C146-E466

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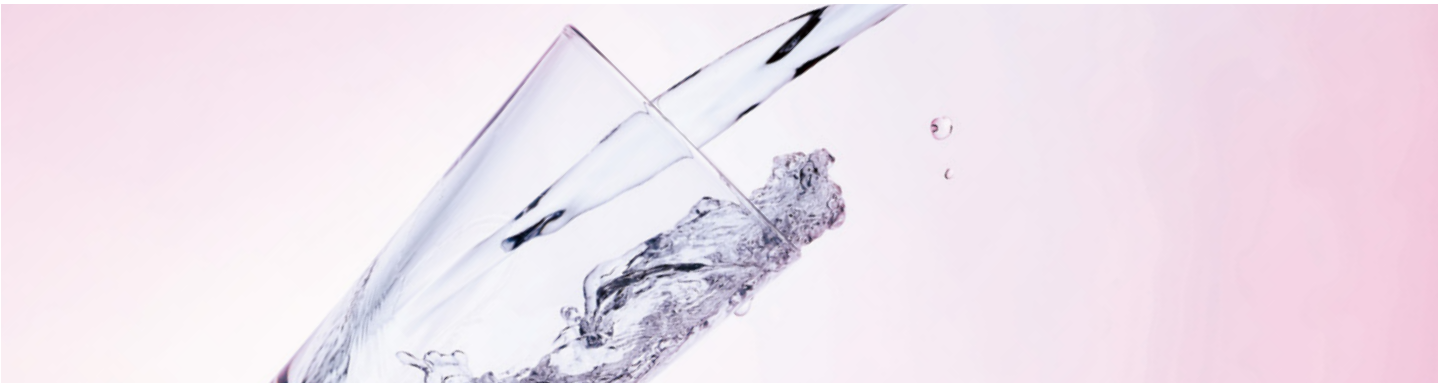
Handbook of Analysis Procedures for EPA method 537.1



Outline

EPA method 537.1

In EPA methods 537.1, 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 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)
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. (9.2)

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

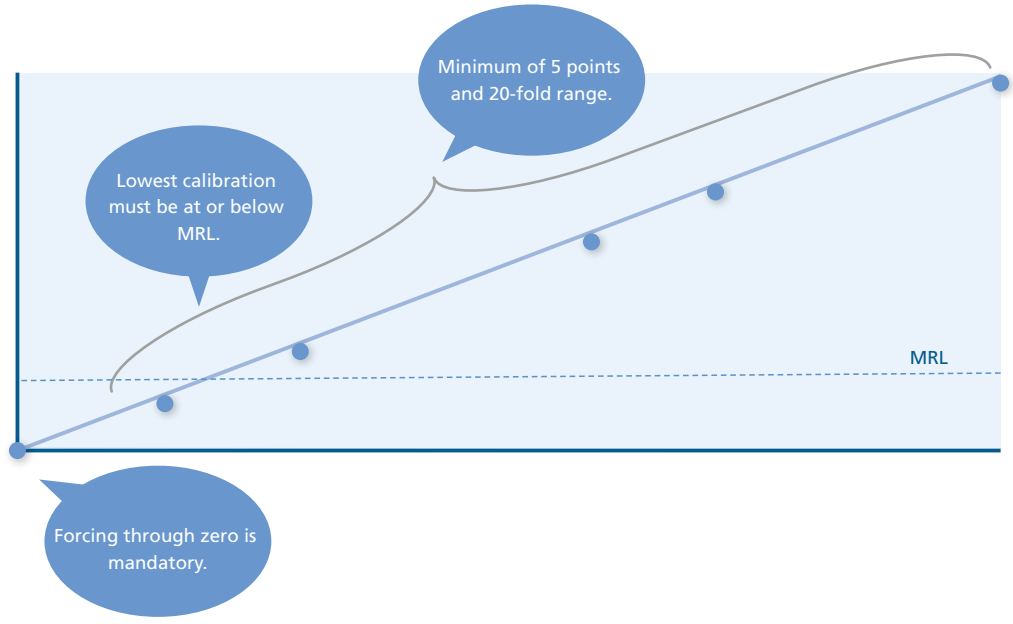
Determine the Minimum Reporting Level (MRL) according to the purposes or requirements of the analysis. Each analyte has its own MRL.

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. (7.2.4)
- There are at least five calibration points. (7.2.4)
- Concentration range must span at least a 20-fold range. (7.2.4)
- Forcing through the origin. (10.2.6)

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) (7.2.3.2)
- Internal Standard Primary Dilution Standards (IS PDS) (7.2.1.2)
- Surrogate Primary Dilution Standards (SUR PDS) (7.2.2.2)

Purchasing commercial products is acceptable. If not available, suggested concentrations are shown below for IS-PDS, that is intended for a 10 µL fortification to 1 mL of extracted sample. (7.2.1.2) Similarly, suggested concentrations for SUR- PDS are shown and is intended for a 10 µL fortification to 250 mL of sample. (7.2.2.2)

Internal Standards	Acronym	Conc. in IS-PDS (ng/µL)
Perfluoro-[1,2-13C2]octanoic acid	13C2-PFOA	1.0
Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate	13C4-PFOS	3.0
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d3-NMeFOSAA	4.0

Surrogates	Acronym	Conc. in SUR-PDS (ng/µL)
Perfluoro-n-[1,2-13C2]hexanoic acid	13C2-PFHxA	1.0
Perfluoro-n-[1,2-13C2]decanoic acid	13C2-PFDA	1.0
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d5-NEtFOSAA	4.0
Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid	13C3-HFPO-DA	1.0

1-3. Prepare QC samples in the laboratory

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

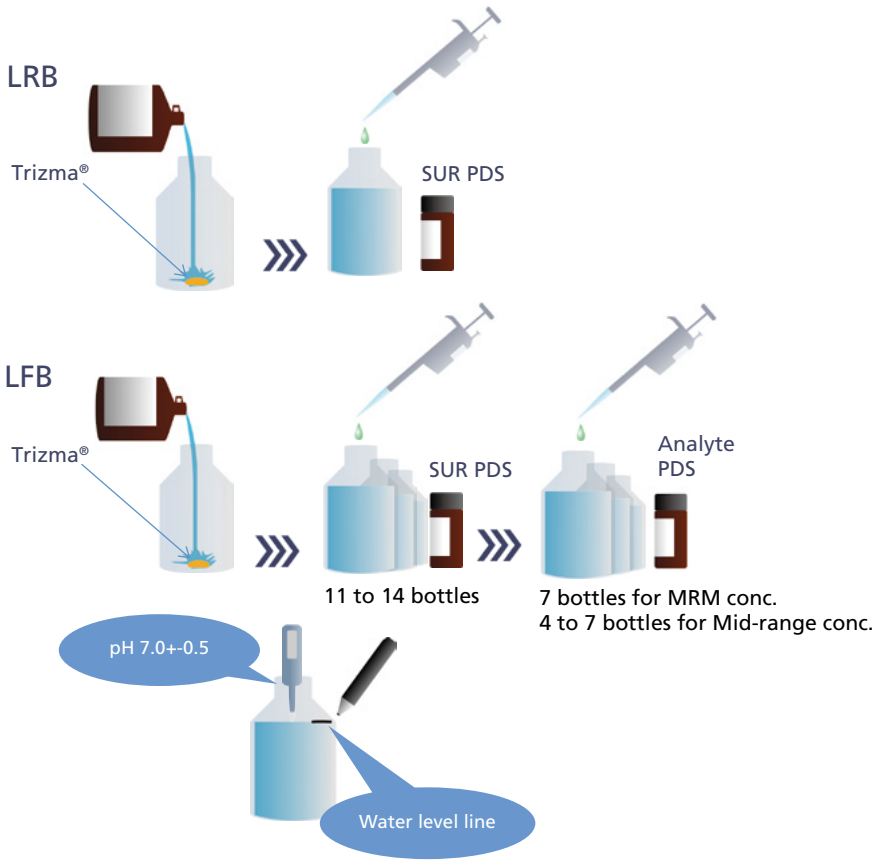
- Laboratory Reagent Blank (LRB)

  Put 1.25 g of Trizma® in the sample bottle and add reagent water until the bottle is almost full, followed by adding 10 µL of SUR-PDS. (3.13)

- Laboratory Fortified Blank (LFB)

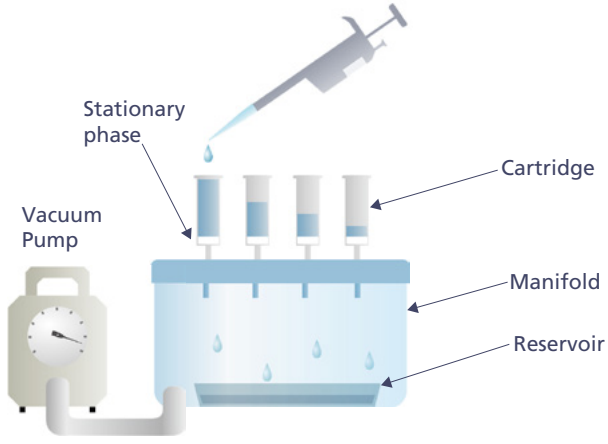
  Put 1.25 g of Trizma® in the sample bottle and add reagent water until the bottle is almost full, followed by adding 10 µL of SUR PDS. Add analyte PDS so that the concentration of solution equals the MRL and mid-range levels in the calibration curve. (3.10) Seven bottles of MRL concentrated LFB must be prepared for MRL confirmation (9.2.6), and four to seven bottles of mid-range concentrated LFB must be prepared for precision and accuracy. (9.2.3, 9.2.4)

Measure the pH of each sample to check the range is between 7.0±0.5, and mark each bottle to indicate the height of the liquid level. (11.3.1)

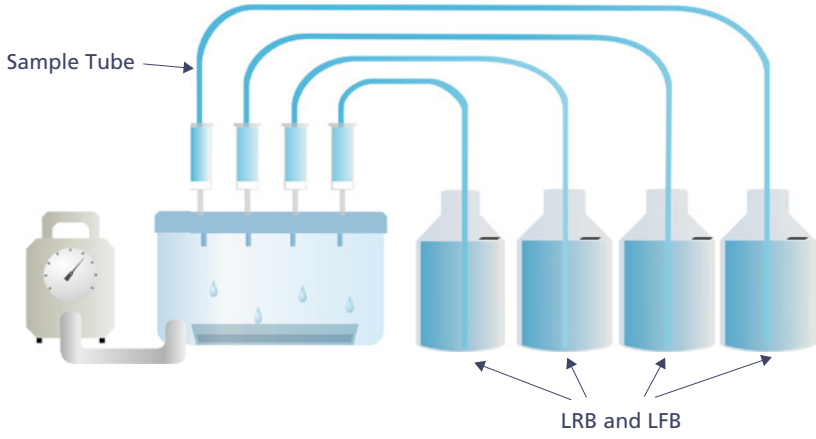


1-4. Extract QC sample

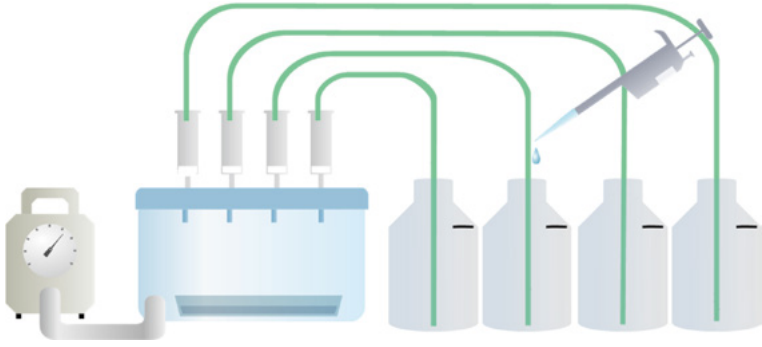
Place the reservoir inside the manifold, close the manifold, and connect the SPE cartridges and vacuum pump. Rinse each cartridge in sequence with 15 mL of methanol and 18 mL of reagent water. (11.4.1)



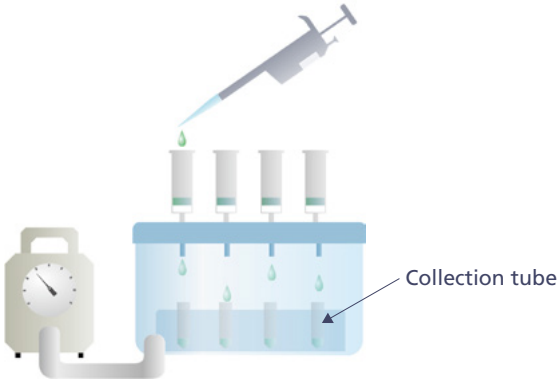
Connect the sample introduction tubes and then start the vacuum operation. Adjust the output of the vacuum pump so that the flow rate is 10 - 15 mL/min. (11.4.2)



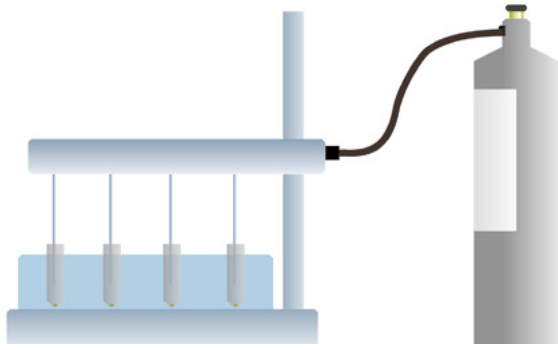
When the sample is depleted, add 7.5 mL of reagent water to each sample bottle to rinse them. Repeat it twice. Dry the cartridges at high vacuum for 5 minutes. (11.4.3)



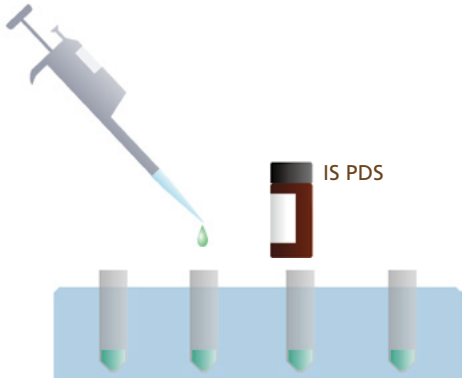
Stop the vacuum, open the manifold lid, remove the reservoir, and insert the collection tubes. Rinse the sample bottles with 4 mL of methanol and elute the analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 4-mL aliquot of methanol. (11.4.4)



Warming the collection tubes in a water bath at 60 to 65 °C, dry the solution collected in the collection tubes by using a nitrogen purge. (11.5)



Add 10 µL of IS PDS and the 96/4 methanol/water solution to obtain a final volume of 1 mL. (11.5)



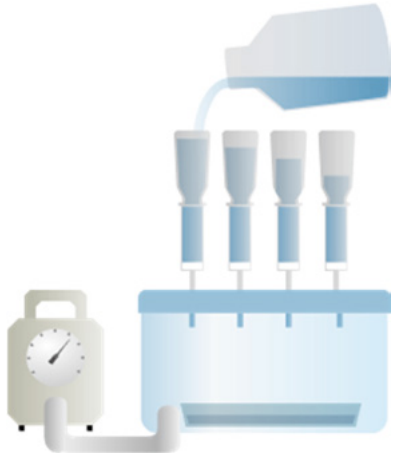
Fill the empty sample bottle with water up to the mark indicating the height of the liquid level prior to extraction, then pour this water into a measuring cylinder to determine the sample volume. It is also possible to measure the weight to determine the sample volume. Assume a sample density of 1.0 g/mL. (11.6)

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.



1-5. Prepare the calibration curve sample

Prepare the Calibration Standards and Quality Control Samples (QCS). In both cases, use 96/4 methanol/water solution for the sample solvent.

- Calibration Standards

  Dilute the Analyte PDS prepared in 2-1-2 to obtain the sample concentration determined in 2-1-1, and then add IS PDS and SUR PDS for designated concentration. (Typically 10 µL for both) (7.2.4)

- 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. Different vendors or lot of the product should be used. (9.3.10)

1-6. 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.

1-7. Caluculate and verify the calibration curve

Run all of the Calibration Standards and QCS prepared in 2-1-5, and calculate a calibration curve.

- Calibration curve must be calculated with the internal standard calibration technique.
- Forcing the calibration curve through the origin is required.
- Weighting may be used.

✓ QC check

Initial Calibration (10.2.7)

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). And all surrogates must be 70 – 130%.

Quality Control Sample (9.3.10)

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

1-8. Verify the system background

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

✓ QC check

Demonstration of low system background (9.3.1)

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

1-9. Verify the MRL

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

✓ QC check

MRL confirmation (9.2.6)

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*

1-10. Verify the reproducibility and accuracy

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

✓ QC check

Demonstration of precision (9.2.3)

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

Demonstration of accuracy (9.2.4)

Make sure the mean recovery is within ± 30% of the true value.

1-11. Check the peak asymmetry factor

✓ QC check

Initial Demonstration of Peak Asymmetry Factor (9.2.5)

The peak asymmetry factor for the first two eluting peaks in a mid-level CAL standard.

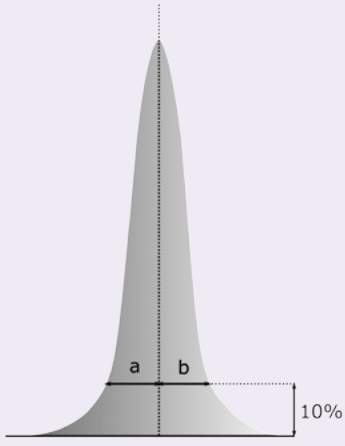
$$As = \frac{b}{a}$$

where:

As = peak asymmetry factor

b = width of the back half of the peak measured (at 10% peak height) from the trailing edge of the peak to a line dropped perpendicularly from the peak apex.

a = the width of the front half of the peak measured (at 10% peak height) from the leading edge of the peak to a line dropped perpendicularly from the apex.

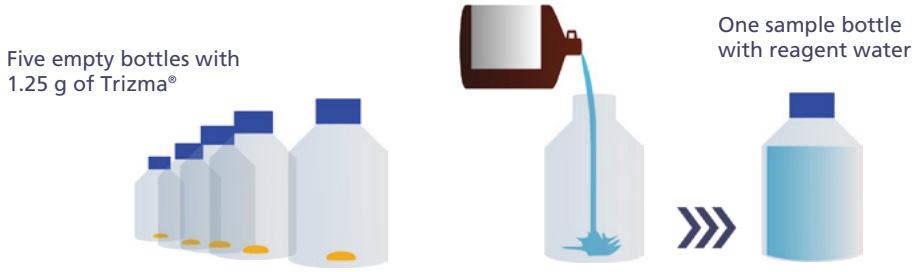


Make sure the asymmetry factor falls in the range of 0.8 to 1.5.

2 Sample Analysis

2-1. Prepare for sampling

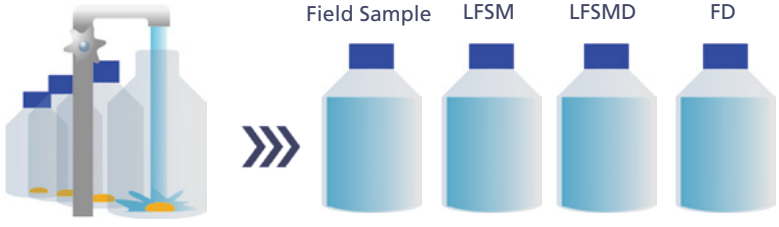
Prepare six 250 mL sample bottles and caps made of polypropylene, and put 1.25 g of Trizma® in five of these bottles. (8.1, 8.2) Fill one sample bottle with reagent water that was confirmed with no contamination in the LRB analysis. (8.3.3) Ship all six sample bottles to the sample collection site.



2-2. Collect the samples

Open the faucet and wait until the temperature is stable. And then, flow the water into the four empty bottles until they are almost full. (8.2)

Use one bottle as field sample, and three remaining botlles are used as Laboratory Fortified Sample Matrix (LFSM), Laboratory Fortified Sample Matrix Duplicate (LFSMD) and Field Duplicate (FD). (3.11, 3.12, 3.7)



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). (8.3.3)

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 10 µL of SUR PDS.

- LFSM and LFSMD

  Add 10 µL of SUR 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.

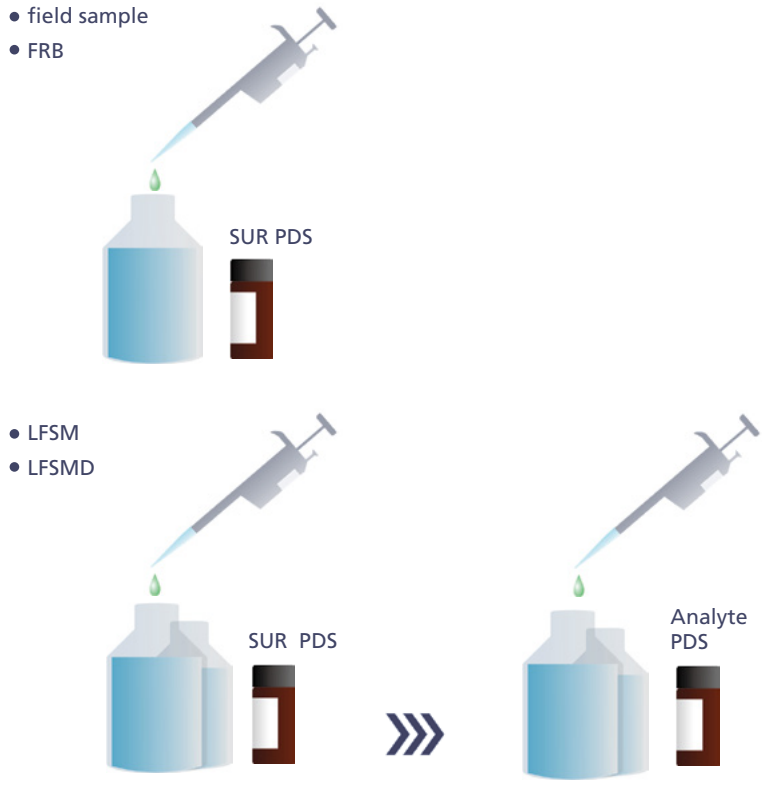
- LFB and LRB

  Prepare them as the same manner described in 2-1-3.

- Continuing Calibration Check (CCC)

  Prepare low, mid, and high concentration calibration standards in the same manner as Calibration Sandards described in 2-1-6. When Calibration Standards remain, they may be used as CCC. (7.2.4)

Measure the pH of each sample to check the range is between 7.0 ± 0.5, and mark each bottle to indicate the height of the liquid level. (11.3.1)



2-4. Extract the QC samples

Extract the QC samples(Field Sample, FRB, LFSM, LFSMD) following the same procedure in 2-1-4. Refer to the relevant sections listed below for details on the necessity of performing extraction other than for the sample.

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)	- Start batch always with CCC (10.3) - CCC concentration needs to be MRL or lower when beginning batch (10.3) - CCC can be substituted with Calibration Standards (7.2.4)
2	LRB	- Need LRB in each extraction batch (9.3.1)
3	LFB	- Need at least one LFB in an extraction batch (9.3.3) - The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch (9.3.3)
4	LFSM	- Need at least one LFSM in an extraction batch (9.3.6)
5	LFSMD or FD	- Need at least one LFSMD in an extraction batch (9.3.7)
6	Field Sample	
⋮		
15	Field Sample	
16	CCC	- CCC needs to be analyzed after every 10 samples (10.3)
17	Field Sample	
⋮		
26	Field Sample	
27	CCC	- CCC needs to be analyzed after every 10 samples (10.3) - One analysis batch must be within a 24-hour period and no more than 20 field samples (3.1)

2-6. Check QC while running the batch

✓ QC check

Continuing Calibration Check (CCC) (10.2.7)

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) (9.3.1)

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) (9.3.3)

For analytes fortified at a concentration near the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified for the other concentrations.

✓ QC check

Laboratory Fortified Sample Matrix (LFSM) (9.3.6)

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 a concentration near the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified for the other concentrations.

✓ QC check

Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD) (9.3.7)

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 is near the MRL).

✓ QC check

Internal Standard (9.3.4)

For all samples, Peak area counts for each internal standard must be within 50–150% of the average peak area in the initial calibration and 70-140% from the most recent CCC.

✓ QC check

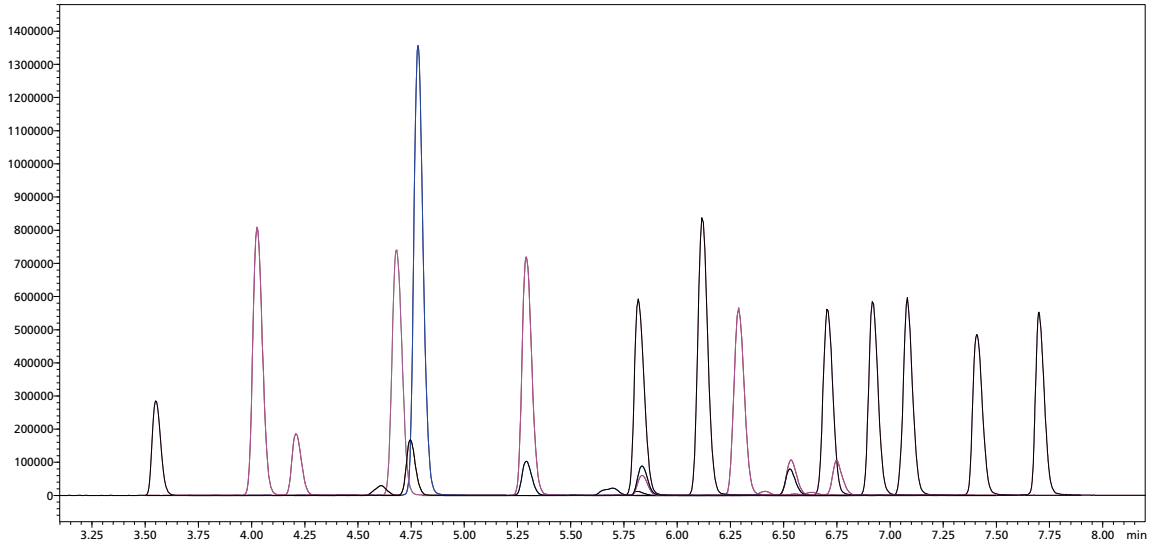
Surrogate Standards (9.3.5)

For all samples, make sure all recoveries are 70% - 130% of the true value.

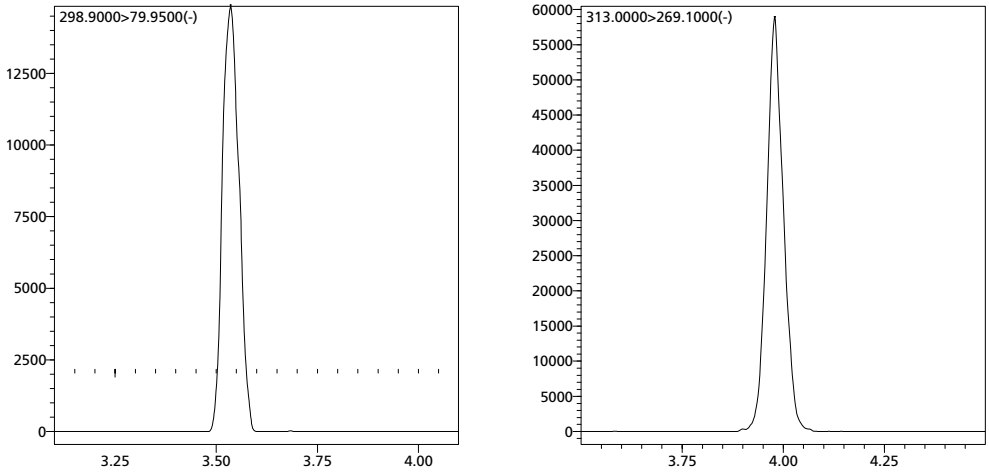
Chromatogram Examples

Shown below are the chromatogram examples.

100 ppb of standards.



Peak shape of first two eluting analytes (PFBS (left) and PFHxA (right)). The asymmetry factors are 1.167 and 1.175, respectively.



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