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Per- and Polyfluoroalkyl Substances (PFAS): Reviewing Analytical Methods Data for Environmental Samples

Tips for reviewing LC/MS/MS data generated from PFAS analytical methods

Background

Interpreting data from analysis of per- and polyfluoroalkyl substances (PFAS) in a variety of environmental sample types can be challenging due to variations in analytical protocols, quality control types and criteria, and data review procedures across laboratories. Analytical chemistry reference methods, both prescriptive and performance-based, are available for laboratories to use as a basis to create standard operating procedures (SOPs) to test for these chemicals; however, results can be difficult to assess, compare, and apply to make site-based decisions.

Data reviewers should consider a variety of information when assessing data quality from liquid chromatography/tandem mass spectrometry (LC/MS/MS) analyses, regardless of the method or procedure used. Data quality reviewers should always defer to site and/or state specific data review requirements, where available.

Scope of the Review

The level of detail required for the reviewer to adequately assess data quality will depend on how the data will be used. For example, site-specific enforcement actions will generally require a much higher level of scrutiny compared to pilot-level projects or general screening for site characterization.

Documentation Needed for Review

Quality Assurance Project Plan (QAPP)

A Quality Assurance Project Plan (QAPP) captures information that identifies project-specific data quality objectives (DQOs) and measurement performance criteria, including analytes and concentration levels of interest and how the analytical data will be used to achieve any stated project goals[1]. The QAPP should be reviewed by data reviewers and used to determine whether the data collection effort is successful in achieving project DQOs.

Laboratory Procedures & Quality System Documents

The laboratory's SOPs and other quality systems documents (e.g., Quality Management Plan) should be included in the data review to understand how the laboratory does the following:

- Maintains sample integrity, including measures to minimize PFAS contamination during handling and transport to the laboratory
- Manages personnel qualifications (e.g., initial demonstration of capability, or IDOC).
- Prepares and analyzes samples.
- Establishes and verifies detection and quantitation limits [e.g., method detection limit (MDL), limit of detection (LOD), limit of quantitation (LOQ), etc.]. The analyst's method for dealing with branched and linear PFAS isomer detection limits should also be documented.
- Assures measurement traceability.
- Evaluates quality controls (QC) to assess measurement performance (including QC types, frequencies, acceptance criteria, and use of second source PFAS standards for some QC criteria, if required).
- Handles out-of-control data, including client notification and corrective and preventive actions.
- Reviews data internally prior to release.
- Performs internal audits.

Laboratories that are accredited to quality systems standards[2] are generally required to have these quality assurance (QA) elements in place. If the project required accreditation, the review should verify that the PFAS analysis (method, media type, analytes) is included in the scope of the laboratory's accreditation.

Laboratory Data Deliverables

Depending on the scope of the review, information requested from the laboratory can include the following:

- **Summary reports of results**, including measured concentrations of any PFAS of interest found in the field samples, associated dates of preparation and analysis, quantitation limits and detection limits (as applicable), QC samples, definitions of assigned data qualifiers, and narratives specific to the samples analyzed that identify any data quality issues in the data package and any potential impact on data usability, potential sample integrity issues, including any unforeseen events (e.g., broken/leaky sample containers and known or suspected PFAS contamination sources).
- **Supporting information for sample preparation**, including preparation bench sheets for field samples and associated method blanks, blank spikes, matrix spikes, laboratory duplicates, and any field blanks, trip blanks, field spikes, and field replicates, as applicable. For isotope dilution methods, it will be critical for the reviewer to identify the point of addition for radiolabeled isotopic analogues.
- **Supporting information for instrumental analysis**, including mass spectrometer optimization reports, initial calibration reports, calibration verification reports, and associated quantitation reports/graphic displays for initial calibration standards, calibration verification standards, and instrument blanks. Calibration data for PFAS analysis should clearly list the concentrations of calibration standards, surrogates, and internal standards and the type of calibration used (e.g. linear, 1/X weighted, quadratic).
- **Detailed quantitation reports** for field samples and associated preparation and instrument QC samples, including graphical displays (e.g., extracted ion current profiles) of quantitation ion and confirmation ion transitions, chromatographic retention times, peak areas, signal to noise (S/N) ratios, ion ratios (as applicable), and evaluation criteria for ion ratios.
- **Other relevant supporting data**, including a list of field samples tested with chain-of-custody documentation; summaries of surrogate and/or internal standard (including sources, concentrations, and point at which they are added in the method used); and spike recoveries and precision, including associated control limits, analyst notes, calibration standard and spiking solution preparation records (including sources and lot numbers), instrument sequences, and dilution records.
- **Examples of calculations** used to derive final results from the raw data. Documentation should establish whether reported results were based on the calibration of the acid, ionic, or salt PFAS species and summations of whether results for PFAS compounds with branched and linear isomers were integrated or reported separately.

General Data Quality Considerations

Laboratories should provide sufficient documentation to assure reviewers that SOPs were followed, data calculations and any transcriptions were accurate, QC samples were prepared and analyzed in the same manner as field samples (as applicable), corrective actions were taken (and documented) as appropriate, and that communications with the client were made, especially if problems were encountered. The specific QA activities used to ensure data integrity and the QC checks used to evaluate and document data quality should be reviewed in the QAPP, SOP, and/or reference method. Depending on the scope, review elements supplied by the testing laboratory can vary. Some data quality elements for LC/MS/MS analysis of PFAS are common to other chromatography and mass spectrometry methods, and some are specific to this class of analytes (e.g. co-occurrence of branched and linear isomers for some PFAS compounds). The data reviewer can use these critical elements to identify the types of documentation needed to verify the following:

- Completeness of the data deliverables.
- Compliance with the identified QC criteria.
- Acceptable performance of analytes of interest throughout preparation and analysis under the conditions used.
- Appropriateness of qualitative PFAS identifications through review of the data and corresponding chromatograms. The reviewer should establish that automated software programs (if used) correctly identify qualitative peaks for specific PFAS analytes.
- Correctness of calculations and conversions from raw instrument data to the final results being reported.

Standard Operating Procedures (SOPs)

The data reviewer should use the laboratory PFAS SOPs to reconstruct how samples were prepared and analyzed at the facility, and how data was verified, qualified, and reported. Other supporting SOPs (e.g., field sample collection, sample receiving/log-in) might also be useful to evaluate possible points of PFAS contamination. If an SOP is used based on an existing reference method (e.g., EPA Method 537 for drinking water), modifications to the reference method should be clearly stated and documented in the SOP. The reviewer should confirm whether modifications made to procedures are allowed within the scope of the reference method, including matrix type and QC sample types, chromatography columns, mobile phase solvents, solids content, mass spectrometer settings, calibration frequency, and

associated acceptance criteria. The data reviewer is also advised to compare the QC categories, frequency, and criteria in the method to those employed in other established methods and data review guidelines to ensure all critical data quality elements are addressed[3]

Demonstrations of Capability

The laboratory should provide the data used to establish acceptable performance for the SOPs under the same conditions used for testing of the chemicals and matrices of interest (initial demonstration of capability, or IDOC) and matrix-specific quantitation limit studies (e.g., MDL, LOD, LOQ, etc.).

PFAS-Specific Data Quality Considerations

Sample Collection, Subsampling, & Preparation

Sample containers can be a source of PFAS contamination. The reviewer should verify the containers used for sample collection were thoroughly tested for PFAS contamination prior to use. Reagents used for sample preservation, preparation, and analysis should also be tested to ensure they are PFAS-free. Evaluation of field blanks that were prepared from reagent water shown to be clean in the laboratory and using the same lot of containers used for field sample collection can help the reviewer determine the extent of any PFAS contamination during sampling.

In addition, longer chain PFAS analytes in aqueous samples are known to not stay in solution; they may migrate to surfaces such as the interior of sample containers, which can lead to low bias measurement if subsamples are removed for testing. The reviewer should confirm if any subsampling was performed by the laboratory and note the potential for low bias on larger PFAS analytes (e.g., > C10 perfluorinated carboxylic acids, > C8 perfluorinated sulfonic acids). This potential source of measurement bias for aqueous matrices is mitigated if the laboratory prepared the whole sample as received from the field, added a sufficient proportion of water-miscible organic co-solvent (>50%)[4a] prior to subsample or transfer, or rinsed the sample containers with solvent during preparation to ensure complete quantitative transfer[4]. Sample preparation documentation and field blank results can help the data reviewer evaluate the impact of these potential sources of measurement bias.

Calibration

PFAS reference methods for LC/MS/MS use either external standard calibration (EC), internal standard calibration (IC), or isotope dilution calibration (ID) for calculating target analyte concentrations[3a,4]. EC procedures typically rely on surrogates added to samples prior to preparation (and target compound additions for matrix spikes of select samples) to monitor for losses and matrix effects, but the additions are not used for correcting sample concentration calculations. IC procedures typically rely on surrogate and target compound standard additions to monitor for losses during preparation in the same manner as for EC, but internal standards are also added to sample extracts prior to analysis; these internal standards can correct for analytical matrix enhancement or suppression of target analyte peak responses. ID procedures include an isotopically labeled internal standard specific to each target analyte (where available or used), and these internal standards are added to each field sample prior to preparation; target analyte concentrations are recovery-corrected based on performance of the internal standards to account for both matrix effects and losses during sample preparation.

There are limitations to using surrogates or internal standards that are not mass-labeled analogs of the target analytes to evaluate or compensate for matrix effects and losses during sample preparation. Differences in physicochemical properties and chromatographic retention times can lead to differential performance of even closely-related PFAS target analytes, resulting in measurement bias when one chemical is normalized to performance of another. However, the laboratory may be unable to use a mass-labeled surrogate or internal standard for each target analyte depending on commercial availability or cost. Standard additions of native target analytes (e.g., added to samples prior to preparation as matrix spikes or as post-preparation spikes of extracts) can help the reviewer evaluate these potential sources of measurement bias, if necessary.

The reviewer should verify that all calibrations meet established acceptance criteria and, if not, associated data qualified appropriately. Initial calibrations should include adequate (e.g., at least five) data points, an appropriate calibration range, and the reviewer should confirm the calibration method used (e.g., linear, 1/X weighted, quadratic). Continuing calibration check (CCC) standards should be analyzed at appropriate concentrations, and at established intervals to verify system stability. The use of second source standards, where available, can strengthen confidence in CCC results. When exceedance of acceptance criteria indicates that instrument performance is out of control, verify any corrective actions taken (e.g., system was recalibrated, sample batch re-analyzed) and the potential consequences to data quality.

Quality Control (QC)

The reviewer should verify that data for QC samples such as procedural blanks, blank spikes, matrix spikes, performance evaluation samples, and surrogates/ internal standards are present, as required in the QAPP or SOP, and that QC samples meet established criteria. Analytical sequences containing field samples and associated preparation batch QC samples should be

clearly associated with specific calibration curves and CCC standards. Blank results should be reviewed carefully. Levels of PFAS analytes present in consumables and reagents may vary considerably depending on the source.

Contaminants in reagents used to prepare calibration standards can lead to low bias or false negative measurement of target analytes in field samples, particularly for low concentration sample analytes. Instrument blank responses above established criteria suggest a source of contamination in the analytical process and indicate potential contamination from eluents and/or the sample delivery system. Method blank concentrations above acceptance criteria indicate contamination from sample preparation procedures particularly if associated instrument blank results are acceptable. If method and instrument blanks meet acceptance criteria but field and trip blanks do not, the data reviewer must decide if the data is usable, how it should be qualified, and/or if re-sampling is warranted.

Chromatography Considerations

In general, the reviewer should verify that chromatographic peaks are clear and distinct, symmetrical, and meet any method-defined performance criteria (e.g., signal-to-noise, quantitation-confirmation ion ratio requirements), especially at the limit of quantitation or near any identified level of interest for the project. Broad, split, or fronting peaks (i.e., peak shoulders) and erratic baselines may indicate deterioration of chromatographic performance.

Qualitative Identifications

The reviewer should confirm reported targets meet established qualitative criteria. Chromatographic peak retention times of reported targets and surrogates/internal standards in field samples are consistent with initial calibration standards and CCC standards.

Quantitative Considerations

The reviewer should confirm reported targets meet established qualitative criteria. Chromatographic peak retention times of reported targets and surrogates/internal standards in field samples are consistent with initial calibration standards and CCC standards. Monitored MS/MS transitions from parent to daughter ions include at least two ions specified, one which is used for calibration/quantitation, and the other(s) used to evaluate/confirm ion ratios against established acceptance criteria. Some PFAS analytes may not produce more than one clear MS/MS transition, so some peaks may lack confirmation ions (e.g., perfluorobutanoic acid [PFBA], perfluoropentanoic acid [PFPeA], N-MeFOSAA, N-EtFOSAA). Reporting of these chemicals should be considered more carefully, particularly in the absence of any related target analytes and when measured at low signal-to-noise ratios.

Matrix Effect Considerations

Analyzed field samples may exhibit matrix effect bias, resulting in enhanced or suppressed target analyte measurements. Data users should evaluate potential matrix bias by carefully reviewing the following:

- Target analyte recoveries from field sample matrix spikes and matrix spike duplicates.
- Surrogate recoveries from all samples analyzed.
- Internal standard recoveries (IC and ID only).

Low recoveries for isotopically labeled internal standards or surrogates for any field samples (e.g., <50% recovery) should be reviewed carefully to determine project-specific acceptability. Pronounced negative matrix bias can result in false negatives when typically observed low level target analyte response is suppressed below instrument sensitivity limits.

Reporting Concentrations as Salts or Free Acids

The data reviewer may also need to determine whether measurements were based on standard concentrations for the acid, ionic, or salt species and convert to the appropriate reference concentration as necessary. For example, it may be necessary to convert results to the free acid form of a sulfonic acid if the certified concentration of the salt species was used as a basis for prepared calibration standard concentrations.

Assessment of Data Quality

The reviewer should verify that the final results and the quantitation/detection limits provided in laboratory data deliverables were calculated correctly from the raw data (including any dilutions), that the reported results were measured within the calibration range, and that all information necessary to calculate the results was provided by the laboratory. When supported by the monitoring program to meet project DQOs, the laboratory or data reviewer can make use of data qualifiers (e.g., “J” flags) to identify where pre-defined QC criteria are not met that can affect any samples and/or target analytes. Qualified data might be useful, but the magnitude of the QC exceedance(s) and impact on measurement uncertainty or bias in any affected samples requires careful consideration and cautious review, especially when sample results are near any identified concentration levels of interest for the project.

Laboratories that frequently apply data qualifiers to analytical results, particularly due to chronic QC criteria failures involving calibration verification, blanks, or spiked blanks, should provide corrective action narratives detailing steps taken to minimize these recurring QC failures. If some QA/QC elements are not available for review, or if evaluation criteria are not specified in the reference method, the reviewer should apply their best

professional judgement as to the impact on data usability. If the QC criteria used by the laboratory are insufficient for the project application or are incomplete, performance criteria in published methods with similar calibration models may be used to evaluate the data presented. The reviewer should determine if sufficient evidence is available to demonstrate that the laboratory followed the method/QAPP. If not, any method modifications and any suspected impacts on data quality should be noted. Site-specific QC sample results (including IDOC and MDL studies, field duplicates, field blanks and matrix spikes) may provide the clearest evidence regarding method performance in the sample matrix. The reviewer should document any findings and carefully consider how they may impact the usability of the data to meet the DQOs.

References

Please note that citations in the text that reference a number refer to the collective set of documents under that number.

1a. Guidance on Systematic Planning Using the Data Quality Objectives Process, epa.gov/quality/guidance-systematic-planning-using-data-quality-objectives-process-epa-qag-4

1b. Guidance for QA Project Plans, epa.gov/quality/guidance-quality-assurance-project-plans-epa-qag-5

2a. National Environmental Laboratory Accreditation Program, nelac-institute.org/content/NELAP/index.php

2b. General Requirements for the Competence of Testing and Calibration Laboratories ISO/IEC 17025:2017, iso.org/standard/66912.html

2c. Department of Defense Environmental Laboratory Accreditation Program, denix.osd.mil/edqw/accreditation/

3a. SW-846 Method 8000D, Determinative Chromatographic Separations, epa.gov/hw-sw846/sw-846-test-method-8000d-determinative-chromatographic-separations

3b. National Functional Guidelines (NFG) for Organic Superfund Methods Data Review, epa.gov/clp/national-functional-guidelines-organic-superfund-methods-data-review-som024

3c. Guidance on Environmental Data Verification and Validation, epa.gov/quality/guidance-environmental-data-verification-and-data-validation

3d. Data Validation and Laboratory QA for Region 9, epa.gov/quality/data-validation-laboratory-quality-assurance-region-9

3e. New England Environmental Data Review Program Guidance, epa.gov/quality/epa-new-england-environmental-data-review-program-guidance

3f. Training Courses on Quality Assurance and Quality Control Activities, epa.gov/quality/training-courses-quality-assurance-and-quality-control-activities

4a. Improved Analysis of Polyfluorinated Alkyl Substances in Environmental Samples Using Optimized ASTM Method 7968/7979, chromatographyonline.com/improved-analysis-polyfluorinated-alkyl-substances-environmental-samples-using-optimized-astm-method

4b. Analyzing PFAS in Wastewater, Solids, and Soils: State of the Science Webinar, static1.squarespace.com/static/54806478e4b0dc44e1698e88/t/59baa9f7e5dd5ba4b0f0a2d2/1505405465878/AnalyzingPFASInWWSolidsSoils-WEBINAR-AllSlides-14Sept2017v2.pdf

4c. Method 537.1: Determination of Selected PFAS in Drinking Water by Solid Phase Extraction and (LC/MS/MS), cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab=NERL

4d. Site Characterization Considerations, Sampling Precautions, and Laboratory Analytical Methods for PFAS, pfas-1.itrcweb.org/wp-content/uploads/2018/03/pfas_fact_sheet_site_characterization_3_15_18.pdf

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Additional Information

- **PFAS in Your Environment Website:** epa.gov/pfas
- **PFAS Technical Briefs:** epa.gov/water-research/water-research-fact-sheets