C146-E465

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Handbook of Analysis Procedures for EPA method 533

Outline

EPA method 533

In EPA methods 533 the validity of the analysis performed by each laboratory needs to be confirmed by Quality Control (QC).

> **Note**
> Confirming QC compliance is outside the scope of this method package. The section below is intended for reference use only.

The section below shows the general analytical procedure for fulfilling QC requirements. Note that the procedure could be modified depending on the situation in the lab and analysts.

Analysis Procedures

Reference

Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry
Chapters of reference information in the document are shown in brackets.

1 Initial Demonstration of Capability (IDC)

IDC must be carried out when a lab starts the analysis for the very first time , or when a method modification happens. (9.1)

1-1. Determine Minimum Reporting Level (MRL) and calibration curve range

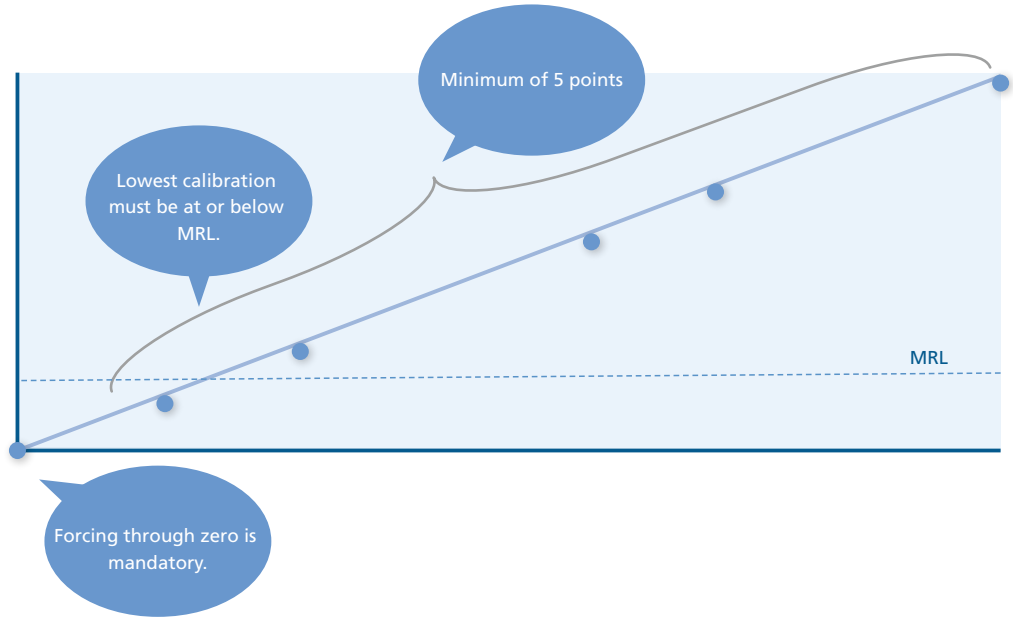
Determine the Minimum Reporting Level (MRL) according to the purposes or requirements of the analysis. Each analyte has its own MRL.

Along with the determined MRLs, the calibration curve concentration should be determined under the conditions below:

- The point of minimum concentration is equal to or less than the MRL. (7.17.5)
- There are at least five calibration points. (7.17.5)
- Forcing through the origin. (10.3.2)

Hint

The Minimum Reporting Level (MRL) is the minimum level of concentration to report as a reliable value. The reliability must be validated with analytical reproducibility.



1-2. Prepare the standard solutions

Prepare the following three types of standard solutions:

- Analyte Primary Dilution Standard (Analyte PDS) (7.17.4)
- Isotope Performance Standard PDS (7.15)
- Isotope Dilution Analogue PDS (7.16.1).

Suggested concentrations for the Isotope Performance Standard PDS is intended for a 10 µL fortification to 1 mL of extracted sample. (7.15.1) Similarly, suggested concentrations for the Isotope Dilution Analogue PDS is intended for a 20 µL fortification to 250 mL of sample. (7.16.1)

Isotope Performance Standards	Abbreviation	Conc. in PDS (ng/µL)
Perfluoro-n-[2,3,4-13C3]butanoic acid	13C3-PFBA	1.0
Perfluoro-[1,2-13C2]octanoic acid	13C2-PFOA	1.0
Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate	13C4-PFOS	3.0

Isotope Dilution Standards	Abbreviation	Conc. in PDS (ng/µL)
Perfluoro-n-[1,2,3,4-13C4]butanoic acid	13C4-PFBA	0.50
Perfluoro-n-[1,2,3,4,5-13C5]pentanoic acid	13C5-PFPeA	0.50
Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate	13C3-PFBS	0.50
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]hexane sulfonate	13C2-4:2FTS	2.0
Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	13C5-PFHxA	0.50
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-13C3-propanoic acid	13C3-HFPO-DA	0.50
Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	13C4-PFHpA	0.50
Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate	13C3-PFHxS	0.50
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate	13C2-6:2FTS	2.0
Perfluoro-n-[13C8]octanoic acid	13C8-PFOA	0.50
Perfluoro-n-[13C9]nonanoic acid	13C9-PFNA	0.50
Sodium perfluoro-[13C8]octanesulfonate	13C8-PFOS	0.50
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-decane sulfonate	13C2-8:2FTS	2.0
Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	13C6-PFDA	0.50
Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	13C7-PFUnA	0.50
Perfluoro-n-[1,2-13C2]dodecanoic acid	13C2-PFDoA	0.50

Isotope Dilution Standards	Abbreviation	Conc. in PDS (ng/μL)
Sodium perfluoro-[13C8]octanesulfonate	13C8-PFOS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-13C2]-decane sulfonate	13C2-8:2FTS	2.0
Perfluoro-*n*-[1,2,3,4,5,6-13C6]decanoic acid	13C6-PFDA	0.50
Perfluoro-*n*-[1,2,3,4,5,6,7-13C7]undecanoic acid	13C7-PFUnA	0.50
Perfluoro-*n*-[1,2-13C2]dodecanoic acid	13C2-PFDoA	0.50

Use methanol for the these standard solution. (7.13.1)

If base is not already present, add an aqueous solution of sodium hydroxide to the three PDS solutions to prevent esterification of fluorinated carboxylic acids. Use the equation shown below to calculate the amount of sodium hydroxide to add. (7.13.1)

$$Mass\ of\ NaOH\ Required\ (g) = \frac{Total\ PFASS\ mass\ (g)\ \times 160\ (\frac{g}{mol})}{250\ (\frac{g}{mol})}$$

1-3. Prepare QC samples in the laboratory

Using 250 mL sample bottles and caps made of polypropylene, prepare the samples for QC.

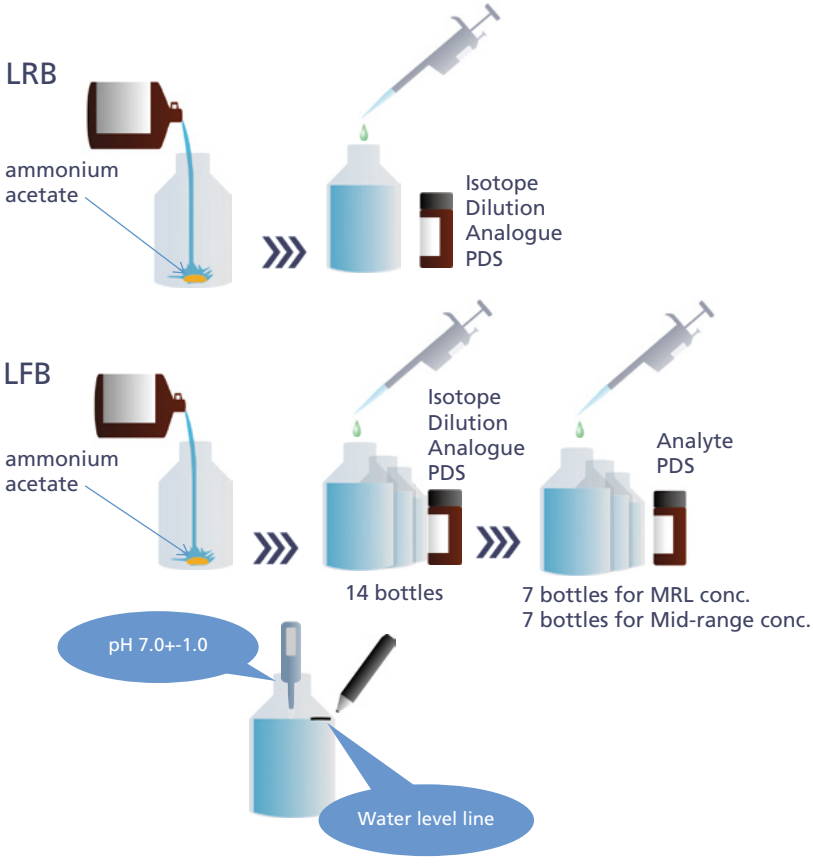
- Laboratory Reagent Blank (LRB)

  Put 250 mg of ammonium acetate in the sample bottle and add reagent water until the bottle is almost full, followed by adding 20 μL of Isotope Dilution Analogue PDS. (3.13)

- Laboratory Fortified Blank (LFB)

  Put 250 mg of ammonium acetate in the sample bottle and add reagent water until the bottle is almost full, followed by adding 20 μL of Isotope Dilution Analogue PDS. Add analyte PDS so that the concentration of the solution equals MRL and mid-range concentrations in the calibration curve. (3.10) Seven bottles of MRL concentrated LFB should be prepared for MRL confirmation (9.1.4), and seven bottles of mid-range concentrated LFB should be prepared for precision and accuracy. (9.1.2, 9.1.3)

Measure the pH of each sample to check that the range is between 7.0±1.0 (11.3.2), and mark each bottle to indicate the height of the liquid level. (11.3.1)



1-4. Prepare the reagents for sample extraction and mobile phase

- 0.1 M phosphate Buffer pH 7.0 (7.8)

  [1]: Prepare an aqueous solution of 0.1 M sodium dihydrogen phosphate.

  [2]: Prepare 0.1 M disodium hydrogen phosphate.

  [3]: Mix 500 mL of [2] and 275 mL of [1], and check that the pH is 7.0.

  Use this solution to rinse solid-phase extraction (SPE) cartridges.

- 1 g/L Ammonium Acetate (7.3.2)

  Dissolve the required amount of ammonium acetate in reagent water. (7.3.2)

  Use this solution also to rinse SPE cartridges.

- Solution of Ammonium hydroxide in Methanol (7.5)

  Add 2 mL of commercially available concentrated ammonium hydroxide (approx. 28.8%, 14.5 N, 56.6% (w/w)) to 100 mL of methanol.

  Use this solution to elute PFAS from SPE cartridges.

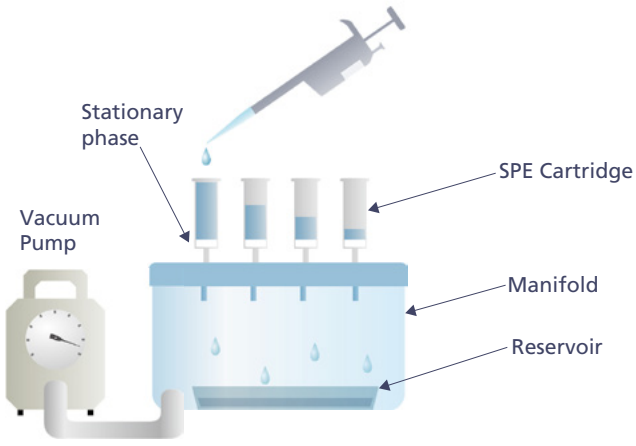
- 20% reagent water in methanol (11.4.6)

  Use this solution for the final sample solvent.

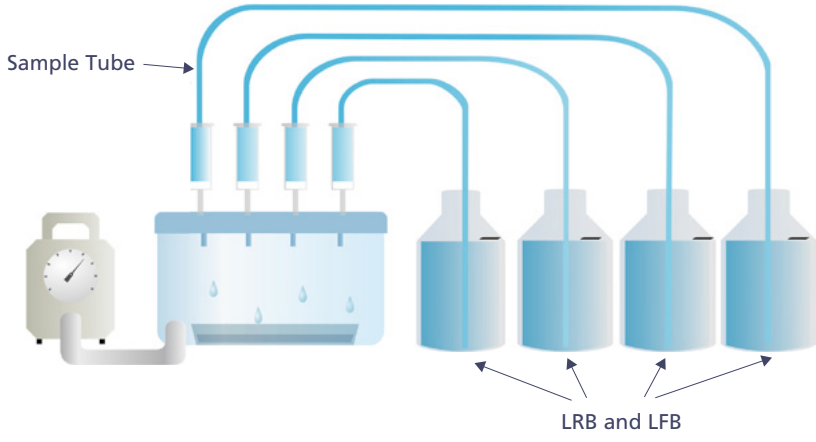
1-5. Extract QC samples

Place the reservoir inside the manifold, close the manifold, and connect the SPE cartridges and vacuum pump.

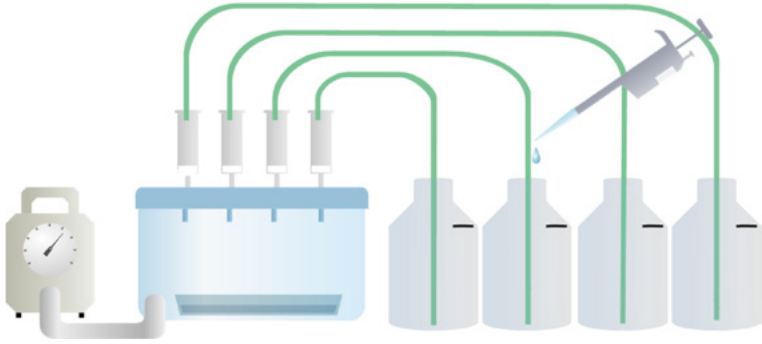
Rinse each cartridge in sequence with 10 mL of methanol and 10 mL of 0.1 M phosphate buffer pH 7.0. (11.4.1)



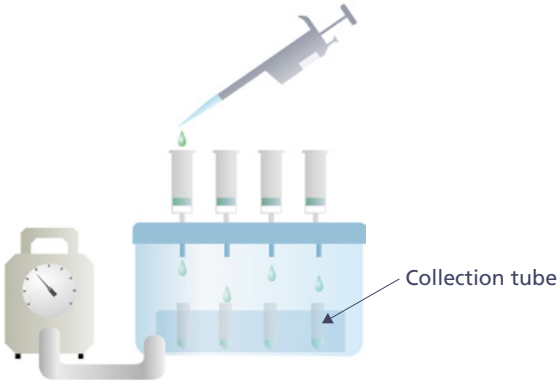
Connect the sample introduction tubes and then start the vacuum operation. Adjust the output of the vacuum pump so that the flow rate is 5 mL/min. (11.4.2)



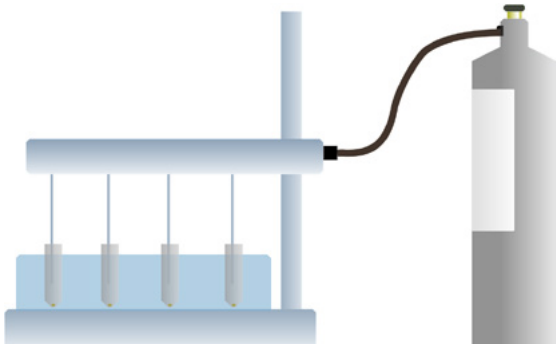
When the sample is depleted, add 10 mL of 1 g/L ammonium acetate to each sample bottle to wash them, and then add 1 mL of methanol. Dry the cartridges at high vacuum for 5 minutes. (11.4.3, 11.4.4)



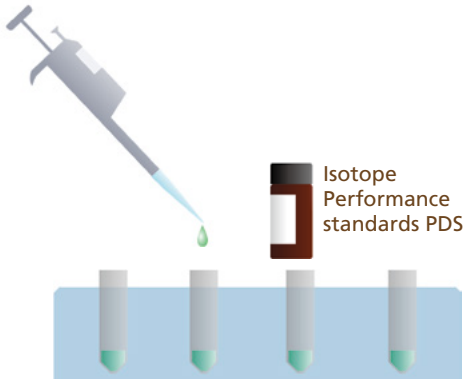
Stop the vacuum, open the manifold lid, remove the reservoir, and insert the collection tubes. Place 5 mL of ammonium hydroxide methanol solution in the bottles. Adjust the output of the vacuum pump so that the ammonium hydroxide methanol solution passes through slowly. Add another 5 mL of the same solution and pass it through. (11.4.5)



Warming the collection tubes in a water bath at 55 to 60 °C, dry the solution collected in the collection tubes by using a nitrogen purge. (11.4.6)



Add 1.0 mL of the 80/20 methanol/water solution to reconstitute the extract. Add 10 µL of Isotope Performance Standards PDS and vortex. (11.4.6)



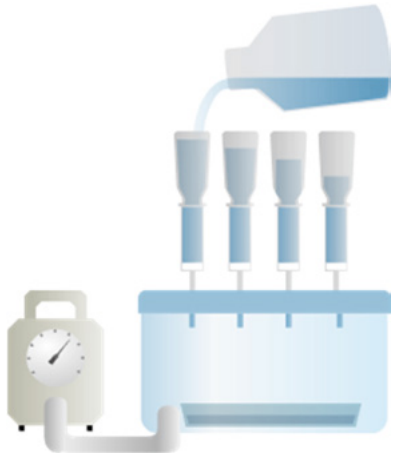
Fill the empty sample bottles (LRB and LFB) with water up to the mark indicating the height of the liquid level prior to extraction, then pour this water into a measuring cylinder to determine the sample volume. It is also possible to measure the weight to determine the sample volume. Assume a sample density of 1.0 g/mL. (11.5)

Hint

When the sample is collected in a 250 mL sample bottle, the sample volume is approximately 250 mL. However, the sample volume is not measured accurately at the time of sampling.
Therefore, the sample volume measured with this procedure needs to be used when making fine adjustments to the final concentration of the target compound.

Hint

Sample introduction tubes may cause contamination. (6.8.3) Using reservoirs may prevent contamination.



1-6. Prepare the calibration curve sample

Prepare the Calibration Standards and Quality Control Samples (QCS). In both cases, use 80/20 methanol/water solution.

- Calibration Standards

  Dilute the Analyte PDS prepared in 1-2 to obtain the sample concentrations determined in 1-1, and then add a constant amount of Isotope Dilution Analogue and Isotope Performance Standard to each calibration standard at the designated concentration. (Typically, 10 µL of Isotope Dilution Analogue PDS and 20 µL of Isotope Performance Standard PDS when the PDSs are at the suggested concentration) (7.17.5)

Hint

The concentration of the added Isotope Dilution Analogue should be 250 times greater than the concentration added to the field sample before solid-phase extraction. Considering that the concentration becomes 250 times greater through solid-phase extraction, this matches the concentration contained in the sample extract, assuming a 100% recovery rate through the solid-phase extraction operation.

- Quality Control Samples

  Prepare standard samples with a concentration near the midpoint of the calibration range in the same manner as Calibration Standards. Quality Control Samples must be prepared independently from the Calibration Samples. Preparation by another analyst is recommended. (9.2.9)

1-7. Prepare LC-MS system

Startup LC-MS and carry out auto-tuning. Place the bottles of mobile phase and deliver the mobile phase. Make sure no leakage or liquid delivery problems occur.

1-8. Calculate and verify the calibration curve

Run all of the Calibration Standards and QCS prepared in 1-6, and calculate a calibration curve.

- Calibration curve must be calculated with the internal standard calibration technique.
- Internal standard for the analytes is the isotope dilution analogue, and the internal standard for the isotope dilution analogue is the isotope performance standard.
- Forcing the calibration curve through the origin is required.
- Weighting may be used.

✓ QC check

Initial Calibration (10.3.5)

Make sure that the result for each analyte is within 70–130% of the true value. (50 – 150% is acceptable if the calibration level is equal to or less than the MRL)

Calibration Verification (9.1.5)

Make sure that the QCS results are within 70–130% of the true value.

1-9. Verify the system background

Analyze a LRB after the highest standard in the calibration range.

✓ QC check

Demonstration of low system background (9.1.1)

Make sure the analytes are less than one-third of the MRL.

1-10. Verify the MRL

Analyze the seven replicates of LFB at the proposed MRL concentration.

✓ QC check

MRL confirmation (9.1.4)

Calculate Upper Prediction Interval of Results (PIR) and Lower PIR. Make sure the results fulfill the below:

$$Upper\ PIR = \frac{Mean + 3.963S}{Fortified\ Concentration} \times 100 \leq 150\%$$

$$Lower\ PIR = \frac{Mean - 3.963S}{Fortified\ Concentration} \times 100 \geq 50\%$$

S : *The standard deviation*

1-11. Verify the reproducibility and accuracy

Analyze the seven replicate of LFB near the midpoint of the calibration curve range.

✓ QC check

Demonstration of precision (9.1.2)

Make sure the percent relative standard deviation is ≤ 20%.

Demonstration of accuracy (9.1.3)

Make sure the mean recovery is within 70–130% of the true value.

1-12. Check the branched isomer-derived peaks of the target compound

✓ QC check

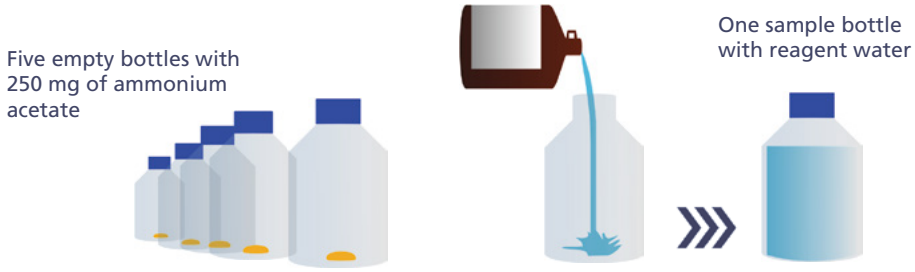
Establish retention times for branched isomers (10.2.2)

Make sure all isomers of each analyte elute within the same MRM window.

2 Sample Analysis

2-1. Prepare for sampling

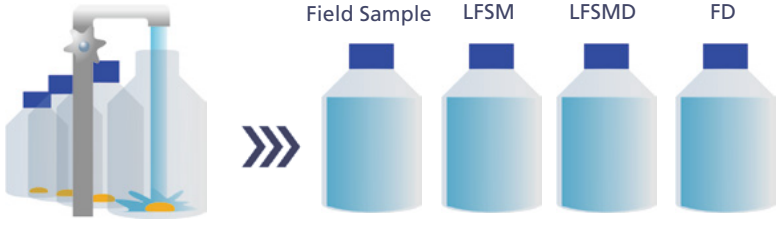
Prepare six 250 mL sample bottles and caps made of polypropylene, and add 250 mg of ammonium acetate to five of these bottles. (8.1, 8.2) Fill one sample bottle with reagent water that was confirmed with no contamination in the LRB analysis. (8.4.1) Ship all six sample bottles to the sample collection site.



2-2. Collect the samples

Open the faucet and wait until the temperature is stable. And then, flow the water to the four empty bottles until they are almost full. (8.3.2)

Use one bottle as field sample, and remainig three botlles are used as Laboratory Fortified Sample Matrix (LFSM), Laboratory Fortified Sample Matrix Duplicate (LFSMD) and Field Duplicate (FD). (3.11, 3.12, 3.5)



Pour the reagent water shipped from the laboratory into the remaining empty bottle at the water collection site, and use this as the Field Reagent Blank (FRB). (8.4.2)

Ship these bottles back to the laboratory. The samples should be kept at less than 10 °C during shipment, and store them at 6 °C or less in the laboratory. Perform extraction within 28 days of collection. Samples must not be frozen.

2-3. Prepare the QC samples

Prepare the QC samples.

- Field Sample and FRB

  Add 20 µL of Isotope Dilution Analogue PDS.

- LFSM and LFSMD

  Add 20 µL of Isotope Dilution Analogue PDS. Add analyte PDS at higher concentration than the original concentration of the field sample. For selecting the value of spiking, previous data may be used if available.

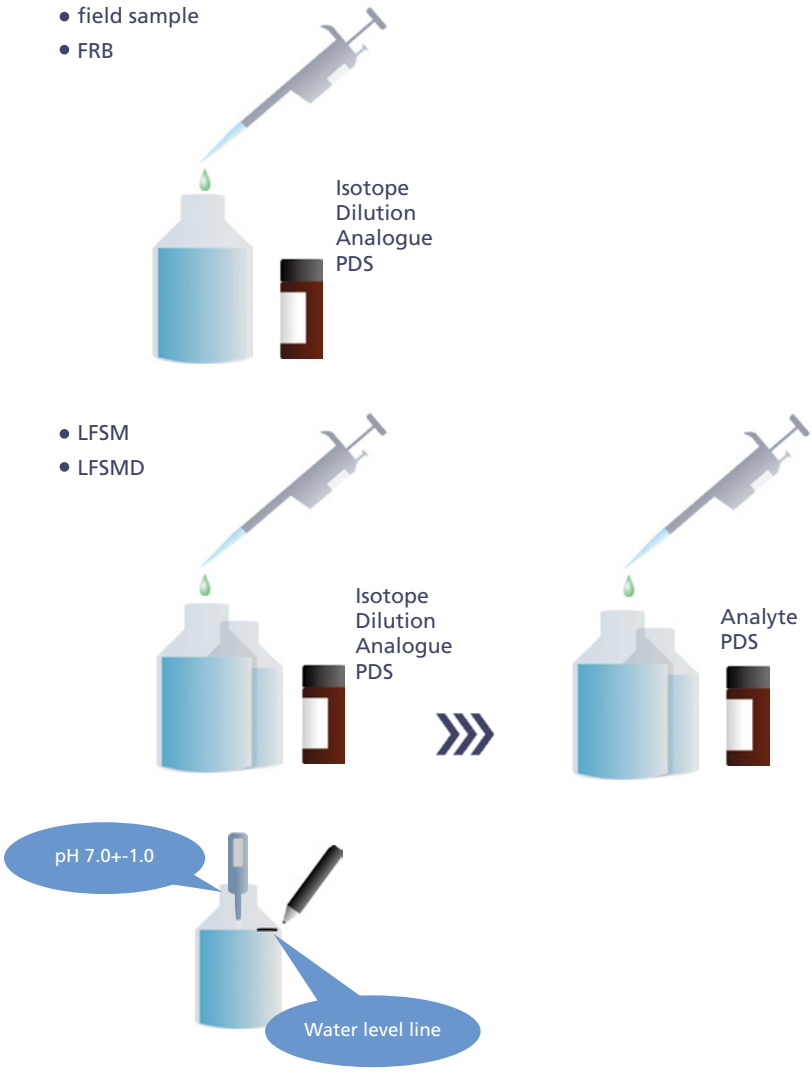
- LFB and LRB

  Prepare them as the same manner described in 1-3.

- Continuing Calibration Check (CCC)

  Prepare low, mid, and high concentration calibration standards in the same manner as Calibration Standards described in 1-6. (3.3) When Calibration Standards remain, they may be used as CCC. (7.17.5)

Measure the pH of each sample to check that the range is between 7.0 ± 1.0 (11.3.2), and mark each bottle to indicate the height of the liquid level. (11.3.1)



2-4. Extract QC samples

Extract the QC samples (Field Sample, FRB, LFSM, LFSMD) following the same procedure in 1-5.

2-5. Determine analysis batch

Determine the analytical batch depending on the situation. An example is shown below.

#	Sample	Note
1	CCC (Calibration Standards level 1)	- Always start batch with CCC (10.4) - CCC concentration needs to be MRL concentration or lower when beginning batch. (10.4) - CCC can be substituted with Calibration Standards (7.17.5)
2	LRB	- Need LRB in a batch (11.7.2)
3	LFB	- Need at least one LFB in a extraction batch (9.2.3) - The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. (9.2.3)
4	LFSM	- Need at least one LFSM in a extraction batch (9.2.6)
5	LFSMD or FD	- Need at least one LFSMD in a extraction batch (9.2.7)
6	Field Sample	
⋮		
15	Field Sample	
16	CCC	- CCC needs to be analyzed after every 10 samples (10.4)
17	Field Sample	
⋮		
26	Field Sample	
27	CCC	- CCC needs to be analyzed after every 10 samples (10.4) - CCC concentration needs to be mid- or high-level when finalizing batch. (11.7.2) - One analysis batch must be within a 24-hour period and no more than 20 field samples. (11.7)

2-6. Check QC while running the batch

✓ QC check

Continuing Calibration Check (CCC) (10.4)

Make sure the lowest level CCC is within 50–150% of the true value. All other levels must be within 70–130% of the true value.

✓ QC check

Laboratory Reagent Blank (LRB) (9.2.1)

Make sure that all method analytes are below one-third the Minimum Reporting Level (MRL), and that possible interferences from reagents and glassware do not prevent identification and quantitation of method analytes.

✓ QC check

Laboratory Fortified Blank (LFB) (9.2.3)

For analytes fortified at concentrations less than 2x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2x the MRL.

✓ QC check

Laboratory Fortified Sample Matrix (LFSM) (9.2.6)

Calculate percent recovery (%R) below:

$$\%R = \frac{A\text{-}B}{C} \times 100$$

where,

A = measured concentration in the fortified sample,

B = measured concentration in the unfortified sample, and

C = fortification concentration.

For analytes fortified at concentrations less than 2x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2x the MRL.

✓ QC check

Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD) (9.2.7)

Calculate Relative Percent Difference (RPD) below:

For LFSMDs,

$$RPD = \frac{|\, LFSMD - LFSM \,|}{(LFSMD + LFSM)/2} \times 100$$

For FDs,

$$RPD = \frac{|\, FD_2 - FD_2 \,|}{(FD_2 + FD_2)/2} \times 100$$

RPD must be ≤30% (≤50% if analyte concentration ≤2 x the MRL).

✓ QC check

Isotope performance standards (9.2.4)

For all samples, Peak area counts for each isotope performance standard must be within 50–150% of the average peak area in the initial calibration.

✓ QC check

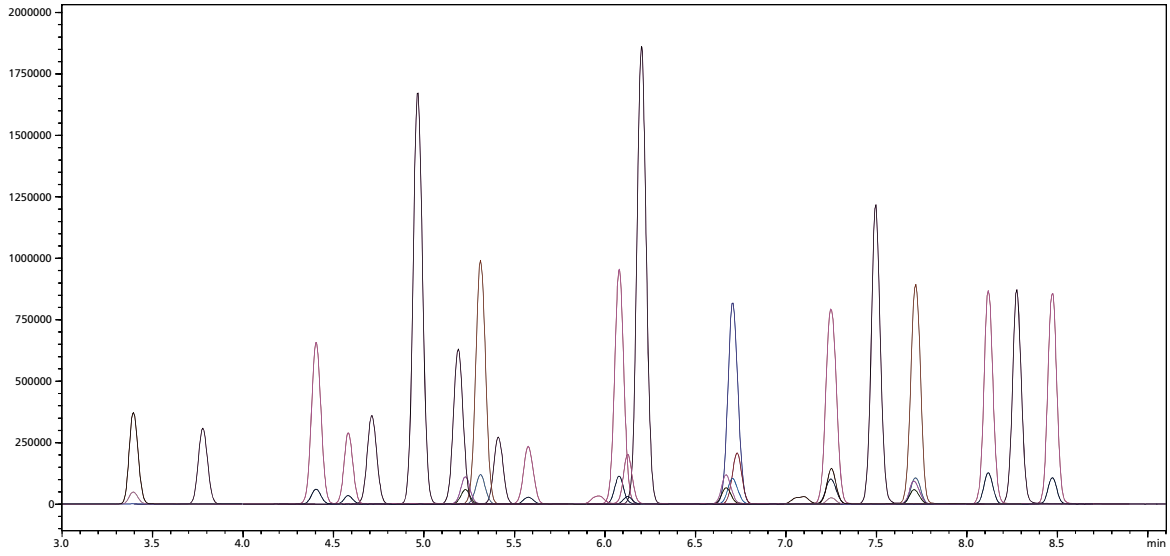
Isotope dilution analogues (9.2.5)

For all samples, make sure 50%–200% recovery for each analogue.

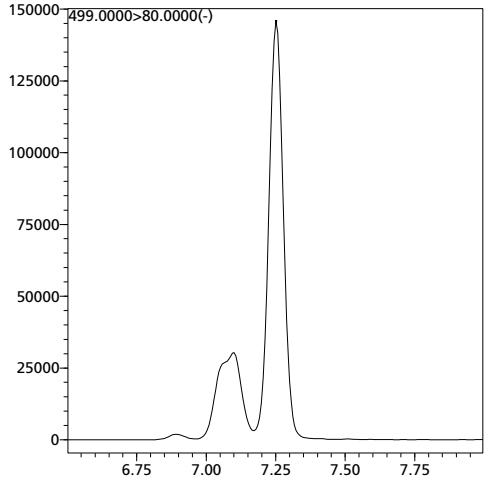
Chromatogram Examples

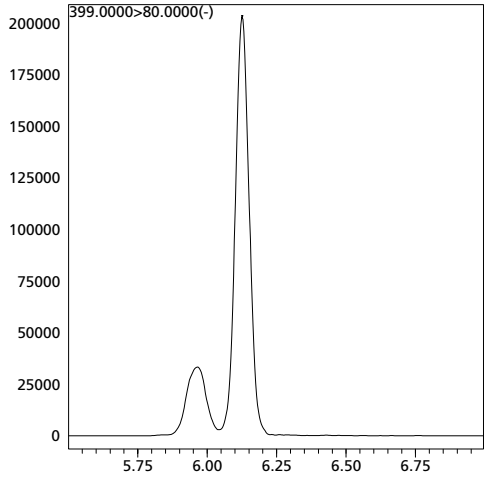
Shown below are the chromatogram examples.

100 ppb of standards.



Branched isomer separation in PFOS (left) and PFHxS (right).





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