support Reporting Limits (and MDLs) that are less than 1/5 of applicable numerical standards. The method is not fit-for-purpose if an MDL exceeds a guideline, standard, or regulatory criteria against which it will be used for evaluation of compliance.

Performance Requirement for Seawater Matrix: If the method will be used for seawater matrix, independent method validations must be conducted on seawater matrix samples.

Performance Requirements

Quality Control

Summary of QC Requirements		
QC Component	Minimum Frequency	Minimum Data Quality Objectives
Method Blank (MB)	One per batch (max 20 samples)	Less than reported DL
Laboratory Control Sample (LCS)	One per batch (max 20 samples)	70-130%
Laboratory Duplicate (DUP)	One per batch (max 20 samples)	≤30% RPD [or within 2x reported DL for low level results]

Matrix Spike (MS)	One per batch (max 20 samples)	70-130%
Peak Asymmetry Factor	Calculate the peak asymmetry factor for the first two eluting chromatographic peaks in a mid-level calibration standard every time a calibration curve is generated.	0.8–1.5 (Ref: EPA 537)
Injection Internal Standard (IIS) (not used in isotope dilution calculations)	All samples	Peak area counts for all internal standards in all injections must be within $\pm 50\%$ of the average peak area calculated during the initial calibration and 70-140% from the most recent CCV (Ref: EPA 537).
Isotope Dilution Standard(s) (IDS)	All samples, all regulated PFAS analytes	Absolute recovery of all isotope dilution standards used for recovery correction must be 10-130%
Calibration Verification Standard (CVS)	Minimum 1 per initial calibration	70-130%
Continuing Calibration Verification (CCV)	Every 12 hours within an instrument run and at the end of each run	70-130%
If DQOs are not met, repeat testing or report qualified test results. DQOs do not apply to MS results where sample background exceeds spike amount.		

Method Blank (MB): Required. An aliquot of reagent water that is treated exactly like a sample including exposure to equipment, solvents and reagents, sample preservatives, and the internal standards (IDS and IIS) that are used in the analysis batch.

Laboratory Duplicate (DUP): Required. Data quality objectives are listed above.

Laboratory Control Sample (LCS): Required. Prepare a Laboratory Control Sample by fortifying deionized water with known concentrations of target the analytes.

Matrix Spike (MS): Required. Spike a duplicate field sample with known concentrations of the target analytes.

Injection Internal Standard (IIS): One or more injection internal standards are added to extracts at the end of the extraction process. IIS's should be stable isotopically-labelled PFAA substances representative of method analytes. They are used to monitor the integrity of each injection, and to calculate absolute recoveries of isotope dilution standards in samples and QC.

Isotope Dilution Standards (IDS): Isotope dilution standards must be stable isotopically-labelled analogues of the parameters of interest. Isotope Dilution Standards are added to the sample prior to extraction and are used for correction of extraction recoveries and matrix effects. Isotope Dilution is required for all regulated PFAS analytes (PFOS, PFOA, PFBS), and is recommended for other PFAS analytes. If suitable labelled isotopes are unavailable for non-regulated PFAS analytes, then the use of the most chemically similar available IDS is recommended.

Calibration Verification Standard (CVS): Required. A CVS from a source separate from the calibration standard must be analyzed with each initial calibration to monitor calibration accuracy.

Continuing Calibration Verification (CCV): Required. A mid-point calibration standard must be analyzed throughout the instrument run at least every 12 hours and at the end of the run to monitor calibration drift. A CVS may serve the same purpose.

Prescribed Elements The following components of this method are mandatory:

1. Analysis must be by LC/MS/MS. At least two MRM transitions are required to be monitored for PFOS and PFOA (only one suitable transition exists for PFBS). Labs must define appropriate criteria for confirmation of analyte identity by secondary transitions.
2. Initial calibrations must include at least 5 points.
3. PFOS test results must represent and include the sum of all branched and linear isomers that are identifiable from technical grade PFOS standards or material.
4. Test method utilized must include extraction and analysis of a representative portion of any solids or particulate matter contained in the sample. Where samples contain more than ~1% solids, the solids must be separated and extracted separately with extracts or test results combined to represent total sample concentrations.
5. The 499 to 80 m/z MRM transition must be used for the quantitation of PFOS, unless subject to sample specific interferences.
6. Isotope dilution calibration and recovery correction must be used for all regulated PFAS analytes.
7. Blank correction is not permitted for this method.
8. If this method is utilized for seawaters or brine samples, method validation for that matrix must be conducted prior to use using Matrix Spikes and/or Reference Materials.
9. All Performance Requirements and Quality Control requirements must be met.
10. Sample container materials, preservation, storage, and hold time requirements may not be modified. Samples analyzed beyond the stated holding time must be qualified. Refer to latest version of "BC MOE Sample Preservation and Hold Time Requirements" for updates.

Apart from these limitations, and provided performance requirements are met, laboratories may introduce modifications to this method in order to improve quality or efficiency.

References

1. US EPA Method 537, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), version 1.1, Sept/2009.
2. ASTM D7979-15. Standard Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), ASTM International, Nov 2015.

Revision History

Sept 15, 2017 First version added to BC Lab Manual in support of 2017 CSR updates.