UNITED STATES DEPARTMENT OF DEFENSE

Data Validation Guidelines Module 6:

Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-24

Environmental Data Quality Workgroup

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Data Validation Guidelines Module 6

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Module 6: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by Quality Systems Manual for Environmental Laboratories (QSM) Table B-24

1.0 Purpose

This document provides guidance on the validation of data generated by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis for per- and polyfluoroalkyl substances (PFAS) by EPA Method 1633 compliant with DoD/DOE Quality Systems Manual (QSM) Table B-24 criteria in solid, biota, aqueous film forming foam (AFFF), and aqueous matrices. The objective of this procedure is to provide the end user with a clear understanding of the quality and limitations of the data through documented validation procedures and to encourage consistency in the validation and reporting of PFAS data generated for Department of Defense (DoD) projects when analyzed by EPA Method 1633. The designation of EPA Method 1633 in this document refers to the most recent version of the method published by the EPA, including draft versions, if applicable. The users of this document should apply these data validation procedures to definitive data only.

Project-specific requirements as identified in the Quality Assurance Project Plan (QAPP) should always supersede the requirements of this document.

This document assumes the user is familiar with data validation conventions and qualifiers used in the *DoD General Data Validation Guidelines (**Rev. 1, 2019**).* This document is not intended to obviate the need for professional judgment during the validation process. If a validator feels that the data cannot be reported as required by the QAPP in a scientifically defensible manner, they should use the QAPP point of contact to discuss their concerns.

This document references the *Uniform Federal Policy for Quality Assurance Project Plans (UFP-QAPP) Optimized Worksheets (March 2012)*. Other QAPP formats are equally acceptable as determined by the project team.

2.0 Procedure

2.1 Introduction

This document was written with primary consideration to EPA Method 1633 compliant with QSM, Table B-24. Appendix A summarizes these additional QC criteria included in QSM Version 5.4. It does not include all of the QC criteria included in EPA Method 1633. Validation should proceed using the acceptance criteria for the QSM version specified in the laboratory data deliverable or in the QAPP.

2.2 Deliverables

Laboratory data deliverables consist of a combination of forms and raw data. The manner in which laboratories label their forms is not dictated nor specified. **The labeling convention below is used for simplicity.**

- Cover Sheet
- Table of Contents
- Case Narrative
- Transition Ion Summary
- Sample Results Summary or equivalent Laboratory Report
- Chain of Custody (CoC) forms, Laboratory Receipt Checklists, and other supporting records
- Field QC forms and supporting records
- Sample Ion Ratio Summary
- Extracted Internal Standard Recovery and Retention Time Summary
- Non-Extracted Internal Standard Recovery and Retention Time Summary
- Laboratory Control Sample/Low-Level Laboratory Control Sample Recovery Summary
- Matrix Spike/Matrix Spike Duplicate Recovery and Relative Percent Difference Summary
- Matrix Duplicate Recovery and Relative Percent Difference Summary
- Method Blank Summary
- Sample and Extract Dilution and Reanalysis Summary
- Bile Salt Interference Check Summary
- Qualitative Identification Standard Summary
- Sequence and Preparation Logs (or equivalent to include Instrument Blanks)
- Instrument Performance Check Summary (mass calibration and mass calibration verification)
- Initial Calibration Summary (any equivalent to include the Initial Calibration Analyte and Extracted Internal Standard (EIS) Responses, Analyte and EIS Concentrations, Isomeric Profiles, Response Ratios, Response Factors, Relative Standard Deviation or Relative Standard Error of RR and RFs)
- Initial/Continuing Calibration Verifications and Instrument Sensitivity Check Summary
- Instrument Blank Summary
- Standards traceability forms and worksheets (including Manufacturer provided Certificate of Analysis for Standards)
- Raw Data- including quantitative and confirmation transition ion chromatograms, peak areas, and ion ratios

2.3 Validation Stages

The types of laboratory data deliverables, staged data validation, and the relationship between the two are outlined in the *DoD General Data Validation Guidelines*.

Stage 1 data validation consists of a review of sample results forms, associated sample receipt summaries (chain of custody), and field QC data.

Stages 2A and **2B** data validation consist of review of summary forms only.

Stages 3 and **4** data validation require review of both summary forms and all associated raw data.

Both the laboratory deliverable and the stage of validation should be specified in the QAPP or other planning documents. Data review guidelines and how they apply to the different validation stages are indicated in the following sections.

Note: Any required stage of validation that reveals significant deviations from project requirements will require a higher stage of validation to uncover the source. Data validators are encouraged to communicate with their points of contact identified in the project QAPP (such as the UFP-QAPP Worksheet #6) to resolve discrepancies.

3.0 Stage 1 Validation

To ensure that the analytical method protocols outlined in the QAPP were performed (*representativeness*); to verify sampling and reporting *completeness*; to evaluate the performance of field blanks; and to verify compliance with project *sensitivity* needs, the following documents should be reviewed:

- Cover Sheet
- Table of Contents
- Case Narrative
- Transition Ion Summary
- Sample Results Summary or equivalent Laboratory Report
- Chain of Custody (CoC) forms, Laboratory Receipt Checklists, and other supporting records
- Field QC forms and supporting records

Stage 1 is the validation of investigative and field QC samples**.**

3.1 Sample Results

Examine the Laboratory Report sample results and verify the following information, ensuring that:

- Holding times have been met, as applicable
- All sample identification labels are unique, and match the chain of custody
- All project analytes identified in the QAPP and listed on the chain of custody have been analyzed and are reported. All laboratory reported Detection Limits (DLs), Limits of Detection (LODs), and Limits of Quantitation (LOQs) are equal to or less than QAPP required DLs/LODs/LOQs (before adjustment for sample-specific conditions, such as dilution)
- All project required Detection Limits (DLs) have been met and are lower than the LODs
- All project required LOQs have been met and those LOQs are less than the project required action levels for both detects and non-detects
- All reported units (e.g., ng/L) are accurate and reflect the requirements of the project and that units are consistent with the type of sample matrix
- All required field QC samples (such as equipment blanks, reagent blanks, and field duplicates) have been included in the Laboratory Report at the frequency specified in the QAPP
- Soil, sediment, and biosolid samples have been reported on a dry weight basis, unless specified by the QAPP to report on a wet weight basis

- Tissue samples have been reported on a wet-weight basis, unless specified by the QAPP to report on a dry weight basis
- Each laboratory report has a case narrative that explains all non-conformities with the data
- All ion transitions used for quantitation and confirmation are identified
- All project target analytes whose quantitation includes branched and linear isomers are identified

Evaluation of the Laboratory Report

Any samples received for analysis that were not analyzed should be noted in the data validation report, along with the reason(s) for failure to analyze the samples, if the reason(s) can be determined; conversely, samples that were analyzed but were not requested should also be noted.

Analytes that have project action levels less than the laboratory's LOQ may reveal a severe deficiency in the data and a failure to meet project goals and should be noted in the data validation report. Analytes that have LODs or LOQs (before adjusting for sample-specific factors) that differ substantially from those presented in the QAPP may also have an impact on the ability to meet the project goals and should be noted in the data validation report. Errors in reported units and case narrative non-conformities that call into question the quality of the data should also be discussed in the data validation report.

Errors in quantitation limits or missing and misidentified samples may require a higher than Stage 1 validation. Data validators are encouraged to reach out to their point of contact identified in the project QAPP (such as the UFP-QAPP Worksheet #6) when preparing the data validation report.

For sample results (assuming no other qualifications due to data quality issues):

Qualification of data is based upon the reporting requirements of the project QAPP.

The QSM requires reporting non-detects as **U**-qualified at the LOD and requires reporting detects between the DL and LOQ with a **J** qualification. There are several ways that a project team may change these reporting requirements for project-specific reasons which are outlined in the QAPP. These changes are not recommended for typical projects and must be technically justified in the QAPP. They could include reporting non-detects as **U**-qualified at the DL; reporting non-detects and detects below the LOD as non-detects with **U** qualification at the LOD; or reporting non-detects and detects below the LOQ as non-detects with **U** qualification at the LOQ. These varying reporting conventions are summarized in the following table.

Table I: Reporting Requirements

Reporting Requirements (listed below)	Non-detects or results Below (<) DL	Results Below (<) LOD	Results Below (<) LOQ
Standard QSM Reporting	LOD value **U**	Reported Result **J**	Reported Result **J**
*Reporting results to DL	DL value **U**	Reported Result **J**	Reported Result **J**
Reporting results to LOD	LOD value **U**	LOD value **U**	Reported Result **J**
Reporting results to LOQ	LOQ value **U**	LOQ value **U**	LOQ value **U**

***Note:** non-detects reported at the DL have a 50% false negative rate. For further discussion please see Fact Sheet: Detection and Quantitation – What Project Managers and Data Users Need to Know, DoD Environmental Data Quality Workgroup, October 2017.

The transitions listed in the Sample Transition Ion Summary should be compared to those in Table 2 of EPA Method 1633. If a qualitative or quantitative standard containing an isomeric mixture (branched and linear isomers) of an analyte is commercially available for an analyte, the quantification ion used must be the quantification ion identified in Table 2 of EPA Method 1633 unless interferences render the product ion unusable as the quantification ion. In these cases, project approval is required before using the alternative product ion. The case narrative should contain documentation of the project approval as well as an explanation of the technical justification for using the alternative product ion. If a technical justification is not provided, or the explanation provided does not provide a technical justification for the change, use professional judgment to qualify the data, and all affected results must be noted in the data validation report.

3.2 Chain of Custody (CoC)

Examine the CoC form (some information may be included on Laboratory Receipt Checklists) for legibility and check that all PFAS by LC/MS/MS analyses requested on the CoC have been performed by the laboratory. Ensure that the sample identification on the laboratory sample results form (Form I [or equivalent]) matches the sample identification on the CoC. Read the laboratory case narrative for additional information.

Evaluation of the CoC

Any discrepancies in sample naming between the CoC and sample results form should be noted in the data validation report with the correct sample name being identified in the report and on the appropriate summary form, if the correct sample name can be determined. These edit corrections should also be verified in any associated electronic data deliverables (EDDs).

If the receiving laboratory transferred the samples to another laboratory for analysis, both the original CoC and transfer CoC should be present. If the transfer CoCs are not present or if there is missing information (such as location of the laboratory), it should be documented in the data validation report. Make note in the data validation report when signatures of relinquish and receipt of custody were not present.

3.2.1 Sample Preservation, Handling, and Transport

Evaluate sample handling, transport, and laboratory receipt from the CoC and laboratory receipt checklists to ensure that the samples have been properly handled. The project quality assurance project plan (such as UFP-QAPP Worksheet #19) should provide specific preservation requirements. The following are general guidance if project specifications were not stipulated.

- AFFF samples are to be shipped in HDPE containers with an unlined cap.
- Samples are shipped in coolers that are maintained at the temperature required by the QAPP. The recommended sample shipment temperature requirement is 0 - 6 °C, although it is recommended to freeze tissue samples upon collection and ship on dry ice. See EPA Method 1633 for details.
- The recommended sample storage temperature requirement at the laboratory is ≤ -20 °C. The holding time may vary per matrix depending on holding temperature; see EPA Method 1633 for details.

Evaluation of Preservation, Handling, and Transport

If the temperature of receipt is greater than that required by the QAPP, detects should be flagged as estimated **J** and non-detects as estimated **UJ**.

On occasion, the samples may be delivered to the laboratory within a few hours of collection and before the temperature of the cooler can reach the required temperature. For those instances, if cooling has begun, but the temperature is greater than the required temperature, special note should be made but no qualification should be required.

If the temperature of receipt is below that required by the QAPP, special note should be made but no qualification should be required.

In the event that both a cooler temperature and a temperature blank were measured, the temperature blank should be evaluated for temperature compliance as it best represents the condition of the samples; however, both temperatures shall be noted in the data validation report.

If the temperature upon receipt at the laboratory was not recorded, note this in the data validation report and assume that a temperature non-conformance occurred. Detects should be flagged as estimated **J** and non-detects as estimated **UJ**. Review any log-in check sheets for indication that the samples were at least received on ice and note in the data validation report. If the receiving laboratory transferred the samples to another laboratory for analysis, apply the same temperature criteria to both laboratories.

3.2.2 Holding Times

Holding times for PFAS are measured from the time of collection (as shown on the CoC) to the start time of sample extraction and analysis (as shown on the sample results form or extraction log). Based on input from the DoD Environmental Data Quality Workgroup (EDQW), holding time exceedances are calculated as follows:

For a test with a recommended maximum holding time measured in hours, the holding time shall be tracked by the hour. For a test with a recommended holding time measured in days, the holding time shall be tracked by the day. For a test with a recommended maximum holding time measured in months, the holding time shall be tracked by the month. One month is defined as 30 days.

For example, an exceedance of holding time for a sample with a 48-hour holding time will occur when the 49th hour is reached (e.g., a sample with a 48-hour holding time collected at 830 AM on April 4th must be analyzed or extracted by 9 AM on April 6th, or an exceedance will be considered to have occurred). An exceedance of holding time for a sample with a 14-day holding time will occur when the 15th day is reached (e.g., a sample with a 14-day holding time collected at 840 AM on April 4th must be analyzed or extracted by 12AM on April 19th, or an exceedance will be considered to have occurred). An exceedance of holding time for a sample with a 6- month holding time will occur when 6 months have passed (e.g., a sample with a 6-month holding time collected at 830 AM on April 5th must be analyzed or extracted by 12AM on October 2nd, or an exceedance will be considered to have occurred).

The holding time for aqueous, solid, and tissue samples depends on the temperature they are stored at (Table II). No chemical preservation is needed. Sample extracts should be stored at 0 - 4°C, protected from light for up to 90 days from extraction, however, ether sulfonate concentrations become elevated after 28 days and if NFDHA is a target analyte, samples should be analyzed as soon as possible. The QAPP should specify the storage temperature and holding time requirements.

Table II. Sample Storage and Holding Time Requirements

Matrix Type	Stored at 0 - 6°C, protected from light		Stored at ≤ -20°C, protected from light	
	Holding Time	**Caveat**	**Holding Time**	**Caveat**
Aqueous	28 days	Precursor degradation occurs after 7 days	90 days	None
Solid and Tissue	90 days	Should be prepared as soon as possible if NFDHA is a target analyte	90 days	Should be prepared as soon as possible if NFDHA is a target analyte
Biosolid	90 days	Not recommended due to the production of gases due to microbiological activity	90 days	None

Evaluation of Holding Times

If the holding time is exceeded, qualify all associated detects as estimated **J** and all associated non-detects as estimated **UJ** and document that holding times were exceeded.

If holding times are grossly exceeded (defined as two times the holding time), detects should be qualified as estimated **J** and non-detects as **X**, exclusion of data recommended.

3.3 Field QC

Field QC can consist of various blanks, field duplicates, and field replicates.

3.3.1 Field Blanks

The purpose of blanks is to identify potential cross-contamination at different stages of sampling and cleaning of equipment for reuse. Not every field blank type may be utilized during any given sampling event and there may be more blank types than described in this document. Field blanks may be varied throughout the sampling events of a project. The types of blanks and their collection frequency should be stipulated in the QAPP. Generally, the blanks are collected once a day or one per twenty field investigative samples, by each sampling team, and may be matrix dependent.

Below are the common types of field blanks for PFAS by LC/MS/MS analysis.

Note: PFAS-free water is a project specific definition and must be defined in the QAPP. If project-specific direction is not provided, use the requirement that all analyte detections are ≤ ½ the LOQ or ≤ 1/10th of the screening level for that analyte.

A field blank is a sample of PFAS-free water supplied by the laboratory that is transferred from one sample container directly into another sample container in the field. Analytes detected in field blanks indicate the possibility of cross-contamination between the ambient environment and the matrix collected for testing.

If water other than the PFAS-free water supplied by the laboratory is used during sampling, a source blank should be collected from each of these sources of water. Due to the ubiquitous presence of PFAS, any source water that has not been verified as PFAS-free should be collected as a separate QC sample and analyzed to assess whether the chemical nature of the water used in decontamination may have affected the analytical results of site samples. A source blank is collected once per source prior to sample collection.

An equipment blank (also called a rinse or rinsate blank) is an aliquot of PFAS-free water, subjected to all aspects of sample collection (usually poured over or through the sample collection device). Analytes detected in equipment blanks indicate the possibility of cross-contamination between samples due to improper equipment decontamination. Equipment blanks are usually collected at a frequency of one per twenty investigative samples (per matrix per sampling technique), or as specified in the QAPP.

Evaluation of Field Blanks

Determine which field blanks apply to samples in the sample delivery group (SDG) from the CoC or any QC sample associative listing. If the applicability of multiple field blanks cannot be determined, communicate with the point of contact identified in the project QAPP to inquire if applicability can be determined.

Note: SDGs can be called different names such as SEDD Lab Reporting Batch, depending on the project.

Ensure that units are correct when applying field blank qualifications.

Note: it may not be appropriate to make a direct quantitative comparison for aqueous field blanks (such as equipment blanks reported as ng/L) to a solid parent sample (such as a soil sample reported as µg/kg). At best, only a qualitative comparison can be made during a Stage 1 assessment, as raw data and/or preparation logs would be needed for unit conversion.

Generally, when multiple blank type contaminations are present, the evaluation should not involve a 'hierarchy' of one blank type over another. Each blank is evaluated separately and independently. The final validated result should be assessed on the blank with the highest value (i.e., greatest effect on sample analyte concentration).

If analytes (as appropriate) are detected in the field blanks, the procedure for the qualification of associated sample results is summarized below.

Compare the results of each type of blank with the associated sample results. The reviewer should note that the blank analyses may not involve the same units, weights, volumes, percent moistures, or dilution factors as the associated samples. These factors may be taken into consideration when applying the 5X criteria discussed below, such that a comparison of the total amount of contamination is actually made. Care should be taken to factor in the percent moisture or dilution factor when doing comparisons between detects in the sample and the blank. If an analyte is detected in the field blank, but not in the associated samples, no action is taken.

If field blanks were not collected at the proper frequency required by the QAPP, then use professional judgment to qualify the data, and make note of this in the data validation report.

If an analyte is detected in the field blank (at any concentration) and in the associated samples, the action taken depends on both the blank and sample concentrations (Table III).

Table III: Sample Qualification in the Presence of Blank Contamination

	Sample		
Row Number	**Result**	**Validated Result**	**Validation Qualifier**
1	**Non-detect or detect ≤ LOD**	**Report at LOD**	**U**
2	**> LOQ but ≤ 5x blank**	**Report at Sample Result**	**J+**
3	**> LOQ and > 5x blank**	**Report at Sample Result**	**None**

LOD = Limit of Detection

***Note** 1: The laboratory blank contamination qualifier (typically, B) is a part of the laboratory report. The validation qualifier is identified in the validation report with reason codes for the qualifiers traceable to the blank contamination. See the General Data Validation Guidelines appendices 5 and 7 for examples. During the data usability assessment, the DUA team has both sets of information available.*

***Note** 2: The Data Validation Subgroup acknowledges the differences in the QSM requirements for qualification of the method blank by the laboratory and qualification of all blanks by the validator. The method blank, having gone through only the laboratory processing steps and not the field sample handling, should be the most controlled of the blanks. Additionally, the laboratory may reprocess the method blank and samples in order to address the contamination. The laboratory does not evaluate the results of or qualify data based upon field, equipment, trip, or other blanks.*

The Data Validation Subgroup encourages project development teams to set acceptance requirements for blanks based upon project DQOs. In the absence of those project-specific

requirements, these guidelines are written to allow for a higher blank contamination tolerance resulting in a more conservative approach to qualification based upon potential contamination. In other words, the assumption that detects in samples are attributed to contamination rather than true sample concentration is minimized, thus minimizing the assumption of false positives.

It is expected that during data usability analysis, the DUA team will review qualifications from the laboratory and from the validator as well as comments contained in the laboratory case narrative and the validation report. The DUA team can then take into consideration whether they believe it more appropriate to consider a result qualified as biased high as a non-detect based upon decision criteria and other quality measures within the data set.

In situations where the QAPP requires the determination of an LOQ for the sum of a set of PFAS, (e.g., sum of concentrations of Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), and Perfluorononanoic acid (PFNA) and the sum of the detects of those analytes in a blank exceeds this value, use professional judgement to qualify the sample results and note all affected results in the data validation report.

3.3.2 Field Duplicates (Replicates)

Field duplicates consist of either colocated or subsampled (split) samples. Field duplicates for groundwater and surface water samples are generally considered to be colocated samples. Soil duplicate samples may be split samples or colocated, as specified in the QAPP. Field duplicate results are an indication of both field and laboratory precision; the results may be used to evaluate the consistency of sampling practices.

Evaluation of Field Duplicates

Check to ensure that field duplicates were collected and analyzed as specified in the QAPP. If the sampling frequency is less than the frequency stated in the QAPP, no qualification of the associated sample results is necessary, but the incident should be discussed in the data validation report.

The QAPP should describe the manner in which field duplicates will be evaluated. This should include the acceptance criteria for Relative Percent Difference (RPD) or absolute difference and when it is appropriate to use RPD or absolute difference. For example, the QAPP may specify that RPD be calculated when detected results are reported for the duplicates(s) and both results are greater than or equal to the LOQ or specify that absolute difference should be calculated when results for one or more of the duplicates are below the LOQ. The QAPP should also specify how to evaluate duplicates when one or more results are not detected. For example, the QAPP may specify the use of the LOD as the value for determining absolute difference when one or more results are not detected.

Additionally, the QAPP should define what is considered a major or minor exceedance of the RPD or absolute difference criteria. For example, RPD greater than 50% in aqueous matrices and 100% in soil matrices or absolute difference greater than 2x LOQ in aqueous matrices and 4x LOQ in soil matrices may be considered a major exceedance.

For field duplicate results, if the RPDs or absolute differences are greater than the criteria stated in the QAPP, qualify the associated sample results for detects as estimated **J** and for

non-detects as **UJ**. If the RPDs or absolute differences are greater than the QAPP-defined value for a major exceedance, qualify the associated results as **X,** recommended for exclusion. Any non-conformities should be noted in the data validation summary.

The associated sample results may include samples in the SDG which are similar to the parent sample or be limited to the parent and field duplicate samples if no other samples in the SDG are sufficiently similar to warrant qualification. The validator should note their reasoning for applying qualifications (e.g., the samples are contained "in the same SDG, collected on the same day, prepared together [and] contained in the same analytical sequence" (NFG 2017)).

Some sampling schemes (such as Incremental Sampling Methodology (ISM) if used to collect metals soil samples) require specific replicate calculations (e.g., relative standard deviation), which should be specified in the QAPP.

It should be noted that RPDs or absolute differences for field duplicates are generally not calculated or reported by the laboratory and should be calculated by the validator.

4.0 Stage 2A Validation

Note: Stage 2A includes all of Stage 1

Stage 2A requires the review and qualification of the following summary documents:

- Sample Ion Ratio Summary
- Extracted Internal Standard Recovery and Retention Time Summary
- Non-Extracted Internal Standard Recovery and Retention Time Summary
- Laboratory Control Sample/Low-Level Laboratory Control Sample Recovery Summary
- Matrix Spike/Matrix Spike Duplicate Recovery and Relative Percent Difference Summary
- Matrix Duplicate Recovery and Relative Percent Difference Summary
- Method Blank Summary
- Sample and Extract Dilution and Reanalysis Summary
- Bile Salts Interference Check Summary
- Qualitative Identification Standards Summary

Stage 2A is the validation of preparation batch specific QC data in addition to any sample specific parameters included in Stage 1.

Generally, a "preparation batch" of samples consists of up to twenty field samples (maximum) along with a method blank, laboratory duplicate or matrix spike/matrix spike duplicate, and laboratory control sample. They are meant to be analyzed together on a single instrument. However, laboratories may choose to split up a batch over multiple instruments to save time. In this case, if the use of multiple instruments is uncovered in a Stage 2A validation, the validator should request from their point of contact identified in the project QAPP a Stage 2B validation to review sequence logs. The use of multiple instrumentation should be noted in the data validation report.

4.1 Ion Ratio

Ion ratios can be used to help determine if the sample matrix or isomer composition of the sample has resulted in a bias in the data. A laboratory should calculate ion ratios in accordance with EPA Method 1633, which is outlined in Appendix B. To determine if a bias has potentially occurred, the ion ratio is evaluated against the ion ratio of standards, which do not contain matrix interferences. Ion ratios should be within ion ratios acceptance criteria specified in EPA Method 1633. For analytes that are reported at concentrations at or greater than the LOQ, the ion ratio of the analyte should be within 50-150% of the ion ratio of the analyte observed in the mid-point initial calibration standard. For analytes that are reported at a concentration between the detection limit (DL) and LOQ, the ion ratio of the analyte should be within 50-150% of the ion ratio of the analyte observed in the initial daily continuing calibration verification standard. The Sample Ion Ratio Summary should contain the sample ion ratios and the applicable criteria (i.e., the ion ratios of the mid-level calibration standard and daily continuing calibration verification standard).

Evaluation of Ion Ratios

Verify analytes are within their required criteria. Verify the ion ratios are within acceptance limits. If detects are reported with ion ratios outside of the 50-150% acceptance criteria, qualify the sample results as estimated **J** and note all affected results in the data validation report. Ion ratio failures could be caused by matrix interference and/or be the result of the presence of isomers in the sample at different ratios than the ratio of isomers present in the calibration standards. A full evaluation (Stage 4 validation) of the raw data and quantitation report is necessary to fully evaluate the potential cause of the failure.

4.2 Extracted Internal Standard (EIS) Recovery

Extracted Internal Standard (EIS) recoveries are used to correct for bias associated with matrix interferences and sample preparation efficiencies, injection volume variances, chromatographic behavior, and mass spectrometry ionization efficiency. All samples, standards, blanks, and QC samples are fortified with EIS compounds. EIS compounds are added to the solid sample prior to extraction and to an aqueous sample in the original sample container prior to extraction. For instances requiring a subsample of the original sample be prepared (e.g., AFFF samples or very high concentration samples), EIS compounds are added to the prepared subsample, prior to solid phase extraction. EIS recoveries are quantitated with respect to Non-Extracted Internal Standard (NIS) recoveries using the equation in Appendix B.

The EIS recoveries and acceptance limits should be reported for all field samples, batch QC samples, standards, and instrument blanks.

Sample and batch QC EIS percent recoveries should be within control limits specified in the project QAPP; otherwise, QSM acceptance criteria should be met.

If any EIS percent recovery is out of specification, then a reextraction (if applicable) and reanalysis should be performed and reported. The laboratory should have reported both runs if the first was unsuccessful.

The laboratory does not have to reanalyze a sample if a matrix spike/matrix spike duplicate or sample/sample duplicate was performed on the sample with out-of-control EIS percent recoveries showing the same matrix effects, as long as the batch QC display acceptable EIS percent recoveries.

The EIS retention times (RTs) for all samples and batch QC samples should be within 0.40 minutes of the retention time of the midpoint standard in the initial calibration, or on days when an initial calibration is not performed, the initial continuing calibration verification can be used instead. Analytes calibrated using isotope dilution (i.e., those with corresponding isotopically labeled analogs) should elute within +/- 0.1 minutes of their associated EIS.

Evaluation of Extracted Internal Standards

If isotopically labeled analogs of analytes are not used, but were commercially available, then justification should be noted in the laboratory case narrative. If justification is not noted, the point of contact identified in the project QAPP should be reached for further guidance.

Verify that samples or batch QC EIS percent recoveries meet criteria. If EIS percent recoveries are out of specification with no evidence of re-extraction (if applicable) and reanalysis, justification should be noted in the laboratory case narrative (e.g., limited sample volume prevented reanalysis). If justification is not noted, the point of contact identified in the project QAPP should be reached for further guidance.

If the EIS percent recovery control criteria displayed in the deliverable are not the same ranges stipulated in the QAPP or the DoD QSM, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The project team should be informed to implement changes to the current deliverables or those to be created in the future. Please follow the notification protocols outlined in the QAPP (such as the UFP-QAPP Worksheet #6).

Detects for analytes quantitated using an EIS percent recovery > 200% should be qualified estimated with a negative bias **J-**. Non-detects should not be qualified.

If the EIS recovery is < 10%, associated detects and non-detects should be qualified **X**.

Large retention time variations may call into question peak identifications. If an EIS retention time varies by more than 0.40 minutes, use professional judgment to qualify the sample results and note all affected results in the data validation report.

If the retention time of an analyte quantified by isotope dilution varies by more than 0.10 minutes from their associated EIS, use professional judgment to qualify the sample results and note all affected results in the data validation report.

Analyte concentrations should only be reported when within the calibration range. Some extracts may require dilution to bring analyte concentrations within the calibration range. If analyte concentrations exceeded the calibration range and the extract was not diluted to bring the concentration within range or the sample was not reextracted using a smaller aliquot of sample, detects should be qualified as estimated **J**. The responses for the EISs associated with analytes reported from a dilution should meet the signal to noise (S/N) and

retention time requirements of EPA Method 1633 and the EIS recoveries should be > 5%. If this criteria is not met, detects associated with unacceptable EIS should be qualified as estimated **J**. Non-detects in the diluted extract should be reported from less-diluted or undiluted extract results. EIS results may not be reported as "diluted out" since they are used to quantify analytes. A full evaluation (Stage 4 validation) of the sample, chromatogram, mass spectral ions, and quantitation report may be necessary to determine that diluted analytes are quantified correctly.

In the special case of a blank analysis with EIS percent recoveries out of specification, the reviewer should give special consideration to the validity of associated sample data. This nonconformance could represent an isolated problem with the blank alone or a fundamental problem with the analytical process. For example, if the samples in the batch show acceptable EIS percent recoveries, the reviewer may determine the blank problem to be an isolated occurrence for which no qualification of the data is required.

4.3 Non-Extracted Internal Standard (NIS) Recovery

Non-Extracted Internal Standard (NIS) peak areas are used to quantify EIS recoveries. NIS analytes are labeled PFAS compounds spiked into the extract prior to injection of an aliquot of the extract into the LC-MS/MS. The NIS recovery is the ratio of the NIS peak area in the sample relative to the mean are of the corresponding NIS in the initial calibration, as defined by EPA Method 1633.

Verify that NIS recoveries and acceptance limits were reported for all field samples, batch QC samples, standards, and instrument blanks.

Sample and batch QC NIS peak areas should be within control limits established in the QAPP or the QSM. Verify that no samples or batch QC have NIS peak areas outside the criteria.

If any NIS peak area is out of specification, then a re-extraction (if applicable) and reanalysis should be performed and reported. The laboratory should have reported the first run if the second was still unsuccessful. If the second run did not confirm the failure, it should have been reported.

The laboratory does not have to reanalyze a sample if a matrix spike/matrix spike duplicate or sample/sample duplicate was performed on the sample with out-of-control NIS peak area showing the same matrix effects, as long as the batch QC display acceptable NIS percent recoveries.

The NIS retention times (RTs) for all field and QC samples should be within 0.40 minutes of the retention time of the midpoint standard in the initial calibration, or on days when an initial calibration is not performed, the initial CV is used.

Evaluation of Non-Extracted Internal Standards

If NIS peak areas are out of specification, justification should be noted in the laboratory case narrative (e.g., limited sample volume prevented reanalysis). If justification is not noted, the point of contact identified in the project QAPP should be reached for further guidance.

If the criteria displayed in the deliverable are not the same ranges stipulated in the QAPP or the QSM, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The project team should be informed to implement changes to the current deliverables or those to be created in the future. Please follow the notification protocols outlined in the QAPP (such as the UFP-QAPP Worksheet #6).

Verify area counts are within acceptance criteria. If low area counts are reported (< 30%), detects and non-detects should be qualified **X**.

If an NIS retention time varies by more than 0.40 minutes, use professional judgment to qualify the sample results and note all affected results in the data validation report.

NIS results may not be reported as "diluted out" since they are used as the internal standard for calculation of the EIS recoveries. A full evaluation (Stage 4 validation) of the sample, chromatogram, mass spectral ions and quantitation report may be necessary to determine that diluted analytes are quantified correctly.

4.4 Laboratory Control (LCS) and Low-Level Laboratory Control Sample (LLLCS)

An LCS (equivalent to OPR in EPA Method 1633) is an analyte free sample matrix spiked with known amounts of the analytes of interest and taken through all sample preparation, cleanup and analytical steps. LCSs establish the method precision and bias for a specific batch of samples. LLLCSs (equivalent to LLOPR in EPA Method 1633) verify the LOQ. An LLLCS is an LCS spiked at low concentration (2x the LOQ), while the LCS is spiked at mid-level concentration relative to the calibration range.

LCS and LLLCS recoveries should be within QC limits established in the QAPP or as listed in the QSM.

An LCS and LLLCS are prepared in every preparation batch of 20 environmental samples.

Evaluation of LCS and LLLCS

Verify that an LCS and LLLCS were analyzed with each batch of samples.

Verify that results (from appropriate summary form), percent recoveries, and acceptance limits were reported for all target analytes.

If the spike percent recovery control criteria displayed in the deliverable are not the same range (i.e., outside or wider than) as those stipulated in the QAPP or the QSM, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The project team should be informed to implement changes to the current deliverables or those to be created in the future.

In-house control limits are acceptable for any analytes not specified in the QAPP or DoD QSM. No qualification is necessary for any reported in-house control limit that is within its control range.

If the LCS or LLLCS percent recoveries were greater than the upper control limit, qualify detects for the analyte in associated samples as estimated with a positive bias **J+**. Non-detects should not be qualified.

If the LCS or LLLCS percent recoveries were less than the lower control limit, qualify detects for the analyte in associated samples as estimated with a negative bias **J-** and non-detects as **X**, exclusion of data is recommended.

In the event the biases associated with a sample conflict due to LCS and LLLCS recoveries (i.e., one is **J+**, the other, **J-**), the qualification should be **J** without bias.

If the LCS or LLLCS was not spiked with all target analytes, notify the project team by following the notification protocols outlined in the QAPP (such as UFP-QAPP Worksheet #6) and qualify detects and non-detects for those analytes not spiked as **X**, exclusion of data is recommended.

Professional judgment should be utilized in qualifying data for circumstances other than those listed above.

4.5 Matrix Spike/Matrix Spike Duplicate (MS/MSD)

MS/MSD data are used to determine the effect of the matrix on a method's recovery efficiency and precision for a specific sample matrix. If the QAPP does not specify a statistical sampling design, each preparatory batch should have one site specific MS and MSD. For sample designs that rely on Incremental Sampling Methodology (ISM), three or more replicates may be specified by the QAPP. Field blanks should not be used as the parent sample for the MS/MSD or LD analyses.

Note: If a field blank was used for the MS/MSD, the information must be included in the data validation report, but the data should not be qualified. Sample matrix effects should not be observed with field blanks; therefore, no site-specific matrix effects can be determined from a field blank.

The MS and MSD should be spiked per QSM requirements with all target analytes. If the parent sample for the MS/MSD was from another site or project (for example, not enough sample collected, or multiple site samples analyzed within a single batch), the reason should be documented in the data validation report, and sample results should not be qualified due to any non-conformities noted in non-site-specific matrices.

Evaluation of MS/MSD

Verify that MS/MSD analyses were performed at the specified frequency.

Verify that the MS/MSD were spiked with all target analytes, and that percent recoveries and RPDs were reported for all target analytes. If the MS/MSD was not spiked with all target analytes, notify the project team by following the notification protocols and qualify all detects and non-detects in the parent sample for those analytes not spiked as **X**, exclusion of data recommended.

Recovery criteria for MS and MSD are applicable where the spike concentration is at least 3 times greater than the native analyte concentration, or as defined in the QAPP. If this is not the case, the MS and MSD percent recovery criteria do not apply. This should be noted in the data validation report.

If the MS/MSD or MD results do not meet the technical criteria, apply the action to all samples in the same preparation batch of the same matrix, if the samples are considered sufficiently similar. Exercise professional judgment in determining sample similarity when making use of all available data, including: samples of the same matrix from the same project site with similar analyte concentrations; site and sampling documentation (e.g., location and type of sample, descriptive data, and soil classification); field test data; and laboratory data for other parameters. If no samples in the SDG are sufficiently similar to the parent sample, only the parent sample should be qualified. This should be noted in the data validation report.

Compare the percent recovery for each analyte with LCS control limits established by the QAPP or DoD QSM. If the spike percent recovery control criteria displayed in the deliverable are not the same range (i.e., outside or wider than) as those or stipulated in the QAPP or the DoD QSM, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The project team should be informed to implement changes to the current deliverables or those to be created in the future. Follow the notification protocols outlined in the QAPP (such as UFP-QAPP Worksheet #6).

If the MS or MSD percent recoveries were greater than the upper control limit, qualify detects for the analyte in the associated parent sample as estimated **J+**. Non-detects should not be qualified.

If the MS or MSD percent recoveries were less than the lower acceptance limit but ≥ 10%, qualify detects for the analyte in the associated parent sample as estimated **J-** and non-detects as estimated **UJ**. If the percent recoveries were < 10%, qualify detects for the analyte in the associated parent sample as estimated **J-** and non-detects as **X**, exclusion of data is recommended.

If the MS/MSD RPDs were greater than the acceptance limits, qualify detects for the analyte in the associated sample(s) as **J**. Non-detects should be qualified as estimated **UJ**.

Failures of the MS/MSD due to the presence of analyte(s) in the parent sample at > 3X the spike concentration or failures due to matrix spike requiring dilution to less than the LOQ should not result in qualification. The incident should be noted in the data validation report.

4.6 Matrix Duplicate

Matrix duplicate (MD) sample data are used to verify the instrument was capable of accurately quantifying PFAS in the sample's matrix at the reported LOQ. Each AFFF sample prepared using an aliquot of the field sample must be prepared in duplicate. AFFF samples must be subsampled in duplicate in accordance with DoD AFFF01, Section 11.2.1 through 11.2.9.

Note: DoD AFFF01 equivalent to the MD is the Sample Duplicate (SD).

Evaluation of Matrix Duplicate Sample

Matrix duplicate sample data should be reported on a matrix duplicate sample summary form (or equivalent). Verify that the MD was performed for each AFFF sample prepared using an aliquot of the field sample.

Compare the RPD between sample and corresponding MD for each analyte. For concentration of analytes that are equal to or greater than the LOQ, the RPD between the sample and the corresponding MD must be ≤ 30%. If this criterion is exceeded, then the sample and associated SD must be re-extracted.

If the RPDs were greater than the acceptance limits, qualify detects for the analyte in the associated sample(s) as estimated **J**. Non-detects should not be qualified.

Professional judgment should be utilized in qualifying data for circumstances other than those listed above.

There are instances where an RPD is not calculable (for example, when one result is a non-detect and the other is > LOQ). In those cases, the RPDs are not calculated but the non-conformity should be noted in the data validation report. The reported concentrations should be carefully examined to determine what conditions would permit one result to be reported at or above the LOQ/Reporting Limit (RL), and the other to be reported below the LOQ/RL or as a non-detect.

The equation for RPD calculations is given in Appendix B.

4.7 Method Blanks

A method blank is used to identify systemic contamination originating in the laboratory that may have a detrimental effect on project sample results. The validator should identify samples associated with each method blank using a method blank summary form (or equivalent). Verify that the method blank has been reported per batch.

Compare the results of each method blank with the associated sample results. The reviewer should note that the blank analyses may not involve the same weights, volumes, percent moistures, or dilution factors as the associated samples.

These factors should be taken into consideration when applying the 5x criteria (discussed in section 3.3.1), such that a comparison of the total amount of contamination is actually made. Care should be taken to factor in the percent moisture or dilution factor when doing comparisons between detects in the sample and the method blank.

In the method blank, no analytes should be detected > ½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.

Evaluation of Method Blanks

If no method blank was analyzed, qualify detects in samples with no associated method blank as **X**, exclusion of data recommended. Non-detects do not require qualification.

If gross contamination exists (defined as greater than a Project Action Limit) in the method blanks, all analytes affected in all associated samples in the preparation batch should be qualified as **X** due to interference. This should be noted in the data validation comments.

If an analyte is detected in the method blank, but not in the associated samples, no action is taken.

If an analyte is detected in the method blank and in the associated samples, the action taken depends on both the method blank and sample concentrations. Table III and Section 3.3.1 discussions on evaluations of results from the LOD to LOQ is also applicable to the method blank.

Additionally, there may be instances where little or no contamination was present in the associated method blanks, but qualification of the sample was deemed necessary. Contamination introduced through dilution water is one example. Although it is not always possible to determine, instances of this occurring can be detected when contaminants are found in the diluted sample result, but are absent in the undiluted sample result. It may be impossible to verify this source of contamination. However, if the reviewer determines that the contamination is from a source other than the sample, the data should be qualified. In this case, the 5X rule does not apply. The sample value should be reported as a non-detect and the reason should be documented in the data validation report.

Multiple blank contaminations (such as a batch with field blanks and a method blank) does not establish a 'hierarchy' of one blank over another. Each blank must be evaluated individually. Blanks should not be qualified due to the results of other blanks.

4.8 Sample and Extract Dilution and Reanalysis

EPA Draft Method 1633 requires aqueous samples to be prepared using the entire sample volume received unless prescreening of the sample indicates high concentrations of analytes. In those cases, the laboratory is required to notify the client before proceeding with subsampling according to EPA Method 1633. AFFF samples must be prepared using an aliquot of the sample received in accordance with the requirements of the QSM. Dilutions of sample extracts are required when concentrations of target analytes exceed the quantification range or EIS failures are associated with a sample and matrix interference is suspected. Reanalysis of samples is required when NIS or EIS compounds fail to meet the acceptance criteria.

Evaluation of Sample and Extract Dilution and Reanalysis

If the entire sample received by the laboratory (with the exception of AFFF samples) was not prepared and the client approval of subsampling was not documented, document the nonconformance in the data validation report. If project-specific subsampling requirements are defined, qualify associated data as prescribed in the QAPP. If project-specific subsampling requirements are not defined qualify all associated data as **J**.

When sample results are reported at more than one dilution due to analyte concentrations exceeding the calibration curve, the dilution that results in the lowest DL/LOD/LOQ should be used for each target analyte unless a QC criterion has been exceeded.

The data validation report should indicate the reason for all reported dilutions resulting in elevated sensitivity limits for non-detected results. When reanalysis has occurred due to quality control non-conformities, the validator should ensure that the non-conformity was corrected during the reanalysis. If that is not the case, then the appropriate qualifier should be placed on the reported results.

In some cases, using professional judgment, the validator may determine that an alternate result was more appropriate than the one reported. In those cases, explain the rationale for accepting the alternate result in the data validation report.

In some cases, reanalysis may lead to exceedances of holding time. Use professional judgment to evaluate the results and apply the appropriate qualifiers (if required).

4.9 Bile Salt Interference Check

A bile salt interference check summary should provide, for each analytical sequence, the retention times of each bile salt included in the bile salt interference check standard and the retention time window of PFOS, from the first daily continuing calibration verification standard analyzed on the same day. A bile salt interference check standard consisting of taurodeoxycholic acid (TDCA) when the mobile phase used for analysis is acetonitrile, or taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA) when an alternate mobile phase is used, must be analyzed daily, prior to analysis of all matrix types (aqueous, solid, tissue, and AFFF). During the retention time calibration process, conditions are adjusted to ensure that bile salt peaks do not coelute with any of the target analytes, EIS, or NIS standards. Analytical conditions should be set to allow a separation of at least 1 minute between retention time of the bile salts and the retention time window of PFOS.

All EPA Draft Method 1633 requirements for evaluation of the relationship of the retention time of the bile salt peaks to the retention time of PFOS must be met. The retention time of PFOS applies to the retention time of all isomers of PFOS.

This standard can also include the Qualitative identification Standard analytes.

Evaluation of the Bile Salt Interference Check

If a bile salt interference check standard was not analyzed, did not include all bile salts required, or the required separation was not achieved and PFOS was detected in the sample, and its ion ratio did not meet criteria and was **I** qualified, qualify the detects as **J**. Otherwise, discuss the nonconformance in the data validation report.

4.10 Qualitative Identification Standard

A qualitative identification standard(s) containing a mixture of the branched and linear isomers of PFOA, PFNA, Perfluorooctanesulfonamide (PFOSA), N-methyl perfluorooctanesulfonamide (NMeFOSA), N-ethyl perfluorooctanesulfonamide (NEtFOSA), N-ethyl perfluorooctanesulfonamidoethanol (NEtFOSE), and N-methyl perfluorooctanesulfonamidoethanol (NMeFOSE) must be analyzed daily, prior to analysis of all samples. NMeFOSA is an impurity of the NMeFOSE qualitative standard and NEtFOSA is an impurity of the NEtFOSE qualitative standard. This qualitative standard should be used to determine the retention time of branched isomers of these target analytes in

samples. Branched isomers of a target analyte are included in the quantitation of a target analyte only when their retention times match those determined by a qualitative standard(s) or quantitative standard that contained an isomeric mixture of the target analyte that was used to create the calibration standards (PFOS, Perfluorohexanesulfonic acid (PFHxS), N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA), and N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)).

This standard can also include the bile salt interference check analytes.

Evaluation of the Qualitative Identification Standard

If the required qualitative identification standards were not analyzed once daily prior to sample analysis, discuss the nonconformance in the data validation report and qualify all associated data as **X**.

If the target analyte quantitation included branched isomers not identified in the qualitative identification standard, discuss the nonconformance in the data validation report and qualify the associated detects as **J**.

If the target analyte quantitation did not include branched isomers identified in the qualitative identification standard and present in the sample, discuss the nonconformance in the data validation report and qualify the associated detects as **J-.**

5.0 Stage 2B Validation

Note: Stage 2B includes all of Stage 1, and Stage 2A

Stage 2B requires the review and qualification of the following summary documents for each instrument.

- Sequence and Preparation Logs (or equivalent to include Instrument Blanks)
- Mass Calibration and Mass Calibration Verification Summary
- Initial Calibration Summary (any equivalent to include the Initial Calibration Analyte and EIS Responses, Analyte and EIS Concentrations, Isomeric Profiles, Response Ratios (RRs) or Response Factors (RFs), RR or RF Relative Standard Deviation or Relative Standard Error)
- Initial/Continuing Calibration Verification and Instrument Sensitivity Check Summaries
- Instrument Blank Summary

Stage 2B is the validation of instrument specific QC data.

5.1 Sequence and Preparation Logs

Sequence logs are reviewed by the data validator to ensure all QC samples (both batch and instrument specific) have been analyzed within a specific batch, in the correct order. Preparation logs are reviewed by the data validator to ensure that samples had the proper extraction performed, within specified holding times. The logs themselves do not require validation. However, non-conformities uncovered in the review of the logs may point the

validator to specific samples that require further review. Non-conformities uncovered in preparation or sequence logs should be noted in the data validation report.

Sequence logs are helpful in identifying when multiple instrumentation is used to analyze a batch of samples. For example, it is not uncommon to analyze a single batch of twenty samples at the same time on two or more different instruments. At a minimum, each instrument must be tuned and calibrated independently. Batch QC should be reviewed on each instrument, as appropriate. Non-conformities involving the use of multiple instruments should be noted in the data validation report.

5.2 Mass Calibration and Mass Calibration Verifications

A mass calibration of the LC/MS/MS instrument is required prior to analysis of an initial calibration curve. The mass calibration should meet all requirements of EPA Method 1633. A mass calibration of the LC/MS/MS instrument is required prior to analysis of an initial calibration curve. A mass calibration verification should be performed after the mass calibration to ensure mass resolution, identification, and to some degree, sensitivity are all within criteria. The peak apex for each mass should not shift more than 0.2 Da (or amu) from the expected masses for each target analyte.

Conformance should be determined using reference standards; therefore, acceptance criteria should be met in all circumstances.

The mass calibration and mass calibration verification should be performed prior to the initial calibration used. The peak apex for each mass should not shift more than 0.2 Da (or amu) from the expected masses for each target analyte.

Evaluation of Mass Calibration and Mass Calibration Verifications

Verify a mass calibration and mass calibration verification was performed prior to analysis of the initial calibration curve. Verify the peak apex for each mass did not shift more than 0.2 Da (or amu) from the expected masses for each target analyte.

Careful consideration should be given to any reported results that accompany a mass calibration verification that does not meet criteria. Based on EPA Method 1633 requirements, the samples should not have been analyzed. All associated data should be qualified as **X**, exclusion of data is recommended.

5.3 Initial Calibration

The objective of initial calibration is to ensure that the instrument is capable of producing acceptable qualitative and quantitative data. Initial calibration demonstrates that the instrument is capable of acceptable performance prior to sample analysis and of producing an acceptable calibration curve.

The instrument should be calibrated for all target analytes and isotopically labeled analogs of target analytes (EIS compounds and NIS compounds) with at least six solutions, with at least five of the six calibration standards being within the quantification range (LOQ to highest calibration standard that meets criteria). (If a second-order calibration model is used, then one additional concentration is required.) Isotope dilution quantitation should be used when an EIS analyte of the target analyte is commercially available. In instances

when not available for a target analyte, the EIS analyte with the closest retention time or chemical similarity to the target analyte should be used for quantitation. Under no circumstances should external calibration quantitation be used. If isotopically labeled analogs of analytes become commercially available for any analytes that did not have isotopically labeled analogs commercially available during validation of EPA Method 1633, they must be used.

The instrument calibration summary should identify which analytes were calibrated using standards that contained branched and linear isomers of the analyte. Branched and linear isomers should be used for calibration standards when they are commercially available as a certified standard. Table IV lists standards that are commercially available and used. The target analyte response for analytes containing branched and linear isomer should be result of the summation of peaks from all isomers. If a certified standard is not available, a technical standard may be used to identify retention time and ion transition ratios, but may not be used for calibration. In these instances, a certified linear standard should be used to build the calibration curve, and the samples must be quantified for all isomers that meet the technical grade standard identification for retention time and ion transitions.

Table IV: Available Certified PFAS Standards Containing Branched and Linear Isomers
Perfluorohexanesulfonic acid (PFHxS)
Perfluorooctanesulfonic acid (PFOS)
2-(N-methylperfluorooctanesulfonamido) acetic acid (NMeFOSAA)
2-(N-ethylperfluorooctanesulfonamido) acetic acid (NEtFOSAA)

Evaluation of Initial Calibration

If target analytes were not calibrated, qualify associated non-detects and detects as **X**, exclusion of data is recommended. Samples should not have been run without a valid calibration in accordance with EPA Method 1633 requirements. If less than the required minimum number of calibration standards were used, qualify all associated data as **X**.

If the laboratory has analyzed more than the required number of calibration standards and picked out the "best" set (e.g., analyzed seven calibration standards and picked the five "best" to pass calibration criteria), make note of this in the data validation report and qualify the data as **X**.

Any other manipulation of calibration points (such as 'dropping' calibration levels at the ends of the calibration curve) should have a technical justification documented in the laboratory report. It is not acceptable to 'drop' a calibration point in between two points that are used. Use professional judgment to evaluate the data. If no technical justification is provided, then make note of this in the data validation report and qualify the data as **X**.

The lowest calibration standard should be at or below the LOQ. If the LOQ is below the lowest calibration standard, then the LOQ has been reported in a manner that is inconsistent with QSM requirements. If the concentration of the lowest calibration standard was greater than the LOQ and the concentration of the associated Instrument Sensitivity Check (ISC) is at the LOQ and meets its acceptance criteria, no qualification is needed. If the concentration of the lowest calibration standard was greater than the LOQ and the

associated ISC concentration is greater than the LOQ or the ISC fails to meet acceptance criteria, qualify all associated data that are at a concentration below the concentration of the lowest calibration standard that meets acceptance criteria as **X** and make note of this in the data validation report.

Inform the point of contact (QAPP Worksheet #6) for further instruction in those instances of unwarranted manipulation of calibration curves. As an example, calibration curves generated with excessive calibration points that are misapplied to achieve passing criteria (without any technical justification) requires prompt notification of the project team. If the issue cannot be resolved with the laboratory, make note of this in the data validation report and qualify all affected data as **X**, exclusion of data is recommended.

Verify isotope dilution quantitation was used for all target analytes where isotopically labeled analogs are commercially available and EISs were used for target analytes when they are not. If isotopically labeled analogs were not utilized when commercially available, make note of this in the data validation report and qualify the associated data as **X**, exclusion of data is recommended.

In order to produce acceptable sample results, the response of the instrument must be within the quantification range established by the initial calibration. Any sample detections above the quantification range of the calibration curve should be accompanied by a dilution that is within the quantification range. If dilutions were not performed, qualify all detections above the initial calibration working range as estimated **J,** and make note of the lack of dilution(s) in the data validation report.

If dilution(s) were performed that were within the quantification range of the initial calibration, then qualification of the data is not necessary. Make note in the data validation report that dilution(s) were performed.

If branched isomers were not included in the summed result reported, qualify associated detects as **J-.**

5.3.1 Response Ratios (RRs), Response Factors (RFs), Relative Standard Deviation (%RSD), and Relative Standard Error (RSE)

The response ratio (RR) for each unlabeled compound calibrated by isotope dilution should be calculated. The response factor (RF) for each unlabeled compound calibrated by extracted internal standard should be calculated. The response factor (RFs) should be calculated for each isotopically labeled compound. The equations for these calculations can be found in EPA Method 1633 and Appendix B.

One of two of the following approaches should be used to evaluate the linearity of the instrument calibration.

- The relative standard deviation (RSD) of the RR or RF values of the six initial calibration standards for each native compound and isotopically labeled compound should be calculated. The RSD should be $\leq 20\%$ to establish instrument linearity.

- The relative standard error (RSE) of the six initial calibration standards for each native compound and isotopically labeled compound should be calculated. The RSE for all method analytes should be ≤ 20% to establish instrument linearity.

All target analytes should either have an associated %RSD or %RSE of ≤ 20% for an average calibration fit. Second order fits should use a %RSE of ≤ 20% criteria.

Evaluation of RRs/RFs, %RSD, and %RSE

Evaluate the %RSD or %RSE for all target analytes. If any target analyte has a %RSD or %RSE > 20% and ≤ 30%, flag detects for the affected analytes as **J** and non-detects as **UJ** in the associated samples.

If the %RSD or %RSE for any target analyte is excessively high (defined as > 30%), qualify associated sample results as **X**, exclusion of data is recommended.

5.4 Initial (Secondary Source), Continuing Calibration Verification, and Instrument Sensitivity Check

The initial calibration curve should be verified with a standard that has been purchased or prepared from an independent source each time initial calibration is performed. This standard is called the secondary source or Initial Calibration Verification (ICV). The ICV should contain all of the PFAS target analytes. Note that multiple ICVs may be analyzed to encompass all of the target analytes.

The LOQ should be verified with a standard that is prepared at the concentration of the LOQ and analyzed following the initial calibration and daily at the beginning of the analytical sequence, prior to sample analysis. This standard is called the Instrument Sensitivity Check (ISC). To verify the LOQ of each target compound, the ISC should contain all of the PFAS target analytes at a concentration that is equal to their LOQ concentration.

After the initial calibration has been verified with a second source, samples may be run continuously until the initial calibration fails. To verify this, a Continuing Calibration Verification (CCV) containing all PFAS target compounds should be analyzed at the beginning of every analytical sequence, prior to sample analysis, after every ten field samples, and at the end of the analytical sequence. The end of the analytical sequence CCV should have an injection time prior to the end of the twelve-hour tune period. Continuing calibration checks satisfactory performance of the instrument on a day-to-day basis.

These ISCs, CCVs, and ICVs verify satisfactory performance of the instrument on a day-to-day basis.

Note: The CCV is equivalent to the CV in EPA Method 1633.

Evaluating the ICV, CCV, and ISC

Verify the ICV was analyzed following the initial calibration and contained all target analytes. Verify the CCVs and ISCs have been run at their proper frequency. When a new initial calibration is performed, the ICV can serve as the first CCV if samples are being run

afterwards. The CCVs after the first ICV are not required to be a second source.

If the ICV (second source) has not been performed successfully after an initial calibration or if samples have been analyzed prior to a valid ICV, qualify **X**, exclusion of data recommended all associated data. No samples should have been analyzed in accordance with QSM requirements

If the ISC has not been analyzed daily, prior to sample analysis, qualify **X**, exclusion of data recommended for all associated data. No samples should have been analyzed without a valid ISC.

If the CCV has not been analyzed as required (first, continuing, or end-of-run), qualify **X**, exclusion of data recommended all associated data.

Verify that the recoveries are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated **J+** when the recovery is higher than acceptance criteria and **J-** when below acceptance criteria. Non-detects are qualified as **UJ** in all associated samples for recoveries outside of acceptance criteria.

For gross exceedances of recoveries (defined as< 50% or >150% ISC, ICV, CCV) qualify all associated data as **X**.

If the ICV, CCV and/or ISC have not been analyzed (either continuing or end-of-run), qualify all associated data as **X**. No samples should have been analyzed without a valid ICV, CCV and ISC.

If CCVs have been analyzed at a frequency less than every ten field samples, qualify the associated sample detects as **J** and the non-detects as **UJ**.

5.5 Instrument Blanks

Instrument blanks (IBs) are used to ensure that the LC/MS/MS system does not contribute unacceptable concentrations of a target analyte into a sample result. The IB should be analyzed immediately following the highest standard analyzed, daily prior to analyzing standards, after each CV, and immediately following samples with PFAS concentrations exceeding the quantification range. In order to quantify contamination, the IBs should contain EIS and NIS compounds. Each analyte in the IB should meet the acceptance criteria defined in the QAPP. The QSM Table B-24 requires this acceptance criteria to be set at a minimum for each target analyte not to exceed ½ LOQ. QAPP defined criteria may be more stringent, especially in cases where there is a project-specific action level associated with the sum of a group of PFAS.

Evaluation of Instrument Blanks

If acceptance criteria are not met after the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met.

If a sample concentration exceeds the highest calibration standard and the subsequent sample(s) exceed the IB acceptance criteria of (i.e., are > ½ LOQ), they must be

reanalyzed using a fresh aliquot of the sample extract. Flagging is only appropriate in cases where the extract cannot be reanalyzed and re-extraction is not possible. Careful consideration should be given to any reported results that accompany an instrument blank that does not meet criteria. Based on QSM requirements the samples should not have been analyzed. Associated data should be flagged in accordance with Table III.

6.0 Stage 3 Validation

Note: Stage 3 validation includes all of Stage 1, Stage 2A and Stage 2B

The following documents are used for a Stage 3 validation:

- Standards traceability forms and worksheets (including Manufacturer provided Certificate of Analysis for Standards)
- Raw data (including any laboratory forms, instrument outputs, spreadsheets, or handwritten calculations necessary for recalculation and re-quantification)
- Method Detection Limit Studies Summaries (optional)
- Limit of Quantitation Verification Studies Summaries (optional)
- Initial Precision and Recovery Determinations Summaries (optional)

Stage 3 validation includes the recalculation and re-quantification of selected samples, and method and instrument QC. The types of results that should be recalculated and re-quantified include target analytes, analytes with detects above the LOQ, and field QC samples (blanks and duplicates). For method QC results, spiked recoveries and method blanks should be considered. For instrument QC, calibrations (including response factors and regressions), calibration verifications, and EIS recoveries should be recalculated and re-quantified. Some calculations may include the need to review standards preparation and serial dilutions.

6.1 Samples and Field QC Recalculation

When choosing samples, field QC and analytes for re-quantification and recalculation, consideration should be given to the laboratory's batching scheme to ensure a representative subsample of recalculations is performed. Additionally, if priority contaminants or contaminants of concern are identified in the QAPP, those analytes should be selected for recalculation. To ensure analytes are reported in the correct form (acid), analytes that are chosen for recalculation should include, at a minimum, at least one analyte where the manufacturer's certificate of analysis provides both the salt and acid concentration of the analyte. Recalculation should include ion ratios as well as concentrations. Other circumstances that should be prioritized for re-quantification and recalculation are diluted samples, manual integrations, re-runs of samples due to QC failures, and field QC blank failures.

Re-quantification and recalculation should be performed on the designated percentage of the samples per SDG (or however defined in the QAPP, such as percentage of total project samples) per analytical suite. At a minimum, it is recommended that 10% of the data should be re-quantified and recalculated unless specific instructions are given in the QAPP.

Sample recalculations should include the raw instrument result, re-quantified from the instrument response against the calibration function, and the final reported sample result,

including any dilution, preparation factor, or percent moisture (if applicable). The equations in Appendix B can be used to calculate a sample result from the corresponding reported calibration or regression function, as appropriate.

Verify that one or more of the laboratory's reporting limits (such as limit of quantitation) are calculated correctly for the non-detects and reported accordingly. If a detection limit study was identified by the QAPP, recalculate one or more analyte detection limits.

Re-quantitate all detected target analytes (concentrations and ion ratios) in the 10% sample data chosen. For some samples, all results may be non-detects, therefore recalculation would not be necessary. Verify that sample-specific results have been adjusted correctly to reflect percent solids, original sample mass/volume, and any applicable dilutions.

Re-quantitate all detects found in the field QC blanks (such as trip blanks, field blanks, or equipment blanks). Field QC sample duplicate recalculations should include re-quantification of the same detected analyte sample/duplicate pair and verification of the %D or RPD as reported.

When recalculations require rounding of data, that rounding should be completed only once at the end of all calculations to minimize rounding errors. Calculations should be rounded to the significant figures of the underlying criteria. For example, an LCS criteria of 80 - 117% would still be considered acceptable if the recalculation was 117.4%.

Evaluation of Sample and Field QC Recalculations

If the laboratory's quantitation or reporting limits (however defined) are calculated incorrectly, then continue to recalculate limits until it is determined that the problem is systemic (such as incorrect equations used) or isolated (such as a transcription or rounding errors).

For systemic (defined as widespread and major in nature) issues that cannot be corrected through a revised laboratory report, qualify all results as **X**, exclusion of data recommended.

For isolated cases, use professional judgment. It may be necessary to engage the point of contact as identified in the project QAPP to communicate with the laboratory, so they can provide revised (corrected) results. In all cases, if calculation errors affect project target analytes, the point of contact should be notified, and all affected results noted in the data validation report, including listing the calculation errors.

6.2 Method QC Recalculation

Re-quantification of batch QC sample results should use raw instrument response in tandem with the reported calibration factor or response factor; the preparation information; and percent moisture for solid samples to recreate the reported result.

6.2.1 EIS Compounds Spike

Verify the concentrations of EIS compounds from the raw data. Verify that the EIS compound result and percent recovery were calculated and reported correctly by re-calculating all EIS compounds in the 10% of chosen sample data and method QC that were originally selected.

6.2.2 NIS Compounds Spike

Verify the peak areas of NIS compounds from the raw data. Verify that the NIS compound result and percent recovery were calculated and reported correctly by re-calculating all NIS analyte peak areas in the 10% of chosen sample data and method QC that were originally selected.

6.2.3 LCS/LLLCS

To check that the spike percent recovery was calculated and reported correctly, using the equation in Appendix B, re-quantitate and then recalculate all contaminants of concern as outlined in the UFP-QAPP Worksheet #12 or #15. Use a random 10% of the analytes in the LCS, LLLCS, and LCSD (if applicable). Recalculate RPDs (if applicable) from LCS/LCSD pairs that would result in the qualification of a sample.

6.2.4 MS/MSD

Re-quantitate 10% of the target analytes as listed in the UFP-QAPP Worksheet #12 or #15 for both the MS and the MSD. Use a random 10% of the analytes in the MS and MSD if contaminates of concern have not been identified. The RPDs of the recalculated MS/MSD pairs should be calculated from the MS/MSD concentrations, not from the recoveries.

6.2.5 Matrix Duplicate

Re-quantitate 10% of the target analytes as listed in the UFP-QAPP Worksheet #12 or #15 for both the sample and the corresponding MD. Use a random 10% of the analytes in the sample and the corresponding MD if contaminants of concern have not been identified. The RPDs of the recalculated sample/MD pairs should be calculated from the sample/MD concentrations, not from the recoveries.

6.2.6 Method Blanks

Method blank analytical results are assessed to determine the existence and magnitude of contamination problems associated with sample extraction (if applicable) and analysis. If problems with any method blank exist, all associated data should be carefully evaluated to determine whether there is any bias associated with the data, or if the problem is an isolated occurrence not affecting other data. Results may not be corrected by subtracting any blank values.

Re-quantitate one or more detects found in the method blank (if applicable) from the reported average RF (or higher order regression, if used) per each batch of samples.

Evaluation of all EIS Compound Spike, NIS Compound Spike, LLLCS, LCS, MS, MSD, MD, and Method Blank Recalculations

If transcription errors (or other minor issues such as rounding errors) are found in method QC results, use professional judgment to qualify the data. It may be necessary to engage the point of contact as identified in the UFP-QAPP to contact the laboratory so they can provide revised (corrected) results. In all cases, if method QC calculation errors affect project target analytes, the point of contact should be notified, and all affected results noted in the data validation report, including listing the calculation errors.

For systemic (defined as widespread and major in nature) problems with LCS/LLLCS or calculations, qualify all affected analytes in associated samples as **X**, exclusion of data recommended.

For systemic problems with method blanks, MD, or MS/MSD calculations qualify all affected analyte detects in associated samples as estimated **J** and non-detects as estimated **UJ**.

6.3 Instrument QC Recalculations

6.3.1 Response Ratios, Response Factors, Instrument Sensitivity Checks, Calibration Verifications

Initial calibration recalculations should use the raw instrument response for the target analytes and associated EIS and NIS compounds, to recreate the calibration curve from the individual calibration standards. If multiple types of calibration curves (e.g., first order or second order curve fit) are employed in a data package, at least one analyte per curve type should be recalculated.

Commercial PFAS standards available as salts are acceptable, providing the measured mass is corrected to the neutral acid concentration. Results shall be reported as the neutral acid with appropriate CAS number. If sample results were not corrected to the neutral acid but reported from the salt, qualify detects as **J+.**

Re-quantitate and recalculate the individual and average RRs/RFs for at least 10% of target analytes.

Re-quantitate and recalculate the ICV, CCV, ISC, %D, %RSD, or %RSE for at least 10% of the target analytes, proportionally selecting analytes based on calibration curve types used in each initial calibration.

The laboratory may employ a linear or weighted linear least squares regression. The low standard should be recalculated using the calibration curve and evaluated. RRs/RFs should not be evaluated for analytes with linear or higher order regression curves. If the ICAL included refitting of the data back to the model (relative standard error), then recalculate 10% of the target analytes for the relative standard error in each ICAL.

Evaluation of Instrument Performance Checks, ICAL, Calibration Factors, Regressions, ICV/CCV/ISC, and EIS Recalculations

If the files provided do not match the quantitation report, the RRs and RFs reported are likely to be from another initial calibration and the laboratory report should be revised. The point of contact (UFP-QAPP Worksheet #6) should be reached to get a revised (corrected) report from the laboratory. For calculation errors for RFs or any other regression equations that cannot be corrected in a revised report, qualify all the data as **X**.

In all cases where instrument QC are calculated incorrectly, the UFP-QAPP point of contact should be notified and noted in the data validation report.

6.4 Standards Traceability

Evaluate the calibration standards used for the analytes of concern. From the Certificate of Analysis (however named), verify that the "true values" of each analyte of concern were correctly applied to create the calibration curve, that all analytes of concern were in the calibration mix, and contained both branched and linear isomers, if commercially available. Some standards are made by manufacturers using the salt of a PFAS. In these cases, the concentration of those PFAS should be corrected to the neutral acid concentration. Results should be reported as the neutral acid with appropriate CAS number.

All initial instrument calibrations should be verified with a standard obtained from a second manufacturer prior to analyzing any samples. From the standard Certificate of Analysis, verify that a second source was used for the Initial Calibration Verification (ICV). The use of a standard from a second lot obtained from the same manufacturer (independently prepared from different source materials) is acceptable for use as a second source standard.

Check that the stock standards were diluted properly into working standards by recalculating the dilutions of one or more calibration standards. Recalculate one or more method QC sample dilutions (such as LCS or MS/MSD) from the stock to the working standard.

Note: It is not the role of the data validator to evaluate the Certificate of Analysis for compliance with the *ISO-17034 Standard*, but to verify that stock and working standards were correctly applied in the creation of calibration curves.

Evaluation of Standards

Professional judgment should be used when evaluating errors in standards preparation. The point of contact identified in the QAPP (UFP-QAPP Worksheet #6) should be reached to get a revised (corrected) report from the laboratory.

For systemic (widespread) issues that cannot be corrected by the laboratory, or issues that affect the results of target analytes, the data should be qualified as **X**, exclusion of data recommended. Issues that do not affect the results of any target analytes should be noted in the data validation report.

For ICV standards that were not verified to be from a second source, qualify all affected data as **X**, exclusion of data recommended. No samples should have been run without a valid second source standard (per QSM requirements).

For expired standards, per QSM requirements, a laboratory cannot use a standard beyond its expiration date. All associated data should be qualified as **X** if expired standards were used. The expiration date of any working standard is based on the expiration date of the primary or stock standard.

6.5 Method Detection/Quantitation Limit Studies (Optional)

In some cases, a project QAPP may specify the review and validation of a detection/quantitation limit study. This could include studies such as Method Detection Limits (MDLs), quarterly LOD verifications, or LOQ verifications. The QAPP should specify

the criteria for evaluating the study. As a minimum, at least 10% of the raw data in the study should be recalculated.

Evaluation of Detection Limit Studies

The criteria for evaluating a detection/quantitation limit study should be listed in the project QAPP. The following guidance should be enacted if the QAPP does not specify the evaluation criteria.

If transcription errors (or other minor issues such as rounding errors) are found in detection/quantitation limit studies, use professional judgment to qualify the data. It may be necessary to engage the point of contact as identified in the project QAPP to communicate with the laboratory, so they can provide revised (corrected) results. In all cases, if calculation errors affect project detection or quantitation limits, the point of contact should be notified, and all affected results noted in the data validation report, including listing the calculation errors.

When calculation errors are uncovered that cannot be corrected by the laboratory and that affect detection/quantitation results, consideration should be given to qualify the study as **X**, exclusion of data recommended.

7.0 Stage 4 Validation

Note: Stage 4 validation includes all of Stage 1, Stage 2A, Stage 2B and Stage 3.

Raw Data (including any instrument outputs, mass spectra, chromatograms, or instrument parameters such as mobile phases and mobile phase gradients)

Stage 4 is a qualitative review of non-detected and detected results from instrument outputs. Chromatograms are checked for peak integration (10% of automated integration and 100% of manual integrations), baseline, and interferences; mass spectra are checked for minimum quantitative ion and qualitative ion signal-to-noise ratio, ion ratios, retention times or relative retention times are within method requirements for analyte identification. Raw data quantitation reports and ion transition chromatograms are required to perform review of the instrument outputs.

7.1 Target Compound Identification

The objective of the criteria for LC/MS/MS qualitative analysis is to minimize the number of erroneous identifications of target compounds. An erroneous identification can either be false positive (reporting a compound present when it is not) or a false negative (not reporting a compound that is present).

The identification criteria can be applied more easily in detecting false positives than false negatives. More information is available for false positives because of the requirement for submittal of data supporting positive identifications. Negatives, or non-detects, on the other hand represent an absence of data and are therefore more difficult to assess.

If a bile salt interference check standard was analyzed, the peaks of the bile salts should elute outside of the 1-minute retention time window of PFOS.

If a quantitative standard containing an isomeric mixture of an analyte or a qualitative identification standard of an analyte was analyzed, the peak area of branched isomers in a sample, if present, should be summed with the peak area integration of the linear isomer. Branched isomers elute prior to the linear isomer of a target analyte. If either standard was not analyzed, suspect branch isomer peaks should not be summed with the peak area integration of the linear isomer.

Target analyte detections should display a signal-to-noise of ≥ 3:1 for the quantitative and qualitative ions, have proper peak integration, and display all ions at the correct retention times with passing ion ratios (50 - 150%).

The retention time of each target analyte and EIS should be within ± 0.40 minutes of the predicted retention and updated with the latest daily CCV. If the analyte concentration was quantified using isotope dilution, the target analyte should be within ± 0.10 minutes of its associated EIS. On occasion where branched isomers peak height is higher than the linear isomer, the assigned RT may differ from EIS >0.1 minutes. Confirm that the branched isomer RT match the expected RT as confirmed by the qualitative or daily quantitative identification standard. Check a minimum of 10% of the reported target analyte detects for retention time. RT performance in samples with only non-detects can be evaluated by reviewing the EIS times.

Evaluation of Target Compound Identification

The application of qualitative criteria for LC/MS/MS analysis of target analytes requires professional judgment. It is up to the reviewer's discretion to obtain additional information from their point of contact identified in the project QAPP, if qualitative identification problems are uncovered. The point of contact should arrange with the laboratory to obtain a revised (corrected) laboratory report. All qualitative identification problems should be discussed in the data validation report. If it is determined that incorrect identifications were made, or if a confirmed positive detect was made, but the confirmation ion was not detected (when available), then all affected data should be qualified as **X**, exclusion of data recommended.

Professional judgment should be used to qualify the data if it is determined that cross-contamination has occurred. If it is determined that cross-contamination has occurred, all affected data should be qualified as **X**. Any changes made to the reported analytes or concerns regarding target analyte identifications should be clearly indicated in the data validation report.

If evaluation of the ion ratios, retention times, or signal-to-noise for a detected target analyte is considered invalid, confer with the point of contact to identify in the project QAPP to consider changing the reported detect to a non-detect for the affected analyte.

While retention time windows are usually less critical to mass spectrometry systems, retention times have an acute effect on LC/MS/MS using Multiple Reaction Monitoring (MRM) mode. For example, retention time window drift on an MRM system can have a direct impact on the reported results. Professional judgment should be used to qualify the data.

7.2 Manual Integrations

For Stage 4, the reviewer should examine and verify the validity of all manual integrations.

Performing improper manual integrations, including peak shaving, peak enhancing, or baseline manipulation to meet QC criteria or to avoid corrective actions is unwarranted manipulation and misrepresents the data. All manual integrations should be reviewed by the data validator. When manual integrations are performed, raw data records should include a complete audit trail for those manipulations (i.e., the chromatograms obtained before and after the manual integration should be retained to permit reconstruction of the results). This requirement applies to all analytical runs including calibration standards and QC samples. The person performing the manual integration should sign and date each manually integrated chromatogram and record the rationale for performing manual integration (electronic signature is acceptable). Any manual integration should be fully discussed in the case narrative, including the cause and justification.

Evaluation of Manual Integrations

Some level of manual integration is considered necessary for the normal operation of chromatographic systems. Instances of properly integrated peaks do not require qualification, but should be noted in the data validation report. However, excessive manual integrations may show a lack of routine maintenance by the laboratory, a rush to complete samples, or the results of analyzing excessively 'dirty' samples. Excessive manual integrations may also be the result of faulty software peak/baseline integration.

The data validator should use professional judgment in the review of manual integrations. All instances of manual integrations should be noted in the data validation report. Instances of incomplete information for manual integrations (such as failure to provide justification) should be reported to the point of contact identified in the project QAPP to obtain a revised (corrected) laboratory report. Instances of excessive manual integrations that cannot be corrected by the laboratory (such as 'dirty' samples that cannot undergo further cleanup procedures) should be qualified as **X**.

If, in the professional judgment of the validator, there are instances of unwarranted manipulation of data (such as multiple manual integrations used to 'pass' QC criteria), then those cases should be reported to the project team as soon as practical (UFP-QAPP Worksheet #6).

Appendix A: Method QC Tables

Note: The following table provides the requirements listed in the QSM Version 5.4 Table B-24. The Table does not include all the QC elements from the methods or as listed in this guidance document.

QC Check	Sample Type, QSM Frequency, and Acceptance Criteria
AFFF Samples	Each AFFF sample. **Note**: This does not include AFFF samples that are to be evaluated for Military Performance Specification 14385 (MIL-PRF-14385) compliance. Those AFFF samples must be performed in compliance with DoD AFFF01, not EPA Draft Method 1633. AFFF samples must 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. All AFFF samples must be processed in duplicate in the same manner as whole sample aqueous samples (solid phase extraction (SPE), carbon cleanup) per EPA Draft Method 1633.
Ion Transitions (Precursor-> Product)	Every field sample, standard, blank, and QC sample. In addition to the requirements of EPA Method 1633, the following must be met: 1) If a qualitative or quantitative standard containing an isomeric mixture (branched and linear isomers) of an analyte is commercially available for an analyte, the quantification ion used must be the quantification ion identified in Table 2 of EPA Draft Method 1633 unless interferences render the product ion unusable as the quantification ion. 2) In cases where interferences render the product ion unusable as the quantification ion, project approval is required before using the alternative product ion.
Ion Ratio	All analytes detected in a sample. Must meet all of the requirements of EPA Method 1633.
Instrument Sensitivity Check (ISC)	Daily. At the beginning of each analytical sequence, prior to sample analysis. In addition to the requirements of EPA Method 1633, the following must be met: All analyte concentrations must be within ± 30% of their true values.
Initial Calibration Verification (ICV)	Once after each ICAL, prior to sample analysis. Must be made from a second source standard. All analyte concentrations must be within ± 30% of their true values.

QC Check	Sample Type, QSM Frequency, and Acceptance Criteria
Instrument Blanks	Immediately following the highest standard analyzed in the calibration, daily prior to analyzing standards, after each CCV, and immediately following samples with PFAS concentrations exceeding the quantification range. In addition to the requirements of EPA Method 1633, the following must be met: Concentration of each analyte must be ≤ ½ the LOQ.
Extracted Internal Standard (EIS) Compounds	Every field sample, standard, blank, and QC sample. In addition to the requirements of EPA Method 1633, the following must be met: 1) Isotopically labeled analogs of analytes must be used when they are commercially available. 2) QC samples and field samples must recover within in-house limits if project limits are not provided; otherwise, project limits must be met. Preliminary in-house acceptance criteria of 20-150% must be used until in-house limits are generated in accordance with Sections 9.4.1 and 9.4.2 of EPA Draft Method 1633. 3) The lower limit of in-house acceptance criteria cannot be < 20%.
Non-extracted Internal Standard (NIS) Compounds	Every field sample, standard, blank, and QC sample. In addition to the requirements of EPA Method 1633, the following must be met: 1) NIS areas must be greater than 30% of the average area of the calibration standards in undiluted sample extracts and sample extracts that required additional NIS to be added. 2) NIS areas corrected for the dilution factor must be greater than 30% of the average area of the calibration standards in diluted samples when additional NIS was not added post dilution of the extract.
Method Blank (MB)	One per preparatory batch. In addition to the requirements of EPA Method 1633, the following must be met: No analytes detected > ½ LOQ or > 1/10th the amount measured in any associated sample or 1/10th the regulatory limit, whichever is greater.
Matrix Duplicate (MD)	Each AFFF sample prepared using an aliquot of the field sample must be prepared in duplicate. In addition to the requirements of EPA Method 1633, the following must be met: RPD ≤ 30% (between sample and MD)

QC Check	Sample Type, QSM Frequency, and Acceptance Criteria
Bile Salt Standards	Daily, prior to analysis of all matrix types (aqueous, solid, tissue, and AFFF). All EPA Draft Method 1633 requirements for evaluation of the relationship of the retention time of the bile salt peak(s) to the retention time window of PFOS must be met for all matrix types. The retention time window of PFOS applies to the retention time of all isomers of PFOS.
Laboratory Control Sample (LCS) and Low-Level Laboratory Control Sample (LLLCS)	One set per preparatory batch. In addition to the requirements of EPA Method 1633 the following must be met: 1) Analyte recoveries must be within in-house limits if project limits are not provided; otherwise, project limits must be met. Preliminary in-house acceptance criteria of 40-150% must be used until in-house limits are generated in accordance with Section 14.5.4 of EPA Draft Method 1633. 2) The lower limit of in-house acceptance criteria cannot be < 40%.
Matrix Spike (MS) and Matrix Spike Duplicate (MSD)	One pair per preparatory batch. In addition to the requirements of EPA Method 1633, the following must be met: Analyte recoveries must be within in-house LCS limits if project limits are not provided; otherwise, project limits must be met. RPD ≤ 30% (between MS and MSD or sample and MD).

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Appendix B: Formulas used in Stages 3 and 4 Data Validation

Calibration:

Response Ratio (RR) of PFAS Calibrated by Isotope Dilution:

$$RR = \frac{Area_n\, M_l}{Area_l\, M_n}$$

Where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS added to the sample before extraction

M_l = The mass of the isotopically labeled compound in the calibration standard

M_n = The mass of the native compound in the calibration standard

Response Factor (RF) of PFAS Calibrated by Extracted Internal Standard:

$$RF = \frac{Area_s\, M_{EIS}}{Area_{EIS}\, M_s}$$

Where:

$Area_s$ = The measured area of the Q1 m/z for the target (unlabeled) PFAS

$Area_{EIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the extracted internal standard (EIS)

M_{EIS} = The mass of the isotopically labeled PFAS used as the extracted internal standard (EIS) in the calibration standard

M_s = The mass of the target (unlabeled) PFAS in the calibration standard

Response Factor (RF) of EIS Compounds:

$$RF = \frac{Area_l\, M_{NIS}}{Area_{NIS}\, M_l}$$

Where:

$Area_l$ = The measured area of the Q1 m/z for the isotopically labeled PFAS standard added to the sample before extraction

$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the non-extracted internal standard (NIS)

M_{NIS} = The mass of the isotopically labeled compound used as the non-extracted internal standard (NIS) in the calibration standard

Mean Area Responses of NIS Compounds:

$$Mean\,Area_{NISi} = \frac{\Sigma\,Area_{NISi}}{n}$$

Where:

$Area_{NISi}$ = Area counts for the *ith* NIS, where *i* designates the individual NIS
n = the number of ICAL standards used

Relative Retention time:

$$RRT = \frac{Retention\ time\ of\ the\ analyte}{Retention\ time\ of\ the\ extracted\ internal\ standard}$$

Percent Difference:

$$\%D = \frac{C_s - C_k}{C_k} \times 100$$

Where:

C_s = Concentration, reported

C_k = Concentration, known

Sample Concentration:

Target Analyte Reported Values:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_n\ M_l}{Area_l\ (\overline{RR}\ or\ \overline{RF})} \times \frac{1}{W_S}$$

Where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

M_l = The mass of the isotopically labeled compound added (ng)

$\overline{RR}$ = Average response ratio used to quantify target compounds by the isotope dilution method

$\overline{RF}$ = Average response factor used to quantify target compounds by the extracted internal standard method

W_S = Initial sample volume (L) or weight (g) (wet weight for tissue, dry weight for solids)

EIS Compound Reported Values:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_l\ M_{nis}}{Area_{nis}\overline{RF_s}} \times \frac{1}{W_S}$$

Where:

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

$Area_{nis}$ = The measured area of the Q1 m/z for the non-extracted internal standard (NIS)

M_{nis} = The mass of the added non-extracted internal standard (NIS) compound (ng)

W_S = Initial sample volume (L) or weight (g) (wet weight for tissue, dry weight for solids)

$\overline{RF_s}$ = Average response factor used to quantify the isotopically labeled compound by the non-extracted internal standard method

EIS, LCS, or LLLCS Percent Recovery:

$$Percent\ Recovery = \frac{C_s}{C_K}\ x\ 100$$

Where:

C_s = Concentration, Reported

C_K = Concentration, Known

MD, MS, or MSD Percent Recovery:

$$Percent\ Recovery = \frac{C_M - C_s}{C_K}\ x\ 100$$

Where:

C_M = Concentration, MD, MS, or MSD

C_s = Concentration, Sample

C_K = Concentration, Known

MS/MSD or Sample/MD Relative Percent Difference (RPD):

$$RPD = \frac{|C_s - C_d|}{(C_s + C_d)/2}\ x\ 100$$

Where:

C_s = Concentration, Sample or MS

C_d = Concentration, Duplicate or MSD

Ion Ratio of Standard:

$$IRstd = \frac{Area_{Q1}}{Area_{Q2}}$$

Where:

IRstd = Ion Abundance Ratio of standard

$Area_{Q1}$ = The measured area of the Q1 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration

$Area_{Q2}$ = The measured area of the Q2 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration

Q1 m/z= The quantitation ion

Q2 m/z= The confirmation ion

Ion Abundance Ratio of Sample:

$$IRs = \frac{Area_{Q1}}{Area_{Q2}}$$

Where:

IRs = Ion Abundance Ratio of sample

$Area_{Q1}$ = The measured area of the Q1 m/z for the analyte in the sample

$Area_{Q2}$ = The measured area of the Q2 m/z for the analyte in the sample

Q1 m/z= The quantitation ion

Q2 m/z= The confirmation ion

Ion Ratio Percent Recovery:

$$Percent\ Recovery = \frac{IR_s}{IR_{STD}}\ x\ 100$$

Where:

IRs = Ion Abundance Ratio of sample

IR_{std} = Ion Ratio of standard