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HOW TO
Set Up Your Lab for PFAS Analysis by Method 537.1

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 537.1 requires sample preconcentration by solid phase extraction (SPE)
  - ☐ Use SPE cartridges containing styrenedivinylbenzene (SDVB) single polymer (copolymers not allowed)
  - ☐ 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, Surrogates and Internal Standards
- ☐ Reagents (solvents, buffers and other chemicals)
  - ☐ Reagent water – LCMS reagent grade
  - ☐ Methanol – LCMS reagent grade
  - ☐ Isopropanol – LCMS reagent grade
  - ☐ Ammonium acetate – LCMS reagent grade
  - ☐ Sodium hydroxide
  - ☐ Acetic acid
  - ☐ Trizma® pre-set crystals pH 7
  - ☐ 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 537.1

Reagents

- ☐ 4% 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
- ☐ Calibration standards
  - ☐ Dilute the analyte PDS to the selected concentration levels
  - ☐ Add surrogates and internal standards 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 (from a second vendor or different lot of standard); preparation by a second analyst is recommended
- ☐ **Continuing calibration check (CCC)** – Calibration standards may be used as CCCs

When preparing calibration standards, remember to add the surrogates 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 covering a 20-fold range.

Laboratory Quality Control Samples

For initial demonstration of capabilities

- ☐ Laboratory reagent blank (LRB) – Reagent water, Trizma pre-set crystals, surrogates and internal standards
- ☐ Laboratory fortified blank (LFB) – Reagent water, Trizma pre-set crystals, analytes, surrogates and internal standards
- ☐ 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, Trizma pre-set crystals, surrogates and internal standards
- ☐ Laboratory fortified blank (LFB) – Reagent water, Trizma pre-set crystals, analytes, surrogates and internal standards

Field Quality Control Samples (for routine analysis only)

These samples are collected in the field, with Trizma pre-set crystals 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 surrogates and internal standards; 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), Trizma pre-set crystals, surrogates and internal standards
- ☐ Laboratory fortified sample matrix (LFSM) – FD, with added analytes, surrogates and internal standards
- ☐ Laboratory fortified sample matrix duplicate (LFSMD) – FD of the sample used for LFSM, with added analytes, surrogates and internal standards

PREPARE YOUR SAMPLES FOR PFAS Analysis by Method 537.1

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Run your initial demonstration of capabilities when your lab starts the analysis of PFAS by EPA 537.1 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.

† Initially analyzed as LRB to ensure there is no contamination from reagent water and sampling bottles. Afterwards, 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 14 days of sample collection; keep at 6 °C until extraction without freezing Process	Initial Demonstration of Capabilities			Routine Analysis						
		LRB	LFB for MRL	LFB for P&A	LRB	LFB*	FRB†	Field Samples	FD††	LFSM	LFMSD††
	NUMBER OF SAMPLES REQUIRED	1	7	7	1	≥ 1	1	≥ 1 (max 20)	≥ 1 or LFMSD	≥ 1	≥ 1 or FD
1	Confirm pH is 7 ± 0.5 (add acetic acid to decrease pH if needed)										
2	Mark height on bottle or weigh it to determine actual extracted volume (refer to Step 18)										
3	Add standards										
4	Add surrogates										
	RINSE SPE CARTRIDGES										
5	Add 15 mL methanol										
6	Add 18 mL reagent water										
7	Add 2–3 mL of reagent water										
8	**EXTRACT SAMPLES** @ 10–15 mL/min										
	RINSE BOTTLES (once sample load is completed; pass through cartridges, as during sample loading)										
9	Add 7.5 mL of reagent water										
10	Repeat adding 7.5 mL of reagent water										
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 4 mL methanol (rinse sample bottle before pouring it into cartridge)										
13	Repeat adding 4 mL methanol (rinse sample bottle before pouring it into cartridge)										
14	**DRY ELUTION SOLVENT** (under gentle nitrogen stream in a water bath at 60–65 °C)										
15	**RECONSTITUTE** (with required volume of 96%:4% [methanol:water])										
16	**ADD INTERNAL STANDARD 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 or weight of empty bottle [see 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 leach targeted PFAS. Replace with LLDPE lines or reservoirs whenever possible.

Combined volume must equal 1 mL.

ANALYZE YOUR PFAS SAMPLES IN LC-MS/MS by Method 537.1

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

The recovery of surrogate must be within 70–130% for all injections.

- ☐ Tuning of instrument is only required when significant sensitivity loss is observed.
- ☐ Always use fresh mobile phases in clean bottles.
- ☐ For compounds with linear and branched isomers, quantitate field samples by integrating and summing all peaks identified in the standards.
- ☐ 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.

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

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 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

Initial Demonstration of Capabilities	Requirements → Acceptance Criteria
Demonstrate low system background	Analyze LRB prior to any other step → **Concentration of each analyte ≤ 1/3 MRL**
Calculate peak asymetry factor	Calculate peak asymmetry factor for the first 2 eluting compounds in a mid-level calibration standard → **0.8–1.5**
Establish retention times for branched isomers	When chromatographic conditions change → **Qualitative approach for determining retention time**
Initial calibration	At least 5 calibration levels. Lowest concentration ≤ MRL. Calculate with internal standard calibration techniques (linear or quadratic fitting). 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**
Demonstrate of 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 as part of IDC and when preparing new standards or 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; confirm peak asymmetry factor → **Targets: 70% ≤ Recovery ≤ 130% of true value; 50% ≤ Recovery ≤ 150% for lowest standard**
1	CCC	Initial CCC (concentration ≤ MRL) → **Targets: 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 ~MRL; Duplicated samples: %RPD ≤ 30% (or ≤ 50% if target concentration is ~MRL)**
3	Field sample	
12	Field sample	
13	CCC	Analyze every 10 field samples, alternating mid and high calibration level → **Targets: 70% ≤ Recovery ≤ 130% of true value**
14	Field sample	
23	Field sample	
24	CCC	Closing CCC (mid or high calibration level) → **Targets: 70% ≤ Recovery ≤ 130% of true value**