

## Environmental

## Techniques for successful implementation of U.S. EPA Method 1621

### Screening of PFAS compounds in aqueous matrices using adsorbable organic fluorine (AOF) with combustion ion chromatography

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#### Keywords

AOF, CIC, GAC, Dionex IonPac AS24 column

#### Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds. The Organization of Economic Co-operation and Development defines a PFAS compound as a compound containing a fully fluorinated methyl or methylene group on an alkane functional group.<sup>1</sup> The U.S. Environmental Protection Agency (EPA) definition states that the fluorinated compound must have at least two adjacent carbons with one (alkane) carbon fully fluorinated and the other alkane carbon at least partially fluorinated (*unit R-CF<sub>2</sub>-CF(R')* (*R''*), where *R*, *R'*, and *R''* do not equal "H" and the carbon-carbon bond is saturated).<sup>2</sup> For example, the EPA classification excludes trifluoroacetic acid (CF<sub>3</sub>-COOH) as a PFAS compound because of the absence of an adjoining fluorinated carbon. PFAS have been used extensively for more than 80 years, causing concerns about their toxicity, as they are persistent and bioaccumulate. The PFAS classification from the U.S. EPA CompTox PFAS database contains almost 15,000 known fluorinated compounds, which can be challenging to speciate and quantify.<sup>3</sup> U.S. EPA Method 1621 is an effective screening method for PFAS; the method outlines the determination of adsorbable organic fluoride (AOF) using pyrohydrolytic combustion ion chromatography (CIC).<sup>4</sup> A wastewater aliquot (100 mL) is pumped through a granular activated carbon (GAC) column, then rinsed with an aqueous sodium nitrate solution and DI water to remove the inorganic salts. The sorbent and the adsorbed PFAS are pyrolyzed at high temperatures under inert gas, hydrolyzed with oxygen and water vapor, absorbed in DI water, and analyzed by IC. The ng/mL (ppb) sensitivity needs, continuous cross-contamination issues from the environment, and, in some cases, the user's unfamiliarity with these techniques could make EPA Method 1621 challenging. This technical note will discuss the sources of contamination, recommended cleaning processes, and analytical techniques for successful results. A separate application note, Thermo Fisher Scientific AN002748,<sup>5</sup> discusses our laboratory's results from the EPA Method 1621 collaboration study.

## Experimental

### Equipment, software, and reagents

Thermo Fisher Scientific Application Note AN002748 describes the application of EPA Method 1621 in detail.<sup>4,5</sup> The acronyms are defined in Appendix A.

### Equipment

- Any Thermo Scientific™ Dionex™ High Pressure Ion Chromatography (HPIC) system with conductivity detection
- Nittoseiko AQF-2100 combustion system with a Nittoseiko TXA-04 adsorption unit or equivalent equipment
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3.1 or later

### Reagents

- ASTM Type I deionized (DI) water with 18 MΩ·cm resistivity<sup>6</sup>
- Ammonium hydroxide, 28–30 w/w%, Certified ACS, Fisher Scientific™ P/N A669S-500 (CAS 1336-21-6)
- Methanol, Optima™ grade, Fisher Scientific™ P/N A456-500 (CAS 67-56-1)
- Sodium nitrate, ACS Certified, Fisher Scientific P/N S343-3 (CAS 7631-99-4)
- Ar, UHP grade
- O<sub>2</sub>, UHP grade

### Samples and sample preparation

EPA Method 1621 is for use in the Clean Water Act (CWA) to estimate the concentration of adsorbable organic fluorine (AOF) in aqueous matrices, including wastewater by CIC.

The sample matrix can impact the adsorption process and require different workflows for different matrices (Figure 1). Potential interferences include high fluoride and chloride concentrations and high total suspended solids (TSS) levels. High concentrations of fluoride and chloride, >8 mg/L and >500 mg/L, respectively, can overload the capacity of GAC columns and prevent the adsorption of PFAS. These samples require dilution before adsorbing onto the GAC columns. The sample's total fluoride content is calculated considering this dilution factor.

High TSS concentrations can clog the GAC columns, reducing or preventing PFAS adsorption. However, since the solids may have adsorbed PFAS, they must be captured. Insert a 50 mg aliquot of previously cleaned ceramic wool in the flow path before the GAC columns and treat it like the sample. Analyze the ceramic wool by CIC in a separate boat from the GAC columns. Correct the total fluoride results by subtracting the ceramic wool and method blank.

Due to its high surface area and exposure to air, ceramic wool can cause high and variable contamination. Clean it with methanol and combustion before use.

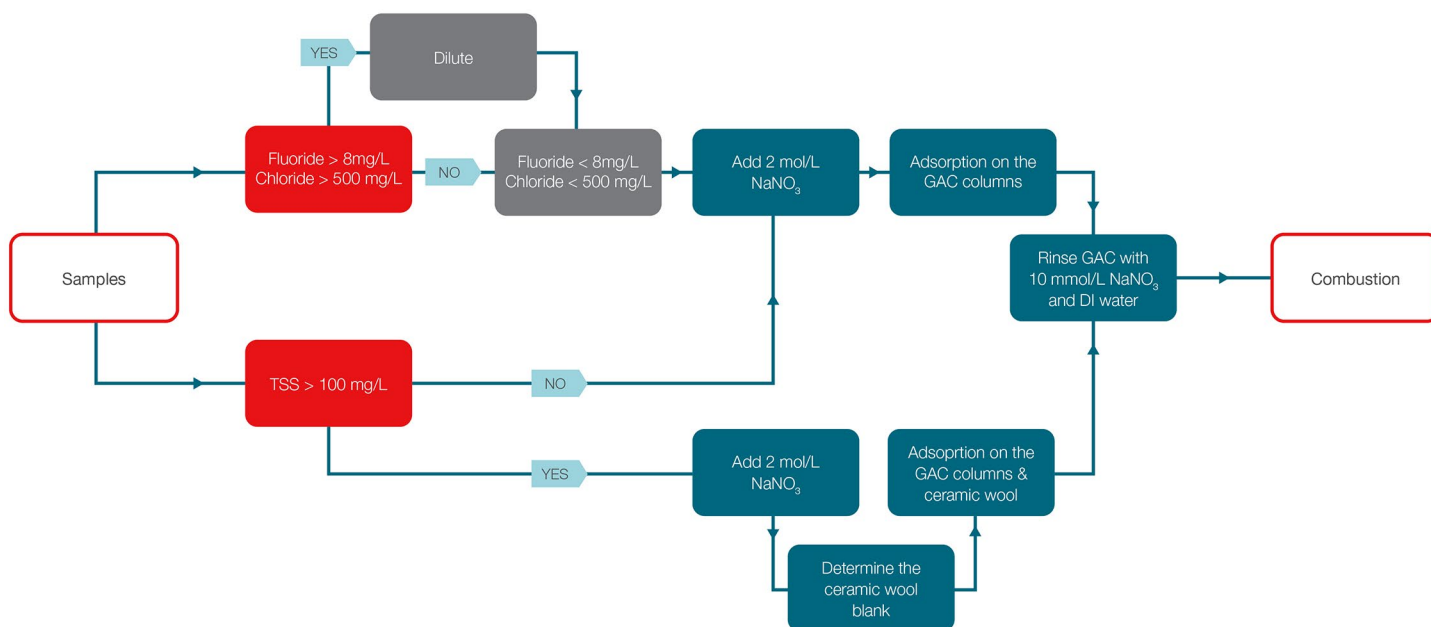


Figure 1. Schematic of the sample pretreatment workflow (dependent upon sample composition)

Figure 2 outlines EPA Method 1621's workflow. Low TSS wastewater samples and standards (MB, MDL, IPR, OPR) undergo sodium nitrate treatment. They are adsorbed onto GAC, then rinsed with sodium nitrate and DI water to remove inorganic salts. High TSS wastewater samples receive additional treatment with ceramic wool to capture suspended solids, with pre-cleaned ceramic wool inserted before the GAC during adsorption. Both low and high TSS samples and the standards are prepared for combustion and analyzed by CIC.

### Sources of contamination

Contamination can occur throughout the analytical process of EPA Method 1621 (Figure 2). To minimize cross-contamination, clean bottles and tools before use, and include cleaning stages after processing each batch of samples and standards in the adsorption phase. Additionally, maintain a continuous process of using clean boats for combusting samples and standards.

Contamination from different sources can lead to overestimated method analysis results and needs to be avoided. Potential sources may include:

- **CIC instruments:** The adsorption unit flow path and the CIC until conditioned and stable

- **CIC consumables:** GAC columns, ceramic boats, ceramic wool, ceramic inserts in the combustion oven, and new combustion tubes
- **Solvents:** Solvents with purities lower than ASTM Type I DI water or Optima LC/MS grade methanol. Lower grades of methanol can cause fluorine/fluoride contamination. Use either high-grade methanol or follow methanol rinses with extra DI water rinses.
- **Tubing:** The large internal surface area of excessively long tubing in the adsorption unit can harbor impurities.
- **Containers:** All plasticware, bottles, vials, and caps. Rinse all high-density polyethylene (HDPE) containers, bottles, and tools with methanol followed by DI water before and after use to eliminate potential fluorine/fluoride contamination.
- **Tools:** Syringes, plastic containers, graduated cylinders, pipettes, and gloves
- **Environment:** Personal products (makeup, hair products, moisturizers, etc.), carryover from samples, inorganic fluoride, and standards

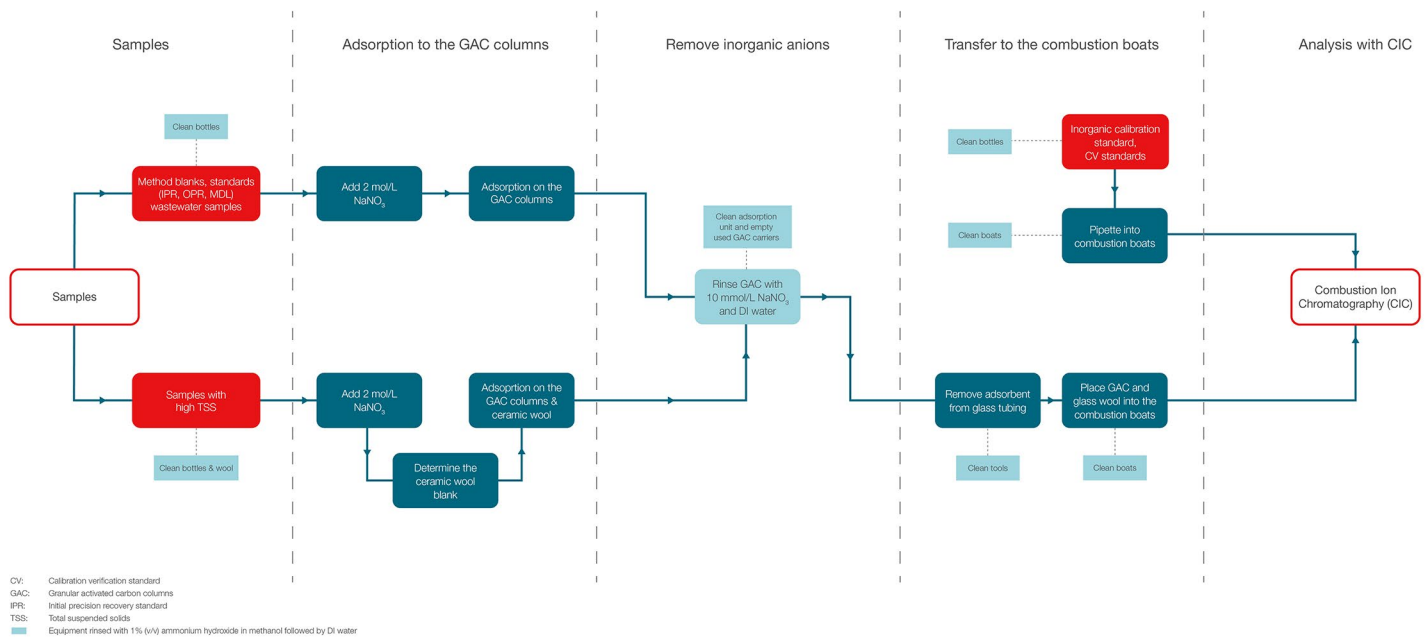


Figure 2. Schematic of the workflow for EPA Method 1621

## Consumables impacting baseline contamination

The ceramic wool, GAC columns, and combustion boats are the consumables most likely to cause falsely high results due to cross-contamination. The cleanliness of GAC columns and combustion boats is crucial for method sensitivity, while uncleaned ceramic wool introduces random, highly variable contamination.

### Ceramic wool

Ceramic wool is a major contamination source due to its composition and high surface area. It captures suspended solids from high-TSS samples during adsorption. To clean ceramic wool, rinse several grams with methanol followed by DI water, shake off excess solvent, and bake to dryness. Store in a desiccator. Measure initial cleanliness by combusting multiple 50 mg aliquots in separate clean ceramic boats.

Repeat cleaning cycles until combustion results stabilize to a non-impactful concentration. Cleanliness can be maintained by baking the boats at 700 °C in a muffle furnace and storing them under vacuum.

### GAC columns

Initially designed for mg/L concentrations of adsorbable organic halogen compounds (AOX), GAC columns are not typically certified for cleanliness. However, EPA Method 1621 and other PFAS applications require cleanliness at single-digit ng/mL (ppb) levels. Achieving low sensitivities depends on consistently low baseline contamination in GAC columns. Significant variability in cleanliness levels has been reported, so each GAC column lot should be examined before use. Validate each lot by combusting 3 to 5 dry GAC columns and comparing results to the expected method blank (MB) range (Table 1).

Keep GAC column packages sealed until use. Analyze adsorbed GAC columns from samples, MBs, and standards within 24 hours. Testing of cleaning procedures for GAC columns was not performed; consult the manufacturer for further instructions.

### Combustion boats

Consistently low baseline contamination in ceramic or quartz combustion boats is essential. Clean ceramic boats by combusting them as empty boat samples at the end of each sequence for use the following day. Note that 2 to 3 cycles may be necessary for adequate cleaning, as some boats are more challenging to clean. Discard boats that maintain unacceptable fluoride residues after 3 cycles.

Cleanliness can be maintained by baking in a muffle furnace and storing under vacuum. EPA Method 1621 recommends soaking boats in methanol for 1 hour, scrubbing with methanol and cotton swabs followed by DI water, baking at 450 °C for 1 hour, and storing in a desiccator. After 3 days, the boats must be cleaned by baking or combusting. Only pre-cleaned stainless-steel tweezers are used to handle the combustion boats.

## Initial cleaning process

### Cleaning the adsorption unit

Clean the adsorption instrument flow path before use to eliminate contamination. Follow these steps:

1. Pump 10–50 mL of 1% ammonium hydroxide in methanol, followed by DI water, through empty GAC column carriers and the adsorption system channels.
2. Install empty GAC column carriers and repeat the cleaning process for 10 cycles.
3. To minimize cross-contamination, number the GAC column carriers and record the channel and carrier for each sample, blank, or standard adsorbed.

### To assess background contamination

1. Clean one bottle and prepare a method blank (MB) for each channel (100 mL DI water, 0.5 mL 2 M sodium nitrate).
2. Install two GAC columns in series into the cleaned GAC column carriers.
3. Connect the GAC column carrier and MB sample to the adsorption unit.
4. Load 100 mL from the MB bottle onto the GAC columns and rinse with 25 mL of 10 mM sodium nitrate and 20 mL DI water.
5. Empty the adsorption syringe. Fill the adsorption syringe with 5 mL of air and dispense to remove excess liquid.
6. Combust individual carbon columns in cleaned combustion boats. Repeat until results stabilize and are <1 ng/mL fluoride.

Verify the adsorption unit is clean before starting EPA Method 1621 by alternating cleaning steps (1% ammonium hydroxide in methanol followed by DI water) with MBs. Depending on the initial contamination level, cleaning may take several days.



## Cleaning the CIC system

To clean a newly installed or idle combustion system and exhaust contamination:

1. Sources of contamination: Common sources include ceramic wool, ceramic sleeves inside the combustion tube, and ceramic boats.
2. Rinse DI water containers: Rinse wash, absorption, and hydrolysis solution containers three times with DI water and refill with fresh DI water, particularly for absorption and hydrolysis containers. Start with fresh DI water daily.

## Cleaning steps

1. Prepare combustion tube:
  - Install the combustion tube with the ceramic sleeve and ceramic wool.
  - Disconnect the flow path from the combustion and adsorption units to prevent contamination spread.
2. Combustion process:
  - Turn on Ar and O<sub>2</sub> gases and heat the combustion furnace to 1,000 °C.
  - Maintain this temperature and gas flow for at least 9 hours.
  - To shut down, turn off the furnace while keeping gas flow until the temperature drops to ≈300 °C, then turn off the gases.
3. Restart and continuous cleaning:
  - The next day, restart by heating the combustion furnace with the gas flow and U-tube disconnected.
  - Connect the U-tube to the furnace as it reaches 1,000 °C.
  - To continue cleaning, run a sequence overnight testing 10 empty boats to ensure each is <1 ng/mL fluoride.

The combustion furnace maintains robustness and cleanliness best when the furnace and gases remain on continuously.

## Continuous cleaning process

### Sample bottles and tools

Rinse all tools and HDPE containers, bottles, and sample containers with methanol followed by DI water after each use to eliminate fluorine/fluoride contamination. HDPE bottles used for MBs can be reused, but those previously containing standards or samples should not be reused.

Periodically rinse stainless steel tweezers with methanol followed by DI water and store them upright with the handle pointing down between uses. Clean the stainless steel rod used to push carbon out of GAC tubes after each use by removing residual carbon particles and rinsing with methanol followed by DI water.

## Adsorption system

The adsorption unit is cleaned each morning before preparing MBs and after each use to prepare samples or standards. Discard any GAC column accidentally washed with methanol or 1% ammonium hydroxide in methanol, as the sample is likely washed away. Ensure the carrier has two GAC columns to prevent sample breakthrough.

## CIC system

The CIC system typically does not need continuous cleaning after the initial stage. However, maintaining the combustion system at ≈500 °C when not in use ensures the best cleanliness. During the initial heat-up, disconnect the combustion unit from the adsorption unit to prevent contamination and reconnect before starting the sequence.

To maintain system cleanliness, monitor the fluoride concentration from combusting empty boats. Due to contamination risks, avoid introducing high-concentration samples (>50 mg of 100 mg/kg fluorine) during EPA Method 1621 analysis.

Regularly check DI water levels in all containers for eluent, wash, hydration, and absorption solutions to prevent them from running empty. Replace the hydration and absorption DI water daily to minimize airborne contamination.

## Adsorption and combustion process

Verify results from the MB and calibration verification (CV) standards before generating adsorbed samples and standards. Combust loaded GAC columns within 24 hours.

1. Clean and label 125 mL HDPE bottles.
2. Add 100 g of DI water for MBs, IPR, OPR, MDL standards, or 100 g of mixed sample.
3. Add 0.5 mL of 2 M sodium nitrate to each bottle and mix.
4. Rinse the microsyringe with methanol and DI water three times. Add PFHxS standard using the microsyringe, mix, and rinse the microsyringe again.

5. Load two new GAC columns into the cleaned GAC carrier.
  6. Attach GAC carrier to each previously cleaned adsorption unit channel.
  7. Add 50 mg of pre-cleaned ceramic wool above GAC columns for high TSS samples. For ceramic wool blank, treat similarly.
  8. Load samples onto each channel at 3 mL/min.
  9. Record channel number, GAC carrier number, and associated samples in the log.
  10. Rinse GAC columns and ceramic wool blanks with 25 mL of 10 mM sodium nitrate and 20 mL of DI water.
  11. Fill the adsorption syringe with 5 mL of air and dispense the air through the GAC to reduce moisture.
  12. Push out GAC columns and ceramic wool using a stainless-steel rod into cleaned combustion boats.
  13. Add samples to the Chromeleon CDS sequence and analyze by CIC as described in EPA Method 1621 and Thermo Fisher Scientific application note AN002748.
  14. Clean and prepare the empty combustion boats for reuse.
  15. Clean the adsorption unit and empty the GAC carriers.
2. Calibration:
    - Prepare six to seven fluoride standards (1–100 mg/L) from a 1,000 mg/L stock.
    - Combust 200 µL of each standard in separate boats (no ceramic wool).
    - Plot concentration vs. peak area and calculate relative standard error (RSE) for each curve. Choose the curve with the lowest RSE using Chromeleon CDS (Appendix B).
  3. Prepare fluoride CV standards from a second 1,000 mg/L stock, combust 200 µL in individual boats, and calculate percent recovery.
  4. Determine baseline contamination for 3–5 dry GAC columns by CIC. Repeat for each new lot.
  5. Analyze seven MBs and 5 µg/L MDL standards over three days by CIC. Calculate MDL(b) and MDL(s) per 40 CFR 136 Appendix B instructions.
  6. Analyze five 15 µg/L fluoride IPR standards by CIC for percent recovery. Additionally, check baseline contamination on 3–5 dry GAC columns.

### Sample analysis

1. Add 100 g of sample to a precleaned bottle.
2. Pipette 0.5 mL of 2 M sodium nitrate into each bottle and mix.
3. Follow the described adsorption procedure.
4. Bracket samples as outlined in Table 2.

The 15 µg/L fluoride (PFHxS) OPR standard must fall within 80–120% of average IPR results from the IDC. Reject any sequence and associated samples if control standards (MB1, low CV, OPR, MB2, medium CV) fail to meet specifications. Additional cleaning steps, discussed in Appendix C, may be required.

### Initial demonstration of capability (IDC) tests

The EPA mandates IDC tests before sample analysis and EPA Method 1621 implementation. Specifications are listed in Table 1.

1. Retention time window for fluoride (n=3): Ensure fluoride retention time (peak apex) is over 1 min post water dip and 1 min pre-chloride retention time. Calculate mean and standard deviation; then retention time window (mean ± 10·SD).

**Table 1. Specifications for IDC tests**

Parameter	Criteria	Typical value
1	Establish fluoride retention time (n=3)	Fluoride must be >1 min from the water dip and >1 min from the chloride retention time
	Establish fluoride retention time window	Fluoride retention time ± 10 × SD
2	Establish best calibration curve (n=7)	RSE <20%
3	Fluoride CV standards	Low CV at beginning of each sequence; medium CV at the end of each sequence. 80-120% of theoretical value
4	Recommended: Dry GAC (n=3–5 per lot)	Ranged from 0.3 to 1.1 µg/L fluoride for 2 GAC columns
5	MB (n=7 over 3 days)	Ranged from 0.3 to 1.1 µg/L fluoride
	MDL: 5 µg/L fluoride (PFHxS)	80–120% recovery of theoretical value
6	IPR: 15 µg/L fluoride (PFHxS)	80–120% recovery of theoretical value

**Table 2. Starting and ending brackets in Chromeleon sequences for EPA Method 1621**

Bracket process checks	Combustion type	Adsorbed wastewater samples
Empty boat	System blank	
Empty boat	System blank	
Low CV standard	Calibration check	
Empty boat	System blank	
Quartz wool blank	Adsorbed, process blank check	
Empty boat	System blank	
Method blank, MB1	Adsorbed, process blank check	
OPR (spec.: ± 20% of average IPR)	Adsorbed, recovery process check	
	Adsorbed	Quartz wool blank for high TSS samples
	Adsorbed	Samples, initial PFAS content without or with quartz wool
	Adsorbed	Sample duplicates without or with quartz wool
	Adsorbed	Samples with added PFAS without or with quartz wool
	Adsorbed	Duplicates of samples with added PFAS without or with quartz wool
Method blank, MB2	Adsorbed, process blank check	
Medium CV	Calibration check	
Empty boat	System blank	

## Conclusion

Per- and polyfluoroalkyl substances (PFAS) are prevalent environmental contaminants with significant toxicity, making them a critical concern for regulatory agencies. The Environmental Protection Agency (EPA) Method 1621 effectively identifies PFAS at nanogram per milliliter (ppb) concentrations using Adsorbable Organic Fluorine (AOF) combined with combustion ion chromatography (CIC). In this document, we detail the precise trace analysis techniques necessary to achieve such sensitivity, emphasizing the importance of meticulous cleanliness and stringent methods to minimize cross-contamination. Adhering to these protocols is essential to maintain the detection limits required by this method and to ensure reliable and accurate analysis of PFAS compounds in various environmental samples.

## References

1. U.S. Center of Disease Control (CDC) / Agency for Toxic Substances and Disease Registry. [Potential health effects of PFAS chemicals | ATSDR \(cdc.gov\)](https://www.cdc.gov/atsdr/pfas/) [viewed Jan 18, 2024]
2. National Toxic Programs (NTP). [Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid \(PFOA\) and Perfluorooctane Sulfonate \(PFOS\)](#). Office of Health Assessment and Translation Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, **2016**. [viewed Jan 18, 2024]
3. U.S. EPA CompTox PFAS database. <https://www.epa.gov/comptox-tools/comptox-chemicals-dashboard> [accessed June 2024].
4. U.S. Environmental Protection Agency (EPA). [Method 1621 Determination of Adsorbable Organic Fluorine \(AOF\) in Aqueous Matrices by Combustion Ion Chromatography \(CIC\)](#), EPA 821-R-24-002. U.S. Environmental Protection Agency, Office of Water (4303T), Office of Science and Technology Engineering and Analysis Division, Washington, DC, USA, January 2024.
5. Christison, T. and Rumachik, N. Application Note AN002748, Screening of PFAS compounds in wastewater using adsorbable organic fluorine with combustion ion chromatography (CIC), **2024**. Thermo Fisher Scientific, Sunnyvale, CA, USA.
6. ASTM International, ASTM D1193 - 99e1 Standard Specification for Reagent Water. <https://www.astm.org/d1193-06r18.html>
7. United States Environmental Protection Agency, Office of Water. [Report on the Single-laboratory Validation of Clean Water Act Method 1621 for Adsorbable Organic Fluoride \(AOF\)](#). EPA 820-R-22-003. April 2022.
8. U.S. Federal Register: 40CFR136, Appendix B, Sections ii, and iii.

## Appendix A

Table A1. Definitions

Term	Definition	Description
AOF	Adsorbable organic fluorine	Fluorocarbon and perfluorocarbon compounds in water or solvent that adhere to carbon
CD	Conductivity detector	IC detector
CIC	Combustion IC (includes adsorption unit, sampler, furnace, absorption unit and IC)	The adsorption unit, an off-line system, facilitates adsorption of organic compounds from the sample onto GAC. A combustion system converts halogen and sulfur compounds to halides and ionic sulfur species at 1,000–1,100 °C using pyrolysis and combustion with oxygen and water vapor.
CV	Calibration verification standard	Check standards prepared from an inorganic fluoride standard that are from a different (second) source than the calibration standards. Added directly to combustion cups. Run at the beginning and the end of each sequence.
DI water	Deionized water, >18 MΩ-cm resistivity and <10 ppb total organic carbon	Diluent for rinsing solutions, calibration standards, MDL, MB, all QC standards and source for IC eluent, and combustion system (absorption, washing, and water vapor)
EPA	U.S. Environmental Protection Agency	United States (U.S.) agency responsible for developing EPA Method 1621
GAC	Granular activated carbon	Carbon material used for adsorbing organic compounds
HPIC	High pressure ion chromatography system	High pressure ion chromatography system
IC	Ion chromatography system	Liquid chromatography system using ion-exchange separations, typically with suppressed conductivity detection.
IDC	Initial demonstration of capability	Initial tests required for qualification prior to the implementation of EPA Method 1621.
IPR	Initial Precision Recovery standard	Used to determine the expected process check value (OPR). Prepared by adding PFHxS standard to DI water. Processed through adsorption, combustion, absorption, and IC analysis.
MB	Method blanks	DI water processed through adsorption, combustion, absorption, and IC analysis. Indicates the lowest actual measurements possible and the baseline contamination of the CIC and the adsorption module. Always run at the beginning and the end of each sequence.
MDL	Method detection limit	The lowest reliable measurement, typically 3x S/N. In this test, the standard is processed through the adsorption on carbon, combusted, absorbed, and analyzed by IC.
MDL(b)	Method detection limit of blanks, n=7	Defined as: (Mean of method blanks) plus (standard deviation x <i>Student t-test</i> factor)
MDL(s)	Method detection limit of 5 ng/mL PFHxS standard, n=7	Defined as: (standard deviation) x ( <i>Student t-test</i> factor)
OPR	Ongoing Precision Recovery standard	Check standard run at the beginning of each sequence. Prepared by adding PFHxS standard to 100 mL of DI water. Processed through the adsorption on carbon, combusted, absorbed and analyzed by IC.
PFHxS	Perfluorinated sulfonate standard	Sodium perfluoro-1-hexanesulfonate standard. Used for MDL and QC check standards.
RFIC	Reagent-Free IC	An IC system using electrolytically-generated eluent
RSE	Relative Standard Error	Error from calibration line. Defines the best fit of a calibration curve.
TSS	Total Suspended Solids	Includes any loose particulates or precipitated solids in the water sample



## Appendix B

### Using Chromeleon CDS to determine RSE

1. Open the Chromeleon CDS sequence containing the calibration data.
2. Double-click on calibration data.
3. Open interactive results tables
4. To select only calibration data, double-click on the interactive results table or a column in the table.
5. Select *Filter Injections*, and *Calibration Standard*. Close.

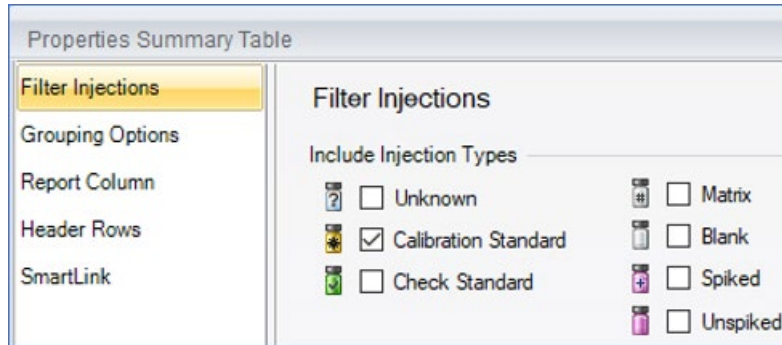


Figure A1. Selecting only calibration standards for the interactive results table

6. Select a column in the interactive results table next to the amount column (Rel. Area percent). Right-click, select *Insert a Column*, and Report Column tab. Click on the *three dots button* in the *Formula* tab.
7. Select *Peak Calibration* and *Relative Standard Error*. Click OK.

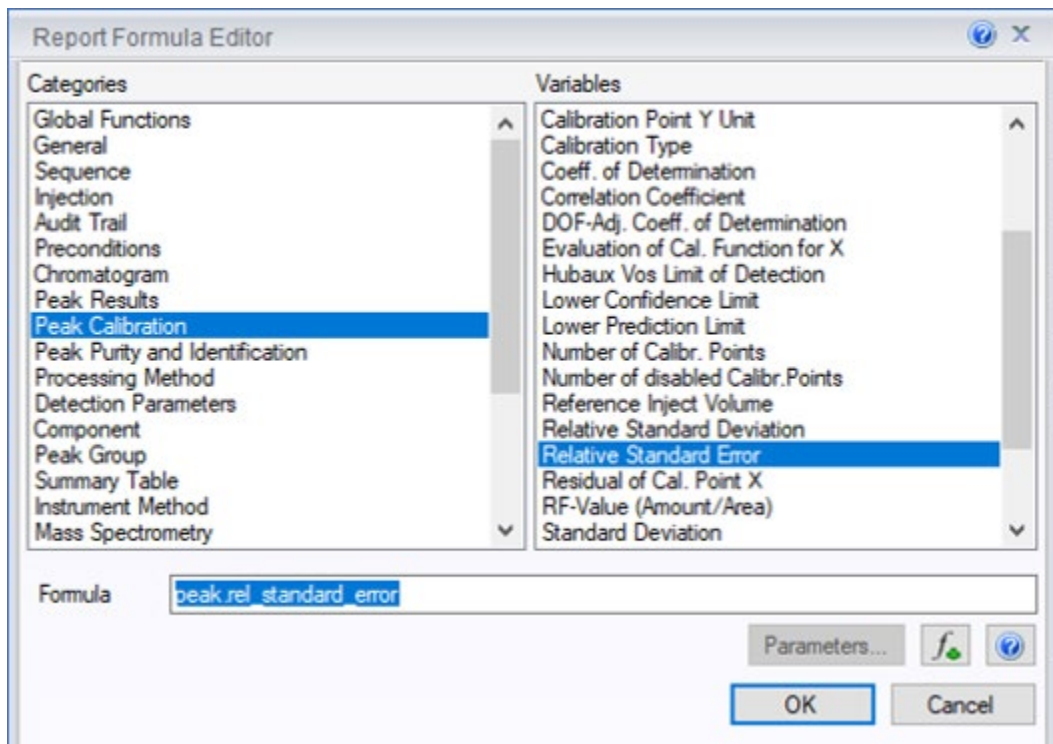


Figure A2. Selecting Relative Standard Error variable for results table

8. To add the calibration type into the interactive results table, select a column in the interactive results table next to the RSE column. Right-click, select *Insert a Column*, and *Report Column* tab. Click on the *three dots button* in the *Formula* tab.
9. Select *Peak Calibration* and *Calibration Type*. And OK.

The Interactive Results Table will display Relative Standard Error (Rel.Std.Err) and Calibration Type.

Inj. No.	Injection Name Selected Peak:	Position	Ret.Time min CD_1_Total Fluoride	Amount ng CD_1_Total Fluoride	Rel.Std.Err. % CD_1_Total Fluoride	Cal.Type CD_1_Total Fluoride
60	calibration 1 mg/L -200 ng	12	5.773	197.9543	0.774	Lin, WithOffset, 1/A
61	calibration 2.5 mg/L -500 ng	17	5.777	509.5516	0.774	Lin, WithOffset, 1/A
62	calibration 5 mg/L -1000 ng	18	5.780	1034.3498	0.774	Lin, WithOffset, 1/A
63	calibration 12.5 mg/L -2500 ng	19	5.800	2554.7185	0.774	Lin, WithOffset, 1/A
64	calibration 25 mg/L -5000 ng	20	5.837	4909.7852	0.774	Lin, WithOffset, 1/A
65	calibration 37.5 mg/L -7500 ng	21	5.837	7499.1116	0.774	Lin, WithOffset, 1/A
66	calibration 50 mg/L -10000 ng	23	5.867	9552.5655	0.774	Lin, WithOffset, 1/A
Maximum			5.867	9552.5655		
Average			5.807	3294.7270		
Minimum			5.773	99.7793		
Standard Deviation			0.035	3638.2417		
Relative Standard Deviation			0.61%	110.43%		

Figure A3. Interactive results table displaying RSE and calibration type

10. Open processing method. Select Component Table tab, highlight fluoride peak row, double-click on Cal. Type.

Detection		Component Table			
Component Table					
Group Area		Drag			
#	Level "1"	Level "2"	Cal.Type	Conc.Unit	
1	100.00	200.00	Quad, WithOffset	ng	
2			Lin, WithOffset, 1/A	ng	

Figure A4. Selecting the calibration type in processing method

11. Select calibration type and weighting to determine RSE for all calibration types.

Repeat for Linear, 1/A weighting and 1/A<sup>2</sup> weighting, quadratic, no weighting, 1/A<sup>2</sup> weighting and 1/A<sup>2</sup> weighting. Select the best fit, lowest RSE.

Compare the curve fit (RSE) of no weighting, 1/A weighting and 1/A<sup>2</sup> weighting.

Figure A5. Process the data with linear curve fit and quadratic curve fit

Inj. No.	Injection Name Selected Peak:	Position	Ret. Time min CD_1_Total Fluoride	Amount ng CD_1_Total Fluoride	Rel. Std. Err. % CD_1_Total Fluoride	Cal. Type CD_1_Total Fluoride
60	calibration 1 mg/L -200 ng	12	5.773	197.9543	0.774	Lin, WithOffset, 1/A <sup>2</sup>
61	calibration 2.5 mg/L -500 ng	17	5.777	509.5516	0.774	Lin, WithOffset, 1/A <sup>2</sup>
62	calibration 5 mg/L -1000 ng	18	5.780	1034.3498	0.774	Lin, WithOffset, 1/A <sup>2</sup>
63	calibration 12.5 mg/L -2500 ng	19	5.800	2554.7185	0.774	Lin, WithOffset, 1/A <sup>2</sup>
64	calibration 25 mg/L -5000 ng	20	5.837	4909.7852	0.774	Lin, WithOffset, 1/A <sup>2</sup>
65	calibration 37.5 mg/L -7500 ng	21	5.837	7499.1116	0.774	Lin, WithOffset, 1/A <sup>2</sup>
66	calibration 50 mg/L -10000 ng	23	5.867	9552.5655	0.774	Lin, WithOffset, 1/A <sup>2</sup>

Figure A6. Interactive results table showing the best calibration fit

## Appendix C

### Troubleshooting tips

Table C1. Troubleshooting tips. See Table C2 for further discussion.

Parameter	Failure code	Impact on sample sequence
Fluoride retention time (n = 3)	A	Rare if it fails during the IDC: Check IC system.
Fluoride retention time window	B	Established for all subsequent sequences. This is an indicator of column aging. If it fails: See column manual for washing instructions or replace columns.
Calibration (n = 7)	C	If it fails: Reject calibration sequence.
Fluoride CV standards	D	If it fails: Reject sequence and samples in sequence.
Dry GAC per lot	E	If it fails: confirm analysis with clean boat. If it fails again: report to supplier.
MB	F	If it fails: Reject sequence. Clean adsorption unit and CIC. Repeat MB and sequence.
MDL: 5 µg/L Fluoride (PFHxS)	F, G	If it fails (high): Reject MB and MDL sequence. Clean syringe, adsorption unit and CIC. Use a fresh vial of the PFHxS stock standard. If it fails (low): Reject MB and MDL sequence. Check for CIC leaks and refill DI water containers.
IPR: 15 µg/L Fluoride (PFHxS)	F, G	If it fails (high): Reject sequence. Clean tools and modules. Use a fresh PFHxS standard. If it fails (low): Reject MB and MDL sequence. Check for CIC leaks and refill DI water containers.
OPR: 15 µg/L Fluoride (PFHxS)	F, G	If it fails (high): Reject sample data and sequence. Clean syringe, adsorption unit, and CIC. Repeat OPR and sequence. If it fails (low): Reject sample data and sequence. Check for CIC leaks and refill DI water containers.

**Table C2. Causes and actions**

Code	Suggested causes and actions	
A	Rare failure during IDC. Check for leaking connections on the IC. Common error with aging columns. Clean column according to column manual or replace guard column or both the guard and separation columns.	
B	Requires recalibration. This is a rare failure during IDC but is an indicator of an aging or dirty column. Suggest replacing the guard column or washing or replacing both the guard and analytical columns.	
	Rare failure for experienced EPA Method 1621 users with a properly operating CIC system.	
	<i>Potential causes</i>	<i>Suggested solutions</i>
	Ceramic wool added to combustion boats/cups.	Discontinue adding ceramic wool to combustion boats/cups.
	Incorrect preparation of calibration standards.	Prepare a new set of calibration standards.
C	Contamination was introduced: combustion boats, storage containers, volumetric flasks, and pipette tips.	Retest combustion boats by CIC for baseline contamination. Pipette tips, volumetric flasks, and storage bottles can have residual contamination. Rinse with methanol three times followed by DI water three times prior to use.
	Leak in CIC system.	Verify that the fittings in the CIC are secure and reset fittings.
	DI water containers are empty.	Refill water containers with DI water.
	This is a rare failure for initial use.	Prepare a new set of CV standards.
D	CV are used as check standards to indicate when it is time to recalibrate.	Reject sequence and all MB, MDL, IPR, OPR, and sample results in sequence. Prepare a new set of calibration and CV standards. Recalibrate and verify that CV standards are correct. Repeat samples and sequence.
E	Dirty boat(s) and/or lot is contaminated.	Clean combustion boats and repeat analysis. Contact supplier for solutions.
		Reject sequence and all MB, MDL, IPR, OPR, and sample results in sequence.
F	Cross contamination can cause high results.	Clean syringe, adsorption unit, and CIC. Some testing lab environments may contribute contamination. Enclosures of the autosampler and boat/cup covers have proven useful in this case. Repeat sequence with freshly adsorbed MB, MDL, and OPR standards and wastewater samples.
F	Methanol in stock PFAS (PFHxS) standard evaporated, which will cause high results.	Reject sequence and all MB, MDL, IPR, OPR, and sample results in sequence. Use a fresh PFHxS standard. Check for leaks for low results. Repeat samples and sequence.
G	Gas leaks or empty DI water containers in the CIC flow path can cause low results.	Reject sequence and all MB, MDL, IPR, OPR, and sample results in sequence. Check for gas leaks in the CIC flow path. Refill the CIC DI water containers. Repeat samples and sequence.

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