EPA Response to Public Comments on SW-846 Update VII, Phase 2 – Method 8327 for Per- and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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INTRODUCTION

On June 21, 2019, the U.S. EPA announced the availability of Method 8327 as part of Update VII to the Third Edition of EPA publication SW-846, "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods". EPA asked the public to submit comments on the new analytical method, which included Method 3512, a sample preparation procedure in Appendix B that was proposed to be published as a separate preparation method. EPA followed its streamlined approval process for non-regulatory SW-846 methods.[1]

The Agency has received public comment on Method 8327, and after consideration of these comments, is placing Method 8327 and Method 3512 in the SW-846 methods compendium. EPA is issuing this update as guidance. This response-to-comment document provides comment summaries and EPA's responses. Complete copies of all received comments can be found in the docket associated with this addition to the SW-846 method compendium (Docket EPA-HQ-OLEM-2018-0846). Appendix A at the end of this document cross-references commenter numbers used throughout this document with the commenter/organization names in each of the sets of comments.

Comments are organized into a general section (pages 4-10) and sections for Method 8327 (pages 10-95) and the three summary reports (pages 96-116) that were included in the docket when the method was proposed for public comment. The method and summary reports were posted in the proposed docket in June 21, 2019 and are identified as follows:

Document name in Proposed Docket	**Document ID in Proposed Docket**	**Referred to in Response to Comments document (below) as:**
Proposed Method 8327 Procedure	EPA-HQ-OLEM-2018-0846-0002	Method 8327
Method 8327 Executive Summary	EPA-HQ-OLEM-2018-0846-0003	Executive Summary
Method 8327 Statistical Analysis Report	EPA-HQ-OLEM-2018-0846-0004	Statistical Summary Report
Method 8327 Validation Summary Report 060719 final	EPA-HQ-OLEM-2018-0846-0005	QC Summary Report

Revised versions of these documents are included as separate files in the final docket.

[1] https://www.epa.gov/hw-sw846/streamlined-procedure-publishing-non-regulatory-sw-846-methods

GENERAL COMMENTS

1. Comment:

A number of commenters (8, 9, 13, 14, 16, 19, 23, and 24) expressed concerns about precision, bias, and/or sensitivity of Method 8327 resulting from procedural steps in the methods and/or the outcomes of the validation study, which are described in more detail in the comments by section for the method and summary reports. Many of these commenters concluded that EPA's validation study did not demonstrate the methods are capable of producing definitive data for the target chemicals in the tested matrices (e.g., for regulatory purposes) and recommended withdrawal or significant revision to address performance issues.

Response:

EPA disagrees with these commenters' view of the performance of these methods and finds the validation study data to be supportive of final publication. The validation study data met EPA's Data Quality Objectives (DQOs) for measurement precision, bias, and sensitivity for 23 of the 24 target analytes that were evaluated. The Executive Summary in the final docket has been revised to provide a clear overview of EPA's DQOs and a summary of the validation data that was evaluated against each of them. The validation data demonstrated that Methods 3512 and 8327 produced measurements with defined precision and bias across the tested sample matrices and participating laboratories at 60 and 200 ng/L (nominal) concentrations. Most laboratories met the preliminary acceptance for Lower Limit of Quantitation (LLOQ) verification at 10-20 ng/L (nominal) for most target analytes using LC/MS platforms from a variety of instrument manufacturers. The validation data also demonstrated that the quality controls included in the methods are well-suited for identifying measurement bias or other performance issues and will prove valuable for laboratories and data reviewers for evaluation of data quality and usability per relevant project Data Quality Objectives. Based on the outcomes of the validation study, EPA anticipates these methods will be well-suited for sample testing in a production laboratory environment.

2. Comment:

Regarding method sensitivity, a number of commenters (8, 10, 13, 16, 19 and 24) expressed concern that the Lower Limits of Quantitation (LLOQs) evaluated in the validation study were insufficient to meet Data Quality Objectives for some project applications, including some state cleanup levels.

Response:

EPA agrees with the commenters that Methods 3512 and 8327 as validated may not provide data of sufficient sensitivity for every project application. Preparation of aqueous samples by Method 3512 involves a two-fold dilution with organic solvent rather than a concentration step as might be included for other sample preparation techniques such as solid phase extraction. Sensitivity needs are expected to be very application-dependent and should be considered as

part of project planning, potentially in consultation with the testing laboratory. As with other non-required SW-846 test methods, Methods 3512 and 8327 are provided as tools for use by government and the regulated community to support their project-specific data needs, where appropriate. These methods are also performance-based and do not have a required sensitivity, and they can be modified without prior approval by EPA to meet specific project needs provided that the laboratory demonstrates acceptable performance for the intended application and the methods used and any modifications thereto are acceptable to the end data user. The Agency recommends consulting with the decisionmaker or regulatory authority before method modifications are made. Please refer to Section 9.0 of Method 8327 for more information about quality controls, demonstrations of proficiency, and LLOQ verifications, and refer to the SW-846 Policy Statement for more information regarding flexibility of SW-846 methods.

3. Comment:

A number of commenters (5, 8, 9, 13, 19, and 21) pointed out the lack of clarity in Method 8327 regarding calibration, identification and quantitation of PFAS target analytes containing both branched and linear isomers. These commenters indicated that the text is unclear regarding whether Method 8327 requires branched and linear standards to be used for quantitative analysis. Some commenters requested that EPA incorporate into Method 8327 the approach used in Method 537.1 for calibration, identification and quantitation of target analytes composed of multiple isomer peaks.

Response:

EPA concurs with the commenters and revised Sections 7.4, 11.3, and 11.6, and the figures in Method 8327 to provide a clearer description of calibration, identification and quantitation of target analytes composed of linear and branched isomers. EPA acknowledges there are limitations related to optimizing analytical conditions for individual branched PFAS isomers due to limited availability of certified reference materials and limited ability to separate isomers chromatographically. However, the general recommended approach outlined in Method 8327 is similar to that presented in the drinking water methods, and it can be modified as needed to suit project-specific applications. More specifics are provided by section in the method below.

4. Comment:

Several commenters (8, 9, 11, 16, 21, and 22) requested that EPA validate and publish additional or alternative preparation and cleanup methods for aqueous and solid sample matrices, including Solid Phase Extraction (SPE) for aqueous samples, solvent extractions for solid samples, and carbon cleanup for sample extracts. Commenters noted an urgent and unmet need for validated EPA PFAS test methods for matrices such as soil, sediment and waste. Commenters also noted that Sections 4.2 and 11.2 of Method 8327 state that cleanup may be necessary to reduce matrix interferences in highly contaminated matrices, but no validated cleanup procedures are included.

Response:

EPA agrees with the commenters regarding the need for additional validated sample preparation and cleanup methods. At the time of this writing, the only SW-846 PFAS preparative and determinative methods for which validation has been completed are Methods 3512 and Method 8327 for aqueous sample matrices. Method 8327 is written in the same modular format as many other SW-846 reference methods, and EPA intends to publish additional SW-846 sample preparation and cleanup methods for use in conjunction with this determinative method, including for solid matrices. Section 1.0 of Method 8327 in the final docket clearly identifies the preparation method and sample types that have been evaluated by EPA. Development and validation of new SW-846 test methods take time, and EPA intends to publish additional validated SW-846 PFAS test methods as soon as they can be made available to the public.

5. Comment:

Several commenters (5, 8, 9, and 22) mentioned that Method 8327 refers to solid matrices in several sections, but no solid sample preparation methods have been validated for this preparation method. The commenters recommended that EPA remove reference to solid matrices in Sections 1.0, 2.1, 8.2, and/or 11.1 of Method 8327. Commenter 9 also recommended revision of Method 8327 to remove references specific to aqueous sample preparation to be consistent with EPA's stated intent to make this determinative method applicable to other matrices.

Response:

EPA concurs with these comments. References to solid matrices have been removed from the final version of Method 8327. EPA also moved references appropriate to aqueous sample preparation to Method 3512.

6. Comment:

Commenter 1 stated "Daily, millions of people across the country are exposed to PFAS. These are man-made toxic chemicals that are used in everyday products such as waterproof jackets, nonstick pans, food wrappers, and personal care products. They persist in the environment, readily spread from sources of contamination, and can remain in humans for decades. For decades, chemical manufacturers have known exposure to PFAS was linked with serious health harms (e.g., cancer, infertility, and impaired fetal development) but hid the truth from their workers, fence line communities and regulators. Even after the EPA learned about PFAS dangers, it failed to take appropriate action. At present, no enforceable rules exist to protect us from these forever chemicals. People living in the U.S. need tougher protections against PFAS immediately. Please ban all manufacturing of and uses of PFAS now. Also, please make all communities, local governments and workers aware should PFAS be present. Additionally, please cleanup all PFAS in the environment and hold PFAS manufacturers accountable for the costs of cleanup.

Response:

Thank you for expressing your concern about exposure to chemicals that can cause serious health effects. Although the Agency's mission as a whole is to protect human health and the environment, addressing this comment is outside the scope of this method project.

7. Comment:

Commenter 3 stated "For a long time, chemical manufacturers (e.g., 3M, DuPont) knew that exposure to PFAS was linked with serious health harms (e.g., cancer, infertility, impaired fetal development) but hid the truth from their workers, fenceline communities, and regulators. Every day, millions of people across the country are exposed to food and drinking water contaminated with PFAS, a class of some 5,000 man-made toxic chemicals which were first developed during efforts to create the atomic bomb. Now PFAS are used in everyday products, e.g., waterproof jackets, nonstick pans, food wrappers, and personal care products. They persist in the environment, readily spread from sources of contamination, and can remain in bodies for decades, which is why they are known as forever chemicals. Perversely, no enforceable rules exist to protect people living in the U.S. from PFAS. Even after the EPA learned about the dangers, it failed to act. Rather than doing what was needed, the EPA recently issued a useless action plan which promised that it will take the necessary steps to ensure our drinking water is safe or to notify communities when PFAS are released in their backyards. Chemical manufacturers shouldn't be allowed to continue poison us. Please take real action to end ongoing pollution from PFAS chemicals."

Response:

Thank you for expressing your concern about exposure to chemicals that can cause serious health effects. Although the Agency's mission as a whole is to protect human health and the environment, addressing this comment is outside the scope of this method project.

8. Comment:

Commenter 4 recommended adding guidance related to estimating relative measurement uncertainty for target analyte concentrations reported with Method 8327 and suggested using control charts of LCS data as a basis for these estimates.

Response:

Providing confidence limits or other estimates of uncertainty for individual measurements is outside the scope of this method; different organizations may have different approaches or requirements regarding estimating measurement uncertainty, and the decision about how to represent measurement uncertainty is more appropriate for project planning documents such as a Quality Assurance Project Plan that directly addresses project-specific data quality objectives.

9. Comment:

Commenter 5 stated “The current analysis of PFAS in water by Method 537.1 is quite expensive. None of the documents posted discuss the cost of Method 8327 compared to the cost of Method 537.1. Please provide a cost analysis discussing the additional cost (or savings) per sample so that it can be evaluated by the regulated community. Was this draft method reviewed to identify any opportunity for cost savings (e.g. identifying less expensive reagents and solvents)?”

Response:

The evaluation or comparison of cost with other reference methods is beyond the scope of the methods workgroup, but it is important to note that the application of Method 537.1, which is required for drinking water compliance testing, does not overlap with SW-846 Methods 3512 and 8327. With regard to cost, EPA expects that the use of Method 3512 for aqueous sample preparation will result in some cost savings for commercial laboratories compared to more labor-intensive sample preparation procedures like solid-phase extraction due to reduced labor and consumables needed for sample preparation.

10. Comment:

A number of commenters (4, 8, 9, 10, 11, 13, 14, 15, 18, 19, 20 and 23) stated that EPA should revise Method 8327 to include the use of isotope dilution calibration, either exclusively or in addition to external standard calibration. These commenters consider isotope dilution calibration to be superior to external standard calibration because it accounts for measurement bias and/or variability introduced during sample preparation and analysis from sources such as transfer losses and matrix interferences. Commenters 8 and 23 noted that electrospray ionization is susceptible to matrix interferences that can result in signal enhancement and suppression and indicated that isotope dilution is well-suited to account for these types of interferences using this technology. Commenter 8 noted that PFAS analysis by LC/MS/MS is well-suited for isotope dilution calibration due to the availability of isotopically-labeled analogs of most of the target analytes in this method. This commenter also noted that isotope dilution calibration is consistent with PFAS test methods used in industry and published by EPA and other federal agencies such as FDA and CDC. A few commenters (8, 9, and 15) also supported using internal standard calibration to quantify target analytes without isotopically labeled structural analogs (Note: Section 11.4.5 of SW-846 Method 8000D refers to this as ‘extracted internal standard calibration’).

Response:

EPA recognizes that the use of isotope dilution calibration for PFAS analysis by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) is consistent with widely accepted industry practice and with several other PFAS test methods published by EPA and other federal agencies. EPA also generally concurs with the commenters’ statements regarding the potential usefulness of isotope dilution calibration for LC/MS/MS as long as the appropriate isotopically labeled chemicals are available and used. However, EPA cannot readily accommodate these

commenters' request to include isotope dilution calibration in Method 8327 without the appropriate supporting data.

Method 8327 was proposed to EPA and validated as an external standard calibration method. EPA met its Data Quality Objectives (DQOs) for precision, bias and sensitivity for 23 of 24 target analytes in the Method 8327 validation study using external standard calibration; it was not necessary to use isotope dilution calibration to achieve these outcomes. Isotope dilution calibration would not have resolved the principal problem EPA had meeting its DQOs, which was related to 6:2 FTS contamination in study data reported by half of the laboratories.

EPA did not pursue recalculating the study data with an isotope dilution calibration model because both the target analytes and the isotopically labeled analytes were calibrated at variable concentrations. Other published EPA methods that use isotope dilution calibration models specify a constant concentration of isotopically labeled analytes in the calibration standards. As with other method modifications, EPA considers the use of an isotope dilution calibration model to be a reasonable modification to Method 8327 provided that the laboratory generates data of acceptable quality for the intended application and the modification is acceptable to the end data user (e.g., regulatory authority). EPA will consider adding isotope dilution calibration as an option to this method or to a future PFAS determinative method.

EPA expects bias will only be reduced with isotope dilution or extracted internal standard calibrations relative to external standard calibration to the extent that the signals for both the native chemical and isotopically labeled chemical are affected in the same way. Factors influencing this covariance include not only matrix effects but also the relative magnitudes of the signals and signal-to-noise ratio. Method 533 is the first low resolution isotope dilution mass spectrometry method published by EPA, and at the time of this writing its performance has not yet been formally evaluated by EPA for more complex matrices. Other EPA methods that use isotope dilution calibrations are high resolution mass spectrometry methods where the mass accuracy helps to reduce interferences. EPA will provide more information about advantages and disadvantages of various calibration models as more data becomes available. Because Method 8327 is guidance and is not required for compliance with the Resource Conservation and Recovery Act, the regulated community has the choice in the selection of which methods to use from EPA's program or other appropriate sources as stated in the policy statement and the Methods Innovation Rule.

11. Comment:

Commenter 9 stated that Method 8327 utilizes a direct aqueous injection sample introduction to the instrument, which negatively impacts the sensitivity of the procedure, and suggested that using a larger sample injection volume to compensate for this lower sensitivity can lead to additional sample matrix interferences. (Note: The sample preparation procedure in Method 3512 produces a prepared sample in 1:1 methanol-water + 0.1% acetic acid).

Response:

EPA respectfully disagrees with the suggestion that the injection volumes used in the validation study or suggested in the method will necessarily lead to matrix interferences. Sensitivity was evaluated as one of the Data Quality Objectives (DQOs) for the validation study, and Lower Limits of Quantitation (LLOQs) for most target analytes were verified at concentrations of 10-20 ng/L by most laboratories. Whether these LLOQs are sufficient for a given application depends on the end use of the data. Recovery of surrogates and target analytes in the study samples demonstrated that matrix interferences were generally manageable in the tested sample matrices. Other sample types may have more severe matrix interferences that will have to be managed on a case-by-case basis. The injection volume in the method is not intended to be fixed; Laboratories that participated in the validation study used injection volumes of 10-30 uL. Other injection volumes may be appropriate provided the laboratory demonstrates acceptable performance as described in Section 9.0 of Method 8327. Please refer to the Executive Summary for more details about validation study DQOs.

12. Comment:

Commenter 22 recommended allowing the use of isotope dilution calibration because many laboratories find it is more sensitive.

Response:

It is not clear to EPA how changing the calibration model would improve sensitivity, which is more closely related to signal-to-noise ratio.

METHOD 8327

Please note that some sections in the final methods were reorganized to improve clarity and organization and are different than the version that was posted for public comment. Where these sections were reorganized, section references in the comments refer to the version of the method that was posted for public comment, and section references in the response refer to the final version of the method.

DISCLAIMER

13. Comment:

Commenter 9 expressed concern that the disclaimer in this method regarding method flexibility would lead to a lack of standardization in the commercial laboratory industry and resulting in highly variable data with poor comparability between laboratories. Commenter 9 also stated that Method 8327 and future PFAS methods developed by US EPA should have definitive performance metrics (acceptance criteria) and concluded that, without these changes, “Use of

Draft Method 8327 will at best lead to semi-quantitative and semi-qualitative screening data in its present form, rendering efforts to reach ppt levels ineffective and unsuitable for regulatory use."

Response:

EPA interprets the commenter's meaning of the term "definitive" to be fixed and not statistically based. EPA disagrees with the commenter regarding the need for fixed acceptance limits to generate useful and reliable data. Many SW-846 determinative methods rely on statistically based acceptance limits for general use for a variety of sample preparation quality controls. Sections 9.0 and 11.0 and Table 6 of Method 8327 in the final docket provide defined acceptance criteria for instrument quality controls and statistically based acceptance criteria for most categories of sample preparation quality controls. Fixed limits may be perfectly appropriate for and may be used for specific project applications, and these details should be included in project planning documents, where appropriate, and discussed with the laboratory that will be generating the data. Regarding usability of data generated with Method 8327, please refer to the Executive Summary in the final docket, which demonstrated the appropriateness of these methods for quantitative analysis of parts-per-trillion (ng/L) concentrations in the tested matrices.

SECTION 1.0: SCOPE AND APPLICATION

14. Comment:

Two commenters (4 and 9) expressed concerns about whether the flexibility described in Section 1.0 of Method 8327 is warranted given that EPA provided laboratories with supplies and materials for the validation study, including PFAS target and surrogate stock standards, glass Luer-lock syringes, filters, a liquid chromatography column, and autosampler vials. The commenters were concerned that providing supplies and stock solution undermines the intent of an inter-laboratory validation study intended to evaluate performance across a variety of commercial, industrial, and government laboratories.

Response:

EPA disagrees with the commenters and does not consider the list of provided supplies to have affected its evaluation of the multi-laboratory validation data relative to the Data Quality Objectives (DQOs) that EPA defined for the study. EPA's intent of providing supplies was to defray some costs for laboratory participants, who were not paid for their participation, and to limit the number of factors evaluated as part of the validation study. The most critical factors that EPA identified as DQOs and evaluated in the validation study were measurement precision, bias and sensitivity of these sample preparation and analysis methods across multiple laboratories. Comparability of certified standards from different manufacturers and differences in PFAS background or performance in different laboratory supplies could be important variables, but they were not the focus of the validation study. The benefit of including these additional

variables was limited, and the variables that were controlled are unlikely to present problems for implementing the methods or interpreting data. Laboratories used a variety of reagents, supplies, equipment and instrumentation that were not supplied by EPA and may have been unique to their laboratory, representing a number of important variables that were integrated for comparing performance within and between laboratories. The final versions of Methods 3512 and 8327 list reagents, supplies and standards used during method development, but the list is not all-inclusive. The methods state that other sources or supplies may be used, and this flexibility is consistent with other SW-846 reference methods. It is the responsibility of the laboratory to demonstrate that whatever materials are used are suitable for their intended purpose, which includes demonstrating that acceptance criteria can be met for all categories of quality controls in Section 9.0. Refer to Section 6.0 of these methods for more information.

15. Comment:

Two commenters (2 and 9) expressed concern about the allowed flexibility in Section 1.0 of Method 8327 given the problems that some of the laboratories had producing reliable data when they deviated from steps in the study protocol. Commenter 2 requested that EPA add a warning to the beginning of the method stating, “The procedure must be followed without deviation to generate acceptable data unless a laboratory can demonstrate that a change improves method performance.” Commenter 9 concluded that no flexibility or modifications should be permitted for determination of PFAS at parts-per-trillion (ng/L) concentration levels.

Response:

EPA disagrees with the conclusion that the flexibility in the methods is unwarranted just because some method modifications resulted in unacceptable performance for the validation study. EPA does not intend Methods 3512 or 8327 to be interpreted as being prescriptive, as described for these and other non-required SW-846 methods in the 'Disclaimer' in the SW-846 Compendium. They are considered guidance. EPA acknowledges that laboratories have to consider the effect of modifications carefully. No warning was included at the beginning of the method, but EPA added information to Methods 3512 and 8327 to inform users where specific deviations led to non-representative data. Laboratories are responsible for demonstrating acceptable performance for all categories of quality controls when implementing these methods in their laboratory, including completing an Initial Demonstration of Proficiency (IDP), periodically verifying Lower Limits of Quantitation (LLOQs), and performing other quality assurance activities as described in Section 9.0 of Methods 8000D and 8327.

16. Comment:

Commenter 20 requested that Method 8327 emphasize reporting of results below the Lower Limit of Quantitation (LLOQ) with a qualifier even if they are above the lowest calibration standard concentration, and that non-detects be reported at the LLOQ. Commenter 20 also asked why Method 8327 uses the term LLOQ instead of LOQ.

Response:

EPA considers the emphasis provided in Section 9.9 of Method 8327 to be sufficient to address appropriate qualification of results reported below the LLOQ. This section also recommends documenting in a standard operating procedure or project planning document how reporting of results below the LLOQ will be managed. EPA respectfully disagrees with the commenter regarding the need to change the acronym for LLOQ in Method 8327. To keep all methods consistent with the SW-846 Style Guide and the base method, Method 8000D, the use of LLOQ is more appropriate than LOQ.

17. Comment:

Several commenters (5, 8, 12, 13, and 19) requested that EPA add target analytes to Method 8327, including chemicals in EPA Method 537.1 such as GenX (hexafluoropropylene oxide dimer acid; HFPO-DA) and ADONA (4,8-dioxa-3H-perfluorononanoic acid). Two additional commenters (14 and 18) recommended adding language to Section 1.0 of Method 8327 providing the flexibility to add PFAS compounds in addition to the 24 target analytes specified in the method, consistent with similar wording in Appendix B.

Response:

EPA cannot accommodate the commenters' requests to add specific target analytes to Method 8327 until the agency has acquired and evaluated appropriate validation data. The validation study for Method 8327 began before Method 537.1 was published, and at that time certified reference materials containing at least some of these chemicals were not readily commercially available. However, EPA concurs with the suggestion to more clearly identify method flexibility to accommodate additional target analytes. Methods 3512 and 8327 both state in Section 1.0 that they may be applicable to additional target analytes as long as the laboratory demonstrates adequate performance for the intended application in representative sample matrices, and they refer to Section 9.0 of the determinative method or to project-specific acceptance criteria for definition of adequate performance. EPA will consider adding specific target analytes to these methods in a future revision. Please note that Methods 3512 and 8327 were validated for non-potable waters, not finished drinking water like Method 537.1.

18. Comment:

Commenter 22 pointed out that the acronyms used for some target analytes in Section 1.0 of Method 8327 are inconsistent with those used for drinking water in EPA 537 and EPA 537.1, and recommended using the acronyms in Method 537.1, including for Perfluoroundeconoic acid (recommended using PFUnA instead of PFUdA) and Perfluorotetradeconoic acid (recommended using PFTA instead of PFTeDA).

Response:

EPA recognizes that using different acronyms for the same target analytes can lead to confusion in communicating with stakeholders inside and outside EPA. However, confusion can also result from using an inconsistent acronym naming convention. Chemical Abstracts Service Registry Numbers (CAS RNs) and the system for chemical nomenclature used by the International Union of Pure and Applied Chemistry (IUPAC) provide unambiguous identification of chemical structures, and any commonly accepted abbreviations for these target analytes can be used as long as they are cross-referenced to either of these systems. EPA changed the acronyms of some PFAS target analytes in the final versions of Methods 3512 and 8327 and in most supporting documents to be consistent with acronyms used in other published references. For example, all acronyms for PFAS target analytes used in the final versions of Methods 3512 and 8327 are also found on the Interstate Technology and Regulatory Council's PFAS Acronyms website: https://pfas-1.itrcweb.org/acronyms/. Other acronyms used by EPA or other organizations can be used and may be completely appropriate as long as they are also clearly associated to unambiguous identification of specific chemicals (e.g., CAS RNs, IUPAC nomenclature).

19. Comment:

Commenter 8 pointed out that the term "sample extracts" that was used in Section 1.0 was inconsistent with the preparation procedure, which did not involve extraction but rather dilution of aqueous samples with a water-miscible solvent. The commenter recommended removing references to extracts and solid matrices.

Response:

EPA partially agrees with these comments and included "prepared samples or sample extracts" in Section 1.0 and removed reference to solid matrices. EPA also edited this section to state that Method 8327 "may also be applicable to other PFAS target compounds and other matrices, provided that the laboratory can demonstrate adequate performance". The term 'extracts' was retained in this section to be compatible with this wording. To clarify the scope and outcomes of this validation study, Section 1.0 of Method 8327 was revised to clearly state which matrices EPA included in the validation study, and a reference to Method 3512 was included in the Section 1.0 target analytes table.

20. Comment:

A number of commenters (8, 9, 13, 14, 19, and 23) interpreted the '#' designation in the table in Section 1.0 and associated information in Section 1.3 addressing quality assurance issues for specific subsets of analytes to mean that these chemicals performed poorly during method validation. These commenters suggested removing these target analytes from the methods or revising and revalidating the methods to address the identified performance issues.

Response:

EPA recognizes that the '#' designation for specific target analytes in Section 1.0 and the quality assurance considerations included in Section 1.3 for specific subsets of target analytes led to confusion among commenters about the agency's intent. These designations were not intended to indicate that these chemicals are poor performers or that they were unsuitable for testing by this method; rather, the purpose of including this information was to help laboratories and data users more readily identify potential root causes if they encounter certain performance problems. Including this information in Section 1.0 is consistent with other published SW-846 determinative methods.

The only target analyte listed in Method 8327 that did not meet EPA's Data Quality Objectives for the validation study was 6:2 FTS. The table and definitions have been revised to make it clear which compounds met the validation study criteria ("✓"), which met criteria but may need special attention to meet laboratory or project needs ("✓*"), and which did not meet the criteria ("*"). EPA revised the analyte designations and definitions in this table to be consistent with the outcomes of the validation study and with other SW-846 determinative methods. EPA also revised the presentation of information in Section 1.3. Three of the four quality assurance considerations identified in this section were retained in the final version of the method. Please refer to responses to comments 21-28 for responses specific to each of the statements in Section 1.3, including for 6:2 FTS.

21. Comment:

Several commenters (2, 4, 6, and 15) stated that the '#' designation was inconsistent with the outcomes of the multi-laboratory validation study for any target analytes except 6:2 FTS or was otherwise inconsistent with their experience using this technique. Commenters 2 and 6 also stated that they suspected EPA may have used data from excluded laboratories that had deviated from the study protocol as a basis for the information in Section 1.3. Commenter 2 stated that inclusion of the '#' designation and information in Section 1.3 gives the impression that data for these chemicals is suspect or unreliable. These commenters requested that this designation and the associated information in Section 1.3 be removed except for 6:2 FTS.

Response:

As described in the response to comment 20, EPA recognizes that many commenters misunderstood the purpose of the '#' designation. As a result, EPA changed the definitions and designations for target analytes in the table in Section 1.0 to clarify its intent. EPA retained three of the four quality assurance considerations in Section 1.3 for reasons described in responses to comments about these specific statements below, but EPA agrees with the commenters regarding 'low response and/or high background' and removed it from Section 1.3. EPA removed this statement from Section 1.3 because Method 8327 does not have a required sensitivity. Each laboratory is responsible for establishing Lower Limits of Quantitation (LLOQs) at which level all method QC acceptance criteria can routinely be met using the equipment, instrumentation, and supplies specific to their laboratory.

The only statement EPA included in Section 1.3 that was supported with data provided by laboratories that deviated from the study protocol was related to 'reduced solubility'. The method

deviations EPA used as a basis for this statement were associated with spiking solution and aqueous sample preparation steps. A comprehensive list of compounds affected by these deviations is not included in Section 1.3, but the identified target analytes were among the most-affected. EPA retained this statement in the final version of Method 8327 because the method is performance-based, and EPA expects that highlighting these quality assurance issues will lead method users to more carefully consider method modifications related to decreasing organic solvent content in standards or samples or storage in containers made from different materials. EPA also added or strengthened information and cautions in Sections 4, 8, and/or 11 of Methods 3512 and 8327 related to specific method deviations that led to measurement bias in the validation study.

22. Comment:

Regarding the statement in Section 1.3 related to compounds that "showed potential for reduced solubility", Commenter 8 stated that the low bias for the longer-chain PFAS observed during method development was attributable to not maintaining a sufficiently high proportion of organic solvent in the samples themselves ("≥95%") and to not rinsing the sample containers with solvent as required in other PFAS reference methods such as Method 537.1 and ISO/FDIS 21675(e). The commenter further stated that the validation study data had a very high percentage of unacceptable recoveries for the identified target analytes (PFUnDA, PFDoDA, PFTrDA, PFTeDA, N-EtFOSAA, and N-MeFOSAA). The commenter concluded that, "As written, it is clear by this statement, this method is not capable of accurately quantitating these analytes" and requested that EPA revalidate the method after including a methanol rinse step for the sample containers.

Response:

EPA disagrees with the commenter's assertions that maintaining ≥95% solvent content and rinsing sample containers with methanol are essential for accurate quantitation of the indicated target analytes. EPA met its Data Quality Objectives for measurement precision and bias for 23 of 24 target analytes in the tested aqueous sample matrices, including for these six target analytes; please refer to the Executive Summary for more detail. The study samples were prepared at a central location and shipped to study laboratories where they were tested days to weeks later, so the validation study data provide adequate demonstration that these methods are capable of recovering these chemicals even if some fraction adsorbed to interior surfaces of sample containers after collection of aqueous samples.

EPA changed the wording in Section 1.3 of Method 8327 to be consistent with observations of "loss from solution" rather than "reduced solubility". EPA also added information and cautions to Sections 4, 8, and/or 11 of Methods 3512 and 8327 related to specific deviations from the study protocol that resulted in measurement bias during method validation, including avoiding subsampling from aqueous sample containers prior to adding sufficient organic solvent and avoiding storage of standard solutions and prepared samples in 1:1 methanol-water+0.1% acetic acid stored in glass containers. More detail about these deviations is included in Appendix E of the Statistical Summary Report.

23. Comment:

Commenter 9 noted that Section 1.3 identifies six compounds as having "reduced solubility in stocks and intermediate calibration standards unless there is > 95% organic cosolvent to keep the compounds in solution." The commenter questioned why EPA would include these target analytes in the method.

Response:

As described in the response to the comment 22, EPA met its Data Quality Objectives for precision, bias and sensitivity in the validation study for these target analytes. EPA included this supporting information in Section 1.3 to point laboratories and data reviewers to potential causes if they identify quality assurance problems during routine use. The quality controls included in the methods are generally well-suited for this purpose. As described in the QC Summary Report, an upper limit was not tested for quantitative analysis.

24. Comment:

Commenter 6 noted that the PFAS target analytes PFBA and PFPeA that are identified in Section 1.3 of Method 8327 as "difficult" due to lack of secondary transitions have a higher reporting limit in ASTM D7979-17 (50 ng/L instead of 10 ng/L for the other target analytes). The commenter did not agree that PFHxA should be considered together with PFBA and PFPeA. The commenter also stated that laboratories should be allowed to modify the method only if the modifications improve performance, such as lowering detection limits, but the method should specify a minimum sensitivity requirement that is not based upon the worst performing laboratories or instruments.

Response:

EPA agrees with the commenter that PFHxA presents different challenges for qualitative identification than PFBA and PFPeA because PFHxA makes a secondary product ion with relatively low abundance, while PFBA or PFPeA produce no identifiable secondary product ion using this analytical technique. EPA edited the wording in this section to address target analytes that either lack or have low relative abundance secondary product ions. PFOSA is also included under this header in Section 1.3 because no secondary product ion is identified in the method for this analyte. As mentioned above, SW-846 methods do not have a required sensitivity, and EPA removed information from the final version of Method 8327 related to initial calibration concentration ranges or suggested Lower Limits of Quantitation (LLOQs). Each laboratory is responsible for establishing and verifying LLOQs appropriate for the instrumentation, equipment, reagents, and supplies used at their facility. Please refer to Section 9.0 of Methods 8000D and 8327 for more detail.

25. Comment:

Several commenters (5, 8 and 9) found the statement in Section 1.3 of Method 8327 identifying chemicals with no secondary product ion as potentially "difficult at low concentrations" to be unclear. (Note: the version of Method 8327 that was posted for public comment used the term 'qualifier transition' instead of 'secondary product ion'). Commenters 8 and 9 did not consider lack of a secondary product ion to be relevant for quantitative analysis of these target analytes but noted it could lead to less confident qualitative identification. Commenters 8 and 9 also mentioned that solid phase extraction and cleanup (e.g., with EnviCarb) could eliminate interferences that affect quantitation. Commenter 8 suspected EPA included target analytes under this header due to interferences and concluded that "it is clear by this statement; this method is not capable of accurately quantitating these analytes". Commenter 9 asked why EPA had not made more of an effort to remove interferences like had been done for drinking water samples in Method 537 by preparing samples with solid phase extraction. Commenter 8 requested that EPA validate solid phase extraction and cleanup methods. Commenters 5 and 8 also noted "due to" was repeated in this section.

Response:

EPA agrees with the commenters regarding the lack of clarity in this statement in Section 1.3 and revised it in the final version of Method 8327 to clarify its intended meaning: "interferences may make qualitative identification more difficult". EPA recognizes that cleanup techniques can reduce certain types of matrix interferences that would otherwise be problematic but notes that EPA met its precision and bias Data Quality Objectives (DQOs) for these target analytes in the aqueous matrices used for method validation. Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) has high specificity, and the validation data demonstrated that cleanup is not a prerequisite for laboratories to generate defensible data. EPA expects that these same target analytes will have no or low relative abundance secondary product ions for other test methods that use negative electrospray ionization tandem mass spectrometry as well. Even so, laboratories have a number of tools they can use to support qualitative identification, including evaluating retention times, peak shapes, and signal-to-noise ratio of target analytes and isotopically labeled surrogates in prepared samples, matrix spikes, post-preparation spikes, and dilutions. Refer to Section 11.6 of Method 8327 for more information related to qualitative identification. EPA also edited the statement in Section 1.3 to address chemicals with low relative abundance secondary product ions, which more clearly applies to PFHxA, and included PFOSA under this header, for which no secondary product ion is identified in Method 8327. EPA also addressed the typographical error noted by these commenters. As described in response to comment 4 above, EPA will consider validating and publishing additional SW-846 sample preparation and cleanup methods at a future date.

26. Comment:

Commenters 14 and 18 requested that EPA correct the information provided in Section 1.3 related to PFHxA. The commenters noted that EPA identified PFHxA as lacking qualifier transitions and pointing out that Table 3 listed two transitions for this analyte: m/z 313→ 269 and m/z 313→119.

Response:

EPA recognizes that this statement in Section 1.3 of Method 8327 was inaccurate; two product ions were listed for PFHxA in Table 3 when method 8327 was posted for public comment, but the statement in Section 1.3 only referred to target analytes that lack a secondary product ion. EPA revised the statement in Section 1.3 to clarify that the identified target analytes either lack or have a low relative abundance secondary product ion which may provide limited information to support qualitative identification as described in Section 11.6 of the method. EPA also identified PFOSA in Section 1.3 as another Method 8327 target analyte that lacks or has a low relative abundance secondary product ion.

27. Comment:

Two commenters (6 and 8) considered the basis EPA used for identifying target analytes in Section 1.3 as having "quality control failures at concentrations of 40 ng/L and below due to low response and/or high background " to be unclear. Commenter 6 noted that EPA included no study samples spiked at 40 ng/L and below and suspected that EPA used LLOQ verification quality control samples as the basis for this statement. Commenter 6 also remarked that Table 5 in the QC Summary Report showed that these target analytes were recovered acceptably in LLOQ verifications prepared at 10 ng/L by the majority of study laboratories. Commenter 8 remarked that this statement was inconsistent with the other information in this section because it identified "no suspected cause or solution". Commenter 8 was critical of this statement and remarked that "it is clear by this statement, this method is not capable of accurately quantitating these analytes." Commenter 8 noted that laboratories using solid phase extraction and cleanup can achieve accurate quantitation at concentrations below 40 ng/L for these analytes and recommended that EPA modify the method to include these preparation and cleanup methods for PFAS and re-validate it.

Response:

EPA removed this statement related to "low response and/or high background" and the list of associated target analytes from Section 1.3 because EPA did not identify a clear benefit to method users from including it and did not intend for it to be interpreted to mean that measurement of these target analytes will be problematic below a fixed concentration limit. EPA also removed Table 1 with Suggested LLOQs and calibration ranges. Method 8327 does not have a required sensitivity or LLOQ, consistent with other SW-846 reference methods. As described in Section 9.9 of Method 8327 and in Section 9.7 of Method 8000D, each laboratory using this method is responsible for establishing LLOQs at which all method QC acceptance criteria can consistently be met using equipment, instrumentation, and supplies specific to that laboratory. EPA added Table 5b in the QC Summary Report to provide a more detailed summary of LLOQ verification data for each target analyte by laboratory and preparation batch, and a link to this table and other supporting information is included on the SW-846 website along with the published version of Method 8327. Please refer to the Executive Summary for more details regarding EPA's Data Quality Objective for sensitivity.

28. Comment:

Three commenters (5, 9 and 16) noted that EPA's Executive Summary identified performance of 6:2 fluorotelomer sulfonate (6:2 FTS) as erratic in the study samples, with higher frequencies of some QC failures (including for its isotopically labeled surrogate M2-6:2 FTS) due to high or variable 6:2 FTS background in multiple laboratories, including in method blanks and calibration standards. Based on its performance in the validation study, these commenters and others (13, 19) recommended either resolving the observed 6:2 FTS performance issues before publishing the method or removing it from the method completely. Three other commenters (2, 6 and 15) also remarked on performance of 6:2 FTS in the validation study and recommended retaining the 'special care' designation for 6:2 FTS in Section 1.0 of Method 8327. Commenter 15 stated that 6:2 FTS was the only target analyte listed in the method that performs inconsistently in their laboratory.

Response:

The Executive Summary identifies background contamination at some laboratories as the most likely source of performance problems for 6:2 FTS in the validation study. Four of eight laboratories reported background contamination and had related problems meeting preliminary acceptance criteria for instrument and/or sample preparation quality controls used for the validation study. However, EPA retained 6:2 FTS in the method because it performed acceptably in study samples in the four laboratories that did not report laboratory contamination problems and that consistently met acceptance criteria for all categories of quality controls evaluated in the validation study for this analyte. Based on the study data, EPA expects that laboratories that can strictly control 6:2 FTS background contamination and consistently meet the acceptance criteria for all categories of quality controls in Section 9.0 of Method 8327 will be capable of reporting quality-assured measurements of 6:2 FTS. The method does not require laboratories to report all of the listed target analytes, and laboratories that cannot routinely meet the method QC criteria for 6:2 FTS (or other target analytes) should not report quantitative measurements until any source(s) of contamination are identified and minimized.

The product ion that laboratories used for calibration and quantitation of the M2-6:2 FTS surrogate in the validation study has a positive interference from the M+2 ion from native 6:2 FTS due to the natural abundance of the ^{34}S isotope from the native compound. This positive interference led to enhanced response and correspondingly high bias recovery of M2-6:2 FTS when the concentration of 6:2 FTS was relatively high. High bias recovery of the surrogate was only observed when the 6:2 FTS concentration was also high, and it is unlikely to have resulted from another sort of matrix interference. Similar interferences are expected for the M2-4:2 FTS and M2-8:2 FTS surrogates when high concentrations of 4:2 FTS and 8:2 FTS, respectively, are present. EPA revised Method 8327 to provide a detailed explanation of this interference in Section 4.5 and recommended using a secondary product ion for quantitation of the corresponding surrogates to avoid this interference, as needed. Secondary product ions for these surrogates are listed in Table 2 of the method.

Please refer to the QC Summary Report in the final docket for more information about 6:2 FTS and M2-6:2 FTS performance for each quality control category by laboratory and preparation batch, as applicable, and refer to the Statistical Summary Report for summary information about performance in the study samples by laboratory, matrix type and spiking level.

29. Comment:

Commenter 20 stated that “quantifier and qualifier transitions” are not standard terms for LC/MS/MS analysis. This commenter recommended using “quantitation ion and either secondary product ion or confirmation ion” and referred to SW-846 Method 8290A and DOD’s Quality Systems Manual Table B-15 for examples.

Response:

EPA partially agrees with the comment. Method 8290A does not include tandem mass spectrometry, so the terminology will not be directly analogous. EPA revised Method 8327 to use the terms ‘precursor ion’ and 'product ion', consistent with IUPAC terminology (https://www.msacl.org/documents/cms_guidance/Mass_Spectrometry_Definitions_and_Terms_IUPAC_2013.pdf). EPA also added the terms ‘primary product ion’ and 'secondary product ion,' which follow from the term ‘product ion’ based on how they are used in the method.

SECTION 2.0: SUMMARY OF METHOD

30. Comment:

Commenter 6 stated that acetic acid is added to improve chromatography and keep all samples at the same pH, not to improve sensitivity as stated in Section 2.1 of Method 8327.

Response:

EPA revised Section 2.1 to accurately reflect the purpose of acetic acid addition (to improve chromatography) but left out any statement about expected pH to avoid implying that the pH is intended to be measured, which it is not.

31. Comment:

Commenters 14 and 18 suggested eliminating addition of acetic acid to prepared samples and standards. These commenters noted that Section 2.1 states acetic acid is added "because it improved the sensitivity for some target analytes," but the method does not indicate which compounds’ sensitivity are enhanced by the addition of acetic acid, nor the level of signal enhancement. The commenters suggested this step could be eliminated if the increase in sensitivity is negligible (as observed in their facilities).

Response:

As mentioned in the response to comment 30, EPA revised Section 2.1 to state that the addition of acetic acid improves chromatography rather than sensitivity *per se*. As with other method modifications, the laboratory may omit this step as long as it demonstrates that it can generate

data of acceptable quality for the intended application and the methods used and any modifications thereto are acceptable to the end data user.

32. Comment:

Regarding Section 2.2, commenter 4 requested that EPA clarify whether the method allows the response of all Multiple Reaction Monitoring (MRM) transitions to be summed for quantification, which the commenter suggests would increase the signal to noise and improve the detection limit.

Response:

EPA did not revise the method as the commenter requested. Other 8000-series SW-846 mass spectrometry-based determinative methods generally do not recommend using multiple ions for quantitation. Quantitation is generally performed using the highest abundance ion, and other secondary ions are used to support qualitative identification.

SECTION 4.0: INTERFERENCES

33. Comment:

Regarding Section 4.1, Commenter 5 remarked that a Reagent Blank (RB) is not essential to be included with each sample batch and is redundant if a method blank (MB) is also included because a MB is exposed to all reagents and all sample processing steps. The commenter suggested RBs should only be required under the conditions described in Section 9.5.4, when "new reagents, chemicals, or materials that will come in contact with the sample are received". The commenter further stated that RBs are typically only for identifying contamination in a single reagent or solvent.

Response:

EPA partially agrees with the commenter. Due to ubiquitous nature of PFAS, reagents should be monitored regularly by analysis of reagent blanks for PFAS rather than at receipt, which is typical of most methods. EPA revised Section 9.5 to clearly identify that RBs are strongly recommended to be analyzed daily but are not required ("should" instead of "must"). A RB is recommended to be prepared and analyzed each day in addition to the MB that is required to be prepared along with field samples in every preparation batch. Section 9.5.7 of Method 8327 was also revised to identify that a RB serves a different purpose than a MB, which is to identify contamination in reagents (no surrogates are required), and it is only recommended to be analyzed at a minimum frequency of once per day unless reagents are changed during sample preparation. Section 11.4.4 requires either a MB or RB to be analyzed after initial calibration or continuing calibration verification standards, so daily analysis of a RB is not an absolute

requirement, but if blank contamination is found this section does recommend analyzing additional blanks to help determine the source of the contamination.

34. Comment:

Commenter 12 noted that Sections 4.1 and 4.3 state "Careful selection of reagents and consumables is necessary…" but noted that PFAS are used in machinery in the chemical industry and considered it improbable that solvents or salts produced by these manufacturers would be free of PFAS. The commenter provided some of the testing specifications for solvents produced by different manufacturers and noted that pesticide grade solvents and LC/MS grade solvents have no testing specifications for the target analytes in Method 8327.

Response:

As far as EPA is aware there is currently no "PFAS free" industry standard or testing specification for maximum PFAS content in existing grades of solvents or other reagents. Nevertheless, laboratories that participated in the validation study generally did not have significant problems with blank contamination. 6:2 FTS was the exception, but it is not clear that this contamination came from solvents. Section 9.5 specifies that reagent blanks and method blanks are used for evaluating PFAS content and that reagents and supplies should be free from contaminants *that prevent identification or bias the measurement of an analyte*. Please see response to comment 52 for further information regarding solvent grade.

35. Comment:

Commenter 9 noted that Section 4.2 mentions matrix interferences will vary from sample source to sample source and noted that Methods 3512 and 8327 included no cleanup procedures. The commenter identified these cleanup procedures as essential for testing complex aqueous samples and recommended that EPA publish cleanup methods as soon as possible.

Response:

EPA agrees with the commenter. Cleanup methods are under consideration for validation and inclusion in SW-846.

36. Comment:

Commenter 8 noted that Section 4.3 states supplies should be verified prior to use but provides no guidance on maximum PFAS concentrations to use for this evaluation. The commenter requested inclusion of acceptance criteria for evaluating cleanliness of supplies.

Response:

EPA respectfully disagrees with the commenter regarding the need for this level of detail to be included in the method. Cleanliness of supplies can be demonstrated by meeting the acceptance criteria for reagent blank and method blank quality controls in Section 9.5.

37. Comment:

Commenters 5 and 6 expressed concerns about the wording in Section 4.3.4 regarding loss of PFAS chemicals from solutions of 50:50 methanol-water containing 0.1% acetic acid during storage in glass containers. Commenter 5 stated "polypropylene or HDPE containers should be required, not recommended." Commenter 6 stated that shelf life of standard solutions of the same target analytes was evaluated in 50:50 methanol-water containing 1% acetic acid stored in polypropylene and glass auto-sampler vials during development of ASTM D7979-17, and no significant difference was found. Commenter 6 further stated that glass autosampler vials may vary, and laboratories that use glass vials should conduct their own studies to evaluate loss.

Response:

EPA disagrees with commenter 5 about the need to require polypropylene and/or HDPE sample containers to be used with this method. Glass autosampler vials were successfully used during analysis of samples and standards in the validation study. EPA has not conducted a designed study similar to the that described by commenter 6, and the information included in Section 4.3.4 is limited to performance issues identified during method validation. Section 4.3.4 identifies the container materials that were used during method validation, and it states that other materials, including HDPE, should be tested prior to use to ensure method performance is not adversely affected. EPA will consider conducting a study to evaluate loss of target analytes in different container types and solvent compositions. Until such work is completed and reaches definitive conclusions, container materials used during method validation are recommended, and alternative materials should be tested. EPA modified Section 4.3.4 of Methods 3512 and 8327 to only recommend short term storage in glass autosampler vials.

38. Comment:

Commenters 5 and 9 noted that Sections 4.3.5 and 4.3.6 state that materials other than polyethylene may be used for LC vial caps and pipettes if the blank criteria are met; however, other sections state that loss of some PFAS target analytes may occur as a result of exposure to other materials like glass. Both commenters suggested including evaluation of loss rather than just using the blank criteria as a guideline for equivalent performance.

Response:

EPA partially agrees with the commenters. These sections were removed from Method 8327, but the statement about polyethylene pipettes was retained as Section 4.3.5 of Method 3512. Section 6.0 of both methods was revised to specify evaluation of alternate materials with all

quality controls in Section 9.0 of the determinative method rather than just the blank criteria in Section 9.5.

39. Comment:

Commenter 12 noted that Method 8327 recommends the use of disposable materials (e.g., polyethylene pipettes) and suggested emphasizing reuse of materials to reduce plastics consumption, consistent with SW-846 method practices to consider “green chemistry” alternatives.

Response:

EPA does not have many “green” alternatives to propose other than the small recommended sample size already included in the preparation method (Method 3512) and smaller volumes needed for the associated reagents. Most transfer pipettes are disposable, and polyethylene may be less of a concern for loss or introduction of PFAS target analytes than other types of materials.

40. Comment:

Commenter 9 recommended making the use of an isolator column in Section 4.3.7 a requirement if it minimizes contamination and interference.

Response:

EPA disagrees with this comment and notes that a laboratory could avoid using an isolator column if all categories of method-defined and any project-defined acceptance criteria can be met for the system without it. However, many current liquid chromatography systems have sources of PFAS background that can lead to problems meeting the method QC acceptance criteria, and EPA strongly suggests use of an isolator column for this method.

41. Comment:

Commenter 9 noted that Section 4.4 indicates that “the laboratory should inform the data user of any suspected contamination but does not require any additional corrective action.” The commenter requested that EPA add corrective actions when contamination is observed.

Response:

EPA disagrees with the need for corrective action requirements to be included in the method. This language is common to other SW-846 methods as well. Corrective actions are beyond the scope of this method and should be addressed in a laboratory's Standard Operating Procedure or Quality Management Plan or in project planning documents.

SECTION 6.0: EQUIPMENT AND SUPPLIES

42. Comment:

Commenter 4 noted that Section 6.1 only specifies liquid chromatography columns with stationary phases of ~2 µm diameter particle size, which will limit injection volume and column flow. The commenter recommended allowing the use of stationary phases with larger (5 µm) particle size to accommodate larger injection volumes (up to 80 µL) and higher flow rates. The commenter noted this suggestion is also relevant to the LC conditions and injection volume in Table 5C.

Response:

EPA disagrees with the commenter regarding the need to add this information to the method. The analysis conditions included were used during method development and are recommendations. Laboratories are allowed to modify supplies (column dimensions, phases), equipment and analytical conditions suggested in the method as long as the acceptance criteria for the categories of quality controls in Sec 9.0 are met, performance is not adversely affected, and the requirements for a specific project (e.g., action levels, Data Quality Objectives) are also met. Section 6.0 requires that laboratories demonstrate and document that alternative supplies, equipment and conditions used are also appropriate for the intended test.

43. Comment:

Commenter 12 noted that there appeared to be a section formatting problem for Section 6.1.3 compared to Sections 6.1.2 and 6.1.4.

Response:

EPA did not make any changes to formatting based on this comment. Section 6.1.3 was present in Method 8327 but was formatted to be consistent with all SW-846 Methods following the 2017 SW-846 Method Style Guide and was difficult to distinguish from Section 6.1.2.4.

44. Comment:

Commenters 10 and 12 noted that Method 8327 did not indicate what type of tandem mass spectrometer was to be used. Commenter 12 noted that it was likely a triple quadrupole because that is commonly used for selected reaction monitoring (SRM). Commenter 10 recommended including more specific and quantifiable criteria for minimum performance specifications for the mass spectrometer

Response:

EPA purposely left the mass spectrometer specifications generic to avoid limiting the types of instruments that can be used for this method. EPA did revise Section 6.1.4 to state that the system must be capable of documenting the performance of the mass spectrometer against manufacturer specifications for mass resolution, mass assignment, and sensitivity using the internal calibrant. This section also indicates that a triple quadrupole mass spectrometer with an electrospray ionization source was used during method development.

45. Comment:

Commenter 6 noted that Section 6.1.2 lists several columns that were used during method development and remarked that only one of them was provided for the method validation study. The commenter further noted that no data was provided to support equivalency of these columns to validation data produced with the liquid chromatography column used in the validation study. The commenter was concerned that pooling data from laboratories that was generated under different conditions could have resulted in higher variability for the validation study as a whole and recommended that laboratories first demonstrate equivalent performance prior to modifying the method. (Note: Commenter 6 provided a similar comment for the QC Summary Report).

Response:

EPA did not combine data from different HPLC columns for evaluation of the validation study data. Comparison of results generated with different HPLC columns was not one of the factors evaluated as part of the validation study Data Quality Objectives. Some participants chose to reanalyze samples using different HPLC columns, but the data from these columns were not pooled with data from other laboratories for evaluation against the validation study Data Quality Objectives. SW-846 methods do not require laboratories to demonstrate equivalency to a validation study or to another laboratory's performance data, but they do require that laboratories demonstrate and document that supplies, equipment and conditions used are appropriate for the intended application. Please refer to Section 6.0 of Method 8327 and comment 42 for additional information.

46. Comment:

Commenter 9 noted that Section 6.1.2 provides options for columns that can be used “provided that method performance is appropriate” but remarked that the method does not provide a “chromatographic performance criterion for separation and resolution,” without which the commenter considered the method to be unclear regarding how appropriate performance was to be evaluated. Commenter 9 further stated that Method 8327 and future PFAS methods developed by EPA should include chromatographic resolution criteria to improve reproducibility and comparability of data from different laboratories.

Response:

Method 8327 does not provide chromatographic peak resolution criteria because the determinative technique is tandem mass spectrometry, and coelutions of target analytes generally do not lead to interferences. Branched and linear isomers may be an exception, but coelutions of these isomers may not lead to measurement bias compared to chromatographically separated peaks when the isomers are integrated together for the purpose of quantitation. At the time of this writing the availability of reference materials for individual structural isomers is limited, which presents a hurdle for evaluating chromatographic separation of structural isomers. If the commenter intended to address chromatographic performance (e.g., peak symmetry check), the higher water content of standards and samples limits peak distortions of early eluting peaks that might be more common when injecting higher proportions of organic solvent. Section 11.3.1 was revised to state "chromatographic peaks should be inspected to ensure they are symmetrical, and significant peak tailing should be corrected." Appropriate performance for alternative liquid chromatography columns should be demonstrated by meeting acceptance criteria in Section 9.0.

47. Comment:

Commenters 5 and 12 stated that Section 6.2.2 should specify a more sensitive/precise balance for preparation of standards from neat materials. Commenter 12 provided an example of needing a balance to weigh 20 µg of material to preparing a 2000 ng/mL solution at a final volume 10 mL.

Response:

EPA agrees with the commenter and added a balance tolerance of weighing 0.0001 g (100 µg) for preparation of analytical standards from neat materials.

48. Comment:

Commenter 12 noted that labware cleaning instructions in Sections 6.2 and 6.2.4 and reagents in Section 7.1 should emphasize that traces of compounds should be reduced to a minimum.

Response:

EPA agrees with the commenter and added a related statement to Section 6.2.4. Section 7.1 was also revised to state that reagents should be verified prior to use to ensure the blank acceptance criteria can be met. Section 9.5 states that the analyst must demonstrate that equipment and supplies are free from contaminants and interferences that would prevent identification or bias measurement of the target analytes.

49. Comment:

Commenter 6 remarked that Sections 6.2.3.1 and 6.2.3.3 mention supplies made from materials that were not used in the validation study, including autosampler vials made from HDPE, polypropylene or silanized glass, and syringes used for filtration of prepared samples made from HDPE or polypropylene. The commenter noted that study laboratories were provided with glass vials with polyethylene septumless caps and glass syringes. The commenter stated the only supplies that had been demonstrated for use with this method were those required in ASTM D7979-17, and the laboratory should first use method-specified materials and establish equivalency before using different vial materials.

Response:

EPA disagrees with the commenter regarding the need to demonstrate equivalency prior to using different materials, which is inconsistent with the flexibility in these and other SW-846 methods. Section 4.3.4 identifies that polypropylene sample container materials and glass autosampler vials were used during method validation, and it states that other materials, including HDPE, may be used if it can be shown that method performance is not adversely affected. Laboratories are allowed to modify supplies, equipment and analytical conditions suggested in the method as long as the acceptance criteria for the categories of quality controls in Sec 9.0 are met, performance is not adversely affected, and the requirements for the specific project (e.g., action levels, Data Quality Objectives) are also met. Demonstration of equivalent performance is not an SW-846 requirement. Section 6.0 also requires that laboratories demonstrate and document that alternative supplies, equipment and conditions used are appropriate for the intended test. Please note that ASTM D7979-20 does not specify a part number for glass autosampler vials or glass syringes. EPA revised the method to remove the term ‘silanized.’

50. Comment:

Commenter 5 remarked that the discussion of filters in Section 6.2.3.8 is insufficient. The commenter noted that filters can result in a loss of PFAS and asked whether the filtration step had been thoroughly tested and whether loss had been evaluated to prevent low bias in addition to introduction of contamination from the filters.

Response:

EPA evaluated loss of target analytes through the filtration step as part of method validation. Prepared samples were filtered while standards were not, therefore, the validation study demonstrated acceptable performance and negligible loss from filtration. Aqueous samples are first diluted 1:1 with methanol prior to filtration, which aids in keeping chemicals in solution, and contact time during filtration is minimal. No significant low bias was observed in the study that was attributable to the filtration step, as evident in average % recoveries of the longer-chain target analytes near 100% in Table 1 of Method 8327. Filtration was moved from Method 8327 to Method 3512 because the filters are not utilized for the determinative method but rather the preparatory method.

51. Comment:

Commenter 22 noted that Section 6.2.3 states all supplies should meet blank criteria. This commenter recommended changing “should” to “must” to indicate meeting blank criteria was a requirement. The commenter further stated that, if glass or other supplies are used, the laboratory should prove there are no interferences.

Response:

EPA disagrees with this comment. Sections 9.5 and 11.4.4 require blanks to be analyzed at a minimum frequency, but the blank criteria are considered guidance. The laboratory is responsible for demonstrating and documenting that supplies, equipment and conditions used are appropriate for the intended application and that the supplies do not adversely affect performance. For more information about demonstration of acceptable performance please refer to EPA’s response to comment 42.

SECTION 7.0: REAGENTS AND STANDARDS

52. Comment:

Two Commenters (5 and 12) noted that the solvent grades in Method 8327, specified as “pesticide residue purity or higher”, are inappropriate for PFAS analysis because they are not appropriately tested or certified by the manufacturer for these target analytes. Since LC/MS/MS is used for analysis, these commenters recommended specifying the solvents as LC grade, where available. Additionally, commenter 12 stated that PFAS are used in the chemical industry and it is very improbable that a manufacturer will be able to guarantee that a solvent or salt is “PFAS/PFOS free.”

Response:

EPA agrees with the comment regarding Liquid Chromatography (LC) grade being more appropriate than pesticide grade for this application, but EPA recognizes that manufacturers of these solvent grades do not typically provide testing specifications for any PFAS target analytes included in Method 8327. Regardless of the specified grade, the laboratory is responsible for demonstrating that solvents and reagents are free from identifiable Method 8327 target analytes that would interfere with identification or bias measurement, as described in Section 9.5. The lack of industry standards for PFAS content in any solvent grades tested options means the grade on the container is less critical than the performance of the product. However, the term ‘pesticide grade, or equivalent’ was replaced with ‘LC/MS grade, or equivalent’ in the final version of the method. Note that similar comments were provided for the Appendix B sample preparation method and are addressed in Method 3512 in the same fashion.

53. Comment:

Commenter 4 recommended revising Section 7.4 to require all stock solutions and intermediate standards to be prepared in 99% solvent.

Response:

EPA disagrees that this change is needed. Section 7.4.3 states that spiking solutions should be prepared in 95:5 acetonitrile-water, which was tested during method validation, and it states that alternative solvents (e.g., 96:4 methanol-water) may be used provided that method performance is not adversely affected and the QC criteria in Section 9.0 can be met. EPA 500 Series PFAS methods use standards prepared in 96:4 methanol-water with ~ 4 mol equivalents of base. The EPA Method 537 Research Summary included as Reference 3 in the final version of Method 8327 determined that a small amount of water was necessary to retain some carboxylic acid target analytes in solution.

54. Comment:

Commenter 5 asked why stock solutions are prepared with acetonitrile, but samples are prepared with methanol.

Response:

EPA used preparation instructions provided by the method developer as a basis for the validation study protocol and included them in the reference method.

55. Comment:

Commenter 5 asked why Section 7.0 allows 96:4 methanol-water to be used if esterification is a known problem, since it could be a problem whenever mixtures of methanol and water are used.

Response:

EPA revised Section 7.4 to address this concern when standards are prepared from neat source materials. This section specifies addition of base to stock solutions to prevent esterification, consistent with EPA Methods 537.1 and 533.

56. Comment:

Commenter 6 remarked that Section 7.4 states HDPE containers can be used for storage of standard solutions, and that this section mentions spiking solutions can be prepared in alternate solvents such as 96:4 methanol-water as long as method performance is not affected. The commenter noted that the developers of ASTM D7979 – 17 have no data to support the use of

HDPE containers or preparation of spiking solutions in an alternate solvent and stated that both should be removed from the method.

Response:

Reference to HDPE was removed from Section 7.4, but it was retained in Section 6. As described in comments 49 and 53, other materials, including HDPE, and other spiking solution solvent compositions may be used provided the laboratory demonstrates that all categories of quality controls in Section 9.0 can routinely be met, performance is not adversely affected, and the requirements for a specific project (e.g., action levels, Data Quality Objectives) are also met.

57. Comment:

Commenter 9 remarked that Section 7.4 allows laboratories to recertify spiking solutions after a one-year shelf-life for continued use if the analytes are within ±20% of expected concentrations compared to a freshly opened stock. The commenter was concerned that the total bias could be larger than ±20% due to allowable % error for a continuing calibration verification standard. The commenter also recommended including a clear procedure for re-verification frequency after the initial one-year accuracy check.

Response:

EPA removed criteria for re-verification of spiking solution concentrations and instead stated that laboratories could either document their own quality control practices for determining expiration dates or use the manufacturer’s expiration date or a 1-year shelf-life for prepared standards, whichever was sooner. Using standards beyond a manufacturer's or method's recommended expiration date should be addressed in a laboratory's Standard Operation Procedure or Quality Management Plan.

58. Comment:

Commenters 5, 8, 9 and 21 noted that Sections 7.3.8 and 7.4.4 are unclear regarding how standards composed of linear and branched isomers are intended to be used for qualitative identification and/or quantitative analysis. Commenter 5 recommended clarifying the text regarding whether purchasing of standards containing both linear and branched isomers is recommended. Commenter 8 stated the method should require standards with branched isomers to be used for instrument calibration and/or defining retention time ranges in the same manner as Method 537.1. Commenter 9 mentioned that commercially available mixtures of PFOS are composed largely of methyl and dimethyl isomers, which may be different than in samples. Commenter 21 suggested requiring the use of linear and branched isomers for calibration to avoid underestimating target analytes concentrations in samples.

Response:

EPA did not provide more specifics about quantitative analysis of PFAS composed of linear and branched isomers in Section 7.4, but the note after Section 11.3.3 and Sections 11.6 and 11.7 were revised to provide more specificity regarding calibration, qualitative identification and quantitation of target analytes composed of mixtures of linear and branched isomers. The note after Section 11.3.3 also states the choice of calibration and quantitation options must be made clear to the data user and refers to Method 533 as an example.

59. Comment:

Commenter 9 requested that EPA resolve the discrepancy between Section 7.4.4, which mentions storage of certified solutions in accordance with manufacturer's temperature recommendations, and Section 7.4, which directs laboratories to store standard solutions at ≤6ºC.

Response:

EPA agrees with the commenter and revised Section 7.4.1 to include "or according to manufacturer's recommended storage conditions."

60. Comment:

Commenter 7 recommended calibrating to ≤½ the LLOQ to avoid the need for extrapolation when quantitating LLOQ verification QC samples. The Commenter noted that Section 7.4.3 states LLOQ verifications can be prepared at concentrations from 0.5 to two times the LLOQ.

Response:

While EPA agrees that calibrating to below the Lower Limit of Quantitation (LLOQ) is an excellent practice, EPA did not make this revision to the method because it would be inconsistent with Method 8000D and other SW-846 determinative methods. Test methods published by other EPA programs (e.g., 500 series, 600 series) also generally do not require calibrating to below the Minimum Reporting Level (MRL) or Minimum Level (ML). The minimum requirement in 8000-series SW-846 determinative methods is to calibrate to ≤ LLOQ, and more restrictive requirements should be documented in a laboratory's Standard Operating Procedure or Quality Management Plan or in project planning documents.

61. Comment:

Commenter 9 remarked that Section 7.4.4.1 does not provide a shelf-life for a Primary Dilution Standard (PDS) solution despite the concern raised in the QC Summary Report and Appendix E of the Statistical Summary Report regarding loss of some target analytes from solutions of 1:1

methanol-water+0.1% acetic acid in glass containers. The commenter asked whether similar losses might also occur from the PDS, calibration standards, and sample extracts and whether this loss could have contributed to the variability observed in the study. The commenter also considered preparation of the PDS and calibration standards to be tied to Method 3512 and questioned whether it would be appropriate for Method 8327 applied to other sample preparation methods. The commenter requested that EPA resolve these discrepancies in the method.

Response:

EPA disagrees with the commenter and considers the information included in Sections 4.0 and 7.0 of Method 8327 to be sufficient regarding container materials, storage conditions and shelf-life for standards, including for the PDS. EPA did not observe loss of target analytes in standard solutions prepared in a higher proportion of organic cosolvent or in 1:1 methanol-water + 0.1% acetic acid stored in polypropylene containers. EPA revised Method 8327 to add a section entitled 'Storage and Shelf Life' (Section 7.4.1) to distinguish this information from the rest of the text in Section 7.4. This section retained the manufacturer's expiration date or 1 year shelf-life for prepared standard solutions, but it was also revised to provide laboratories the option to establish their own QC practices for determining expiration dates, consistent with methods from other EPA programs (e.g., Method 533). This section was also revised to state that stock solutions should be checked frequently for signs of degradation or evaporation, consistent with language in other SW-846 methods. EPA added information from Section 4.3.4 of Method 8327 to Method 3512 as well.

62. Comment:

Commenter 20 requested that EPA clarify sections on calibration for PFAS target analytes composed of linear and branched isomers, including how these analytes are to be calibrated, qualitatively identified (e.g., evaluating chromatographic retention times), quantitated, and reported. The commenter observed that laboratories commonly report concentrations of integrated branched and linear isomers based on calibration of only the linear isomer that could result in bias. The commenter expressed concern about comparability of data between laboratories if they used different approaches to calibration and quantitation that might not be apparent unless laboratories explain these details in a narrative associated with the data.

Response:

EPA concurs with the commenter and revised the note in Section 11.3.3 and Sections 11.6 and 11.7 to provide additional specificity regarding calibration, qualitative identification, and quantitation of multi-component analytes. The note in Section 11.3.3 requires that the choice of calibration (linear vs branched) be clear to the data user. Please see responses to comments 3 and 123 for additional information.

63. Comment:

Commenter 5 considered the statement in Section 7.4.4.2, "a second lot number from the same manufacturer may be adequate to meet this requirement," to be too vague and recommended not allowing a "second lot number" to be used for Initial Calibration Verification (ICV) to avoid ambiguity.

Response:

EPA made no changes to the method based on this comment, as this same language is used in other SW-846 methods. The first sentence of this section states the same manufacturer may be used if the "batch is prepared independently from the batch used for calibration." The laboratory should verify that the source materials for the calibration standards are not the same as for the ICV. Given potential for limitations in sources of certified reference materials for PFAS, EPA does not intend to prohibit laboratories from using a 2nd lot from the same supplier.

SECTION 8.0: SAMPLE COLLECTION, PRESERVATION, AND STORAGE

64. Comment:

Commenter 22 noted that Section 8.0 states all sample collection, preservation and storage language are guidance and recommended that the EPA identify sample collection, preservation and storage specifications as requirements that must be met. The commenter expressed concern that an accredited laboratory could take months to prepare and analyze samples without clear requirements.

Response:

EPA agrees with this commenter that holding time information based on a properly designed study would be useful, but at the time of this writing a formal holding time study has not yet been completed. Until data are available from this study, information in this section is considered guidance. Please note that the holding times in Chapters 3 and 4 of SW-846 are guidelines, and the project team should consider all available sources of information regarding holding times and sample preservation options to ensure measurements are representative and suitable for their intended use.

65. Comment:

Commenter 22 recommended that the EPA require collection of field blanks and addition of trizma to remove residual chlorine from aqueous samples as described in EPA Methods 537 and EPA 537.1.

Response:

EPA respectfully disagrees with the commenter's suggestions to require field blanks and to preserve aqueous samples with trizma. Section 8.0 states that field blanks may be required, but identification of field quality controls needed to support a given project is more appropriate for project planning documents or field sampling guidance and is outside the scope of these reference methods. Trizma may be useful for removing residual chlorine from aqueous samples, but it is not recommended for use with this method because it was not tested during method development, and the impact of residual chlorine on stability of the PFAS target analytes was not evaluated.

66. Comment:

Several commenters (4, 11 and 21) noted that 5 mL sample sizes recommended in Section 8.1 may be difficult to collect using typical field sampling equipment (e.g., ISCO samplers), and these commenters recommended specifying or allowing for larger sample sizes (125 or 250 mL). Commenter 11 stated that allowing larger sample sizes would enable commercial laboratories to continue using 250 mL High Density Polyethylene (HDPE) sample containers. Commenter 4 was concerned that sample characteristics (color, particulates, foam) could lead to inaccurate volume measurement, and commenters 4 and 11 also expressed concern that over-filling sample containers and/or manual transfers could lead to adsorption-related losses or bias for some target analytes. Commenter 21 noted that it would be difficult for field samplers to collect 5 mL ± 5% volumes and suggested that adjusting the amounts of isotopically labeled surrogates/internal standards would be cumbersome for laboratories to manage.

Response:

EPA included 5 mL sample size and 15 ml sample containers as examples. Sample containers may be of any size as long as the entire sample is processed, whether in the original container or after transfer with adequate solvent rinsing. The recommended sample size is intended to minimize waste and cost associated with diluting larger sample sizes 1:1 with methanol and adding appropriate amounts of isotopically labeled surrogates. Method 3512 also states that alternate sample volumes are acceptable; please refer to Section 11.0 of Method 3512 for more information.

67. Comment:

Commenter 5 recommended deleting "where practical" from the statement in Section 8.1 that sample containers and reagent water used for field blanks should be pre-verified to be clean prior to sample collection.

Response:

EPA disagrees with the commenter's suggested change. Sample collectors may not always have access to pre-verified water and containers from the analysis laboratory. While this is an excellent practice, it may not always be practicable.

68. Comment:

Commenter 6 stated suggested deleting "high density polyethylene (HDPE)" from Section 8.1 as an acceptable container type for aqueous sample collection, noting that "The ASTM D7979 – 17 test method developers have no data to support HDPE containers, nor did the multi-lab study use HDPE containers."

Response:

EPA partially agrees with the commenter and revised the wording in Section 8.1 to specifically identify that HDPE sample containers may be used if acceptable performance is demonstrated. As described in EPA's responses to comments 45, 49 and 56, laboratories are allowed to modify supplies, equipment and analytical conditions in the method as long as the acceptance criteria for the categories of quality controls in Sec 9.0 are met, performance is not adversely affected, and any requirements for the specific project (e.g., action levels, Data Quality Objectives) are also met. Section 4.3.4 also states other materials, including HDPE, may be used if adequate performance is demonstrated. Note that other PFAS test methods allow the use of HDPE containers for collection of aqueous samples, including EPA Method 533.

69. Comment:

Commenter 9 expressed concern that 5 mL samples collected as described in Section 8.1 would be imprecise, and the commenter stated that the sample preparation procedure should explicitly allow for solvent volumes to be scaled to be equivalent to aqueous sample volumes "(e.g., 5.2 mL of sample to 5.2 mL of methanol)". This commenter further stated that the method was only validated for 5 mL volumes and expressed concern that small sample volumes could "exacerbate precision error particularly in complex sample matrices with no cleanup procedures."

Response:

EPA moved the information pertaining to sample volume and dilution solvent volume to Sections 11.1 and 11.2 of Method 3512. Please refer to EPA's response to comment 190 for more detail.

70. Comment:

Commenter 9 remarked that the note after Section 8.1 referred to Section 11.1.1 that did not exist in Method 8327, and Section B11.1.5 of Method 3512 appeared to be a more appropriate reference.

Response:

EPA agrees with the commenter and removed the section reference from Section 8.1. Related information was included as a “caution” after Section 11.0 of Method 3512.

71. Comment:

Commenter 10 stated that the sample collection containers, volumes and handling in Section 8.0 should be specified if they are critical to method performance, not suggested.

Response:

EPA considers the critical specifications related to aqueous sample collection to be included: container materials are specified, the entire sample must be prepared, and 50% solvent content must be achieved prior to subsampling, or the associated data must be qualified appropriately; and, extra replicate sample containers are recommended to be collected. Container size, volume, and headspace were not identified as critical for performance of the method and are at the discretion of the laboratory or project.

72. Comment:

Regarding the note after Section 8.1, commenter 11 remarked that addition of isotopically labelled chemicals and the use of isotope dilution calibration would allow for more accurate quantitation of the native compounds because isotope dilution calculations would account for subsampling losses and differences in sample volume. This commenter noted that isotope dilution would enable the method to more readily accommodate a wider variety of sample volumes and allow laboratories to subsample aqueous matrices prior to diluting 1:1 with methanol as long as the isotopically labeled analogs are added before removing subsamples, as detailed in Method 3512 Section B2.0. Commenter 4 also recommended adding isotopically labeled standards to sample containers prior to collection, which would avoid the need for additional replicate sample containers that are otherwise required if samples require re-preparation and/or reanalysis as described in Section 9.5.3.

Response:

EPA did not acquire the appropriate data to add isotope dilution calibration as an option to this method. EPA also did not evaluate kinetics of loss of PFAS target analytes from aqueous solution or determine how to use recovery of isotopically labeled chemicals to account for this loss. EPA notes that replicate containers would only be needed for duplicate and Matrix

Spike/Matrix Spike Duplicate QC samples or if re-preparation of a sample is needed. Sufficient sample volume should be available for reanalysis if the default 5 mL sample volumes are prepared as described in Method 3512. Please refer to EPA's response to comment 10 for more information about isotope dilution.

73. Comment:

Commenters 14 and 18 recommended using pipettes to collect 5 mL sample volumes as described in Section 8.1. The commenters stated that precise measurement of initial sample volumes during collection would prevent under/overfilling of containers and eliminate the need for determination of volume or adjustment of internal standard/surrogate additions. These commenters further recommended collection of a minimum of four replicate containers for each sample for MS/MSD and re-extraction, as needed.

Response:

EPA considers these suggestions to be potentially useful for sample collection, but addressing this comment is outside the scope of the method. Sample collection practices and minimum numbers of replicate sample containers are more appropriate for project planning documents and laboratory standard operating procedures or quality management plans, respectively. Volumetric measuring devices such as pipets should be thoroughly tested prior to use in the field for sample collection to ensure samples are representative.

74. Comment:

Commenter 22 recommended that EPA revise Section 8.1 to require that laboratories not subsample due to the potential for low bias measurement. The commenter noted that "the drinking water methods also do not allow subsampling for the same reason".

Response:

EPA agrees with the commenter regarding the potential for low bias measurement but notes that there may be circumstances in which subsampling is unavoidable and/or useful for specific projects. Rather than requiring that subsampling not be performed, EPA revised Section 8.0 in Method 8327 and Section 11.0 in Method 3512 to state "If subsampling is performed prior to achieving 50% organic cosolvent content, i.e., when preparing the entire water sample is not possible or practical, the data must be qualified appropriately."

75. Comment:

Several commenters (5, 9, and 12) provided suggestions or requested additional information about holding times in Section 8.2. Commenter 5 asked whether there was sufficient evidence to require collected samples to be maintained below 6°C after collection as specified in Section

8.2, noting that “PFAS are very stable and being able to ship samples without cooling would be a great cost savings for a number of remote Alaskan sites”. Commenter 9 noted that Section 8.2 included a recommended holding time for solid samples but without including an extraction method or holding time study as a basis. Commenter 9 also remarked that EPA should provide a schedule for completion of holding time studies in various matrices. Commenter 12 noted that studies in the literature have shown some PFAS have the potential to transform during storage (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3214619) and recommended reducing maximum holding times to 14-15 days.

Response:

EPA revised Section 16.0 of Method 8327 to include a reference to a published article (Woudneh, et. al 2018) that suggested some PFAS can transform even during refrigerated storage of aqueous samples. EPA agrees with commenter 12 and revised the recommended maximum holding time for aqueous samples to 14 days for aqueous samples maintained at ≤6°C. The note after Section 8.2 was also revised to indicate frozen storage may reduce transformation of some PFAS, and additional references were provided for PFAS stability in aqueous matrices. EPA also revised Section 8.2 to removed reference to holding times for solid matrices. Please see comments 4 and 5 for more detail. At the time of this writing, EPA has not completed a formal holding time study, and the holding times and sample preservation specifications in Method 8327 are recommendations. Section 4.1.2 of SW-846 Chapter 4 states “selection of preservation techniques and applicable holding times should be based on all available information”, including the project Data Quality Objectives. Please refer Section 8.0 and Table 6 in the final version of Method 8327 and to Chapter 4 for more details.

76. Comment:

Commenter 20 recommended that Section 8.0 be revised to indicate that extracts should be mixed prior to analysis because “PFAS can float to the top of the extract if it's sitting around waiting for LC-MS/MS analysis”.

Response:

EPA added a statement to Section 11.5 regarding warming of prepared samples to room temperature and mixing prior to transferring to autosampler vials.

SECTION 9.0: QUALITY CONTROL

77. Comment:

Commenter 5 expressed concern that the allowance in Section 9.1 for laboratories to generate their own statistically based acceptance limits would lead to high variability in evaluation of data

between laboratories and suggested specifying a minimum threshold for acceptable performance.

Response:

EPA disagrees with this comment and notes that all 8000-series SW-846 methods recommend calculation of statistically-based acceptance limits for certain types of sample preparation quality controls once sufficient data has been acquired. Please refer to Section 9.0 of Method 8000D for more information.

78. Comment:

Commenter 5 remarked that analysis of only four QC samples in Section 9.4 for an Initial Demonstration of Proficiency (IDP) study was “insufficient for such a sensitive and difficult analytical method” and recommended that EPA revise the method to require 10 such samples for IDP.

Response:

EPA respectfully disagrees with this commenter regarding the need to require a higher number of replicate samples for IDP for this method. All chromatographic SW-846 methods have a four-sample minimum requirement for IDP (see Method 8000D). The laboratory is free to add more samples to demonstrate capability; however, requiring more would be inconsistent with other SW-846 methods.

79. Comment:

Commenter 22 noted that Section 9.4 does not set requirements for an Initial Demonstration of Proficiency (IDP) and recommended requiring 70-130% average recovery and <30% RSD for IDP. The commenter concluded that the analytes should not be run by this method if a laboratory cannot meet these IDP requirements.

Response:

EPA respectfully disagrees with the commenter regarding the need to include ‘required’ language for IDP in this section. This section refers to Method 8000D, which provides a general approach for IDP that includes comparison of laboratory-generated mean and standard deviation of % recovery to the performance data in the method. This general approach is relevant for all 8000-series SW-846 methods. ‘Preliminary’ acceptance criteria for IDP were removed from Section 9.4 of Method 8327 and were instead incorporated by reference to Method 8000D. This change is intended to make this section more broadly applicable and to improve consistency with other recently published reference methods (8260D, 8270E). Alternative acceptance criteria may be used as long as they are acceptable to the end data user (e.g., regulatory authority), and these criteria should be documented in the laboratory's SOP or

in project planning documents. Consistent with other SW-846 methods, some analytes may not perform as well as others, and data users will have to address data limitations when quality controls do not meet method- or project-specified acceptance criteria.

80. Comment:

Commenter 9 remarked that the acceptance criteria for Initial Demonstration of Proficiency (IDP) in Section 9.4 of 70-130% recovery is wide and suggested that method is "not determinative" without incorporating isotope dilution calibration. The commenter encouraged EPA to "reconsider the quantitative problems with external standard calibration".

Response:

Please refer to EPA's response to comment 79 for an explanation of the changes made to Section 9.4 and to comments 1 and 10 regarding method performance and isotope dilution calibration, respectively.

81. Comment:

Commenter 4 considered the minimum analysis frequency specified for Reagent Blanks (RBs) as once per day in Section 9.5.7 to be insufficient. This commenter recommended including multiple reagent blanks throughout an analysis sequence and as needed to demonstrate lack of carryover. The commenter further recommended revising the method to specify a minimum RB frequency of one per 10 field samples and quality control samples and additionally after any CCV and at the end of the analysis sequence.

Response:

EPA did not change the minimum frequency of RBs in Section 9.5.7 based on this comment but agrees that more frequent analysis of RBs is a good quality assurance practice. Please see EPA's response to comment 33 for more information about RBs.

82. Comment:

Regarding Section 9.5.1, commenter 5 noted that Reagent Blanks (RBs) should be analyzed prior to samples rather than Method Blanks (MBs). The commenter considered RBs to be more appropriate for pinpointing sources of contamination because MBs are exposed to more potential sources of contamination. The commenter also considered analysis of MBs to be more appropriate than RBs on a continuing basis during sample analysis.

Response:

EPA did not change the wording in Section 9.5.1 based on this comment but notes that MBs and RBs are both useful for identifying and isolating potential sources of contamination. EPA considers the statement in Section 7.1 that reagents should be tested prior to use to ensure that the blank criteria can be met to be sufficient to address the commenter's concern about identifying potential sources of contamination. EPA agrees with the commenter that MBs integrate more variables than RBs. EPA considers the minimum MB frequency described in Section 9.6 to be sufficient for evaluation of contamination during sample preparation and analysis. EPA also considers analysis of RBs and/or MBs on a continuing basis as described in Sections 9.5.1 and 11.4.4 to be sufficient to evaluate whether background of any PFAS target analytes in the analytical system and reagents is low enough for analysis to continue. The allowance to analyze RBs rather than MBs on a continuing basis is also consistent with other SW-846 methods.

83. Comment:

Commenter 7 noted that Section 9.5.2 states blanks are generally considered acceptable if concentrations are <½ the Lower Limit of Quantitation (LLOQ). The commenter remarked that quantitative measurements extrapolated outside the calibrated concentration range should not be relied upon, even in blank samples, because the calculated concentrations are highly uncertain. The commenter further noted that Section 9.9 refers to the LLOQ as the lowest concentration that is quantitated.

Response:

EPA agrees with the commenter that quantitative measurements below the lowest initial calibration standard can be highly uncertain. However, quantitation of concentrations below the LLOQ in blanks is a common and necessary practice associated with most analytical methodology, including this and other SW-846 methods. Data users must understand the limitations of such data, and professional judgement can be an important part of interpreting the impact of blank concentrations below the LLOQ on measured concentrations in samples. Method 8327 provides some related guidance. For example, Section 9.5.2 identifies that response can be used as an alternative to concentration for assessing background if quantitation accuracy <LLOQ is concerning. Please refer to EPA's response to comment 60 for related discussion.

84. Comment:

Commenter 22 noted that Section 9.5.2 does not set a specific blank acceptance criterion and recommended that EPA require blank concentrations to be < ½ the LLOQ to limit interferences from supplies.

Response:

EPA did not change the blank evaluation criteria in Section 9.5.2 or state that the criteria are required in order to avoid creating an inconsistency with other 8000-series SW-846 determinative methods. Section 9.5.3 states that corrective action may be required if blanks do not meet the acceptance criteria established by the laboratory or for the project. If more specificity is needed it should be included as a project-specific requirement.

85. Comment:

Commenter 5 remarked that Section 9.5.3 is vague about the circumstances under which sample re-extraction or re-analysis is required when blank contamination is found; specifically, the commenter requested that EPA better define the meaning of “well below” the action or regulatory limit. The commenter recommended revising the statement to include more specific guidelines.

Response:

EPA made no changes to the wording in Section 9.5.3 based on this comment. The same language is used in other SW-846 methods, including Method 8000D. The laboratory and/or project team should use judgment for interpreting the effect that blank contamination may have on meeting action or regulatory limits or other Data Quality Objectives for the project. These procedures should be documented in the laboratory's SOP or in project planning documents.

86. Comment:

Commenter 5 expressed support for the discussion of blank subtraction in Section 9.5.5, noting “As a regulatory agency we have had to provide guidance on this topic and seeing it in the method is a welcome addition”.

Response:

EPA concurs with the comment. As described in Section 9.5.5, the laboratory should not subtract the results of the Method Blank (MB) from those of any associated samples, consistent with Method 8000D and other SW-846 determinative methods.

87. Comment:

Two commenters (8 and 9) remarked that Section 9.5.6 is unclear regarding the circumstances under which more than one method blank (MB) may be needed and how EPA intends sample data to be interpreted when samples are associated with multiple MBs. Both commenters requested that EPA revise this section to clarify how it intends for sample results associated with multiple blanks to be interpreted. Commenter 8 noted that analyzing multiple MBs or

reagent blanks (RB) could result in laboratories reporting the 'best' result, which the commenter did not consider to be a good laboratory practice. Commenter 8 further remarked that target analytes that do not meet the acceptance criteria in any blanks must be qualified in the samples, or the samples must be re-prepared and re-analyzed. Commenter 8 stated that if the MB criteria are not achievable for 6:2 FTS or another target analyte then the LLOQ may be set too low and should be re-evaluated for that compound. Commenter 8 supported including an additional MB and LCS when the lot of a supply was changed during preparation of a single batch of samples.

Response:

EPA partially agrees with the commenters and removed the note after Section 9.5.6 that suggested multiple MBs or RBs may be needed for "commonly observed laboratory contaminants… or for applications in which very low levels (i.e., at or near the LLOQ) are of interest." The wording in Section 9.5.4 was retained, which recommends preparation of an additional MB when supplies are changed during preparation of a batch of samples. EPA notes that MB and RB results can both be applicable to and relevant for analysis of field samples, and laboratories or data reviewers may have to consider results from more than one blank during data evaluation. EPA did not include guidelines for interpreting sample results associated with multiple blanks in Method 8327 or in Method 8000D but generally agrees with Commenter 8 that using the highest blank concentration is preferable. More detailed procedures for evaluation of blank contamination than are provided in the method should be documented in a laboratory's Standard Operating Procedure or Quality Management Plan or in project planning documents. EPA did not revise Section 9.0 to recommend including an additional LCS when the lot of a supply is changed while preparing a batch of samples as this is inconsistent with other SW-846 methods but agrees it is a good practice. Each laboratory using this method is required to establish and periodically verify LLOQs at which level acceptance criteria for all categories of quality controls in Section 9.0 can routinely be met using the instrumentation, equipment, reagents, consumables and personnel at that laboratory. EPA considers this requirement to be sufficient to address the concern Commenter 8 identified regarding when MB acceptance criteria are not achievable.

88. Comment:

Commenter 9 remarked that Sections 9.5.7 and 9.6.4 were not clear regarding whether surrogates are included in reagent blanks (RB) and suggested surrogates should be added to monitor instrument performance.

Response:

EPA revised Section 9.5.7 to state that surrogates are optional for RBs. However, EPA notes that the purpose of RBs is to assess sources of PFAS contamination, not to evaluate performance of surrogates.

89. Comment:

Commenter 10 noted that Matrix Spike/Matrix Spike Duplicate (MS/MSD) or MS/duplicate quality control samples (Section 9.6) only monitor the effect of the sample matrix on that one sample and stated that the labeled compounds monitor matrix effects in each sample.

Response:

EPA agrees with the commenter but notes that MS/MSD or MS/duplicate quality control samples are required in most SW-846 methods as long as sufficient amounts of samples are made available to the laboratory, and all methods have the same limitation: matrix effects and other sources of variability are only monitored in an individual sample and only for the target analytes that are present or are added. MS/MSD and MS/duplicate QC samples can also provide additional information about measurement precision integrated across sample collection and sample processing steps. EPA agrees that surrogates are spiked into chromatographic methods to monitor matrix effects in each sample; however, isotopically labeled analogs of all target analytes were not available when the method was validated, and some target analytes may not perform the same as structurally dissimilar surrogates. Matrix spikes are still considered to be a valuable tool.

90. Comment:

Commenter 4 remarked that Section 9.6.1 does not provide guidance on matrix spike concentrations and noted that spiking levels that are too high or too low may be less relevant or may lead to difficulties interpreting data. The commenter recommended using matrix spike concentrations “between 0.5X to 10X the endogenous level for a meaningful assessment of matrix spike recovery”.

Response:

EPA revised Section 9.6 to provide guidance on matrix spike and LCS concentrations (“near the middle of the calibration range, when appropriate”) and notes that appropriate matrix spike and LCS concentrations may also be a project-specific decision. Section 9.4.2 of Method 8000D recommends matrix spike concentrations at the action level or at one to five times the background sample concentration (if known), whichever is higher. In the absence of project-specific requirements, please refer to Sections 7.4 and 9.6 in Method 8327 for guidance on concentration levels for LCS and MS/MSD QC samples.

91. Comment:

Commenter 4 considered the minimum frequency of one Matrix Spike/Matrix Spike Duplicate (MS/MSD) or MS/duplicate sample pair per batch of 20 or fewer samples in Section 9.6.1.1 to be insufficient. The commenter stated that their laboratory runs a matrix spike duplicate for each sample and reduces the frequency to 1 per 5 samples for sampling locations that have been

tested before. The commenter suggested increasing the frequency of matrix spikes to 1 in 5 samples “[g]iven the relatively poor performance of the method.”

Response:

EPA did not revise the minimum matrix spike frequency in Section 9.6.1.1 based on this comment. Changing the MS/MSD frequency would create an inconsistency with Method 8000D and other SW-846 methods that specify the same minimum frequency for MS/MSD, LCS, and method blank sample preparation quality controls. Laboratories and project managers are free to increase the frequency for these quality controls as needed to meet their goals. Please see EPA’s response to comment 1 for more information regarding EPA’s evaluation of method precision, bias, and sensitivity.

92. Comment:

Commenter 8 noted that the acronym MS is defined in the glossary as mass spectrometer, but Section 9.6 uses MS to refer to matrix spike. The commenter requested that EPA revise the method to include a different acronym for mass spectrometer.

Response:

EPA agrees with the commenter and revised the glossary in Method 8327 to define MS as Matrix Spike and to define MS/MS as tandem mass spectrometry.

93. Comment:

Commenter 5 noted that Section 9.6.1.1 discusses including Matrix Spike/Matrix Spike Duplicate (MS/MSD) quality control samples "when required" but provides no explanation regarding when an MS/MSD is or is not required.

Response:

EPA agrees with the commenter and revised Section 9.6.1.1 to remove "when required". Generally, ‘required’ referred to project requirements, but the method specifies preparation of MS/MSD or MS/duplicate quality control samples as long as sufficient sample amounts are made available to the laboratory.

94. Comment:

Commenter 9 noted that Section 9.6.1.1 refers to Section 11.1.5, but this section does not exist.

Response:

EPA agrees with the commenter and revised Section 9.6.1.1 to remove reference to Section 11.1.5.

95. Comment:

Commenter 4 noted that "Section 9.6.1.2 addresses the statistical approach used for calculating the LMS recoveries. Section 1.3.1 indicates that the background was calculated for the target analytes before calculating the recoveries for the LMS samples. The mean background concentration was then subtracted from each measured concentration to give a modified concentration. It is not clear if the background concertation was subtracted regardless of whether or not the measured concentration of the background sample (identified as the Blank Sample in Tables A-1 through A-24) was below the laboratory's LLOQ." (Note: EPA assumes the commenter used the acronym "LMS" interchangeably with "Matrix Spike" or "Spiked study sample" and that Section 1.3.1 refers to the Statistical Summary Report). The commenter further noted that Section 9.6.1.2 does not provide guidance on how to calculate matrix spike recovery when the measured concentration in the un-spiked sample is below the established Lower Limit of Quantitation (LLOQ). The commenter did not consider it to be appropriate to subtract the un-spiked sample concentration if the measured concentration was below the LLOQ.

Response:

EPA made no changes to Section 9.6.1.2 based on this comment. SW-846 Method 8000D does not provide guidance regarding whether to subtract unspiked sample concentrations below the LLOQ for determination of matrix spike recovery. EPA notes that the difference in % recovery should be negligible when the matrix spike concentration is high relative to the LLOQ. If matrix spike concentrations selected for a specific project are relatively near a laboratory's LLOQ, the issue may be best addressed in project planning documents. EPA subtracted the average concentration of a target analyte in replicate unspiked samples of the same matrix type reported by an individual laboratory from each replicate spiked sample concentration for that matrix reported by the laboratory prior to calculating % recovery without considering whether the average concentration in the unspiked matrix type was above or below the Lower Limit of Quantitation (LLOQ).

96. Comment:

Commenter 4 noted that Section 9.6.2 does not recommend a concentration for LCS quality control samples and recommended preparing LCS samples "at a minimum of 5x the low-LOQ and 80% of the high-LOQ, each level in triplicate". The commenter noted that this approach would "provide for good coverage of LCS levels relative to sample levels and provide for statistical evaluation of accuracy and precision of LCS results."

Response:

EPA revised Section 9.6.2 to recommend that LCS samples are spiked at the same concentration as any Matrix Spike/Matrix Spike Duplicate (MS/MSD) quality control samples and at a concentration near the middle of the calibration range unless otherwise specified. SW-846 methods specify preparation of a minimum of one LCS per batch of 20 or fewer samples. The laboratory is free to include more LCS samples, but the change recommended by the commenter is not consistent with other SW-846 methods or methods published by other EPA programs (e.g., 537.1.).

97. Comment:

Commenter 8 requested that EPA include the limitation noted in section 9.6.2 ("Statistically-derived acceptance limits or project defined acceptance limits may be necessary for some targets, including PFTriA, PFBA, and 6:2 FTS, as 70-130% default limits may be too narrow") in Section 1.0 as well. The commenter also suggested that EPA revise Section 9.6.2 to include performance information for these analytes to help laboratories determine if they have optimized implementation of this method. The commenter also noted that the acronym PFTriA is not listed in the method and presumed EPA intended to refer to PFTrDA. The commenter requested that EPA review the method to ensure the acronyms used are consistent.

Response:

EPA disagrees with the commenter's statement that statistically-derived acceptance limits constitute a limitation of the method and also disagrees with the suggestion to add related information to Section 1.0. EPA revised Section 9.6 to recommend the use of statistically based acceptance limits for recovery of all surrogates in field samples and of all target analytes in Lower Limit of Quantitation (LLOQ) verification, LCS, and Matrix Spike/Matrix Spike Duplicate (MS/MSD) quality control samples once the laboratory has generated sufficient data, consistent with Method 8000D. Section 9.6.1.2 was also revised to indicate that project-defined acceptance limits are preferred for evaluation of MS/MSD performance, which is also consistent with Method 8000D and other SW-846 methods. Please refer to Method 8000D for guidance regarding the use of performance data in reference methods for evaluation of Initial Demonstration of Proficiency data that might help a laboratory determine when their implementation of a method has been optimized. EPA addressed the noted discrepancy in acronyms in the final versions of Methods 3512 and 8327. Please refer to EPA's response to comment 18 for more information about the acronyms that are used.

98. Comment:

Commenter 9 noted that statements included in Sections 9.6.2 and 9.6.4 regarding using statistically based acceptance limits for several target analytes in LCS quality control samples and for some surrogates in field samples were related to performance using Method 3512 for

sample preparation. The commenter asked whether these sections should be edited so Method 8327 was more clearly independent of the preparation method.

Response:

EPA agrees with the commenter that these statements were specific to Method 3512, and the sections were revised to remove this information. Please refer to EPA's response to comment 97 for more detail regarding revisions to acceptance criteria in these sections.

99. Comment:

Several commenters (6, 9 and 11) requested that EPA revise wording in Section 9.6.2 that combined fixed and statistically based recovery limits for target analytes in LCS samples. Commenter 11 stated that there was no published data to demonstrate that the acceptance criteria are routinely achievable and requested that EPA "allow statistically-derived acceptance limits or project defined acceptance limits for all PFAS targets". Commenter 9 did not support including statistically based acceptance limits for LCS recovery and asserted that there is little difference between an LCS and a Continuing Calibration Verification (CCV) standard for this method. Commenter 6 did not consider the statement that statistically based recovery limits "may be necessary" to be supported by the data from the validation study.

Response:

EPA revised Section 9.6 to recommend the use of statistically derived or project defined acceptance limits for recovery of all target analytes in Matrix Spike/Matrix Spike Duplicate (MS/MSD) and LCS samples, consistent with Method 8000D. Please refer to Tables 6a and 6b in the QC Summary Report, which contain summaries of LCS recovery data across all laboratories and by laboratory, respectively.

100. Comment:

Commenter 5 considered the default Relative Percent Difference (RPD) limit of ≤30% to be reasonable for aqueous samples but too stringent for soil samples. This commenter expects that "most soil MS/MSD samples will fail the 30% RPD, requiring flagging of data and/or resampling of a site" and considered this limit to be unnecessarily stringent.

Response:

EPA revised Section 9.6.1.3 to specify that RPD limits can be statistically based or project-defined, as for matrix spike and LCS recovery. This approach is also consistent with Method 8000D. EPA also removed references to solid matrices in Method 8327. Please note that ≤30% RPD is a guideline and is preliminary. EPA will consider addressing precision for testing of PFAS target analytes in soil and other solid samples in a future revision of this method or in a new method.

101. Comment:

Commenter 9 noted that allowed variations in sample volumes will lead to errors when concentration is used as a basis to determine Relative Percent Difference (RPD) between Matrix Spike and Matrix Spike Duplicate (MS/MSD) QC samples in Section 9.6.3. The commenter noted that Section B11.2.1 allows for sample volume to vary between 4.75-5.25 mL without adjusting the volume of methanol added. The commenter also noted that Section 7.4.2 does not mention scaling the amounts of standards added when the sample volume differs from the nominal (expected) volume. The commenter recommends using % recovery as a basis for RPD because % recovery is corrected for sample volume differences and is more directly comparable than calculated concentrations in MS/MSD samples.

Response:

EPA partially agrees with the commenter and removed the ±5% tolerance for volume from Section 11.0 of Method 3512 but retained the use of concentration as a basis for calculating RPD in Section 9.6.1.3 of Method 8327 rather than % recovery, consistent with Section 9.4 of Method 8000D. EPA recognizes that using % recovery as a basis for estimating RPD can provide some advantages, but it can also lead to interpretation problems when the concentrations of target analytes in the sample are high compared to the equivalent matrix spike concentration based on the amount of standard added even when the concentrations are similar. Using concentration as a basis for RPD allows the same approach to be used for evaluation of measurement precision in sample/duplicate and matrix spike/matrix spike duplicate QC samples. EPA added a note after Section 9.6.1.3 that states using approximately the same sample size or scaling the spike amount to the sample size will minimize bias in the determination of RPD, which should address the circumstance the commenter described (i.e., when the actual volumes of containers are significantly different). This note is also included in Section 9.4.3 of Method 8000D.

102. Comment:

Three commenters (14, 18 and 23) considered the default acceptance criterion of 70-130% for surrogate recovery in Section 9.6.4 to be too narrow to be useful and recommended widening the range to 50-150%. Commenters 14 and 18 recommended including the option of using statistically based recovery limits to accommodate more complex matrices such as wastewater.

Response:

EPA partially agrees with the commenters and revised Section 9.6.3 to recommend using statistically based recovery limits for all surrogates once laboratories have acquired sufficient data, consistent with Method 8000D. Preliminary surrogate recovery limits of 70-130% were used as guidelines for the validation study and were not intended to be absolute requirements. Laboratories are encouraged to develop their own statistically based acceptance limits, and project-specific requirements should be considered a primary source for acceptance criteria.

103. Comment:

Commenter 8 noted that Section 9.6.4 identified a high percentage (8 of 19) of surrogates as potentially needing wider limits than 70-130% recovery, which the commenter interpreted to mean that 70-130% recovery limits did not "reflect the accuracy of the method." The commenter recommended repeating the statement in Section 9.6.4 about 70-130% recovery limits potentially being too narrow for some surrogates in Section 1.0, along with other limitations of the method. The commenter further recommended including more information in Section 9.6.4 about a typical range for recovery of these surrogates to aid laboratories in determining whether their implementation of the method had been properly optimized.

Response:

EPA did not make any changes to the method based on this comment, but EPA did revise Section 9.6.3 to recommend the use of statistically based or project defined acceptance limits for surrogate recovery, consistent with Method 8000D for this and other categories of sample preparation quality controls. Please refer to EPA's response to comments 77 and 97 for more information.

104. Comment:

Commenter 9 recommended revising Section 9.6.4 to use fixed acceptance limits for surrogate recovery and to require corrective action when recovery limits are not met, which the commenter stated would standardize performance between laboratories and improve comparability of data.

Response:

EPA did not make changes to the method based on this comment, but EPA did revise Section 9.6.3 to recommend the use of statistically based or project defined acceptance limits for surrogate recovery, consistent with Method 8000D for this and other categories of sample preparation quality controls. Please refer to EPA's response to comments 77 and 97 for more information. Required corrective actions should be documented in a laboratory's Standard Operating Procedure or Quality Management Plan or in project planning documents and are beyond the scope of this method.

105. Comment:

Commenter 6 did not consider the statement in Section 9.6.4 that statistically based recovery limits for some surrogates "may be necessary" to be clearly supported by the data from the validation study. The commenter requested that EPA remove wording in Section 9.6.4 related to using statistically based recovery limits for surrogates.

Response:

EPA revised Section 9.6.3 to recommend the use of statistically based or project defined acceptance limits for recovery of surrogates, consistent with Method 8000D for this and other categories of sample preparation quality controls. Please refer to Tables 7a-d in the QC Summary Report, which contain summaries of surrogate recovery data in study samples or in quality control samples in individual laboratories and across laboratories. Please refer to EPA's response to comments 77 and 97 for more information.

106. Comment:

Commenter 2 noted that Method 8327 is performance based and recommended allowing laboratories to establish Lower Limits of Quantitation (LLOQs) at a lower level than specified in the method as long as the laboratory demonstrates it can meet performance requirements, which the commenter considered to be dependent on instrument sensitivity and method blank and reagent blank concentrations.

Response:

Method 8000D defines the LLOQ as the lowest concentration at which quantitative and qualitative requirements are consistently and reliably met and is ≥ the lowest calibration point. Each laboratory is responsible for establishing and periodically verifying LLOQs using instrumentation, equipment, supplies, reagents and personnel specific to that laboratory. Laboratories are free to establish the LLOQ at any level that meets their and their clients' needs as long as the laboratory demonstrates that is can consistently and reliably meet the acceptance criteria for all categories of quality controls in Section 9. See Section 9.9 of Method 8327 for more information.

107. Comment:

Several commenters (5, 9, 11, and 20) remarked about a discrepancy they perceived between the frequency for Lower Limit of Quantitation (LLOQ) verifications in Section 9.9, which states they are required annually, and Section 9.9.1.4, which states they are recommended to be included with every batch of 20 or fewer field samples. Commenter 9 also noted that Section 9.9.1.4 recommends qualifying sample results if the LLOQ verification does not meet the acceptance criteria, which the commenter stated is inconsistent with a "recommended" frequency. Commenters 8, 11 and 20 also noted that Table 7 specifies a minimum frequency for LLOQ verifications of one per preparation batch of 20 or fewer field samples, which they also considered to be inconsistent with the "annual" required frequency in Section 9.9. The commenters requested that EPA clarify the frequency for LLOQ verification. Commenter 5 further recommended revision of the method to require an LLOQ verification QC sample to be included in each sample preparation batch, and commenter 9 recommended analysis of LLOQ verifications at the beginning and end of each analysis sequence.

Response:

EPA agrees with these commenters regarding inconsistent wording related to LLOQ verification frequency and revised Sections 9.6, 9.9 and Table 6 accordingly. However, the minimum required annual frequency and recommended frequency per batch of 20 or fewer samples, as needed for the project, is consistent with Method 8000D and is unchanged. EPA revised Section 9.6 to include LLOQ verifications with other types of sample preparation quality controls, but on an "as needed" basis. EPA revised Table 6 to provide different required and recommended frequencies, consistent with Section 9.9. EPA also revised Section 9.9.4 to remove the recommendation to qualify data under certain circumstances when LLOQ verifications do not meet the acceptance criteria because this quality control type is not required to be included. Project-specific LLOQ verification requirements should be identified in project planning documents and communicated to the laboratory. Interpretation of sample results based on LLOQ verification performance is also not defined in the method, as it may depend on project-specific Data Quality Objectives.

108. Comment:

Commenter 8 noted that Section 9.0 does not require analysis of a calibration standard at or below the limit of quantitation on a continuing basis after initial calibration (i.e., as a low level Continuing Calibration Verification standard) like EPA Method 537.1. The commenter considered this check to be important because Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) system sensitivity can be affected by matrix interferences that can be present in the types of samples for which the method is intended to be used. Commenter 8 considered the annual check described in Section 9.9 to be insufficient to ensure acceptable sensitivity is maintained on a daily basis. Commenter 8 noted that Method 537.1 requires daily analysis of a calibration standard at or below the Minimum Reporting Level (MRL) at the beginning of the analysis sequence and requested that EPA modify the method to include this same requirement.

Response:

EPA did not make the commenter's requested change to the method. Method 8327 recommends preparation and analysis of Lower Limit of Quantitation (LLOQ) verification quality control samples "as needed" on a project-specific basis to evaluate method performance near the LLOQ, consistent with Method 8000D. LLOQ verifications incorporate all sample preparation and analysis steps. The laboratory is free to include Continuing Calibration Verification (CCV) standards at the LLOQ in their analysis sequences, and this modification may be useful for evaluation of sample results when action levels are near the established LLOQ; however, requiring analysis of CCV standards at the LLOQ would be inconsistent with other SW-846 methods and may not be useful or necessary depending on the concentration levels of interest for the project.

109. Comment:

Commenter 8 interpreted the note after Section 9.9 identifying target analytes for which "LLOQ verifications at higher concentration may be needed" to mean that the 50-150% recovery limits would be difficult to meet at a quantitation level of 10 ng/L for 8 of 24 target analytes. The commenter suggested that EPA state this as a limitation in Section 1.0 along with an "observed acceptance range" for these compounds in LLOQ verifications. The commenter also noted that the suggested LLOQs in Table 1 did not include higher LLOQs for all of the target analytes listed in Section 9.9. The commenter concluded that "With only 10 out of 24 target compounds achieving the 10 ppt quantitation level, this method should not be endorsed as a method that, in general, is capable of achieving a 10 ppt quantitation level for target compounds."

Response:

EPA removed the note after Section 9.9 in part to address this commenter's and other commenters' perception that these compounds performed poorly during method validation; that was not EPA's intent. EPA included Table 5b in the QC Summary Report to provide a more detailed summary of performance of target analytes in LLOQ verifications in each laboratory for the validation study. The lowest evaluated LLOQ in the validation study was 10 ng/L, and Table 5b shows that the upper 95% confidence interval of the median verified LLOQ across laboratories was 10-20 ng/L for 22 of the 24 target analytes that were evaluated. This table and other tables are included as 'additional information' linked to the Method 8327 web page. EPA also removed Table 1, "Suggested LLOQs," from Method 8327 because SW-846 methods generally do not include this information. As described in Section 9.9, each laboratory is required to establish and periodically verify LLOQs at levels at which the QC criteria in Section 9.0 can routinely be met using equipment, reagents, supplies, and instrumentation specific to their laboratory. With technological improvements in instrumentation and appropriately clean supplies, EPA expects that laboratories' ability to achieve LLOQs ≤10 ng/L with these methods will likely become increasingly common.

110. Comment:

Commenter 9 noted that Section 9.9 refers to Section 11.6.4, which does not exist.

Response:

EPA agrees with the commenter and revised Section 9.9 to refer to Section 11.6.

111. Comment:

Commenter 9 noted that Section 9.9 specifies that the calibration standard at the LLOQ should have a calculated signal to noise ratio (S/N) ≥3, but calculation of S/N is not defined in Sections 9.9 or 11.3.2, nor is it included in Section 11.6 as a criterion for qualitative identification of a target analyte. Commenter 9 also noted that S/N is not included with other quality control acceptance criteria in Table 7. Commenter 9 requested that EPA include more specificity about

how S/N is to be calculated, reported, and used to support qualitative identification of target analytes in the method.

Response:

EPA did not make the requested changes related to calculation of S/N or inclusion as a criterion to support qualitative identification. Method 8327 purposely does not define how to calculate S/N because the calculation may be software dependent and not apparent to the user, and all software packages may not have the same configuration options. EPA did revise the recommended minimum S/N ratio for the primary product ion in the LLOQ-level calibration standard to be ≥10 and for secondary product ions to be ≥3 and made clear that it is not an absolute requirement (listed as a 'should'). Refer to the note after Section 11.3.2 in Method 8327 for more detail. S/N may be useful for evaluation of qualitative identification, but SW-846 reference methods generally do not include a minimum S/N criterion. Professional judgment can be an important part of qualitative identification, and all available information should be considered, including but not limited to the qualitative identification criteria in Section 11.6.

112. Comment:

Commenter 9 remarked that the statement in Section 9.9 that "The LLOQ should be less than the desired decision level" is not a practical consideration for commercial laboratories and recommended deleting it. The commenter further noted that the LLOQ should be set at a concentration at or below the lowest calibration point that meets the specified qualitative and quantitative requirements.

Response:

EPA agrees with the commenter's statement related to how a laboratory should establish LLOQs but disagrees with the recommendation to remove 'desired decision level' from Section 9.9. Project specified action levels or decision levels are important considerations during project planning and important context for evaluating whether data are of sufficient quality for the intended application. Project planning documents such as QAPPs or SAPs should contain decision or action levels for a specific project, and project managers may need to consult with laboratories to determine whether LLOQs are sufficient to meet the needs of a given project. This is common language throughout SW-846 methods and is also addressed in Chapter 1.

113. Comment:

Commenter 9 considered the statement in the note after Section 9.9 to be unclear regarding the recommendation to include Lower Limit of Quantitation (LLOQ) verifications at higher concentrations for target analytes with no or low relative abundance secondary product ions (e.g., PFBA, PFPeA, PFHxA). The commenter noted that the explanation provided was to "provide higher confidence in confirmation" but considered the statement to only be clearly applicable to a target analyte with a low relative abundance secondary product ion. The commenter recommended that EPA revise this section to clarify the applicability of this statement. The commenter also noted that the statement in Section 9.9 was unclear regarding

recommendations to verify LLOQs at higher concentrations for compounds with “higher variability, lower response and/or high background” and noted that commercial laboratories commonly use the low initial calibration standard concentration as their basis for LLOQs.

Response:

EPA agrees with the commenter that the note after Section 9.9 was unclear and removed recommendations to verify LLOQs for specific target analytes at higher concentrations. Please refer to EPA’s responses to comments 24 and 27, which address similar considerations in Section 1.3. Method 8327 specifies using the same approach for establishing LLOQs for all target analytes; Section 9.9 states “The laboratory shall establish LLOQs at concentrations where both quantitative and qualitative requirements can consistently be met.” EPA expects that there will be some variation in LLOQs between laboratories due to differences in instrumentation, equipment, reagents and supplies. Secondary product ions are simply not available to support qualitative identification of selected target analytes, and this issue is expected to be common to any current test method that is based on negative electrospray ionization tandem mass spectrometry.

114. Comment:

Several commenters (5, 8, 9 and 22) considered acceptance limits of 50-150% recovery for Lower Limit of Quantitation (LLOQ) verification in Section 9.9 to be too wide to adequately control for measurement bias at this level. These commenters also did not consider statistically based acceptance limits to be appropriate for LLOQ verification; Commenter 5 stated that “control charting of bad data would allow the reporting of inaccurate results”, and Commenter 9 stated that statistically based limits would lead to “detection” being used as the criterion for LLOQ verification in commercial laboratories. Commenter 5 recommended narrowing the acceptance criteria to 70-130% and suggested that sample results should be qualified when the LLOQ verification acceptance criteria are not met. Commenter 8 considered 50-150% recovery limits proposed for Lower Limit of Quantitation (LLOQ) verifications to be inappropriate because of a perceived allowance for “a sliding scale of uncertainty associated with concentrations within the quantitation range”. Commenters 5, 9 and 22 further stated that LLOQ verification quality control samples should be required to be prepared and analyzed with samples.

Response:

EPA disagrees with the commenters’ assertions that a preliminary acceptance range of 50-150% is too wide for LLOQ verification for general purposes and made no changes to Section 9.9 based on these comments. Methods 8000D, 8260D and 8270E also recommend the use of 50-150% for preliminary acceptance limits for recovery of target analytes in LLOQ verification QC samples, and they recommend application of statistically based acceptance limits once the laboratory has acquired sufficient data. Laboratories and project managers are free to specify alternative/tighter LLOQ verification acceptance limits as appropriate to meet the needs of the project. EPA agrees with commenter 5 that it is important to identify data usability concerns for compounds that do not meet established performance criteria, including for LLOQ verification, but how this issue is managed is outside the scope of this method because an LLOQ verification QC sample is not required to be prepared and analyzed with every set of samples. How non-conforming LLOQ verifications are managed should be addressed in a standard operating

procedure, quality management plan, or project planning document. Please refer to EPA's response to comment 107 for more information related to 'required' and 'recommended' use of LLOQ verifications.

115. Comment:

Commenter 9 did not agree with the allowance in Section 9.9.1.2 for Lower Limit of Quantitation (LLOQ) verifications to be prepared at a concentration up to two times the nominal LLOQ. The commenter did not consider the rationale to be clear for this allowance and requested that the method be revised to limit LLOQ verification concentrations to be equivalent to or below the LLOQ.

Response:

EPA did not make changes to the method based on this comment, as this wording is common to many SW-846 methods and is taken from Method 8000D. A laboratory may establish LLOQs at different concentrations for different target analytes. Providing a range for the LLOQ verification concentrations of 0.5-2 times the expected LLOQ may reduce the number of QC samples a laboratory needs to analyze. The laboratory is free to perform LLOQ verifications using a tighter concentration range.

116. Comment:

Two commenters (5 and 8) considered the statement in Section 9.9.1.4 to be inaccurate regarding conditions under which compounds could be reliably reported as non-detects when LLOQ verifications fail low. Commenter 5 stated that the described approach should not be used because actual concentrations in the samples are likely to be higher than the measured concentrations and did not consider "raising the LLOQ" to be an acceptable corrective action. Commenter 8 considered the purpose of LLOQ verification to be to demonstrate that the reported LLOQ is appropriate and concluded that, if the acceptance criteria are not met, then the reported LLOQ is not appropriate. Commenter 8 requested that this sentence be deleted.

Response:

EPA removed this statement from Section 9.9.4 of the method because LLOQ verifications are not required to be included in every sample preparation batch, therefore it is not necessary and perhaps would be confusing for the method to include guidelines for qualification of data based on performance of LLOQ verifications. Section 9.9 refers to Method 8000D for guidance regarding LLOQ verification, and Section 9.0 also refers to method 8000D for guidance on evaluation and reporting of sample results when QC acceptance criteria are not met. Defining corrective actions or data qualifiers for each category of quality control when the acceptance criteria are not met is beyond the scope of this method. The laboratory or data user should define how to handle QC failures in their SOP, QMP, or project planning documents. EPA expects LLOQ verifications will produce periodic QC failures, as will other types of quality controls. For a particular batch of samples, raising the LLOQ to a level that can be confidently reported as not found is reasonable if that LLOQ is sufficient for the project application, even if it

is the LCS level. If the failure is frequent and/or not random, then the laboratory should establish the LLOQ at a concentration at which the acceptance criteria can routinely be met. Project planning documents such as QAPP or SAP should define quantity and quality of data needed to achieve DQOs, and QC failures at a given LLOQ for a given analyte may not be critical for establishing data usability.

117. Comment:

Commenter 9 noted that Section 9.9.1.5 states, “Analytes below the LLOQ that are reported should meet most or all of the qualitative identification criteria in Section 11.6.4.” The commenter asked what qualitative identification criteria EPA considered to be most important since this section does not require all criteria to be met. The commenter also considered reporting of target analyte concentrations to be inappropriate when all qualitative identification criteria are not met and recommended removing “most or” from this sentence.

Response:

EPA revised this section to be consistent with the commenter’s request but notes that the specification here is a recommendation (i.e., “should”). EPA does not expect that all qualitative identification criteria will necessarily be met at concentrations below the LLOQ; professional judgment may be important, and the need for careful evaluation of qualitative identification may depend on the intended use of the data. In general, meeting the RT criterion is probably the most important qualitative factor. Ion ratios are also important for evaluation of qualitative identification, but some target analytes do not produce a secondary product ion, and other target analytes with low relative abundance secondary product ions may not meet the acceptance criterion at concentrations <LLOQ.

118. Comment:

Commenter 5 requested that EPA revise Section 9.10 to include more specifics regarding additional Quality Assurance (QA) practices instead of leaving it to the laboratory. For example, the commenter recommended including a required frequency for participating in performance evaluation studies.

Response:

EPA did not make changes to this section based on this comment because the wording in Section 9.10 is common to many SW-846 methods. It is beyond the scope of the method to define a complete quality management system that may include the analysis of certified reference materials and participation in performance evaluation studies. The laboratory is encouraged to include additional QA elements beyond the minimum requirements of this method and document them in the laboratory's SOP or QMP. For more information about quality systems please refer to Method 8000D and Chapter 1 in SW-846, to EPA guidance and requirements for Quality Management Plans, and to consensus standards for quality systems for testing laboratories such as ISO17025:2017.

SECTION 11.0: PROCEDURE

119. Comment:

Two commenters (9 and 12) recommend including more preparation or extraction methods in Section 11.1 (e.g., soil/sediment, biota, and landfill leachate).

Response:

EPA agrees with the commenters and recognizes the need for additional preparation methods and/or performance data for other matrices. At the time of this writing EPA is evaluating extraction methods for solids, and EPA will consider including landfill leachate in a future revision of this method. Please refer to EPA's response to comment 4 for more information.

120. Comment:

Commenters 8 and 22 requested that EPA revise Section 11.3.1 to require the instrument manufacturer's mass spectrometer performance criteria to be met. Commenter 8 noted that the instrument manufacturer's performance criteria may not include evaluation of low masses and requested that this section specify evaluation across the entire mass range of interest for both precursor and product ions. Commenter 22 additionally recommended including requirements for instrument setup from Method 537.

Response:

EPA disagrees that additional specification is necessary in Section 11.3.1 regarding evaluation of mass spectrometer optimization. This section does not indicate that evaluation of mass spectrometer performance is optional. The wording in this section is not intended to require changes to instrument manufacturers' performance specifications to address low m/z values but to rely on their performance specifications to ensure the system is in good general working order. LC/MS instrument manufacturers have optimization procedures and performance specifications for mass spectrometer settings that are compound-independent, and they are the focus of the wording in this section. In addition, the analyst optimizes various MS settings for the chemicals being analyzed. An example of these categories of settings for a specific instrument is provided in Table 4 of Method 8327. Instrument settings are optimized to establish accurate mass assignments for all product ions, including for lower m/z values.

121. Comment:

Four commenters (8, 9, 21, and 22) remarked that the note after Section 11.3.2 states that sulfonate salts are typically corrected to the concentration as an anion. Commenters 8 and 9 stated that correcting to the neutral acid concentration would be more appropriate. Commenters 21 and 22 recommended including an equation for correction. Additionally, commenters 9 and

21 recommended revising the method to require conversion of these sulfonate salts to the acid concentrations for reporting purposes.

Response:

The EPA partially agrees with these commenters. The note from Section 11.3.2 was revised and moved to Section 7.4.2, and reporting results as the free acid concentrations is not listed as optional. However, an equation for conversion of the salt form to the free acid form was not included. The supplier of the standards for the Method 8327 validation study provided a certified concentration of each target analyte as a salt and as an anion, and an example is provided in the method.

122. Comment:

Commenter 4 requested revision of Section 11.3.2 to allow the use of isotope dilution calibration, which would enable the method to correct for sampling losses and interferences.

Response:

Please refer to EPA's response to comment 10.

123. Comment:

A number of commenters (5, 8, 9, 13, 19 and 21) requested that EPA revise Section 11.3.3 and the note thereafter to clearly specify how to establish chromatographic retention times of branched isomers. Commenters 9, 13, 19 and 21 stated that the use of a qualitative standard for identification of branched isomers should be required if a quantitative standard is not available, consistent with EPA Method 537.1. Commenters 13 and 19 further stated that prescribing the same calibration approach as in Method 537.1 would reduce variability between methods and laboratories. Commenter 9 noted that Method 537.1 and DoD's Quality Systems Manual Table B-15 state that branched isomers should not be reported if no standard is available to identify them. Commenter 8 requested that the method also include guidance for establishing retention times of branched isomers when a quantitative or qualitative standard is unavailable. Commenter 9 also requested that linear and branched isomers be reported separately to provide more information about their proportions in samples and to allow for "appropriate qualification" when a quantitative standard containing branched isomers is unavailable.

Response:

EPA revised the wording in this section to clearly allow the same approach specified in Methods 533 and 537.1 to be used in Method 8327 for calibration of PFAS target analytes composed of linear and branched isomers. The method retains the flexibility to allow alternative approaches to calibration, which could accommodate separate reporting of linear and branched isomers, as long as the following requirements are met:

- A qualitative or quantitative standard must be used to identify chromatographic retention times for branched and linear isomers that will be quantified;
- A quantitative standard must be used for calibration; and,
- The data must be reported such that calibration and quantitation choices are clear to the end data user.

EPA could not provide guidelines for establishing retention times of branched isomers when a quantitative or qualitative standard is unavailable, so this information was not included. Please refer to EPA's response to comment 3 for references to other changes made to the method to accommodate PFAS chemicals composed of linear and branched isomers.

124. Comment:

Commenter 5 considered the information provided in the note after Section 11.3.3 related to summing linear and branched isomers to conflict with Method 537.1, which the commenter interpreted as requiring quantitation of individual isomers where standards are available. The commenter was concerned that summing isomer concentrations is inconsistent with Method 537.1 and with common practice in commercial laboratories, and that this approach would "create data comparability and trending anomalies."

Response:

EPA disagrees with the commenter and notes that Method 537.1 specifies quantitation of linear and branched isomers as a single analyte. Please refer to EPA's response to comment 123 for related discussion.

125. Comment:

Commenter 9 remarked that the note after Section 11.3.3 indicates all branched isomers are to be integrated but pointed out that integrations in Figures 1 and 2 for PFOS did not include the low relative abundance branched isomers at ~8.22 minutes. The commenter stated that some commercially available standards containing mixtures of linear and branched isomers of PFOS have low relative concentrations of the dimethyl PFOS isomers and noted that they can be found at higher relative concentrations in samples from some sources. The commenter was concerned that laboratories may not consistently include integration of these peaks in standards even though they are present due to their low relative concentrations. The commenter recommended increasing the relative concentrations of these dimethyl isomers in mixed standards or using standards of individual isomers to help laboratories consistently identify, integrate and quantitate these peaks in samples, which the commenter suggested would improve consistency in data reporting across laboratories.

Response:

Addressing the proportions of structural isomers in commercially available standards is outside of the scope of the method and may be more appropriate to raise with standard manufacturers. At the time of this writing the availability of certified reference materials for individual structural isomers of PFAS target analytes is limited, so EPA cannot provide more specific recommendations for establishing retention times for branched isomers. EPA expects the reference materials for individual PFAS isomers will become more commonly available over time. Please note that EPA replaced Figures 1-4 with a single figure that shows elution patterns of branched and linear PFOS isomers in a standard and in a sample.

126. Comment:

Commenter 9 noted that Section 11.3.3 specifies the use of standards containing branched and linear isomers for calibration of some target analytes but without chromatographic resolution criteria for structural isomers. The commenter expressed concern that chromatographic separation of structural isomers is inconsistent across laboratories and noted inconsistencies in results for specific target analytes from split samples analyzed by different laboratories that they attributed to differences in chromatographic separation. The commenter requested that EPA revise the method to include chromatographic resolution criteria. The commenter also considered provision of standards to participating laboratories to have eliminated a potentially important source of variability in analysis of PFAS target analytes composed of multiple isomers.

Response:

Please refer to EPA's response to comments 3 and 123 for discussion regarding calibration, identification and quantitation of linear and branched isomers. Please refer to EPA's response to comment 14 for discussion regarding provision of supplies for the validation study. Please refer to EPA's response to comment 46 for discussion regarding chromatographic resolution criteria.

127. Comment:

Commenter 9 requested that EPA revise the note after Section 11.3.3 to state that the product ions listed in Table 3 have only been optimized for the linear isomers even though they are also used for the branched isomers. The commenter noted that some branched isomers will not contain the listed transitions.

Response:

EPA partially disagrees with the commenter and notes that laboratories optimized mass spectrometer settings during infusion of mixed standards, so settings are not necessarily optimized only for the linear isomers. However, EPA agrees with the commenter that the specified secondary product ions may not be found for some structural isomers or may be at

different proportions relative to the primary product ions when compared to linear isomers. EPA did not include the information requested by the commenter in Section 11.3.3, but Section 11.6 includes information about differences in retention times and ion ratios that can result from different proportions of linear and branched isomers in samples and standards.

128. Comment:

Commenter 9 expressed a preference for reporting concentrations of individual isomers, where possible, or otherwise for branched and linear isomers to be reported separately. The commenter expressed concern that reporting summed linear and branched isomers under the Chemical Abstracts Service (CAS) number for the linear isomer would be misleading and result in more potential for quantitative error. The commenter further stated "Toxicologically, reporting the branched and linear compounds together renders toxicity assessment virtually meaningless, as the toxicity of the branched and linear compounds are uncertain." The commenter considered separate reporting of linear and branched isomers to be potentially more useful for assessment of exposure risk and for fate and transport. The commenter identified a need for further evaluation of elution patterns and ion ratios and for chromatographic resolution criteria for linear and branched isomers that could help support quantitative analysis of isomers. Commenter 9 further suggested that, if results for compounds with branched and linear isomers are quantified together, that they should be reported as "total" (e.g., "total PFOS").

Response:

No changes were made to Methods 3512 or 8327 based on these comments. The approach described in Method 8327 to quantify linear and branched isomers together and report as a single analyte is relatively straightforward for general use. Separate quantitation of branched and linear isomers is a reasonable alternative that could be used as needed. Please refer to EPA's response to comment 123 for related discussion. At the time of this writing commercial availability of certified reference materials for individual structural isomers is limited, and these materials would be needed to perform the additional evaluations or separate reporting of individual structural isomers that the commenter suggests.

129. Comment:

Commenter 22 did not support EPA's decision to include the requirement to quantitate both linear and branched isomers in a note after Section 11.3.3 and recommended moving it to a subsection in the method.

Response:

EPA did not move the information in this note but considers a 'must' in a note to be equivalent to any other requirement in a section.

130. Comment:

A number of commenters (5, 8, 9, 14, 18 and 23) requested that EPA revise Section 11.3.6 to remove the recommendation to force calibrations through the origin "when background PFAS are present to better estimate background concentrations." Several commenters considered forcing calibrations through the origin to be inappropriate and stated that laboratories should instead find and eliminate sources of background contamination and use other mitigation measures for instrument background (e.g., by using a delay column, replacing fluoropolymer containing components with PEEK, etc.). Commenter 9 stated that forcing the calibration through the origin may be necessary for PFAS and noted it is required by Method 537, but this commenter interpreted the wording in this section to mean it was used to address background contamination and did not consider this approach to be correct. Commenter 8 also requested that the method expressly prohibit forcing the calibration through zero, which the commenter considered to be consistent with the initial calibration guidance in Method 8000D.

Response:

EPA agrees with these commenters' suggestion regarding the wording in Section 11.3.6 and revised it to remove mention of forcing calibrations through the origin under the identified circumstances. However, as long as the initial calibration criteria are met, forcing calibrations through the origin is permitted, as described in Section 11.5.2.1 of Method 8000D. Section 11.3.4 refers to Method 8000D for more information regarding calibration options. Some laboratories that participated in the validation study forced calibrations through the origin, and there was no clear impact on performance in the study samples or for other categories of quality controls evaluated in the validation study. EPA agrees with these commenters regarding the need to find and eliminate sources of background and to use other measures to mitigate interferences.

131. Comment:

Commenters 6 and 22 requested that EPA revise Section 11.3.6 to remove reference to initial calibration acceptance criteria based on correlation coefficient (r) or coefficient of determination (r^2). Commenter 6 stated these measures of calibration fit "provide no real value and can mislead analysts into believing their calibration curve is adequate." Commenter 22's reasoning for removing r and r^2 was that new EPA methods use Relative Standard Error (RSE) instead. This commenter also recommended revising the method to make the initial calibration acceptance criteria required.

Response:

EPA did not make these changes to the method because the calibration options and acceptance criteria are all referenced from Method 8000D, including the use of correlation coefficient (r) or coefficient of determination (r^2). Section 11.3.4.2 also recommends using % error to assess calibration fit in addition to r or r^2, especially near the Lower Limit of Quantitation (LLOQ), and mentions that weighted regressions may improve calibration fit at low

concentrations. The other three options presented in this section for evaluation of initial calibration fit can be applied independently. EPA did revise Section 11.3.4 to more clearly identify options for fitting and evaluation of initial calibrations and included references to specific sections of Method 8000D for each of them. These options provide stakeholders some flexibility for evaluating acceptability of calibration fit while emphasizing the importance of managing and minimizing calibration-related measurement bias.

132. Comment:

Several commenters (5, 8, and 9) considered the allowance for reporting of estimated concentrations of target analytes that fail to meet initial calibration acceptance criteria in Section 11.3.8 to be unacceptable. These commenters stated that any compounds that do not meet the initial calibration acceptance criteria should not be reported. Commenter 8 requested that EPA revise the method to require the initial calibration acceptance criteria to be met. Commenters 8 and 9 also considered the allowance in Section 11.3.8 for up to 10% of target analytes to not meet the initial calibration acceptance criteria to be unacceptable. Commenter 8 stated that laboratories are capable of meeting the initial calibration acceptance criteria, “as exhibited by multiple other validation studies,” and expected that the acceptance criteria would be easier to meet if isotope dilution calibration is used. Commenters 5 and 8 also noted that Section 11.3.8 provides no upper limit for Relative Standard Deviation (RSD, for average calibration factor calibration models) and/or Relative Standard Error (RSE). Commenter 5 was concerned that “the risk of data comparability and trending issues for the analytes that are more difficult to determine by this method is high” without an upper limit for % RSD. Commenter 8 stated that, without an upper limit for RSD or RSE in the initial calibration section, the method allows for extreme QC failures and “an unwarranted level of uncertainty to be associated with reported concentrations.” This commenter also noted that the Continuing Calibration Verification (CCV) section provides a limit of 50% recovery below which corrective action is required.

Response:

EPA did not revise the method as requested by these commenters and notes that the same guidance for initial calibration is provided in other multi-analyte SW-846 determinative methods. EPA did revise Section 11.3.6 to refer to Method 8000D for recommended corrective actions when initial calibration acceptance criteria are not met, and Section 11.5.6 of Method 8000D provides a number of options to address poor calibration fit. More stringent acceptance criteria may be appropriate and necessary for specific project applications and should be included as project-specific requirements (e.g., for specific analytes of primary concern). Whether a maximum %RSD is useful or should be implemented as a requirement may also depend on the data needs for a specific project. If the analyte in question is not one that is of primary interest for the specific project application, corrective action may not be warranted. If a calibration model other than average Calibration Factor (CF) or RSE is used it may not make sense to set a maximum RSD and/or require corrective action when the criterion is not met.

133. Comment:

Commenter 8 considered the acceptance criteria for evaluation of calibration fit for the initial calibration standard at the Lower Limit of Quantitation (LLOQ) in Section 11.3.9 (50-150% of expected concentration) to be unnecessarily wide given that the % error criteria for the higher concentration calibration standards is ±30%. The commenter stated this difference "allows for a sliding scale of uncertainty associated with concentrations within the quantitation range" and requested that EPA revise the method to specify the same ±30% error limit for all initial calibration standards, including at the LLOQ. This commenter was also concerned with the statement in this section regarding actions to be taken when the acceptance criteria are not met, namely "the analytes should be reported as estimated near that concentration, or the LLOQ should be reestablished at a higher concentration." The commenter considered these actions to be inappropriate because "LLOQs are typically near the required project limits," and these actions "would typically result in data that does not meet the stated project needs."

Response:

EPA disagrees that ±50% error is unreasonable for the calibration standard at the LLOQ and did not make the requested change to the method. EPA considers the ±50% error limit to be reasonable for general purposes and notes that other commonly used SW-846 determinative methods (e.g., Methods 8260D and 8270E) and other PFAS test methods published by EPA (e.g., Method 533) use the same criterion. More stringent limits may be needed for specific project applications and should be included as project-specific requirements.

134. Comment:

Commenter 9 considered the wording in Section 11.3.9 regarding evaluation of calibration fit at the Lower Limit of Quantitation (LLOQ) for each initial calibration to be appropriate "if followed." However, the commenter was concerned that the allowance for laboratories to use alternate acceptance criteria in this section would permit them to use less stringent criteria and "basically freelance" regarding acceptance of initial calibrations.

Response:

EPA disagrees with the commenter that the flexibility in this section is inappropriate; the same guidance is provided in other commonly used SW-846 determinative methods. EPA revised Section 11.3 to clarify the options presented for evaluation of initial calibration fit and associated acceptance criteria, but wording regarding "alternate acceptance criteria" for initial calibration is retained in Section 11.3.4. Evaluation of calibration error for individual initial calibration standards particularly at the LLOQ is strongly recommended in Section 11.3.4.2 for regressions when correlation coefficient (r) or coefficient of determination (r^2) is used for evaluation of calibration fit. Section 11.3.6 of Method 8327 also refers to Section 11.5.6 of Method 8000D for recommended corrective actions when the initial calibration acceptance criteria are not met. More stringent criteria may be needed for specific project applications and should be included as project-specific requirements.

135. Comment:

Commenters 9 and 22 remarked that Section 11.3.10 provides suggested acceptance criteria for Initial Calibration Verification (ICV), and they recommended making the ±30% acceptance limit a method requirement. Commenter 9 further remarked that this section allows for alternate ICV acceptance criteria based on project-specific Data Quality Objectives (DQOs), and this commenter recommended leaving discussion of project-specific requirements to Quality Assurance Project Plans or other project planning documents to avoid variability.

Response:

EPA did not revise the method as suggested by these commenters. The 'suggested' wording in this section for ICV acceptance criteria is consistent with other commonly used SW-846 determinative methods, as is the statement that "alternate acceptance limits may be appropriate based on project-specific DQOs." A number of sections in the method mention application of alternative acceptance criteria based on project needs and reflect the project-specific focus in SW-846 Chapter 1.

136. Comment:

Commenter 5 requested that the method clearly state whether surrogates are intended to be included in Continuing Calibration Verification (CCV) standards.

Response:

EPA made no changes to the method based on this comment and considers the wording in Section 7.4.4.3 to be sufficient, which states that CCV standards should be prepared in the same manner as initial calibration standards. The 'example preparation' for the calibration standards stock in Section 7.4.4.1 specifies addition of target compounds and surrogates.

137. Comment:

Commenters 6 and 9 requested revision or clarification of Continuing Calibration Verification (CCV) frequency in Section 11.4.1. Commenter 6 remarked that EPA provided "no basis to require a continuing calibration verification (CCV) after every 10 samples" and stated that CCVs should be analyzed at the end of every batch or per 24-hour period. Commenter 6 also stated "the test methods should be calibrated daily." Commenter 9 requested that the method better define the meaning of "field samples" in relation to CCV frequency or to just specify a 12-hour frequency for CCV analysis.

Response:

EPA partially agrees with commenter 6 regarding CCV frequency and revised section 11.4 to specify analysis of CCV standards at a minimum frequency of one per every 20 samples of every 12 hours, whichever is shorter, and at the end of the analysis sequence. For the external phase of the validation study, EPA specified that laboratories were to analyze a CCV at a

frequency of one per 10 field samples and found no clear benefit compared to a CCV frequency of one per 20 field samples. The method still requires a CCV to be analyzed after the last sample as well. Analysis of CCVs at higher frequency may prevent rework, but it is not required. One of the laboratories that participated in the interlaboratory validation study used successive CCVs instead of new initial calibrations to demonstrate that the initial calibration was still acceptable, and this laboratory's performance was similar to other laboratories that ran fresh initial calibrations. EPA did not further define the term "field samples" in this section because it is used in the same manner and has the same intended meaning as in Section 9.6, which describes frequency of sample preparation quality controls per batch of "20 or fewer field samples." EPA does not intend for method users to consider quality control samples to evaluate whether the minimum CCV frequency of one per 20 field samples is met.

138. Comment:

Commenter 22 recommended that EPA revise Section 11.4 to state that the Continuing Calibration Verification (CCV) acceptance criterion of ≤±30% is a method requirement.

Response:

EPA did not revise this section because requiring all CCV acceptance criteria to be met would be inconsistent with other published SW-846 determinative methods. As with other quality controls, some marginal failure rate is permitted. However, Section 11.4.3 specifies that laboratories must qualify affected sample results or refer to Section 11.7 of Method 8000D, which discusses related corrective actions. The note after Section 11.4.3 also prompts the analysts to look for signs in the CCVs that performance has degraded (e.g., unusual tailing, loss of resolution, loss in response) and corrective action is needed. As with other types of quality controls, more stringent acceptance criteria may be needed for specific project applications (e.g., for specific analytes of interest) and should be included as project-specific requirements.

139. Comment:

Commenter 5 remarked that Section 11.4.3 states Continuing Calibration Verification (CCV) standards are acceptable as long as ≤10% of the analytes fail to meet the acceptance criteria. The commenter considered this allowance to be reasonable but recommended establishing a maximum allowable % difference or % drift limit for analytes before corrective action is required, which would limit the risk of "data comparability and trending issues for the analytes that are more difficult to determine by this method." This commenter referred to the note after this section, which the commenter interpreted as providing a similar threshold (50%) for surrogate recovery.

Response:

EPA did not revise the method according to the commenter's suggestion because establishing a maximum % difference or % drift criterion would be inconsistent with Method 8000D. Whether a

maximum % difference or % drift criterion is useful or should be implemented as a requirement may also depend on the data needs for a specific project. If the analyte in question is not one that is of primary interest for the specific project application, corrective action may not be warranted. The note after Section 11.4.3 is intended to provide indications that system performance has degraded to the point that system maintenance is needed. (e.g., unusual tailing, loss of resolution, loss in sensitivity). EPA revised the wording in the note after Section 11.4.3 to include 50% recovery as an example of 'significant' loss, along with other laboratory-defined criteria or degradation in chromatography. Please note that this specification is for an instrument quality control rather than for recovery of a surrogate in a field sample.

140. Comment:

Commenter 9 interpreted the allowance for up to 10% of target analytes to not meet the Continuing Calibration Verification (CCV) acceptance criteria in Section 11.4.3 to mean that "analytes with recoveries outside of the CCV recovery limits would be acceptable to report," which the commenter considered to conflict with Section 11.4.2. The commenter recommended revising the method to either remove the allowance for CCVs to be considered acceptable when target analytes do not meet the acceptance criteria or to indicate that instrument maintenance and calibration is needed when the acceptance criteria are not met.

Response:

EPA did not revise the method as the commenter suggested. The intent of the wording in Section 11.4.3 is to allow some limited margin of exceedances for CCVs and other quality controls. Section 11.4.3 does require corrective action if > 10% of the reported compounds do not meet the CCV acceptance criteria. More stringent requirements may be necessary and can be used for specific applications, and these requirements should be included in project planning documents and addressed with the testing laboratory, as needed.

141. Comment:

Commenter 20 remarked about the wording in Section 11.4.3 related to qualification of sample results when Continuing Calibration Verification (CCV) acceptance criteria are not met. This commenter stated that non-detects may be biased low when measured CCV standard concentrations are below their lower acceptance limits. The commenter further stated that "it's not possible to assess adequacy of sensitivity unless an LLOQ verification standard is analyzed following that CCV" and requested that this section be clarified.

Response:

EPA revised Section 11.4.3 of Method 8327 to state that results for target analytes that do not meet CCV acceptance criteria are qualified in any affected field samples or to refer to Method 8000D for guidance. The approach to continuing calibration verification in Method 8327 is consistent with other multi-analyte organic SW-846 methods where limited QC failures may not

adversely affect whether the data meet the project requirements. Section 9.6 of Method 8327 recommends including an LLOQ verification QC sample in every batch of 20 or fewer field samples, however it is not required. If batch-specific LLOQ verifications and/or more stringent acceptance criteria for other quality controls are needed they should be included in project planning documents and discussed with the testing laboratory.

142. Comment:

Commenter 22 stated that the note after Section 11.4.3 includes requirements related to "monitoring responses and demonstrating sensitivity" and recommended moving them from a note to the text of the method.

Response:

EPA did not move the information in this note as requested and considers a requirement in a note to be equivalent to any other requirement in a section.

143. Comment:

Commenters 8 and 9 recommended revision of Section 11.4.4 to include corrective actions when contamination is found in a blank analyzed after a Continuing Calibration Verification (CCV) standard. Commenter 8 recommended specifying that samples should be re-prepared and re-analyzed if the associated Method Blank (MB) concentration is >½ the Lower Limit of Quantitation (LLOQ). Commenter 8 also recommended requiring the system to be checked and any carryover contamination to be eliminated if a Reagent Blank (RB) concentration is >½ the LLOQ. Commenter 9 recommended reanalysis of bracketed samples as an appropriate corrective action when blank contamination is found. Commenter 8 also recommended root cause analysis to determine the source and impact on any samples.

Response:

EPA did not revise this section based on these comments and considers the corrective actions related to blank contamination in Section 9.5 to be sufficient. How laboratories should address blank contamination may depend on the project application, which is described in this section. Root cause analysis is an essential component of a laboratory's quality system and is beyond the scope of any one method.

144. Comment:

Commenter 8 noted that Section 11.5.1 provide a suggested analysis sequence order that includes a Lower Limit of Quantitation (LLOQ) verification QC sample, which the commenter considered to be misleading since the method only requires LLOQ verification QC samples to be analyzed annually. The commenter requested that EPA either require LLOQ verifications on

a daily basis or at least distinguish between QC that are and are not required in this suggested analysis sequence. The commenter also noted that no blank sample is included in the suggested analysis sequence after the last Continuing Calibration Verification (CCV) standard, and the commenter requested that EPA revise the method to require analysis of a blank after the last CCV.

Response:

EPA did not revise the method as the commenter suggested. An LLOQ verification QC sample is listed in the suggested sequence order in Section 11.5.1 because Section 9.6 recommends including a prepared LLOQ verification QC sample in each batch of 20 or fewer field samples. Batch-specific LLOQ verifications are not required in Method 8327, consistent with other SW-846 reference methods, but they are required on a periodic basis and may be included for specific project applications. The value of requiring a blank to be analyzed at the end of the analysis sequence after the ending CCV is unclear. Such a requirement would be inconsistent with other SW-846 reference methods and, as far as we are aware, would also be inconsistent with PFAS test methods published by other EPA programs.

145. Comment:

Several commenters (5, 9 and 22) remarked about lack of requirements or corrective actions for surrogate recovery in Section 11.5.2. Commenter 5 requested that EPA establish a fixed lower acceptance limit for surrogate recovery below which corrective action is required and recommended using 50% recovery as a lower limit, consistent with the note after Section 11.4.3. Commenter 9 stated that corrective action should be required if acceptance criteria are not met for surrogate recovery in Method Blanks, Reagent Blanks, LCS samples, and LLOQ Verification QC samples. Commenter 9 further stated that samples with unacceptable surrogate recovery should be reprepared and/or reanalyzed to confirm any matrix interferences. Commenter 22 stated the acceptance limits for surrogate recovery in Section 11.5.2 should be required.

Response:

EPA disagrees with these commenters' requests to include fixed or required acceptance limits for surrogate recovery or to include recommended or required corrective actions when surrogate recovery criteria are not met. The wording in Section of 11.5.2 is consistent with other SW-846 methods: surrogates must be added to samples; surrogate recoveries must be monitored; and, surrogate recoveries should meet the acceptance criteria. Defining corrective actions for each category of quality control when the acceptance criteria are not met may depend on the specific project application and is outside the scope of this method. Requiring corrective action when a surrogate is not recovered within acceptance limits may be onerous for a method that includes many surrogates, and, depending on the cause of low recovery, could result in significant rework for the laboratory with limited benefit. Laboratories are free to establish more stringent acceptance criteria and should define corrective actions when acceptance criteria for quality controls are not met in the laboratory's standard operating procedures or quality management plan. Alternate acceptance criteria and corrective action requirements may also be useful or

necessary for specific project applications, and these requirements should be included in project planning documents and addressed with the testing laboratory, as needed.

146. Comment:

Commenter 9 noted that Section 11.5.2 indicates surrogates should be added to MB, LCS, and LLOQ verification samples and recommended including surrogates in Reagent Blanks (RBs) as well.

Response:

As described in EPA's response to comment 88, surrogates are optional in RBs, which serve a different purpose than Method Blanks (MBs). Section 11.5.2 was revised to state that the laboratory must monitor recovery of surrogates in samples.

147. Comment:

Commenters 8 and 21 noted that Section 11.5.2 refers to Section 7.3.11, but this section does not exist.

Response:

EPA agrees with the commenters and revised Section 11.5.2 to include the proper reference (Section 7.4.2.2).

148. Comment:

Commenter 8 noted that Section 11.5.2 refers to monitoring of isotopically labeled surrogates and requested that EPA modify the method to specify the use of these chemicals as isotope dilution standards.

Response:

Please refer to EPA's response to comment 10 for discussion related to isotope dilution calibration.

149. Comment:

Commenters 14 and 18 noted that Section 11.5.3 recommends dilution of prepared samples with 1:1 methanol-water+0.1% acetic acid when PFAS concentrations exceed the calibration range. The commenters considered this procedure to be problematic because it also dilutes the

isotopically labeled analytes added to the samples. The commenters recommended performing dilutions on a replicate sample that has not yet been processed by transferring to a new container, quantitatively rinsing the original container, diluting the sample with solvent, and scaling the internal standard/surrogate spiking volume to maintain the same labeled compound concentration as is added to the calibration standards.

Response:

EPA did not revise the method as recommended by these commenters. The procedure the commenters proposed for preparing a dilution from a replicate sample container may be useful or necessary for isotope dilution or extracted internal standard calibrations, but dilutions are more straightforward when external standard calibration is used, and they can be made directly from the original prepared samples.

150. Comment:

Commenters 9 and 21 recommended including a minimum signal to noise ratio (S/N) as a criterion for qualitative identification of target analytes in Section 11.6. Commenter 9 suggested using S/N > 3.

Response:

EPA did not revise the method to include a S/N specification in Section 11.6 for qualitative identification. Meeting a minimum signal to noise criterion is not included as part of the qualitative identification criteria in other mass spectrometry-based SW-846 determinative methods based on unit mass resolution instruments. Section 11.7 only suggests quantitation of peaks that meet qualitative identification criteria. Peak shape and background noise can be important considerations for qualitative identification and the professional judgment of the analyst or data reviewer may be an important part of avoiding misidentification of chemicals and avoiding false positive or false negative results. Signal to noise ratio can be a helpful consideration to support qualitative identification, but it is not explicitly included here.

151. Comment:

Commenters 20 and 23 remarked about the ion ratio criteria used for qualitative identification in Section 11.6.1. Commenter 20 noted that Section 11.6 is the first place that ion ratios are mentioned and recommended including related information in the Initial Calibration (ICAL) and Continuing Calibration Verification (CCV) sections. Commenter 20 also considered an ion ratio acceptance criterion of ≤±30% to be very tight for more difficult matrices and noted that the US Department of Defense (DoD) recommends using ≤±50%. Commenter 20 also noted that the method does not mention evaluation of retention times for the quantitation ion and confirmation ion, which DoD states should maximize within ± 2 sec of each other. Commenter 23 requested that EPA revise the ion ratio criteria to be dependent on the relative intensities of primary and secondary ions. Commenter 23 stated that their laboratory uses “limits provided by the

European Commission decision implementing the performance of analytical methods (2002/657/EC), summarized in the following table." Commenter 23 recommended using these limits to reduce the possibility of misidentification.

Relative intensity (% of base peak)	Relative ion intensities for LC-MS/MS
> 50%	±20%
>20% to 50%	±25%
>10% to 20%	±30%
≤10%	±50%

Response:

EPA revised Section 11.6 to increase the product ion ratio criterion to ± 50%. This change will accommodate target analytes with lower relative abundances of secondary ions for which a narrower limit may be harder to meet and may also better accommodate differences in proportions of signals for primary and secondary product ions in linear and branched isomers of the same target analyte. EPA did not address evaluation of ion ratios in the initial calibration and continuing calibration verification standards and did not include acceptance criteria for evaluation of retention times of primary and secondary product ions. These considerations may be useful for evaluation of qualitative identification, but this level of detail is not included in other SW-846 organic determinative methods and is not explicitly included here. EPA also did not recommend different ion ratio limits for different ranges of relative responses for primary and secondary product ions because this approach has not been sufficiently evaluated, but it may be useful, and EPA will consider it for a future revision.

152. Comment:

Commenter 8 interpreted the statement in Section 11.6.1 regarding the use of professional judgment to prevent misidentification of peaks to imply that "the analyst has the ability to not report results (i.e. report as ND) when ratios are off." The commenter noted that an ion ratio may not meet the acceptance criteria even though the analyte is present. The commenter recommended that laboratories report appropriately qualified results when the ion ratio criteria are not met so they can be evaluated more carefully by the project team. The commenter requested that EPA modify the method to state that target compounds should be qualified as potentially biased when the ion ratio criteria are not met.

Response:

EPA did not revise the method as the commenter requested, but EPA did increase the ion ratio acceptance criterion in Section 11.6.1 from ≤±30% to ≤±50% to address a similar concern. Please refer to response to comment 151 for related discussion. EPA recognizes the importance of limiting misidentification of target analytes, either as false positives or false negatives, and considers target analytes composed of multiple structural isomers to present additional challenges. Section 11.6.1 does mention the use of professional judgment to support qualitative identification, but it does not state who is responsible for making those judgments, and EPA considers assigning this responsibility to be outside the scope of the method. The need for detailed review of qualitative identification may depend on the nature and level of contamination in the samples and on the specific project goals, and these considerations should be included in project planning documents and addressed with the testing laboratory, as needed.

153. Comment:

Commenter 9 remarked that Section 11.6.1 indicates that ion ratios may be affected if samples have different proportions of linear and branched isomers and noted that it is difficult to tell if an ion ratio failure is caused by the presence of branched isomers without more information about chromatographic retention times and elution patterns of linear and branched isomers. The commenter noted that only linear isomers are available for some target analytes, so the analytical community's ability to evaluate coelutions under a given set of analytical conditions is limited. The commenter concluded that "US EPA needs to conduct further research on how ion ratios are impacted when peaks consist of mixtures of branched and linear isomers and set appropriate qualitative criteria."

Response:

EPA revised Section 11.6.1 to allow for wider ion ratio limits, which should reduce but will not eliminate the potential for false negative identification based solely on differences in product ion ratios in branched and linear isomers. The note after Section 11.6.1 addresses differences in proportions of branched and linear isomers in general terms. Until certified reference materials of individual structural isomers are more readily available it is unclear how to determine elution patterns of structural isomers under different chromatographic conditions or to optimize chromatographic separations. Please refer to EPA's responses to comments 151 and 152 for related discussion.

154. Comment:

Commenter 21 noted that Section 11.6.1 references Figures 1-4 in reference to differences in ion ratios in standards and samples and recommended adding a note to indicate that "Figures 1 and 2 have different ion ratios between standard and sample for PFOS and Figures 3 and 4 illustrate the different ion ratios between the standard and sample for PFHxS."

Response:

EPA received multiple comments about the figures in the method and revised them to more clearly illustrate the proportions of primary and secondary product ions in a standard and a sample. Please refer to EPA's response to comments in Section 17 for more information.

155. Comment:

Commenter 9 stated that the last sentence of Section 11.6.2 "clearly assumes the normal and branched isomers are separately resolved" but noted that the method provides no chromatographic resolution criteria. The commenter concluded that "resolution checks need to be defined and resolution criteria need to be included."

Response:

EPA disagrees with the commenter regarding the purpose of the last sentence in Section 11.6.2. There is no presumption that the peaks are resolved, it is only intended to ensure that the time segments during which the appropriate ions are acquired include all of the chromatographic peaks in the quantitative and any qualitative standards as described in Section 11.3. Again, as this commenter has described, without single isomer standards it is unclear how to clearly evaluate chromatographic resolution or establish useful criteria.

156. Comment:

Commenters 9 and 22 remarked about the evaluation of chromatographic Retention Time (RT) in Section 11.6.2 to support qualitative identification of target analytes in samples. Commenter 9 noted that Section 11.6.2 provides qualitative identification criteria based on comparison of chromatographic Retention Time (RT) of a target analyte in a sample to a mid-level initial calibration standard or continuing calibration verification standard. Commenter 9 also noted that this section recommends evaluation of the delta Retention Time (RT) of a target analyte in the sample compared to its corresponding mass labelled analog (surrogate) "should also be considered to confirm target analytes". The commenter recommended making the delta RT (±10 seconds) a requirement for qualitative identification and stated that RT windows of ±10 seconds "will likely result in false positives." Commenter 22 stated that EPA should make the ±10 seconds retention time window used for evaluation of chromatographic retention time a requirement.

Response:

EPA revised Section 11.6.2 to recommend the evaluation of chromatographic retention time of each target analyte relative to its structurally identical isotopically labeled surrogate in the same sample, where available. If no isotopically labeled analog is available, evaluation is based on comparison of a target analyte's retention time in the sample to that in the midpoint Initial Calibration (ICAL) standard, average of ICAL standards, or preceding Continuing Calibration Verification standard. As with other QC criteria, this retention time evaluation does not include 'required' language, as some matrix effects are likely to be outside of the scope of the testing

done during method development. Evaluation of relative retention time (in %) was retained as an option in Section 11.6.2, and this section lists other factors that may influence retention time, like differences in relative proportions of structural isomers for some target analytes. This section also states that other means may also be useful to support qualitative identification, such as standard additions (e.g., matrix spikes).

SECTION 16.0: REFERENCES

157. Comment:

Commenter 6 requested that EPA replace the reference to ASTM D7979 – 15 with ASTM D7979 – 17. The commenter considered EPA's conclusions about the outcomes of the collaborative study to reflect poorly on performance ASTM D7979, but the commenter's own evaluation of the study data supported "the robustness and Precision and Accuracy of ASTM D7979 – 17." The commenter noted that ASTM D7979 – 17 specifies that "instruments of specific sensitivity, considered equivalent to the instrument used in method development MUST be used," and that "modifications may be made only if they IMPROVE performance." The commenter stated that EPA allowed laboratories to participate in the validation study that had insufficiently sensitive instrumentation or that made unacceptable modifications to the procedural steps in the method, which negatively influenced EPA's interpretation of the study data. The commenter requested that EPA either remove the interpretation that ASTM D7979 performed poorly or remove reference to ASTM D7979 from the method.

Response:

EPA agrees with the commenter that method performance was acceptable based on EPA's own analysis of the supporting data and considers the validation data to be supportive of method publication. EPA excluded data from laboratories that deviated from critical steps in the study protocol or that identified having instrument stability problems, as described in Appendix E of the Statistical Summary Report, and the laboratories whose data are included in the statistical analysis were found to have substantially complied with the study protocol. Some laboratories that participated in the study did not have much prior experience with the method, which as described in ASTM E691-19 can influence comparability of data between laboratories. Nevertheless, EPA met its goals for the validation study, as described in the Executive Summary. Please refer to this report for more details. EPA updated the reference to ASTM D7979-20 in Section 16.0 of Methods 3512 and 8327 and revised the methods and supporting documents to address concerns expressed by a number of commenters regarding perceptions of poor performance.

SECTION 17.0: TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

Please note that some of the summary tables that were included in the version of Method 8327 that was posted for public comment were removed from the method and are posted as links on the EPA website, and the validation study data are presented differently, as described in response to comment 8.

158. Comment:

Commenter 20 stated that the footnote in table 1 should use the term "straight-chain" or "linear" instead of "normal-chain," which the commenter noted was not a chemical term.

Response:

EPA agrees with the commenter and replaced any instances of “normal-chain” with “linear”. EPA removed Table 1 from the method as described in comment 27.

159. Comment:

Commenters 4 and 9 remarked about the suggested LLOQs and calibration ranges for target analytes in Table 1. Commenter 4 considered the suggested Lower Limits of Quantitation (LLOQs) and calibration ranges presented in Table 1 to represent a best-case scenario since data from nearly 1/3 of the participating laboratories were not included. Commenter 9 recommended raising the suggested LLOQs to 40 ng/L for all PFAS analytes and increasing the corresponding calibration ranges.

Response:

As described in EPA’s response to comment 27, EPA removed Table 1 from the method. SW-846 methods generally don’t provide suggested Lower Limits of Quantitation (LLOQs) or calibration ranges, and each laboratory is responsible for establishing and periodically verifying LLOQs using instrumentation, reagents, equipment, supplies and personnel specific to their laboratory. As described in EPA’s response to comment 218, EPA excluded data from laboratories that deviated from a critical step in the validation study protocol or that reported having instrument stability problems; please refer to this comment and to Appendix E of the Statistical Summary Report for more information. EPA revised the QC Summary Report to provide more detail about initial calibration ranges with acceptable % error (Table 2c) and verified LLOQs (Table 5b) by laboratory and preparation batch. Table 5b from this report is included in the ‘additional data’ link on the Method 8327 web page. Most laboratories consistently met acceptance criteria for initial calibration and LLOQ verification at 10-20 ng/L for most target analytes.

160. Comment:

Several commenters (6, 8, 10, 13, and 19) considered the Lower Limits of Quantitation (LLOQ) presented in Table 1 of Method 8327 to be insufficient for some PFAS applications (e.g., state regulatory limits or action levels). Commenters 10, 13, and 19 indicated that some states have action limits for groundwater as low as 10 ng/L. Commenter 8 also recommended removing Table 1 since Table 4 provides calibration ranges.

Response:

EPA removed Table 1 from Method 8327 because SW-846 methods generally do not provide suggested LLOQs. Please refer to EPA's response to comment 2 for more discussion related to method sensitivity.

161. Comment:

Three commenters (9, 21, and 22) identified what they considered to be a typographical error in Table 2A. The column title is "# of LCS/LCSD pairs with RPD > 30%" and all the commenters stated that it should be "# of LCS/LCSD pairs with RPD < 30%."

Response:

EPA agrees with the commenters and replaced this table with a more detailed summary of LCS performance by laboratory consistent with Table 6b in the QC Summary Report. This table can be found at the 'additional data' link on the Method 8327 web page.

162. Comment:

Commenter 4 stated that 11 of 24 analytes (nearly ½) listed in Table 2A are identified in the method as being poor performers for reproducibility, response, recovery, stability and/or chromatography, but their laboratory "regularly measures PFBA, PFPEA, PFHxA, PFUdA, PFDoA, PFTeDA, N-EtFOSAA, N-MEFOSAA at detection limits nominally in the range 10 ng/L to 25 ng/L with recoveries typically within 70 to 130% and RSDs less than 25%."

Response:

As described in EPA's responses to comments 1 and 20, EPA did not intend to identify these chemicals as poor performers. EPA met the Data Quality Objectives it defined for the validation study for these target analytes. Consistent with other recently published SW-846 method revisions, most performance data will be removed from methods and available on the SW-846 website. Only a summary of performance data for the target analytes in the study samples that supports the table in Section 1.0 was retained. Other useful summaries of performance data for different categories of quality controls are included in tables in the QC Summary Report, and some of these tables and more detailed summaries of validation study data are included in the 'additional data' link on the Method 8327 web page.

163. Comment:

Commenter 8 noted that Table 2B did not include LLOQ verifications for the 50 ppt LLOQs listed in Table 1 for PFPeA and PFBA and requested that EPA modify Table 2B to include this data.

Response:

EPA removed tables 1 and 2B from Method 8327 as described in responses to comments 27, 162, and 208. EPA included a more detailed summary of LLOQ verifications as Table 5b of the QC Summary Report, and this table is also included in the 'additional data' link on the Method 8327 web page.

164. Comment:

Commenter 8 remarked that the footnote of Table 2C states that the averaged unspiked concentration in that matrix type was subtracted from spiked concentrations if the average un-spiked concentration was >5 ng/L. The commenter stated that the unspiked sample concentration should only be subtracted if it was above the Lower Limit of Quantitation (LLOQ) because concentrations below the LLOQ are not considered to be quantitative. The commenter also considered averaging unspiked sample concentrations across all laboratories to be "not defensible" and considered it more appropriate to subtract average background concentration determined by an individual laboratory from spiked sample results from the same laboratory. The commenter requested that EPA correct these calculations and include the new calculations in the method.

Response:

EPA respectfully disagrees with the commenter's assertion that only sample concentrations > LLOQ can be subtracted from matrix spike samples for the purpose of calculating recovery. Method 8000D and other 8000-series SW-846 methods do not provide guidance on whether sample concentrations below the LLOQ should be ignored or subtracted from spiked sample concentrations prior to calculating matrix spike recovery. Bias resulting from subtracting a measured concentration < LLOQ from a matrix spike should be negligible for determination of recovery when the spike level is high relative to the LLOQ. Please refer to EPA's response to comment 95 for additional discussion. EPA subtracted the mean measured concentration of a given target analyte in the unspiked samples of the same matrix determined by an individual laboratory from the measured concentration in each of the spiked samples reported by that laboratory prior to calculating % recovery, which is consistent with the commenter's suggestion (if EPA interpreted the comment correctly). Please note that Table 2C was replaced, and the validation study data are presented as Table 1 in Method 8327. Please refer to EPA's response to comment 208 for explanation. The footnote of Table 2C had a minor error in that the % recovery data provided in the Statistical Summary Report was background subtracted without considering whether the average unspiked sample concentrations were above or below a default or laboratory-determined LLOQ, but only sample concentrations >5 ng/L (half the lowest

LLOQ evaluated in the validation study) were used to calculate the average unspiked concentration in that matrix type at that laboratory. Please refer to the tabular validation study sample data in the final docket for the measured concentrations of target analytes in each individual study sample and the background-subtracted concentrations used for calculation of % recovery.

165. Comment:

Commenter 20 stated that the Liquid Chromatograph (LC) operating conditions used to generate the chromatographic Retention Times (RTs) in Table 3 was important context, without which the RTs were not useful.

Response:

EPA incorporated LC operating conditions in this table by reference to Table 3. Note that EPA reorganized the tables in the method and renumbered this table as Table 2.

166. Comment:

Commenter 8 considered the sentence in the footnote of Table 4 that discussed the effect of the solvent dilution sample preparation procedure on the reporting range to be unnecessary and confusing and requested that EPA remove it.

Response:

EPA determined the information related to standards preparation provided in Table 4 to be unnecessary and removed it. EPA also removed the sentence the commenter mentioned in the footnote, but other relevant information in the footnote related to conversion of sulfonate salt concentrations was moved to a note after Section 7.4.2.1.

167. Comment:

Commenter 20 asked what the purpose of associating target analytes to specific isotopically labeled surrogates in Table 6 if the method does not use isotope dilution calibration.

Response:

EPA included this table in the method to identify which target analytes had corresponding isotopically labeled analogs during method development. This information could also be useful to support evaluation of retention time in Section 11.6 and for interpretation of data for samples for which no matrix spikes are performed. Please note that EPA reorganized the tables in Method 8327 and this is now Table 5.

168. Comment:

Commenter 5 requested that EPA add an acceptance criterion for correlation coefficient ($r \geq 0.995$) for initial calibration in Table 7 and to correct the criterion for coefficient of determination ($r^2 \geq 0.99$ rather than >0.99), consistent with Section 11.3.8.

Response:

EPA agrees with the commenter and revised Table 6 to include appropriate acceptance criteria for both r and r^2. EPA also revised Section 11.3.4 to more clearly identify options for evaluation of initial calibration fit and associated acceptance criteria.

169. Comment:

Commenter 8 noted that Table 7 did not provide acceptance criteria for recovery of surrogates in samples (70-130%). This commenter also noted that the surrogate recovery row in Table 7 references Section 9.10, which does not pertain to surrogates. This commenter requested that EPA modify the method to address these issues.

Response:

EPA agrees with the commenter and revised Table 6 to include the correct section reference and preliminary surrogate recovery limits from Section 9.6.3.

170. Comment:

Commenter 8 noted that Table 7 does not include the limitation on recoveries <50% for CCVs noted in Section 11.4.3 and requested that EPA revise Table 7 to include this information.

Response:

EPA did not make the requested change to the method because the note after Section 11.4.3 is about system maintenance rather than quality control acceptance criteria. Please refer to EPA's response to comment 139 for more information.

171. Comment:

Commenter 9 noted that Table 7 states the acceptance criteria for holding time are "TBD" for and pending the outcome of a holding time study. The commenter requested that EPA revise the method to state that the sample data is qualified if the holding times are exceeded.

Response:

EPA revised this row of Table 6 to provide a recommended 14 day holding time for aqueous samples, consistent with changes made to Section 8, and replaced “TBD” with “use professional judgment to qualify”. EPA also added references for holding time studies to Section 16.0 that were the used as a basis for the recommended holding times for aqueous samples in Section 8. SW-846 methods do not generally specify how to qualify data if holding time is exceeded, and Table 6 does not include related statements for other categories of quality controls, so EPA did not include the statement requested by the commenter.

172. Comment:

Commenter 9 considered the statement in Table 7 indicating that surrogates are added to each sample to be vague and suggested including quality control samples: “initial calibration standards, ICV, CCV, LCS, MB and RB.”

Response:

EPA revised the surrogates row in Table 6 to include prepared quality control samples (i.e., method blanks, LCS, MS/MSD, and LLOQ verifications), consistent with Section 9.6.3.

173. Comment:

Commenter 21 noted that method blank frequency is not found in the sections cited in Table 7. The commenter requested that EPA include the method blank frequency (“one per preparation of 20 or fewer samples”) in the body of the method.

Response:

EPA updated the section reference in Table 6 of Method 8327 to refer to Section 9.5.6 for minimum frequency for preparation of method blanks. Section 11.4.4 describes analysis frequency for blanks, and either a method blank or reagent blank may be used for that purpose, so the section reference is pertinent for method blank frequency.

174. Comment:

Commenter 8 pointed out a typo in the note associated with Figure 2 “that was not observed in the calibration standard for used for quantitation...” and recommended changing to “that was not observed in the calibration standard used for quantitation…”

Response:

EPA concurs with the comment but revised the figures which eliminated the need for this note.

175. Comment:

Several commenters (13, 19, 20, and 21) mentioned the PFOS peak at 8.22 minutes in Figure 2 that was provided as an example of a potential PFOS isomer in a sample that was not integrated because it was not clearly present in the standard. Commenters 13 and 19 suggested revising the text in Sections 11.6.1 and 11.7 to indicate that only isomers found in calibration standards are to be integrated in samples. Commenter 20 asked whether the PFOS isomer at 8.22 minutes in the sample should be included in the quantitation but flagged by the lab and discussed in their narrative. Commenter 21 noted that this peak may be at small relative abundance in the calibration standard chromatogram in Figure 1 and suggested it might be more clearly identified if the chromatogram was scaled differently. Commenter 21 considered Figures 1 and 2 to be ambiguous and recommended using a different example and labeling the branched and linear isomers. Commenter 21 further recommended adding the information in the footnote in Figure 2 to the body of the method "if it's EPA's intention to only integrate branched isomers of PFASs if the peaks are found in the calibration standards."

Response:

EPA revised Section 11.3.3 and 11.6.1 to clarify that a qualitative and/or quantitative standard must be used to define chromatographic retention times of branched isomers. Method 8327 does not address how a laboratory should manage data for a chromatographic peak that is outside the retention time range determined with quantitative or qualitative standards. Figures 1-4 were also replaced with a simplified figure, and the branched and linear isomer peaks are labeled. Please refer to EPA's responses to comments 3, 58, 123, 125, 152, and 153 for more information related to calibration and identification of PFAS target analytes composed of branched and linear isomers.

APPENDIX B (future Method 3512) – AQUEOUS SAMPLE PREPARATION

Please note that Appendix B has been formatted and included in the final docket as SW-846 Method 3512.

SECTION B1.0: SCOPE AND APPLICATION

176. Comment:

Two commenters (4 and 5) remarked that Sections 1.2, 2.1, and 7.3.4 in Method 3512 mention the use of isotopically labelled chemicals as internal standards for use with the method. The commenters found this to be confusing as Method 8327 is not an isotope dilution method, and commenter 4 added that using these isotopically labeled analogs as internal standards in an external calibration method would eliminate their use as surrogates for quality assurance purposes. Additionally, commenter 4 raised the concern that the method provides no guidance on the selection of a labeled chemical as a surrogate or IS or whether they are used.

Response:

Many SW-846 methods are written in modular format, meaning sample preparation methods are separate from determinative methods, which allows the user to select the preparation method and determinative method of their choice. Method 3512 was written so that it could be used with external standard or isotope dilution calibration. As such EPA expects that isotopically labeled analogs of target analytes may be used for slightly different purposes depending on the determinative method. Language was included to allow these isotopically labeled chemicals to be used as surrogates or isotope dilution internal standards once the appropriate validation data have been evaluated.

177. Comment:

Commenter 5 noted that Section 1.2 refers to Method 8328, which the commenter considered to be intended for analysis of solid samples.

Response:

EPA removed reference to Method 8328 from Method 3512.

SECTION B2.0: SUMMARY OF METHOD

178. Comment:

Commenter 20 noted that Sections B2.1 and B11.2.1 describe spiking “prior to dilution of sample and level adjusted by spiking in more surrogate after dilution or using methanol spiked with surrogate as the diluent.” the commenter remarked that DOD only requires the spike be added to final dilution of sample and asked why this method does not use the same approach, which the commenter considered to potentially eliminate errors generated by adding multiple spikes of surrogates.

Response:

EPA did not validate the method by adding isotopically labeled chemicals at the end of the sample preparation process and considers recovery of surrogate and target analyte standards added prior to sample processing to be potentially important for assessing process performance and variability. Whether used as surrogates or isotope dilution internal standards, isotopically labeled chemicals are not intended to be added twice; they are only intended to be added prior to sample preparation. EPA received multiple comments about the note after Section 11.2.1 and removed it from the final version of Method 3512.

SECTION B4.0: INTERFERENCES

179. Comment:

Commenter 5 requested clarification in Sections B4.1, B9.4.1, and B9.4.2 for how Reagent Blanks (RBs) are intended to be used and how they differ from Method Blanks (MBs). Commenter 5 also asked whether surrogates are intended to be added to Sections B9.6 and B9.7 about surrogate spiking applied to RBs as well as MB and QC samples.

Response:

EPA revised Section 9.0 of Method 3512 to better reflect the organization and information included in Section 9.0 of method 8327; and Section 9.5 clearly describes how MB and RB quality control samples are intended to be prepared and used. RB quality control samples are used to assess cleanliness of reagents and consumables and are not intended to be subjected to sample preparation procedures like MBs. Surrogates are not required to be added to RBs, but they are required for MBs. Sections 9.6 and 9.7 do not apply to RBs.

180. Comment:

Commenter 12 noted that Section B4.3 mentions disposable labware and noted that SW-846 methods should provide “green chemistry” alternatives. The commenter considered the statement in Section B4.3.4 regarding re-use of materials to be important to emphasize reduction in consumption of plastics.

Response:

Please refer to EPA’s response to comment 39 for more information.

181. Comment:

Commenter 5 noted that Section B4.3.3 refers to polyethylene pipettes and asked if it referred to low-density polyethylene, high-density polyethylene, or both. The commenter stated that “low-density polyethylene can cause loss of PFAS.”

Response:

Pipettes are low density polyethylene and can be used to transfer sample into autosampler vials. Because the prepared samples are 50% organic solvent when these pipettes are used and contact time is minimal, sorption losses should be minimal, and any losses of native target analytes should be reflected in loss of the added isotopically labeled analogs.

182. Comment:

Commenter 22 noted that Section B4.3.4 states the blank criteria can be used as a guideline for evaluating cleanliness of reusable labware and recommended making the use of the blank criteria a requirement. The commenter noted that "the blank, by definition, is what determines the "cleanliness" of the entire test."

Response:

The blank criteria included in Section 9.0 of Methods 3512 and 8327 are intended to evaluate introduction of contaminants during sample preparation and analysis. Reagent Blanks and Method Blanks are included as quality controls to evaluate introduction of contaminants during sample preparation and analysis. The importance of evaluating contamination in reusable labware may depend on how this labware is used, and details regarding how background contamination is evaluated in reusable labware is more appropriate for the laboratory's Standard Operation Procedure or Quality Management Plan.

SECTION B6.0: EQUIPMENT AND SUPPLIES

183. Comment:

Commenter 5 stated that the analytical balance in Section B6.2 should be capable of weighing to 0.0001g rather than to 0.01g.

Response:

EPA respectfully disagrees with the commenter and considers the specification of ±0.01 g to be sufficient, as the balance is intended for determining sample masses on the order of 5 g. A ±0.01 g tolerance for a 5 g mass is comparable to the tolerance for a Class A volumetric flask of 5.00±0.02 mL (refer to Table 9 of https://www.nist.gov/system/files/documents/calibrations/circ602.pdf).

184. Comment:

Commenter 6 noted that Section B6.3.1 includes syringes made from High Density Polyethylene (HDPE), polypropylene or glass and remarked that "ASTM D19.06 has no data to support the use of HDPE and polypropylene syringes." The commenter also referred to the QC Summary Report, which states that all laboratories were provided with glass syringes. The commenter understood this statement to mean that the study data was produced with glass syringes. The commenter stated that "test methods should not allow use of untested materials unless the test method plainly states that equivalency must first be established."

Response:

EPA revised Section 4.3.4 to identify that polypropylene sample containers were used during method validation and that other materials such as HDPE can be used as long as the laboratory can show that the target analytes are not adversely affected. The contact time between the sample and the syringe is relatively short. One of the laboratories that participated in the validation study indicated in a post-study survey that they used polypropylene syringes for sample filtration, and no evidence of adverse impact was found in the study sample data or associated quality controls from this laboratory. These surveys are in the docket. Please see comment 50 for related discussion.

185. Comment:

A number of commenters (4, 9, 13, 14, 18, 19 and 23) remarked about the potential for PFAS analytes to adsorb onto particle filters. Commenter 4 recommended clarifying whether the filter has been shown to adsorb PFAS analytes, which the commenter has observed for polyether sulfone filter membranes. Commenter 9 noted that the filters specified in the method contain glass fiber and recommended utilizing a different type of filter for sample filtration. Commenters 14 and 18 considered loss during filtration likely even in 50% organic solvent and suggested it could have led to poor reproducibility observed for some compounds in the validation study. Commenters 14 and 18 requested that EPA replace filtration with centrifugation. Commenter 23 noted that PFAS are generally surface active, so filtration through a high surface area medium presents potential for loss. Commenter 23 also noted that filters have to be cleaned or demonstrated to be free of PFAS prior to being used, which is an additional burden on the laboratory. Commenter 23 suggested making the filtration step “as needed” and supported adding centrifugation to the method as an alternative to filtration.

Response:

EPA did not change the recommended filter type in the method and considers the validation study data to have provided sufficient evidence that loss during filtration was minimal. EPA did not explicitly evaluate filtration-related loss by itself during method validation, but EPA found that the average recovery of target analytes and surrogates in the study samples and in LCS quality control samples was near 100% across the validation study laboratories. No clear trend in low bias measurement was observed particularly for the higher molecular weight target analytes that might more readily sorb to surfaces. Please refer to Table 1 in Method 8327, the Statistical Summary Report, the QC Summary Report, and the Executive Summary for supporting data. Other types of particle filters and other liquid-solid separation techniques like centrifuging samples may be used provided the laboratory demonstrates acceptable performance for the intended application and can consistently meet the acceptance criteria for the categories of quality controls in Section 9.0 of Method 3512 and the associated determinative method. EPA revised Section 11.2.4 of Method 3512 to specifically mention that centrifugation may aid in removal of particulates.

186. Comment:

Commenter 12 stated that the labware cleaning instructions in Section B6.4 and reagents in Section B7.1 should emphasize that traces of compounds should be reduced to a minimum.

Response:

EPA agrees with commenter and made the same revisions to Method 3512 as were made to Method 8327. Please refer to EPA's response to comment 48 for more information.

SECTION B9.0: QUALITY CONTROL

187. Comment:

Commenter 9 noted that Section B9.7 allows isotopically labeled analogs of target analytes to be used as internal standards and asked why this allowance was not included in Method 8327.

Response:

EPA validated Method 8327 as an external standard calibration method. EPA wrote this option into Method 3512 to support validation with an isotope dilution calibration determinative method and will consider conducting this validation study in the future. Please refer to EPA's responses to comments 10 and 176 for related discussion about isotope dilution.

SECTION B11.0: PROCEDURE

188. Comment:

Commenter 9 noted that some of the subsections in Section B11.1 make references to subsections in an earlier draft that appear to be out of sequence.

Response:

EPA reviewed these subsections in Method 3512 to ensure they were properly organized.

189. Comment:

Commenter 8 noted that Section B11.1.1 presents determination of sample volume by mass with an assumed density of 1 g/mL, and the commenter considered this assumption to be potentially incorrect due to the wide variety of sample types for which this method will be used. The commenter also noted that the method presents marking the meniscus on the sample

container for later determination of volume or using certified graduation marks on the sample tubes as options for estimating sample volume, and the commenter considered these to be qualitative rather than quantitative determinations. The commenter further noted that the method requires no adjustment of the amount of methanol added unless the determined sample volume differs from the expected volume (5 mL) by >5%. The commenter considered maintaining the minimum 50% solvent content to be critical to keeping all target compounds in solution and requested that EPA modify the method to require quantitative measurement of sample volume and to ensure the ratio of sample to solvent is maintained for all prepared samples.

Response:

EPA partially agrees with the commenter and revised Sections 11.1.1 and 11.2.1 to include an option to directly measure sample volume and to remove the 5% tolerance for sample volume before adjusting the volume of methanol added. Estimating sample volume without transferring to another sample container is ideal because it minimizes opportunities for loss or contamination. EPA considers the use of sample mass and a presumed density of 1 g/ml for aqueous samples to be an accepted and common practice for estimating aqueous sample volume in other EPA methods and in voluntary consensus standards, and, in general, EPA expects that this approach will provide a reasonably accurate determination of aqueous sample volume especially for small volume containers. EPA recognizes that a different approach such as direct determination of sample volume or density may be useful and appropriate for some sample types. EPA considers marking the meniscus of a sample container for later determination of volume to be reliable as long as it is done carefully. EPA did not understand the commenter's specific concern with using certified graduation marks on sample containers to estimate sample volume.

190. Comment:

Two commenters (9 and 11) noted their concerns with collecting small sample sizes (i.e., 5 mL samples in 15 mL tubes) in the field. Commenter 9 was concerned that small volumes of samples may be non-representative and may be difficult to measure accurately. Commenter 11 was concerned that significant resources would be required to prepare and track pre-tared sample containers and recommended allowing the use of 250 mL HDPE sample bottles. The commenter also stated that larger sample containers could be rinsed with methanol to minimize the potential for adsorption.

Response:

EPA revised Section 11.0 to explicitly allow for preparation of alternative sample sizes as long as the entire sample is prepared and the proportions of reagents and standard additions are maintained. Small aqueous sample size presents several potential advantages, including ease of handling in the field and reduction of waste generated and associated disposal cost. Determination of appropriate or minimum sample size to ensure representativeness may be a project-specific consideration and is outside the scope of the method. EPA also revised Section 11.1.1 to provide options to measure sample volume if sample containers are not pre-weighed and do not have certified graduation marks.

191. Comment:

Commenter 9 noted that the Section B11.1.1 provides an option for determining sample volume that requires transfer of the sample from the original sample container, while Section B11.2.1 states that methanol volume and spiking solution volume may need to be adjusted depending on the sample volume. The commenter asked how these adjustments can be made if sample volume is not determined until the container has been emptied.

Response:

EPA revised Section 11.1.1 to include several options for determination of sample volume. If the containers are not pre-weighed and do not have certified graduation marks, the other options provided in Section 11.1.1 for determination of sample volume require transfer to a different container and including a solvent rinse of the original sample container to ensure quantitative transfer.

192. Comment:

Commenter 5 asked whether the note after Section B11.1.3 applies to the Lower Limit of Quantitation (LLOQ), and, if so, recommended revising the note to state that. (Note: EPA presumes the commenter means 'LLOQ verification', which was addressed in the following subsection).

Response:

EPA agrees with the commenter and revised these subsections to indicate the note clearly applies to LLOQ verifications as well.

193. Comment:

Commenter 22 recommended including the note after Section B11.1.3 in the body of the method because it is indicated as a requirement.

Response:

EPA did not revise the method as the commenter suggested and considers a method requirement included in a note to be equivalent to a requirement in a section of the method.

194. Comment:

Commenter 5 stated that the first sentence in Section B11.1.5 is missing the word "temperature" after "room."

Response:

EPA concurs with this comment and added the word "temperature" to the updated Section 11.1.1 in Method 3512.

195. Comment:

Commenter 9 remarked that the first note after Section B11.1.5 states that aqueous subsampling is not recommended unless the sample is first diluted ≥ 50% with methanol, but the method validation study report noted loss of target analytes from solution in 50:50 methanol-water+0.1% acetic acid in glass sample containers. The commenter concluded that this statement indicates “loss is still possible with ≥ 50% organic cosolvent present.”

Response:

EPA considers the commenter’s conclusion to be overly broad because the same issue was not observed during the validation study for standards stored in polypropylene containers. EPA revised Section 11.0 in Method 3512 to remove the statement that storage of prepared samples in glass containers was “not recommended” and revised Section 4.0 of Method 3512 to include the information from Section 4.3.4 of Method 8327 regarding materials of construction for sample containers and potential for loss under certain conditions during storage in glass containers.

196. Comment:

Commenter 9 remarked that the 2nd note in Section B11.1.5 states samples should be transferred to larger containers if the original sample containers are not large enough to hold the sample plus dilution solvent but noted that the surrogate is not specified to be added until after this step is complete. The commenter asked why surrogates are not added prior to transfer “to reflect the entire process,” and the commenter also asked if the batch quality control samples should be prepared in a similar manner.

Response:

EPA did not change the order of these processing steps based on this comment because determination of sample volume is necessary to ensure the appropriate volumes of methanol and surrogates are added, and under some circumstances this determination may require transfer of the sample from the original sample container to another container. EPA agrees that addition of surrogates at the beginning of sample preparation is the most straightforward approach but recognizes it is not always practicable. Other SW-846 sample preparation methods also allow for spiking of surrogates after the sample has been transferred. The sample preparation procedure in Section 11.2 of Method 3512 Section 11.2.2 requires the spiking of surrogates and any target compounds at the same point for field samples and associated prepared quality control samples.

197. Comment:

Commenter 22 recommended revising the method to move the quantitative transfer step described in the note after Section B11.1.5 to the body of the method.

Response:

EPA revised Sections 11.1 and 11.2 in Method 3512 and moved this information in this note to Section 11.1.1, but EPA considers specifications in the notes in the method to be equivalent to those in the body of the method.

198. Comment:

Commenter 9 remarked that Section 11.2.1 allows for a 5% tolerance in sample volume (4.75-5.25 mL) without adjusting the volume of methanol. The commenter considered this 5% allowance to be inconsistent with the method requirement to maintain a 1:1 ratio of methanol and water and recommended that the preparation procedure be revised to explicitly allow solvent volumes to be scaled to be equivalent to aqueous sample volumes "(e.g., 5.2 mL of sample to 5.2 mL of methanol)". This commenter also stated that the method was only validated for 5 mL volumes.

Response:

EPA concurs with this comment and revised Section 11.2 to remove the indicated tolerance for sample volume. EPA expects the performance of this preparation method will be unaffected by variations in initial sample volume as long as the proportions of reagents and standards are maintained.

199. Comment:

Several commenters (6, 8 and 9) remarked about the note after Section B11.2 that provides the option to add isotopically labeled surrogates (and target analytes for LCS and matrix spike quality control samples) to the dilution solvent rather as a separate spiking solution prior to solvent dilution. Commenter 6 noted that, while this practice has been used in other test methods, it was not evaluated during development of this method, and the commenter asked whether EPA had data to support the use of this technique. Commenter 8 considered the practice of adding surrogates and target compounds to the dilution solvent rather than directly to the sample to contradict Section 1.1.4.6 of SW-846 Chapter 1, which states that analytical bias is evaluated by determining recovery of a known amount of contaminant spiked into a sample. This commenter did not consider adding surrogates to the dilution solvent to be representative of the bias associated with the sample preparation process and requested that EPA modify the method to state that "surrogates and target compound spikes must be spiked directly into the

sample (or reagent water in the case of MB and LCS) and mixed thoroughly before any solvent is added." Commenter 9 asked whether addition of surrogates to samples in the dilution solvent was "likely to improve surrogate recovery in a manner that is not reflective of target analytes."

Response:

EPA partially agrees with these comments and removed the option to add isotopically labeled chemicals to the dilution solvent from Section 11.0 of Method 3512. EPA did not evaluate this approach for adding standards during validation of Methods 3512 and 8327 but considers dilution of an aqueous sample with a water-miscible organic solvent to be different from a process standpoint than extraction with a water-immiscible solvent.

200. Comment:

Commenter 12 remarked that replicate sample containers may be needed for each field sample when dilutions are required because the polyethylene vial caps have no septa to limit solvent evaporation from prepared samples.

Response:

EPA did not revise the method to address the commenter's concern because no sealing problem was identified for the polypropylene sample containers and caps used for method validation. If the suggested volume for sample collection in this method is used, 10 ml of prepared sample is available for analysis and can be used to prepare dilutions or for reanalysis as needed (<2 ml might be added to an autosampler vial). Section 7.4.4.1 of Method 8327 does identify volatile loss as a concern after the autosampler vial caps are punctured, but Section 4.3.4 also cautions against storage of standards and prepared samples in glass containers except during analysis. More detailed sample collection requirements are beyond the scope of this method and should be defined by the laboratory or for the project.

201. Comment:

Commenter 9 noted that B11.2.4 includes filtration to remove particulates in samples, but Section B1.4 states that the method may not be appropriate for samples with high levels of suspended solids. The commenter asked whether the method validation study included samples with particulates to evaluate the impact of filtration or whether EPA had defined an upper limit for particulates content. The commenter also asked how samples are to be treated as multi-phase samples given that "a solid extraction method has yet to be published."

Response:

As described in the response to comment 217, EPA did not evaluate particulates content of the sample matrix types used for the validation study. The tested matrix types were not considered to have significant solids content, and EPA did not determine what solids content would affect

performance or trigger treatment as a multi-phase sample. Most aqueous SW-846 sample preparation methods have not been tested for maximum particulate content. EPA revised Section 1.3 of Method 3512 to indicate that collecting larger sample volumes or centrifugation may aid in phase separation. EPA will consider evaluating the effects of particulates type and content on method performance in the future.

202. Comment:

Three commenters (14, 18, and 23) recommended either replacing the filtration step in Section B11.2.4 with centrifugation or removing the requirement for the filtration step altogether.

Response:

EPA revised Section 11.2.4 in Method 3512 to include centrifugation as an option to "aid in removal of particulates." However, centrifugation was not tested during method validation, and laboratories should exercise care if they use centrifugation as an alternative to filtration to avoid damaging instrumentation by introducing particulates and to ensure the method quality control criteria can routinely be met with this approach. Please refer to EPA's response to comment 185 for additional discussion related to filtration and centrifugation.

203. Comment:

Commenter 9 remarked that Section B11.2.5 states that sample pH is adjusted to approximately 3-4 after filtration, but the method does not indicate whether sample pH should be measured or whether corrective action is required if the specified pH is not achieved. The commenter also asked about the basis for addition of glacial acetic acid.

Response:

EPA concurs with this comment and removed the specified pH range to avoid any confusion about whether the method intends for laboratories to measure sample pH after addition of acid, which it does not. EPA also added a note after Section 7.4.4.1 of Method 8327 that states the primary intent of adding acetic acid is primarily to improve chromatography for some target analytes.

Executive Summary

204. Comment:

Commenter 5 stated "The executive summary does not actually summarize the results of the report."

Response:

EPA revised the Executive Summary to more clearly identify the Data Quality Objectives (DQOs) for precision, bias and sensitivity used for the validation study along with a summary of the study data showing that the DQOs were met for 23 of 24 target analytes.

205. Comment:

Commenter 2 stated "The multi-laboratory validation study of Method 8327 in four water matrices showed excellent recoveries, accuracy and precision for 23 of 24 compounds included. The exception was 6:2FTS. Eight of the twelve laboratories participating did very well. The other four did not follow the method properly and their data was not included. The procedure was straightforward with few preparation steps."

Response:

EPA thanks the commenter for their input.

206. Comment:

Commenter 9 noted that the Executive Summary indicates that data from 12 laboratories were evaluated but noted that the QC Summary Report and Statistical Summary Report both mention that 13 laboratories participated in the study. The commenter also noted that only four laboratories are identified in Appendix E of the Statistical Summary Report as having been removed from the final data analysis set. The commenter stated that "apparently some laboratories did not return results in the needed timeframe and were excluded" and requested that EPA clearly state the number of laboratories that participated in the validation studies.

Response:

EPA sent study samples to 13 laboratories to participate in the Method 8327 validation study, and 12 of those laboratories submitted data. Of those 12 labs, EPA excluded data from 4 individual laboratories leaving eight laboratories whose data EPA presented in the Executive Summary, Statistical Summary Report, and QC Summary Report.

207. Comment:

Commenter 18 stated "We disagree with EPA's finding in the Executive Summary that, despite nearly half the samples indicating analytical errors, the method is "generally acceptable." If an analytical method reveals errors or inconsistencies to this extent – in particular in situations where the method may ultimately be used for determining compliance with the Clean Water Act or another environmental statute – the method must be rejected until it can provide the scientific confidence needed. EPA must, at a minimum, revise the draft methodology and propose a new

draft method with sufficient data to qualify performance of the method for public comment before considering approval of its use in regulatory programs."

Commenter 18 further stated "It is clear from EPA's Statistical Report and the Data Validation Summary that significant errors are present with nearly half of the analytes tested (n=11 of 24), revealing serious issues with reproducibility, response, recovery, stability and chromatography. The imprecisions found for long-chain PFAS compounds, short-chain PFAS compounds, and PFAS precursors all expose problems with this methodology. With the 3,000 plus known PFAS compounds in the environment, public wastewater utilities must have the confidence that their sampling and analysis accurately reflects the true concentrations and are not misrepresented by unacceptable uncertainty. If there is variability in precision across the board for PFAS compounds, as the Statistical Report and Data Validation Summary demonstrates, EPA must reconsider and revise this methodology."

Response:

EPA respectfully disagrees with the commenter's conclusions. Please refer to EPA's response to comment 1 for discussion related to method performance and to comment 20 for discussion related to the '#' designation for target analytes in Section 1.0 of Method 8327. Addressing questions and comments related to compliance with the Clean Water Act is outside of the scope of this document. For further information on Clean Water Act methods, please consult the Clean Water Act Methods Team, which can be reached by pressing the "CONTACT US" link on the webpage at: https://www.epa.gov/cwa-methods.

208. Comment:

Commenter 6 stated "The EPA statistical summary of the data does not provide repeatability or reproducibility statements. This is inconsistent with the general consensus standard organization practice for evaluating results from multi-laboratory studies. The EPA SW-846 document, *Guidance for Method Development and Method Validation for the RCRA Program*, suggests the use of Youden Pairs such as described in ASTM D2777, *Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water*. The Forum for Environmental Measurements (FEM) document, *Validation and Peer Review of U.S Environmental Protection Agency Chemical Methods of Analysis,* references ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1 General principles and definitions,* which provides definitions for repeatability and reproducibility and references for conducting interlaboratory studies, such as using ASTM E691, *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*. As far as we can tell, neither of these ASTM standards, nor the ISO standard, has been used in developing EPA's study plan or data evaluation of SW-846 Method 8327."

Response:

EPA partially agrees with this comment. EPA did not use references from ISO or ASTM as a basis for developing Data Quality Objectives (DQOs) for the multi-laboratory validation study, but, as described in the revised Executive Summary in the final docket, EPA did develop DQOs

for precision, bias and sensitivity for the validation study, and the study data met these DQOs for 23 of 24 target analytes. Please refer to this document for more information.

EPA recognizes that many published SW-846 methods provide estimates of repeatability (within-laboratory variability, as single-laboratory Percent Relative Standard Deviation, or %RSD) and/or reproducibility (between-laboratory variability, as reproducibility %RSD), and we recognize the value of using a consistent approach. However, estimates of repeatability and reproducibility calculated according to an appropriate reference such as ASTM E691 or AOAC International's "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis" are based on measured concentration and assume that replicate samples are as similar as possible. This assumption did not always hold true for the Method 8327 validation study because it was conducted in two phases. Aqueous samples were collected from the same sources but in sampling events separated by more than a year, and the wastewater matrix used for the external phase of the validation study contained shorter-chain carboxylic acids with measurable concentrations that were not present in samples collected for the internal phase, some of which approached the low spike concentration (60 ng/L, nominal). Pooling data from the internal and external phases of the validation study would result in artificially high estimates of variability for this matrix that were unrelated to method performance. Therefore, to provide some measure of consistency with presentation of multi-laboratory performance data with past and future SW-846 reference methods, EPA included estimates of bias and within- and between-laboratory precision in Method 8327 that were based on calculations in the Clean Water Act guidance document "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program" (February 2018). These precision and bias calculations are based on recovery of standard additions after subtracting background concentrations in the respective unspiked samples, thereby accounting for differences in background and providing a consistent comparison of measurements across internal and external phases of the validation study.

209. Comment:

Commenter 6 stated that, even though some laboratory deviations led to bias that reflected poorly on precision of the test method, based on their own evaluation of the collaborative study results using ASTM E691 that the data was suitable for testing of parts-per-trillion (ng/L) concentrations.

Response:

EPA excluded data from laboratories that were identified to have deviated from critical steps in the study protocol or otherwise identified having instrument problems, so these issues did not negatively impact EPA's evaluation of the validation study data or otherwise exaggerate estimates of measurement precision between laboratories. The laboratories whose data are included in the Executive Summary, Statistical Summary Report and QC Summary Report substantially followed the validation study protocol.

Statistical Summary Report

210. Comment:

Commenter 9 remarked that the "The Statistical Analysis Report describes using a mean value for RSD calculation, but a median value for the overall %R calculation" and considered the use of "two different measures of central tendency to calculate the two critical decision criteria" to be arbitrary. The commenter requested that EPA provide a more detailed explanation of "the calculations and decision criteria."

Response:

EPA revised the Executive Summary to provide more information about EPA's Data Quality Objectives for precision, bias and sensitivity. The summarized data presented in the Executive Summary draw from the Statistical Summary Report and QC Summary Report. Please refer to these documents and to EPA's response to comment 208 for more information about descriptive statistics presented for the validation study data and associated quality controls.

211. Comment:

Commenter 9 noted that the Statistical Summary Report uses the Shapiro-Wilk statistic to evaluate whether study data are normally distributed, but the report states this statistic was not mentioned in the Quality Assurance Project Plan (QAPP). The commenter further noted that the Shapiro-Wilk statistic was not used further, and "the lower control limit (LCL) and upper control limit (UCL) at 95% limits were calculated using the mean value, presumably as to account for non-normality." The commenter asked why the Shapiro-Wilk statistic was included if it was not used to "determine confidence intervals or other statistical metrics." The commenter also noted that the Statistical Summary Report includes the Student's t-test, which was not mentioned in the QAPP. The commenter concluded that "the use of these statistical approaches as a post-planning analysis appears arbitrary and indicates insufficient planning and QAPP development" and requested that EPA provide additional detail about the statistical approaches that were used.

Response:

EPA revised the Executive Summary to provide more information about EPA's Data Quality Objectives (DQOs) for precision, bias and sensitivity and to present a summary of the study data for evaluation against each of them. The only DQO that relied on the presumption that data was normally distributed was the Relative Standard Deviation (RSD). The Shapiro-Wilk statistic is a very common statistic that is used to determine if data are non-normally distributed, but the statistical power of this test is low when the number of data points on which it is based is limited. Five replicate samples were analyzed by each laboratory for each matrix and spike level, which was insufficient to apply the Shapiro-Wilk statistic to confidently identify non-normally distributed data. Therefore, the outcome of the Shapiro-Wilk test was not applied to EPA's assessment of whether the study data met EPA's DQOs. Similarly, the Student's t-test is a very common

statistical test used to test a hypothesis that the mean of a dataset is not effectively different from a hypothesized mean. The Student's t-test was used as an extra test to more fully analyze the data, but it was not used to evaluate the multi-laboratory validation data against EPA's study DQOs. The 95% LCL and UCL, as stated in the statistical report, were calculated based on the median. The median is not dependent on the type of distribution. Please refer to the revised Executive Summary for more information.

212. Comment:

Commenters 5 and 9 remarked about performance of 6:2 FTS in the Statistical Summary Report. Commenter 5 noted that this report lists performance of 6:2 FTS as "fair," while performance of all other target analytes and surrogates is described as "good." Commenter 5 asked why 6:2 FTS is still included in the method and what basis EPA used to decide whether "a particular analyte cannot or should not be analyzed by this method." Commenter 9 noted that the Statistical Summary Report provided an acceptance criterion of <50% Relative Standard Deviation (RSD), which Table 1 of the Executive Summary indicates was not met for 6:2 FTS at either spiking level. Commenter 9 concluded that "EPA did not follow its QAPP rule, instead using an arbitrary determination to keep this analyte as acceptable for the method" and requested that EPA address why 6:2 FTS performance was determined to be acceptable.

Response:

Please refer to EPA's response to comment 28 and to the Executive Summary for further discussion of 6:2 FTS.

213. Comment:

Commenter 9 noted that the tables in Appendix A presented summary statistics for recovery and precision in spiked samples based on "modified concentration", which the commenter presumed to mean the unspiked sample concentrations had been subtracted. The commenter also noted that Section 1.3.1 of the report describes background subtraction for evaluation of recovery, but not for Relative Standard Deviation (RSD). The commenter stated that subtracting a mean value across a set of data influences the variance and "no longer captures the original precision." The commenter requested that EPA include a justification in the Statistical Summary Report.

Response:

The commenter is correct that Section 1.3.1 does not describe using the modified RSD. However, both the unmodified RSD and modified RSD are presented in the tables. The standard deviation does not change with the use of unmodified and modified values, only the denominator of the RSD equation causes the change between the two RSDs. EPA revised the Executive Summary to include the range in RSDs determined for each target analyte in each laboratory by matrix type and spike level. The RSDs presented in the Executive Summary are

the unmodified RSDs from the Statistical Summary Report and are based on measured concentration (i.e., without background subtraction), which addresses the commenter's concern and is similar to how precision is calculated for target analyte concentrations in sample and duplicate or Matrix Spike and Matrix Spike Duplicate quality control samples in Method 8000D and in Method 8327.

214. Comment:

Commenter 9 noted that the combined mean and maximum for PFTeA in the groundwater (GW) matrix in Table 1 were non-zero numbers even though zeros were reported for every laboratory on the table, and the commenter stated "it appears that Lab 11, that typically detected almost everything in every matrix, may have been left off the PFTeA table for GW."

Response:

EPA concurs with the comment and revised the table to correct the information presented.

215. Comment:

Commenter 9 noted that Appendix E of the Statistical Summary Report included justifications for why four laboratories' data were from statistical analysis, but the commenter did not consider these justifications to be adequate "unless the QAPP included details with respect to how closely the draft method was to be followed, and what level of precision and bias would be required by a laboratory to be included in the final data set." The commenter mentioned that no data were provided for laboratory 15 and considered statements describing Laboratory 15's data in Appendix E to be contradictory. The commenter requested that EPA include data from all participating laboratories in the Statistical Summary Report, even as appendices, and provide the validation study Quality Assurance Project Plan (QAPP) with details about the criteria EPA used to exclude laboratory data. The commenter also noted that the Data Validation Report for Laboratory 3 in the docket cited two versions of the validation study QAPP, and the commenter requested that EPA include all versions of the QAPP so the statistical analysis could be "compared against the validation study planning rules" and to provide details about how the study data were validated across both phases of the validation study. The commenter further stated that "The statistical approaches used appeared to be arbitrary and lacked transparency."

Response:

EPA did not include a summary of Laboratory 15's data in the Statistical Summary Report because EPA did not intend to compare study sample results from laboratories that did not follow the validation study protocol to results from laboratories that did. EPA did include summary data spreadsheets from laboratory 15 and all other laboratories in the docket with Method 8327 when it was posted for public comment. The commenter is welcome to examine those results, but they are not relevant to EPA's purpose for conducting or assessing the outcomes of the validation study. Appendix E provides a general description of Laboratory 15's

deviation from the study protocol as well as their initial reported results and reprepared and reanalyzed sample results, and the statements mentioned by the commenter were related to two different data sets and were not contradictory. Nevertheless, EPA revised the wording in this section to more clearly reflect observations about the data the laboratory provided.

The Quality Assurance Project Plan (QAPP) for this validation study is an internal document, and EPA has not provided similar planning documents in the docket for past SW-846 method validation studies, so it is not included in the docket. Instead, EPA included additional information in the final docket to provide all necessary context from the QAPP regarding EPA's Data Quality Objectives (DQOs) for the validation study, including:

- Data Quality Objectives for precision, bias, and sensitivity used to evaluate the validation study data included in the Executive Summary
- The study protocol and study instructions for the external phase of the validation study
- A summary of differences in preliminary acceptance criteria for internal and external study phases, included as Appendix B in the QC Summary Report

EPA's validation study QAPP stated that laboratories were required to request advance approval from the study manager for any deviations from the study plan that were not expressly permitted. All individual laboratories' study data were evaluated against a common set of QC acceptance criteria, and the same approach to data qualification was used in data validation reports for data from all laboratories. These data validation reports are included in the docket, but EPA did not validate data from all laboratories listed in Appendix E.

216. Comment:

Commenter 9 stated that "The statistical analysis performed by US EPA shows high variability and bias for many of the PFAS target analytes listed in Draft Method 8327 (11 of 24 PFAS, noting these analytes, "may require special care to ensure analytical performance will meet the needs of the project and, where necessary, may also require the use of appropriate data qualification")."

Response:

EPA respectfully disagrees with the commenter's interpretation of the validation study results. Please refer to EPA's responses to comments 1 and 20 for additional discussion.

QC Summary Report

217. Comment:

Commenter 9 requested more information about the sample matrices used for the validation study such as pH, oxidation-reduction potential and mineral content. The commenter also noted

that the method states it may not be appropriate for aqueous samples with high levels of suspended solids and asked whether any of the study sample matrices contained particulates "in order to evaluate what level of suspended solids would be of issue and the impact of the filtration step when particulates are present."

Response:

EPA agrees that more detailed characterization of samples would have provided useful supporting information, but the aqueous sample matrices used for method validation were not characterized in this manner at the time the study was conducted. EPA did not explicitly evaluate suspended solids content during method validation, but EPA expects that at least the wastewater matrix contained some level of suspended solids, and method performance in this matrix was similar to other matrices. EPA does not have a clear basis to recommend an upper limit for solids content in Method 3512, but related wording in Section 1.3 of Method 3512 is similar to other EPA sample preparation methods. EPA will consider conducting further evaluation of the impact of suspended solids composition and content on method performance at a future date. Please refer to EPA's response to comment 185 for more discussion related to filtration.

218. Comment:

Two commenters (4 and 9) expressed concern that when laboratories deviated from specific procedural steps in the study protocol, which is allowed under the flexibility described in Section 1.1 of Method 8327, that their results were considered unacceptable and were excluded from the statistical analysis. The commenters were also concerned with the statement in the QC Summary Report "The majority of the analytes met the acceptance criteria for precision, bias and method DQIs" because one third of the laboratories' data were excluded for failure to meet LLOQ recoveries, initial and continuing calibrations, surrogates and LCS recoveries.

Response:

EPA respectfully disagrees with the commenters' interpretation regarding how exclusion of laboratory data for not following certain procedural steps in the study protocol reflects on the flexibility described in Method 8327. Methods 3512 and 8327 state that the laboratory is required to demonstrate acceptable performance for the intended application. Laboratories that deviated from certain critical procedural steps in the study protocol had much more significant problems meeting the preliminary acceptance criteria established for certain categories of instrument and/or sample preparation quality controls in the validation study. The study data from these laboratories could not be directly compared to data from other laboratories, which is why they were excluded from statistical analysis. EPA either used all of the data generated by a laboratory or none of it based on this information. Cautions and other statements related to sample handling and materials compatibility were added or strengthened in Sections 1.0, 4.0, 8.0, and/or 11.0 of Methods 3512 and 8327 based on laboratory deviations from the study protocol, and this information will benefit method users and data reviewers. Please refer to Appendix E of the Statistical Summary Report for more information regarding exclusion of laboratory data.

219. Comment:

Commenter 9 remarked that laboratories did not prepare Matrix Spike/Matrix Spike Duplicate (MS/MSD) quality control samples as part of the validation study even though they are listed as a quality control category in Method 8327 and are typically required by "CERCLA QA programs." The commenter also stated "If US EPA omitted MS/MSDs because the spiked study samples provided by US EPA are similar to MS/MSD samples, then the report should clearly state that fact and further indicate interlaboratory variability is likely higher than represented by the study samples since multiple Analysts performing spiking activities were not reflected in the study data."

Response:

EPA respectfully disagrees with the presumption that poorer precision would result from laboratory-prepared Matrix Spike/Matrix Spike Duplicate (MS/MSD) QC samples than from blind samples. At best the commenter's assertion is untested. MS/MSD QC samples are used to assess measurement precision and bias with a relatively short equilibration time between spiked target analytes and the sample matrix and container. Providing spiked samples to participating laboratories enabled EPA to assess integrity of standard solutions, stability of chemicals in the study samples during shipping and storage, and reliability of the sample preparation and analysis procedures to recover the target analytes, some of which may not remain in aqueous solution over time. These sources of variability may not be captured with MS/MSD data due to the relatively limited equilibration time of standard additions with the sample matrix. EPA expects measurement errors related to spiking in the laboratory to be relatively small compared to these other potential sources of bias. LCS samples were prepared and analyzed by each laboratory along with the blind study samples, and isotopically labeled surrogates were added to all study samples and associated QC samples. EPA revised the QC Summary Report to include surrogate and LCS precision and recovery data by laboratory (Tables 6b and 7c-d), and Table 6b also includes descriptive statistics for pooled recovery and within- and between-laboratory standard deviations for target analytes in LCS samples that can be compared with the study sample data presented in Table 1 of Method 8327. Please refer to the QC Summary Report for additional information about measurement variability resulting from spiking activities performed at individual laboratories.

220. Comment:

Commenter 9 noted that Section 7.4.4 of Method 8327 describes preparation of an initial calibration verification (ICV) standard but remarked that the method validation study participants were not required to analyze ICVs. The commenter requested that EPA comment on this omission.

Response:

At the time the internal phase of the multi-laboratory validation study began, a certified second source standard containing all target analytes was not readily available. Therefore, comparability of sources of PFAS target analytes was not included in the scope of the validation study (note the Initial Calibration Verification requirement in the method includes the caveat "if available"). Comparability of PFAS target analyte concentrations in certified reference materials from different sources can be an important aspect of quality assurance, but it was not included as a factor in the validation study design.

221. Comment:

Commenter 9 noted that EPA did not include or summarize “initial demonstration of competency (IDOC) or lower limit of quantitation (LLOQ) trial results” from Phase 1 of the validation study and considered this information to be important for understanding the participating laboratories’ initial successes and failures using the method.

Response:

EPA’s primary interest in collecting Initial Demonstration of Proficiency (IDP) data was to ensure the instrumentation used in the participating laboratories was sufficiently sensitive to provide reliable data for the spiking levels planned for the study and to ensure laboratories were familiar with the electronic data deliverable format. EPA’s validation study Data Quality Objectives (DQOs) were focused on the study sample results and associated instrument and sample preparation quality controls, and these DQOs did not include evaluation of Initial Demonstration of Proficiency (IDP) data. Each of the summary spreadsheets from the study laboratories is included in the docket, and each includes a complete set of quality controls for use in evaluating the sample results therein. Laboratories’ IDP data is not included in the final docket because it does not reflect on the outcomes of the validation study.

222. Comment:

Commenter 9 asked EPA to clarify whether laboratories were blinded to the identity of the unspiked study samples and trip blanks in Phase 2 of the validation study. The commenter noted that EPA did not include study data from five laboratories in the statistics report (one laboratory for not providing results by the submittal deadline and four laboratories as described in Appendix E of the Statistical Summary Report), and the commenter did not consider EPA’s explanation of those disqualifications to be sufficiently detailed. The commenter requested that EPA “elaborate on the difficulties encountered by the laboratories that were eliminated since it is indicative of the problems likely to occur by laboratories trying to implement the method in the future.”

Response:

Participating laboratories were blind to the concentrations of all study samples provided by EPA, but trip blanks were labeled as such. Laboratories were also provided with a randomized analysis order in which to test study samples. EPA made a couple of clarifications to the explanations for why some laboratories' data were excluded in Appendix E of the Statistical Summary Report but considers the information presented therein to be sufficient to support the cautions included in the final versions of Methods 3512 and 8327 in the docket. Data summary spreadsheets submitted by all laboratories are also provided in the docket, including laboratories whose data were excluded. Cautions already present in the method were strengthened in the final versions of Methods 3512 and 8327 to clearly identify the potential for measurement bias resulting from subsampling prior to adding methanol and storing prepared samples and standards in 1:1 methanol-water+0.1% acetic acid in glass containers. The only other issue that was identified in Appendix E was related to instrument stability problems, which could arise from a number of potential sources. The laboratory did not identify a root cause to the study team.

223. Comment:

Commenter 9 noted that the QC Summary Report mentions that laboratories were provided with a draft method to be used for sample preparation and analysis and separate study instructions. The commenter stated "to remain compliant with the review process, the study instructions should have been posted for review/comment."

Response:

EPA has included the Phase IIb study instructions and Phase IIb study protocol in the final docket.

224. Comment:

Commenter 9 remarked that the QC Summary Report states "each laboratory was tasked with striving to meet the recommended acceptance criteria for sample preparation and analysis in the method", which the commenter considered to be "biased direction… that likely… resulted in laboratories optimizing, repeating and undertaking extraordinary steps that will not be undertaken in normal day-to-day operations."

Response:

EPA disagrees with the assertion that study laboratories were asked to do anything inappropriate or extraordinary as participants in this validation study. EPA provided participating laboratories with study instructions, a study protocol with preliminary QC acceptance criteria, and a reporting template, none of which contained biased direction. Laboratories were not asked to repeat steps or take any extraordinary measures to meet the preliminary QC

acceptance criteria EPA provided. Instead, laboratories were asked to report all of the data they generated, regardless of whether the preliminary QC acceptance criteria were met. Participating in the validation study included a number of steps that involved serious effort, including optimizing instrument settings and sample processing steps and finding and eliminating potential sources of background contamination. These steps are also an important part of day-to-day operations in a testing laboratory.

225. Comment:

Commenter 9 remarked that the QC Summary Report notes laboratories received 5-mL aqueous study samples, but laboratories using the method are likely to receive samples at different volumes that are not in pre-weighed containers and will have to precisely determine sample volume in order to determine the appropriate volume of methanol and surrogates to add. The commenter also remarked that laboratories may receive sample containers that are too full to hold a 1:1 dilution. The commenter considered it likely that real-world laboratories will commonly encounter these situations that were not evaluated in the validation study.

Response:

EPA recognizes that laboratories may not consistently receive 5 mL samples for use with this method. However, as long as the provided samples are spiked with a proportional volume of surrogates and diluted 1:1 with methanol, the basic chemistry of the method is the same. Surrogate recovery in every sample provides some evidence that methanol and surrogate additions were scaled appropriately, and Section 11.1 of Method 3512 provides several options for measuring sample volume. If sample containers are more than half full and samples need to be transferred to larger sample containers prior to dilution, solvent rinsing the original sample containers as described in the method will aid in quantitative transfer. As long as the laboratory takes care to blank-check supplies and monitor introduction of contaminants during sample processing with reagent blanks and method blanks, EPA does not anticipate problems for laboratories to process samples like those the commenter described. The number of variables EPA could evaluate in the validation study was limited. In EPA's view, the benefits of including these additional variables was minimal, and the variables that were controlled are unlikely to present problems for implementing the methods or interpreting data.

226. Comment:

Commenter 9 remarked that all target analytes were spiked at the same concentration (either 60 or 200 ppt) for the validation study, which the commenter did not consider to support EPA's claim that the method is appropriate for measurement of PFAS in non-potable waters at concentrations as low as 10 ng/L. The commenter also remarked that matrix suppression interferences cannot be evaluated when all analytes are at similar concentrations as was the case for the study samples. The commenter did not consider the study samples to adequately represent contamination typically encountered in real-world environmental samples, which can have different relative concentrations of target analytes. The commenter also remarked that

EPA did not identify whether a different source of target analytes was used for spiking samples or whether the ratio of branched and linear isomers added to the samples was different from the calibration standards, which the commenter suggested would better represent typical environmental samples.

Response:

The 60 and 200 ng/L spiking levels for the validation study were designed to provide a reasonable test of method performance and generate meaningful data at concentrations near the lifetime health affects levels established for PFOA and PFOS. These spiked sample matrices were not intended to evaluate method performance at or near the Lower Limit of Quantitation (LLOQ). EPA instead evaluated sensitivity based on performance data for LLOQ verifications in each laboratory. Spiking sample matrices at the LLOQ has not been a common practice in other SW-846 method validation studies.

EPA agrees that different isomeric compositions and relative concentrations of target analytes could lead to bias in quantitative analysis, but EPA disagrees with the commenter that these additional variables were essential for the validation study. Varying sources or relative concentrations of target analytes as factors in a multi-laboratory study design is not straightforward and could require a large number of additional samples, with little added benefit. Suppression or enhancement of responses from coeluting target or non-target chemicals is a well-known problem associated with electrospray ionization LC/MS analysis, and Method 8327 recommends including an isotopically-labeled analog of every target analyte to monitor for these types of matrix effects. Where isotopically labeled standards are not available, the method also recommends preparation and analysis of matrix spike QC samples. EPA did not observe signal suppression or enhancement in the study samples, but EPA considers the quality controls included in the method to be generally well-suited for identifying these types of matrix effects when they are encountered in real-world samples.

227. Comment:

Commenter 9 remarked that the QC Summary Report states EPA provided supplies and standards to laboratories “to minimize variables”, but the commenter considered different sources of standards and liquid chromatography columns to be important variables that should have been included as part of the validation study if EPA intended the study to “simulate day-to-day laboratory operation and variability.” The commenter remarked that providing laboratories with the same stock solutions eliminated variation in composition of branched and linear isomers for applicable target analytes, and providing laboratories with the same liquid chromatography (LC) column may have minimized variability in chromatographic resolution and separation of target analytes, especially those with branched and linear isomers.

Response:

The number of factors EPA included in the validation study design was limited to ensure the amount of data laboratories generated was manageable and to focus evaluation of validation data on the most critical aspects of method performance, as with any validation study. Available

sources of the certified reference materials for PFAS target analytes were limited when the validation study began. Several participating laboratories analyzed standards and study samples with both the provided liquid chromatography column and alternative LC columns, but EPA did not provide a detailed evaluation because this comparison was not part of the scope of the validation study. Please refer to EPA's response to comment 14 for related discussion.

228. Comment:

Commenter 9 did not consider the Method 8327 validation study to have included a thorough evaluation of method ruggedness. The commenter stated "Ruggedness testing is the carefully ordered testing of an analytical method while making slight variations in test conditions (as might be expected in routine use) to determine how such variations affect test results. If a variation affects the results significantly, the method restrictions are tightened to minimize this variability." The commenter concluded that "ruggedness testing has not been performed that accommodates the lack of restriction and flexibility in the method. Based on the study performed, method restrictions should be tightened."

Response:

The level of ruggedness testing requested by the commenter is beyond the resources of the program for a multi-laboratory validation study. The method developer conducted a number of tests that were used to inform the options and restrictions included in the validation study protocol and in the final versions of the reference methods. Robustness was tested in that instructions critical for the execution of the method were not included in the initial instructions. Where critical procedural steps were identified that had a negative impact on method performance, they were added into the method text as cautions. Adding more samples would have been an additional burden with limited benefit. Non-conforming data provided by laboratory intercomparison study participants and associated deviations from the study protocol were also used to assess method robustness, and several cautions were added to the method to address known or likely sources of these specific data quality problems.

229. Comment:

Commenter 9 recommended that EPA include an explanation in the QC Summary Report of the "evaluation rubric and data quality objectives used to determine that Draft Method 8327 is fit for the purpose US EPA intended."

Response:

EPA revised the QC Summary Report to provide a more detailed summary of performance of each category of quality control by laboratory and preparation batch; refer to the tables in this report for more information. Please also refer to the Executive Summary in the final docket, which explains EPA's Data Quality Objectives (DQOs) for this validation study and presents a summary of data from the validation study.

230. Comment:

Commenter 9 noted that the QC Summary Report provides a range in laboratory-reported Lower Limits of Quantitation (LLOQs) of "10-20 ng/L …for most target analytes, with higher ranges (up to 40-80 ng/L)," and the commenter also noted the report states that "LLOQ verification standards did not always support the reported LLOQs." The commenter concluded that "Considering the variability and sensitivity issues noted in the report despite how controlled this study was and that four laboratories' data were excluded, this draft method cannot be relied upon for generation of definitive quantitative data used for risk-based decisions."

Response:

EPA respectfully disagrees with this comment. EPA's intent of including the statement cited above in the QC Summary Report was to indicate that laboratories' Lower Limits of Quantitation (LLOQs) in their summary data spreadsheets for the validation study were not always consistent with the LLOQ verification data they provided. Please refer to Table 5b in the revised QC Summary Report, which provides a more detailed summary of verified Lower Limits of Quantitation (LLOQs) by laboratory and preparation batch. Please also refer to the Executive Summary, which describes EPA's Data Quality Objectives for the validation study, including for sensitivity. Laboratories were not required to establish LLOQs of 10 ng/L for all target analytes as a prerequisite for participation in the validation study. Each laboratory using these methods is required to establish and periodically verify LLOQs at which the acceptance criteria for the categories of quality controls in Section 9.0 can consistently be met using the instrumentation, equipment, reagents, supplies and personnel specific to that laboratory. The project team should consult with the laboratory to make decisions about whether the laboratory's implementation of these methods is sufficiently sensitive to achieve the project goals.

231. Comment:

Commenter 9 noted that "Laboratories did meet r^2 criteria and/or % error criteria for 12 of the 24 PFAS at the "lower calibration points," and met the "no or low-abundance qualifier transitions" for three PFAS." The commenter concluded that "Considering the variability and sensitivity issues noted in the report despite how controlled this study was and that four laboratories' data was excluded, this draft method cannot be relied upon for generation of definitive quantitative data used for risk-based decisions." (Note: Given the conclusion, EPA interprets the commenter to have intended to state that the initial calibration "did *not* meet r^2 criteria").

Response:

EPA respectfully disagrees with the commenter's assertion that the initial calibration data were insufficient to support the use of Method 8327 for quantitative analysis. EPA added Tables 2b-c to the revised QC Summary Report to provide a more detailed summary of initial calibration performance by laboratory. EPA also included additional discussion in this report comparing a couple of options in Method 8327 for evaluation of initial calibration fit. As mentioned in the comment above, each laboratory is required to establish and verify Lower Limits of Quantitation

(LLOQs) at levels at which the laboratory can consistently meet the quality control criteria, and whether these LLOQs are sufficient may depend on the specific project application.

232. Comment:

Commenter 9 noted that the QC Summary Report did not include a summary of concentrations at which the participating laboratories met the signal to noise ratio (S/N) criterion of >3 for the 24 PFAS target analytes and requested that EPA "report this important information."

Response:

EPA did not report a summary of S/N for initial calibrations across study laboratories because the concentration of the calibration standard for which S/N was reported was not always apparent in the summary data provided by laboratories. Please refer to EPA's response to comment 111 for discussion related to S/N.

233. Comment:

Commenter 9 remarked that they considered detections of target analytes in blanks from each participating laboratory to be important information that was not included in the Quality Control Summary. The commenter also noted that summary data for trip blanks was not included and considered this information to be important. The commenter further noted that Table 4 included frequencies at which Method Blank (MB) and Reagent Blank (RB) concentrations were <50% of the laboratory reported Lower Limit of Quantitation (LLOQ), and the commenter stated the LLOQs reported by each laboratory were needed to properly understand this summary. The commenter also stated that Table 4 "does not differentiate between not detects and detections at concentrations below < 50% LLOQ," which the commenter considered important to support EPA's statement in the report that "blank contamination was infrequent."

Response:

EPA did not provide a summary of trip blank data, but a summary of unspiked reagent blank samples is included in the Statistical Summary Report, and these unspiked samples were prepared and analyzed in the same manner as trip blanks. Complete results for all trip blanks, reagent blanks and method blanks analyzed by participating laboratories are provided in the summary data spreadsheets included in the docket for each laboratory and preparation batch. EPA also revised the QC Summary Report to include a summary of maximum method blank and reagent blank concentrations by laboratory in Table 4b and a summary of verified LLOQs by laboratory and preparation batch in Table 5b. Tables 4a and 4b focus on method blank and reagent blank concentrations >5 ng/L because the lowest LLOQ evaluated in the validation study was 10 ng/L, and Method 8000D only specifies evaluation of blank contamination > ½ the LLOQ. No attempt was made to differentiate between detects and non-detects below half the LLOQ. Section 9.7.4 of Method 8000D states that the procedure for reporting results below the LLOQ should be specified in an appropriate project planning document, and any results reported below the LLOQ should be qualified appropriately.

234. Comment:

Commenter 9 noted the QC Summary Report states that laboratories met Lower Limit of Quantitation (LLOQ) verification acceptance criteria at a higher frequency at 20 ng/L than at 10 ng/L. The commenter also noted that some laboratories prepared LLOQ verification quality control samples at concentrations of 40 and/or 80 ng/L instead of 10-20 ng/L as recommended. The commenter considered it "reasonable to assume that even after dropping 4 of the 12 laboratories that were invited to participate and returned results within the needed timeframe, the 8 remaining could not meet the target 10 ng/L LLOQ even with biased direction to do so."

Response:

The conclusion above is based on mistaken assumptions and speculation and is not consistent with how the validation study was conducted. The Method 8327 validation study instructions did not require all laboratories to establish Lower Limits of Quantitation (LLOQs) at 10 ng/L as a precondition for participating in the study. Please refer to the revised QC Summary Report for more information about acceptable initial calibration ranges (Table 2c) and LLOQ verifications (Table 5b) by laboratory. Variations in laboratory-verified LLOQs did not lead to problems evaluating the study data against EPA's Data Quality Objectives (DQOs). Please refer to the Executive Summary for more details regarding the validation study DQOs and a summary of study data that was evaluated against each of these DQOs. Please refer to EPA's response to comment 215 for additional discussion related to exclusion of laboratory data. Please refer to EPA's response to comment 224 for additional discussion related to "biased direction."

235. Comment:

Commenter 9 noted that the QC Summary Report states two laboratories "did not meet the LLOQ verification criteria for 6:2 FTS in any batch," which the commenter identified as "a problem with variability and sensitivity with 6:2 FTS that needs further evaluation."

Response:

EPA included Table 5b in the QC Summary Report to provide a summary of verified LLOQs by laboratory and preparation batch. This table shows that the quoted statement from EPA was incorrect. One laboratory did not meet LLOQ verification criteria for 6:2 FTS in any preparation batch, and two laboratories only met the LLOQ verification criteria at the LCS level (160 ng/L, nominal). This statement was corrected in the final report. 6:2 FTS is identified in Method 8327 as not having met the validation study Data Quality Objectives (DQOs), and more detail about the study DQOs and outcomes of the validation study are provided in the Executive Summary. Refer to comment 28 for additional discussion related to 6:2 FTS.

236. Comment:

Commenter 9 considered the stated acceptance limits of 70-130% for LCS recovery to be excessive. The commenter also remarked that the QC Summary Report lists several chemicals for which statistically-derived acceptance limits would be recommended for LCS recovery in Method 8327 because 70-130% recovery may be too narrow. The commenter considered statistically based recovery limits to provide laboratories a "license to fail… without corrective action". The commenter also considered the LCS concentration of 160 ng/L used for the study "to be notable spike concentration that could not consistently be met at a wide criterion of 70-130%." The commenter stated that "much tighter criteria and superior accuracy" could be expected from internal standard or isotope dilution calibration, which could achieve 80-120% or tighter recovery for direct aqueous injection.

Response:

EPA respectfully disagrees with the commenter that preliminary acceptance limits of 70-130% or statistically based acceptance limits for LCS recovery are inappropriate for general use. Many SW-846 determinative methods rely on statistically based acceptance limits for a variety of sample preparation quality controls. The final version of Method 8327 in the docket recommends statistically-derived acceptance limits for recovery of surrogates in field samples and for target analytes in LCS, matrix spike, and LLOQ verification QC samples once the laboratory has generated sufficient performance data, consistent with Method 8000D and other SW-846 determinative methods in which calibration standards do not undergo all of the same preparation steps as field samples. Alternate acceptance criteria may also be useful or necessary for specific project applications, and these requirements should be included in project planning documents and addressed with the testing laboratory, as needed. Please refer to EPA's response to comment 99 for additional discussion related to LCS recovery limits. Please refer to EPA's response to comment 10 for additional discussion related to isotope dilution calibration. Please refer to the Executive Summary for more information related to EPA's Data Quality Objectives (DQOs) for the validation study and a summary of the study data that was evaluated against each of those DQOs.

237. Comment:

Commenter 9 considered the stated 70-130% acceptance limits for surrogate recovery to be excessive. The commenter also remarked that the QC Summary Report listed several surrogates for which statistically-derived acceptance limits would be recommended in Method 8327 because 70-130% recovery may be too narrow. The commenter considered statistically based recovery limits to provide laboratories a "license to fail… without corrective action". The commenter stated that "much tighter criteria and superior accuracy" could be expected from internal standard or isotope dilution calibration, which could achieve 80-120% or tighter recovery for direct aqueous injection. The commenter also noted that the report states 36% of samples had at least one surrogate with recovery outside 70-130% and that surrogate recoveries were similar in study samples and in laboratory prepared QC samples. The commenter interpreted these statements to indicate the method has "a problem with stability and accuracy."

Response:

EPA respectfully disagrees with the commenter that preliminary acceptance limits of 70-130% or statistically based acceptance limits for surrogate recovery are inappropriate. The preliminary acceptance criterion of 70-130% recovery used for surrogates in study samples and target analytes in LCS samples was based on single-laboratory performance data. Statistically-derived acceptance limits are recommended in the final version of Method 8327 for recovery of surrogates in field samples and for target analytes in LCS, matrix spike, and LLOQ verification QC samples, consistent with Method 8000D and with other SW-846 determinative methods in which calibration standards do not undergo all of the same preparation steps as field samples. Please refer to the response to comment 236 for additional discussion related to LCS recovery.

238. Comment:

Commenter 9 noted that the QC Summary Report states that cautions will be included in Method 8327 related to "minimum organic solvent content of higher concentration solutions of target compounds and/or surrogates and avoiding storage of calibration standards and sample extracts in glass containers." The commenter stated, "If these PFAS compounds are not stable in the water medium and being stored in glass at the sub-ppb range, one needs to question the validity of analysis for these compounds in typical environmental media."

Response:

EPA respectfully disagrees with the assertion that stability problems observed in solutions stored in glass containers call into question validity of data generated with these test methods. Study samples that were prepared at a central location were shipped to laboratories and analyzed in days to weeks after preparation, and 23 of 24 target analytes met EPA's Data Quality Objectives for measurement precision and bias. Please refer to the Executive Summary in the final docket for more information. These data demonstrated that limited exposure of standards and prepared samples to glass autosampler vials did not lead to problems meeting the method quality control criteria. The final versions of Methods 3512 and Method 8327 emphasize this caution.

239. Comment:

Commenter 9 stated "the lack of reproducibility of the results for some of the analytes shown on Statistical Report Tables A1-A24 has not been addressed anywhere. For example, for PFHxA in WW, the final eight study laboratory results varied from 0 to 60.896 ng/L. Of the four internal laboratories (where results ranged from 0 to 10.931 ng/L), one laboratory did not detect PFHxA in any of the five replicate WW samples, two more laboratories did not detect PFHxA in at least one of the five replicate WW samples (as their minimum result was 0), and the last internal laboratory had a maximum detection of 8.82 ng/L. This is completely at odds with the results generated by the four external laboratories, where the results were between 41.371 ng/L and 60.896 ng/L. Similar issues were observed for some of the other analytes – even PFOS in WW

varied from 0 to 42.474 ng/L, with similar discrepancies between the internal and external laboratories in the study." The commenter asked how these discrepancies demonstrate method ruggedness, and why the results of the background samples were just summarized in the Statistical Summary Report without being evaluated. The commenter "assumed that the background samples represent the closest thing to real-world samples represented by the study."

Response:

As described in the QC Summary Report, the validation study was conducted in two phases. The wastewater matrix in the external phase of the validation study had identifiable background of some target analytes in the unspiked samples, including PFPeA and PFHxA measured in the range of 40-60 ng/L and lower concentrations of PFOA and other short-chain perfluorinated carboxylic acids. The true values of these target analytes in this matrix were not established, and evaluation of background concentrations of target analytes in unspiked sample matrices or comparison of results between laboratories was not part of the Data Quality Objectives EPA defined for the validation study. However, these data provide additional support for comparability of low concentration measurements of some target analytes in the wastewater matrix across multiple laboratories.

Appendix A: Cross-Reference of Commenter Numbers with Commenter/Organization Names

Commenter #	Commenter Name/Organization
Commenter 1	Anonymous (1)
Commenter 2	Anonymous (2)
Commenter 3	Anonymous (3)
Commenter 4	Brian Mader, The 3M Company
Commenter 5	John Halverson, Program Manager, Contaminated Sites Program, Division of Spill Prevention and Response, Alaska Department of Conservation
Commenter 6	Katharine E. Morgan, President, ASTM International
Commenter 7	Michael F. Delaney, Laboratory Consultant
Commenter 8	Maureen Sullivan, Deputy Assistant of Secretary of Defense (Environment), Office of the Assistant Secretary of Defense
Commenter 9	Rock Vitale, Technical Director of Chemistry/Principal, *et. al.,* Environmental Standards, Inc.; Prepared for American Petroleum Institute
Commenter 10	Charles Neslund, Scientific Officer, Eurofins Lancaster Laboratories Environmental
Commenter 11	Karla Buechler, Corporate Technical Director, Eurofins TestAmerica
Commenter 12	Hilda Arellano, Technical Services, Environmental Quality Office, Ford Motor Company
Commenter 13	Integral Consulting, Inc.
Commenter 14	Naoko Munakata, Supervising Engineer, Reuse and Compliance Section, Sanitation Districts of Los Angeles County
Commenter 15	Isaak Murshak, Vice President, Merit Laboratories, Inc.
Commenter 16	Steve Silver, Executive Director, Michigan PFAS Action Response Team, Department of Environment, Great Lakes, and Energy, State of Michigan
Commenter 17	Not used
Commenter 18	Emily Remmel, Director, Regulatory Affairs, National Association of Clean Water Agencies

Commenter 19	Jeffery S. Hannapel, The Policy Group, On Behalf of the National Association for Surface Finishing
Commenter 20	Nancy C. Rothman, New Environmental Horizons, Inc.
Commenter 21	Dana M. Maikels, Bureau of Technical Support, Division of Environmental Remediation, New York State Department of Environmental Conservation
Commenter 22	Patrick McDonnell, Secretary, Pennsylvania Department of Environmental Protection
Commenter 23	Agustin Pierri, Technical Director, Weck Laboratories, Inc.
Commenter 24	Andy Horn, Westwater Hydrology LLC