United States Environmental Protection Agency

Office of Water

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Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program

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U.S. Environmental Protection Agency
Office of Water
Engineering and Analysis Division
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Washington, DC 20460

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Foreword

This document ("protocol") provides guidance on how the U.S. Environment Protection Agency (EPA) will evaluate certain test procedures under its National Alternate Test Procedure program for inclusion as a new, approved 40 CFR Part 136 method ("new method"). The protocol applies to new alternate test procedures (ATP) for measuring an organic or inorganic analyte for which there is already an existing Part 136 method when the new method uses a *different* determinative technique to measure the analyte. The protocol outlines in substantial detail the kind of information and evidentiary showing EPA would expect is necessary to demonstrate the suitability of a new method for approval and inclusion in Part 136 (§ 136.4(c)). This protocol also includes guidance regarding obtaining approval of new methods for measurement of method-defined analytes or parameters (MDPs) that use a determinative technique that is different than the existing Part 136 methods.

The protocol provides guidance for validation, submission, and EPA review of ATP applications under EPA's National ATP Program to use new methods, including new MDP methods, for inorganic and organic analytes for which there is an approved Part 136 method. The protocol provides supplementary information for complying with the ATP requirements at 40 CFR 136.4 and 136.5

This protocol supersedes the 2016 version of the *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program.* With respect to ATP applications for new methods that measure MDPs, this guidance recommends side-by-side comparison studies to validate that there are no systematic differences in performance between the new method and the EPA-approved methods. This protocol continues the recommended current practices for new method ATP applications involving other types of methods for measurement of organic and inorganic analytes (i.e., applicants should conduct validation studies in the recommended number of laboratories depending upon the type of approval being sought to develop quality control (QC) acceptance criteria associated with target analyte(s) and the determinative technique identified in the new method).

Under EPA's ATP program, in certain circumstances, a method developer may apply for approval for the use of a new method to test for a specific regulated constituent. The recommended procedures described herein will likely expedite the approval of new methods for organic and inorganic analytes, encourage the development of innovative technologies, and enhance the overall utility of the EPA-approved methods for compliance monitoring under the National Pollution Discharge Elimination System (NPDES) permit program.

Disclaimer

This guidance generally describes the approval process for EPA's program for establishing test procedures for organic and inorganic analytes that are used in Clean Water Act programs and codified at 40 CFR Part 136. It describes EPA's conclusions about the types of data and information EPA will need in order to evaluate whether to approve any particular ATP for such analytes. It includes a model application form for use when requesting EPA approval for ATPs for such analytes. Although the guidance provides additional explanation of EPA's requirements, it does not alter or substitute for any of the regulations at 40 CFR Part 136. The guidance, including the model application form, is not a rule and is not legally enforceable. It does not confer legal rights or impose legal obligations on any federal, state agency or any member of the public. It does not create any rights, substantive or procedural, enforceable at law by a party to litigation with EPA or the United States. In the event there is an apparent conflict between the guidance and any statute or regulation, the guidance is not controlling. EPA has made every effort to ensure the accuracy of information in the guidance, but the requirements for EPA approval of test

procedures for use in its CWA programs are determined by the relevant statutes, regulations or other legally binding requirements.

This protocol represents EPA's "best thinking" about the information that is useful in making the determination of whether or not to approve use of any new method for organic and inorganic analytes. This guidance document reflects EPA views about what data and information sound scientific practice would require for approval of a new method or an alternate test procedure for such analytes. Where the guidance uses the word "should" or in some cases "must," this is only intended to apprise the applicant of the kind of information that, in EPA's view, will demonstrate the adequacy of a given method for use under the CWA and thus its suitability for EPA approval. Applicants may provide other data or information for use in EPA's determination and remain free to deviate from the recommendations EPA has provided here. EPA will make the decision to approve or disapprove any new method for such analytes based on the record before it, and that decision is subject to challenge and judicial review.

40 CFR 136.4 and 136.5 establish the procedures and regulatory requirements for applying for and for EPA approval of alternate test procedures for nationwide use and for limited use. The regulations require submission of an application that, among other things, provides comparability data for the performance of the alternate test procedure as compared to the performance of the approved Part 136 method for which it is a proposed alternative. (40 CFR 136.4(a)(4) and 40 CFR 136.5(a)(5)). This guidance explains in more detail the information that EPA expects will be necessary for EPA to determine comparability or justify using the alternate test procedures instead of the approved Part 136 method for organic and inorganic analytes.

EPA may decide to revise the guidance without public notice. The public may offer suggestions to EPA for clarifications at any time.

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Questions or comments regarding this document or the ATP program should be directed to:

Lem Walker
Clean Water Act ATP Coordinator
U.S. Environmental Protection Agency
Office of Science and Technology
Engineering and Analysis Division (EAD)
1200 Pennsylvania Avenue, NW
Mail Code - 4303T
Washington, DC 20460
Fax: (202) 566-1053
walker.lemuel@epa.gov

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1.0 INTRODUCTION

1.1 Background and Objectives

In accordance with section 304(h) of the Clean Water Act (CWA), the U.S. Environmental Protection Agency (EPA) promulgates guidelines establishing test procedures (analytical methods) for the analysis of pollutants. EPA regulations require the use of these methods where measurements of waste constituents are required in applications for National Pollutant Discharge Elimination System (NPDES) permits or for reports required under NPDES permits. 40 CFR 136.1. EPA has codified these approved test procedures in the Code of Federal Regulations (CFR) at 40 CFR Part 136. For the purposes of this protocol, these test procedures are referred to as "EPA-approved" methods, regardless of whether they were developed by EPA, a voluntary consensus standards body (VCSB) such as ASTM International or Standard Methods or by another government entity such as the U.S. Geological Survey (USGS).

EPA's regulations at 40 CFR 136.4 and 136.5 also establish procedures for EPA to review and approve the use of an alternate test procedure (ATP) in place of an EPA-approved method. These regulations govern the Agency's Alternate Test Procedure (ATP) program for CWA methods[1]. Section 136.4 describes the process for obtaining approval for nationwide use of an ATP. Section 136.4(a) first requires a written application for review of an ATP for nationwide use. Required elements of that application include, among other things, a detailed description of the proposed ATP and studies confirming the general applicability of the ATP for analysis of the pollutant or parameter for which approval is requested. The applicant must also provide comparability data for the performance of the ATP as compared to the existing approved method (§ 136.4(a)(4)). The National Coordinator of the ATP program reviews the application and notifies the applicant of its suitability for use in CWA programs (§136.4(c)). If approval is recommended, EPA will propose to amend Part 136 to include the ATP and following public comment make a final decision on approving the ATP. In the event that the National Coordinator recommends against approval, the Coordinator will specify what additional information might lead to a recommendation for approval. These requirements are the basis for EPA's CWA ATP program administered by the Office of Water, Office of Science and Technology, Engineering and Analysis Division (EAD). Section 136.5 describes the process for obtaining approval for limited use of an ATP. Section 136.5 first requires a written application for review of an ATP for limited use to be submitted to the director of the State agency having responsibility for issuance of NPDES permits in cases where the request for use of an ATP concerns use in a State with an NPDES permit program approved pursuant to Section 403 for the Clean Water Act. In cases where the request is made in a State that has not been grated authority to administer the NPDES permit program or in cases where the State is the applicant, the request is submitted directly to the Regional ATP Coordinator who has the final authority to approve or reject applications for use of an ATP. Limited use approval may be restricted to use by a single facility on one or more discharges. In cases where the National ATP Coordinator has approved an applicant's request for nationwide use of an ATP, an applicant may request limited use approval of the method under §136.5. In these instances, limited use approval maybe extended all dischargers or facilities (and their associated laboratories) specified in the approval for the Region at the discretion of the Regional ATP Coordinator. The Regional ATP Coordinator will forward a copy of every approval and rejection notification to the National Alternate Test Procedure Coordinator.

In addition, as specified at 40 CFR 136.6, EPA allows users to make certain modifications to an approved method to address matrix interferences without the extensive review and approval process specified for an alternate test procedure at 40 CFR 136.4 and 136.5. Acceptable reasons for an analyst to modify a method include analytical practices that lower detection limits, improve precision, reduce interferences,

[1] EPA also promulgates analytical methods under the Safe Drinking Water Act (SDWA) and has a similar ATP program. This protocol only addresses the CWA ATP program and does not apply to the SDWA ATP program.

lower laboratory costs, and promote environmental stewardship by reducing generation of laboratory wastes. Acceptable modifications may use existing or emerging analytical technologies that achieve these ends provided that they do not depart substantially from the underlying chemical principles in methods currently approved in Part 136. The flexibility to modify methods without the need for approval as an ATP and the associated requirements that must be met before such modified methods may be used for CWA compliance monitoring are described in more detail at 40 CFR 136.6.

This protocol sets out EPA's views about what information and data will support approval of a new method for organic and inorganic analytes under the ATP program for use in NPDES compliance monitoring. As such, it provides a detailed explanation of the kinds of information and studies that generally will support a finding of a method's comparability to an existing approved method and thus its appropriateness for approval as an ATP for such analytes. The primary feature of a "new method" addressed in this protocol that distinguishes it from other ATPs is that a new method employs a different determinative technique than any of the existing EPA-approved methods for the parameter. Specifically, a new method for purposes of this protocol is defined as a test procedure that:

- is written in standard EPA format as detailed in Appendix D of this protocol
- contains standardized QC elements with associated QC acceptance criteria
- employs a determinative technique for an analyte or parameter of concern that differs from determinative techniques employed for that analyte in methods previously approved at 40 CFR Part 136, and
- employs a determinative technique that is at least as sensitive and/or selective as the determinative techniques in all methods previously approved for the analyte or parameter.

The Office of Water treats new methods from sources other than EPA, VCSBs and other federal government entities as a subset of ATPs and reviews such new methods under the framework of the existing CWA ATP program. The ATP approval program provides chemists with the opportunity to utilize their best professional judgment to enhance compliance monitoring. Approval for a new method may be sought when, for example, the new method reduces analytical costs, overcomes matrix interference problems, improves laboratory productivity, or reduces the amount of hazardous materials used and/or produced in the laboratory. The ATP approval program thus may serve as a mechanism for gaining approval of innovative technologies for use in compliance monitoring programs. An applicant may apply to gain approval for the use of a new method for determination of an analyte of interest to the NPDES monitoring program by developing and validating the new method and developing QC acceptance criteria for the new method, for example by following procedures such as those described in this document. This version of the new method protocol describes validation processes for modifying methods that measure MDPs. Details regarding these MDP validation procedures are found in Appendix H of this document.

Note: New methods developed by voluntary consensus standard bodies (VCSBs) and other federal agencies are *not* processed for approval under the ATP Program. Instead EPA has developed a separate path to approval for these keeping with the National Technology Transfer and Advancement Act (NTTAA). EPA considers VCSB methods and those from other agencies in regulatory actions when periodically updating the list of approved methods at 40 CFR Part 136. EPA's *"Checklist for Methods to be Considered by EPA for Use in Compliance Monitoring Programs under the Clean Water Act"* (Appendix I) provides a list of items and information EPA considers in evaluating all new, updated, and ATP methods for use in wastewater compliance monitoring for approval.

1.2 Tiered System for Validation of New Methods

EPA recognizes that a formal interlaboratory method validation may not be necessary to demonstrate suitability for approval for all situations and may be prohibitively costly to implement, especially for small laboratories and regulated entities. Therefore, the protocol describes a three-tiered, cost-effective approach to method validation that would tailor the validation study to reflect the intended use for the method. When considering how to demonstrate whether a new method for organic and inorganic analytes is suitable for approval, an applicant should review the tiers below and decide what the most appropriate tier for the applicant's new method is based on its intended use and develop QC acceptance criteria accordingly. Appendix G of this protocol describes procedures that may be used for developing these QC acceptance criteria. The three method validation tiers are listed below.

Tier 1: New methods for use only by a single laboratory. These types of new methods should be validated for use in one or more matrix type(s). EPA approval of a Tier 1 new method would generally require successful single-laboratory testing in the matrix type(s) of interest. Tier 1 new methods are reviewed by the State issuing the NPDES permit, where the State is not the requesting party, and forwarded to EPA Regional staff, along with a recommendation for or against approval. Where the State is the requesting party, applications for Tier 1 new methods are sent directly to the EPA Regional staff.

Tier 2: New methods for use by all laboratories for nationwide use for only one matrix type. The application for approval should generally demonstrate successful testing of the new method in a three-laboratory validation study. Tier 2 new methods will be reviewed by the National ATP staff at EPA Headquarters and if positively reviewed, will be recommended for approval. These methods are then proposed for promulgation in the CFR

Tier 3: New methods for use by all laboratories (nationwide use) for all matrix types. The application for approval should generally demonstrate successful testing of the new method in a nine-laboratory validation study. Tier 3 new methods are reviewed by the National ATP staff at EPA Headquarters and if positively reviewed are recommended for approval. These methods are then proposed for promulgation in the CFR As specified at 40 CFR 136.4(c)(5), whenever the National Coordinator has approved an applicant's request for nationwide use of an alternate test procedure, any person may request an approval of the method for limited use under § 136.5 from the EPA Region. In these instances, a Regional ATP Coordinator may choose to use the authority specified at 40 CFR 136.5(d) to all dischargers or facilities (and their associated laboratories) specified in the approval for the Region.

Note: Matrix type, in the context of these tiers, is defined as a sample medium (e.g., air, soil, water, sludge) with common characteristics across a given industrial subcategory. For example, C-stage effluents from chlorine bleach mills, effluent from the continuous casting subcategory of the iron and steel industrial category, publicly owned treatment works (POTW) sludge, and in-process streams in the Atlantic and Gulf Coast Hand-shucked Oyster Processing subcategory are each a matrix type. (A list of industrial categories with existing effluent guidelines can be found at: https://www.epa.gov/eg/industrial-effluent-guidelines).

1.3 Method-Defined Analytes

As specified at 40 CFR 136.6, the term *"method-defined analyte"* means an analyte (or parameter) that is defined solely by the method used to determine the analyte (generically referred to in this document as a method-defined parameter or MDP). Such an analyte may be a physical parameter, a parameter that is not a specific chemical, or a parameter that may be comprised of a number of substances. Examples include, but are not limited to:

- Acidity,
- Alkalinity,
- Biological oxygen demand (BOD),
- Chemical oxygen demand (COD),
- Color,
- Oil and grease,
- pH (hydrogen ion),
- Conductivity (specific conductance)
- Temperature,
- Total dissolved solids (TDS),
- Total organic carbon (TOC),
- Total suspended solids (TSS),
- Total phenolics, and
- Turbidity.

Modifications to methods that measure MDPs have the potential to change what is being measured. Therefore, *any* modifications to those methods beyond that specifically allowed in the approved methods require EPA review and approval as alternate test procedures by the appropriate approval authority (see Table 1).

In order to more clearly distinguish the new method requirements for MDPs from those for the more traditional type of analytes, the discussion of the data and information that, in EPA's view, will generally demonstrate the suitability of the new method for measurement of MDPs has been placed in Appendix H of this document.

2.0 OVERVIEW OF THE NEW METHOD ATP APPROVAL PROCESS

The process for obtaining approval of a new method for organic and inorganic analytes is summarized in Figure 1. Depending on the tier, new methods may be reviewed by (1) the State authority that issues the NPDES permit, and/or by EPA Regional staff, or (2) EPA Headquarters staff. The relevant authority will review the application, including the justification for the new method provided by the applicant, a plan for validation of the new method and all of the application materials. Where the State is not the requesting party, the State will review Tier 1 new method applications and forward these to EPA Regional staff with a recommendation for or against approval. Where the State is the requesting party, the EPA Regional staff will review the Tier 1 new method applications. If, after initial review, EPA Headquarters accepts a Tier 2 or Tier 3 application, the applicant should move forward with preparing a method development and validation study plan in consultation with National ATP staff.

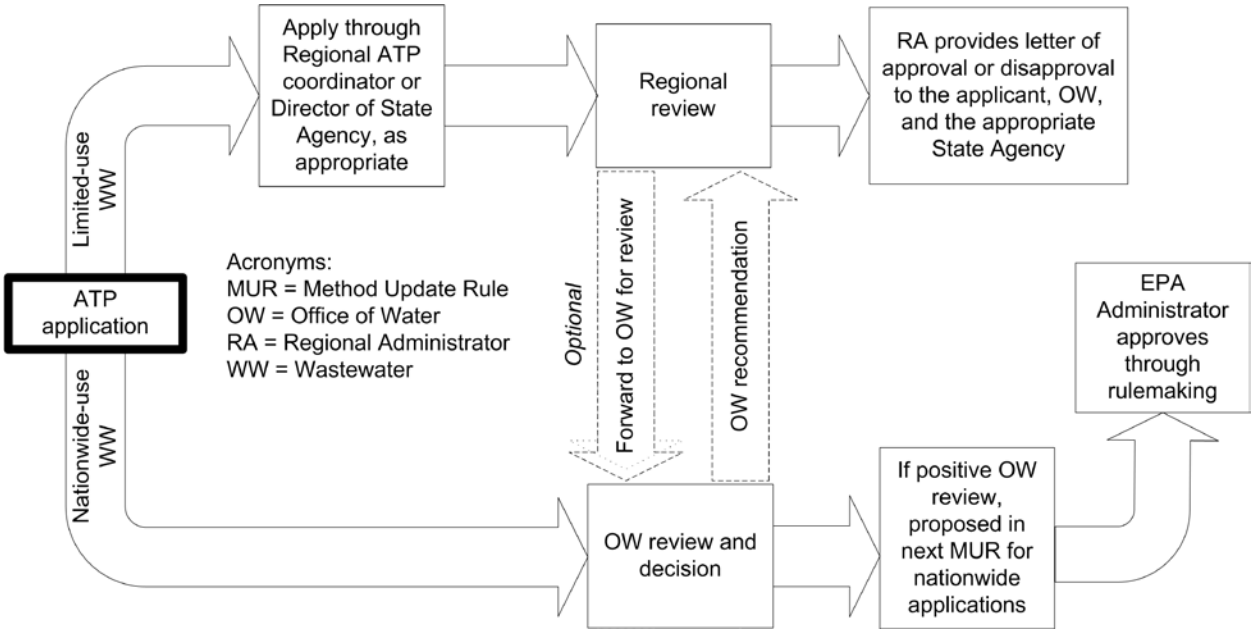

Figure 1. Flowchart summarizing the new method ATP application process for methods under the CWA Program

In order to expedite the approval process, the applicant should submit to EPA its plan for developing the necessary data to demonstrate the suitability of the new method for approval. For Tier 2 and Tier 3 new methods, once the applicant has received EPA's view concerning its study plan, the applicant should move forward with the study and submit the study report to the ATP staff. If the validation study results confirm that the applicant's method is sufficiently rugged and provides data of comparable quality, EPA will generally notify the applicant that it intends to pursue approval via the rulemaking process. If this is not the case, ATP staff may identify additional information or data required. If the laboratory studies fail to satisfactorily verify the comparability of the applicant's method, the applicant should address the problems encountered and follow-up with further laboratory validation studies.

3.0 APPLICATION

This section describes the information that EPA would expect in an application for approval of a new method for organic and inorganic analytes to demonstrate its appropriateness for approval and provides information on the approval authorities for the three tiered approach described in Section 1.2 of this protocol. This section also describes how to treat any proprietary information submitted with an application.

Note: Where the State is not the requesting party, Tier 1, Limited Use new methods are subject to State authority review prior to EPA Regional approval. State authorities may have additional requirements and/or authority-specific application forms that are beyond the scope of this protocol. Therefore, applicants for Tier 1 new methods should consult such authorities regarding Tier 1 ATP requirements.

Applications may be submitted by email, in hardcopy, or on electronic media by U.S. mail or other carrier. Hard copy applications and supporting documentation should be submitted in triplicate. Applicants are advised to consult the recipient before submitting large files via email.

3.1 Submission Addresses

A summary of where to submit new method applications and the approval authorities for each tier is provided in Table 1.

Table 1: Submission of Applications for Approval of New Methods

Tier	Level of Use	Typical Applicant	Submit Application to[1]	Approval Authority
Tier 1	Limited Use for Wastewater[2]	EPA Regional laboratories, States, commercial laboratories, individual dischargers, or permittees in States that do not have authority to implement the NPDES permit program or in cases where the State is the applicant.	EPA Regional ATP Coordinator[3]	EPA Regional ATP Coordinator (as designated by the EPA Regional Administrator)
		Commercial laboratories, individual dischargers, or permittees in States that have authority to implement the NPDES permit program	Director of State or Agency issuing the NPDES permit[4]	
Tier 2	Nationwide Use in a Single Wastewater Matrix Type	All applicants	National ATP Coordinator, EPA Headquarters	EPA Administrator
Tier 3	Nationwide Use in All Wastewater Matrix Types	All applicants	National ATP Coordinator, EPA Headquarters	EPA Administrator

[1] See Appendix C for EPA addresses.

[2] Per 40 C.F.R 136.4(c)(5): "Whenever the National Coordinator has approved an applicant's request for nationwide use of an alternate test procedure, any person may request an approval of the method for limited use under § 136.5 from the EPA Region." In these instances, limited use approval maybe extended all dischargers or facilities (and their associated laboratories) specified in the approval for the Region (limited use approval under § 136.5) at the discretion of the Regional ATP Coordinator.

[3] The Regional ATP Coordinator may choose to request assistance with the Tier 1 (limited use) applications from the National ATP Coordinator for an approval recommendation.

[4] Per 40 CFR 136.5, in States with authority to issue NPDES permits, the State agency has primary responsibility for reviewing Tier 1 new method applications. The State agency will forward the application to the Regional ATP Coordinator with a recommendation for or against approval. Where the State is the applicant for the ATP, the application goes directly to the Regional ATP Coordinator.

On receipt of the application, the ATP Coordinator will assign an identification number to the application. The applicant should use the identification number in all future communications about the application.

3.2 Application Information

A copy of a model new method application form is included in Appendix A. The information requested on the application form includes the following:

- Name and address of the applicant,
- Application submission date,
- Method number and title of the proposed new method,
- Analytes(s) for which the new method is proposed,
- Level of use desired (i.e., limited use or nationwide use),
- Tier at which the proposed new method will be validated, and
- Applicant's NPDES permit number, issuing agency, type of permit, and the discharge serial number (if applicable).

In addition, the applicants should provide the following items:

- The proposed new method prepared in standard EPA method format,
- A validation study plan,
- The method validation study report, including supporting data,
- For nationwide applications that will undergo rulemaking, method development information and documentation that EPA can use in preparing the preamble and docket for the proposed rule, and
- For limited use applications, applicants should identify the NPDES permit numbers for all discharges for which the applicant is seeking approval to apply the new method (if applicable).

Note 1: Not all of these documents would need to be submitted with the initial application. The applicant should submit a validation study plan for EPA review and comment before proceeding with new method validation. Recommended study plan elements are described in Appendix E of this protocol.

Note 2: As stated in Section 1.3, the information that should demonstrate the suitability for approval of new methods that measure MDPs may be found in Appendix H of this document.

The elements that should be provided for an application at each tier are presented in Table 2. For Tier 2 and 3 applications, the National ATP Coordinator at EPA Headquarters will not process an application until the Coordinator determines that the applicant has submitted adequate information to evaluate the application. As noted at the beginning of Section 3.0, Tier 1 applicants should consult the relevant State authority issuing the NPDES permit to determine if there are also State requirements for those applications.

Table 2. Recommended Application Elements

Tier	Level of Use	Application Elements
Tier 1	Limited Use	• Completed application form submitted to the EPA Regional ATP Coordinator or the Director of State Agency issuing the NPDES permit • Justification for the New Method • Method in EPA format • Validation Study Plan[1] • Validation study report
Tier 2	Nationwide Use	• Completed application form submitted to National ATP Coordinator, EPA Headquarters • Justification for the New Method • Method in EPA format • Validation Study Plan[1] • Validation study report • Method information and documentation
Tier 3		

[1] The applicant should submit a validation study plan with the initial application for EPA review and comment before proceeding with the study.

3.2.1 Justification for the New Method

Because EPA review and evaluation of new methods can entail considerable effort, EPA strives to minimize the submission of unnecessary new methods or new methods that EPA has declined to approve. Therefore, the entity that proposes a new method should provide a brief justification for why the new method is being proposed. Examples of useful justifications include, but are not limited to:

- The new method successfully overcomes some or all of the interferences associated with the approved methods,
- The new method reduces the amount of hazardous wastes generated by the laboratory,
- The cost of or time required for analyses is reduced, or
- The quality of the data is improved.

The Agency acknowledges that there may be some trade-offs between strict QC acceptance limits and encouraging use of potentially beneficial alternate methods. For example, a proposed new method may be far more rapid and less expensive to perform, but have slightly lower precision than the currently approved methods for a given analyte. Depending on the chemical being measured, ATP staff may consider the new method application because the new method could allow more frequent monitoring with no added cost. More frequent monitoring may result in enhanced information quality for that chemical. The Agency may consider less strict QC acceptance limits for a given new method, depending on the analyte and the benefits likely to be realized.

It is highly recommended that the method developer consult with ATP staff concerning their proposed candidate method and its justification prior to extensive method development. Candidate methods that are insufficiently justified will not be considered further.

3.2.2 EPA Method Format

In accordance with the standard EPA format originally developed by EPA's Environmental Monitoring Management Council in 1996 (Reference 4), methods should contain 17 specific topical sections in a designated order. These 17 sections are listed in Appendix D. Any additional numbered sections should be inserted starting with Section 11.0, *Procedure*, as appropriate for a particular method. For detailed

information on the EPA format for proposed methods, refer to *Guidelines and Format for Methods to Be Proposed at 40 CFR Part 136 or Part 141* (Guidelines and Format document), EPA-821-B-96-003.

3.2.3 Validation Study Report

The applicant should conduct a validation study of the new method that meets the validation study design described in Section 4.2 of this protocol. Once the validation study is complete, the applicant should prepare a comprehensive report on the validation study and submit a copy of that report with the new method application. The validation study report should include the following elements, which are described further in Appendix E:

- Background
- Study Design and Objectives
- Study Implementation
- Data Reporting and Validation
- Results
- Data Analysis/Discussion
- Conclusions
- Appendix A - The Method
- Appendix B - Validation Study Plan
- Appendix C - Supporting Data (Raw Data and Example Calculations)

3.2.4 Method Information and Documentation

For Tier 2 and 3 applications, a successful new method will be approved by the EPA Administrator following rulemaking. In these cases, in order to expedite the approval process, the applicant should provide information and documentation that will aid EPA in preparing the preamble and docket for publication of a proposed rule in the *Federal Register*. Specifically, it will be useful for the applicant to submit information that:

- Defines the purpose and intended use of the method.
- States what the method is based upon, noting any relationship of the method to other existing analytical methods and indicating whether the method is associated with a sampling method.
- Identifies the matrix type(s) for which the method has been found satisfactory.
- Describes method limitations and indicates any means of recognizing cases where the method may not be applicable to the specific matrix types.
- Outlines the basic steps involved in sample and data analysis.
- Lists options within the method, if applicable.
- Describes and discusses the validation study in a study report that includes study design and objectives, study limitations, study management, technical approach, data reporting and validation, results, data analysis discussion (including, development of QC acceptance criteria), and conclusions.
- Copies of all relevant supporting documents used in developing the new method (including any other studies conducted during method development and validation), for EPA's possible inclusion in the rule docket.

Previous method rules that may serve as examples of the type of information and the appropriate level of detail necessary include: 49 FR 43234, October 26, 1984; 56 FR 5090, February 7, 1991; 60 FR 53988, October 18, 1995; and 61 FR 1730, January 23, 1996.

3.3 Proprietary Information in Applications

All information provided to the Federal government is subject to Freedom of Information Act requirements. Therefore, any information submitted with the proposed new method application that the applicant considers proprietary **must** be marked as "business confidential." EPA staff will handle such information according to the regulations in subparts A and B of 40 CFR Part 2.

In accordance with 40 CFR 2.203, a business that submits information to EPA may assert a business confidentiality claim covering the information by placing on (or attaching to) the information at the time it is submitted to EPA, a cover sheet, stamped or typed legend, or other suitable form of notice employing language such as *trade secret*, *proprietary*, or *company confidential*.

Note: Confidential Business Information (CBI) must be submitted as hard copy and must not be emailed.

Confidential claims to portions of otherwise non-confidential documents should be clearly identified by the business, and may be submitted separately to facilitate identification and handling by EPA. If the business desires confidential treatment only until a certain date, or until the occurrence of a certain event, the notice should state this. However, applicants are advised that any methods to be proposed in the *Federal Register* cannot involve claims of confidential business information.

If a claim of business confidentiality is not made at the time of submission, EPA will make such efforts as are administratively practicable to associate a late claim with copies of previously submitted information in EPA files. However, EPA cannot ensure that such efforts will be effective in light of the possibility of prior disclosure or widespread prior dissemination of the information.

4.0 METHOD VALIDATION

4.1 Introduction

New method validation is the process by which an applicant demonstrates that the new method accurately measures the concentration of an analyte in an environmental sample. The validation recommendations described below were developed to reflect the level of intended use of the new method. This is accomplished through a three-tiered approach, as shown in Table 3.

Table 3. Tiered Validation Strategy

Tier	Laboratory Use	Applicable to . . .
Tier 1	Limited use[1]	One or more matrix types from one or more industries. Approved in Regions for use within the Region[2]
Tier 2	All Laboratories (Nationwide use)	One matrix type[3] within one industrial subcategory
Tier 3	All Laboratories (Nationwide use)	All matrix types[3] from all industrial subcategories

[1] Whenever the National Coordinator has approved an applicant's request for nationwide use of an alternate test procedure, any person may request an approval of the method for limited use under 40 CFR 136.5 from the EPA Region. (40 CFR 136.4(c)(5)). In these instances, limited use approval may be extended to all dischargers or facilities (and their associated laboratories) specified in the approval for the Region at the discretion of the Regional ATP Coordinator (40 CFR 136.5(d)).

[2] See 40 CFR 136.5.

[3] Section 4.2 provides more information on the matrix types applicable to each tier.

Please contact the appropriate Regional ATP Coordinator for specific method validation recommendations applicable to Tier 1 new method ATPs. Methods intended for multi-laboratory use in a given industrial subcategory (Tier 2), or for multi-laboratory use for all industrial subcategories (Tier 3), should be validated through interlaboratory testing as described in the Section 4.2.

4.2 Summary of Validation Study Designs

Approval of new methods will require the applicant to show that ATP performs comparably to an existing Part 136 method. That is, the applicant should validate that the ATP is capable of yielding reliable data for compliance monitoring purposes. Applicants should demonstrate acceptable method performance and to develop QC acceptance criteria associated with different combinations of regulated analyte and determinative technique based on the results of their validation study. Method performance including ruggedness, sensitivity and QC acceptance criteria developed for the new method will be evaluated by EPA to determine if it meets the needs of the NPDES compliance monitoring program.

All validation study results should be documented in accordance with the validation study designs outlined below. Table 4 and Sections 4.2.1 – 4.2.3 below summarize the validation study designs for new methods that measure non-MDPs in wastewater at each of the three tier levels.

All new method ATPs must be approved by the appropriate approval authority before they can be used or reported for compliance monitoring.

Table 4. Summary of Recommended Validation Approaches for Non-MDP Wastewater New Method Procedures[(1)]

	Number of		Number of Analyses						
Method Application	**Labs**	**Matrix types**	**Back-ground Analysis**	**IPR-reagent water[(2)]**	**IPR-sample matrix[(3)]**	**PT Sample[(4)]**	**MS/ MSD**	**MDL[(5)]**	**Total**
Tier 1 - Single-lab First matrix type	1	1	1	4	4	1	0	14	24
Each additional matrix type (8 max.)	1	1-8	1-8	0[(6)]	0	0	2[(7)] (16 max)	0[(6)]	3 (24 max)
Tier 2 - Multi-lab, single matrix type	3	1	3	12	0	3	6[(7)]	42	66
Tier 3 - Multi-lab, all matrix types	9	9	9	36	0	9	18[(7)]	126	198

Notes:

(1) Numbers of analyses in this table do not include additional QC tests such as calibration, blanks, etc. Nine is the maximum number of matrix types to validate a modified wastewater method at Tier 1 or Tier 3.

(2) Initial precision and recovery (IPR) reagent water analyses are used to validate a method modification. The number of IPR analyses is four times the number of laboratories used to validate a method modification because each laboratory performs a four-replicate IPR test.

(3) IPR sample matrix analyses are used to establish QC acceptance criteria for matrix spike/matrix spike duplicate (MS/MSD) recovery and precision for a Tier 1 new method only. IPR sample matrix analyses are not needed for validation of Tier 2 or 3 new methods because this variability data would be obtained from MS/MSD tests in multiple labs.

(4) The proficiency testing (PT) sample should be obtained from a third-party vendor and should be analyzed by each laboratory participating in the study. If sewage sludge or ocean water are matrices of interest, PT samples for those matrices are required as well.

(5) A method detection limit (MDL) test would be performed in each laboratory, using the alternate test procedure. As of August 2017, 40 CFR Part 136 Appendix B requires analysis of a minimum of seven spiked samples and seven blanks per laboratory to determine an MDL. Validation studies will comply with most recent MDL study requirements published in Appendix B of 40 CFR Part 136.

(6) The MDL and reagent water IPR tests do not have to be repeated after the first matrix type is validated.

(7) The MS/MSD analyses would be used to establish MS/MSD recovery and precision for the new method. The number of MS/MSD analyses is two times the number of matrix types tested (i.e., one MS/MSD pair per laboratory).

4.2.1 Tier 1 Validation Studies for Wastewater

Any person may request the Regional Alternate Test Procedure (ATP) Coordinator to approve the use of an alternate test procedure in the Region. The primary intent of Tier 1 is to allow use of a new method by a single laboratory. Tier 1 is expected to be used by commercial laboratories, dischargers, and state and municipal laboratories repetitively testing samples from the same site(s) on a routine basis. Tier 1 can be applied to one or more matrix types. Additional Information regarding the application for and approval of limited use ATPs may be found at 40 CFR 136.5. Please contact the appropriate Regional ATP Coordinator for additional information regarding specific method validation study designs for these types of new method ATPs. See Appendix C for a list of Regional ATP Coordinators.

Tier 1 - Single Matrix Type

Tier 1 - Single Matrix Type validation studies are performed in a single laboratory on a single matrix type plus analysis of a proficiency testing (PT) sample (see Section 4.3.12). Results of the validation study

and the method modification are applicable in the laboratory that validated the new method for this matrix type, and the results may not be used by another laboratory or for another matrix type.

Tier 1 - Multiple Matrix Types

If a laboratory intends to apply the method to fewer than nine matrix types, the laboratory should validate the method on each matrix type. Results of the validation study and use of the new method are applicable in the laboratory that validated the new method for these matrix types; the results may not be used by another laboratory or for another matrix type. The maximum number of matrix types to which the new method should be applied to demonstrate that it will likely be successful for all other matrix types is nine. The specific tests to be conducted on the first matrix type and for each additional matrix type are shown in Table 4.

4.2.2 Tier 2 Validation Studies for Wastewater

The primary intent of Tier 2 is to allow all regulated entities and laboratories to apply a new method to a single sample matrix type from a single industry. EPA has determined that Tier 2 will encourage the development and application of techniques that overcome matrix interference problems specific to effluents of certain industrial subcategories, lower detection limits, improve the reliability of results, lower the costs of measurements, and/or improve overall laboratory productivity when analyzing samples from a given industry.

Tier 2 validation studies are performed in a minimum of three laboratories. Samples of the same matrix type (e.g., final effluent, extraction-stage effluent) are collected from one or more facilities in the same industrial subcategory. Once a new method has been validated under Tier 2, the results can be used by other laboratories as long as it is applied to samples from the validated matrix type within the industrial subcategory, and as long as the other laboratories meet all of the method's QC acceptance criteria. If the new method is to be applied to another matrix type, the modification should be validated separately on that matrix type.

4.2.3 Tier 3 Validation Studies for Wastewater

The primary intent of Tier 3 is to allow nationwide use of a new method by all regulated entities and laboratories for all matrix types. Tier 3 validation studies are performed in a minimum of nine laboratories, each with a different matrix type, for a total of nine samples. Suggested sample matrix types that should be used in the validation study are given in Table 5.

Table 5. Matrix Types Recommended for Multiple Matrix Type Validation Studies

1. Effluent from a POTW
2. ASTM D 5905 - 98 (Reapproved 2013), *Standard Specification for Substitute Wastewater*
3. Sewage sludge, if sludge will be in the permit
4. ASTM D 1141 - 98 (Reapproved 2013), *Standard Specification for Substitute Ocean Water*, if ocean water will be in the permit
5. Untreated and treated wastewaters up to a total of nine matrix types (see https://www.epa.gov/eg/industrial-effluent-guidelines for a list of industrial categories with existing effluent guidelines)
At least one of the above wastewater matrix types should have at least one of the following characteristics: • Total suspended solids (TSS) greater than 40 mg/L • Total dissolved solids (TDS) greater than 100 mg/L • Oil and grease greater than 20 mg/L • NaCl greater than 120 mg/L • $CaCO_3$ greater than 140 mg/L

4.3 Detailed Procedures for Conducting Validation Studies

When validating new methods, laboratories must adhere to the standardized QC elements detailed in the proposed new method. The results of the QC tests will be used to develop QC acceptance criteria for the new method as detailed in Appendix G.

Laboratories should use both a reference matrix (usually reagent water) and field samples for the validation study. For multi-lab validation studies (e.g., Tiers 2 and 3), the applicant is responsible for ensuring that each laboratory in the study fulfills the validation study design specifications detailed in Sections 4.3.2 to 4.3.11 and provides all of the data that support the new method application. However, it is important that the validation study accurately reflect the ruggedness of the new method and any limitations regarding clarity of the new method procedures. Therefore, a vendor or other applicant should not directly assist laboratories participating in the validation study with implementation of the new method or equipment during the course of the study (e.g., the vendor or applicant may provide training and advice to participant laboratories regarding the equipment or methodology *prior to* the start of the study, but the study samples are to be analyzed by the study participants under "routine" conditions). Direct participation by the vendor or applicant will compromise the results of the study. The applicant also is responsible for the **technical and statistical evaluation** of the validation study results in order to produce the validation study report.

4.3.1 Method Compilation

Prior to conducting a validation study, the applicant responsible for development of the new method should detail the full method in accordance with EPA's *Guidelines and Format for Methods to Be Proposed at 40 CFR Part 136 or Part 141* (Guidelines and Format document), EPA-821-B-96-003. The documented method should be distributed to each laboratory participating in the validation study to ensure each laboratory is validating the same set of procedures.

4.3.2 Method Detection Limit Study

Each laboratory participating in the Tier 1, 2, or 3 validation study must perform a method detection limit (MDL) study in accordance with the procedure given at 40 CFR Part 136, Appendix B while using the procedures specified in the new method. The final results for each MDL study aliquot must be provided by each laboratory in the validation study, along with the details of the spiking levels and MDL

calculations, and each laboratory should keep the raw data that supports those MDL study results on file and available for review.

An allowance for an MDL higher than that of other methods approved for each of the analyte(s) of interest, but that supports a regulatory compliance limit, may be made to recognize that a new method that overcomes interferences may not achieve an MDL that is as low as the MDL achieved by the other approved methods, but is potentially more valuable in allowing determination of the analyte(s) of interest at the regulatory compliance limit in a complex sample matrix.

4.3.3 Calibration

Each laboratory participating in the validation study must perform a calibration in accordance with the procedures specified in the new method. Calibration requirements and development of acceptance criteria for the new method are discussed further in Appendix G.

4.3.4 Initial Precision and Recovery

Each laboratory participating in the study must obviously perform initial precision and recovery (IPR) analyses using only the procedures specified in the new method. The IPR test is performed by analyzing four replicates of reagent water spiked with the analytes of interest. The method developer must use the results of these IPR analyses to develop precision and recovery QC acceptance criteria. The concentration of the IPR samples must be stated in the method. This concentration should be between one and five times the minimum level (ML) of quantitation of the new method. For more detail regarding the ML and how it is established see Appendix G, Section 3.1.1, of this document.

4.3.5 Field Sample Collection and Analyses

After laboratories participating in the Tier 1, 2, or 3 validation study have successfully completed the IPR analyses, the method modification should be validated on the matrix type(s) chosen for the validation study. The numbers of analyses required are described below.

Samples of each matrix type should be properly collected in sufficient quantity to support the validation study. The volume required will vary by tier, and by the volume required in the new method. Because the composition of many treated effluents may vary over time, composite sampling equipment may be used to minimize that temporal variability. When a regulation specifies collecting grab samples for compliance monitoring, it still may be feasible to use composite sampling equipment to collect a bulk effluent sample for use in a validation study. Alternatively, multiple grab samples may be collected and combined to create a bulk sample of sufficient quantity to support the validation study.

Note: The validation study plan should describe the sample collection procedures that will be employed and the homogenization procedures that will be used to produce replicate aliquots of the bulk sample for distribution and/or testing by the study participants.

All field samples should be analyzed by the laboratory as received to determine the background concentration of the target analyte prior to preparation of the MS and MSD aliquots. This will ensure that the MS and MSD aliquots are fortified at an appropriate concentration. That is, the MS/MSD pair shall be fortified with the target analyte a concentration equal to the regulatory limit, if the new method is for use to demonstrate compliance with a specific permit, or at one to five times the background concentration of the sample, *whichever is higher*.

Note: Analyzing field samples *before* preparing the MS/MSD aliquots may contradict the specific requirements in some methods that stipulate that the MS/MSD aliquots be prepared and analyzed in the same batch as the field samples. However, for the purposes of validating a new method, it is essential that the MS/MSD aliquots generate meaningful data about the performance of the new method in the matrix of interest.

4.3.5.1 Tier 1 - Single Matrix Type Validation Studies

In a Tier 1- single matrix type study performed to validate a new method, the laboratory should analyze 4 spiked replicates of the matrix type to which the new method will be applied. The replicate samples should be spiked with the analyte(s) of interest at a concentration one to five times the background concentration of the analyte(s) in the sample or at one to five times the ML, whichever is greater. In other words, the laboratory will perform an IPR test in the matrix type of interest. Prior to spiking the replicate samples, the laboratory should determine the background concentration of an unspiked aliquot. In all, Tier 1- single matrix type validation studies for new method ATPs will require, at minimum, analysis of 14 MDL samples (7 spiked samples and seven method blanks), 4 IPR reagent water samples, 1 PT sample and 5 field samples (one background and 4 IPR sample matrix), for a total of 24 analyses. The organization responsible for developing the method should use the results of the IPR sample matrix sample analyses to develop MS/MSD precision and recovery QC acceptance criteria as outlined in Appendix G, Section 3.1.5, of this document. The organization should also develop QC acceptance criteria for other required QC tests as outlined in Appendix G, Section 3.1, of this document.

4.3.5.2 Tier 1 - Multiple (Additional) Matrix Type Validation Studies

In Tier 1- multiple matrix type studies performed to validate new methods, the laboratory should determine MS/MSD precision and recovery QC acceptance criteria using a single matrix of interest as outlined in Appendix G, Section 3.1.5, and determine the background concentration and analyze an MS/MSD pair for each additional matrix type being tested, up to a total of 8 additional matrix types. For a method to be validated for each additional matrix type, the results of the background/MS/MSD samples should fall within the QC acceptance criteria determined in the single matrix. In all, Tier 1- multiple matrix type validation studies for new method ATPs will require, analysis of 14 MDL samples (seven spiked samples and seven method blanks), 4 IPR reagent water samples, 1 PT sample and between 8 and 26 field sample analyses (1 background and 4 IPR sample matrix for the first matrix type and one background, one MS, and one MSD for each additional matrix type to a maximum of 9 total matrix types). A Tier 1- multiple matrix type validation study will require a minimum of between 27 and 48 total analyses since between 2 and 8 additional matrix types may be tested.

4.3.5.3 Tier 2 Single Matrix Type Validation Studies

In a Tier 2 validation study, each of the 3 laboratories will determine the background concentration and analyze an MS/MSD pair on the field sample it receives. Because there are 3 laboratories, each of which performs analysis of 14 MDL samples (7 spiked samples and 7 method blanks), 4 IPR reagent water samples, 1 PT sample and 3 field samples (1 background, 1 MS, and 1 MSD), Tier 2 validation studies will require a minimum of 66 total analyses. The organization responsible for developing the new method should use the results of the samples analyses to develop QC acceptance criteria for required QC tests as outlined in Appendix G, Section 3.2, of this document.

4.3.5.4 Tier 3 Validation Studies

In a Tier 3 validation study, each of the 9 laboratories participating in the study will perform an MDL study, an IPR in reagent water study, analyze a PT sample, determine the background concentration and

analyze an MS/MSD pair on the field sample it receives. Because there are 9 laboratories, each of which performs analysis of 14 MDL samples (7 spiked samples and 7 method blanks), 4 IPR reagent water samples, 1 PT sample and 3 field samples (1 background, 1 MS, and 1 MSD), a Tier 3 validation study will require a minimum of 198 total analyses. The organization responsible for developing the new method should use the results of these samples analyses to develop QC acceptance criteria for required QC tests as outlined in Appendix G, Section 3.3, of this document.

4.3.6 Ongoing Precision and Recovery

If the field samples discussed in Section 4.3.5 are analyzed as a batch with the IPR samples, analysis of an OPR sample is unnecessary in the validation study. If, however, field samples are analyzed in a different batch or batches, then each laboratory participating in the Tier 1, 2, or 3 validation study should analyze an OPR sample with each batch. The concentration of the OPR sample should be as stated in the method being validated. The organization responsible for developing the method should use the results of the IPR tests described above in Section 4.3.4 to develop OPR recovery criteria as described in Appendix G.

4.3.7 Calibration Verification

If the field samples discussed in Section 4.3.5 are analyzed on the same shift or in the same set of instrumental determinations as the initial calibration sequence, calibration verification is unnecessary. However, if field samples are analyzed on a different shift or in a different instrument batch, each laboratory participating in the Tier 1, 2, or 3 validation study should verify calibration as described in the method. The organization responsible for developing the method should use the results of the calibration described above in Section 4.3.3 to develop QC acceptance criteria for the calibration verification analyses as described in Appendix G.

4.3.8 Method Blanks

Each laboratory that participates in a Tier 1, 2, or 3 validation study should prepare and analyze at least one method blank with the sample batch during which the matrix samples are prepared and analyzed. The actual number of blank samples analyzed by each laboratory should meet or exceed the frequency specified in the method. For validation of a new method, the laboratory responsible for the development of the new method must use the results of these sample analyses to develop QC acceptance criteria for allowable blank contamination.

4.3.9 Surrogate or Labeled Compound Recovery

For methods that use surrogates or labeled compounds, each laboratory participating in the Tier 1, 2, or 3 validation study should spike all field and QC samples with the surrogates/labeled compounds at the concentrations specified in the method. The laboratory responsible for the development of the new method must use the results of these sample analyses to develop surrogate or labeled compound recovery QC acceptance criteria.

4.3.10 Absolute and Relative Retention Time

Each laboratory participating in a Tier 1, 2, or 3 validation study of a chromatographic method must determine the absolute and/or relative retention times of the analytes of interest, where required by the method. For validation of a new method, each laboratory participating in the study should use the results of these sample analyses to develop absolute and relative retention time QC acceptance criteria if applicable.

4.3.11 Further Validation Studies

After completing the Tier 1, 2, or 3 validation studies of new methods, the organization responsible for developing the method should document the study results and submit them to EPA. If, based on its review of the method, EPA concludes that the method is not sufficiently rugged or reliable for its intended use; EPA may require further method development and further testing to define the stability and reliability of the method. The tests and studies that should be performed in this case are dependent upon the analyte(s) and the analytical system, and will be determined on a case-by-case basis as these situations arise.

4.3.12 Proficiency Testing Results

Each laboratory participating in a Tier 1, 2, or 3 validation study should include analysis of a proficiency testing (PT) sample obtained from an approved vendor. An example list of approved vendors can be found at: http://www.nelac-institute.org/ptproviders.php (other lists may exist as well). This PT sample will be analyzed in addition to each of the matrix types required to be analyzed as part of the validation study and will be analyzed as it is received from the vendor. The same PT sample or samples obtained from the same vendor with the same lot number or preparation batch number will be analyzed by each laboratory participating in the validation study. The concentrations of the target analytes in the PT sample should be relevant to any regulatory limits associated with the matrix type(s) of interest. PT vendors that prepare samples for periodic Discharge Monitoring Report Quality Assurance (DMRQA) studies may be able to provide assistance with selection of concentrations for the PT samples.

The study coordinator will be responsible for obtaining the PT sample from the vendor, along with the certificate of analysis that specifies the certified value and acceptance limits for reporting results. The study coordinator will also be responsible for distributing the sample to the laboratories that will be performing the analyses for the validation study (or in the case of a Tier 1 study to the analyst responsible for performing the analyses) without providing them with the certificate of analysis (e.g., "blind" as to the expected results). The study coordinator is also responsible for informing each laboratory participating in the validation study (or in the case of a Tier 1 study, the analyst responsible for performing the analyses) that the sample is to be analyzed only once just as it is received and is not to be diluted or fortified for analysis as an MS/MSD pair. In addition, the study coordinator should include a copy the certificate of analysis as an addendum to the validation study report.

5.0 EPA REVIEW AND APPROVAL

5.1 EPA's Office of Water Review of New Method ATP Applications

All requests for approval of new methods must undergo review and approval by the organization(s) listed in Table 1 of Section 3.1. New methods intended for limited-use (Tier 1) will be approved by the EPA Regional ATP Coordinator. New methods intended for nationwide use (Tiers 2 and 3) will be approved through rulemaking. New methods prepared under this protocol should demonstrate an improvement when compared to the other methods approved for analyte(s) of concern that offers one or more of the following advantages: better method sensitivity or selectivity, lower analytical costs, fewer matrix interference problems, improvement in laboratory productivity, or reduction in the amount of hazardous materials used and/or produced in the laboratory.

EPA's Office of Water (OW) will review all Tier 2 and Tier 3 ATP applications for approval of new methods for nationwide use and will review limited-use (Tier 1) applications if requested by the EPA Regional Office or state agency. OW may be assisted in its technical review by contractor personnel. When a formal new method ATP application is received, it will be checked for completeness. If the documentation is incomplete, OW will contact the applicant and request missing documentation before proceeding with its review.

At a minimum, an application should include a completed new method application form, the new method written in EPA standard format, and the method validation study plan, before OW will review the package. If these elements are present, OW will assess the written method and the validation study plan to determine if changes are required and will then notify the applicant of these changes. In all cases, a written method and validation study plan should be agreed upon prior to beginning a full new method validation study.

Once all elements of the new method application are present, including the validation study report and supporting data, OW will begin its internal review of the new method for scientific merit, consistency, and appropriateness. The internal review may involve multiple programs and workgroups. Should any problems or questions arise during the review, OW or its technical support contractor will communicate with the applicant to resolve outstanding issues. Depending on the circumstances, OW may return the application to the applicant for revision. OW review of new method applications will involve the three steps briefly described below.

The first step of OW's technical review will evaluate the description of the new method and assess the new method's applicability for approval at 40 CFR Part 136. If the new method is not applicable to Part 136, OW will notify the applicant and describe the basis for rejection of the application. If this information is acceptable, the evaluation will proceed.

In the second step of OW's review, the performance of the new method is compared to the performance of other methods approved for the analyte(s) of concern. At a minimum, results produced using the new method must be determined to meet the needs of the NPDES compliance monitoring program (for methods addressing non-method-defined parameters) or demonstrate that there are no systematic differences in performance between the new method and the corresponding EPA-approved method (for methods addressing method-defined parameters). If method performance is acceptable, the review will continue.

As the third and final step, OW will perform a detailed audit of the new method test data. The evaluation of test data in applications can be accomplished more quickly if machine-readable files of test data (and

analysis software where different from EPA software) are provided with the application. Data files should be in a PC-compatible format, suitable for input directly into statistical analysis software.

Note: Although EPA will review the data from the validation study and conduct its own statistical test on the study results, the applicant is responsible for the technical and statistical evaluation of the validation study results *prior to* submitting the study report.

5.2 Approval Recommendation

EPA will complete its review and notify the applicant of its approval recommendation as expeditiously as practicable after receipt of an application containing the information necessary for EPA's evaluation. For limited-use applications (Tier 1), the Regional ATP Coordinator will notify the applicant and the appropriate State agency of approval or rejection of the use of the alternate test procedure. The EPA Region will issue the formal approval for use of the Tier 1 new method. The approval may be restricted to use only with respect to a specific discharge or facility (and its laboratory) or, at the discretion of the Regional ATP Coordinator, to all dischargers or facilities (and their associated laboratories) specified in the approval for the Region.

For all nationwide-use new method applications for use in Clean Water Act programs (Tiers 2 or 3), OW will notify the applicant of EPA's recommendation, and if the new method is recommended for approval, will initiate the rulemaking process through which the new method is formally approved by the EPA Administrator.

5.3 Rulemaking Process

EPA periodically updates the lists of analytical methods approved for Clean Water Act compliance monitoring at 40 CFR 136 to provide increased flexibility to the regulated community and laboratories in their selection of analytical methods for use in Clean Water Act programs. EPA also uses these periodic "method update rules" (MURs) to formalize the approval status of nationwide ATPs and new methods which have been positively reviewed. Using the information provided with the new method application to develop the justification and record support, EPA will prepare the proposed rule for approval of wastewater methods, compile the rule docket, pass the proposed rule through internal and/or external review at EPA, and submit it to the Office of the Federal Register (OFR) for publication. *Preparation, approval, and publication of a proposed rule generally requires a minimum of nine months, but may take longer, depending on the number of the methods involved in the rulemaking effort.* When published, the proposed rule requests public comment and allows a specified comment period. At the end of the comment period, EPA may forward any significant comments to the new method applicant with a request that they provide technical assistance to EPA in drafting responses to comments. All comments that have scientific or legal merit, or raise substantive issues with the proposed rule, must be answered to complete the rulemaking process.

EPA will review any technical responses provided by the applicant and complete the response-to-comments document for the final rule. EPA will then prepare the final rule, compile the rule docket, and submit the final rule to the OFR for publication. The final rule will state the date that the rule becomes effective, typically 30 days after rule publication. As of this effective date, the method is approved by EPA and will be included in the appropriate table(s) at 40 CFR Part 136 in the next CFR update. *It generally requires a minimum of fifteen months, but may take longer, after the proposed rule is published to receive and respond to comments, prepare and process the final rule through internal EPA review, and publish the final rule in the Federal Register.*

6.0 REFERENCES

1. ASTM, 1994. *Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water.* Designation D-2777-13. *Annual Book of ASTM Standards,* Vol. 11.04.

2. Youden, W.J. and E.H. Steiner, 1975. *Statistical Manual of the AOAC.* AOAC- International. 1111 N. 19th Street; Suite 210, Arlington, VA 22209.

3. Wernimont, G.T., 1985. *Use of Statistics to Develop and Evaluate Analytical Methods.* AOAC-International.

4. USEPA, 1996. *Guidelines and Format for Methods to Be Proposed at 40 CFR Part 136 or Part 141* (Guidelines and Format document). U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. Washington, DC EPA-821-B-96-003.

5. USEPA, 1999. *Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water*. U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. Washington, DC EPA 821-B-98-003.

6. USEPA, 1999. *Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water.* U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. Washington, DC EPA 821-B-98-002.

7. USEPA, 2018. *Protocol for Review and Validation of Alternate Test Procedures for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*. U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. Washington, DC EPA 821-B-18-002.

APPENDIX A SAMPLE NEW METHOD APPLICATION FORM

EPA Office of Water New Method Application Form for Chemical Analytes			
Applicant Name and Address:			***EPA Use Only*** **ATP Case No.**
Date Application Submitted:			
New Method: ***(Method number & title*)**			
Analyte(s):		**Is this a Method-Defined Parameter (Yes/No)?**	
Type (WW or WW/DW):			
Level of Use: **(Limited Use or Nationwide Use)**		**Validation Tier: (1, 2 or 3)**	
FOR LIMITED-USE APPLICATIONS ONLY:			
ID number of existing or pending permit:			
Issuing agency:			
Type of permit:			
Discharge serial number:			
ATTACHMENTS: Each item below includes a reference to the section of the new method protocol that describes the material in detail			
____ **Justification for New Method *(Sec. 3.2.1)***			
____ **New Method (Method in standard EPA format) *(Sec. 3.2.2)***			
____ **Validation Study Plan *(Appendix E)***			
____ **Validation Study Report *(Sec. 3.2.4)***			
____ **Method Information and Documentation for Preamble and Docket *(Sec. 3.2.5)***			
____ **Other** _____			
Submit Application and Attachments in Triplicate			

APPENDIX B DATA COLLECTION CERTIFICATION

It is the expectation of the ATP program that all data will be collected as outlined in the validation study plan. If a data set needs to be recollected (e.g., QC failure, instrument failure, matrix effects etc.) this should be clearly be documented in the final report and the initial data along with the recollected data should be submitted. It is not permissible to collect multiple data sets and submit the "best one". Occasionally, blind samples (performance evaluation samples) will be distributed by the ATP program to assess method performance. Successful analysis of these samples will be required as part of the candidate method approval process. Laboratory fraud is a serious issue and applicants must attest on the application that the data collection was performed as outlined in the validation study plan.

The applicant hereby certifies that the data included with this application was collected as outlined in the validation study plan.

_____

Applicant (print name)

_____ _____

Applicant (signature) **(Date)**

Questions, comments or applications should be directed to:

Lem Walker
Clean Water Act ATP Coordinator
USEPA
Office of Science and Technology
Engineering and Analysis Division (EAD)
1200 Pennsylvania Avenue, NW
Mail Code - 4303T
Washington, DC 20460
Fax: (202) 566-1053
walker.lemuel@epa.gov

APPENDIX C HEADQUARTERS AND REGIONAL ATP CONTACTS

Headquarters
Lem Walker
Clean Water Act ATP Coordinator
USEPA
Office of Science and Technology
Engineering and Analysis Division (EAD)
1200 Pennsylvania Avenue, NW
Mail Code - 4303T
Washington, DC 20460
walker.lemuel@epa.gov

Region 1
Steve DiMattei
QA Chemist
USEPA Region 1
11 Technology Drive (EQA)
North Chelmsford, MA 01863-2431
dimattei.steve@epa.gov

Region 2
Donna Ringel
USEPA Region 2
Raritan Depot
2890 Woodbridge Avenue (MS-220)
Building 10
Edison, NJ 08837-3679
ringel.donna@epa.gov

Region 3
Terry Simpson
Regional Quality Assurance Manager
USEPA Region 3
Environmental Science Center
701 Mapes Road
Fort Meade, MD 20755-5350
simpson.terry@epa.gov

Region 4
Jeffrey Wilmoth
Chemist/ATP Coordinator
USEPA Region 4
Room: SESD, QA Section
980 College Station Road
Athens, GA 30605-2720
wilmoth.jeffrey@epa.gov

Region 5
Kenneth Gunter
USEPA Region 5
77 W. Jackson Blvd.
WC-15J
Chicago, IL 60604-3507
gunter.kenneth@epa.gov

Region 6
David Stockton
USEPA Region 6 Laboratory
Houston Branch
10625 Fallstone Road (6MD)
Houston, TX 77099
stockton.david@epa.gov

Region 7
Robert Nichols
USEPA Region 7 ENST/LTAB
Science and Technology Center
300 Minnesota Avenue
Kansas City, KS 66101
nichols.robert@epa.gov

Region 8
Paul Garrison
Regional Quality Assurance Officer
USEPA Region 8
1595 Wynkoop Street (8WP-CWW)
Denver, CO 80202-1129
garrison.paul@epa.gov

Region 9
Roseanne Sakamoto
USEPA Region 9
75 Hawthorne Street, MTS-3
San Francisco, CA 94105
sakamoto.roseanne@epa.gov

Region 10
Donald M. Brown
Office of Environmental Assessment
USEPA Region 10
1200 6th Avenue, Suite 900, OEA-40
Seattle, Washington 98101
brown.donaldm@epa.gov

Note: The names and addresses in this list are current as of the date of this document, and are subject to change. Please consult with the individual EPA Regional Office for the current ATP contact.

APPENDIX D STANDARD EPA METHOD FORMAT

The following is a listing of the 17 elements of the standard EPA method format. Applicants should consult the Guidelines and Format document (USEPA, 1996, Reference 4 in Section 6 of the main body of this document) for a detailed description of the content for each section and other formatting guidelines and conventions.

1.0 Scope and application

This section outlines the purpose, range, limitations, and intended use of the method, and identifies target analytes.

2.0 Summary of Method

This section provides an overview of the method procedure and quality assurance.

3.0 Definitions

This section includes definitions of terms, acronyms, and abbreviations used in the method. If preferred, definitions may be provided in a glossary at the end of the method or manual. In this case, the definitions section should still appear in the method, with a notation that definitions are provided in a glossary at the end of the method. Refer to the specific section number of the glossary.

4.0 Interferences

This section identifies known or potential interferences that may occur during use of the method, and describes ways to reduce or eliminate interferences.

5.0 Safety

This section describes special precautions needed to ensure personnel safety during the performance of the method. Procedures described here should be limited to those which are above and beyond good laboratory practices. The section should contain information regarding specific toxicity of analytes or reagents.

6.0 Equipment and Supplies

This section lists and describes all non-consumable supplies and equipment needed to perform the method.

7.0 Reagents and Standards

This section lists and describes all reagents and standards required to perform the method, and provides preparation instructions and/or suggested suppliers as appropriate.

8.0 Sample Collection, Preservation, and Storage

This section provides requirements and instructions for collecting, preserving, and storing samples.

9.0 Quality Control

This section cites the procedures and analyses required to fully document the quality of data generated by the method. The required components of the laboratory's quality assurance (QA) program and specific quality control (QC) analyses are described in this section. For each QC analysis, the complete analytical procedure, the frequency of required analyses, and interpretation of results are specified.

10.0 Calibration and Standardization

This section describes the method/instrument calibration and standardization process, and required calibration verification. Corrective actions are described for cases when performance specifications are not met.

11.0 Procedure

This section describes the sample processing and instrumental analysis steps of the method, and provides detailed instructions to analysts.

12.0 Data Analysis and Calculations

This section provides instructions for analyzing data, and equations and definitions of constants used to calculate final sample analysis results.

13.0 Method Performance

This section provides method performance criteria for the method, including precision/bias statements regarding detection limits and source/limitations of data produced using the method.

14.0 Pollution Prevention

This section describes aspects of the method that minimize or prevent pollution known to be or potentially attributable to the method.

15.0 Waste Management

This section describes minimization and proper disposal of waste and samples.

16.0 References

This section lists references for source documents and publications that contain ancillary information. Note: Each method should be a free-standing document, providing all information necessary for the method user to perform the method may be found. References within a method should be restricted to associated or source material. Procedural steps or instructions should not be referenced as being found elsewhere, but should be included in total within the method.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

This section contains all method tables and figures (diagrams and flowcharts), and may contain validation data referenced in the body of the method.

APPENDIX E Validation Study Plan and Study Report

1.1 Development of a Validation Study Plan

Prior to conducting Tier 1, 2, or 3 validation studies, the new method applicant (e.g., the organization responsible for conducting the study) should prepare and submit a detailed study plan. As noted earlier, for new methods that measure method-defined parameters, a detailed validation study plan should be submitted and agreed upon prior to conducting the study (see Appendix H).

The validation study plan should contain the elements described in Sections 1.1.1 through 1.1.6.

1.1.1 Background

The Background section of the validation study plan should:

- Identify the new method being validated
- Identify intended use of the new method (Tier 1, Tier 2 or Tier 3)
- Include a summary of the new method
- Cite the organization or individual responsible for development of the new method
- Describe the reasons for and the logic behind the technical approach to the new method
- Identify the matrices, matrix types, and/or media to which the new method is believed to be applicable
- List the analytes measured by the new method including corresponding CAS Registry numbers (if applicable)
- Indicate whether any, some, or all known metabolites, decomposition products, or known commercial formulations containing the analyte are included in the measurement. For example, a method designed to measure acid herbicides should include the ability to measure the acids and salts of these analytes; a total metals method should measure total metals.

1.1.2 Objectives

The Objectives section of the validation study plan should describe overall objectives and data quality objectives of the study.

1.1.3 Study Management

The Study Management section of the validation study plan should:

- Identify the organization responsible for managing the study
- Identify laboratories, facilities, and other organizations that will participate in the study
- Delineate the study schedule

1.1.4 Technical Approach

The Technical Approach section of the validation study plan should:

- Indicate at which tier the study will be performed
- Describe the approach that will be followed by each organization involved in the study
- Describe how sample matrices and participating laboratories will be selected
- Explain how samples will be collected and distributed
- Specify the numbers and types of analyses to be performed by the participating laboratories
- Describe how analyses are to be performed

1.1.5 Data Reporting and Evaluation

This section of the validation study plan should explain the procedures that will be followed for reporting and validating study data, and should address statistical analysis of study results.

1.1.6 Limitations

The Limitations section of the validation study plan should explain any limiting factors related to the scope of the study.

1.2 Validation Study Report

Applicants responsible for developing new methods at Tiers 1, 2, or 3 should document the results of the validation study in a formal validation study report that contains the elements described in this section and presents these elements in the same order described in this section. In all cases, a copy of all required validation data should be maintained at the laboratory or other organization responsible for developing the new method.

The information and supporting data required in the validation study report should be sufficient to enable EPA to support a claim of acceptable performance of a method modification. If data are collected by a contract laboratory, the organization responsible for using the method (e.g., permittee, POTW, or other regulated entity) is responsible for ensuring that all method-specified requirements are met by the contract laboratory and that the validation study report contains all required data.

Like the validation study plan, the validation study report contains background information and describes the study design. In addition, the validation study report details the process and results of the study, provides an analysis and discussion of the results, and presents study conclusions. If a validation study plan was prepared, it should be appended to and referenced in the validation study report. The validation study report should identify and discuss any deviations from the study plan that were made in implementing the study.

The validation study report must contain a signed Data Collection Certification form (see Appendix B of this document) and the elements described in Sections 1.2.1 through 1.2.10 below.

1.2.1 Background

The Background section of the validation study report should describe the new method that was validated and identify the organization responsible for developing the new method. The background section of the validation study report should:

- Include a method summary
- Cite the organization and method number and title for the new method
- Describe the reasons for development of the new method and the logic behind the technical approach to method development
- Identify the matrices, matrix types, and/or media to which the modified method is intended to apply
- List the analytes measured by the modified method including corresponding CAS Registry numbers (Alternatively, this information may be provided on the data reporting forms in the Supporting Data appendix to the validation study report.)
- Indicate whether any, some, or all known metabolites, decomposition products, or known commercial formulations containing the analyte are included in the measurement. (For example, a method designed to measure acid herbicides should include the ability to measure the acids and salts of these analytes.)
- State the purpose of the study.

1.2.2 Study Design and Objectives

The Study Design and Objectives section of the validation study report should describe the study design, and identify overall objectives and data quality objectives of the study. Any study limitations should be identified. The validation study plan may be appended to the validation study report to provide the description of the study design.

1.2.3 Study Implementation

The Study Implementation section of the validation study report should describe the methodology and approach undertaken in the study. This section should:

- Identify the organization that was responsible for managing the study
- Identify the laboratories, facilities, and other organizations that participated in the study; describe how those participants were selected; and explain the role of each organization involved in the study
- Indicate at which Tier level the study was performed
- Delineate the study schedule that was followed
- Describe how sample matrices were chosen, including a statement of compliance with Tier specific validation study specifications for matrix type selection
- Explain how samples were collected and distributed
- Specify the numbers and types of analyses performed by the participating laboratories
- Describe how analyses were performed
- Identify any problems encountered or deviations from the study plan and their resolution/impact on study performance and/or results

1.2.4 Data Reporting and Validation

This section of the validation study report should describe the procedures that were used to report and validate study data. While EPA does not require the use of a standard format for analytical data submission, a validation study data reporting form may be found in Appendix F of this document.

1.2.5 Results

This section of the validation study report presents the study results. Raw data and example calculations are required as part of the results and shall be included in an appendix to the validation study report (see Section 1.2.10 below).

1.2.6 Data Analysis/Discussion

This section of the validation study report should provide a statistical analysis and discussion of the study results. The discussion should address any discrepancies between the results and the QC acceptance criteria of the EPA-approved reference method.

1.2.7 Conclusions

The Conclusions section of the validation study report should describe the conclusions drawn from the study based on the data analysis discussion. The Conclusions section should contain a statement(s) regarding achievement of the study objective(s).

1.2.8 Appendix A - The Method

A written version of the modified method prepared in accordance with EPA's Guidelines and Format document, should be appended to the validation study report (see Reference 4 in Section 6 of the main body of this document).

1.2.9 Appendix B - Validation Study Plan

If a validation study plan was prepared, it should be appended to the validation study report.

1.2.10 Appendix C - Supporting Data

The validation study report should be accompanied by raw data and example calculations that support the results presented in the report.

1.2.10.1 Raw Data

The Results section of the validation study report should be supported by an appendix containing all raw data that will allow an independent reviewer to verify each determination and calculation performed by the laboratory. This verification consists of tracing the instrument output (peak height, area, or other signal intensity) to the final result reported. Raw data are method-specific and may include any of the following:

- Sample numbers or other identifiers used by the both the new method applicant and the laboratory(ies) that participated in the study
- Sample preparation (extraction/digestion) dates
- Analysis dates and times
- Sequence of analyses or run logs
- Sample volume
- Extract volume prior to each cleanup step
- Extract volume after each cleanup step
- Final extract volume prior to injection
- Digestion volume
- Titration volume
- Percent solids or percent moisture
- Dilution data, differentiating between dilution of a sample and dilution of an extract or digestate
- Instrument(s) and operating conditions
- GC and/or GC/MS operating conditions, including detailed information on
  - Columns used for determination and confirmation (column length and diameter, stationary phase, solid support, film thickness, etc.)
  - Analysis conditions (temperature programs, flow rates, etc.)
  - Detectors (type, operating conditions, etc.)
- Chromatograms, ion current profiles, bar graph spectra, library search results
- Quantitation reports, data system outputs, and other data to link the raw data to the results reported. (Where these data are edited manually, explanations of why manual intervention was necessary should be included)
- Direct instrument readouts; i.e., strip charts, printer tapes, etc., and other data to support the final results
- Laboratory bench sheets and copies of all pertinent logbook pages for all sample preparation and cleanup steps, and for all other parts of the determination

Raw data are required for all samples, calibrations, verifications, blanks, matrix spikes and duplicates, and other QC analyses. Data should be organized so that an analytical chemist can clearly understand how the analyses were performed. The names, titles, addresses, and telephone numbers of the analysts who performed the analyses and of the quality assurance officer who will verify the analyses should be provided. For instruments involving data systems (e.g., GC/MS), raw data should be made available in appropriate electronic formats upon request.

1.2.10.2 Example Calculations

The validation study report should provide example calculations that will allow the data reviewer to determine how the laboratory used the raw data to arrive at the final results. Useful examples include both detected compounds and undetected compounds. If the laboratory or the method employs a standardized reporting level for undetected compounds, this should be made clear in the example, as should adjustments for sample volume, dry weight (solids only), etc.

APPENDIX F SAMPLE NEW METHOD DATA REPORTING FORM

This appendix provides an example of a data reporting form to be provided during the approval review. The form illustrates those aspects of data reporting which EPA would expect, regardless of the specific format used in order to evaluate the new method. Specifically, data should be presented in a clear and logical format, and should be labeled clearly.

In addition to using an appropriate data reporting format, submitting the data in an appropriate electronic format can be very helpful in expediting the review of a new method. Data files should be in PC-compatible format, suitable for input directly into statistical analysis software.

Sample New Method Data Reporting Form[1]

New Method Title*		**Revision Date**	__/__/__

*Include Method Number and Revision Number

Please record all data and quality control (QC) performance results (for comparison against QC acceptance criteria) from your validation study using this data form. If you have additional data, please attach it to this form in a tabular format, being sure to label all columns and rows clearly.

For Tier 2 (Nationwide Use; Single Matrix) or Tier 3 (Nationwide Use; Multiple Matrices): Complete **1** form for each participant laboratory.

Linear Calibration Data

Units of Concentration:_____ Units of Response: _____ Number of Points:_____

Analyte Conc.							
Response							
RF/CF/RR*							

*Response Factor/Calibration Factor/Relative Response

Method Detection Limit (MDL) Data

Spiking Concentration used for MDL Study (include units): _____

MDL Data							

Initial Precision Recovery (IPR) Data

Spiking Concentration used for IPR Study (include units): _____

IPR Data				

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

Spiking Concentration used for MS/MSD Study (include units): _____

MS Concentration	
MSD Concentration	
Background Concentration	

New Method QC Performance Results

Calibration		Spike	IPR Recovery and Precision			OPR Data Precision		MS/MSD Recovery and RPD			MDL/ML	
Points	Lin	Conc	Low	High	Precision	Low	High	Low	High	RPD	MDL	ML

[1]For multi-analyte methods, present additional data and QC acceptance criteria for each analyte in a tabular format, making sure to include proper labels, and attach to this form.

APPENDIX G DEVELOPING QUALITY CONTROL ACCEPTANCE CRITERIA

1.0 Introduction

All new methods should contain standardized QC tests and specify QC acceptance criteria for each test. The person or organization that develops a new method for a particular combination of analyte and determinative technique will be responsible for validating the method and for developing the QC acceptance criteria. QC acceptance criteria will be based on data generated during the method validation study. Under this protocol, EPA expects a method validation study that reflects the level of intended use for a method. Because QC acceptance criteria will be developed from validation studies and because the validation study designs vary with each tier, the statistical procedures used to develop the criteria will vary by tier.

This appendix lists and describes the standardized QC tests required in all new methods, and outlines procedures for developing QC acceptance criteria for new methods at Tiers 1, 2, and 3.

2.0 Standardized Quality Control Tests

Under this protocol, standardized QC tests are a mandatory component of all new methods. The following standardized QC tests should be included in new methods, as appropriate to the technology:

- Calibration linearity
- Calibration verification
- Absolute and relative retention time windows (for chromatographic analyses)
- Initial precision and recovery
- Ongoing precision and recovery
- Analysis of blanks
- Surrogate or labeled compound recovery
- Matrix spike and matrix spike duplicate precision and recovery (for non-isotope dilution analyses)
- Method detection limit demonstration
- Analysis of a performance testing (PT) sample

These tests are described in Sections 2.1 to 2.10 below.

2.1 Calibration

Calibration is the process of establishing the relationship between the concentration or amount of an analyte and the response of an analytical instrument or system to the analyte. The process begins by measuring instrument responses to known concentrations or amounts of the analyte. The calibration equation is then established by fitting a line or curve through the calibration data. Concentration is the independent variable and the corresponding instrument response, which will include some random variation, is the dependent variable. The most common calibration model is a straight line through the origin (zero response at zero concentration).

Analyte concentrations in future samples are estimated by measuring instrument response and applying the inverse of the calibration equation. To achieve the overall goal of obtaining the most accurate estimates of concentrations in future samples, the most effective calibration procedures involve:

- Selecting the proper response relationship
- Calculating the most precise estimates of the parameters of the calibration equation through regression.

2.1.1 Unweighted and Weighted Regression

Simple linear regression is based on the assumption that the standard deviation of the dependent variable is constant over the range of the regression. This simple regression is also termed "unweighted regression," and it treats all of the calibration points (e.g., concentrations) equally. Many of the methods currently approved for Clean Water Act (CWA) compliance monitoring use an unweighted regression as the model for the instrument calibration. However, in order to do so, they usually limit the overall calibration range to a small portion of the range that might be utilized otherwise, perhaps covering a factor of 20 to 100 times the lowest calibration point. That approach represents a trade-off between the analytical range of the instrumentation and the ease of assessing the calibration model. This trade-off originated in the days before sophisticated instrument data systems were readily available and the unweighted regression offered calculations that were simple enough to be accomplished by the analyst or a data reviewer with a hand-held calculator.

In reality, for nearly all analytical instruments and systems, the variability of the response is not constant over the analytical range but increases with increasing concentration. The most accurate statistical method for estimating the best calibration line or curve with non-constant standard deviation is "weighted regression." In contrast to the unweighted regression that treats all calibration points equally, the weighted regression applies a "weight" to the assumed variability at each concentration that is a function of the concentration itself.

Fortunately, modern analytical instrument data systems often include several different statistical options for analysts, including both unweighted and weighted regressions. An unweighted or simple regression is often the default choice, but as noted above, involves a trade-off between simplicity and overall analytical range. A weighted regression allows the calibration points with the lowest variability (typically the lower level standards) to more heavily influence the resulting calibration curve.

Regardless of the capabilities of an instrument data system, the choice of the calibration model should be an informed decision that is based on knowledge of the instrumentation and the range of sample concentrations of interest. Application of weighted regression to a straight line through the origin is discussed below. Application of weighted regression to straight lines not through the origin and to second and higher order quadratic equations is beyond the scope of this protocol.

In the simplest case of calibration using a proportional model ($y = mx$), the weighted regression estimate for the proportionality coefficient m (e.g., the slope) is as follows:

$$m_{est} = \frac{\sum_{i=1}^{n}\left(\frac{y_i x_i}{w_i}\right)}{\sum_{i=1}^{n}\left(\frac{x_i^2}{w_i}\right)}$$

where:
y_i = Instrument response for the *ith* standard
x_i = Analyte amount for the *ith* standard
n = The number of calibration points, and
w_i = The variance or squared error of response y_i.

With the assumption that the response error is proportional to concentration (i.e., $\sigma [y_i, x_i] = kx_i$ and $w_i = x_i^2$), the weighted regression estimate of the proportionality coefficient becomes:

$$m_{est} = \frac{\sum_{i=1}^{n}\frac{y_i}{x_i}}{n}$$

where:
y_i = Instrument response for the *ith* standard
x_i = Analyte amount for the *ith* standard, and
n = The number of calibration points

The proportionality coefficient *m* is estimated as the mean ratio between instrument response and standard concentration. For external standard calibration, this ratio is often termed the "calibration factor" (CF). For internal standard calibration, terms for the response and concentration or amount of the internal standard are included and the mean ratio is termed the "response factor" (RF). For isotope dilution, the internal standard is a labeled compound and EPA has designed the mean ratio as "relative response" (RR). For details of use of "calibration factor," "response factor," and "response ratio," see the respective methods in which they are used; e.g., the organic methods at 40 CFR Part 136, Appendix A (Ref. 1)

2.1.2 Calibration Linearity

The calibration linearity specification establishes the metric by which one judges the performance of a calibration model based on a straight line through the origin or the other forms of calibration. The metric used is the relative standard deviation (RSD) of the:

- Calibration factor (CF) for external standard calibration,
- Response factor (RF) for internal standard calibration, or
- Relative response (RR) for isotope dilution calibration.

When the RSD is less than or equal to the maximum RSD value, the calibration data are well enough modeled by a straight line through the origin, and the slope of the line, *m*, represented by the mean CF, RF, or RR, may be used to calculate the concentration from an analysis. If the RSD is greater than the limit specified, then the data are not well modeled by a straight line through the origin and another form of calibration must be used.

The equations below illustrate the calculations of the mean, standard deviation (s), and relative standard deviation (RSD) of any of these "factors" (i.e., CF, RF, or RR) for each analyte from the results of the initial calibration:

$$Mean\ Factor = \overline{Factor} = \frac{\sum_{i=1}^{n} Factor_i}{n}$$

where:
Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and
n = The number of calibration points.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(Factor_i - \overline{Factor})^2}{n}}$$

$$RSD = 100\ x\ \frac{s}{\overline{Factor}}$$

The number of calibration points required for calibration is dependent on the error of the measuring technique. During method development, measurement technique error is determined by:

- Calibrating the instrument with a standard at the minimum level (ML) of quantitation and a minimum of two additional non-zero standards.
- Determining the RSD of the CFs, RFs, or RRs.

Depending on the resulting RSD, calibration during the subsequent validation study must be performed at the minimum number of points shown in Table G-1. Additional criteria for RSDs are obtained from the results of the validation study; following procedures specific to Tiers 1, 2 and 3 (see subsections on calibration linearity under Section 3).

Table G-1. Minimum Number of Points Required for Calibration

RSD Range[2]	Minimum Number of Calibration Points[1]
≤ 2	1[3]
> 2 but ≤ 10	3
> 10 but ≤ 25	5
>25	7

[1] Based on EPA's validation of Method 1625.
[2] Percent RSD shall be determined from the calibration linearity test.
[3] Assumes linearity through the origin (0,0). For analytes for which there is no origin (such as pH), a two-point calibration shall be performed.

Calibrations other than a straight line through the origin are required when the RSD linearity criterion cannot be met. For most instruments and analytical systems, these calibrations are straight line not through the origin ($y = mx + b$) or a second-order quadratic equation ($y = ax^2 + bx + c$). A second or higher order calibration may be justified when an analyte can only be determined with a method that uses a determinative technique with a nonlinear response over the calibration range. For example, enzyme-linked immunoassay methods typically use log-log or similarly shaped curves for calibration. A second or higher order calibration may be used provided that the response function increases or decreases monotonically with concentration. A monotonic calibration function is one that produces a unique (i.e., only one) analyte amount or concentration for a given instrument response.

Most instruments and analytical systems are linear over a range large enough to preclude the need for second order or higher calibration. If the linear range of any of these systems is limited, sample dilution and reanalysis should be performed to bring the concentration within the linear range, rather than extend the calibration into a nonlinear region of the response. EPA discourages use of higher order calibrations, where possible, because responses in the nonlinear region can mask curvature that may be attributable to preparation of an inaccurate standard. EPA requires that all calculations of concentrations of analytes in blanks, field samples, QC samples, and samples prepared for other purposes be based on the mean CF, RF, or RR, on a straight line not through the origin, or on a calibration curve.

2.2 Calibration Verification

This test is used to periodically verify that instrument performance has not changed significantly from calibration. Verification is based on time (e.g., working day, 12-hour shift) or on the number of samples analyzed in a batch (e.g., after every 10th sample). The terms "shift" and "batch" should be specified in the method. If not, the general rule has been that calibration verification is performed every 12-hour shift on instruments used for determination of organic analytes and every 10th sample on instruments used for determination of metals. However, the over-riding rule should be that verification is performed frequently enough to ensure that the response of the instrument or analytical system has not drifted significantly from calibration.

Calibration verification tests are typically performed by analyzing a single standard in the concentration range of interest for the target analyte(s). In most methods, this standard is in the range of 1 - 5 times the

minimum level (ML) of quantitation and is at the same level as one of the standards used for calibration. The calibration verification standard concentration should be within 1 - 5 times the ML rather than at a "midpoint" concentration because specifying the midpoint can be interpreted as one-half (1/2) the highest calibration point. Using a concentration that high when the calibration covers orders of magnitude may lead to erroneous results, because this midpoint standard may be far removed from the range where most measurements will be made.

If the calibration is linear through the origin (in other words, it meets the linearity criteria), specifications for calibration verification may be developed for:

- The allowable deviation of the CF, RF, or RR calculated from the calibration verification standard from the mean CF, RF, or RR of the initial calibration,
- The range of acceptable concentration values calculated for the analytes in the calibration verification standard, or
- The range of acceptable recoveries calculated for the analytes in the calibration verification standard, based on the known concentrations in the standard

If a line with a non-zero intercept or a higher-order curve is used for calibration, the specifications for calibration verification must be defined as a maximum allowable deviation of the verification standard from the calibration line or curve. These specifications may be based on either concentration or recovery, as above.

For calculation of analyte concentrations in field samples, the mean CF, RF, or RR, or the calibration curve is always used; i.e., the calibration is **not updated** to the RR, RF, CF of the single point verification standard. Updating the calibration to a single point after establishing a mean CF, RF, or RR, or a calibration curve is equivalent to performing a single-point calibration. This updating procedure, which is sometimes termed "continuing calibration," is unacceptable and shall not be used because it nullifies the statistical power of the full calibration.

2.3 Absolute and Relative Retention Time Precision

Absolute retention time (RT) and relative retention time (RRT) are the QC criteria used in chromatographic analyses to aid in the identification of each detected analyte and to confirm that sufficient time was allowed for the chromatographic separation of the analytes in complex mixtures. These criteria also prevent laboratories from accelerating the analysis in an effort to reduce costs, only to find that complex mixtures cannot be adequately resolved.

A minimum RT specification is developed for those methods in which a minimum analysis time must be established to ensure separation of the analytes in complex mixtures including known or expected interferences. An RT precision specification (e.g., a retention time window) is developed for identification of an analyte by external standard measurements.

RRT is a unitless quantity and the general formula for RRT is as follows:

$$\frac{Retention\ time_{analyte}}{Retention\ time_{reference\ compound}}$$

and an RRT precision specification is developed for:

- Each analyte relative to an internal standard for internal standard measurements,
- Each analyte relative to its labeled analog by isotope dilution measurements, and
- Each labeled compound relative to its internal standard for isotope dilution measurements.

2.4 Initial Precision and Recovery

The initial precision and recovery (IPR) test, also termed a "startup test" or the initial demonstration of capabilities (IDC) is used to demonstrate a laboratory's capability to produce results that are at least as precise and accurate as results from practice of the method by other laboratories. The IPR test also is used to demonstrate that a method modification will produce results that are as precise and accurate as the reference method. The IPR test consists of analyzing at least four replicate aliquots of a reference matrix spiked with the analytes of interest and with either surrogate compounds or, for isotope dilution analysis, labeled compounds. The concentration of the target analytes in the spike solution may vary between one and five times the concentration used to establish the lowest calibration point (e.g., one to five times the ML). The spiked aliquots are carried through the entire analytical process. The IPR test is performed by the laboratory before it utilizes a method for analysis of actual field samples. Specifications are developed for the permissible range of recovery for each analyte and for an upper limit on the standard deviation or RSD of recovery.

2.5 Ongoing Precision and Recovery

The ongoing precision and recovery (OPR) test, sometimes termed a "laboratory control sample," "quality control check sample," or "laboratory-fortified blank," is used to ensure that the laboratory remains in control during the period that samples are analyzed, and it helps separate laboratory performance from method performance in the sample matrix. The test consists of a single aliquot of reference matrix spiked with the analyte(s) of interest and carried through the entire analytical process with each batch of samples. Typically, the concentration of the target analyte(s) is the same as the concentration used in the IPR test. Specifications are developed for the permissible range of recovery for each analyte.

2.6 Analysis of Blanks

Blanks are analyzed either periodically or with each sample batch to demonstrate that no contamination is present that would affect the analysis of standards and samples for the analytes of interest. The period or batch size is defined in each method. Typical periods and batch sizes are one per shift or one for every 10 or 20 samples, but more or fewer may be required, depending upon the likelihood of contamination.

For most methods, QC acceptance criteria for blanks are given in each method and are specified as the concentration or amount of the analyte allowed in each type of blank. The source of contamination in a blank must be identified and eliminated before the analysis of standards and samples may begin. Samples analyzed with an associated contaminated blank must be reanalyzed. Methods for which blank contamination cannot be eliminated should use a "$y = mx + b$" calibration model, i.e., a straight line not through the origin.

2.7 Surrogate or Labeled Compound Recovery

The surrogate or labeled compound recovery is used to assess the performance of the method on each sample. Surrogates, or stable, isotopically labeled analogs of the analytes of interest, are spiked into the sample and the recovery is calculated. Specifications are developed for the permissible range of recovery for each surrogate and/or labeled compound from each sample.

2.8 Matrix Spike and Matrix Spike Duplicate

The matrix spike and matrix spike duplicate (MS/MSD) test is used in non-isotope dilution methods to assess method performance in the sample matrix. In most cases, analytes of interest are added to a field sample aliquot that is then thoroughly homogenized and split into two spiked replicate aliquots.

One of these replicates is identified as the matrix spike sample and the other is identified as the matrix spike duplicate sample. The recoveries of the analytes, relative to the spike, are determined in each sample. The precision of the determinations also is assessed by measuring the relative percent difference (RPD) between the analyte concentrations measured in the MS and MSD. The MS and MSD should each be spiked at a level that results in the concentration of the target analyte(s) being:

- At the regulatory compliance limit
- One to five times the background concentration of unspiked field sample, or
- At the level specified in the method, whichever is greater.

If the background concentration in the field sample is so high that the spike will cause the calibration range of the analytical system to be exceeded, the sample is spiked after the field sample is diluted by the minimal amount necessary for this exceedance not to occur. This dilution of the sample to stay within the calibration range of the analytical system for the target analyte is necessary to verify that the sample matrix has not prevented reliable determination of the analyte. Specifications are developed for the permissible range of recovery and RPD for each analyte.

2.9 Demonstration of Method Detection Limit

Nearly all of the methods in 40 CFR Part 136, Appendix A contain method detection limits (MDLs), although few of the methods explicitly require laboratories to demonstrate their ability to achieve these MDLs. Each laboratory that intends to practice a new method will be required to demonstrate that it can achieve an MDL that meets acceptable criteria. The MDL must be determined according to the most recent procedures specified at 40 CFR Part 136, Appendix B (Ref. 2). The Appendix B MDL calculation and analytical procedure is described in Section 3.1.1.

2.10 Performance Testing Sample Analysis

At one time, EPA routinely provided QC materials such as whole volume samples or concentrates to laboratories performing CWA compliance monitoring, and organized periodic performance evaluation studies of laboratory performance by distributing blind samples to laboratories. More recently, EPA has relinquished this role in favor of a system of performance testing (PT) samples and studies run by commercial vendors.

New methods must include the analysis of some form of PT sample as part of the routine QC operations. An example list of approved vendors can be found at: http://www.nelac-institute.org/ptproviders.php (other lists may exist as well). The PT sample must be prepared by the vendor in a sample matrix that represents the matrix to which the method is applicable (e.g., methods for wastewater analysis must use a PT samples made in a wastewater matrix, not a solid matrix, or even a drinking water matrix). The concentrations of the target analytes in the PT sample should be relevant to any regulatory limits associated with the matrix type(s) of interest. PT vendors that prepare samples for periodic Discharge Monitoring Report Quality Assurance (DMRQA) studies may be able to provide assistance with selection of concentrations for the PT samples.

Alternatively, the use of Standard Reference Materials (SRMs) from the National Institute of Standards and Technology (NIST), or Certified Reference Materials (CRMs) from another recognized source, may be included in a method if such materials are available in the relevant sample matrix.

Note: The relevance of the matrix of the reference material to the field sample matrices to which the method is to be applied cannot be emphasized enough. For example, performance in a freeze-dried sewage sludge reference material is only relevant when the new method includes freeze-

drying of solid samples before extraction or digestion, but that performance sheds little light on the new method's performance in a wet sludge, and has no relevance to wastewater.

Providers of PT samples, SRMs, and CRMs will also provide information on the concentrations of target analytes in the material, along with acceptance limits for those concentrations. Whenever such information is provided, it must be used as the basis for the QC acceptance criteria in the new method.

3.0 Development of Quality Control Acceptance Criteria

The procedures for developing QC acceptance criteria for Tier 1, Tier 2, and Tier 3 methods are described in Sections 3.1, 3.2, and 3.3, respectively. EPA's expectation is that interlaboratory study data will be generally necessary to develop QC acceptance criteria for Tier 2 and Tier 3 methods. Although these studies are not necessary for Tier 1 methods, interlaboratory study data may be available. If interlaboratory data are available for a Tier 1 method, these data should be used to develop QC acceptance criteria for Tier 1 methods by following the Tier 2 or Tier 3 procedures described in Section 3.2 or 3.3, respectively. Where possible, interlaboratory study data used for development of QC acceptance criteria should be derived from study designs that follow the basic principles outlined in References 3 and 4, or other well-established and documented principles.

The statistical procedures described in Sections 3.2 and 3.3 for Tier 2 and Tier 3 are based on the use of interlaboratory multipliers. These multipliers were derived from a comparison of intralaboratory versus interlaboratory variability in the development of EPA Method 1625. The variation in the interlaboratory multiplier used is directly related to the number of laboratories used at each of the two tiers. The general relationship follows the concept that an increase in the number of laboratories used results in a decrease in the interlaboratory multiplier.

If the method being developed is applicable to a large number of compounds, the organization responsible for developing QC acceptance criteria for the method may wish to consider the use of statistical allowances for simultaneous compound testing. Procedures associated with simultaneous compound testing and the development of applicable QC acceptance criteria can be found at 49 FR 43242 (Ref. 3).

3.1 Quality Control Acceptance Criteria Development for New Methods at Tier 1

Method validation at Tier 1 consists of (1) using the new method to perform an MDL study in accordance with the procedure described at 40 CFR Part 136, Appendix B, (2) using the results of this MDL study to establish an ML, and (3) running, in a single laboratory, a test of four spiked reference matrix samples and four spiked samples of the matrix type(s) to which the method is to be applied. The spike level of these reference matrix and real-world matrix IPR samples must be in the range of one to five times the ML, or at the regulatory compliance level, whichever is higher.

3.1.1 Method Detection (MDL) Limit and Minimum Level (ML)

An MDL must be determined for each target analyte using the procedure detailed at 40 CFR Part 136, Appendix B. As of August 2017, this procedure involves processing seven spiked samples and seven method blank samples through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates and analyzed on three separate calendar dates. (Preparation and analysis may be on the same day.) The spiked samples must include all of the analytes of interest at a concentration of two to ten times the estimated MDL. The fourteen aliquots are then carried through the entire analytical process. Two calculations are performed on the resulting data: the MDL_s is calculated for the spiked samples, and the MDL_b is calculated for the method blanks, using the equations below.

Calculate the MDL_s (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\propto=0.99)} S_s$$

where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1, 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom

S_s = sample standard deviation of the replicate spiked sample analyses.

Calculate the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \overline{X} + t_{(n-1,1-\propto=0.99)} S_b$$

where:

MDL_b = the MDL based on method blanks

$\bar{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha = 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses.

The ML is established by multiplying the MDL by 3.18 and rounding the value to the number nearest to (1, 2, or 5) x 10^n where n is positive or negative integer. The purpose of rounding is to allow instrument calibration at a concentration equivalent to the ML without the use of unwieldy numbers. The use of 3.18 results in an overall standard deviation multiplier of 10, which is consistent with the American Chemical Society definition of the limit of quantitation (LOQ) (Refs. 4 and 5).

3.1.2 Calibration Linearity

Once the ML is established, the instrument or analytical system is then calibrated with one standard at the ML and at least two additional higher concentration standards to calculate an initial RSD_{CAL} for the response factor and to determine the number of points required for subsequent calibrations. The highest point should be at, but not exceed, the upper end of the analytical range for the instrument and the remaining point should be mid-way between the ML and highest point on a logarithmic scale. For example, if the ML is 1.0 and the highest point is 100, the mid-point is 10. If the initial RSD_{CAL} is < 2%, a one- or two-point calibration can be used (see Section 2.1.2) and it is unnecessary to establish a limit for calibration linearity.

If three or more calibration points are required, the maximum allowable RSD (RSD_{max}) for the CFs, RFs, or RRs is determined as follows:

1. Determine the mean factor (i.e., $\overline{CF}$, $\overline{RF}$, or $\overline{RR}$) for each analyte from the results of the initial calibration:

$$Mean\ Factor = \overline{Factor} = \frac{\sum_{i=1}^{n} Factor_i}{n}$$

where:

Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and

n = The number of calibration points.

2. Determine the standard deviation (s) of the $\overline{CF}$, $\overline{RF}$, or $\overline{RR}$ for each analyte from the initial calibration.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(Factor_i - \overline{Factor})^2}{n-1}}$$

3. Determine the RSD for the $\overline{CF}$, $\overline{RF}$, or $\overline{RR}$ based on the standard deviation, s, as follows:

$$RSD = 100\ x\ \frac{s}{\overline{Factor}}$$

4. Develop RSD_{max} as the smaller of 35% and:

$$RSD_{max} = k(RSD)$$

where:

k = The square root of the 95th percentile of an *F* distribution with degrees of freedom corresponding to the number of points in the initial calibration minus 1 in both the numerator and denominator (e.g., a 5-point calibration curve has 4 degrees of freedom).

For a three-point calibration, the value of *k* is 4.4, and for a five-point calibration, the value of *k* is 2.5. The maximum allowable specification for RSD_{max} is 35%.

3.1.3 Calibration Verification

As noted in Section 2.2., acceptance limits for calibration verifications can be determined in three different ways, each of which is described below.

Calibration verification criteria may be specified as allowable percentage deviations from the $\overline{CF}$, $\overline{RF}$, or $\overline{RR}$ obtained from the initial calibration. The upper and lower QC acceptance criteria for the calibration verification as follows:

1. Calculate a multiplier, *k*, as the 97.5th percentile of a Student's *t* distribution with *n* - 1 degrees of freedom times the square root of (1 + 1/*n*), where there are *n* points in the calibration. For a three-point calibration, the *n* - 1 Student's *t* value is 4.3, and for a five-point calibration, the Student's *t* value is 2.8, resulting in values for *k* of 5.0 for a three-point calibration and 3.0 for a five-point calibration.

2. Calculate the upper and lower QC acceptance criteria for the factors for each analyte by developing a window around the mean factor found in the initial calibration as:

$$Lower\ limit\ (\%) = \frac{\overline{Factor} - ks}{\overline{Factor}}\ x\ 100$$

$$Upper\ limit\ (\%) = \frac{\overline{Factor} + ks}{\overline{Factor}}\ x\ 100$$

where:
k = The multiplier determined in Step 1 and s is the standard deviation determined in 3.1.2, Step 2.

Calibration verification criteria may be specified as the range of acceptable concentration values calculated for the analytes in the calibration verification standard. The calculations are very similar to those used for the "factor" limits shown above.

3. Calculate the upper and lower QC acceptance criteria for the known concentration of the analyte in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above:

$$Lower\ limit = \ (Lower\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

$$Upper\ limit = \ (Upper\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

Alternatively, calibration verification criteria may be specified as the range of acceptable recoveries calculated for the analytes in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above to create a window around 100% recovery.

3.1.4 Initial and Ongoing Precision and Recovery

For Tier 1 methods, an IPR test must be performed in both a reference matrix (usually, reagent water) and the sample matrix of interest. Results of the reference matrix IPR tests are used to generate QC acceptance criteria for IPR and OPR tests as described in this subsection. Results of the sample matrix IPR test are used to develop QC acceptance criteria for the MS/MSD tests (see Section 3.1.5 below). The reference matrix IPR test is performed by analyzing four aliquots of the reference matrix spiked with the target analyte(s) at the concentration determined in Section 2.4.

Calculate the QC acceptance criteria for the IPR and OPR tests using results of the test of the reference matrix per the following steps:

1. For each analyte, calculate the mean percent recovery ($\overline{X}$), the standard deviation of recovery (s), and the relative standard deviation (RSD_{IPR}=100s/$\overline{X}$) from the four IPR results.

2. QC acceptance criteria for IPR recovery - Calculate the QC acceptance criteria for recovery in the IPR test by constructing a window around the mean percent recovery ($\overline{X}$). The width of the acceptance window is based on the 97.5th percentile of the *t*-distribution for 3 degrees of freedom and a factor that accounts for interlaboratory variability. For the 4 IPR aliquots, that factor is:

$$t_{(0.975,n-1)}\left(\sqrt{1.15(1+1) + \left(\frac{1}{4} + \frac{1}{n}\right)}\right) = 5.3$$

(Based on EPA's interlaboratory validation study of Method 1625, the additional variance due to interlaboratory variability is estimated as 1.15 *s*.)

For the data from the four IPR aliquots, the factor simplifies to 5.3(*s*), and the lower and upper limits are as follows:

$$Lower\ limit\ (\%) = \bar{X} - 5.3s$$

$$Upper\ limit\ (\%) = \bar{X} + 5.3s$$

3. QC acceptance criterion for IPR precision - The maximum acceptable RSD for the four IPR results is approximated as a 95% upper confidence limit around the observed RSD. The RSD_{IPR} is multiplied by the square root of the 95th percentile of an *F* distribution with 3 degrees of freedom. The resulting multiplier on RSD_{IPR} for four data points simplifies to 3.0, and the QC acceptance criterion is calculated as follows:

$$Maximum\ RSD_{IPR} = (3.0)x\ RSD_{IPR}$$

4. QC acceptance criterion for OPR recovery - A similar multiplier is used as for the IPR test, but the second factor is:

$$t_{(0.975,n-1)}\left(\sqrt{1.15(1+1)+\left(1+\frac{1}{n}\right)}\right) = 6.0$$

and the limits for the OPR recovery are calculated from the mean percent recovery in the IPR test as:

$$Lower\ limit\ (\%) = \bar{X} - 6.0s$$

$$Upper\ limit\ (\%) = \bar{X} + 6.0s$$

Note: For highly variable methods, it is possible that the lower limit for recovery for both the IPR and OPR analyses will be a negative number. In these instances, the data should either be log-transformed and the recovery window recalculated, or the lower limit established as "detected," as was done with some of the methods in 40 CFR Part 136, Appendix A.

3.1.5 Matrix Spike and Matrix Spike Duplicate

As noted above, during method development an IPR test must be performed in both an appropriate reference matrix *and* the sample matrix of interest for Tier 1 new methods. The results of the sample matrix IPR test are used to develop acceptance criteria for the MS/MSD analyses. Sample matrix IPR tests are performed by:

- Determining the background concentration of the sample matrix,
- Spiking four replicate aliquots of the sample matrix at a concentration equal to the regulatory compliance limit, one to five times the ML determined in Section 3.1.1, or one to five times the background concentration of the sample, whichever is greater, and
- Analyzing each of these spiked replicate samples.

Calculate the QC acceptance criteria for the recovery of MS and MSD samples as follows:

1. Calculate the mean percent recovery ($\bar{X}$) and the standard deviation of recovery (s) of each target analyte in the sample matrix IPR aliquots.

2. Calculate the QC acceptance criteria for recovery in the MS and MSD tests by constructing a ± 6.0s window (assuming 4 sample matrix IPR aliquots) around the mean percent recovery ($\bar{X}$) in the sample matrix as:

$$Lower\ limit\ (\%) = \bar{X} - 6.0s$$

$$Upper\ limit\ (\%) = \bar{X} + 6.0s$$

Note: For highly variable methods, it is possible that the lower limit for recovery for both the IPR and OPR analyses will be a negative number. In these instances, the data should either be log-transformed and the recovery window recalculated, or the lower limit established as "detected," as was done with some of the methods in 40 CFR Part 136, Appendix A (49 FR 43234).

Calculate the QC acceptance criterion for the relative percent difference (RPD) between the MS and MSD as follows:

3. Calculate the relative standard deviation (RSD) of the recoveries of each target analyte in the sample matrix IPR aliquots (IPR_{SM}) as follows:

$$RSD_{IPR_{SM}} = \frac{100s}{\bar{X}}$$

where:

$RSD_{IPR_{SM}}$ = Observed RSD of the results for the sample matrix IPR aliquots
s = Standard deviation of the results for the sample matrix IPR aliquots, and
$\bar{X}$ = Mean percent recovery of the analyte in the sample matrix IPR aliquots

4. Calculate the maximum allowable relative percent difference (RPD_{max}) by multiplying the $RSD_{SM\text{-}IPR}$ by the square root of 2 times the square root of the 95th percentile of an *F* distribution with 1 and *n* - 1 degrees of freedom, where n is the number of IPR data points. For 4 sample matrix IPR data aliquots, the calculation simplifies to:

$$RPD_{max} = 4.5\ x\ RSD_{IPR_{SM}}$$

3.1.6 Absolute and relative retention time

Determine the mean retention time, $\overline{RT}$ (and/or the mean relative retention time $\overline{RRT}$) and the standard deviation (s) of the RT and/or RRT for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits (e.g., retention time windows) as follows:

$$Lower\ limit = \overline{RT} - \left(ts\sqrt{1 + \frac{1}{n}} \right)$$

$$Upper\ limit = \overline{RT} + \left(ts\sqrt{1 + \frac{1}{n}} \right)$$

where:

t = The 97.5th percentile of a *t* distribution with n - 1 degrees of freedom, and
n = The number of retention time or relative retention time values used.

The relative retention time upper and lower limits are determined by replacing $\overline{RT}$ with $\overline{RRT}$ in the equations above.

3.1.7 Blanks

Establish the QC acceptance criteria for blanks. The usual requirement is that the concentration of an analyte in a blank must be below the ML or below one-third (1/3) the regulatory compliance level,

whichever is higher. In instances where the level of the blank is close to the regulatory compliance level or the level at which measurements are to be made, it may be necessary to require multiple blank measurements and establish the QC acceptance criteria based on the mean of the blank measurements plus two standard deviations of the blank measurements.

3.1.8 Surrogate or Labeled Compound Recovery

Where surrogates or labeled compounds are used in a method, establish the QC acceptance criteria for the recovery of the surrogates or labeled compounds. Historically, two approaches have been used to establish QC acceptance criteria: consensus-based limits, and statistically derived limits.

For Tier 1 new methods, consensus-based limits are the most appropriate starting point. Ideally, the limits will be round number multiples of 5 or 10, centered around 100% recovery, e.g., 75 - 125%, 60 - 140%, etc.

However, as more routine samples are analyzed, more recovery data become available for use in deriving more robust limits. A minimum of 20 data points should be used to generate meaningful criteria. Inclusion of additional data should result in more robust criteria that better describe variance in method performance and result in fewer outliers.

Note: A common error in developing acceptance criteria is discarding data that do not meet a preconceived notion of acceptable performance. Do not discard specific results simply because they do not meet one's expectations. This practice results in a censored data set, which when used to develop acceptance criteria, will lead to unrealistically narrow criteria. Rather, employ a statistical test for outlier values, or at least calculate the acceptance limits both with and without the results considered suspect. Then, observe the effect of deleting suspect data.

1. Calculate the mean recovery ($\bar{R}$) for each surrogate or labeled compound and the standard deviation (s) of the observed recoveries.

2. Calculate the lower and upper limits of the recovery as follows:

$$Lower\ QC\ limit = \bar{R} - 3s$$

$$Upper\ QC\ limit = \bar{R} + 3s$$

3. In-house QC limits must be examined for reasonableness. For example, if the calculated lower limit of the acceptance range is <10%, it should be set to 10%. It may be useful to compare QC limits generated in the laboratory with performance data listed in similar determinative methods. However, be aware that performance criteria generated with data from a single laboratory tend to be significantly narrower than those generated from multi-laboratory studies. In addition, it is not EPA's intent to legitimize poor recoveries due to incorrect choice of surrogate spiking levels.

3.1.9 Performance Testing Sample

As noted in Section 2.10, providers of PT samples, SRMs, and CRMs will provide information on the concentrations of target analytes in the material, along with acceptance limits for those concentrations. Whenever such information is provided, it must be used as the basis for the QC acceptance criteria in the new method.

3.2 Quality Control Acceptance Criteria Development for New Methods at Tier 2

Method validation at Tier 2 consists of running tests on a single matrix type collected from three different facilities in the same industrial subcategory, with the sample being analyzed in three separate laboratories (see 40 *CFR* Parts 405 - 503 for industrial categories and subcategories).

Each of the three laboratories will need to run a full suite of tests, beginning with an MDL study to determine the appropriate ML, followed by calibration, IPR, OPR, and blank analyses, along with a pair of MS/MSD analyses for each sample matrix. Results from each laboratory will be submitted to the organization responsible for developing the method. That organization will use the laboratory results to develop QC acceptance criteria as described in the following subsections.

3.2.1 Method Detection Limit and Minimum Level

Each laboratory participating in the validation study must perform an MDL test as described in Section 3.1.1. The organization responsible for developing the new method must establish an MDL for the method, using a pooled MDL from the three laboratories. The precautions concerning blanks and the effect of the matrix, and the detailed steps in 40 CFR Part 136, Appendix B must be observed to arrive at a reliable MDL.

A pooled MDL is calculated from *m* individual laboratory MDL values by comparing the square root of the mean of the squares of the individual MDL values and multiplying the result by a ratio of *t*-values to adjust for the increased degrees of freedom.

Note: The MDL values used in this calculation are those determined in each of the three laboratories. If one laboratory reports an MDL_s (from spiked samples), that value is used in conjunction with the MDL values from the other laboratories, including any values reported as MDL_b (from blanks).

$$MDL_{pooled} = \sqrt{\frac{d_1\left(\frac{MDL_{Lab\,1}}{t_{(0.99,d_1)}}\right)^2 + d_2\left(\frac{MDL_{Lab\,2}}{t_{(0.99,d_2)}}\right)^2 + \cdots d_1\left(\frac{MDL_{Lab\,m}}{t_{(0.99,d_m)}}\right)^2}{d_1 + d_2 + \cdots d_m}} \; x \; t_{(0.99,[d_1 + d_2 + \cdots d_m]}$$

where:

m = The number of laboratories, and
d_i = The number of replicates used by Lab i to derive the MDL.

In the case of 3 laboratories with 7 replicates per laboratory, the equation simplifies to:

$$MDL_{pooled} = \sqrt{\frac{MDL_{Lab\,1}^2 + MDL_{Lab\,2}^2 + MDL_{Lab\,3}^2}{3}} \; x \; \frac{2.55}{3.14}$$

The organization responsible for developing the method also must use this pooled MDL to develop an ML. Procedures for determining the ML are given in Section 3.1.1.

3.2.2 Calibration Linearity

Once the ML is established, the instrument or analytical system is then calibrated at the ML and a minimum of two additional points to calculate an initial RSD for the response factor and to determine the

number of points required for subsequent calibrations (Section 3.2.3). If the initial RSD is < 2%, a one-or two-point calibration can be used (see Section 3.1.2) and it is not necessary to establish a limit for calibration linearity.

If three or more calibration points are required, the upper limit on the RSD of the CFs, RFs, or RRs is determined as follows:

1. Calculate the mean and standard deviation of the CFs, RFs, or RRs for each laboratory and analyte as:

$$Mean\ Factor = \overline{Factor} = \frac{\sum_{i=1}^{n} Factor_i}{n}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(Factor_i - \overline{Factor})^2}{n-1}}$$

where:

Factor = In the above equation, the "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and

n = The number of calibration points used in each laboratory.

2. Calculate the relative standard deviation of the CFs, RFs, or RRs of each laboratory and analyte as:

$$RSD_i = 100\ x\ \frac{s_i}{\overline{Factor_i}}$$

where s_i and $\overline{Factor_i}$ are the standard deviation and mean of the CFs, RFs, or RRs for laboratory *i*.

3. Calculate the pooled RSD of the CFs,. RFs, or RRs for each analyte from *all* laboratories. The pooled RSD is calculated as the square root of the mean of the squares of the sample RSDs from each individual laboratory. For example, for three laboratories, the pooled RSD is calculated as:

$$RSD_{pooled} = \sqrt{\frac{RSD_1^2 + RSD_2^2 + RSD_3^2}{3}}$$

4. Calculate RSD_{max} as the smaller of 35% and:

$$RSD_{max} = k(RSD_{pooled})$$

where:

k = The square root of the 95th percentile of an F distribution with *n* - 1 degrees of freedom in the numerator and *m*(*n* - 1) degrees of freedom in the denominator, where *m* is the number of laboratories and *n* is the number of calibration points.

For three laboratories using a three-point calibration, (*m* = 3, *n* = 3), the value of *k* is 2.3, and for three laboratories using a five-point calibration (*m* = 3, *n* = 5), the value of *k* is 1.8. The maximum allowable specification for RSD_{max} is 35%.

3.2.3 Calibration Verification

The calibration verification criterion may be expressed as a maximum relative distance between the mean CF, RF, or RR obtained by a future laboratory's initial calibration ($\overline{Factor}$) and the CF, RF or RR

obtained from its calibration verification standard ($Factor_{VER}$), or based on either the known concentration of the calibration verification standard, or based on recovery of the analyte in the standard.

When using the "Factor" approach, the maximum allowable deviation is based on the pooled relative standard deviation (RSD_{pooled}) calculated in Section 3.2.2.

1. Determine k_{VER} by multiplying the 97.5th percentile of a Student's *t* distribution with (m[n-1]) degrees of freedom times the square root of (1+1/*n*), where there are *n* points in the calibration and *m* laboratories:

$$k_{VER} = t\sqrt{\left(1 + \frac{1}{n}\right)}$$

   For a three-point calibration with three laboratories, the *m* (*n* - 1) Student's *t* value is 2.4, and for a five-point calibration, the Student's *t* value is 2.2, resulting in combined multipliers of 2.8 for a three-point calibration, and 2.4 for a five-point calibration.

2. The calibration verification criterion for the new method would then be stated as the maximum percent difference as follows:

$$Percent\ Difference = 100\ x\ \left(\frac{Factor_{VER} - \overline{Factor}}{\overline{Factor}}\right) \leq\ k_{VER}\ RSD_{pooled}$$

   where:
   Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and

   For example, if the calibration verification criterion, calculated as k_{VER} RSD_{pooled}, equals 17%, then the difference between the $\overline{Factor}$ from the initial calibration and the $Factor_{VER}$ from the calibration verification sample must be less than or equal to 17% of the $\overline{Factor}$.

When using either the concentration or the recovery approach, the calculations are very similar to those used for the "factor" limits shown above.

3. Calculate the upper and lower QC acceptance criteria for the known concentration of the analyte in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above:

$$Lower\ limit =\ \ (Lower\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

$$Upper\ limit =\ \ (Upper\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

Alternatively, calibration verification criteria may be specified as the range of acceptable recoveries calculated for the analytes in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above to create a window around 100% recovery.

3.2.4 Initial and Ongoing Precision and Recovery

For the IPR and OPR tests, QC acceptance criteria are calculated using the mean percent recovery and the standard deviation of recovery from the IPR tests on four aliquots of the reference matrix and the OPR test of one aliquot of the reference matrix (for a total of five samples) in the three laboratories, as follows:

1. Calculate the mean percent recovery ($\overline{X}$) for each analyte, based on all data points from all laboratories, the between-laboratory standard deviation (s_b) of the mean results for each of the three laboratories (standard deviation of the three laboratory means $\overline{X_1}$ + $\overline{X_2}$ + $\overline{X_3}$), and the pooled within-laboratory standard deviation (s_w):

$$s_b = \sqrt{\frac{\sum_{j=1}^{m}\left(\bar{X}_j - \bar{X}\right)^2}{m-1}}$$

where:
$\overline{X}_j$ = The mean percent recovery for the *jth* laboratory
m = The number of laboratories, and
$\bar{X}$ = The overall mean of the percent recoveries from all laboratories

The value of s_w is calculated as the square root of the mean of all within-laboratory variances. For example, for three laboratories:

$$s_w = \sqrt{\frac{s_1^2 + s_2^2 + s_3^2}{3}}$$

Note: The organization responsible for developing the method must ensure that all laboratories are spiking IPR and OPR samples at the same concentration.

2. QC acceptance criteria for IPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right)s_b^2 + \left(\frac{1}{4} - \frac{1}{n}\right)s_w^2}$$

where:
m = The number of laboratories, and
n = The number of data points per laboratory.
For 3 laboratories and 5 data points per laboratory, the calculation becomes:

$$s_c = \sqrt{\left(\frac{4}{3}\right)s_b^2 + \left(\frac{1}{20}\right)s_w^2}$$

3. Calculate the QC acceptance criteria for recovery in the IPR test by constructing a ± 3.2 s_c window around the mean percent recovery $\overline{X}$, where 3.2 is the 97.5th percentile Student's *t* value for 3 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 3.2s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 3.2s_c$$

If more than 3 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

4. QC acceptance criterion for IPR precision - The maximum acceptable RSD for the four IPR aliquots is approximated by a 95% upper confidence limit around the observed RSD of the results from all of the laboratories. The RSD_{IPR} (computed as s_w divided by $\overline{X}$) is multiplied by the square root of a 95th

percentile *F* value with 3 degrees of freedom in the numerator and *m* (*n* - 1) degrees of freedom in the denominator, where *m* = the number of laboratories, and *n* is the number of data points per laboratory. For example, the resulting multiplier on the RSD for three laboratories and five data points per laboratory will then be 1.9, and the QC acceptance criterion for precision in the IPR test is calculated as follows:

$$Maximum\ RSD_{IPR} = (1.9)x\ RSD_{IPR}$$

5. QC acceptance criteria for OPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(1 - \frac{1}{n}\right) s_w^2}$$

where:
m = The number of laboratories, and
n = The number of data points per laboratory.

For 3 laboratories and 5 data points per laboratory,

$$s_c = \sqrt{\left(\frac{4}{3}\right) s_b^2 + \left(\frac{4}{5}\right) s_w^2}$$

6. Calculate the QC acceptance criteria for recovery in the OPR test by constructing a ± 2.6 s_c window around the mean percent recovery $\overline{X}$, where 2.6 is the 97.5th percentile Student's *t* value for 5 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.6 s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.6 s_c$$

If more than 3 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

3.2.5 Matrix Spike and Matrix Spike Duplicate

Results of the MS/MSD analyses performed in the validation study are used to develop the MS/MSD QC acceptance criteria for Tier 2. Each laboratory will measure MS and MSD in one sample. Calculate the MS and MSD performance criteria as follows:

1. Calculate the mean and standard deviation of the recoveries of each MS/MSD pair, and then compute the overall mean recovery $\overline{X}$, the between-laboratory standard deviation of the 3 pairwise means (s_b), and the pooled within-laboratory standard deviation (s_w) for each target analyte.

$$s_b = \sqrt{\frac{\sum_{j=1}^{m} \left(\bar{X}_j - \bar{X}\right)^2}{m - 1}}$$

where:
$\overline{X}_j$ = The mean percent recovery for the *jth* laboratory
m = The number of laboratories, and
$\bar{X}$ = The overall mean of the percent recoveries from all laboratories

$$s_w = \sqrt{\frac{s_1^2 + s_2^2 + s_3^2}{2}}$$

2. In order to allow for interlaboratory variability, calculate the combined standard deviation (s_c) for interlaboratory variability and estimation of the mean as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(1 - \frac{1}{n}\right) s_w^2}$$

where:
m = The number of laboratories, and
n = The number of observations per lab.
For an MS/MSD pair analyzed in each laboratory, n = 2, and s_c becomes:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \frac{1}{2} s_w^2}$$

For m =3 (e.g., three labs), this becomes:

$$s_c = \sqrt{\left(\frac{4}{3}\right) s_b^2 + \left(\frac{1}{2}\right) s_w^2}$$

3. QC acceptance criteria for MS/MSD recovery - Calculate the QC acceptance criteria for recovery in the MS/MSD test by constructing a ± 2.6s_c window around the mean percent recovery ($\overline{X}$) using the combined standard deviation. This factor comes from a *t* value for an estimated 5 degrees of freedom (based on this experimental design and variance ratios observed in Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.6 s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.6 s_c$$

If more than 3 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories plus 2 will serve for most situations.

Note: For highly variable methods, it is possible that the lower limit for recovery for the MS/MSD analyses will be a negative number. In these instances, the data should either be log-transformed and the recovery window recalculated, or the lower limit established as "detected," as was done with some of the methods in 40 CFR Part 136, Appendix A.

4. QC acceptance criteria for MS/MSD relative percent difference (RPD) - To evaluate a 95% upper confidence limit for precision, the RSD (computed using the pooled within-laboratory standard deviation s_w of the MS/MSD samples, divided by $\overline{X}$, is multiplied by the square root of the 95th percentile *F* value with 1 degrees of freedom in the numerator and m degrees of freedom in the denominator multiplied by the square root of 2 ($\sqrt{2}$), where m is the number laboratories. The resulting multiplier on the RSD for 3 laboratories will then be 4.5. The QC acceptance criterion for precision in the MS/MSD test (RPD_{max}) is calculated as follows:

$$\mathrm{RPD_{max}} = 4.5\ \mathrm{RSD}$$

3.2.6 Absolute and Relative Retention Time

Establishing QC acceptance criteria for RT and RRT precision is problematic when multiple laboratories are involved because laboratories have a tendency to establish the chromatographic conditions that suit their needs. Calculating mean RTs and RRTs based on different operating conditions will result in the establishment of erroneously wide windows. Therefore, it is advised that the organization developing the method specify to the participating laboratories the chromatographic conditions and columns to be used. Any future laboratories operating under different conditions will need to develop new acceptance criteria for RT and RRT precision.

Determine the mean retention time, $\overline{\text{RT}}$ (and/or the mean relative retention time $\overline{\text{RRT}}$) and the standard deviation (s) of the RT and/or RRT for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits as follows:

$$Lower\ limit = \overline{RT} - \left(ts \sqrt{1 + \frac{1}{n}} \right)$$

$$Upper\ limit = \overline{RT} + \left(ts \sqrt{1 + \frac{1}{n}} \right)$$

where:
t = The 97.5th percentile of a *t* distribution with n - 1 degrees of freedom, and
n = The number of retention time or relative retention time values used.

The relative retention time upper and lower limits are determined by replacing $\overline{\text{RT}}$ with $\overline{\text{RRT}}$ in the equations above.

3.2.7 Blanks

Establish the QC acceptance criteria for blanks. The usual requirement is that the concentration of an analyte in a blank must be below the ML or below one-third (1/3) the regulatory compliance level, whichever is higher. In instances where the level of the blank is close to the regulatory compliance level or the level at which measurements are to be made, it may be necessary to require multiple blank measurements and establish the QC acceptance criteria based on the mean of the blank measurements plus two standard deviations of the blank measurements.

3.2.8 Labeled Compound Recovery

The labeled compound recoveries from the samples analyzed in the validation study can be used to develop the labeled compound QC acceptance criteria. Calculate the labeled compound performance criteria as follows:

1. Calculate the recovery of each labeled compound for the background analysis sample for each laboratory, and then compute the mean recovery $\bar{X}$, and the standard deviation (s_b) of the recoveries across all three laboratories.

2. QC acceptance criteria for labeled compound recovery - Calculate the QC acceptance criteria for recovery the labeled compound as:

$$Lower\ limit\ (\%) = \bar{X} - (t * s_c * \sqrt{1 + \tfrac{1}{n}})$$

$$Upper\ limit\ (\%) = \bar{X} + (t * s_c * \sqrt{1 + \frac{1}{n}})$$

Where:
t = The 97.5th percentile of a *t* distribution with n - 1 degrees of freedom, and
n = The number of samples analyzed across all laboratories.

For three laboratories each analyzing a single background analysis sample, the equation above simplifies to approximately:

$$Lower\ limit\ (\%) = \bar{X} - 5 * s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 5 * s_c$$

3.2.9 Performance Testing Sample

As noted in Section 2.10, providers of PT samples, SRMs, and CRMs will provide information on the concentrations of target analytes in the material, along with acceptance limits for those concentrations. Whenever such information is provided, it must be used as the basis for the QC acceptance criteria in the new method.

3.3 Quality Control Acceptance Criteria Development for New Methods at Tier 3

In Tier 3, a single sample collected from each of a minimum of nine industrial categories is analyzed in nine separate laboratories (one sample analyzed by each laboratory). QC acceptance criteria is developed for the Tier 3 methods in ways that are analogous to development of these criteria at Tiers 1 and 2, with minor modifications described below.

3.3.1 Method Detection Limits and Minimum Levels

Each laboratory participating in the validation study must perform an MDL study as described in Section 3.1.1. The organization responsible for developing the new method must establish an MDL for the method, using a pooled MDL from the nine laboratories. A pooled MDL is calculated from *m* individual laboratory MDLs by computing the square root of the mean of the squares of the individual MDLs and multiplying the result by a ratio of *t*-values to adjust for the increased degrees of freedom.

Note: The MDL values used in this calculation are those determined in each of the nine laboratories. If one laboratory reports an MDL_s (from spiked samples), that value is used in conjunction with the MDL values from the other laboratories, including any values reported as MDL_b (from blanks).

$$MDL_{pooled} = \sqrt{\frac{d_1 \left(\frac{MDL_{Lab\ 1}}{t_{(0.99,d_1)}}\right)^2 + d_2 \left(\frac{MDL_{Lab\ 2}}{t_{(0.99,d_2)}}\right)^2 + \cdots d_1 \left(\frac{MDL_{Lab\ m}}{t_{(0.99,d_m)}}\right)^2}{d_1 + d_2 + \cdots d_m}} \; x\; t_{(0.99,[d_1 + d_2 + \cdots d_m]}$$

where:

m = The number of laboratories, and
d_i = The number of replicates used by Lab i to derive the MDL.

In the case of 9 laboratories with 7 replicates per laboratory, the equation simplifies to:

$$MDL_{pooled} = \sqrt{\frac{MDL_{Lab\,1}^2 + MDL_{Lab\,2}^2 + \cdots MDL_{Lab\,9}^2}{9}}\ x\ \frac{2.41}{3.14}$$

The organization responsible for developing the method must also use this MDL to develop an ML. Procedures for determining the ML are given in Section 3.1.1.

3.3.2 Calibration Linearity

Once the ML is established, the instrument or analytical system is then calibrated at the ML and a minimum of two additional points to calculate an initial RSD for the response factor and to determine the number of points required for subsequent calibrations (Section 2.1.2). If the initial RSD is < 2%, a one-or two-point calibration can be used (see Section 2.1.2) and it is unnecessary to establish a limit for calibration linearity.

The RSD and the RSD limit for the CF, RF, or RR is determined as follows:

1. Calculate the mean and standard deviation of the CFs, RFs, or RRs for each laboratory.

$$Mean\ Factor = \overline{Factor} = \frac{\sum_{i=1}^{n} Factor_i}{n}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(Factor_i - \overline{Factor})^2}{n-1}}$$

where:
Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and
n = The number of calibration points used in each laboratory.

2. Calculate the relative standard deviation of the CFs, RFs, or RRs of each laboratory and analyte as:

$$RSD_i = 100\ x\ \frac{s_i}{\overline{Factor_i}}$$

where s_i and $\overline{Factor_i}$ are the standard deviation and mean of the CFs, RFs, or RRs for laboratory *i*.

3. Calculate the pooled RSD of the CFs,. RFs, or RRs for each analyte from *all* laboratories. The pooled RSD is calculated as the square root of the mean of the squares of the sample RSDs from each individual laboratory. For example, for nine laboratories, the pooled RSD is calculated as:

$$RSD_{pooled} = \sqrt{\frac{RSD_1^2 + RSD_2^2 + RSD_3^2}{3}}$$

4. Calculate RSD_{max} as the smaller of 35% and:

$$RSD_{max} = k(RSD_{pooled})$$

where:
k = The square root of the 95th percentile of an F distribution with n - 1 degrees of freedom in the numerator and $m(n - 1)$ degrees of freedom in the denominator,
m = The number of laboratories, and
n = The number of calibration points.

For nine laboratories using a three-point calibration, (m = 9, n = 3), the value of k is 1.0, and for nine laboratories using a five-point calibration (m = 9, n = 5), the value of k is 1.6. The maximum allowable specification for RSD_{max} is 35%.

3.3.3 Calibration Verification

As noted in Section 2.2., acceptance limits for calibration verifications can be determined in three different ways, each of which is described below.

The calibration verification criterion may be specified as a maximum relative distance between the mean CF, RF, or RR obtained by a future laboratory's initial calibration ($\overline{\text{Factor}}$) and the CF, RF or RR obtained from its calibration verification standard (Factor_{VER}). The maximum allowable deviation is based on the pooled relative standard deviation (RSD_{pooled}) calculated in Section 3.2.2.

1. Determine k_{VER} by multiplying the 97.5th percentile of a Student's t distribution with (m[n-1]) degrees of freedom times the square root of (1+1/n), where there are n points in the calibration and m laboratories:

$$k_{VER} = t\sqrt{\left(1 + \frac{1}{n}\right)}$$

For a three-point calibration with nine laboratories, the m (n - 1) Student's t value is 2.1, and for a five-point calibration, the Student's t value is 2.0, resulting in combined multipliers of 2.4 for a three-point calibration, and 2.2 for a five-point calibration.

2. The calibration verification criterion for the new method would then be stated as the maximum percent difference as follows:

$$Percent\ Difference = 100\ x\ \left(\frac{Factor_{VER} - \overline{Factor}}{\overline{Factor}}\right) \leq\ k_{VER}\ RSD_{pooled}$$

where:
Factor = The "Factor" terms are replaced by the CF, RF or RR terms, based on the quantitation approach described in the method in question, and

For example, if the calibration verification criterion, calculated as k_{VER} RSD_{pooled}, equals 17%, then the difference between the $\overline{\text{Factor}}$ from the initial calibration and the Factor_{VER} from the calibration verification sample must be less than or equal to 17% of the $\overline{\text{Factor}}$.

When using either the concentration or the recovery approach, the calculations are very similar to those used for the "factor" limits shown above:

3. Calculate the upper and lower QC acceptance criteria for the known concentration of the analyte in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above.

$$Lower\ limit = \ (Lower\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

$$Upper\ limit = \ (Upper\ Percentage\ in\ Step\ 2)\ x\ (Known\ Concentration\ in\ Standard)$$

Alternatively, calibration verification criteria may be specified as the range of acceptable recoveries calculated for the analytes in the calibration verification standard, using the lower and upper percentages calculated in Step 2 above to create a window around 100% recovery.

3.3.4 Initial and Ongoing Precision and Recovery

For the IPR and OPR tests, QC acceptance criteria are calculated using the mean percent recovery and the standard deviation of recovery from the IPR tests of four aliquots of the reference matrix and the OPR test of one aliquot of the reference matrix (for a total of five samples) in nine laboratories. The QC acceptance criteria are developed using the following steps:

1. Calculate the mean percent recovery ($\overline{X}$) for each analyte, based on all data points from all laboratories, the between-laboratory standard deviation (s_b) of the mean results for each of the nine laboratories (standard deviation of the nine laboratory means $\overline{X_1}$ + $\overline{X_2}$ + … $\overline{X_9}$), and the pooled within-laboratory standard deviation (s_w). The value of s_w is calculated as the square root of the mean of all within-laboratory variances. For example, for nine laboratories:

$$s_b = \sqrt{\frac{\sum_{j=1}^{m}\left(\bar{X}_j - \bar{X}\right)^2}{m-1}}$$

where:
$\overline{X}_j$ = The mean percent recovery for the *jth* laboratory
m = The number of laboratories, and
$\bar{X}$ = The overall mean of the percent recoveries from all laboratories

$$s_w = \sqrt{\frac{s_1^2 + s_2^2 + \cdots s_9^2}{9}}$$

Note: The organization responsible for developing the method must ensure that all laboratories are spiking IPR and OPR samples at the same concentration.

2. QC acceptance criteria for IPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right)s_b^2 + \left(\frac{1}{4} - \frac{1}{n}\right)s_w^2}$$

where:
m = the number of laboratories, and
n = the number of data points per laboratory.

For 9 laboratories and 5 data points per laboratory, the calculation becomes:

$$s_c = \sqrt{\left(\frac{10}{9}\right)s_b^2 + \left(\frac{1}{20}\right)s_w^2}$$

3. Calculate the QC acceptance criteria for recovery in the IPR test by constructing a ± 2.3 s_c window around the mean percent recovery $\bar{X}$, where 2.3 is the 97.5th percentile Student's *t* value for 10 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.3s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.3s_c$$

   If more than 9 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

4. QC acceptance criterion for IPR precision - The maximum acceptable RSD for the four IPR aliquots is approximated by a 95% upper confidence limit around the observed RSD of the results from all of the laboratories. The RSD_{IPR} (computed as s_w divided by $\bar{X}$) is multiplied by the square root of a 95th percentile *F* value with 3 degrees of freedom in the numerator and *m* (*n* - 1) degrees of freedom in the denominator, where *m* = the number of laboratories, and *n* is the number of data points per laboratory. For example, the resulting multiplier on the RSD for nine laboratories and five data points per laboratory will then be 1.7, and the QC acceptance criterion for precision in the IPR test is calculated as follows:

$$\text{Maximum } RSD_{IPR} = (1.7)\text{x } RSD_{IPR}$$

5. QC acceptance criteria for OPR recovery - Calculate the combined standard deviation for interlaboratory variability and estimation of the mean (s_c) as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(1 - \frac{1}{n}\right) s_w^2}$$

   where:
   m = the number of laboratories, and
   n = the number of data points per laboratory.

   For 9 laboratories and 5 data points per laboratory,

$$s_c = \sqrt{\left(\frac{10}{9}\right) s_b^2 + \left(\frac{4}{5}\right) s_w^2}$$

6. Calculate the QC acceptance criteria for recovery in the OPR test by constructing a ± 2.1 s_c window around the mean percent recovery $\bar{X}$, where 2.1 is the 97.5th percentile Student's *t* value for 19 degrees of freedom (an estimated degrees of freedom based on the variance ratios observed with EPA Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.1s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.1s_c$$

   If more than 9 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories will serve for most situations.

3.3.5 Matrix Spike and Matrix Spike Duplicate

Results of the MS/MSD analyses performed in the Tier 3 validation study are used to develop the MS/MSD QC acceptance criteria for Tier 3. Calculate the MS/MSD performance criteria as follows:

1. Calculate the mean and sample standard deviation of the recoveries of each MS/MSD pair, and then compute the overall mean recovery $\overline{X}$, the between-laboratory standard deviation (s_b) of the mean results for each of the nine laboratories, and the pooled within-laboratory standard deviation (s_w) for each target analyte using the MS and MSD analyses.

$$s_b = \sqrt{\frac{\sum_{j=1}^{m}\left(\bar{X}_j - \bar{X}\right)^2}{m-1}}$$

where:
$\overline{X}_j$ = The mean percent recovery for the *jth* laboratory
m = The number of laboratories, and
$\bar{X}$ = The overall mean of the percent recoveries from all laboratories
In order to allow for interlaboratory variability, calculate the combined standard deviation (s_c) for interlaboratory variability and estimation of the mean as:

$$s_c = \sqrt{\left(1 + \frac{1}{m}\right)s_b^2 + \frac{1}{2}\,s_w^2}$$

where:
m = the number of laboratories.

For nine labs, this becomes:

$$s_c = \sqrt{\left(\frac{10}{9}\right)s_b^2 + \left(\frac{1}{2}\right)\,s_w^2}$$

2. QC acceptance criteria for MS/MSD recovery - Calculate the QC acceptance criteria for recovery in the MS/MSD test by constructing a ± 2.2s_c window around the mean percent recovery ($\overline{X}$) using the combined standard deviation. This factor comes from a *t* value for an estimated 11 degrees of freedom (based on this experimental design and variance ratios observed in Method 1625):

$$Lower\ limit\ (\%) = \bar{X} - 2.2s_c$$

$$Upper\ limit\ (\%) = \bar{X} + 2.2s_c$$

If more than 9 laboratories are used, the degrees of freedom for *t* will increase, but a complete calculation is beyond the scope of this document. An approximation of degrees of freedom equal to the number of laboratories plus 2 will serve for most situations.

Note: For highly variable methods, it is possible that the lower limit for recovery for the MS/MSD analyses will be a negative number. In these instances, the data should either be log-transformed and the recovery window recalculated, or the lower limit established as "detected," as was done with some of the methods in 40 CFR Part 136, Appendix A.

3. QC acceptance criteria for MS/MSD relative percent difference (RPD) - To evaluate a 95% upper confidence limit for precision, the RSD (computed using the pooled within-laboratory standard deviation s_w of the MS/MSD samples, divided by $\overline{X}$, is multiplied by the square root of the 95th

percentile *F* value with 1 degrees of freedom in the numerator and m degrees of freedom in the denominator multiplied by the square root of 2 (i. e., $\sqrt{2}$), where *m* is the number laboratories. The resulting multiplier on the RSD for 3 laboratories will then be 3.2. The QC acceptance criterion for precision in the MS/MSD test (RPD_{max}) is calculated as follows:

$$RPD_{max} = 3.2\ RSD$$

3.3.6 Absolute and Relative Retention Time

Establishing QC acceptance criteria for RT and RRT precision is problematic when multiple laboratories are involved because laboratories have a tendency to establish the chromatographic conditions that suit their needs. Calculating mean RTs and RRTs based on different operating conditions will result in the establishment of erroneously wide windows. Therefore, it is advised that the organization developing the method specify to the participating laboratories the chromatographic conditions and columns to be used. Any future laboratories operating under different conditions will need to develop new acceptance criteria for RT and RRT precision.

Determine the mean retention time, $\overline{RT}$ (and/or the mean relative retention time $\overline{RRT}$) and the standard deviation (s) of the RT and/or RRT for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits as follows:

$$Lower\ limit = \overline{RT} - \left(ts\sqrt{1 + \frac{1}{n}} \right)$$

$$Upper\ limit = \overline{RT} + \left(ts\sqrt{1 + \frac{1}{n}} \right)$$

where:
t = The 97.5th percentile of a *t* distribution with n - 1 degrees of freedom, and
n = The number of retention time or relative retention time values used.

The relative retention time upper and lower limits are determined by replacing $\overline{RT}$with $\overline{RRT}$ in the equations above.

3.3.7 Blanks

Establish the QC acceptance criteria for blanks. The usual requirement is that the concentration of an analyte in a blank must be below the ML or below one-third (1/3) the regulatory compliance level, whichever is higher. In instances where the level of the blank is close to the regulatory compliance level or the level at which measurements are to be made, it may be necessary to require multiple blank measurements and establish the QC acceptance criteria based on the mean of the blank measurements plus two standard deviations of the blank measurements.

3.3.8 Labeled Compound Recovery

The labeled compound recoveries from the samples analyzed in the validation study can be used to develop the labeled compound QC acceptance criteria. Calculate the labeled compound performance criteria as follows:

1. Calculate the recovery of each labeled compound for the background analysis sample for each laboratory, and then compute the mean recovery $\overline{X}$, and the standard deviation (s_b) of the recoveries across all nine laboratories.

2. QC acceptance criteria for labeled compound recovery - Calculate the QC acceptance criteria for recovery the labeled compound as:

$$Lower\ limit\ (\%) = \bar{X} - (\ t * s_c * \sqrt{1 + \frac{1}{n}})$$

$$Upper\ limit\ (\%) = \bar{X} + (\ t * s_c * \sqrt{1 + \frac{1}{n}})$$

Where:
t = The 97.5th percentile of a *t* distribution with n - 1 degrees of freedom, and
n = The number of samples analyzed across all laboratories.

For nine laboratories each analyzing a single background analysis sample, the equation above simplifies to approximately:

$$Lower\ limit\ (\%) = \bar{X} - \ 2.43 * s_c$$

$$Upper\ limit\ (\%) = \bar{X} + \ 2.43 * s_c$$

3.3.9 Performance Testing Sample

As noted in Section 2.10, providers of PT samples, SRMs, and CRMs will provide information on the concentrations of target analytes in the material, along with acceptance limits for those concentrations. Whenever such information is provided, it must be used as the basis for the QC acceptance criteria in the new method.

REFERENCES

1. *Code of Federal Regulations* (CFR), Volume 40, Part 136, Appendix A, originally published in 1984.

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3. *Federal Register*, Volume 49, Number 209, October 26, 1984.

4. MacDougall, D., et al. 1980, "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry," *Analytical Chemistry*, 52: 2242-2249.

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APPENDIX H METHOD-DEFINED PARAMETERS (MDPs)

This appendix provides the recommended validation requirements associated with new method ATPs for a method-defined parameter (MDP). As noted throughout the main document, these details are provided in this appendix to distinguish the validation requirements for new method ATPs for MDPs more clearly from the validation requirements of new method ATPs for the more traditional analytes.

1.1 Definition of a Method-defined Analyte or Parameter

As defined at 40 CFR 136.6 and noted in Section 1.3.4 in the main body of this document, the term *"method-defined analyte"* means an analyte (or parameter) that is defined solely by the method used to determine the analyte (generically referred to in this document as an MDP). Such an analyte may be a physical parameter, a parameter that is not a specific chemical, or a parameter that may be comprised of a number of substances. Examples include, but are not limited to:

- Acidity,
- Alkalinity,
- Biological oxygen demand (BOD),
- Chemical oxygen demand (COD),
- Color,
- Oil and grease,
- pH (hydrogen ion),
- Temperature,
- Total dissolved solids (TDS),
- Total organic carbon (TOC),
- Total suspended solids (TSS),
- Total phenolics, and
- Turbidity.

New method ATPs that measure MDPs have the potential to change what is being measured. Therefore, *all* new method ATPs that measure MDPs require EPA approval prior to use in NPDES compliance monitoring. Furthermore, the three-tiered validation approach to new method ATPs described in the main body of this document for non-MDPs should not be used in the case of new method ATPs for MDPs. Rather, all new methods for MDPs should be validated and reviewed using the process described in this appendix.

1.2 Approaches to Validation of New Method ATPs for MDPs

EPA would not expect to be able to approve any applications for a new method ATP that failed to establish the suitability of the method to measure the MDP through side-by-side comparison studies using the new method and the EPA-approved reference method. These are necessary to ensure there are no systematic differences in method performance and that the comparison data may be evaluated to ensure that any differences in what is being measured are not masked by between-sample variability.

1.2.1 Tier 1: Side-by-side Comparison for Use in a Single Laboratory

For new method ATPs that measure MDPs and are intended for limited use in a single laboratory (Tier 1), the laboratory should perform and document side-by-side comparison of the new method and the EPA approved reference method. This study should include analysis of a minimum of three replicate samples collected on any seven days over a minimum 30-day period using each method. This will require analysis of a total of 42 field samples (21 by the new method and 21 by the EPA approved reference method for a single matrix study). If the laboratory wishes to use the new method for analysis of more than one matrix

type, a similar model should be used for each additional matrix type up to a maximum of nine matrix types. If the laboratory wishes to use the new method for analysis of any matrix type, the study design should be similar to the Phase I single-laboratory study comparison study described in Section 1.2.3.1 for Tier 3 new methods.

If all six results for a given day associated with any sample are less than the minimum level (< ML) of the reference method, these results should not be used in the comparison because it is necessary to have actual measured values to test equivalency. In the event that a test result less than the ML is obtained, samples should be collected on an additional day (i.e., the number of tests should be increased to provide a minimum of seven paired triplicate results for the comparison).

1.2.2 Tier II: Side-by-side Comparison for Nationwide Use in a Single Matrix Type

Similarly, in the case of new method ATPs that measure MDPs that are intended for nationwide use in a single matrix type (Tier 2), in order to establish its suitability for use, the applicant should provide data from validation studies that are conducted in two phases: a single-laboratory phase that includes side-by-side comparison of the new method and the EPA-approved reference method, and a multi-laboratory phase. In the single-laboratory phase, comparability would be established by performing a statistical comparison of the results obtained from the analysis of minimum of three replicate samples of the appropriate matrix type collected on any seven days over a minimum 30-day period by both the new method and the approved reference method. The single-laboratory comparison study should also include analysis of a proficiency testing sample obtained from an approved vendor and analyzed in triplicate using both the new method and the approved reference method. If the new method single-laboratory data are determined to be generally comparable to those from the approved reference method, then a second phase should be conducted to generate method performance data across multiple laboratories and establish applicable quality control (QC) acceptance criteria.

Given the nature of the side-by-side testing, a carefully prepared validation study plan is an essential component of the validation and approval process for new method ATPs that measure MDPs. The applicant may prepare separate study plans for the two phases of the process, or where practical, a single plan may be developed that supports both phases.

1.2.2.1 Phase I: Side-by-side Comparison in a Single Laboratory

In Phase I of the comparison study, a minimum of three replicate samples of the appropriate matrix type collected on any seven days over a minimum 30-day period should be analyzed in a single laboratory by both the new method and the approved reference method, and will be used to assess whether there is a statistically significant difference between the results produced by the new method and the results produced by the approved corresponding reference method.

The design of the side-by-side comparison is up to the applicant. However, a detailed validation study plan **must be** prepared by the applicant and submitted to EPA for review and comment, and the plan **must be** agreed upon by all parties prior to conducting the comparison study. This will ensure that the plan provides the demonstration necessary for EPA to evaluate the new method's suitability for measurement of the MDP. Although EPA may be consulted for additional guidance during the development of the study plan, it is the applicant's responsibility to write the study plan and submit it to EPA for review. The minimum elements to provide the data necessary for EPA's evaluation for the Phase I study are provided below and summarized in Section 1.2.2.3, Table H-1 of this appendix.

- *Number/Types of Real-World Sample Types*: A minimum of three replicate samples of the appropriate matrix type types should be collected on any seven days over a minimum 30-day period and analyzed by each method. If preparation of multiple spike levels is feasible for the method-defined parameter, then use of multiple spike levels is recommended, but a minimum of **seven** samples per spike level is

expected unless the applicant explains why they are unnecessary. However, in most cases, seven samples are the minimum number needed to capture the expected variability. If spiking is not feasible, a range of samples should be targeted that would be expected to yield background concentrations that vary by at least one order of magnitude.

- *Laboratories*: The Phase I Comparison Study should be performed in a single laboratory to minimize the sources of variability. This laboratory should have familiarity with both the approved method and the new method to ensure that any differences in performance are not the result of inexperience with one or both methods. However, it is important that the validation study accurately reflect the ruggedness of the new method and any limitations regarding clarity of the new method procedures. Therefore, the laboratory should not be affiliated with the applicant.

- *Replication*: The recommended number of replicates to be analyzed per method and sample within the side-by-side study is **three**.

To ensure the laboratory can perform both methods acceptably, the laboratory must meet all QC analysis criteria specified in the approved reference method using both, the approved reference method and new method, prior to the statistical comparisons of the method data. Moreover, the specific statistical tests that will be used to compare the results of the new method with those from the reference method **must be** described in the study plan. See Section 1.3 of this appendix for a discussion of the relevant statistical considerations.

If a statistical assessment indicates that Phase I study results produced by the new method are comparable to those produced by the approved reference method based on the statistical test described in the validation study plan, then the new method will be deemed to be sufficiently comparable to proceed to Phase II of the study.

1.2.2.2 Phase II: Interlaboratory Study

In Phase II of the validation study, results of the analyses of synthetic and real-world samples in **three** laboratories will be used to characterize interlaboratory method performance and establish interlaboratory QC acceptance criteria for the new method. The study design and specific QC tests for the Phase II study will generally follow the guidelines presented for Tier 2 validation as described in Section 4.3 of this document, and acceptance criteria will be developed as described in Appendix G of this document. However, not all QC tests will be applicable to all method-defined parameters. For example, matrix spike samples are not applicable to methods that measure method-defined analytes such as pH or temperature.

Despite careful planning, situations may arise in which the results from one of the three laboratories in the study may not represent the performance of the new method or the other laboratories. Applicants may wish to plan for such a contingency in the Phase II study plan by utilizing more than three laboratories, or by documenting relevant corrective action procedures that all laboratories in the study will use *prior to* repeating study analyses.

Outlier testing is not recommended for either the single-lab or multi-laboratory phases of the study. However, if the applicant has reason to believe that some of the results from the validation study truly do not represent the performance of the method, then they should contact EPA to discuss whether and how an outlier test could be applied.

It is important that Phase II accurately reflect the ruggedness of the new method and any limitations regarding clarity of the new method procedures. Therefore, a vendor or other applicant should not directly assist laboratories participating in Phase II of the study with implementation of the new method procedures or equipment during the course of the study (e.g., the vendor or applicant may provide training and advice to participant laboratories regarding the equipment or methodology *prior to* the start of the

study, but the study samples are to be analyzed by the study participants under "routine" conditions). Direct participation by the vendor or applicant will compromise the results of the study.

1.2.2.3 Analyses Recommended for Both Phases of a Tier 2 Validation Study of a New method ATP for a MDP

The following tables summarize the recommended minimum numbers of analyses involved in both phases of the validation study for a new method ATP involving an MDP

Table H-1a Summary of Validation Recommendations for Tier 2 MDP New Method ATPs – Phase I[1]

Study Phase	Procedure	Number of		Number of Analyses Required					
		Labs	Matrix Samples[2]	Replicates per Matrix Sample[3]	IPR in Reagent Water[4]	PT Sample	MS/MSD[5]	MDL[(6)]	Total
Phase I	New Method	1	7	3	4	1	14	14	54
	Reference Method	1	7	3	4	1	14	14	54

Notes:

(1) Numbers of analyses in this table do not include additional QC tests such as calibration, blanks, etc.
(2) In Phase I, the matrix samples are collected on any seven days over a minimum 30-day period and analyzed using each method.
(3) Each laboratory analyzes each matrix sample in triplicate.
(4) The IPR analyses only apply to MDPs where the approved reference method also includes the IPR test.
(5) Each laboratory analyzes one MS/MSD pair for each matrix sample.
(6) A method detection limit (MDL) test would be performed in each laboratory, using the new method and the approved reference method. As of August 2107, 40 CFR Part 136 Appendix B requires analysis of a minimum of seven spiked samples and seven blanks per laboratory to determine an MDL. Validation studies will comply with most recent MDL study requirements published in Appendix B of 40 CFR Part 136.

Table H-1b. Summary of Recommended Validation Approaches for Tier 2 MDP New Method ATPs – Phase II[(1)]

	Number of		Number of Analyses					
Study Phase	Labs	Matrix types	Back-ground Analysis	IPR-reagent water[(2)]	PT Sample[(3)]	MS/ MSD	MDL[(4)]	Total
Phase II	3	1	3	12	3	6[(5)]	42	66

Notes:

(1) Numbers of analyses in this table do not include additional QC tests such as calibration, blanks, etc.
(2) Initial precision and recovery (IPR) reagent water analyses are used to validate a new method in a clean matrix. The number of IPR analyses is four times the number of laboratories used to validate a method modification because each laboratory performs a four-replicate IPR test.
(3) The proficiency testing (PT) sample should be obtained from a third-party vendor and should be analyzed by each laboratory participating in the study. If sewage sludge or ocean water are matrices of interest, PT samples for those matrices are required as well.
(4) A method detection limit (MDL) test would be performed in each laboratory, using the new method. As of August 2017, 40 CFR Part 136 Appendix B requires analysis of a minimum of seven spiked samples and seven blanks per laboratory to determine an MDL. Validation studies will comply with most recent MDL study requirements published in Appendix B of 40 CFR Part 136.
(5) The MS/MSD analyses would be used to establish MS/MSD recovery and precision for the new method. The number of MS/MSD analyses is two times the number of matrix types tested (i.e., one MS/MSD pair per laboratory).

1.2.3 Tier 3: Side-by-side Comparison for Nationwide Use in Any Matrix Type

New method ATPs that measure MDPs and are intended for nationwide use in all matrix types (Tier 3), shall require validation studies that will be conducted in two phases: a single-laboratory phase that includes side-by-side comparison of the new method and the EPA-approved reference method, and a multi-laboratory phase. In the single-laboratory phase, comparability will be established by performing a statistical comparison of the results obtained from the analysis of various sample types by both the new method and the approved reference method, including the analysis of a proficiency testing sample obtained from an approved vendor. If the new method single-laboratory data are determined to be generally comparable to those from the approved reference method, then a second phase will be conducted to generate method performance data across multiple laboratories and to establish applicable quality control (QC) acceptance criteria.

Given the nature of the side-by-side testing, a carefully prepared validation study plan is an essential component of the validation and approval process for new methods that measure MDPs. The applicant may prepare separate study plans for the two phases of the process, or where practical, a single plan may be developed that supports both phases.

1.2.3.1 Phase I: Side-by-side Comparison in a Single Laboratory

In Phase I of the comparison study, a wide variety of synthetic and real-world samples agreed upon (by EPA and the applicant) prior to analysis will be analyzed in a single laboratory, and will be used to assess whether there is a statistically significant difference between the results produced by the new method and the results produced by the approved corresponding reference method.

The design of the side-by-side comparison is left up to the applicant. However, a detailed validation study plan **must be** prepared by the applicant and submitted to EPA for review and comment, and the plan **must be** agreed upon by all parties prior to conducting the comparison study. Although EPA may be consulted for additional guidance during the development of the study plan, it is the applicant's responsibility to write the study plan and submit it to EPA for review. The minimum requirements regarding the design of the Phase I study are provided below and summarized in Section 1.2.3.3, Table H-2 of this appendix.

- *Number/Types of Real-World Sample Types*: A minimum of **nine** real-world sample types must be collected from a variety of sources and analyzed by each method. To better identify any sample-specific differences between the new method and the approved reference method, analyses should be performed across a wide range of sample types (a list of industrial categories with existing effluent guidelines can be found at: https://www.epa.gov/eg/industrial-effluent-guidelines). If preparation of multiple spike levels is feasible for the method-defined parameter, then use of multiple spike levels is recommended, but a minimum of nine sample types per spike level are required. If spiking is not feasible, a range of sample types should be targeted that would be expected to yield background concentrations that vary by at least one order of magnitude.
- *Laboratories*: The Phase I Comparison Study should be performed in a single laboratory to minimize the sources of variability. This laboratory should have familiarity with both the approved reference method and the new method to ensure that any differences in performance are not the result of inexperience with one or both methods. However, it is important that the validation study accurately reflect the ruggedness of the ATP and any limitations regarding clarity of the ATP procedures. Therefore, the laboratory should not be affiliated with the new method applicant.
- *Replication*: The recommended number of replicates to be analyzed per method and sample within the side-by-side study is **three**.

To ensure the laboratory can perform both methods acceptably, the laboratory must meet all QC analysis criteria specified in the approved method using both, the approved method and new method, prior to the statistical comparisons of the method data. Moreover, the specific statistical tests that will be used to compare the results of the new method with those from the reference method **must be** described in the study plan. See Section 1.3 of this appendix for a discussion of the relevant statistical considerations.

If a statistical assessment indicates that Phase I study results produced by the new method are comparable to those produced by the approved reference method based on the statistical test described in the validation study plan, then the new method will be deemed to be sufficiently comparable to proceed to Phase II of the study.

1.2.3.2 Phase II: Interlaboratory Study

In Phase II of the validation study, results of the analyses of synthetic and real-world samples in **nine** laboratories will be used to characterize interlaboratory method performance and establish interlaboratory QC acceptance criteria for the new method. The study design and specific QC tests for the Phase II study will generally follow the guidelines presented for Tier 3 validation as described in Section 4.3 of this document, and acceptance criteria will be developed as described in Appendix G. However, not all QC tests will be applicable to all method-defined parameters. For example, matrix spike samples are not applicable to methods that measure method-defined analytes such as pH or temperature.

Despite careful planning, situations may arise in which the results from one of the nine laboratories in the study may not represent the performance of the new method or the other laboratories. Applicants may wish to plan for such a contingency in the Phase II study plan by utilizing more than nine laboratories, or by documenting relevant corrective action procedures that all laboratories in the study will use *prior to* repeating study analyses.

Outlier testing is not recommended for either the single-lab or multi-laboratory phases of the study. However, if the applicant has reason to believe that some of the results from the validation study truly do not represent the performance of the method, then they should contact EPA to discuss whether and how an outlier test could be applied.

It is important that Phase II accurately reflect the ruggedness of the new method and any limitations regarding clarity of the new method procedures. Therefore, a vendor or other applicant should not directly assist laboratories participating in Phase II of the study with implementation of the new method procedures or equipment during the course of the study (e.g., the vendor or applicant may provide training and advice to participant laboratories regarding the equipment or methodology *prior to* the start of the study, but the study samples are to be analyzed by the study participants under "routine" conditions). Direct participation by the vendor or applicant will compromise the results of the study.

1.2.3.3 Analyses Required for Both Phases of a Tier 3 Validation Study of a New Method ATP for an MDP

The following tables summarize the recommended minimum numbers of analyses for both phases of the validation study for a new method ATP involving an MDP.

Table H-2a Summary of Validation Recommendations for Tier 3 MDP New Method ATPs - Phase I[1]

Study Phase	Procedure	Number of		Number of Analyses Required					
		Labs	Matrix types	Replicates per Matrix Type[2]	IPR in Reagent Water[3]	PT Sample	MS/MSD[4]	MDL[(5)]	Total
Phase I	New Method	1	9	3	4	1	18	14	64
	Reference Method	1	9	3	4	1	18	14	64

Notes:

(1) Numbers of analyses in this table do not include additional QC tests such as calibration, blanks, etc. Nine is the maximum number of matrix types that should be used to validate a modified wastewater method at Tier 1 or Tier 3.
(2) In Phase I the laboratory analyzes each of the nine matrix types in triplicate by each method.
(3) The IPR analyses only apply to MDPs where the reference method also includes the IPR test.
(4) In Phase I, the laboratory should analyze one MS/MSD pair for each of the nine matrix types by each method.
(5) A method detection limit (MDL) test would be performed in each laboratory, using the new method and the approved reference method. As of August 2017, 40 CFR Part 136 Appendix B requires analysis of a minimum of seven spiked samples and seven blanks per laboratory to determine an MDL. Validation studies will comply with most recent MDL study requirements published in Appendix B of 40 CFR Part 136.

Table H-2b. Summary of Recommended Validation Approaches for Tier 3 MDP New Method ATPs – Phase II[(1)]

Study Phase	Number of		Number of Analyses					
	Labs	Matrix types	Back-ground Analysis	IPR-reagent water[(2)]	PT Sample[(3)]	MS/ MSD	MDL[(4)]	Total
Phase II	9	9	9	36	9	18[(5)]	126	198

Notes:

(1) Numbers of analyses in this table do not include additional QC tests such as calibration, blanks, etc.
(2) Initial precision and recovery (IPR) reagent water analyses are used to validate a new method in a clean matrix. The number of IPR analyses is four times the number of laboratories used to validate a method modification because each laboratory performs a four-replicate IPR test.
(3) The proficiency testing (PT) sample should be obtained from a third-party vendor and should be analyzed by each laboratory participating in the study. If sewage sludge or ocean water are matrices of interest, PT samples for those matrices are required as well.
(4) A method detection limit (MDL) test would be performed in each laboratory, using the new method. As of August 2017, 40 CFR Part 136 Appendix B requires analysis of a minimum of seven spiked samples and seven blanks per laboratory to determine an MDL. Validation studies will comply with most recent MDL study requirements published in Appendix B of 40 CFR Part 136.
(5) The MS/MSD analyses would be used to establish MS/MSD recovery and precision for the new method. The number of MS/MSD analyses is two times the number of matrix types tested (i.e., one MS/MSD pair per laboratory).

1.3 Statistical Considerations in Evaluating for MDPs

Demonstrating comparability of the results for a new method for a MDP presents a number of challenges for both the applicant and EPA. By their very nature, the results for method-defined parameters are a direct function of the sum of all of the steps in the method used to generate them. Thus, a new method that achieves "better" results for an MDP is *not* an appropriate goal, and common statistical tests such as the Student's *t*-test of mean results, the *F*-test of variances, or an analysis of variance (ANOVA) are *not* useful for MDPs.

For the purposes of evaluating new methods for MDPs, EPA employs the Root Mean Square Deviation (RMSD). The RMSD measures variations in the new method results *both above and below* the results from the reference method. For example, the average results for the new method across all samples may be close to those obtained with the reference method, yet the variability of the new method data may be quite high (results are accurate on average but are imprecise), or the differences between the methods vary widely from sample to sample. The RMSD computes the squared deviation of the results from the new method from the results of the reference method on the same sample, and sums those squared deviations across all the samples in the validation study to provide an overall measure of agreement between the two sets of results (new method and reference method). A generalized formula for the RMSD applicable to a new method evaluation is shown below:

$$RMSD = \sqrt{\frac{\sum_{j=1}^{J}(\bar{X}_{RMj} - \bar{X}_{ATPj})^2}{J}}$$

where: $\bar{X}_{RMj}$ = The "jth" sample mean from the reference method
$\bar{X}_{ATPj}$ = The "jth" sample mean from the new method, and
J = The total number of samples being analyzed by the methods

The calculated RMSD is then compared to the upper limit $RMSD_{max}$, determined using the formula below:

$$RMSD_{\max} = \sqrt{\frac{MSE}{J} * (\sum_{j=1}^{J}\sum_{k=1}^{2}(\frac{1}{n_{jk}})) * F_{(0.95;J,n_T-(2*J))}}$$

where: J = the total number of samples, and
n_{jk}= the number of replicates for sample j and method k,
n_T = the total number of replicates across all samples and methods,
J = the total number of samples, and

MSE = the mean-squared error, as calculated below:

$$MSE = \frac{1}{n_T - (2*J)}\sum_{j=1}^{J}\sum_{k=1}^{2}(n_{jk} - 1) * s_{jk}^2$$

where: s_{jk} = the standard deviation of the replicates for sample j and method k (i.e., where the approved method is method 1 and the new method is method 2),
n_{jk}= the number of replicates for sample j and method k,
n_T = the total number of replicates across all samples and methods, and
J = the total number of samples

Due to the natural variation in the MDP across samples, it is recommended that all results from both methods be log-transformed prior to calculating the RMSD and $RMSD_{max.}$

Using the RMSD, the goal is to demonstrate whether or not there is a statistically significant difference between the performance characteristics of the new method and the reference method[1]. By its derivation, the RMSD sums the deviations in *both* directions (i.e., new method results above the reference method results and those below), rather than looking at the simple "inequality" of the two sets of results. If no

[1] The significance test used in the RMSD is equivalent to an *F*-test of significant difference that tests the compound null hypothesis that the mean log concentration is equal between the two methods, for each sample in the study.

statistically significant difference is observed with the RMSD, then the results for the new method may be judged acceptable.

Another advantage of the RMSD relative to other common statistical tests is that using the other tests will generate a large number of statistical outcomes that would not produce a clear picture of the overall performance of a new method relative to the reference method. For example, for the Phase I study of a new method ATP application for nationwide use, 27 analyses are required (e.g., 9 separate sample matrices, analyzed in triplicate, in a single laboratory). Using t-tests and F-tests to compare the results across even nine samples could well result in a mix of outcomes across all the samples (i.e., 5 samples with statistically significant differences and 4 without such differences). Such a mix of outcomes for the new method would be difficult, if not impossible, to interpret in the context of comparability with the reference method.

1.4 Other Recommendations for New Method ATPs for MDPs

Despite the more rigorous side-by-side testing warranted for new method ATPs for MDPs, all other aspects of the new method development and approval process still apply. For example:

- The applicant should comply with the application in Section 3.2 of this document, use of the application form in Appendix A of this document, and inclusion of the Data Collection Certification form in Appendix B of this document with their validation study report
- The applicant should follow the procedures for proprietary information in Section 3.3 of this document.
- The applicant should document per Section 4.3 the new method in EPA format, provide MDL data and the routine QC operation data described in Sections 4.3.3 to 4.3.10 of this document.
- EPA review, approval, and rulemaking framework described in Section 5 of this document continue to apply

Note: As noted in Section 1.3 of this appendix, demonstrating comparability of the results for a new method ATP for a MDP presents a number of challenges. However, even if the use of the RMSD demonstrates there are not any statistically significant differences between the performance characteristics of the new method ATP and the reference method, EPA may choose not to consider new method ATPs for MDPs that alter the fundamental chemistry of the overall analytical process, including the determinative technique used for measurement of the MDP.

Note: As for all other new method applications, the applicant is responsible for the technical and statistical evaluation of the validation study results and preparation of the study report.

Table H-3. Standardized QC, QC Acceptance Criteria, and Performance Data for Methods for Method-defined Analytes in 40 CFR Part 136, Table IB [1]

Analyte - Detector	Reference Method	Spike conc	Calibration		Calibration Verification		Precision and Recovery					Matrix Spike/Matrix Spike Duplicate (MS/MSD)			Detection or Quantitation Limit	Method Performance	
							Initial (IPR)			Ongoing (OPR)							
							Recovery (%)		Precision	Recovery (%)		Recovery (%)		Precision			
			# Pt	Linearity	Low	High	Low	High	RSD	Low	High	Low	High	RPD	ML	Rec (%)	RSD
Acidity - endpoint	SM 2310B	20 mg/L														100	9
Alkalinity - endpoint	SM 2320B	120 mg/L														93	4.2
BOD_5 - Iodometric	SM 5210B	300mg/L								56	76				LDL 2 mg/L	66	15.4
COD - Spectrophotometric	EPA 410.4	50 mg/L	3				90	110				90	110		Range 3 mg/L	93	14
Color - Spectrophotometric	NCASI 253	100 CU	6	R^2 >0.991	90	110	80	120	10	75	125				MDC 10 CU		
Hydrogen ion - Electrometric	SM 4500-H^+ B	7.3 pH													0.1 pH		SD 0.26 pH
Oil and grease-HEM - Gravimetry	EPA 1664A	40 mg/L	2		Note 2		83	101	11	78	114	78	114	18	5 mg/L	93	8.7
TOC - Persulfate-UV Oxidation	SM 5310C	10 mg/L														93	7
Total solids - Gravimetry	SM 2540B																SD 6.0
Total dissolved solids - Gravimetry	SM 2540C	293 mg/L															7.2
Total suspended solids - Gravimetry	SM 2540D	24 mg/L															10
Temperature - Thermometer	SM 2550B														0.1 °C		

Note 1. Some QC acceptance criteria may not be appropriate for some analytes in this table.

Note 2 Within ±10% of Class S weight at 2 mg and with ±0.5% at 1000 mg

APPENDIX I Checklist for Methods To Be Considered by EPA for Use in Compliance Monitoring Programs under the Clean Water Act

EPA uses the following checklist to evaluate requests for consideration of Alternate Test Procedures (ATPs), new methods, or modified[1] methods for use in Clean Water Act (CWA) compliance monitoring programs. The checklist addresses minimum submission requirements (documented in 40 CFR 136.6 and 136.7 and in the ATP Protocols), as well as other laws, regulations, and policies that EPA staff must consider when evaluating method submissions. Although the checklist is for internal use by EPA, applicants are encouraged to review the checklist to better understand the Agency's process for reviewing and considering applicant submissions. Two attachments are provided at the end of the checklist to assist EPA users and applicants in understanding the requirements. In addition, the checklist contains references to applicable laws, regulations, and policies that are not explicitly covered at 40 CFR 136.6 and 136.7 and in the ATP Protocols.

Reviewer #1, Name and Organization:_____ Reviewer # 1 Initials and Date: _____

Reviewer #2, Name and Organization:_____ Reviewer # 2 Initials and Date: _____

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.

YES	NO	N/A	ITEM OR QUESTION TO BE ADDRESSED	COMMENTS/NOTES
☐	☐	■	**1a. Is this a completed ATP or a new method that has been reviewed by EPA? If yes, indicate type below.** ☐ ATP ☐ New Method	
☐	☐	■	**1b. If no, is this a request for EPA to consider approval of a method from a Voluntary Consensus Standards Body (VCSB) or other Government Agency (or their designated representative)?** ☐ VCSB Method ☐ Other Government Agency Method	

[1] For the purposes of this checklist, the terms modified method, revised method, and updated method are synonymous and are intended to mean any method changes, updates, or revisions that are being submitted to EPA for review and approval at 40 CFR Part 136 to support CWA programs. Due to the increased flexibility allowed for method modifications under 40 CFR 136.6, most method changes, updates, or revisions submitted to EPA will consist of ATP applications for procedures involving method defined analytes, procedures that involve changes to the chemistry of the method, determinative techniques, or applications for consideration of updated versions of previously approved methods.

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.

YES	NO	N/A	ITEM OR QUESTION TO BE ADDRESSED	COMMENTS/NOTES
☐	☐	■	**2. Is a justification provided for consideration of the ATP, new method, or VCSB or other Government Agency method for use in CWA compliance monitoring programs?** This may include advantages over approved method(s) or may state that the method is a revised or updated version of an already approved method.	
☐	☐	■	**3. Is a copy of the method written in standard EPA format included?** (See the *Guidelines and Format* document at https://www.epa.gov/cwa-methods/alternate-test-procedure-documents) **Alternatively, method(s) may be written in another organization's format but must address and reference the topics specified below in Attachment A and Attachment B.** ☐ EPA Format ☐ Other Format that Addresses topics below and Attachments A and B	
☐	☐	■	**4. Does the method include all appropriate quality control (QC) elements or are they included as part of a compendium and referenced in the method?** *(see 40 CFR 136.7, reprinted as Attachment B to this checklist, for a list of required QC elements*) ☐ Included in method ☐ Included in compendium and referenced in method	
☐	☐	■	**5. Does the method specify acceptance criteria for required QC tests equal to or better than the method currently approved at 40 CFR Part 136?**	
☐	☐	■	**6a. Does the method include a unique method number and date/revision date?**	
☐	☐	☐	**6b. For methods submitted by a VCSB or another Government Agency, does the method contain a revision date or date of approval?** *Enter N/A if the application is not for a VCSB or other Government Agency method.*	
☐	☐	☐	**7. Is a copy of the approved reference method (with red-line strikeouts and additions) enclosed if the application is for a modified method or a revised version of an approved method?** *This applies to method modifications/revisions. Enter N/A if the submission is for a new method application.*	
	☐	☐	**8. Would utilization of the method be practical and comply with existing law and be compatible with agency and departmental missions, authorities, priorities and budget resources? [**stipulated by the National Technology Transfer Advancement Act, 15 USC 3701 et seq. (1996)] *This applies to VCSB applications. Enter N/A if the application is not for a VCSB method.*	

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.				
YES	**NO**	**N/A**	**ITEM OR QUESTION TO BE ADDRESSED**	**COMMENTS/NOTES**
☐	☐	☐	**9. If the method is from a VCSB or other Government Agency is the method in its final form and has it been approved/published by that VCSB or Government Agency?** *This applies to VCSB and other Government Agency methods only. Enter N/A for all other types of applications.*	
			Questions 10a through 10c address method validation study plans. These requirements only apply to new method applications, applications for methods involving method-defined parameters, and other ATP applications that go beyond the modifications explicitly allowed at 40 CFR 136.6. *Enter N/A to questions 10a through 10 c if the application is for an update to a previously approved method and the revisions do not affect the chemistry of the method, determinative technique or QC acceptance criteria.*	
☐	☐	☐	**10a. Was EPA consulted or did EPA participate in the development of the original study plan for validation of the method?**	
☐	☐	☐	**10b. If EPA was consulted or participated in the development of the original study plan for validation of the method, does the application include written documentation of EPA's participation** (e.g., copies of correspondence and records of any verbal communications with EPA staff by phone or in meetings)**?**	
☐	☐	☐	**10c. If EPA was consulted or participated in the development of the original study plan for validation of the method, were all EPA recommendations incorporated into the study plan?** *If yes, this must be documented in writing. If no, the submission should include a written explanation regarding EPA recommendations that were not adopted.*	
☐	☐	☐	**11. Is a copy of the validation study plan with validation study report and reference to the organization's study data requirements provided?** *Enter N/A if the application is for an update to a previously approved method and the revisions do not affect the chemistry of the method, determinative technique or QC acceptance criteria (including the Method Detection Limit).* *A "yes" answer is required for consideration of new method applications, applications for methods involving method-defined parameters, and other ATP applications that go beyond the modifications explicitly allowed at 40 CFR 136.6.*	

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.				
YES	**NO**	**N/A**	**ITEM OR QUESTION TO BE ADDRESSED**	**COMMENTS/NOTES**
			Questions 12a through 12j address method validation study reports and supporting documentation. These requirements only apply to new method applications, applications for methods involving method-defined parameters, and other ATP applications that go beyond the modifications explicitly allowed at 40 CFR 136.6. A yes answer is required for such applications. *Enter N/A to questions 12a through 12j if the application is for an update to a previously approved method and the revisions do not affect the chemistry of the method, determinative technique or QC acceptance criteria.*	
☐	☐	☐	**12a. Are supporting data documenting the Method Detection Limit (MDL) was determined as a part of the method validation study provided?** *Note:* EPA requires that all methods approved at 40 CFR Part 136, including ATPs, be supported by an MDL determined as specified at 40 CFR Part 136, Appendix B. This includes VCSB and other Government Agency methods, even if those organizations normally use other approaches for defining and determining detection limits.	
☐	☐	☐	**12b. Does the method validation include real world samples?** (*see list of effluent guidelines promulgated by EPA, sorted by industry category,* https://www.epa.gov/eg/industrial-effluent-guidelines)	
☐	☐	☐	**12c. Was the method validated to demonstrate compliance with existing analyte concentration ranges, sample collection, preservation, preparation and holding time requirements of the approved method?** (Data demonstrating compliance should be included in the submission)	
☐	☐	☐	**12d. Are quantitation range and limits supporting data provided?** A quantitation range corresponds to the range of analyte concentration (or other quantity) characterized for measurement accuracy (trueness and precision) during method validation**. (***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	
☐	☐	☐	**12e. Are supporting data that address instrument calibration provided?** The performance characteristic is sometimes referred to as "instrument linearity." **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.				
YES	**NO**	**N/A**	**ITEM OR QUESTION TO BE ADDRESSED**	**COMMENTS/NOTES**
☐	☐	☐	**12f. Are supporting data that address bias/trueness provided?** Trueness is a performance characteristic that addresses sources of known systematic error and bias is a measure of trueness. **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	
☐	☐	☐	**12g. Are supporting data that address precision (repeatability and reproducibility) provided?** Precision is a performance characteristic that reflects sources of random error in a measurement process. Methods designed for demonstrating compliance with regulatory requirements should be evaluated for both repeatability (within lab) and reproducibility (among labs). **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	
☐	☐	☐	**12h. Are data demonstrating method selectivity provided?** Selectivity is a performance characteristic that demonstrates the ability of the method to yield useful data for the analytes, analytes levels and matrices defined within the scope of the method. Selectivity is demonstrated by providing information that substantiates the identity of the analyte in presence of expected matrix constituents. **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	
☐	☐	☐	**12i. Are data demonstrating method ruggedness provided?** Ruggedness refers to the capacity of analytical method to remain unaffected by small variations in operating conditions or environmental conditions. The changes should reflect expected, reasonable variations that are likely to be encountered in different labs. **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	
☐	☐	☐	**12j. Are interlaboratory study/studies as defined in the ATP and New Method Protocols documents provided?** Interlaboratory studies determine whether an analytical method can be transferred for use in other laboratories and used for regulatory testing. Data from the interlaboratory study should be reported in tabular form and the raw data should be maintained and available for review. If appropriate, there should be a discussion describing the details of, and rationale for, any changes made to the method resulting from the interlaboratory study. **(***see* "Chemical Methods Validation and Peer Review Guidelines (PDF)" at https://www.epa.gov/measurements/method-validation-and-peer-review-policies-and-guidelines)	

Use the check boxes to identify if following items were submitted or indicated. The Not Applicable (N/A) box may not be used to answer a question if it is blacked out.				
YES	**NO**	**N/A**	**ITEM OR QUESTION TO BE ADDRESSED**	**COMMENTS/NOTES**
			Questions 13a through 13g address applications for methods involving method-defined parameters. A yes answer is required for such applications. *Enter N/A to questions 13a through 13g if the application does not involve method defined parameters.*	
☐	☐	☐	**13a. Is the request for one or more well-defined analytes that are NOT a § 136.6 Method Defined Parameter? The following is a list of some Method Defined Parameters: Acidity, Alkalinity, BOD5, COD, Color, Oil & Grease, Total Solids, Total Dissolved Solids, Total Organic Carbon, Total Suspended Solids, Total Phenols, Temperature, pH. Other parameters may be added at EPA's discretion.**	
☐	☐	☐	**13b. If the request is for a § 136.6 Method Defined Parameter, does the application include 1) comparative raw data resulting from side-by-side split sample or grab sample analyses performed in triplicate using both the new method and the approved method in a minimum of 9 distinct real world matrix types and 2) data from all required QC analyses performed using each method?**	
☐	☐	☐	**13c. If the request is for a § 136.6 Method Defined Parameter, is the chemistry or determinative step the same as the approved method?**	
☐	☐	☐	**13d. If the request is for a § 136.6 Method Defined Parameter (MDP) AND the chemistry or determinative step is different than the approved method, are the chemistry and determinative step used to identify and measure the MDP well explained and clearly defined as well as any potential interferences or difficulties with the method?**	
☐	☐	☐	**13e. Are data provided from a routinely run, freshly prepared method calibration curve that was used to quantify the analyte(s) in the samples analyzed as part of the validation study, including verification of the calibration curve using independent second source, quality certified, traceable standards?**	
☐	☐	☐	**13f. If the request is for a § 136.6 Method Defined Parameter, do the data submitted demonstrate comparable performance of the new method to the approved method?** *Note:* Comparable performance is determined by comparing the achievement of statistical RMSD comparability between the new method and the approved method from analyses of samples from a minimum of 9 distinct real world matrix types (split or grab - collected and analyzed at the same time), performed in triplicate AND by comparison of the QC acceptance criteria of the two methods.	
☐	☐	☐	**13g. Will the new method and the approved method measure the same forms and species of analyte?**	

Attachment A
Topics to be Covered in Written Method Submission

Scope and Application - This section of the method should clearly state the analyte(s) determined and the types of matrices to which the method is applicable. This section also may list the detection limit of the method and the range of concentrations over which the method is applicable.

Summary - This section briefly states the sample preparation (if any) and the underlying chemistry and determinative technique used in measurement of the target analyte(s). It also may list the method detection limit and the range of concentrations over which the method is applicable.

Definitions - This section should define the terms and abbreviations that are used in the method. The section should include definitions for abbreviations, especially those that relate to quality control, for example: LRB – Laboratory Reagent Blank, LFB – Laboratory Fortified Blank, LFM – Laboratory Fortified Matrix, MS and MSD – Matrix Spike and Matrix Spike Duplicate, MDL – Method Detection Limit, and QCS – Quality Control Sample.

Interferences - This section should identify common interferences, and where applicable, list ways to eliminate, reduce, or overcome them. Of particular note are interferences that may lead to loss or under reporting of target analyte(s).

Safety - This section should adequately address any safety concerns associated with the performance of the method (e.g., toxicity, carcinogenic reagents, or explosion risks).

Equipment and Supplies - This section should list all equipment (apparatus) and supplies to perform the procedures of the method.

Reagents and Standards - This section of the method should clearly list all reagents and standards needed to perform the analysis. It also may detail both preparation and storage of stock standard solutions from neat materials and preparation and storage of working standard solutions.

Sample Collection, Preservation and Storage - This section should list the proper types of sample containers, preservation techniques and holding times per the requirements of 40 CFR 136.3, Table II.

Quality Control - This section should list the minimum QC requirements and acceptance criteria for each of the QC tests applicable to the method (see 40 CFR 136.7 for a listing of QC elements that are required where applicable).

Calibration and Standardization - This section of the method should list the procedures for calibration of the instrument and the type of calibration used (i.e., linear, 2^{nd} order). It should specify a sufficient number of standards used to establish linearity or to clearly define any non-linear portion of the curve. This section also may specify procedures for periodic verification of calibration standards and specify acceptance criteria listed for calibration verification.

Procedure - This section should contain all of the critical steps required to perform the analysis of samples. If sample preparation steps such as distillation, digestion, or pH adjustment are required prior to analysis these steps should also be specified or referenced.

Data Analysis and Reporting - This section should explain how to calculate and report sample results. A statement indicating that only results that fall between the lowest and highest calibration standards should be reported unless the result is flagged as an estimated value. In addition, a statement should be included that samples with results exceeding the highest calibration standard should be diluted and re-analyzed.

Method Performance – This section should present any data or other information that demonstrate or indicate the expected performance characteristics of the method.

Pollution Prevention - This section should contain information on minimizing or preventing pollution known to be potentially attributable to use of the method.

Waste Management - This section should contain information on the minimization and proper disposal of any hazardous wastes known to be generated by use of the method?

References – This section should cite proper references and sources used in the development of the method. References should be restricted to associated or source material.

Tables, Diagrams, and Validation Data - This section of the method should contain all method tables and figures (diagrams and flowcharts). If performance data are included here, they should support the MDL, method range and QC acceptance criteria listed in the method.

Attachment B
40 CFR 136.7 Quality Assurance and Quality Control

The permittee/laboratory shall use suitable QA/QC procedures when conducting compliance analyses with any Part 136 chemical method or an alternative method specified by the permitting authority. These QA/QC procedures are generally included in the analytical method or may be part of the methods compendium for approved Part 136 methods from a consensus organization. For example, *Standard Methods* contains QA/QC procedures in the Part 1000 section of the Standard Methods Compendium. The permittee/ laboratory shall follow these QA/QC procedures, as described in the method or methods compendium. If the method lacks QA/QC procedures, the permittee/ laboratory has the following options to comply with the QA/QC requirements:

(a) Refer to and follow the QA/QC published in the ‘‘comparable’’ EPA method for that parameter that has such QA/QC procedures;

(b) Refer to the appropriate QA/QC section(s) of an approved Part 136 method from a consensus organization compendium;

(c)(1) Incorporate the following twelve quality control elements, where applicable, into the laboratory’s documented standard operating procedure (SOP) for performing compliance analyses when using an approved Part 136 method when the method lacks such QA/QC procedures. One or more of the twelve QC elements may not apply to a given method and may be omitted if a written rationale is provided indicating why the element(s) is/are inappropriate for a specific method.

(i) Demonstration of Capability (DOC);
(ii) Method Detection Limit (MDL);
(iii) Laboratory reagent blank (LRB), also referred to as method blank (MB);
(iv) Laboratory fortified blank (LFB), also referred to as a spiked blank, or laboratory control sample (LCS);
(v) Matrix spike (MS) and matrix spike duplicate (MSD), or laboratory fortified matrix (LFM) and LFM duplicate, may be used for suspected matrix interference problems to assess precision;
(vi) Internal standards (for GC/MS analyses), surrogate standards (for organic analysis) or tracers (for radiochemistry);
(vii) Calibration (initial and continuing), also referred to as initial calibration verification (ICV) and continuing calibration verification (CCV);
(viii) Control charts (or other trend analyses of quality control results);
(ix) Corrective action (root cause analysis);
(x) QC acceptance criteria;
(xi) Definitions of preparation and analytical batches that may drive QC frequencies; and
(xii) Minimum frequency for conducting all QC elements.

(2) These twelve quality control elements must be clearly documented in the written standard operating procedures (SOP) for each analytical method not containing QA.