Data Validation Guidelines Module 3: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-15

Environmental Data Quality Workgroup
05/01/2020
Data Validation Guidelines
Module 3

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Module 3: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by Quality Systems Manual for Environmental Laboratories (QSM) Table B-15

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) compliant with DoD Quality Systems Manual (DoD QSM) Table B-15 criteria in solid, biota, AFFF, and non-drinking water 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 technique and reporting of data generated for Department of Defense (DoD) projects for PFAS when analyzed on LC/MS/MS.

Project-specific requirements as identified in the Quality Assurance Project Plan (QAPP) (also called Sampling and Analysis Plan (SAP)) 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 (2019). This document is also not intended to obviate the need for professional judgment during the validation process.

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

2.0 Procedure

2.1 Introduction

This document was written with primary consideration to Version 5.3 of Table B-15 in the DoD Quality Systems Manual (QSM). Actual validation should proceed using the acceptance criteria for the DoD QSM version specified in the laboratory data deliverable or in the QAPP. Appendix A summarizes the quality control (QC) checks and the required frequency and acceptance criteria for DoD QSM Version 5.3 requirements. This guidance can be applied to PFAS data generated in support of DoD projects that was produced by LC/MS/MS. This guidance should be implemented by personnel familiar with the methodology contained herein.

Data validation personnel are responsible for implementing this procedure for validation of data and generation of data validation reports for LC/MS/MS PFAS contaminant data.

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.
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

The following documents should be reviewed for representativeness (compliance with required analytical protocols outlined in the QAPP), completeness, and project sensitivity needs:

- Cover Sheet
- Table of Contents
- Case Narrative
- Sample Results form or equivalent Laboratory Report
- Transition Ion Summary
- 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 project target analytes have been analyzed and are reported
- All ion transitions used for quantitation and confirmation are identified
- All project target analytes whose quantitation includes branched and linear isomers are identified
- All sample identification labels are unique, and match the chain of custody
- 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
- All project required LODs have been met and are lower than the LOQs
- All reported units (e.g., µg/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 samples have been reported on a dry weight basis, unless specified by the QAPP to report on a wet weight basis
- Each laboratory report has a case narrative that explains all non-conformities with the data

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.
If the project QAPP changes reporting requirements from that specified in the QSM by reporting data down to the DL, then any detects between the DL and LOQ are qualified as estimated J. Values below the DL are considered non-detects and are qualified as U at the stated DL.

If the project QAPP changes reporting requirements from that specified in the QSM by reporting data down to the LOD, then any detects between the LOD and below the LOQ are qualified as estimated \( J \). Values below the LOD are considered non-detects and are qualified as U at the stated LOD.

If the project QAPP changes reporting requirements from that specified in the QSM by reporting data down to the LOQ, then any detects below the LOQ are considered non-detects and are qualified as U at the stated LOQ.

**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 by LC/MS/MS but were not requested should also be noted.

Analytes that have project action levels less than the laboratory’s LOQ should be noted in the data validation report as there is greater uncertainty at values less than the LOQ. 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) and communicate issues when preparing the data validation report.

A minimum of two ion transitions (precursor to quantitative ion and precursor to confirmation ion) should be used for identification of target analytes, when possible. In some cases, such as perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), N-methylperfluorooctanesulfonamidoethanol (NMMeFOSE), and N-ethylperfluorooctanesulfonamidoethanol (NEtFOSE) only one ion transition is possible. In cases where the DoD QSM has specified a particular ion transition to be used for quantitation (Table I), that ion transition should be the one identified for quantitative purposes. When evaluating the Sample Transition Ion Summary, if the DoD QSM specified ion transitions are not used for quantitation, the technical justification provided in the case narrative should be reviewed. 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.
### Table I: DoD QSM Specified Quantitative Ion Transitions

<table>
<thead>
<tr>
<th>PFAS Name</th>
<th>CASRN</th>
<th>Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorooctanoic acid (PFOA)</td>
<td>335-67-1</td>
<td>413 → 369</td>
</tr>
<tr>
<td>Perfluorooctanesulfonic acid (PFOS)</td>
<td>1763-23-1</td>
<td>499 → 80</td>
</tr>
<tr>
<td>Perfluorohexanesulfonic acid (PFHxS)</td>
<td>355-46-4</td>
<td>399 → 80</td>
</tr>
<tr>
<td>Perfluorobutanesulfonic acid (PFBS)</td>
<td>375-73-5</td>
<td>299 → 80</td>
</tr>
<tr>
<td>1H,1H, 2H, 2H-Perfluorohexane sulfonic acid (4:2 FTS)</td>
<td>757124-72-4</td>
<td>327 → 307</td>
</tr>
<tr>
<td>1H,1H, 2H, 2H-Perfluorooctane sulfonic acid (6:2 FTS)</td>
<td>27619-97-2</td>
<td>427 → 407</td>
</tr>
<tr>
<td>1H,1H, 2H, 2H-Perfluorododecane sulfonic acid (8:2 FTS)</td>
<td>39108-34-4</td>
<td>527 → 507</td>
</tr>
<tr>
<td>N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)</td>
<td>2991-50-6</td>
<td>584 → 419</td>
</tr>
<tr>
<td>N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)</td>
<td>2355-31-9</td>
<td>570 → 419</td>
</tr>
</tbody>
</table>

### 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 CoC sample identification on the Laboratory Sample Results form matches the sample identification on the CoC. Ensure the CoC was signed and dated during transfers of custody. 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. Document in the data validation report if the transfer CoC is not present or if there is missing information (such as location of the laboratory). 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.

- Aqueous, solid, and aqueous film forming foam (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

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.

If a temperature non-conformance occurs as well as a holding time non-conformance (see next section), apply professional judgment to qualify the sample results.

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 assimilates 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 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:

Total holding time is based on the time frame (i.e., hours, days, or months) of the requirement. The following example gives guidance on how hold time exceedances are measured:

For a test with a recommended maximum holding time measured in **days**, the holding time is tracked by the **day**.

- An exceedance of holding time for a sample with a 14-day holding time will occur when the 15th day is reached. Therefore, a sample with a 14-day holding time collected at 8:30 AM on April 4th must be analyzed or extracted before 12:00 AM April 19th (midnight, the start of the 15th day), or an exceedance has occurred.

The QAPP should specify the holding time requirements.

*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.

3.3 Field QC

Field QC can consist of various blanks, field duplicates, and field replicates. The purpose of blanks is to identify potential cross-contamination at different stages of sampling and cleaning of equipment for reuse. Duplicates and replicates help a project identify reproducibility among samples at the project site.

3.3.1 Field Blanks

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.

Below are the common types of field blanks utilized.

A **field blank** is a sample of PFAS-free water (as defined by the QAPP) 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** must 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 (as
Learning objects must 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. Analytes detected in equipment blanks indicate the possibility of cross-contamination between samples due to improper equipment decontamination. Equipment blanks should be collected at a minimum, the frequency specified in the QAPP.

**Evaluation of Field Blanks**

Determine which field blanks apply to samples in the sample delivery group (SDG) from the CoC. 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 μg/mL) to a solid parent sample (such as a soil sample reported as mg/kg). At best, only a qualitative comparison can be made.

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).

Water used for field blanks should be PFAS-free (as defined by the QAPP) and provided with the sample bottle kit by the contracted laboratory performing the analysis. To ensure the origin of the water used, consult with the field sampling team leader via appropriate channels identified in the QAPP (such as UFP-QAPP Worksheet #6). If source water was used during sampling, field blanks using each source water should also be PFAS-free (as defined by the QAPP).

If field blank water is used as equipment blanks and both are contaminated, the affected samples are qualified by either the field blank or equipment blank results, whichever has the higher contaminant concentration. The same applies if source water is used as equipment blanks and both are contaminated.

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 II).

**Table II: Blank Qualifications**

<table>
<thead>
<tr>
<th>Row Number</th>
<th>Blank Result</th>
<th>Sample Result</th>
<th>Validated Result</th>
<th>ValidationQualifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \leq \text{DL or LOD} )</td>
<td>( \leq \text{DL or LOD} )</td>
<td>Report as required by QAPP (at DL or LOD)</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>( \text{&gt; DL or LOD} )</td>
<td>( \leq \text{DL or LOD} )</td>
<td>Report at Sample Result</td>
<td>U</td>
</tr>
<tr>
<td>3</td>
<td>( \text{&gt; DL or LOD} )</td>
<td>( \text{&gt; DL or LOD but} \leq \text{LOQ} )</td>
<td>Report at Sample Result</td>
<td>U</td>
</tr>
<tr>
<td>4</td>
<td>( \text{&gt; DL or LOD} )</td>
<td>( \text{&gt; LOQ but} \leq 5x \text{ blank} )</td>
<td>Report at Sample Result</td>
<td>J+</td>
</tr>
<tr>
<td>5</td>
<td>( \text{&gt; DL or LOD} )</td>
<td>( \text{&gt; LOQ and} &gt; 5x \text{ blank} )</td>
<td>Report at Sample Result</td>
<td>None</td>
</tr>
</tbody>
</table>

**LOD = Limit of Detection, LOQ = Limit of Quantitation, DL = Detection Limit**

**Note:** The laboratory B qualifier is maintained, and the validation qualifier is added in addition to the laboratory qualifier. The QAPP should specify reporting non-detects at either the DL, or the LOD.

In situations where the QAPP requires an LOQ for the sum of a number of PFAS, (e.g., sum of concentrations of PFOA, PFOS, and PFNA) and the sum of the detects in blank exceed this value, use professional judgment 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 collocated samples. 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.

For field duplicate results, if the Relative Percent Differences (RPDs or absolute differences are greater than those stated in the QAPP, qualification of the associated sample results is not necessary, but any non-conformities should be noted in the data validation summary.

Professional judgment may be required in instances where the sample and field duplicate results are less than the LOQ or project reporting limits. RPD results can be elevated when low (for example, <5X the LOQ) or estimated concentrations in the samples and duplicates are reported.

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.

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.

4.0 Stage 2A Validation

Note: Stage 2A includes all of Stage 1

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

- Ion Ratio Summary
- Extracted Internal Standard Recovery Summary
- Laboratory Control Sample/Laboratory Control Sample Duplicate Recovery and Relative Percent Difference Summary
- Matrix Spike/Matrix Spike Duplicate Recovery and Relative Percent Difference Summary
- Post Spike Sample Recovery Summary
- Method Blank Summary form
- Dilution/Reanalysis 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 20 field samples (maximum) along with blank, duplicate, and control/matrix type QC samples. 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 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 matrix of the sample has resulted in a bias in the data. A laboratory can calculate ion ratios in a number of ways, which are 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. In-house acceptance criteria for evaluation of transition ion ratios should be used and should not exceed 50-150%.

Evaluation of Ion Ratios

Verify the ion ratio(s) for each detect were reported, met in-house acceptance criteria, and in-house acceptance criteria did not exceed 50-150%. For detects reported with ion ratios exceeding in-house control limits and/or 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 analytes. EIS analytes are added to the solid sample prior to extraction and to an aqueous sample in the original sample container prior to extraction. For aqueous samples prepared by serial dilution (e.g., Aqueous film forming foam (AFFF)), EIS analytes are added to the final dilution of samples prior to analysis.

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

Sample and batch QC EIS percent recoveries should be within control limits established in the QAPP or the QSM. Verify that no samples or batch QC have EIS percent recoveries outside the criteria.
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.

Each EIS percent recovery should be within control limits established in the QAPP or within 50-150% (if DoD QSM criteria is used) of the area of the mid-point standard in the ICAL for associated standards. On days when an ICAL is not performed, the daily initial continuing calibration verification (CCV) can be used.

The EIS retention times (RTs) for all field and QC samples should be within 0.40 minutes (if DoD QSM criteria is used) of the retention time of the midpoint standard in the ICAL, or on days when an ICAL is not performed, the initial CCV is used.

**Evaluation of Extracted Internal Standards**

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 > 150% should be qualified estimated with a negative bias J-. Non-detects should not be qualified.

Detects for analytes quantitated using an EIS percent recovery < 50% but ≥ 20% should be qualified estimated with a positive bias J+ for detects. Non-detects should be qualified estimated UJ.

If extremely low area counts are reported (< 20%), detects and non-detects should be qualified X.

If an EIS retention time varies by more than 0.40 minutes (if DoD QSM criteria is used), use professional judgment to qualify the sample results and note all affected results in the data validation report.

EIS results may not be reported as “diluted out” since they are used as the internal standard for calculation of the native analyte. 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.
Some extracts may require dilution, to bring analytes within the calibration range. This can result in EIS dilution to the point that EIS recoveries may not be sufficiently measurable and would require EIS fortification to the diluted extract. Detects for analytes quantified from this type of diluted extract should be qualified as estimated $J$. Non-detects in the diluted extract should be reported from less-diluted or undiluted extract results.

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. The basic concern is whether the blank problems represent an isolated problem with the blank alone, or whether there is 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 Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)

An LCS 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. Analysis of LCSDs may be required by the QAPP, or may be used as an indication of batch precision in instances where matrix spike duplicate analysis is not possible (e.g., a limited volume of sample).

LCS (sometimes called a “Blank Spike”) and, if analyzed, LCSD recoveries should be within the QC limits specified in the QAPP or as listed in the DoD QSM. If an LCSD was analyzed, the RPDs should be within the QC limits specified in the QAPP.

Evaluation of LCS/LCSD

Verify that results (from appropriate summary form), percent recoveries, RPDs (if applicable) 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 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.

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 (i.e., same or less than) those specified in the QAPP or DoD QSM. If the laboratory’s in-house control limits are wider than those in the QSM but the results remain within the DoD QSM limits, no qualification is necessary. If the laboratory’s in-house control limits are wider than those in the QSM and the results are outside of the DoD QSM limits, qualify the appropriate data as $X$.

If the LCS 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 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.

If the LCS/LCSD 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 for those analytes not spiked as X and non-detects for those analytes not spiked as X, exclusion of data is recommended.

If the LCS/LCSD 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.

4.4 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.

Generally, qualifying action is taken only on the parent sample based on MS/MSD non-conformities. In instances where it can be determined that the results of the MS/MSD affect only the sample spiked, then qualification should be limited to that sample alone. Using informed professional judgment, the data reviewer may use the MS/MSD results in conjunction with other QC criteria (i.e., EIS and LCS) and determine the need for additional qualification beyond that applied to the parent sample when the laboratory is having a systemic problem in the analysis of one or more analytes, which affects all associated samples.

Field QC samples (e.g., field blank, equipment blank, source blank) should not be used for the MS/MSD. If a field blank was used for the MS/MSD, this fact should be included in the data validation summary. Sample matrix effects may not be observed with field blanks; therefore, the recoveries and precision do not reflect the extraction or analytical impact of the site matrix.

The laboratory should spike and analyze an MS/MSD from the specific project site as required by the QAPP for each matrix type and analytical batch. 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**

MS/MSD data should be reported on a MS/MSD summary form (or equivalent). Verify that the MS/MSD were spiked with all target analytes, and that percent recoveries were reported for all target analytes.
Compare the percent recovery and (RPD for each analyte with LCS control limits established by the QAPP. 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 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 UFP-QAPP Worksheet #6).

If the MS/MSD was not spiked with all target analytes, notify the project team by following the notification protocols and qualify detects in the parent sample for those analytes in each batch not spiked as X, and non-detects in the parent sample for those analytes not spiked as X, exclusion of data is recommended.

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 \( \geq 10\% \), 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 not be qualified.

Failure of MS/MSD due to the presence of target analyte(s) in the parent sample at > 4X the spike concentration or if the matrix spikes are diluted to less than the LOQ, matrix spike non-conformities should not result in any qualifications. Note the incident in the data validation report.

4.5 Post Spike Sample

Post spike sample data are used to verify the instrument was capable of accurately quantifying PFAS in the sample’s matrix at the reported LOQ. This QC sample is only applicable to aqueous and AFFF samples that were prepared using serial dilution instead of solid phase extraction (SPE).

A post spike sample should be associated with every PFAS target analyte that is reported as a non-detect or < LOQ in the associated sample.

**Evaluation of Post Spike Sample**

Post spike sample data should be reported on a post spike sample summary form. Verify that the post spike samples were spiked with all target analytes reported as non-detect or < LOQ in the associated sample. The lowest possible dilution while meeting QC criteria (EIS percent recovery and post spike percent recovery) should be reported for these analytes. This dilution should be spiked at a concentration at the LOQ (after dilution is taken into account).
The post spike sample percent recoveries should be within 70 - 130%. 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 DoD QSM, reference the required control ranges instead of the summarized ranges in the deliverable. The client 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 post spike sample percent recoveries were less than the lower acceptance limit but ≥ 20%, qualify non-detects as estimated UJ.

If the post spike sample percent recoveries were < 20%, qualify non-detects as X, exclusion of data is recommended.

If the post spike sample percent recoveries were >130%, qualify detects for the analyte in the associated parent sample as estimated J+. Non-detects should not be qualified.

4.6 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.

Evaluation of Method Blanks

If no method blank was analyzed, qualify detects in samples with no associated method blank as X. 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 should be qualified as X due to interference in all affected samples and this should be noted in the data validation comments.

If target analytes are found at low levels in the method blank(s), it may be indicative of a problem at the laboratory and should be noted in the data validation report.

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 II (Blank Qualifications) and Section 3.3.1 discussions on evaluations of results from the DL/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. Qualification of the data should be performed as given in Table II.

Professional judgment should be applied to any field blank result that was associated with a contaminated method blank. Generally, if the field blank result was qualified as a non-detect due to the method blank, it does not need to be applied to the associated sample results. However, the fact that the field blank was qualified should be noted 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.

4.7 Sample Dilution and Reanalysis

Laboratories may dilute samples due to high analyte concentrations or reanalyze samples due to quality control non-conformities, and document both sets of results. Generally, the laboratory will report the “best” value for a given analyte in the official laboratory report (or equivalent form). The validator should evaluate both the reported and the initial analysis result. In the case of AFFF samples, the laboratory should report the lowest dilution that met all quality control criteria (e.g., post spike recovery and EIS recovery) for each target analyte. The validator should consider the application of appropriate qualifiers to the reported results within the scope of the project due to elevated LODs/LOQs or other quality control anomalies. Qualifiers apply only to the reported results in the official laboratory report.

Evaluation of Sample Dilution and Reanalysis

When sample results are reported at more than one dilution due to analyte concentrations exceeding the calibration curve, the lowest LODs are generally used for the non-detects unless a QC criterion has been exceeded.

Results reported from dilutions lead to elevated LODs for non-detects. The data validation report should indicate the reason for all reported dilutions (including cases where the laboratory did not perform an undiluted analysis) 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).

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)
- Instrument Performance Check Summary (mass calibration verification)
- Initial Calibration Summary (any equivalent to include the Initial Calibration Analyte Responses, Isomeric Profiles, Average Response Factors, and Regression)
- Instrument Blank Summary
- Initial/Continuing Calibration Verification and Instrument Sensitivity Check Summaries (any equivalent to include Initial and Continuing Calibration Verifications and Instrument Sensitivity Checks)

Stage 2B adds for review, 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) had 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 20 samples at the same time on two or more instruments. At a minimum, mass calibration and mass calibration verification documentation should be included for each instrument used. 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 Instrument Performance Checks

LC/MS/MS instrument performance checks (referred to as mass calibration verifications) are performed to ensure mass resolution, identification, and to some degree, sensitivity are all within criteria. Conformance is determined using reference standards; therefore, acceptance criteria should be met in all circumstances. Check that all samples and associated QC analyses are associated with an acceptable mass calibration verification.

Make certain that a mass calibration verification has been performed prior to the initial calibration used. The mass calibration verification should verify a mass range which includes the ion masses of all target analytes. Mass accuracy should be verified to be within ± 0.5 amu of the true value by the acquisition of a full scan continuum mass spectrum of a PFAS stock standard or by following the instrument manufacturer’s instructions for performing a mass calibration verification and using the instrument manufacturer’s recommended standards as long as they verify the mass range of the PFAS ions of interest.

Evaluation of Instrument Performance Checks

Careful consideration should be given to any reported results that accompany a mass calibration verification that does not meet criteria. Based on QSM 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 analytes) with a minimum of five calibration standards depending on the type of curve. More standards are required for higher order regression curves. 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.

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 III lists standards that are currently 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 III: Currently Available Certified PFAS Standards Containing Branched and Linear Isomers

<table>
<thead>
<tr>
<th>Standard</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorohexanesulfonic acid (PFHxS)</td>
<td></td>
</tr>
<tr>
<td>Perfluorooctanesulfonic acid (PFOS)</td>
<td></td>
</tr>
<tr>
<td>2-(N-methylperfluorooctanesulfonamido) acetic acid (NMeFOSAA)</td>
<td></td>
</tr>
<tr>
<td>2-(N-ethylperfluorooctanesulfonamido) acetic acid (NEtFOSAA)</td>
<td></td>
</tr>
</tbody>
</table>

**Evaluation of Initial Calibration**

If target analytes were not calibrated, qualify associated non-detects and detects as X, exclusion of data is recommended.

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

Each calibration standard should recover within 70 - 130% of its true value. If this criteria is not met for an analyte, 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.
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.

In order to produce acceptable sample results, the response of the instrument must be within the working range established by the initial calibration. Any sample detections above the working range of the calibration curve should be accompanied by a dilution that is within the working 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 working 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 reported concentration exceeded the calibration range, qualify detects as estimated J.

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

If the LOD is not 2 to 4 times the DL for target analytes, use professional judgment to qualify the sample results and note all affected results in the data validation report. If the LOQ is not the lowest calibration standard, then any detects that fall between the LOQ and the lowest calibration standard should be qualified as estimated J.

5.3.1 Response Factors (RFs) and Relative Standard Deviation (%RSD)

Evaluate the average response factors (RFs) for all target analytes. RFs are an indicator of the sensitivity of the analyte to detection and quantitation by Mass Spectrometry (the higher the RF the more sensitive the analyte).

All target analytes should have an associated %RSD ≤ 20% for an average calibration fit.

Evaluation of RFs and %RSD

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

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

Each calibration standard should recover within 70 - 130% of its true value. If this criteria is not met for an analyte, make note of this in the data validation report and qualify all affected data as X, exclusion of data is recommended.
5.3.2 Linear Least Squares Regression

The laboratory may employ a linear or weighted linear least squares regression. A minimum of five standards is required for a linear regression. Evaluate the coefficient of determination ($r^2$). The DoD QSM specifies that the $r^2$ value should be ≥ 0.99.

*Evaluation of Linear Least Squares Regression*

If the required number of calibration standards was not used, qualify detects as $X$, exclusion of data is recommended. Apply professional judgment to qualify non-detects based on the concentrations of the standards used.

If the $r^2$ value is < 0.99, qualify detects for the affected analytes $J$ and non-detects $UJ$ in the associated samples.

If the $r^2$ value is excessively low (defined as < 0.90), qualify all associated non-detects as $X$, exclusion of data is recommended, and detects as estimated $J$.

Each calibration standard should recover within 70 - 130% of its true value. If this criteria is not met for an analyte, make note of this in the data validation report and qualify all affected data as $X$, exclusion of data is recommended.

5.3.3 Non-Linear Regression

The laboratory may also generate a higher order curve for the calibration. The calibration curve should not be more than second order. It is a statistical requirement that the instrument response is the dependent variable (Y-axis). Verify that the instrument response is on the Y-axis.

A minimum of six standards is required for a second order (quadratic) curve.

Evaluate the correlation coefficients ($r$) for all applicable target analytes. The coefficient of determination ($r^2$) should be ≥ 0.99.

*Evaluation of Non-Linear Regression*

If the required number of calibration standards was not used, qualify detects as $X$, exclusion of data is recommended. Apply professional judgment to qualify non-detects based on the concentrations of the standards used.

If the $r^2$ value is < 0.99, qualify detects for the affected analytes $J$ and non-detects $UJ$ in the associated samples.

If the $r^2$ value is excessively low (defined as < 0.90), qualify all associated non-detects as $X$, exclusion of data is recommended, and detects as estimated $J$.

Calibration curves that are higher than second order (such as a third order polynomial fit) are not allowed in accordance with QSM requirements. Qualify $X$ all associated data based on third order (or higher) calibration curves.
Each calibration standard should recover within 70 - 130% of its true value. If this criteria is not met for an analyte, make note of this in the data validation report and qualify all affected data 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 an initial calibration is performed. This standard is called the secondary source or Initial Calibration Verification (ICV). The LOQ should be verified with a standard that is prepared at the concentration of the LOQ each time an initial calibration is performed. This standard is called the ISC. Both the ICV and ISC should contain all of the target analytes. Note that multiple ICVs and ISCs may be analyzed to encompass all of the target analytes.

After the initial calibration has been verified with a second source and the ISC has verified the associated LOQ, samples may be analyzed continuously until an on-going calibration verification fails. To verify this, a CCV containing all 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. To ensure the instrument can achieve the LOQ throughout the analytical sequence, an ISC containing all target compounds should be analyzed at the beginning of every analytical sequence prior to sample analysis, every 12 hours thereafter. These continuing calibration checks verify satisfactory performance of the instrument on a day-to-day basis.

Verify the ICV was analyzed following the initial calibration and contained all target analytes. Verify the CCVs have been run prior to sample analysis, every ten field samples, and at the end of the analytical sequence. 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.

Verify the ISC was analyzed following the initial calibration and contained all target analytes. Verify the ISCs have been run prior to sample analysis and every 12 hours thereafter.

The ICV, ISC, and CCV percent difference (%D) or percent drift for each target analyte and EIS analytes should be within ± 30%.

When a new initial calibration is performed, the ICV can serve as the first ISC if the ICV was analyzed at the LOQ. If the initial daily CCV is analyzed at the LOQ, it can also serve as the first ISC of the analytical sequence. The CCVs analyzed after the first ICV and the ISCs are not required to be a second source.

Evaluating the ICV, CCV, and ISC

Verify that the %Ds 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 %D is higher than acceptance criteria and J- when below acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.
For gross exceedances of %D (defined as > 50% for ICV/ISC/CCV) qualify all associated data as X.

If the ICV (second source) and/or ISC have not been performed after an initial calibration or if samples have been analyzed prior to a valid ICV and/or ISC, qualify all associated data as X, exclusion of the data is recommended. No samples should have been analyzed without a valid ICV and ISC.

If the continuing calibration verification 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 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. If ISCs have been analyzed beyond the 12-hour time limit criteria, qualify the associated sample detects as J and the non-detects as UJ. For gross exceedances of the 12-hour time limit (defined as > 16 hours), qualify all associated data as X.

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 and daily prior to sample analysis. In order to quantify contamination, the IBs should contain EIS analytes. Each analyte in the IB should meet the acceptance criteria defined in the QAPP. The DoD QSM 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

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. All associated data should be qualified as X, exclusion of data is recommended.

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:

- Raw data (including any laboratory forms, instrument outputs, spreadsheets, or handwritten calculations necessary for recalculation and re-quantification)
- Standards traceability forms and worksheets
- Detection limit studies (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

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 re-quantification and recalculation. Other circumstances that should be prioritized for re-quantification and recalculation are diluted samples, manual integrations, re-runs of samples, and field QC blank failures.

Re-quantification and recalculation should be performed on the designated percentage of the samples per Sample Delivery Group (or however defined in the QAPP, such as percentage of total project samples) per analytical suite. As 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 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

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

6.2.1 EIS Analytes Spike

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

6.2.2 LCS/LCSD

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/LCSD if contaminants of concern (target analytes) have not been specifically identified. Recalculate RPDs (if applicable) from LCS/LCSD pairs that would result in the qualification of a sample.

6.2.3 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 contaminants 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.

6.2.4 Post Spike

Re-quantitate 10% of the target analytes spiked into the post spike sample. Use a random 10% of the analytes in the Post Spike if contaminants of concern have not been identified.

6.2.5 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 Analyte Spike, LCS, MS, 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/LCSD or Post Spike calculations, qualify all affected analytes in associated samples as X, exclusion of data recommended.

For systemic problems with method blanks, 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

#### 6.3.1 Initial (Response Factors and Regressions) and Continuing Calibration Verifications

Initial calibration recalculation should use the raw instrument response for the target analytes and associated EIS analytes, to recreate the calibration curve from the individual calibration standards. If multiple types of calibration curves are employed in an analytical site, 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 RFs, %RSDs, regression function (if used), and \( r^2 \) values reported for at least 10% of the target analytes per each EIS, preferably analytes of concern which were identified in the QAPP, per initial calibration curve type.

Re-quantitate and recalculate the ICV, CCV, ISC, RF results, and %D for at least 10% of the target analytes, proportionally selecting analytes based on each calibration curve type.

The laboratory may employ a linear or weighted linear least squares regression. The low standard should be recalculated using the calibration curve and evaluated. 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.

Analytes should be within 70 - 130% of their true value for each calibration standard.
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 RFs (equivalent to calibration factors, CFs) 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.

The reprocessed standards of a regression curve should be within 30% of the true value. If the recalculated concentration is not within 30% of the true value, qualify detects (at the LOQ and above) for the affected analytes J and non-detects UJ in the associated samples.

Qualify all associated data as X if the corresponding ICV/CCV/ISC/%D has been calculated incorrectly by the laboratory and cannot be corrected in a revised laboratory report.

Qualify all data as X if the corresponding EIS had been calculated incorrectly (or if the EIS had been assigned to the wrong analyte) by the laboratory and cannot be corrected in a revised laboratory report.

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 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. For minor issues, such as the calculation of a PFAS using the concentration of that PFAS as a salt (i.e. not correcting calculation to the neutral acid concentration), the project management team (UF-P-QAPP Worksheet #6) should be contacted to get a revised (corrected) report from the laboratory. Minor issues (that do not affect the results of any target analytes) should be noted in the data validation report.

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.

For ICV standards that were not verified to be from a second source, qualify all affected data as X. No samples should be analyzed 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 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 project 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.
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, or chromatograms)

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, transition 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.

The peak area of the branched isomers, 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.

Target analyte detections should display a signal-to-noise of ≥ 10:1 for the quantitative ion and ≥ 3:1 for the confirmation ion, 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 ± 0.4 minutes of the predicted retention and updated with the latest daily CCV. 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 is based on the DoD QSM Standard. The Table does not include all the QC elements from the methods or as listed in this guidance document.

<table>
<thead>
<tr>
<th>QC Check</th>
<th>DoD QSM Frequency and Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqueous Sample Preparation</strong></td>
<td>Each sample and associated batch QC samples.</td>
</tr>
<tr>
<td></td>
<td>Solid Phase Extraction (SPE) must be used unless samples are known to contain high PFAS concentrations (e.g., Aqueous Film Forming Foam (AFFF) formulations). Inline SPE is acceptable.</td>
</tr>
<tr>
<td></td>
<td>Entire sample plus bottle rinsate must be extracted using SPE.</td>
</tr>
<tr>
<td></td>
<td>Known high PFAS concentration samples require serial dilution be performed in duplicate.</td>
</tr>
<tr>
<td></td>
<td>Documented project approval is needed for samples prepared by serial dilution as opposed to SPE.</td>
</tr>
<tr>
<td><strong>Solid Sample Preparation</strong></td>
<td>Each sample and associated batch QC samples.</td>
</tr>
<tr>
<td></td>
<td>Entire sample received by the laboratory must be homogenized prior to subsampling.</td>
</tr>
<tr>
<td><strong>Biota Sample Preparation</strong></td>
<td>Each sample and associated batch QC samples.</td>
</tr>
<tr>
<td></td>
<td>Sample prepared as defined by the project (e.g., whole fish versus filleted fish).</td>
</tr>
<tr>
<td><strong>AFFF and AFFF Mixture Samples Preparation</strong></td>
<td>Each sample and associated batch QC samples.</td>
</tr>
<tr>
<td></td>
<td>Each field sample must be prepared in duplicate (equivalent to matrix duplicate).</td>
</tr>
<tr>
<td></td>
<td>Serial dilutions must be performed to achieve the lowest LOQ possible for each analyte.</td>
</tr>
<tr>
<td><strong>Sample Cleanup Procedure</strong></td>
<td>Each sample and associated batch QC samples.</td>
</tr>
<tr>
<td></td>
<td>Not applicable to AFFF and AFFF Mixture Samples.</td>
</tr>
<tr>
<td></td>
<td>ENVI-Carb™ or equivalent must be used on each sample and batch QC sample.</td>
</tr>
<tr>
<td>QC Check</td>
<td>DoD QSM Frequency and Acceptance Criteria</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Mass Calibration</strong></td>
<td>Instrument must have a valid mass calibration prior to any sample analysis.</td>
</tr>
<tr>
<td></td>
<td>Mass calibration is verified after each mass calibration, prior to initial calibration (ICAL).</td>
</tr>
<tr>
<td></td>
<td>Calibrate the mass scale of the MS with calibration compounds and procedures described by the manufacturer.</td>
</tr>
<tr>
<td></td>
<td>Mass calibration range must bracket the ion masses of interest. The most recent mass calibration must be used for every acquisition in an analytical run.</td>
</tr>
<tr>
<td></td>
<td>Mass calibration must be verified to be ±0.5 amu of the true value, by acquiring a full scan continuum mass spectrum of a PFAS stock standard or by following the instrument manufacturer’s recommended standards as long as these standards verify the mass range of the PFAS ions of interest.</td>
</tr>
<tr>
<td><strong>Mass Spectral Acquisition Rate</strong></td>
<td>Each analyte, Extracted Internal Standard (EIS) Analyte.</td>
</tr>
<tr>
<td></td>
<td>A minimum of 10 spectra scans are acquired across each chromatographic peak.</td>
</tr>
<tr>
<td><strong>Calibration, Calibration Verification, and Spiking Standards</strong></td>
<td>All analytes.</td>
</tr>
<tr>
<td></td>
<td>Standards containing both branched and linear isomers must be used when commercially available.</td>
</tr>
<tr>
<td></td>
<td>PFAS method analytes may consist of both branched and linear isomers, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes.</td>
</tr>
<tr>
<td></td>
<td>For PFAS that do not have a quantitative branched and linear standard, identify the branched isomers by analyzing a qualitative standard that includes both linear and branched isomers and determine retention times, transitions and transition ion ratios. Quantitate samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration that uses the linear isomer quantitative standard.</td>
</tr>
<tr>
<td>QC Check</td>
<td>DoD QSM Frequency and Acceptance Criteria</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Sample PFAS Identification</td>
<td>All analytes detected in a sample. The chemical derivation of the ion transitions must be documented. A minimum of two ion transitions (Precursor → quant ion and precursor → confirmation ion) and the ion transitions ratio per analyte are required for confirmation. Exception is made for analytes where two transitions do not exist (PFBA and PFPeA). Documentation of the primary and confirmation transitions and the ion ratio is required. In-house acceptance criteria for evaluation of ion ratios must be used and must not exceed 50 - 150%. Signal-to-Noise Ratio (S/N) must be ≥ 10 for all ions used for quantification and must be ≥ 3 for all ions used for confirmation. Quant ion and confirmation ion must be present and must maximize simultaneously (± 2 seconds).</td>
</tr>
<tr>
<td>Ion Transitions (Precursor -&gt; Product)</td>
<td>Every field sample, standard, blank, and QC sample. In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 → 369 PFOS: 499 → 80 PFHxS: 399 → 80 PFBS: 299 → 80 4:2 FTS: 327 → 307 6:2 FTS: 427 → 407 8:2 FTS: 527 → 507 NEtFOSAA: 584 → 419 NMeFOSAA: 570 → 419 If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).</td>
</tr>
<tr>
<td>QC Check</td>
<td>DoD QSM Frequency and Acceptance Criteria</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Initial Calibration (ICAL)</strong></td>
<td>At instrument set-up and after ICV or CCV failure, prior to sample analysis. The isotopically labeled analog of an analyte (Extracted Internal Standard Analyte) must be used for quantitation if commercially available (Isotope Dilution Quantitation). 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 a labeled analog is not commercially available, the Extracted Internal Standard Analyte with the closest retention time or chemical similarity to the analyte must be used for quantitation. (Internal Standard Quantitation) Analytes must be within 70 - 130% of their true value for each calibration standard. ICAL must meet one of the two options below: Option 1: The RSD of the RFs for all analytes must be ≤ 20%. Option 2: Linear or non-linear calibrations must have $r^2 \geq 0.99$ for each analyte.</td>
</tr>
<tr>
<td><strong>Retention Time window position establishment</strong></td>
<td>Once per ICAL and at the beginning of the analytical sequence. Position shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the midpoint standard of the ICAL curve or the initial CCV may be used.</td>
</tr>
<tr>
<td><strong>Retention Time (RT) window width</strong></td>
<td>Every field sample, standard, blank, and QC sample. RT of each analyte and EIS analyte must fall within 0.4 minutes of the predicted retention times from the daily calibration verification or on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minutes of the associated EIS. This criterion applies only to analyte and labeled analog pairs.</td>
</tr>
<tr>
<td><strong>Instrument Sensitivity Check (ISC)</strong></td>
<td>Prior to analysis and at least once every 12 hours. Analyte concentrations must be at LOQ; concentrations must be within ±30% of their true values.</td>
</tr>
<tr>
<td><strong>Initial Calibration Verification (ICV)</strong></td>
<td>Once after each ICAL, analysis of a second source standard prior to sample analysis. Analyte concentrations must be within ± 30% of their true value.</td>
</tr>
<tr>
<td>QC Check</td>
<td>DoD QSM Frequency and Acceptance Criteria</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Continuing Calibration Verification (CCV)</strong></td>
<td>Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.</td>
</tr>
<tr>
<td></td>
<td>Concentration of analytes must range from the LOQ to the mid-level calibration concentration.</td>
</tr>
<tr>
<td></td>
<td>Analyte concentrations must be within ± 30% of their true value.</td>
</tr>
<tr>
<td><strong>Instrument Blanks</strong></td>
<td>Immediately following the highest standard analyzed and daily prior to sample analysis.</td>
</tr>
<tr>
<td></td>
<td>Concentration of each analyte must be ≤ ½ the LOQ.</td>
</tr>
<tr>
<td></td>
<td>Instrument Blank must contain EIS to enable quantitation of contamination.</td>
</tr>
<tr>
<td><strong>Extracted Internal standards (EIS)</strong></td>
<td>Every field sample, standard, blank, and QC sample.</td>
</tr>
<tr>
<td></td>
<td>Added to solid sample prior to extraction. Added to aqueous samples, into the original container, prior to extraction.</td>
</tr>
<tr>
<td></td>
<td>For aqueous samples prepared by serial dilution instead of SPE, added to final dilution of samples prior to analysis.</td>
</tr>
<tr>
<td></td>
<td>Extracted Internal Standard Analyte recoveries must be within 50% to 150% of ICAL midpoint standard area or area measured in the initial CCV on days when an ICAL is not performed.</td>
</tr>
<tr>
<td><strong>Method Blank (MB)</strong></td>
<td>One per preparatory batch.</td>
</tr>
<tr>
<td></td>
<td>No analytes detected &gt; ½ LOQ or &gt; 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.</td>
</tr>
<tr>
<td><strong>Laboratory Control Sample (LCS); Matrix Spike (MS); Matrix Spike Duplicate (MSD) Relative percent difference</strong></td>
<td>One per preparatory batch.</td>
</tr>
<tr>
<td></td>
<td>LCS: Blank spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration.</td>
</tr>
<tr>
<td></td>
<td>MS/MSD: Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration.</td>
</tr>
<tr>
<td></td>
<td>A laboratory should use the DoD QSM Appendix C Limits for batch control if project limits are not specified.</td>
</tr>
<tr>
<td></td>
<td>If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.</td>
</tr>
<tr>
<td></td>
<td>MSD or MD: RPD of all analytes ≤ 30% (between MS and MSD or sample and MD).</td>
</tr>
<tr>
<td>QC Check</td>
<td>DoD QSM Frequency and Acceptance Criteria</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Post Spike Sample</td>
<td>Only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of &lt; LOQ for analyte(s).</td>
</tr>
<tr>
<td></td>
<td>Spike all analytes reported as &lt; LOQ into the dilution that the result for that analyte is reported from. The spike must be at the LOQ concentration to be reported for this sample as &lt; LOQ.</td>
</tr>
<tr>
<td></td>
<td>When analyte concentrations are calculated as &lt; LOQ, the post spike for that analyte must recover within 70 - 130% of its true value.</td>
</tr>
</tbody>
</table>
Appendix B: Formulas used in Stages 3 and 4 Data Validation

Calibration:

Response Factor (RF):

\[ RF = \frac{A_s \times C_{IS}}{A_{IS} \times C_s} \]

Where:
- \( A_s \) = Area, Standard
- \( C_{IS} \) = Concentration, Extracted Internal Standard
- \( A_{IS} \) = Area, Extracted Internal Standard
- \( C_s \) = Concentration, Standard

Average RF:

\[
\text{mean } RF = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n}
\]

\[
SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n - 1}}
\]

\[ RSD = \frac{SD}{\overline{RF}} \times 100 \]

Where:
- \( RF_i \) = RF for each calibration standard
- \( \overline{RF} \) = mean RF for each compound from the initial calibration
- \( N \) = number of calibration standards
- \( SD \) = standard deviation
- \( RSD \) = Relative standard deviation

Relative Retention time:

\[ RRT = \frac{\text{Retention time of the analyte}}{\text{Retention time of the extracted internal standard}} \]

Percent Difference:

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

Where:
- \( C_s \) = Concentration, reported
- \( C_k \) = Concentration, known
Sample Concentration:

Raw Values:

\[ C_s = \frac{A_s \times C_{IS}}{A_{IS} \times RF} \]

Where:
- \( C_s \) = Concentration, sample
- \( A_s \) = Area, Sample
- \( C_{IS} \) = Concentration, Extracted Internal Standard
- \( A_{IS} \) = Area, Extracted Internal Standard
- \( RF \) = Average RF

Linear Regression: \( y = mx + b \)

\[ C_s = \left( \frac{A_s}{A_{IS}} - b \right) \times C_{IS} \]

Where:
- \( C_s \) = Concentration, Sample
- \( A_s \) = Area, Sample
- \( A_{IS} \) = Area, Extracted Internal standard
- \( C_{IS} \) = Concentration, Extracted Internal Standard
- \( b \) = Intercept
- \( m \) = Slope

Quadratic Regression: \( y = ax^2 + bx + c \)

\[ C_s = -\frac{b \pm \sqrt{b^2 - 4ac \left( c - \frac{A_s}{A_{IS}} \right)}}{2a} \times C_{IS} \]

Where:
- \( C_s \) = Concentration, Sample
- \( A_s \) = Area, Sample
- \( A_{IS} \) = Area, Extracted Internal standard
- \( C_{IS} \) = Concentration, Extracted Internal Standard
- \( a \) = Quadratic Coefficient
- \( b \) = Linear Coefficient
- \( c \) = Constant Term
LCS Percent Recovery:

\[ \text{Percent Recovery} = \frac{C_s}{C_K} \times 100 \]

Where:
- \( C_s \) = Concentration, Reported
- \( C_K \) = Concentration, Known

Post Spike, MS, or MSD Percent Recovery:

\[ \text{Percent Recovery} = \frac{C_M - C_s}{C_K} \times 100 \]

Where:
- \( C_M \) = Concentration, Post Spike, MS, or MSD
- \( C_s \) = Concentration, Sample
- \( C_K \) = Concentration, Known

MS/MSD or Duplicate Relative Percent Difference (RPD):

\[ RPD = \frac{|C_s - C_d|}{(C_s + C_d)/2} \times 100 \]

Where:
- \( C_s \) = Concentration, Sample
- \( C_d \) = Concentration, Duplicate

Transition Ion Ratio:

\[ \text{IR} = \frac{Qq}{Qc} \quad \text{or} \quad \text{IR} = \frac{Qc}{Qq} \]

Where:
- IR = Ion Ratio
- Qq = quantitative ion abundance
- Qc = confirmation ion abundance

Transition Ion Percent Recovery:

\[ \text{Percent Recovery} = \frac{R_s}{R_K} \times 100 \]

Where:
- \( R_s \) = Ion Ratio, Reported
- \( R_K \) = Ion Ratio, Known
Reported Values:

Aqueous

Concentration $\mu g/L = R \times V_f \times D_f / V_i$

Where:
- $R$ = Raw value from above in micrograms per liter (ug/L)
- $V_f$ = Final Volume of extract in liters (L)
- $V_i$ = Initial Volume extracted in liters (L)
- $D_f$ = Dilution Factor

Solids

Concentration $\mu g/Kg$ (Dry weight basis) = $(R \times V_f \times 1,000 \times D_f) / (W_s \times D)$

Where:
- $R$ = Raw value from above in micrograms per liter (ug/L)
- $V_f$ = Final volume of extract in liters (L)
- $W_s$ = Weight of soil/sediment extracted, in grams (g)
- $D_f$ = Dilution factor.
- $D = \frac{100 - \% \text{ moisture}}{100}$