Method 1633

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Objectives

- *Provide an overview of the requirements for analysis of PFAS samples by EPA Method 1633 in accordance with the requirements of the DoD QSM, Table B-24*
  - *Focus on what has changed from Table B-15 compliant methods*
  - *Future of EPA 1633*
  - *Future of DoD ELAP requirements*

EPA 1633 Basics

- *Applicable to all matrices other than DW*
  - *Validated for groundwater (GW), surface water (SW), wastewater (WW), landfill leachate (LC), soil (SS), sediment (SD), biosolid (BS), & tissue (TS)*

- *Currently Version is Revision 2.0 (https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas)*

- *40 PFAS, includes all PFAS applicable to EPA 537.1, 533, & SW-846 Method 8327 and 8 additional analytes:*

NEtFOSA	*3:3FTCA*
NMeFOSA	*5:3FTCA*
NEtFOSE	*7:3FTCA*
NMeFOSE	*PFDoS*

EPA 1633 Basics

- *Media specific sample preparation procedures (aqueous, solid, tissue)*
- *Analysis by LC-MS/MS method using isotope dilution/extracted internal standard quantitation*
- *Validated by the Strategic Environmental Research and Development Program (SERDP)*
  - *Single-laboratory study completed, report published (https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas)*
  - *Multi-laboratory validation nearing completion*

Method Analyte List Comparison

Analyte	EPA 533	EPA 537.1	EPA 1633
11Cl-PF3OUdS	x	x	x
9Cl-PF3ONS	x	x	x
ADONA	x	x	x
HFPO-DA	x	x	x
PFBS	x	x	x
PFDA	x	x	x
PFDoA	x	x	x
PFHpA	x	x	x
PFHxA	x	x	x
PFHxS	x	x	x
PFNA	x	x	x
PFOA	x	x	x
PFOS	x	x	x
PFUnA	x	x	x
4:2FTS	x		x
6:2FTS	x		x
8:2FTS	x		x
NFDHA	x		x
PFBA	x		x
PFEESA	x		x
PFHpS	x		x
PFMBA	x		x
PFMPA	x		x
PFPeA	x		x
PFPeS	x		x
NEtFOSAA		x	x
NMeFOSAA		x	x
PFTeDA or PFTA		x	x
PFTrDA		x	x
NEtFOSA			x
NMeFOSA			x
NEtFOSE			x
NMeFOSE			x
3:3FTCA			x
5:3FTCA			x
7:3FTCA			x
PFOSA			x
PFDoS			x
PFDS			x
PFNS			x

Table B-24 Basics

- *Meet DoD data quality needs*
- *Maintain consistency with EPA 1633*
  - *Include requirements in addition to those in EPA 1633 when more stringent requirements are needed*
  - *Provide temporary QC acceptance criteria until the final method is published*
- *Does NOT include all of the requirements contained in the method*
- *Requirements for DoD ELAP accreditation found in:*
  - *EPA Method 1633, current version*
  - *DoD/DOE QSM, Version 5.4, Table B-24*

Table B-24 Basics

ALL OF THE REQUIREMENTS CONTAINED IN EPA DRAFT METHOD 1633 MUST BE MET. This table contains additional requirements that must be met. Where the name for the QC sample listed in this table differs from EPA Draft Method 1633 terminology, the corresponding EPA Draft Method 1633 terminology is provided in the Comments column.

EPA 1633 Sample Collection

- *Specific requirements for in-field compositing equipment*
  - *HDPE tubing in composting equipment, except for a minimum length of silicon rubber tubing in the pump.*
  - *Integrated flow meter to collect proportional composite samples (Section 6.1.2)*
- *Size, type, & number of sample bottles is specified*
- *Storage & Shipping requirements are provided*
- *Holding times*
  - *Based on results of holding time study*
  - *Dependent on storage temperature and analytes of interest*
- *Project must specify which option is required for their project; NOT the laboratory*

EPA 1633: Sample Collection

Matrices	Type of Container	Sample Size per Sample	Sample Shipping
Groundwater, Surface Water, and Wastewater	One 500 mL HDPE bottle with linerless HDPE or PP caps & one lesser volume (e.g., 125-250 mL) HDPE bottle with linerless HDPE or PP caps, filled to the shoulder of bottle	500 mL for sample analysis & a minimum of 20 mL needed for screening	- Protect from light at 0 - 6˚C from time of collection until shipped. - Maintain 0 - 6˚C during shipping - Receive in lab within 48 hrs of collection
Landfill Leachate	Two 125 mL HDPE bottle with linerless HDPE or PP caps, filled to the shoulder of bottle	100 mL for sample analysis & 100 mL needed for screening	
Soil and Sediment	wide-mouth high density polyethylene (HDPE) jar or bottle with linerless HDPE or polypropylene caps, fill no more than 3/4 full	- 5.0 grams **dry** weight	- Protect from light at 0 - 6˚C from time of collection until shipped. - Maintain 0 - 6˚C during shipping
Biosolids		- 0.5 grams **dry** weight	
Tissue	One 500 mL HDPE bottle with linerless HDPE or PP caps, if whole fish, wrap in aluminum foil or food-grade polyethylene warp	- 2.0 grams homogenized	- Maintain 0 - 6˚C from time of collection until received by lab if received within 24 hrs - If longer transport is needed, recommend freeze prior to shipping and shipment on dry ice, if possible

EPA 1633 Aqueous Sample Holding Times

- *Option 1: Samples Stored at 0 - 6°C, protected from light:*
  - *Microbiological activity can occur when stored at 0 – 6°C*
  - *28 day hold time EXCEPT, when results reflecting the concentrations of PFAS at the site on the day of sample collection are desired, hold time is 7 days*
  - *Precursors (e.g., 6:2 FTS, PFOSA, etc.) transform to other PFAS (e.g., PFOA, PFOS, PFHxS) after 7 days*
- *Option 2: Samples Stored at ≤ - 20°C, protected from light:*
  - *90 day hold time*
  - *Precursor transformation rates are drastically slowed*
  - *Temperature prevents microbial growth that can cause issue when using SPE technique*

EPA 1633 Solid & Biosolids Sample Holding Times

- *Solid Samples Stored at 0 - 6°C or at ≤ - 20°C, protected from light for up to 90 days:*
  - *Analyze as soon as possible if NFDHA is important*
  - *Microbiological activity can occur when stored at 0 – 6°C*
- *Biosolid Samples Stored at 0 - 6°C or at ≤ - 20°C, protected from light for up to 90 days:*
  - *Microbiological activity can occur when stored at 0 – 6°C*

EPA 1633 Sample Extract Holding Times

- *Aqueous sample extracts stored at 0 - 4°C, protected from light for up to 90 days, except analyze up to 28 days from extraction if ether sulfonates (9Cl-PF3ONS, 11Cl-PF3OUdS, and PFEESA) are important analytes*
- *Solid sample extracts stored at 0 - 4°C, protected from light for up to 90 days, except analyze as soon as possible if NFDHA is important analytes*
- *Biosolid sample extracts stored at 0 - 4°C, protected from light for up to 90 days*

Laboratory Holding Time Summary

Matrices	Stored at 0 - 6 ºC, protected from light		Stored at ≤ -20 ºC, protected from light	
	Holding Time	Exceptions	Holding Time	Exceptions
Samples				
Aqueous	28 days	Precursor degradation after 7 days	90 days	None
Solid	90 days	NFDHA - analyze as soon as possible	90 days	NFDHA - analyze as soon as possible
Biosolid	90 days	Microbiological activity likely	90 days	None

Matrices	Stored at 0 - 4 ºC, Protected from light	
	Holding Time	Exceptions
Extracts		
Aqueous	90 days	28 days for ether sulfonates
Solid	90 days	28 days for NFDHA
Biosolid	90 days	

EPA 1633 Sample Preparation

- *Aqueous samples must be screened using a different container (e.g., 125 or 250 mL container) than the container used for preparation and analysis (500 mL for GW, SW, WW, 100 mL for landfill leachates)*
- *Detailed procedures for each matrix type:*
  - *All samples undergo SPE and carbon cleanup, regardless of concentrations of PFAS in sample*
  - *Once QC acceptance criteria is included for a sample matrix in the method, a carbon cartridge may be used for that sample matrix in lieu of loose carbon ONLY IF THE ACCEPTANCE CRITERIA IN THE METHOD CAN BE MET.*

EPA 1633 Sample Preparation

- *Aqueous samples – whole sample is extracted via SPE & extracts undergo carbon clean-up*
- *Solid samples (soil, sediment, biosolids, tissue) – undergo liquid extraction & the extracts are clean-up up via SPE & carbon clean-up*
- *Prescreening indicated high concentration PFAS in samples – an aliquot of sample is diluted (Appendix B of EPA 1633) & the dilution undergoes the same processes as all other aqueous samples (extracted via SPE & extracts undergo carbon clean-up)*

EPA 1633 Sample Preparation

- *AFFF Samples: Table B-24 requires AFFF samples be subsampled in duplicate for analysis in accordance with DoD AFFF01, Section 11.2.1 through 11.2.9. Note: In lieu of the LCSD required in Section 11.2.6 of DoD AFFF01, one MS/MSD pair must be prepared with each batch of AFFF samples.*
- *Batch QC Samples include:*
  - *Method Blank (MB)*
  - *Laboratory Control Sample (LCS),*
  - *Low-Level LCS (LLLCS), spiked at two times the LOQ*
  - *Matrix Spike & Matrix Spike Duplicate (Table B-24)*
  - *Matrix Duplicate (MD), applicable to AFFF samples only (Table B-24)*

EPA 1633 Sample Preparation

- *24 Extracted internal standards (EISs) are spiked into each sample prior to extraction (whole water samples spiked in sample container)*
- *7 Non-extracted internal standards (NISs) are spiked into each extract prior to analysis*

EPA 1633 Sample Analysis

Isomeric Mixtures:

- *If a qualitative or a quantitative standard containing branched and linear isomers of an analyte is commercially available, the sum of the branched and linear isomers of the analyte is reported*
- *If a quantitative standard containing branched and linear isomers of an analyte is commercially available, it must be used in the calibration standards*
- *Quantitative isomeric mixtures currently commercially available for: PFOS, PFHxS, NMeFOSAA, NEtFOSAA, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, & NEtFOSE*

EPA 1633 Sample Analysis

Isomeric Mixtures:

- *If a qualitative standard containing branched and linear isomers of an analyte is commercially available, it must be included in the Qualitative Identification Standard that is analyzed daily*
- *Retention time window must encompass all isomers*
- *Qualitative Isomeric Mixtures currently commercially available for: PFOA & PFNA (previously included PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, & NEtFOSE)*

EPA 1633 Sample Analysis

Bile Salt Interference Check Standard:

- *Analyzed daily, regardless of matrix type of samples (Table B-24)*
- *Includes Taurodeoxycholic Acid (TDCA) only if acetonitrile is a mobile phase*
- *Includes Taurodeoxycholic Acid (TDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA) if acetonitrile is NOT a mobile phase*
- *Elution time of bile salts peaks must be at least one minute from the retention time WINDOW of PFOS (which includes all isomers)*

EPA 1633 Sample Analysis

- *Table B-24 requires quantification ion masses identified in method to be used for analytes that have commercially available isomeric mixtures of the analyte (PFOA, PFNA, PFOS, PFHxS, PFOSA, NMeFOSAA, NEtFOSAA, NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE)*
  - *If interferences render quant ion unusable, project approval is required before using alternative ion*
- *Mass calibration per manufacturer's instructions*
- *Mass calibration verification requires at least mass unit resolution & peak apex to be within 0.2 Dalton (amu) of expected masses*

EPA 1633 Sample Analysis

- *Calibration criteria:*
  - *minimum of 6 calibration levels (7 for non-linear)*
  - *lowest calibration level must meet a signal-to-noise ratio of 3:1 and be set at a concentration at or below LOQ*
  - *5 of the 6 calibration levels (6 of the 7 for non-linear) must be within the quantitation range (i.e., LOQ to highest calibration standard)*
  - *EIS and NIS masses the same in each calibration level*
  - *Evaluate either the RSD of the RFs or RRs of the analytes and isotopically-labeled compounds or the RSE of the analytes and isotopically-labeled compounds is ≤ 20%*

EPA 1633 Sample Analysis

- *Daily analysis of an Instrument Sensitivity Check (ISC); concentration of analytes must be at the LOQ; Table B-24 requires recoveries must be within 70-130% of true value for all analytes*
- *Table B-24 requires Initial Calibration Verification (ICV) is required to be made from a second source standard, must be analyzed after ICAL, & must recover within 70-130% of true value for all analytes*
- *Continuing Calibration Verification (CCV) is prepared at the mid-level of the calibration, must bracket every 10 samples (maximum), & must recover within 70 – 130% of true value for all analytes and EIS compounds*

EPA 1633 Sample Analysis

- *Method Blank (MB) concentrations must be ≤ ½ LOQ or 1/10th the amount measured in the sample or ≤ 1/10th the regulatory limit, whichever is greater (criteria of Table B-24)*
- *LCS spiked at mid-level of calibration*
- *LLLCS spiked at 2 x LOQ*
- *LCS and LLLCS preliminary acceptance limits are 40 -150% recovery (criteria of Table B-24)*
- *LCS and LLLCS inhouse lower limit must be ≥ 40% recovery (criteria of Table B-24)*

EPA 1633 Sample Analysis

- *MS/MSD:*
  - *Spike at 3 to 5 times the native concentration; if sample was not screened, spike at mid-level concentration*
  - *Recoveries must be within LCS/LLLCS limits and RPD of MS/MSD must be ≤ 30% (criteria of Table B-24)*
- *Sample/MD RPD must be ≤ 30% (criteria of Table B-24)*
- *EIS preliminary acceptance limits are 20 -150% recovery (criteria of Table B-24)*
- *EIS inhouse lower limit must be ≥ 20% recovery (criteria of Table B-24)*
- *NIS recoveries must be > 30% of the average areas of the calibration standards (criteria of Table B-24)*

EPA 1633 Analyte Identification

- *Signal-to-noise ratio of analyte must be at least 3:1*
- *Ion Ratio (total quantitation ion: total confirmation ion) of analyte:*
  - *Must fall within ± 50% of ratio observed in mid-level ICAL standard if concentration in sample is > LOQ*
  - *Must fall within ± 50% of ratio observed in initial daily CCV if concentration in sample is > MDL but < LOQ*
- *Analyte concentrations reported in acid form*
- *Soil, sediment, & biosolids reported on dry-weight basis; tissues wet-weight*

Future of EPA 1633

- *Multi-laboratory validation study data will be used to revise the method*
  - *Include modifications/clarifications to procedures*
  - *Include media type-specific acceptance criteria*
- *First revision of method will include WW acceptance criteria*

Future of DoD ELAP Requirements

- *Table B-24 will be revised*
  - *Preliminary and inhouse recovery criteria for LCS, LLCS, MS, MSD, MD, EIS, & NIS will be replaced by criteria in final method as they become available*
  - *Criteria for analytical QC will be updated to be consistent with method*
- *Addition of LCS/LLCS data in Appendix C of QSM*
- *Module 6 PFAS Data Validation Guidelines will be revised*

Thank you for your attention

Questions???

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