

HOW TO

Set Up Your Lab for PFAS Analysis by Method 533

LC-MS/MS Instrument

- Shimadzu's LC-MS/MS 8050 with Nexera HPLC 40 Series UHPLC
- Shimadzu's delay and analytical columns
- Optional PFAS kit (Read about [Essentials for PFAS Analysis](#))
- Computer
- Gases supply
 - Nitrogen (generator or Dewar tanks)
 - Argon
 - Air (generator)
- Dedicated space, with power outlets, exhaust vents, containers for solvent waste and water waste (generator only)
- Training at Shimadzu's Customer & Education Training Center (CETC) or at your site (Attendance Certificate provided)

Supplies for Sample Preparation

- EPA 533 requires sample preconcentration by solid phase extraction (SPE)
 - Use SPE cartridges containing weak anion exchange, mixed-mode polymeric sorbent
 - Use of manual or automated SPE apparatus is allowed

Standards, Other Reagents and Consumables

(See section *What You Need to Prepare Before Running Your First PFAS Samples* for more info)

- Analytical standards: [Target Analytes](#), [Isotope Performance Standards and Isotope Dilution Analogues](#)
- Reagents (solvents, buffers and other chemicals)
 - Reagent water – LCMS reagent grade
 - Methanol – LCMS reagent grade
 - Isopropanol – LCMS reagent grade
 - Ammonium acetate – LCMS reagent grade
 - Concentrated ammonium hydroxide
 - Sodium phosphate dibasic
 - Sodium phosphate monobasic
 - Sodium hydroxide
 - Acetic acid
 - Shimadzu tuning solution
- Consumables
 - Sample containers
 - Propylene vials
 - Propylene centrifuge tubes
 - Autosampler vials
 - Microsyringes and pipettes
 - Propylene volumetric flasks or graduated cylinders



[Access the detailed list of recommended consumables here](#)



It is recommended to always test products for PFAS contamination when starting new lot numbers and manufacturers.

WHAT YOU NEED TO PREPARE BEFORE Running Your First PFAS Samples by Method 533

Reagents

- 0.1 M phosphate buffer pH 7.0
- 1 g/L ammonium acetate in reagent water (solvent for SPE)
- 2% ammonium hydroxide in methanol (solvent for SPE)
- 20% reagent water in methanol (solvent for reconstituting extracts and preparing calibration and QC standards)
- 5 mM ammonium acetate in reagent water (mobile phase for LC-MS/MS)

Calibration Curve and Quality Control Standards

Primary dilution standards (PDS), from stock standards, in basic methanol

Analytes

Isotope performance standard

Isotope dilution analogue

Calibration standards

Dilute the analyte PDS to the selected concentration levels

Add isotope dilution analogue and isotope performance standard PDS to each calibration standard to achieve the desired concentration

Quality control standards (QCS) – Prepare as the calibration standards, with a concentration at midpoint of the calibration range, independently from the primary calibration solution; preparation by a second analyst is recommended

Continuing calibration check (CCC) – Calibration standards may be used as CCCs

i When preparing calibration standards, remember to add the isotope dilution analogue at the equivalent concentration to that in the field samples after SPE.

Typical concentration range of calibration is < 2–200 ng/L; it must include at least 5 calibration levels.

Laboratory Quality Control Samples

For initial demonstration of capabilities

- Laboratory reagent blank (LRB) – Reagent water, ammonium acetate, isotope performance standard and isotope dilution analogue solutions
- Laboratory fortified blank (LFB) – Reagent water, ammonium acetate, analytes, isotope performance standard and isotope dilution analogue solutions
- For method reporting limit (MRL) confirmation: 7 replicates of LFB at MRL concentration
- For determination of precision and accuracy: 7 replicates of LFB at midrange concentration

For routine analysis

- Laboratory reagent blank (LRB) – Reagent water, ammonium acetate, isotope performance standard and isotope dilution analogue solutions
- Laboratory fortified blank (LFB) – Reagent water, ammonium acetate, analytes, isotope performance standard and isotope dilution analogue solutions

Field Quality Control Samples (for routine analysis only)

These samples are collected in the field, with ammonium acetate as preservative, and processed in the lab

- Field duplicate (FD) – Replicated samples collected separately but simultaneously with the field samples; in the lab, they are processed separately from field samples, with isotope performance standard and isotope dilution analogue; they can be used to prepare LFSM and LFSMD
- Field reagent blank (FRB) – Aliquot of reagent water (that travelled to the field with sampling supplies and was treated as a field sample), with isotope performance standard and isotope dilution analogue solutions
- Laboratory fortified sample matrix (LFSM) – FD, with added analytes, isotope performance standard and isotope dilution analogue solutions
- Laboratory fortified sample matrix duplicate (LFSMD) – FD of the sample used for LFSM, with added analytes, isotope performance standard and isotope dilution analogue solutions

PREPARE YOUR SAMPLES FOR PFAS Analysis by Method 533

Run your initial demonstration of capabilities when your lab starts the analysis of PFAS by EPA 533 for the very first time, or when a method modification happens. You can start your routine analysis afterwards.



* Rotate concentration between extraction batches: low, medium, high.

† Analysis is required only when target PFAS are detected in a sample set.

†† Either 1 FD or LFMSD must be used in each extraction batch.

Steps	Extraction Batch Extract within 28 days of sample collection; keep at 6 °C until extraction without freezing	Initial Demonstration of Capabilities			Routine Analysis							
		Process	LRB	LFB for MRL	LFB for P&A	LRB	LFB*	FRB†	Field Samples	FD††	LFMS	LFMSD††
NUMBER OF SAMPLES REQUIRED			1	7	7	1	≥ 1	1	As needed (max 20)	≥ 1 or LFMSD	≥ 1	≥ 1 or FD
1	Confirm pH, between 6 and 8 (add acetic acid to decrease pH when needed)											
2	Mark height on bottle (refer to Step 18)											
3	Add standards											
4	Add isotope dilution analogues											
RINSE SPE CARTRIDGES												
5	Add 10 mL methanol											
6	Add 10 mL aqueous 0.1 M phosphate buffer											
7	Add 2-3 mL of phosphate buffer and fill the cartridge with reagent water											
8	EXTRACT SAMPLES @ 5 mL/min											
RINSE BOTTLES (once sample load is completed; pass through cartridges, as during sample loading)												
9	Add 10 mL 1 g/L ammonium acetate											
10	Add 1 mL methanol											
11	DRY CARTRIDGES (with air or nitrogen for 5 min under high vacuum)											
ELUTE SPE CARTRIDGES (use gentle vacuum if needed; collect solvent into collection tubes)												
12	Add 5 mL 2% ammonium hydroxide in methanol (rinse sample bottle before pouring it into cartridge)											
13	Repeat adding 5 mL 2% ammonium hydroxide in methanol (rinse sample bottle before pouring it into cartridge)											
14	DRY ELUTION SOLVENT (under gentle nitrogen stream in a water bath at 55–60 °C)											
15	RECONSTITUTE (with 1 mL of 20% reagent water in methanol)											
16	ADD ISOTOPE PERFORMANCE STANDARDS AND VORTEX											
17	TRANSFER AN ALIQUOT OF EXTRACT TO AUTOSAMPLER VIAL (and remaining volume to 15 mL collection tube for extended storage)											
18	CALCULATE VOLUME EXTRACTED (measure volume needed to reach height marked in Step 2)											

These steps can be done in parallel, but never let the SPE cartridges dry.

Manual or automated extractors operate differently. Follow your manufacturer's instructions.

Large-volume sampling lines may each targeted PFAS. Replace with LLDPE lines or reservoirs whenever possible.

ANALYZE YOUR PFAS SAMPLES IN LC-MS/MS by Method 533

i For the analysis batch, no more than 20 field samples with the proper QC samples should be analyzed on the same instrument during a 24 h period.

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.

For all samples, the recovery of each isotope performance analogue must be within 50–200%.

- Tuning of instrument is only required when significant sensitivity loss is observed.
- Always use fresh mobile phases in clean bottles.
- Before running a batch, run a null injection to confirm the system is PFAS-free.
- Shimadzu's **LabSolutions Insight** software allows you to customize the data processing windows and QC flags to accelerate data review.

Steps	Analysis Batch by LC-MS/MS Analyze within 28 days of extraction; keep extracts at room temperature
PREPARE LC-MS/MS	
1	Turn on the instrument, according to Shimadzu's recommendations
2	Prepare mobile phases and rinse solutions (see page 2 for more info)
CONFIRM LC-MS/MS STATUS	
3	Purge LC system with mobile phase
4	Confirm gas flow and temperatures are at ready state and there are no leaks
5	Equilibrate the column
ANALYZE SAMPLES	
6	Confirm vials and trays are in the correct position in the autosampler
7	Update batch template in Shimadzu's LabSolutions software
8	Run the analytical batch (see example on right)
REVIEW DATA	
9	Open data file in LabSolutions Insight software
10	Review data from calibration standards and controls
11	Review data from samples
12	Export data in custom template according to your lab's data management protocols
13	COMPLETE DATA PACKAGE – by primary analyst
14	COMPLETE DATA PACKAGE REVIEW – by QA reviewer

Analytical Intelligence features of Shimadzu's LC automate these steps, even for remote operations.

Initial Demonstration of Capabilities	Requirements → Acceptance Criteria
Establish retention times for branched isomers	When chromatographic conditions change → All isomers of each analyte within same MRM window
Initial calibration	At least 5 calibration levels. Lowest concentration ≤ MRL calculated with internal standard calibration technique. Forcing the calibration curve through the origin is required. Weighting may be used. → 70% ≤ Recovery ≤ 130% of true value; 50% ≤ Recovery ≤ 150% for lowest standard if concentration is ≤ MRL
Demonstrate low system background	Analyze LRB after highest calibration standard → Concentration of each analyte ≤ 1/3 MRL
Demonstrate precision and accuracy	Analyze LFB (n=7, midrange concentration) → Precision: %RSD ≤ 20%; Accuracy: 70% ≤ Mean recovery ≤ 130% of true value
Confirm MRL	Analyze LFB (n=7, at proposed MRL concentration) Confirm limits of prediction interval of results → Lower PIR: ≥ 50%, Upper PIR: ≤ 150%
Verify calibration	Analyze mid-level QCs, at least quarterly → 70% ≤ Recovery ≤ 130% of true value

Steps	EXAMPLE Analysis Batch – Routine Analysis	Requirements → Acceptance Criteria
0	Calibration	After the initial calibration, as needed per QC guidance → 70% ≤ Recovery ≤ 130% of true value; 50% ≤ Recovery ≤ 150% for lowest standard if concentration is ≤ MRL
1	CCC	Initial CCC (concentration ≤ MRL) → 50% ≤ Recovery ≤ 150% of true value
2	LRB	≥1 mandatory in each analysis batch → Concentration of each analyte ≤ 1/3 MRL
*	LFB, LFSM, LFMSD or FD	Mandatory in each extraction batch, not in each analysis batch → Fortified samples: 70% ≤ Recovery ≤ 130% of true value; 50% ≤ Recovery ≤ 150% if fortified concentration is ≤ 2xMRL Duplicated samples: %RPD ≤ 30% (or ≤ 50% if target concentration is ≤ 2xMRL)
3	Field sample	
12	Field sample	
13	CCC	Analyze every 10 field samples, alternating mid and high calibration level
14	Field sample	
23	Field sample	
24	CCC	Closing CCC (mid or high calibration level)