# Screening of PFAS compounds in wastewater using adsorbable organic fluorine with combustion ion chromatography (CIC)

## Results from U.S. EPA draft Method 1621 Collaboration Study

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**Keywords**

EPA Method 1621, AOF, CIC, PFOA, PFHxS, GAC, Dionex IonPac AS24 column, PFAS (per- and polyfluoroalkyl substances), combustion ion chromatography (CIC), adsorbable organic fluorine (AOF), granular activated carbon (GAC)

## Introduction

Per- and polyfluoroalkyl substances (PFAS) is the collective name for synthetic fluorinated compounds. The regulatory definition varies. The Organisation of Economic Co-operation and Development (OECD) defines a PFAS compound as one containing a fully fluorinated methyl group or methylene on an alkane functional group.[1] The more restrictive U.S. Environmental Protection Agency (EPA) definition, based on the degradation properties, states the fluorinated compound must have at least two adjacent carbons with one carbon fully fluorinated and the other at least partially fluorinated *(unit R-CF2-CF(R')(R''), where R, R', and R'' do not equal "H" and the carbon-carbon bond is saturated)*.[2] For example, the EPA classification excludes trifluoroacetic acid ($CF_3$-COOH) as a PFAS compound because of the absence of an adjoining fluorinated carbon. Since the 1940s, many products have been composed of or contain PFAS. It is found in many polymers, surfactants, fire-suppression chemicals, industrial products, and consumer products.[3-6] In addition to their extensive presence, PFAS compounds are chemically stable due to their C-F bonds, and therefore, they are persistent and bioaccumulate. Consequently, PFAS compounds are an environmental contamination concern. Extensive toxicity studies of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) indicate varying toxicity effects from acute to chronic, potentially impacting reproductive health and causing epigenetic effects.[7-11] Less is known about the thousands of other compounds grouped under the EPA PFAS classification.[2]

The EPA has defined PFAS analysis methods for select PFAS analytes using LC-MS/MS in drinking water (EPA Methods 533.0 and 537.1) and in aqueous, solid, biosolids, and tissue samples (EPA Method 1633, at the publishing date of this document in the 90-day comment phase prior to promulgation).[12,13] Thermo Fisher Scientific has also previously demonstrated these methods.[14-18] However, a screening method is needed to assess the extent of PFAS contamination in various matrices, including wastewater. Pyrohydrolytic combustion-ion chromatography (CIC) has been previously demonstrated to eliminate the sample matrices.[19,20] More recently, CIC has been proposed as a screening method for PFAS by converting all organofluorine compounds (including PFAS) to HF, and the subsequent fluoride analyzed by ion chromatography with suppressed conductivity detection (IC).[21-27] The draft EPA Method 1621 has completed a single laboratory review and a round-robin collaborative study and was issued as an official method in the 90-day review and promulgation stage at the time of publication of this document.[28-30] In this method, 100 mL of wastewater are adsorbed onto granular activated carbon (GAC) columns. Inorganic fluoride is rinsed from the GAC columns with 10 mM sodium nitrate followed by a deionized (DI) water rinse. The GAC is removed from the glass tube, combusted under hydropyrolytic conditions, absorbed in DI water, and analyzed for fluoride by IC. In this application note, our results from the collaboration study are presented alongside practical advice that supports successful analyses. EPA draft Method 1621 includes method validation tests, calibration, method blanks, method detection limits, multiple process check standards, analysis of wastewater samples, and recovery results of four types of PFAS (PFHxS, PFOS, PFOA, and a blend described as a mixture of PFAS compounds).

## Experimental

### Equipment

Any Thermo Scientific™ Dionex™ RFIC systems with a conductivity detector (CD) combined with any combustion-absorption system and offline adsorption system can be used.

Recommended: Thermo Scientific™ Dionex™ Inuvion™ IC system with RFIC (P/N 22185-60108), Thermo Scientific™ Dionex™ Integrion™ RFIC system (P/N 22153-60305), and Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system.

The Dionex ICS-6000 HPIC system was used in this study, including

- SP Single Pump, isocratic (P/N 22181-60003)
- EG Eluent Generator (P/N 22181-60019)
- DC Detector Column module (P/N 22181-60043)
- CD Conductivity Detector (P/N 079829)

In this study, Nittoseiko Analytech (Nittoseiko Analytech Co., Ltd) AQF-2100H combustion-absorption system with an autosampler for solid samples and an offline adsoprtion unit was used.

### Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3.1 or higher, was used to control the IC. Contact your local Thermo Fisher Scientific specialist for the latest information on combustion unit control.

### Consumables list for EPA Method 1621

- Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting kits for:
  - Dionex Inuvion RFIC system (P/N B51000232)
  - Dionex Integrion RFIC system with CD detection (P/N 088798)
  - Dionex ICS-6000 HPIC system with CD detection (P/N 302965)
- Thermo Scientific™ Dionex™ EGC 500 KOH eluent generator cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 electrolytic trap column (P/N 088662)
- Thermo Scientific™ Dionex™ HP EG degasser kit (P/N 075522)
- Thermo Scientific™ Dionex™ ADRS 600 suppressor (2 mm) (P/N 088667)
- Thermo Scientific™ Dionex™ IonPac™ AS24 analytical column (P/N 064153)

- Thermo Scientific™ Dionex™ IonPac™ AG24 guard column (P/N 064151)
- Hamilton™ 100 µL microsyringe, metal plunger, Fisher Scientific (P/N 14-813-091)
- Fisherbrand™ Elite™ adjustable, 200 µL pipette and pipette tips, Fisher Scientific (P/N FBE00200 and P/N 02-707-409)
- HDPE 1.0 mL vials and caps, Fisher Scientific (P/N NC2038456, P/N 6ERC11PEC)
- Ceramic combustion boats for Nittoseiko Analytec ASC-270LS sample changer, Nittoseiko Analytec TX3SCY
- Granular activated carbon (GAC) columns used to adsorb samples in EPA Method 1621 multi-lab collaboration study with corresponding GAC column carriers, Analytik Jena 402-880.615, 402-880.013
- Quartz wool used in combustion tube and used to accumulate solids in high TSS samples, Nittoseiko Analytec TNQWL5K

## Method

The method includes adsorption, combustion-absorption, and analysis by ion chromatography.

### Adsorption conditions

All standards and samples are processed through the adsorption process discussed in section 11.3, EPA Method 1621. The empty boats, calibration standards, and calibration verification standards (CV) are the exceptions. They skip the adsorption process and enter the workflow at the combustion stage. For CV and calibration standards, pipette 200 µL of the individual µg/mL (mg/L) standard into a clean ceramic boat (resulting in "ng" amounts of fluoride). For example, 200 µL of 2.5 µg/mL fluoride = 500 ng.

| Parameter | Setting |
|---|---|
| Adsorption | Pump 100 mL of each sample or standard onto the GAC columns. |
| Rinse off inorganic fluoride | Pump 25 mL 10 mM sodium nitrate for each sample, MB, Initial Precision Recover (IPR) standard, Ongoing Precision Recovery standard (OPR), or blank ceramic wool onto the GAC columns. |
| Rinse off nitrate | Pump 20 mL of DI water onto the GAC columns or blank ceramic wool. |
| Dry | Pump 5 mL of air onto the GAC columns or blank ceramic wool. |

### Combustion and absorption conditions

| Parameter | Setting |
|---|---|
| Transfer samples | Transfer GAC material from each GAC column or quartz wool from the GAC carrier into a separate ceramic boat. Combust according to the boat program. |
| Boat program | *Position* *Wait time (s)* *Speed (mm/s)*<br>90 mm 20 10<br>End 600 10<br>Cool 60 40<br>Home 120 20 |
| Furnace temp. | 950 °C inlet, 1,000 °C outlet |
| Combustion time | Ar: 10 s; $O_2$: 600 s; Ar replacement: 30 s |
| Absorption sol. | 3 mL DI water |
| Final absorption sol. | 10 mL, mixed |
| Gas | Ar: 200 mL/min; $O_2$: 400 mL/min |
| Hydration | Water/Ar: 100 mL/min |
| Injection volume | 100 µL |

### IC conditions

| Parameter | Setting |
|---|---|
| Columns | Dionex IonPac AG24 guard, Dionex IonPac AS24 analytical anion-exchange columns, 2 mm i.d.<br>Note: Other hydroxide selective columns can be used instead (e.g., Dionex IonPac AS19, AS20, AS30 columns). |
| KOH gradient*** | 8 mM KOH (0 to 3.5 min, curve 5),<br>8–75 mM (3.5 to 9 min, curve 9),<br>75 mM (9 to 10.25 min, curve 5),<br>75–8 mM (10.25 to 15 min, curve 5),<br>8 mM (15 to 20 min, curve 5) |
| Eluent source | Dionex EGC 500 KOH eluent generator cartridge,<br>Dionex CR-ATC 600 trap column,<br>Dionex HP EG degas module |
| Injection vol. | 100 µL |
| Flow rate | 0.30 mL/min |
| Column temp. | 30 °C |
| Detection temp. | 35 °C |
| Suppressor temp. | 20 °C |
| Detection | Suppressed conductivity, Dionex ADRS 600 suppressor, recycle mode, 56 mA |
| Conductance background | <0.5 µS/cm |
| System backpressure | ≈2,600 psi (≈18,000 kPa) |
| Run time | 25 min for the first sample, 20 min in overlap for the following samples |

***Curve 5 is linear; Curve 9 is convex

## Reagents

- DI water ASTM Type 1 (18 MΩ·cm resistivity, TOC < 50 ng/mL)[31]
- Ammonium hydroxide, 28–30 w/w%, Certified ACS, Fisher Scientific™ P/N A669S-500 (CAS 1336-21-6)
- Methanol, UHPLC-MS grade, Fisher Scientific P/N A458-1 (CAS 67-56-1)
- Sodium nitrate, ACS Certified, Fisher Scientific P/N S343-3 (CAS 7631-99-4)
- 1,000 mg/L chloride standard for retention time determinations, TraceCERT™, MilliporeSigma™ Supelco™ P/N 1.19897.0500, Fisher Scientific P/N 11-101-6049
- 1,000 mg/L certified fluoride standard for IC, SPEX™ CertiPrep™, Fisher Scientific P/N AS-F9-2Y or Dionex Sodium fluoride 1,000 mg/L, NIST traceable, P/N 037158
- 1,000 mg/L Fluoride solution, Ricca Chemical™, used as a second fluoride stock standard, Fisher Scientific P/N 3173-6 or Dionex Sodium fluoride 1,000 mg/L, NIST traceable, P/N 037158
- 1,000 ng/mL 30-PFAS mixed spiking standard in methanol (Wellington Laboratories P/N PFAC30 PAR, Lot PFAC300522), (Fluoride equivalence = 18.2 ng/mL)
- 50 µg/mL (in methanol) L-PFOS sodium perfluoro-1-octanesulfonate standard (Wellington Laboratories P/N L-PFOS, Lot LPOS0922), used for spike-recovery experiments (Fluoride equivalence = 61.7%; 30.3 µg/mL fluoride)
- 50 µg/mL (in methanol) L-PFHxS sodium perfluoro-1-hexanesulfonate standard (Wellington Laboratories P/N L-PFHxS, Lot LPFHxS1022), used for spike-recovery experiments (Fluoride equivalence = 64.6%; 29.26 µg/mL fluoride)
- 50 µg/mL (in methanol) L-PFBA perfluoro-n-butanoic acid standard (Wellington Laboratories P/N PFBA, Lot PFBA1022), used for spike-recovery experiments (Fluoride equivalence = 62.1%; 30.45 µg/mL fluoride)

## Preparation of solutions, standards, and samples

Use only ASTM Type I deionized water (DI water) for preparations.

Rinse all glassware, HDPE containers/bottles, and tools with methanol, followed by DI water, to eliminate potential fluoride contamination.

Adsorption solutions: 2 M sodium nitrate stock solution, 10 mM sodium nitrate wash solution, and 1% ammonium hydroxide in Fisher Chemical™ Optima™ LC-MS grade methanol cleaning solution, as described in section 7.1 of EPA Method 1621.[30]

Standards, blanks, and samples: Follow EPA Method 1621 instructions to prepare inorganic fluoride and chloride standards. Prepare inorganic fluoride and chloride retention time standards, fluoride CV standards, and fluoride calibration standards (0.5, 1, 2.5, 5, 10, 12.5, 25, 37.5, and 50 µg/mL) from 1,000 mg/L stock standards. Refer to TN003056 for detailed processes to achieve low background levels and minimize cross-contamination needed for this application.[32] Follow EPA Method 1621, section 11.3 to process the samples through the adsorption step.

PFAS standards in methanol: Use a microsyringe without Teflon™ or fluoropolymers-containing components to dose PFHxS aliquots or PFAS standards for MDL, IPR, OPR, and recovery samples. Clean the microsyringe multiple times with suitable high-purity methanol. Clean between sample- or standard-spiking. Store standards in fluorocarbon-free vials with lids, such as HDPE, at 20 °C. See EPA Method 1621, section 7.3.3 to calculate total F in the PFHxS standard.

## Instrument setup and installation

### Potential sources of contamination

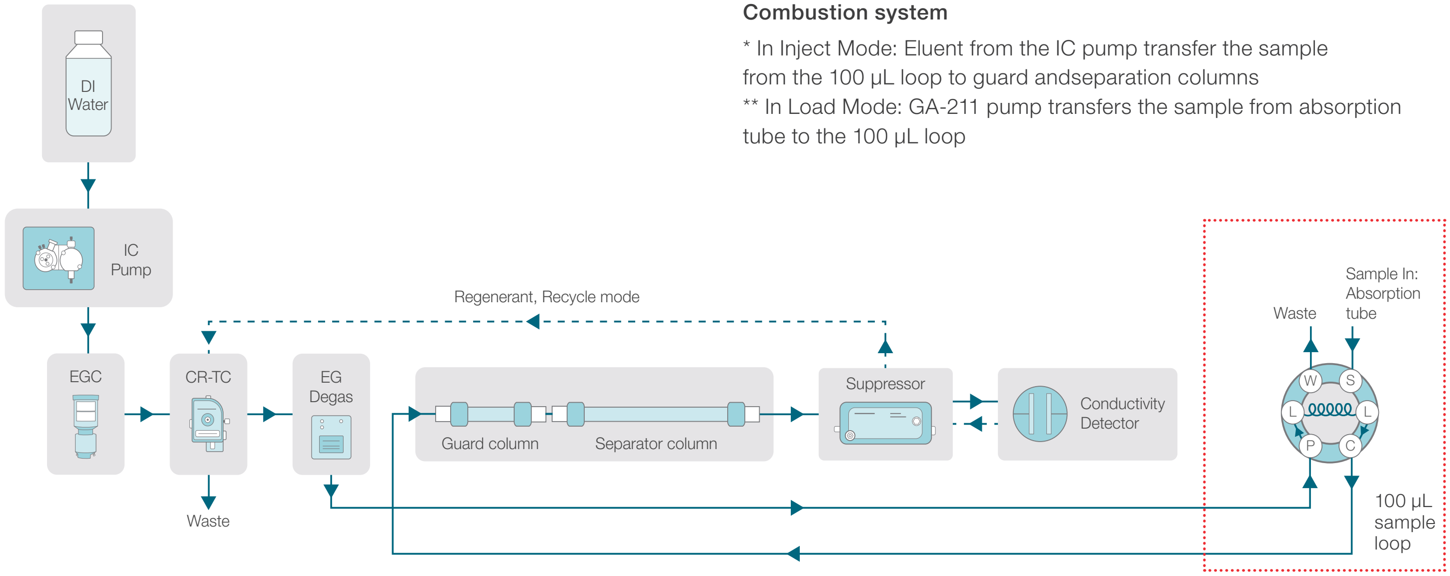
To exploit the full potential of the trace analysis method, potential sources of contamination must be excluded in advance. A detailed discussion on achieving low contamination levels and tips for successful implementation of EPA Method 1621 are discussed in TN003056.[32]

### Adsorption standalone instrument

Set the dials of the instrument to the desired volume. Install empty GAC carriers. Clean the fluid flow with the GAC carriers in position using 1% ammonium hydroxide in methanol rinse followed by DI water rinse.

### CIC system

Install and set up the Dionex HPIC system as described in Figure 1 and the instrument manuals.[33-36] TTL triggers are effective ways to trigger the Dionex IC to start after the combustion-absorption is complete. Refer to the instrument manuals. Thermo Fisher Scientific Technical Note 000767 describes the configuration of CIC with a Dionex Integrion HPIC system using Chromeleon 7 CDS software, which is similar for other Dionex HPIC RFIC systems, such as the Dionex Inuvion RFIC system and the Dionex ICS-6000 HPIC system).[37] Hydrate and condition the IC consumables as described in TN000767.



**Combustion system**

* In Inject Mode: Eluent from the IC pump transfer the sample from the 100 µL loop to guard andseparation columns
** In Load Mode: GA-211 pump transfers the sample from absorption tube to the 100 µL loop

**Figure 1. Flow diagram for CIC system**

## Results and discussion

This application follows EPA draft Method 1621 (similar to EPA Method 1621[30]) statement of work and technical directives. The collaboration study was organized by the managing laboratory, General Dynamics Information Technology company, which provided GAC columns and GAC column carriers, and PFAS, chloride and fluoride stock standards.[38] Definitions are tabulated in the appendix.

### Determining the fluoride retention time and window

Fluoride must be well-resolved from the water dip (void volume) and other peaks eluting near fluoride for accurate determinations. In hydroxide eluent separations with suppressed conductivity, as shown in Figure 2, fluoride is well resolved from the water dip, whereas carbonate eluents can be challenging. The results show that fluoride elutes well after (3.3 min) the water dip. The water dip height is also small (<0.04 µS/cm), characteristic of hydroxide eluents. Additionally, after being suppressed, electrolytically generated and purified hydroxide eluents provide higher signal-to-noise ratios, a measure for increased sensitivity and direct compatibility with gradient elution. Fully automated RFIC Eluent Generation (EG) is known for improved analytical and chromatographic performance. The precise and user-independent gradient formation produces superior separations when paired with high-performance Dionex IonPac columns. All the user has to provide is DI water.

**Table 1. Determining fluoride retention time window**

| Peak | Retention time (min) | Peak window (min) | Separation (min) | Limit |
|---|---|---|---|---|
| **Water dip** | 2.75 ± 0.008 | -- | -- | -- |
| **1,250 ng Fluoride*** | 6.07 ± 0.014 | 5.98–6.14 | 3.32 | >1 min |
| **5,000 ng Chloride**** | 12.48 ± 0.005 | 12.45–12.51 | 6.42 | >1 min |
| **Performance** | PASS | | | |

*0.1 mL of 12.5 µg/mL fluoride, n = 3; **0.1 mL of 50 µg/mL chloride, n = 3

| Sample prep.: | Combustion 10 min at 950 °C to 1,000 °C | Peaks: | | |
|---|---|---|---|---|
| | | 1. Water dip | 2.75 | min |
| Standards: | A: 1,250 ng fluoride | 2. Fluoride | 6.07 | |
| | B: 5,000 ng chloride | 3. Chloride | 12.48 | |

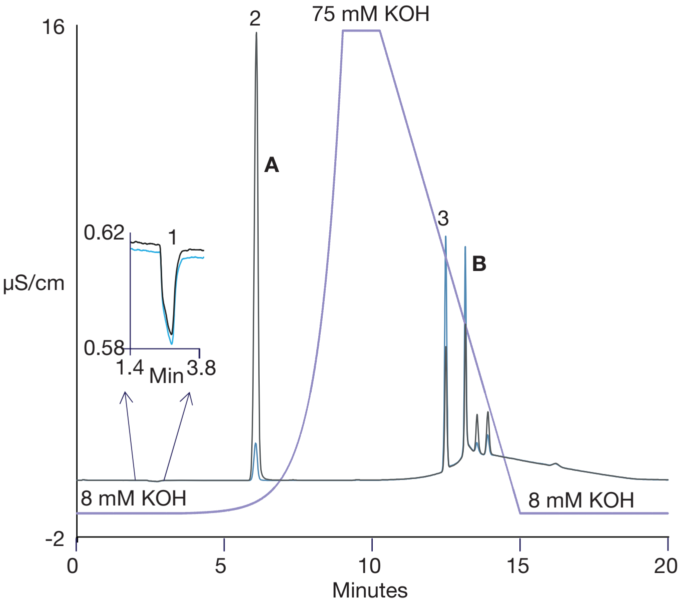


**Figure 2. Determining fluoride retention time window**

## Calibration

Calibration curves were determined according to EPA Method 1621 Step 10.2.2, by the peak area response of fluoride versus concentration, in nanograms, after combusting 200 µL of each mg/L calibration standard in separate ceramic boats. The method requires that the best calibration fit is determined by using a relative standard error (RSE) defined in section 10.3.3 Instrument linearity, EPA Method 1621.[30]

$$\mathrm{RSE} = 100 \times \sqrt{\frac{\sum_{i=1}^{n}\left[\frac{x_i' - x_i}{x_i}\right]^2}{n-p}}$$

with

*$x_i$=Nominal concentration (true value) of each calibration standard*

*$x_i'$=Measured concentration of each calibration standard*

*$n$ = Number of standard levels in the curve*

*$p$ = Type of cure (2 = linear, 3 = quadratic)*

**Equation 1. Definition of the relative standard error to determine instrument linearity.** The RSE must be ≤ 20%.

To determine the RSE, evaluate linear and quadratic fits by allowing offset from zero and calculating the curve fit without weighting and with weighting (1/A, $1/A^2$) by area. Select the calibration curve with the lowest RSE. In our experiments, we used a linear fit with $1/A^2$ area weighting, providing the lowest RSE (0.774) and compliance with the RSE limit of 20% set by the EPA (Table 2). Figure 3 shows the resulting calibration plot.

## Initial demonstration of capability

The collaboration study and Section 9.2, EPA Method 1621 require an initial capability demonstration (IDC) before analyzing wastewater samples. The IDC demonstrates that the instruments' and laboratory's performances suit this method. The accuracy (IDA) and precision (IDP) were determined by preparing and analyzing four replicates of DI water fortified with 15 µg/L fluorine (PFHxS).

**Table 2. Comparison of relative standard error (RSE) and coefficient of determination ($r^2$)**

| | Linear | | | Quadratic | | | RSE limit |
|---|---|---|---|---|---|---|---|
| | No weighting | 1/A | $1/A^2$ | No weighting | 1/A | $1/A^2$ | |
| **RSE$^+$** | 24.8 | 6.01 | 0.774 | 4.09 | 2.05 | 1.49 | 20 |
| **$r^{2++}$** | 0.9991 | 0.9993 | 0.9992 | 0.9996 | 0.9998 | 0.9997 | |

n = 7

$^+$RSE = Relative Standard Error, weighting = 1/A or $1/A^2$

$^{++}r^2$ = Coefficient of Determination

**Type: linear with offset, $1/A^2$ weighting**

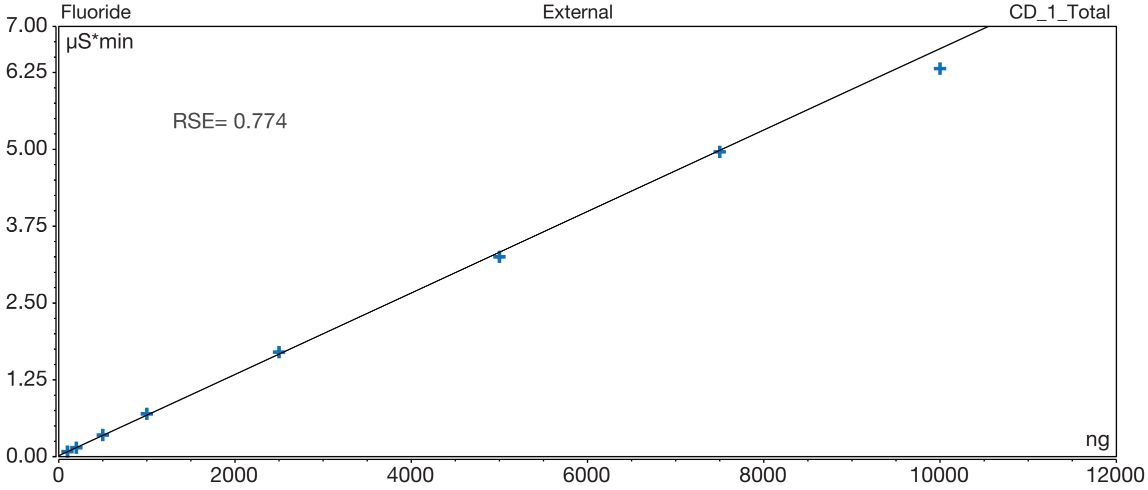


**Figure 3. Calibration curve**

In contrast to sections 9.2 and 10, the collaboration study required seven replicate method blanks (MB) and 5 µg/L method detection limit (MDL) standards to be prepared and analyzed over three days. The IDA and IDP results demonstrate that the method and this laboratory easily meet the IDC requirements (Table 3). Figure 4 shows the chromatography of the four replicate standards.

**Table 3. Initial demonstration of capability**

| IDC Requirement (Section from EPA Method 1621) | Specification | Experimental value |
|---|---|---|
| **IDA (9.2.1)** | Recovery 80% to 120% | Recovery = 98.5% |
| **IDP (9.2.1)** | RSD < 20 | RSD = 4.6 |

Sample prep.: 100 mL adsorbed onto 2 GAC columns
Standards: Chromatograms of only top GAC column
25 ng/mL fluoride equivalence
(PFHxS) IPR standards
Peak: 1. Fluoride 22.97–25.39 ng/mL
Precision: 4.6%
Recovery: 98.5%

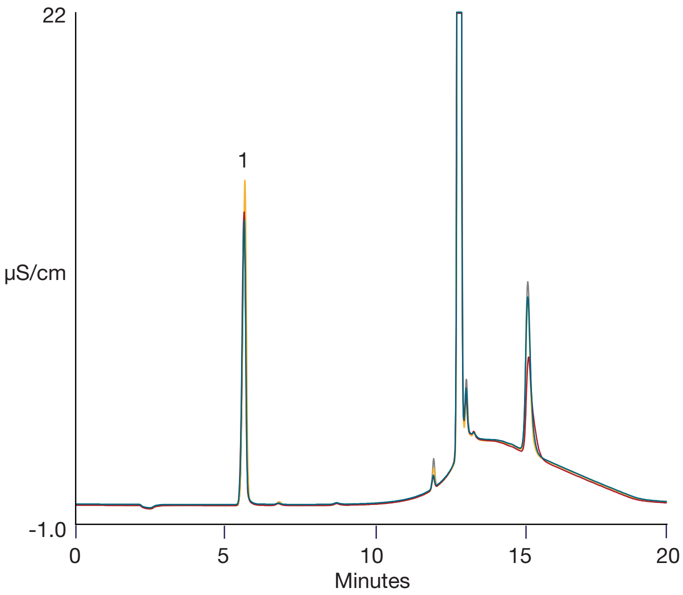


**Figure 4. Determining initial precision and recovery**

After three days of determining MBs and MDLs, the calculated MB was 1.23 ng/mL, and the calculated MDL was 2.5 ng/mL (Table 4). The chromatograms of MB and MDL are compared in Figure 5.

**Table 4. Initial demonstration of determining method blanks and method detection limit standards**

| Day | Method blank Fluoride (ng/mL) | 5 ng/mL MDL standard Fluoride (ng/mL) |
|---|---|---|
| 1 | 0.51 | 3.99 |
| 1 | 0.89 | 4.35 |
| 2 | 0.64 | 5.24 |
| 2 | 0.67 | 4.39 |
| 3 | 0.43 | 5.77 |
| 3 | 0.85 | 3.88 |
| 3 | 0.81 | 5.70 |
| **Average, X** | 0.69 | 4.76 |
| **Standard Deviation (σ)** | 0.17 | 0.80 |
| **RSD** | 25.5 | 16.7 |
| **MDL** | $MDL_{(b)}$^ = 1.23 | $MDL_{(s)}$^^ = 2.50 |

^$MDL_{(b)} = X + (3.14*\sigma)$

Where $MDL_{(b)}$ is the MDL for MBs, X is average of MB, 3.14 is Student's *t*-test value for n = 7, 99 percentile, and σ is the standard deviation.[37]

^^Section ii: $MDL_{(s)} = 3.14*\sigma$

Where $MDL_{(s)}$ is the MDL for spiked MDL standards, 3.14 is Student's *t*-test value for n = 7, 99 percentile, and σ is the standard deviation.[39]

Sample prep.: 100 mL adsorbed onto 2 GAC columns
Standards: Chromatograms of only top GAC column
A: Method blank (DI water)
B: 5 ng/mL fluoride equivalence (PFHxS))

| | | A | B | |
|---|---|---|---|---|
| Peak: | 1. Fluoride | 0.43 | 5.77 | ng/mL |

Calculated $MDL_{(b)}$: 1.23 ng/mL
Calculated $MDL_{(s)}$: 2.50 ng/mL

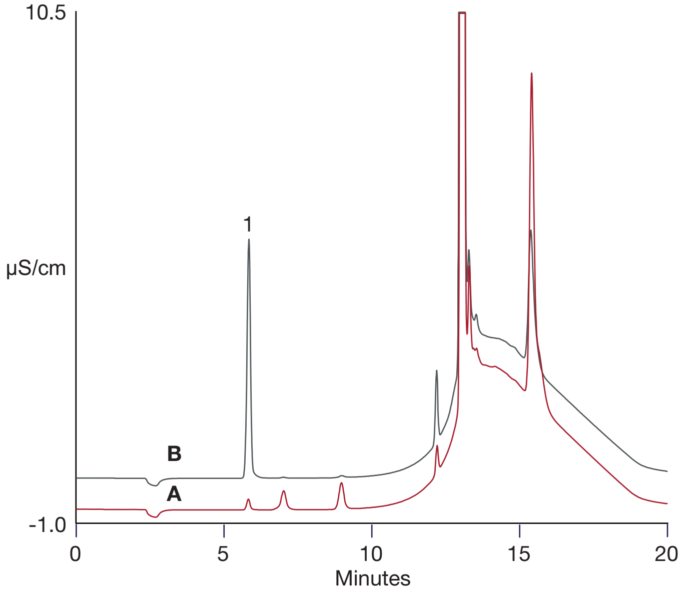


**Figure 5. Determining method blank and MDL**

## Samples

The method was applied to wastewater samples. The results are shown in Table 5. Less than 15 ng/mL of fluoride are present in the samples, ranging from <1 (Sample 7) to 12 ng/mL (Sample 3). The method blanks and process checks show that the method is working well within the expected limits.

**Table 5. Sample results and recovery results of added PFHxS standard**

| Sample | Total fluoride | | Recovered (%) |
|---|---|---|---|
| | Measured (ng/mL) | Added (ng/mL) | |
| **Sample 1** | 9.69 | 30.0 | 87.7 |
| | | 30.0 | 87.1 |
| | | 60.3 | 99.0 |
| | | 60.3 | 95.9 |
| **Sample 2+** | 1.11 | 10.03 | 116 |
| | | 10.03 | 117 |
| | | 25.2 | 98.8 |
| | | 25.2 | 90.4 |
| **Sample 3+** | 12.22 | 30.0 | 92.6 |
| | | 30.0 | 91.2 |
| | | 60.3 | 87.5 |
| | | 60.3 | 88.7 |
| **Sample 4** | 9.69 | 30.1 | 108 |
| | | 30.1 | 105 |
| | | 60.3 | 90.2 |
| | | 60.3 | 102 |
| **Sample 5** | 1.56 | 30.0 | 103 |
| | | 30.0 | 102 |
| | | 60.3 | 106 |
| | | 60.3 | 104 |
| **Sample 6+** | 4.93 | 10.09 | 80.2 |
| | | 10.09 | 81.9 |
| | | 30.0 | 107 |
| | | 30.0 | 105 |
| **Sample 7** | 0.53 | 10.03 | 104 |
| | | 10.03 | 105 |
| | | 30.1 | 102 |
| | | 30.1 | 101 |
| **Sample 8** | 1.71 | 10.03 | 99.1 |
| | | 10.03 | 103 |
| | | 30.1 | 92.4 |
| | | 30.1 | 96.8 |
| **Sample 9** | 3.23 | 30.1 | 98.3 |
| | | 30.1 | 96.6 |
| | | 60.2 | 85.5 |
| | | 60.2 | 95.3 |

After MBs were subtracted

+ Required ceramic wool

## Determining recoveries of added PFHxS standard

To understand method accuracy, recoveries of different volumes of added PFHxS standard were determined at two concentrations and duplicated for all nine samples, including those requiring ceramic wool (Table 5).

The method is accurate, as indicated by the recoveries of added PFHxS standards, 80 to 117%. The chromatogram of Sample 3 is compared with the replicate recovery samples (Figure 6).

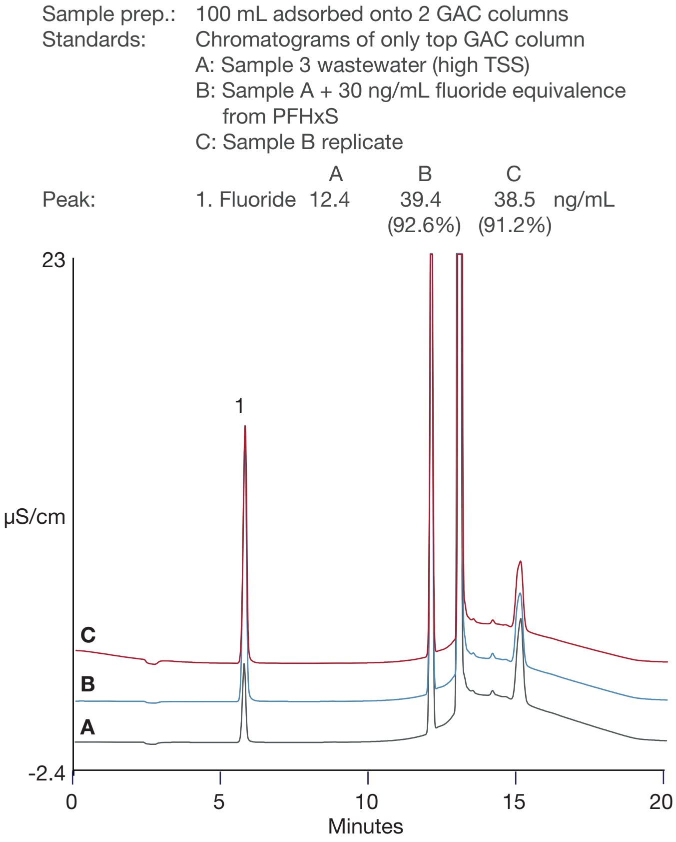


**Figure 6. Determining recovery of PFHxS in Sample 3 wastewater**

## Determining recoveries of PFAS compounds in Sample 7

To evaluate the accuracy of determining other PFAS compounds, similar recovery experiments were conducted with added standards of PFBA, PFOS, and a PFAS mixture (Wellington Laboratories). Approximately 10 ng/mL and 30 ng/mL fluoride from the above standards were added to Sample 7 (defined by the managing laboratory). The results summarized in Table 6 show that the fluoride recoveries of PFBA, PFOS, and PFAS mixture ranged from 76 to 114% of added fluoride as perfluorocarbon. Figure 7 shows representative chromatograms of samples after the addition of PFOS, PFBA, and PFAS mixture to determine compound specific recovery of fluoride after combustion. No compound specific dependency was observed.

**Table 6. Summary of recovery results from added PFBA, PFOS, and PFAS**

| Sample | Total fluorine | | Recovered (%) |
|---|---|---|---|
| | Added (ng/mL) | Measured (ng/mL) | |
| **Sample 7** | – | 0.53 | – |
| **Sample 7 with PFBA** | 10.05 | 10.86 | 103 |
| | 10.05 | 10.63 | 100 |
| | 30.4 | 33.31 | 108 |
| | 30.4 | 23.53 | 76.1 |
| **Sample 7 with PFOS** | 10.0 | 10.19 | 96.8 |
| | 10.0 | 9.08 | 86.2 |
| | 30.3 | 29.16 | 94.6 |
| | 30.3 | 35.2 | 114 |
| **Sample 7 with PFAS mix** | 9.99 | 11.05 | 105 |
| | 9.99 | 8.98 | 85.4 |
| | 30.0 | 25.60 | 83.9 |
| | 30.0 | 24.91 | 81.6 |

Sample prep.: 100 mL adsorbed onto 2 GAC columns
Samples: Chromatograms of only top GAC column
A: Sample 7 wastewater.
Replicates: B) + 10.05 ng/mL fluoride equivalence (PFOS),
C) + 30 ng/mL fluoride equivalence (PFAS mix),
D) + 30 ng/mL fluoride equivalence (PFBA)

| Peak: | | A | B | C | D | |
|---|---|---|---|---|---|---|
| | 1. Fluoride | 0.53 | 9.08<br>(86.2%) | 25.6<br>(83.9%) | 33.3<br>(108%) | ng/mL |

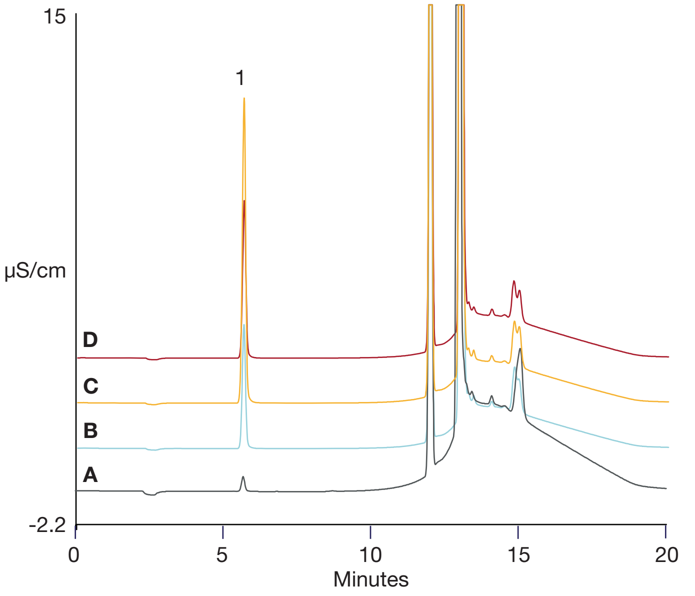


**Figure 7. Determining recovery of different PFAS compounds.** No compound specific dependency is observed.

## Conclusion

This application note demonstrated our results from the multi-laboratory collaboration study of EPA draft Method 1621. This method shows that CIC is an excellent tool to measure AOF and screen for PFAS in wastewater. Along with its ease of use, RFIC provides more accurate results as fluoride is well separated from the water dip, ensuring optimized automated peak integration. The method is sensitive, with MBs of 1 ng/mL and MDLs of 2.5 ng/mL, and accurate, with recoveries of 80–120%. Together, these data highlight the power of CIC in eliminating the sample matrix and measuring only the adsorbable organically bound fluorine content in samples, successfully achieving the goals outlined in EPA draft Method 1621.

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## Appendix

Table A1. Definitions

| Term | Definition | Description |
|---|---|---|
| **AOF** | Adsorbable Organic Fluorine | Fluorocarbon and perfluorocarbon compounds in water or solvent that adhere to a sorbent like activated carbon or ion exchangers. |
| **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. |
| **GAC** | Granular Activated Carbon | Carbon material used for adsorbing organic compounds. |
| **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 Filtration module. Always run at the beginning and the end of each sequence. |
| **MDL** | Method detection limit | The lowest reliable measurement, typically 3× S/N. In this test, the standard is processed through the adsorption on carbon, combusted, absorbed, and analyzed by IC. |
| **$\text{MDL}_{(b)}$** | Method detection limit of blanks, n = 7 | Defined as: (Mean of method blanks) plus (standard deviation × Student *t*-test factor). |
| **$\text{MDL}_{(s)}$** | Method detection limit of 5 ng/mL PFHxS standard, n = 7 | Defined as: (standard deviation) × (Student *t*-test 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. |
| **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. |

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